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Dietary Aspects in the Diagnosis and Treatment of Coeliac Disease

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ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the lecture room of Finn-Medi 5,
Biokatu 12, Tampere, on April 27th, 2007, at 12 o'clock.

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION
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Tampere University Hospital, Department of Gastroenterology and Alimentary Tract Surgery
Finland

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taju@uta.fi
www.uta.fi/taju
http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 1220
ISBN 978-951-44-6891-9 (print)
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 606
ISBN 978-951-44-6892-6 (pdf)
ISSN 1456-954X
http://acta.uta.fi

Tampereen Yliopistopaino Oy – Juvenes Print
Tampere 2007

To My Family

Contents

List of Original Publications

This thesis is based on the following original publications, referred to in the text by the Roman numerals I-IV

I Peräaho M, Kaukinen K, Mustalahti K, Vuolteenaho N, Mäki M, Laippala P, Collin P (2004). Effect of an Oats-Containing gluten-free diet on Symptoms and Quality of Life in Coeliac Disease. A Randomized Study. Scand J Gastroenterol;39:27-31. (Reprinted with permission from Taylor & Francis.) www.tandf.no/gastro

II Peräaho M, Collin P, Kaukinen K, Kekkonen L, Miettinen S, Mäki M (2004). Oats Can Diversify a gluten-free diet in Celiac Disease and Dermatitis Herpetiformis. J Am Diet Assoc;104:1148-1150. (Reprinted with permission from Elsevier Publishing.)

III Peräaho M, Kaukinen K, Paasikivi K, Sievänen H, Lohiniemi S, Mäki M, Collin P (2003). Wheat-starch-based gluten-free products in the treatment of newly detected coeliac disease: prospective and randomized study. Aliment Pharmacol Ther 17;587-594. (Reprinted with permission of Blackwell Publishing.)

IV Kaukinen K, Peräaho M, Collin P, Partanen J, Woolley N, Kaartinen T, Nuutinen T, Halttunen T, Mäki M, Korbonay-Szabo I (2005). Small-Bowel Mucosal Tranglutaminase 2-Specific IgA Deposits in Coeliac Disease without Villous Atrophy: A Prospective and Randomized Clinical Study. Scand J Gastroenterol;40:564-572. (Reprinted with permission from Taylor & Francis.) www.tandf.no/gastro

Abbreviations

AGA	Antigliadin antibodies
ARA	Antireticulin antibodies
BMD	Bone mineral density
CI	Confidence interval
DEXA	Dual energy X-ray absorptiometry
EATL	Enteropathy-associated T-cell lymphoma
ELISA	Enzyme-linked immunosorbent assay
EmA	Endomysium antobodies
ESPGAN	European Society of Pediatric Gastroenterology and Nutrition
GFD	Gluten-free diet
GSRS	Gastrointestinal Symptom Rating scale
HLA	Human leukocyte antigen
IEL	Intraepithelial lymphocyte
PGWB	Psychological General Well-Being
SDS	Self-rating Depression Scale
SF-12	Short Form 12 Healthy Survey
SF-36	Short Form 36 Healthy Survey
STAI	State and Trait Anxiety
tTg	Tissue transglutaminase
Vh/CrD	Villous height and crypt depth ratio

Abstract

Coeliac disease is a chronic inflammatory disorder of the small intestine induced by dietary proteins in wheat, rye and barley. Traditionally, the diagnosis of the disease is made by showing gluten-dependent villous atrophy in small bowel mucosal specimens. The mucosal damage develops from inflammation to crypt hyperplasia and finally to villous atrophy. The treatment of coeliac disease is a strict life-long gluten-free diet.

In Finland oats have been accepted for more than 10 years as part of an otherwise gluten-free diet (GFD), but it has never been shown how patients with coeliac disease have adopted oats, and what is the impact of an oats-containing diet on the quality of life.

Wheat is clearly toxic for coeliac patients, but open questions remain as to its role in the diagnosis and treatment of the disease. It has been debatable whether trace amounts of gluten are harmful; such contaminations are possible for instance in wheat starch-based products which have been rendered gluten-free. It is also evident that demonstrating gluten dependency is an essential part of coeliac diagnosis in borderline cases.

The aim of this study was to establish how oats affects the dietary treatment of coeliac disease, the focus being on quality of life, and how patients nationwide have adopted the use of oats. In addition, the safety of the wheat-starch-based GFD in the treatment of coeliac disease was assessed. Further, the role of gluten or GFD in the diagnosis and treatment of early developing coeliac disease, when mucosal atrophy is not yet evident, was investigated.

To evaluate the effect of oats-containing diet on symptoms, histology and quality of life, 39 biopsy-proven treated coeliac disease patients were randomized to take either 50 g of oats-containing gluten-free products for one year, or to continue their current diet which did not contain oats. Quality of life and gastrointestinal symptoms were measured by the Psychological General Well-Being questionnaire (PGWB) and Gastrointestinal Symptom Rating Scale (GSRS), respectively.

Small bowel mucosal morphology, serum endomysial (EmA) and transglutaminase (tTG) antibodies were assessed. Quality of life did not differ between these study groups, but patients on oats-containing diet suffered significantly more often from diarrhoea. This notwithstanding, there was a simultaneous trend toward more severe constipation in the oats-group than in individuals on the traditional diet. Mucosal integrity was not disturbed by oats. There were more mucosal intestinal intraepithelial lymphocytes (IELs) in the oats group, but no correlation between symptoms and inflammation.

One thousand out of 14 000 members of the Finnish Coeliac Society were randomly chosen to evaluate how they had adopted oat in their GFD, and how they felt that oats diversified the diet: Altogether 710 responded: 73% of coeliac disease and 55% of dermatitis herpetiformis patients were currently using oats. More than 80% of patients using oats appreciated the taste, felt that oats constituted an important part of the diet that the availability of oats was good at low costs, and 94% thought that oats diversified their GFD. Fourteen per cent of patients had stopped taking oats, mostly due to adverse symptoms. The fear of contamination and side-effects was the most common reason in those who had not started to take oats.

Fifty-seven newly detected coeliac disease patients were randomized to receive a wheat-starch-based or naturally GFD. Clinical response, quality of life evaluated by standardized questionnaire, small-bowel mucosal morphology, serology and bone mineral density (BMD) were measured before and after one year on GFD. Wheat-starch-based gluten-free products were well tolerated and there were no differences between the groups in the measured parameters.

A prospective randomized study was carried out to assess whether patients with suspected coeliac disease and increased density of γδ+IELs have a gluten sensitive disorder. Forty-one adults with increased density of γδ+IELs without villous atrophy were randomized to gluten challenge or GFD treatment. Small bowel morphology, serum EmA and tTG-ab were assessed before and after a follow-up of six months. A new clinical score was introduced to measure gluten dependency; it

comprised data on symptoms, associated conditions, family history, serology and histology before and after the dietary intervention. Five patients in the challenge group and six in the gluten-free group were considered to be clinically gluten-sensitive; all of them had human leucocyte antigen (HLA) DQ2 or DQ8. Tissue transglutaminase 2-targeted mucosal IgA deposits have been shown to be a reliable marker of gluten-sensitivity even in the absence of villous atrophy; 10 out of the 11 clinically sensitive subjects had these mucosal antibodies.

According to the prospective study (**I**) and the nationwide inquiry (**II**), oats was widely accepted and well tolerated, and patients felt that oats diversified the GFD. Oats may induce abdominal complaints in some patients, and physicians should be aware of this. Nevertheless, oats can be safely included in a GFD in the majority of coeliac patients.

This study showed that a wheat starch-based GFD was well tolerated despite possible trace amounts of gluten present in the products in question. By gluten-free treatment or challenge it is possible to reveal clinical gluten sensitivity even in patients who do not (yet) fulfil the current diagnostic criteria for coeliac disease. It is time to widen the diagnostic criteria from small bowel mucosal atrophy towards genetic gluten intolerance. The study also showed that the presence of transglutaminase 2-targeted mucosal IgA deposits, being a good diagnostic tool even in developing coeliac disease, is associated with gluten dependency. Which patients with genetic gluten intolerance without villous atrophy will benefit from GFD is a subject for further studies.

Tiivistelmä

Keliakiassa vehnän, ohran ja rukiin valkuaisaineet aiheuttavat ohutsuolen limakalvolle tulehduksen ja suolinukan madaltumisen (villusatrofia). Perinteisesti diagnoosi onkin perustunut ohutsuolen limakalvolta otettuun koepalaaan. Suolivaurio kehittyy kuitenkin asteittain tulehduksesta täydelliseen villusatrofiaan.

Keliakian hoitona on elinikäinen tiukka gluteeniton dieetti, missä tulee välttää vehnää, ohraa ja ruista. Kaura on hyväksytty Suomessa yli kymmenen vuotta osaksi keliakiadieettiä. Ei ole tutkimustietoa siitä, kuinka laajasti kaura on otettu käyttöön ja miten se vaikuttaa potilaiden elämänlaatuun.

Tutkittaessa kauran merkitystä elämänlaatuun, oireisiin ja limakalvomuutoksiin, satunnaistettiin 39 pitkään hoidossa olleita keliakiapotilasta käyttämään vuoden ajan 50 grammaa kauraa tai jatkamaan entisellä kaurattomalla dieetillä. Elämänlaatua selvitettiin Psycholocigal General Well-Being ja suolisto-oireita Gastrointestinal Sympton Rating Scale kyselyillä; lisäksi tutkittiin ohutsuolikoepalat ja keliakiavasta-aineet. Kauran käyttö ei vaikuttanut elämänlaatuun. Kauraa käyttävillä oli tilastollisesti merkitsevästi enemmän ripulia; samalla myös ummetusoireet keskimäärin lisääntyivät, mutta ei merkittävästi. Lisäksi kauraryhmässä oli ohutsuolikoepaloissa nähtävissä enemmän tulehdusta, mutta tulehdus ja oireet eivät korreloineet keskenään. Villusmuutoksia ei kehittynyt kumpaankaan ryhmään seuranta-aikana.

Suomen keliakialiitossa, johon eninosa Suomen keliaakikoista kuuluu, on 14 000 jäsentä, näistä tuhannelle satunnaisesti valikoidulle lähettiin kysely, jolla selvitettiin kuinka laajasti kauraa käytetään ja monipuolistaako kaura dieettiä. Kaikkiaan 710 jäsentä vastasi. Kauraa käytti säännöllisesti 73% keliaakikoista ja 55% ihokeliaakikoista; 94% vastanneista kokivat kauran monipuolistavan gluteenitonta dieettiä ja pitivät kauraa tärkeänä lisänä. Lisäksi yli 85% arvosti kauran saatavuutta, makua ja halpaa hintaa. Tavallisin syy välttää kauraa oli pelko gluteenijäämistä

ja mahdollisista sivuvaikutuksista. Kauran käytön oli lopettanut 14% vastanneista – syynä olivat erilaiset oireet, kuten vatsavaivat, ja osalla myös ihottuma.

On ollut kiistanalaista, voiko gluteeniton dieetti sisältää pieniä jäämiä gluteenia, joita voi esiintyä esimerkiksi vehnätärkkelysjauhoissa, jotka ovat teollisesti puhdistetussa gluteenittomiksi. Tämän tutkimuksen tarkoituksena oli selvittää ovatko vehnätärkkelyksen sisältämät mahdolliset pienet gluteenimäärät haitallisia keliaakikolle. Lisäksi tutkittiin, voidaanko gluteenialtistusta tai gluteenitonta dieettiä käyttää diagnostiikassa, kun villusatrofiaa ei ollut vielä todettu.

Yhteensä 57 uutta keliaakikkoa satunnaistettiin käyttämään pieniä gluteenimääriä sisältävää vehnätärkkelystä tai täysin gluteenitonta dieettiä. Ennen ruokavaliohoidon aloittamista ja vuoden seuranta-ajan jälkeen potilailta katsottiin ohutsuolikoepalat, keliakiavasta-aineet, elämänlaatu ja luuntiheys. Vehnätärkkelyspohjainen gluteeniton dieetti oli hyvin siedetty ja turvallinen, eikä näiden kahden ryhmän välillä ollut eroa mitattaessa edellä mainittuja muuttujia.

Gluteeniherkkyyttä selvitettiin satunnaistamalla 41 potilasta samaan ylimääräistä gluteenialtistusta tai aloittamaan gluteeniton dieettihoito. Näillä potilailla epäiltiin keliakiaa, ohutsuolen limakalvolla olivat epiteelin γδ+tulehdussolut lisääntyneet, mutta villusatrofiaa ei voitu todeta. Ohutsuolen tulehdussolut ja keliakiavasta-aineet tutkittiin ennen ja kuuden kuukauden altistuksen tai hoidon jälkeen. Tutkimuksessa tehtiin kliininen arvio selvittämään gluteeniherkkyyttä – tähän kuului oireiden, vasta-aineiden ja histologian vaste ruokavaliolle, ja lisäksi sukuhistoria sekä liitännäissairaudet. Viisi potilasta altistusryhmässä ja kuusi hoitoryhmässä todettiin kliinisesti ja koepalalla arvioiden gluteeniherkiksi. Kaikilla heillä oli keliakiaan sopiva perimä. Tulokset tarkistettiin uudella menetelmällä, jossa keliakiapotilaan ohutsuolen limakalvolta määritetään transglutaminaasi 2 spesifisiä IgA kertymiä: tutkituista yhdestätoista kaikilla paitsi yhdellä todettiin näitä kertymiä. Löydös yhdessä keliakiaan sopivan perimän kanssa vahvistaa näiden potilaiden sairastavan alkavaa keliakiaa.

Käytettyjen mittarien mukaan kaura ei vaikuta keliakiapotilaiden elämänlaatuun, mutta se on laajasti otettu käyttöön osana gluteenitonta dieettiä, hyvin siedetty ja monipuolistaa dieettiä. Kliinikoiden tulee kuitenkin muistaa, että kaura voi aiheuttaa joillekin potilaille oireita, mitkä eivät kuitenkaan vaikuta liittyvän ohutsuolivaurioon. Kaura voidaan useimmiten turvallisesti liittää osaksi gluteenitonta hoitoa. Vehnätärkkelyspohjainen gluteeniton dieetti on hyvin siedetty. Gluteenittomalla hoidolla tai altistuksella on mahdollista osoittaa gluteeniherkkyys potilailla, joilla ei vielä voida todeta suolinukan madaltumista. Transglutaminaasi-2 spesifiset IgA kertymät ohutsuolen limakalvolla vahvistavat kliinistä arviota. Keliakiamääritelmää on aiheellista laajentaa villusatrofiasta kohti geneettistä gluteeni-intoleranssia.

Introduction

Coeliac disease is an autoimmune condition caused by an inappropriate immune response to gluten proteins in wheat, rye and barley. In genetically susceptible individuals, the ingestion of these proteins results in characteristic small bowel inflammation, mucosal damage and villous atrophy with crypt hyperplasia (Mäki and Collin 1997). The lesion is reversible provided that a GFD is strictly adhered to. It was over 50 years ago when the Dutch paediatrician W.K. Dicke recognized the harmful effect of wheat (Dicke 1950). Dieting is still the only treatment for the disease. It is controversial whether minor dietary transgressions or trace amounts of contaminated gluten are harmful.

Oats were considered toxic and harmful up to the year 1995. This conception was based on sparse evidence only. The difference in cereal chemistry indicated that oats might perhaps not be toxic in coeliac disease. The first prospective and randomized study was carried out by Finnish researchers in Kuopio (Janatuinen et al. 1995). They showed that an oats-containing GFD did not hamper the recovery or induce small-bowel mucosal damage. Subsequently their findings have been supported by other researches indicating that patients with coeliac disease and dermatitis herpetiformis tolerate oats (Reunala et al. 1998). Despite this, the use of oats as part of a gluten–free diet is not widely recommended in the United States or in many European countries. Apart from the fear of contamination, there is some suspicion that oats in itself might induce symptoms or even mucosal damage.

Wheat-starch-based products are used in some European countries as part of the GFD. Industrially purified wheat-starch-based gluten-free products meeting the current Codex Alimentarius Standard are considered gluten-free when they contain less than 0.05 g nitrogen per 100 g of food product on a dry matter basis (Codex-Alimentarius-Commission 1981). This means that these products are allowed to contain residual gluten up to 40-60 mg per 100g (Hekkens 1991, Skerritt and Hill 1992).

The use of these wheat-starch-based gluten-free products has been debated and has remained controversial for many years.

Coeliac disease develops gradually from small bowel mucosal inflammation to crypt hyperplasia and subsequently to overt villous atrophy (Marsh 1992). The current diagnostic criteria for the disease require small bowel mucosal villous atrophy which recovers on a gluten-free diet. An increase in IELs is also a characteristic feature preceding villous atrophy. As a diagnostic tool, intraepithelial lymphocytosis is unspecific, to be found in a variety of disorders (Ferguson and Murray 1971, Kakar et al. 2003). The progress of mucosal deterioration from inflammation to crypt hyperplasia and overt villous atrophy may take many years (Corazza et al. 1996a, Niveloni et al. 2000). It is important to understand this process and to find means of detecting coeliac disease even before the typical small bowel mucosal lesion has developed. Some patients may have gluten-dependent symptoms before the appearance of villous atrophy (Kaukinen et al. 2001). And here the role of gluten challenge and GFD is important. Especially in early developing coeliac disease it is essential to demonstrate that symptoms, minor mucosal lesions and their recovery are dependent on gluten intake or withdrawal in order to avoid over diagnosis (Troncone 1995). In fact, the demonstration of gluten dependence has been included in both earlier and current ESPGAN diagnostic criteria (Meeuwisse 1970, Walker-Smith et al. 1990).

The purpose of this study was to elucidate the role of oats and wheat starch in dietary the treatment of coeliac disease. A further aim was to assess the role of GFD in diagnosis and treatment in early developing coeliac disease, where mucosal atrophy is not yet evident.

Review of the literature

1. Definition of coeliac disease

Coeliac disease, or gluten-sensitive enteropathy, is an autoimmune condition characterised by permanent intolerance to dietary cereals in genetically exposed individuals. The specific protein causing this intolerance is gluten prolamin, the alcohol soluble fraction of wheat and related prolamins in barley and rye (Wieser-98). The typical small-bowel mucosal lesion comprises inflammation in the epithelium and lamina propria, villous atrophy and crypt hyperplasia. The lesions recover when gluten is withdrawn from the diet (Marsh 1992).

The disease does not appear in the small intestine only. The best-known extraintestinal manifestation is dermatitis herpetiformis, an itching papulovesicular skin disease which also responds to GFD (van der Meer 1969, Reunala et al. 1977). A body of evidence has shown that dermatitis herpetiformis to be one manifestation of coeliac disease. Virtually all patients with dermatitis herpetiformis evince small intestinal inflammation or mucosal atrophy indistinguishable from that in coeliac disease (Marks et al. 1966, Reunala et al. 1984). There is also evidence that other extraintestinal organs may be involved in coeliac disease (Collin et al. 2002).

2. Small bowel morphology

The diagnosis of coeliac disease is made from a biopsy specimen of the small bowel mucosa, usually taken by biopsy forceps upon upper gastrointestinal endoscopy (Walker-Smith et al. 1990). The diagnosis is based on small intestinal mucosal atrophy with crypt hyperplasia and inflammation in the epithelium and lamina propria. The mucosal inflammation and damage recover on a GFD (Marsh 1992), the process taking from some months to more than one year. Individual variations

are nevertheless common. In adults a second biopsy is often taken after one year on GFD; at that time the mucosa is not necessarily completely recovered, but unequivocal improvement should be seen.

In subtotal villous atrophy with crypt hyperplasia, the diagnosis is usually easy. In the Western world this finding, especially in adults, is in most cases due to coeliac disease, although there are other conditions where at least partial villous atrophy is possible (Katz and Grand 1979).

3. Current diagnostic criteria

In 1970 the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) issued for the first time the diagnostic criteria for coeliac disease, which required three small-bowel biopsies: the finding of structurally abnormal small-bowel mucosa in a patient ingesting gluten, improvement or normalization of the mucosal lesion on a GFD, and again deterioration during a gluten-containing diet (Weijers et al. 1970). This statement was revised in 1990: the finding of typical small intestinal mucosal atrophy remained mandatory. The effect of a GFD must be proven by clinical response and in asymptomatic coeliac disease patients also by histology. The presence of IgA-class antiendomysial antibodies further supports the diagnosis. Gluten challenge and a third biopsy are needed only exceptionally when the diagnosis has remained uncertain (Walker-Smith et al. 1990). The criteria do not include early mucosal changes, nor do they take into account dermatitis herpetiformis.

4. Widened spectrum of coeliac disease

4.1. Intraepithelial lymphocytes

An increased number of chronic inflammatory cells in the lamina propria and IELs are common in coeliac disease (Roy-Choudhury et al. 1966, Kuitunen et al. 1982). These are not however specific for coeliac disease (Kakar et al. 2003). Ferguson and Murray (Ferguson and Murray 1971) were the first to adopt quantitative measurement, counting IELs per 100 epithelial cells. A level of > 40 IELs per 100 epithelial cells was considered abnormal in the duodenal or jejunal mucosa, but also >25/100 has recently been suggested (Hayat et al. 2002).

Two types of cells populate the normal epithelial layer of the small bowel mucosa, enterocytes and IELs. In a normal mucosa, 80% of the IELs are positive for the cluster design (CD) CD3/CD8+, suggestive of a cytotoxic suppressor cell function, while the remainder are CD 4+ cells, or CD4-, CD8- cells (Tredjosiewics and Howdle 1995). The vast majority of IELs are T cells bearing CD3-TcR complex and 70-85% of these CD3+IELs possess a TcR αβ+, while less than 10% of lymphocytes bear TcR γδ+ (Spencer et al. 1989, Cerf-Bensussan et al. 1997, Groh et al. 1998).

In coeliac disease there is an increased density of IELs, most being CD3+CD8+TcRαβ+ lymphocytes (Brandtzaeg et al. 1989, Cerf-Bensussan et al. 1997). The density of αβ+ IELs decreases to normal on a GFD and increases again upon gluten intake (Savilahti et al. 1990, Savilahti et al. 1992), suggesting that they are activated by gluten peptides.

Several studies have demonstrated a statistically significant increase in the number of IELs bearing TcR γδ+ in coeliac disease and in dermatitis herpetiformis (Halstensen et al. 1989, Spencer et al. 1989, Savilahti et al. 1992). These cells decrease during a GFD, although the process may take even many years (Iltanen et al. 1999a). An increase in the density of TcR γδ+ cells in a patient with normal mucosal morphology have been seen to precede the development of small-bowel villous

atrophy and crypt hyperplasia (Mäki et al. 1991a, Iltanen et al. 1999a). This has been shown in adults with minor small bowel mucosal changes and increased densities of γδ+ IELs to improve clinically and serologically, and there is also histological recovery on a gluten-free diet (Kaukinen et al. 2001). γδ+ IELs were earlier considered disease-specific, but these cells have been evaluated in other intestinal diseases, including cow's milk intolerance and Crohn's disease (Spencer et al. 1989, Söderström et al. 1996), and they are not restricted to HLA DQ2 or DQ8 (Iltanen et al. 1999b).

4.2. The histological spectrum of coeliac disease

The characteristic findings comprise villous atrophy, elongation of the crypts (crypt hyperplasia) and an increase in the number of lymphocytes in the epithelium and lamina propria. This continuing process had been recognized by Alexander (Alexander 1975). A more detailed study of intestinal histology was carried out by Marsh (Marsh 1992), who classified the changes into four grades (Table 1.). Grade 0 is equal to normal small bowel mucosa; grade I comprises normal mucosal architecture with an increased density of IELs (infiltrative type). Minor villous shortening and hyperplastic crypts are typical for grade II (infiltrative-hyperplastic type), and in grade III the mucosal lesion deteriorates to partial or subtotal villous atrophy with crypt hyperplasia (flat destructive type). Grade IV represents the most severe damage to the small-bowel mucosa with subtotal villous atrophy without crypt hyperplasia; this might indicate a premalignant or malignant lesion, namely ulcerative jejunoileitis or lymphoma, respectively. Grade III is considered diagnostic for coeliac disease (Marsh 1992).

Table 1. Marsh classification

	Inflammation	Crypt hyperplasia	Villous atrophy
Marsh 0	-	-	-
Marsh I	+	-	-
Marsh II	+	+	Mild or absent
Marsh III	+	+	Partial or subtotal
Marsh IV	+	-	Subtotal

Evidently these, diagnostic criteria should be revised towards early developing coeliac disease, or genetic gluten intolerance (Table 2.). Individuals possessing the coeliac disease susceptibility genes and evincing normal small bowel mucosal morphology can be gluten-sensitive, and may later develop villous atrophy while continuing on a gluten-containing diet. This concept of latent coeliac disease, where mucosal deterioration has been proved to occur later in life, has been recognized for many years (Weinstein 1974, Mäki et al. 1990, Mäki et al. 1991a, Holm et al. 1992, Ferguson et al. 1993, Corazza et al. 1996a). The term latent coeliac disease is by definition retrospective, and early developing coeliac disease might thus be more descriptive (Salmi et al. 2006a).

To make the diagnosis of early developing coeliac disease without villous atrophy, the patient must have HLA DQ2 or DQ8. An increased density of γδ+ T-cell-receptor bearing IELs is also typical though not pathognomic for the condition (Savilahti et al. 1990, Mäki et al. 1991a, Iltanen et al. 1999a). The counting of IELs in the tips of the villi seems to be as reliable as γδ+ IELs in the diagnosis of early developing coeliac disease (Järvinen et al. 2004).

Dermatitis herpetiformis is a good model for the latent or early developing forms. One in four sufferers do not evince small-bowel villous atrophy, but may subsequently develop typical mucosal

damage, provided that they continue on a normal, gluten-containing diet (Weinstein 1974, Ferguson et al. 1987).

The process of mucosal deterioration from latent form to manifest coeliac disease may take years, even decades (Niveloni et al. 2000, Lähdeaho et al. 2005). However, even in the absence of atrophy these patients may suffer gastrointestinal symptoms which improve upon initiation of a GFD (Cooper 1986, Kaukinen et al. 1998, Kaukinen et al. 2001). The patients may even have osteopenia or osteoporosis (Kaukinen et al. 2001). Though controlled randomized studies are lacking, it may sometimes be advisable to start GFD treatment before villous atrophy has developed.

Table 2. Different types of coeliac disease

Classical coeliac disease	Villous atrophy and crypt hyperplasia Marsh III	
Latent coeliac disease or early developing coeliac disease	No villous atrophy Marsh 0-II	Villous atrophy shown to develop later
Genetic gluten intolerance	HLA DQ2 or DQ8 Gluten dependency demonstrated histologically Coeliac serology often positive	Inflammation alleviates on a gluten-free diet

5. Diagnostic difficulties

The diagnosis of coeliac disease is easy when characteristic symptoms, positive serology and small bowel mucosal atrophy are present. In order to make the diagnosis in this early stage, new methods are nonetheless required, and the gluten dependence of symptoms or inflammation should be proved in one way or another. In fact, the diagnosis has now become more difficult than earlier. Patients may suffer from mild, if any symptoms; these may be atypical and occur outside the gastrointestinal tract. There are also new problems in the interpretation of biopsy specimens (Feighery et al. 1998,

Collin et al. 2005). When the diagnosis is made early, small bowel mucosal damage may be subtle or borderline. The atrophy is sometimes patchy, and the biopsy may have been taken outside the lesion. Biopsy samples are often poorly orientated, while morphometric analysis should be performed in well-orientated samples. In difficult cases the diagnosis must be based on histology, genetics, serology, symptoms and gluten dependency. Of note, there is no single test to identify early developing coeliac disease reliably.

6. Serological tests

Serological tests for coeliac disease are valuable in selecting individuals who should undergo the intestinal biopsy examination to confirm the diagnosis. Serology can be beneficial in verifing the diagnosis when the biopsy finding is unequivocal. The therapeutic effect of GFD can be evaluated by antibody tests, even though negative serology does not necessarily exclude villous damage (Dickey et al. 2000, Kaukinen et al. 2002b, Hill 2005)

Seah and collegues (Seah et al. 1971) were the first to introduce an immunofluorescence method for the detection of serum antireticulin antibodies (ARA), with rat kidney and liver tissue as antigen. Though fairly powerful (Hällström 1989), this test has nevertheless been replaced by newer, less observer-dependent methods. Antigliadin (AGA), antiendomysium (EmA) and tissue transglutaminase antibodies were subsequently introduced as serological markers for coeliac disease (Chorzelski et al. 1983, Savilahti et al. 1983, Hällström 1989, Dieterich et al. 1997).

The predominant method for AgA is the enzyme-linked immunosorbent assay (ELISA) (Vainio et al. 1983). The sensitivity and specificity of the test vary considerably (Table 3). EmA is directed against the “reticulin-like” silver-stain-positive intermyofibrillar substance of the monkey oesophagus smooth muscle (Chorzelski et al. 1983). An improved method, at least in terms of availability, employs human umbilical cord as antigen (Ladinser et al. 1994); this has been shown to

give results comparable in sensitivity and specificity to the monkey oesophagus band method (Volta et al. 1991, Mäki 1995, Volta et al. 1995).

In 1997 Dieterich and associates (Dieterich et al. 1997) showed that serum IgA in coeliac disease reacts against tTG, which is in other words the antigen against which the autoantibodies are targeted. Shortly thereafter the tTG ELISA antibody test was developed, using guinea pig liver tTG as substrate (Dieterich et al. 1998, Sulkanen et al. 1998b) and subsequently human recombinant tTG displaced the guinea pig –based tTg-test as possessing better accuracy (Sblattero et al. 2000, Leon et al. 2001). Nevertheless, the sensitivity and specificity figures for the current tests are highly variable in different studies, as shown in Table 3. There are some obvious reasons for this discrepancy. The study populations to be tested have been different, and results are dependent on the likelihood of coeliac disease in the population tested.

IgA-class tests remain negative in selective IgA deficiency; IgG class EmA or tTg-ab tests can be applied in these cases (Sulkanen et al. 1998a, Korponay-Szabo et al. 1999).The overall prevalence of the condition in Finland is about 1:400 (Koistinen 1975) It has been estimated that patients with selective IgA deficiency deficiency have tenfold risk for coeliac disease (Collin et al. 1992 , Cataldo et al. 1997 , Mäki et al. 2003).

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Table 3. Studies depicting the sensitivity and specificity of IgA-glass antibodies in untreated coeliac disease

Test	Authors	Sensitivity	Specificity
Antigliadin antibodies	Vogelsang et al. (1995);	82%	83%
	Sategna-Guidetti et al. (1995);	55%	100%
	Sulkanen et al. (1998a);	85%	82%
	Rostami et al. (1999)	60%	82%
Endomysial antibodies	Mäki et al. (1991b);	92%	95%
	Vogelsang et al. (1995);	100%	100%
	Sategna-Guidetti et al. (1997);	95%	100%
	Sulkanen et al. (1998a);	93%	99%
	Picarelli et al. (1996)	100%	100%
	Tesei et al. (2003)	86%	100%
Tissue-transglutaminase antibodies	Dieterich et al. (1998);	98%	95%
	Sulkanen et al. (1998b);	95%	94%
	Sblattero et al. (2000);	98%	99%
	Tesei et al. (2003);	91%	96%
	Carroccio et al. (2002)	100%	97%

7. Clinical features

7.1. Typical symptoms

Patients with coeliac disease evince a wide variety of gastrointestinal and extraintestinal manifestations and the clinical features have in fact changed during recent decades, probably due to a better understanding of the condition (Logan et al. 1983). Up to the 1970s, coeliac disease was recognized as a small bowel disease, the diagnosis was based solely on gastrointestinal symptoms, and laboratory tests measured mainly the intestinal absorptive function.

Coeliac disease may manifest itself at any age. Classical symptoms of the disorder in children consist of abnormal stools, iron deficiency anaemia, malabsorption syndrome, underweight and failure to thrive (Visakorpi et al. 1970).Typical gastrointestinal symptoms in adults comprise general tiredness, malaise, diarrhoea, abdominal discomfort and bloating (Cooke and Holmes 1984, Kelly et al. 1990). Steatorrhoea is nowadays rare and occasional loose stools are much more common (Bode and Gudmand-Hoyer 1996). The common signs of malabsorption comprise anaemia (Logan et al. 1983, Cooke and Holmes 1984, Corazza et al. 1993), due to iron or folic acid, less often due to cobalamin deficiency (Stewart et al. 1967); calcium, vitamin-D or any other deficiency of nutrients may also occur (Kemppainen et al. 1995, Kemppainen et al. 1998). Coeliac disease is also related to decreased intestinal disaccharidase enzyme activity (Nieminen et al. 2001), and secondary lactose intolerance is common (Bode and Gudmand-Hoyer 1988).

7.2. Silent coeliac disease

Monosymptomatic or even clinically silent coeliac disease is increasingly frequently detected in both children and adults (Ferguson et al. 1993, Visakorpi and Mäki 1994).Serologic mass screening studies have shown coeliac disease to be highly underdiagnosed (Catassi et al. 1994, Kolho et al. 1998, Mäki et al. 2003). For instance, in Finland the prevalence of screen-detected coeliac disease is at least 1% (Mäki et al. 2003), and the number of detected cases 0.45% (Collin et al. 2007.). Even

though the latter percentage is higher than in most countries, this still means that for every diagnosed patient, two to three remain undetected. Of these screen-detected patients, some may have symptoms consistent with coeliac disease, while others are infact devoid of any symptoms or clinical manifestation of the disease. (Collin et al. 1999, Mustalahti et al. 2002, Mäki et al. 2003). Based on studies in question, clinically silent coeliac disease may even be the most common type of the condition.

7.3. Extraintestinal symptoms and associated conditions

7.3.1. Dermatitis herpetiformis

Dermatitis herpetiformis is the most familiar extraintestinal manifestation in coeliac disease. It is a life-long, gluten-sensitive blistering skin disease, where granular IgA deposits in the uninvolved skin are pathognomic (van der Meer 1969, Reunala et al. 1984). The majority of patients with dermatitis herpetiformis have small bowel mucosal lesion indistinguishable from coeliac disease. However, in 10 % of patients villous shortening cannot be demonstrated (Reunala et al. 1984). The spectrum of intestinal abnormalities in dermatitis herpetiformis ranges from minimal IEL infiltration to complete villous atrophy. In the absence of villous atrophy, a mucosal inflammation compatible with early developing coeliac disease can be seen in virtually all patients; an increase of γδ+ IELs being a typical finding (Savilahti et al. 1992, Vecchi et al. 1992, Sturgess et al. 1993).

7.3.2. Other extraintestinal manifestations and associated conditions

Bone mineral density (BMD) is often decreased in coeliac disease (Mazure et al. 1996, Valdimarsson et al. 1996, Kemppainen et al. 1999b), even when coeliac disease is clinically silent. (Mustalahti et al. 1999). The density can recover on a GFD (Corazza et al. 1996b , Kemppainen et al. 1999b), though not always completely (Valdimarsson et al. 1996, Mustalahti et al. 1999). A low

BMD is a well-known risk factor for bone fractures and this has also been reported in coeliac disease patients (Vazquez et al. 2000), though the additional risk seems to be relatively low (Thomason et al. 2003, West et al. 2003).

Neurological symptoms may accompany the first clinical symptoms in coeliac disease (Luostarinen et al. 1999). Cognitive disorders, ataxia, epilepsy with posterior cerebral calcifications, dementia and brain atrophy have been described (Collin et al. 1991, Hadjivassiliou et al. 1998, Luostarinen et al. 2001). Problems in infertility may arise both in females (Collin et al. 1996, Sher and Mayberry 1996) and male (Farthing et al. 1983). Alopecia areata (Corazza et al. 1995), aphthous ulcerations of the mouth (Ferguson et al. 1980, Jokinen et al. 1998) and dental enamel defects in the permanent teeth (Aine 1996) have been associated with untreated coeliac disease.

Coeliac disease is a common disorder, and many of the reported conditions associated with the disease are probably coincidental. Diseases which commonly occur together with coeliac disease include various autoimmune diseases in general and autoimmune endocrinological diseases in particular (Table 4.).

Table 4. Coeliac disease and associated autoimmune disorders

Type 1 diabetes mellitus
Autoimmune thyroid diseases
Addison´s disease
Primary Sjögren´s syndrome
Alopecia areata
Autoimmune hepatitis and other liver diseases
Multiple autoimmune conditions

(Hagander et al. 1977, Volta et al. 1998, Collin et al. 2002, Kaukinen et al. 2002a, Volta et al. 2002).

8. Quality of life in coeliac disease

Quality of life defined as individuals´ perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is a broad-ranging concept affected in a complex way by the person's physical health, psychological state, level of independence, social relationships, and their relationships to salient features of their environment (WHOQOLGroup 1993).

Coeliac disease as a chronic disorder affects the every day life of patients, and life may be fraught with conflicts between the difficulty of following the diet and the risk of complications. Particularly, in social situations such as travelling or dining out, it may be problematic to follow a strict GFD (Hallert and Lohiniemi 1999).

There are a wide variety of test patterns, scales and evaluation formulas to measure quality of life. Two basic types of instruments are available: generic and disease-specific questionnaires. The effects on the quality of life between different disorders can be evaluated by generic tests, whereas disease-specific tests measure specific clinical changes attending a condition (Bowling 1995). There is contradiction between the view of patients and those of researches in measuring the quality of life: some clinicians are inclined to dismiss patients´ ratings by reason of their subjectivity. A poor correlation between professionals´ and patients´ assessments has been reported (Jachuk et al. 1982) and self administered questionnaires are therefore widely used. Different methods have been applied to evaluate the quality of life in coeliac disease (Table 5.). The tests are mostly generic, but additional disease-specific questions may be included (Zarkadas et al. 2006). A disease specific questionnaire has been created (Hallert et al. 2002).

The psychological General Well-Being (PGWB) questionnaire measures emotional states reflecting a sense of subjective well-being. This comprises 22 items for six domains, giving a score for each, anxiety, depressed mood, self-control, positive well-being, general health and vitality. The total

index is the sum of each domain giving scores between 1 and 6, higher scores indicating better well-being. The questionnaire is well-documented (Dimenäs et al. 1993). Apart from the PGWB, the State and Trait Anxiety (STAI) (Addolorato et al. 2001) and the Self-rating Depression Scale (SDS) (Ciacci et al. 1998, Addolorato et al. 2001) have been used to measure psychological symptoms in coeliac disease patients.

The Gastrointestinal Symptom Rating Scale (GSRS) has been developed to evaluate the severity of abdominal symptoms. The scale comprises altogether 15 items covering 5 different gastrointestinal symptoms describing abdominal pain, gastro-oesophageal reflux, indigestion, diarrhoea and constipation. Each item is graded from 1 to 7. The total index is the mean of the 15 items (from 1 to 7). A higher score indicates more gastrointestinal symptoms (Svedlund et al. 1988).

Health-related quality of life has been measured by the Short Form 36 (SF-36) (McHorney et al. 1994, Hallert et al. 1998) questionnaire. This is a 36-item general health instrument measuring different domains of health. The Short Form 12 (SF-12) (Riddle et al. 2001, Zarkadas et al. 2006) has been developed from the SF-36, and requires less time to complete. The Burden of illness protocol is a disease specific questionnaire evaluating perceived worries, restrictions and subjective outcome in the everyday life of a coeliac patient (Hallert et al. 2002).

In 1970, Goldberg and associates (Goldberg 1970) measured quality of life in treated coeliac disease patients by structured interview, and found that one third suffered from some mental disorder, often depression. In 1982 Hallert and Derefeldt reported that psychic disturbances had been common in untreated coeliac disease, depression being the most common complaint (Hallert and Derefeldt 1982). Since then many studies have evaluated quality of life in both treated and untreated coeliac disease, some studies also concentrating on so-called symptomless patients. Such studies are depicted in Table 6, which shows that the quality of life is often poorer in untreated coeliac disease than in the healthy population. In general, treatment with GFD seems to improve the quality of life. However, in Sweden it became evident that female, but not male patients

experienced poor subjective health and excessive tiredness even though they adhered to a GFD (Hallert et al. 2002, Hallert et al. 2003, Midhagen and Hallert 2003). No such gender difference was obvious in Finland, where quality of life in treated coeliac patients was equal to that in general population (Lohiniemi et al. 2000). Psychological support seems to reduce depression and increase adherence to GFD (Addolorato et al. 2004).

An improvement in the quality of life is also obtainable in seemingly symptomless patients. After long-term follow-up on GFD, quality of life and dietary compliance have been seen to be as good in screen-detected as in symptom-detected patients with coeliac disease (Viljamaa et al. 2005a). Mustalahti et al (Mustalahti et al. 2002) showed that after one year of treatment, quality of life improved in both screen-detected and symptom-detected patients. This finding could not be confirmed in a study by a group under Johnston (Johnston et al. 2004): quality of life in screen-detected patients was equal to that in non-coeliac controls, and subsequent GFD had no effect.

Table 5. Methods for testing gastrointestinal symptoms and quality of life which have been applied in coeliac disease.

Method	**Target of testing**	
PGWB	Quality of life, well-being	22 items, 6-point Likert scale
GSRS	Abdominal complaints	15 items in 5 major gastrointestinal symptoms; 6-point Likert scale
SF-36	Different aspects of quality of life	36 items in 8 major aspects of quality of life
SF-12	Different aspects of quality of life	Shortened from SF-36
STAI	Anxiety	20 items, scores form 1 to 4
SDS	Depression	20 items, scores form 1 to 4
Burden of Illness Protocol	Disease-specific questionnaire	Nine different items

Table 6. Quality of life in adult coeliac disease subjects in different studies. UN=untreated, TR= treated coeliac disease, UN+TR=follow-up data on same patients

Authors	Coeliac disease patients	Control subjects	Methods	Outcome in coeliac disease	Remarks
Ciacci et al. (1998)	92, TR	100, normal controls 48 chronic hepatitis	SDS, modified	Worse	Depressive symptoms
Hallert et al. (1998)	89, TR	5277, general population	1. SF-36 2. GSRS	1. Worse* 2. No data	1 and 2: worse in female patients
Lohiniemi et al. (2000)	58, TR	110, normal controls	1. PGWB 2. GSRS	1. Equal 2. Equal	
Addolorato et al. (2001)	35, UN+TR	59, healthy subjects	1. STAI 2. SDS	1 UN worse, TR improved 2 UN worse, TR worse	Depressive symptoms persisted
Usai et al. (2002)	68, TR	136, healthy subjects	SF-36	Worse	Impaired by poor compliance
Hallert et al. (2002)	68, TR	68, type 2 diabetes mellitus	Burden of illness protocol	Equal	Women worse, correlated with SF-36
Mustalahti et al.(2002)	19 screen-, 21 symptom detected, UN+TR	105, healthy subjects	1. PGWB 2. GSRS	1 and 2. UN worse in symptom-detected	1 and 2 TR: improved in both groups (1year)
Fera et al. (2003)	100, TR	100, healthy subjects 100, diabetes mellitus	SDS SF-36 STA1	Worse in comparison to healthy controls	No difference between coeliac disease and diabetes
Midhagen and Hallert (2003)	51, TR	182, general population	GSRS	Worse	Male equal to controls
Johnston et al. (2004)	14 screen-, 17 symptom-detected, UN+TR	23, healthy subjects 26, healthy controls	SF-36	Screen-detected: equal Symptom-detected: worse	TR: improvement in symptom-detected (1year)
Viljamaa et al. (2005a)	53, screen , 44 symptom-detected TR	110, healthy subjects General population (SF-36)	1. PGWB 2. GSRS 3. SF-36	1. Equal 2. Equal 3. Equal	No difference between screen- and symptom-detected
Zarkadas et al. (2006)	2,681, UN, TR, members of society	General population	SF-12	Equal	Worse for females and newly diagnosed
Roos et al. (2006)	51, TR	182, general population	PGWB	Equal	Worse for females than for males

* for general health and vitality

9. Epidemiology

In the 1980s the prevalence of coeliac disease was estimated to be 58-130:100 000 in adulthood in Europe (Hallert et al. 1981, Logan et al. 1986, Midhagen et al. 1988, Hovdenak et al. 1999). Subsequently prevalence figures for adult coeliac disease patients from population-based screening studies ranged 1:200-1:300 in Europe (Kolho et al. 1998, Ivarsson et al. 1999, Volta et al. 2001). The highest prevalence in the USA was 1:400 in areas to which Europeans had emigrated (Not et al. 1998). Similar figures were seen in Australia, New Zealand and South America (Cook et al. 2000, Gomez et al. 2001, Hovell et al. 2001). The latest figure for Finland shows a seropravalence of 1:67 (Mäki et al. 2003).

The clinical prevalence figures for coeliac disease are much lower than those obtained in serologic screening studies. Amongst patients with classical coeliac disease symptoms a prevalence of 1:6000 has been obtained (Schweizer et al. 2004). In Finland, a prevalence of 1:370 has been shown in adults (Collin et al. 1997), this subsequently increasing to (0.7) in the same area. Mäki et al have evaluated the clinical prevalence 1:99 (Mäki et al. 2003).There is a female predominance of 2:1 in coeliac disease (Collin et al. 2007.).

Among children the prevalence of coeliac disease has been shown to be from 1:70 to 1:82 in population-based screening studies (Csizmadia et al. 1999, Korponay-Szabo et al. 1999, Meloni et al. 1999). The highest prevalence of the disease, 1:20, has been obtained among Saharwi children (Catassi et al. 1999).

10. Pathogenesis

The pathogenesis of coeliac disease involves environmental, genetic and immunologic factors and the precise mechanisms remain unknown. The key environmental factor known to be essential for

the development of coeliac disease is enteric exposure to prolamins in the dietary cereals wheat (gliadin), rye (secalin) and barley (hordein). Apart from gluten, no definite additional environmental factors have been found to be involved in the pathogenesis of the condition (Papadopoulos et al. 2001). It has been concluded that the time of first gluten exposure and the amount of gluten consumed are crucial for the manifestation of coeliac disease (Weile et al. 1995, Ivarsson et al. 2000). A T-cell mediated immune response is indicated by the finding of gluten-specific HLA DQ2 and DQ8 restricted T-cells in the coeliac disease lesions in the small-bowel mucosa (Lundin et al. 1993).

Antigen-presenting cells, macrophages and dendrite cells have specific antigen-binding areas on their surface in genetically susceptible coeliac disease patients. Antigen-binding sites are formed by class II HLA molecules, including a gliadin-binding groove (Molberg et al. 1998). TTG, an enzyme released from macrophages, fibroblasts, red blood cells and epithelial cells, has been suggested as the autoantigen in coeliac disease (Dieterich et al. 1997), and is released from cells during mechanical stress or wounding (Aeschlimann and Paulsson 1991). One of the mechanisms involved is crosslinkage with gliadin, which increases gliadin's immunogenity The enzyme deamidates glutamine residues to negatively charged glutamic acid and further enhances the binding of deaminated gluten peptides to the peptide-binding groove of HLA-DQ2 or -DQ8 on antigen-presenting cells such as dendrite cells (Molberg et al. 2000). This deamidation and introducition to antigen-presenting cells to activate T-helper cells to induce a Th1- and Th2-type reactions. Tumour necrosis factor alpha and interferon-gamma are produced by the type Th1 reaction. These induce matrix metalloproteinase expression and activation in intestinal fibroblasts, this possible leading to matrix breakdown, mucosal destruction and villous atrophy (Schuppan 2000). It has been suggested that intestinal infection or mechanical or chemical factors can tender the mucosal integrity vulnerable, dietary proteins, including gliadins, thus gaining access to epithelial cells in lamina propria in the small bowel mucosa (Fasano et al. 2000).

Recent studies indicate that innate immunity has a central role in the pathogenesis of the intestinal mucosal lesion in coeliac disease. IELs may interact with enterocytes, and express natural killer NKG2D at their surface. The process is not dependent on HLA. Interleukin 15 may have a stimulatory role in this process and T-cells may regulate it (Maiuri et al. 2003, Hue et al. 2004, Meresse et al. 2004, Sollid 2004).

Evidence suggests that humoral immunity may have a pathogenetic role in the development of mucosal lesion (Halttunen and Mäki 1999).

11. IgA deposits

In coeliac disease, extracellular IgA deposition was described many years ago (Shiner and Ballard 1972, Karpati et al. 1988). Dieterich et al showed that tTG is the target for antibodies (Dieterich et al. 1997). Korponay-Szabo et al. demonstrated that these IgA deposits are against tissue transglutaninase specific (Korponay-Szabo et al. 2003, Korponay-Szabo et al. 2004). Coeliac disease autoantibodies are produced in the intestinal mucosa (Marzari et al. 2001). Korponay-Szabo et al have shown that TG2-specific IgA is deposited extracellularly in the small bowel mucosa early in coeliac disease development, even when antibodies are not measured in the circulation (Korponay-Szabo et al. 2004, Salmi et al. 2006b). They are also present in early developing coeliac disease, and are therefore a valuable diagnostic tool in this condition (Salmi et al. 2006a). IgA deposits can be found in the liver, lymph nodes and muscles in coeliac disease patients (Korponay-Szabo et al. 2004, Hadjivassiliou et al. 2006)This phenomenon might explain the extraintestinal manifestations in coeliac disease.

12. Genetics

Coeliac disease has a significant genetic component and is strongly related to HLA class II molecules. The major genetic factors for susceptibility to coeliac disease are located in the HLA gene complex in the short arm of chromosome 6.HLA B8 antigen was found in 80% of coeliac disease patients (Falchuk et al. 1972), since then has been evaluated that coeliac disease is also associated to HLA DR3 (Keuning et al. 1976, Mearin et al. 1983). These HLA II class molecules are found on the surface of antigen-presenting cells; 90% of coeliac disease patients have the HLA DQ2 encoded by alleles DQA1∗0501 and DQB1∗0201 (Sollid et al. 1989, Polvi et al. 1996). DQ2 negative coeliac disease patients have DQ8 encoded by alleles DQA1∗0301 and DQB1∗0302 (Polvi et al. 1998). Only less than 1 % of coeliac disease patients have none of these alleles (Polvi et al. 1998, Karell et al. 2003). On the other hand, among the healthy population 30-40% carry the HLA-DQ2 or HLA-DQ8 (Polvi et al. 1996).

An inherited predisposition to coeliac disease susceptibility has also been demonstrated in twin studies. Concordance for the disorder is in first-degree relatives 10-15% (MacDonald et al. 1965) and increases to 80% in monozygotic twins (Mearin et al. 1983, Hervonen et al. 2000, Greco et al. 2002). It has been estimated that a sibling's relative risk of coeliac disease varies from 30 to 48 (Petronzelli et al. 1997, Bingley et al. 2004).

13. Treatment

13.1. Earlier treatment modes

It is difficult to understand that intolerance to one of the most common components in food (wheat) in our environment can cause such reactions, but man was not originally a gluten-eater. The

cultivation of grains evolved in the ancient Mediterranean culture 10,000 years ago and during thousands of years the skill spread over Europe (Greco et al. 1997). In 1888 Samuel Gee described a disease he called the coeliac affection and noted the classical symptoms of diarrhoea, lassitude and failure to thrive. He thought that the regulation of food was important, and concluded that if the patients can be cured at all, it must be by means of diet (Gee 1888). The link to gluten ingestion was observed in 1950, when the Dutch paediatrician WD Dicke showed that wheat flour was the cause of anorexia, increased faecal output and steatorrhoea in coeliac disease (Dicke 1950). The diagnosis and also monitoring of the dietary response became feasible when a method for small intestinal biopsy sampling was invented. In 1954 Paulley examined full-thickness biopsies taken at laparotomy, and was the first to provide accurate description of the coeliac lesion (Paulley 1954). Subsequently Shiner invented the intestinal biopsy technique for the diagnosis of coeliac disease (Shiner 1957). Green concluded that coeliac disease patients should be maintained on the wheat-free diet for long periods before an adequate response occurred (Green and Freed 1976). The role of GFD in the treatment of dermatitis herpetiformis was recognized in 1969 (Fry et al. 1969).

13.2. Prolamins of cereals

Cereals constitute one of the most important basic components in human nutrition. Prolamins are defined as proteins of cereal endosperm which are soluble in aqueous alcohols without reduction of disulphide bonds (Wieser et al. 1983). Wheat, barley, rye, oats, rice and maize belong to the Gramineae family. Cereals which evidently activate coeliac disease are members of tribe Triticeae and share a close taxonomic relationship.

The proteins of the Gramineae are classified by solubility fractions. The common prolamin fractions of the cereals are called gliadin (wheat), avenin (oats), secalin (rye), hordein (barley), zein (maize) and oryzin (rice). Prolamins differ from each other in their essential amino acid (proline, glutamine) composition, as depicted in Table 7. Gliadin, secalin and hordein are especially rich in proline and

glutamine, which are considered toxic for coeliac disease. By contrast, the prolamins of rice, maize and millet have a low content of proline and glutamine, but are rich in leucine and alanine. Avenin has a higher content of proline and glutamine than maize, but less than wheat, rye or barley (Wieser et al. 1983).

Table 7. Taxonomic relationship of the Gramineae family (Wieser et al. 1983, Schuppan et al. 2002).

Family	Graminae											
Subfamily	Festucoideae						Panicoideae					
Tribe	Triticeae						Avene		Oryzeae		Tripsaceae	
Genus	Triticum		Secale		Hordeu		Avena		Oryza		Zea	
Cereals	**Wheat**		**Rye**		**Barley**		**Oat**		**Rice**		**Maize**	
Prolamin	**gliadin**		**secalin**		**hordein**		**avenin**		**oryza**		**zein**	
Composition	Q	P	Q	P	Q	P	Q	P	Q	P	Q	P
(%)	36	17-23	36	17-23	36	17-23	34	10	20	5	19	10

Q, glutamine; P, praline

13.3. Compliance with GFD

Obviously adherence to a GFD should be as strict as possible. Dietary transgressions constitute a much more significant gluten load than for instance trace amounts of contaminated gluten in gluten-free products. Many factors have been shown to influence dietary compliance in coeliac disease patients, for example female gender, good knowledge of coeliac disease (Ljungman and Myrdal 1993) and education (Ciacci et al. 2002b). Patients whose coeliac disease diagnosis has been

Table 8. Compliance to GFD

Authors	Country	Series	Duration of GFD (range)	Compliance			Remarks
				Good	Intermediate	Poor	
Dissanayake et al. (1974a)	UK	38 adults	2 years	47%	34%	10%	
Colaco et al. (1987)	Italy	37 children	15 years	43%	30%	27%	
Kumar et al. (1988)	UK	102 children	1 year	56%	35%	9%	
Mayer et al. (1991)	Italy	123 adolescents	10 years	65%	24%	11%	
Ljungman and Myrdal (1993)	Sweden	47 adolescents	Over 10 years	81%	13%	6%	
Troncone et al. (1995)	Italy	23 adolescents	Over 10 years	17%	52%	30%	
Greco et al. (1997)	Italy	306 adolescents	-	73%	15%	12%	
Hallert et al. (1998)	Sweden	89 adults	10 years	78%	12%	10%	
Ciacci et al. (2002a)	Italy	390	7 years	42%	32%	24%	
Usai et al. (2002)	Italy	68 adults	Over 2 years	59%	38%	3%	
Fera et al. (2003)	Italy	100 adults	9 years	49%	48%	3%	
Högberg et al. (2003)	Sweden	29 adults	20 years	59%	41%	-	
Ciacci et al. (2003)	Italy	581 adults	8 years	74%	22%	4%	
Kaukinen et al. (1999)	Finland	52 children and adults	8 years	88%	12%	-	
Kemppainen et al. (1999a)	Finland	28 adults	1 year 5 year	96% 82%			
Lohiniemi et al. (2000)	Finland	58 adults	10 years	94%	6%	-	
Kaukinen et al. (2002b)	Finland	87 adults	1 year (1-18)	87%	13%		
Viljamaa et al. (2005a)	Finland	97 adults	14 years (5-21)	93-96%			
Viljamaa et al. (2005b)	Finland	703 children and adults	8 years (0-42)	78%	12%	6%	
Hervonen et al. (2005)	Finland	1104 DH patients	32 years (17-56)	88-94%			

established before four years of age seem to maintain a GFD better even in the adulthood (Högberg et al. 2003). As shown in Table 8, the maintenance of a GFD is often unsatisfactory. The Table 8 also gives the impression that in Finland adherence to diet is better than for instance in Southern Europe. Wheat-starch based gluten-free flours, and more recently oats, have been accepted in the everyday diet of subjects disease in Finland; whether this explains the good compliance remains to be proved.

13.4. Malignant complications and treatment failure

Patients with coeliac disease carry an increased risk of lymphoma and small bowel cancer (Harris et al. 1967, Holmes et al. 1976, Holmes et al. 1989). The lymphoma prevalence has been highly variable, from 0 to 9% (Harris et al. 1967, Holmes et al. 1976, Collin et al. 1994, Egan et al. 1995), this probably depending on the population studied. In reality it is difficult to know the actual prevalence of malignancy in coeliac disease. Evidence suggests that GFD protects from the risk of developing malignant disease, and with adequate treatment the risk is the same as or close to that in the general population (Holmes et al. 1989, Viljamaa et al. 2006).

13.5. Refractory coeliac disease

Refractory coeliac disease is defined as an initial or subsequent failure of a strict GFD to restore the normal intestinal architecture in patients with coeliac-like enteropathy (Trier 1991). In this context it should be noted that compliance with a GFD variables widely in different countries, 17% to 96% maintaining a strict diet (Kumar et al. 1988, Ljungman and Myrdal 1993, Greco et al. 1997, Viljamaa et al. 2005a). Possible failure to adhere to

a strict diet must thus be taken into account in refractory coeliac disease. Prevalence figures for this condition have been as high as 7-8% in some studies(O'Mahony et al. 1996), but are probably underestimated. For instance, in one multicenter study of refractory coeliac disease in France 21 patients were enrolled, although the enrolment area covered the whole country (Cellier et al. 2000). Refractory coeliac disease is associated with aberrant clonal IELs. The clonality encompasses a link between coeliac disease, ulcerative jejunoileitis and enteropathy-associated T-cell lymphoma (EATL) (Cellier et al. 2000). EATL carries a poor prognosis, associated with a high mortality rate (Harris et al. 1967, Holmes et al. 1976, Ryan and Kelleher 2000). The most effective treatment of refractory coeliac disease is prevention, i.e. the exclusion of gluten from the diet (Holmes et al. 1989, Corrao et al. 2001). In some cases immunosuppressive therapy with corticosteroids, azathioprine or cyclosporine has been used, but evidence supporting this approach is based on case reports only (Ryan and Kelleher 2000). There are not any controlled follow-up studies using immunosuppressive therapy or chemotherapeutic agents.

13.6. Wheat starch

Wheat starch based gluten-free products have been used in the UK and Northern Europe for many years. Industrially purified products meeting the current Codex Alimentarius are considered gluten-free when they contain less than 0.05 g nitrogen per 100 g of food product in dry matter (Codex-Alimentarius-Commission 1981), which means that the products may contain 40-60 mg gluten/100 g dry product. This nitrogen limit is valid only for wheat starch. Earlier short-term studies with wheat starch yielded divergent

results (Table 9.). In two short-term challenge studies no small bowel mucosal inflammation was observed after ingestion of 5 mg to 5 g of gluten (Ciclitira et al. 1984, Ciclitira et al. 1985). An increase in IELs and a minor decrease in mean villous height and crypt depth ratio (Vh/CrD) were observed in a short-term challenge with 100 mg or 500 mg of daily gliadin in a study by Catassi et al (Catassi et al. 1993). Chartrand and associates surmised that these products can evoke abdominal symptoms (Chartrand et al. 1997). Ejderhamn et al found no mucosal damage in coeliac patients using wheat-starch-based gluten-free products on average for 10 years (Ejderhamn et al. 1988).

A cross sectional study of 89 adult coeliac disease patients showed that the small amounts of gluten allowed by the Codex were not harmful (Selby et al. 1999). Kaukinen et al (Kaukinen et al. 1999) conducted a cross-sectional study where 41 children and adults with coeliac disease and 11 adults with dermatitis herpetiformis had adhered to gluten-free products for 8 years on average. The response was complete in patients maintaining a strict diet. Forty patients adhered to a strict wheat-starch-based diet and the mean daily intake of gluten was calculated to be 34 mg (5-150 mg). Table 7 summarizes studies with wheat starch. Worldwide, there is an ongoing debate regarding the acceptable threshold for residual gluten in gluten-free products. It can be estimated that 0.5 g daily gluten might be harmful, whereas some milligrams are well tolerated (Table 9). The quality of life in coeliac patients treated with wheat starch was observed to be as good as in non-coeliac controls (Lohiniemi et al. 2000).

Table 9. Studies on the use of wheat starch in coeliac disease

Reference	Number of patients	Age group	Study period	Method	Outcome
	Open challenge studies				
Ciclitira et al. (1984)	7	Adults	1 week	Small-bowel biopsy	No
Ciclitira et al. (1985)	10	Adults	6 weeks	Small-bowel biopsy	Abdominal symptoms
Catassi et al. (1993)	10	Children	4 weeks	Small-bowel biopsy	Increased IEL, decreased Vh/CrD
Chartrand et al. (1997)	17	Children and adults	0.5-10 months	AGA, EmA	Abdominal symptoms
	Cross sectional studies				
Ejderhamn et al. (1988)	11	Adults	10 years	Small-bowel biopsy	No
Kaukinen et al. (1999)	40	Children and adults	8 years	Small-bowel biopsy AGA, ARA, EmA	No
Selby et al. (1999)	39	Adults	0.5-29 years	Small-bowel biopsy AGA, EmA	No
Lohiniemi et al. (2000)	48	Adults	9-11 years	Quality of life	No

13.7. Oats

The common belief that oats are toxic was ill-founded for a long time. The results of some studies from the 1950s and 1970s reported raised levels of faecal fat or reduced xylose excretion in a few coeliac disease patients who consumed large amounts of oats (Dicke et al. 1953, Moulton 1959 , Baker and Read 1976). Two studies again stated that oats can be consumed by coeliac patients without detrimental effects (Sheldon 1955, Dissanayake et al. 1974b). The validity of all these studies can be questioned in that the follow-up time was short, none was controlled and effects of oats were measured using insensitive methods instead of small bowel biopsy. The effects of oats were investigated by obtaining small intestinal biopsies only in study by Dissanayake, which showed no mucosal deterioration in four patients (Dissanayake et al. 1974b).

The first controlled study was carried out by Janatuinen et al in Kuopio, Finland. It comprised 92 adults coeliac disease patients, of whom 40 were newly detected cases, randomized to gluten-free diet with or without oats. The mean daily oats intake was from 47 to 50 g. Participants underwent histology, nutritional assessment and laboratory measurements. No significant differences emerged between the two groups in histology or symptoms (Janatuinen et al. 1995). Based on the same series the investigators further showed that the densities of IELs did not differ between adult coeliac disease patients using or not using oats (Janatuinen et al. 2000). The authors further performed a 5 years' follow-up study involving 23 of the original 45 patients who had continued to take oats. There were no untoward effects on mucosal integrity or increases in serum antibodies to endomysium, reticulin or gliadin (Janatuinen et al. 2002). Störsrud et al found that even

large amounts of oats mean, 93g/day, can be safely consumed by adult coeliac disease patients and that oats improved the nutritional value of the GFD (Störsrud et al. 2003). The first randomized, double-blind oats study was carried out in children with newly detected coeliac disease. It showed that oats caused no untoward effects on clinical, immunological or small-bowel mucosal healing (Högberg et al. 2004).Two studies with dermatitis herpetiformis indicate that most patients tolerated oats and rash did not reappear more often than in patients on GFD alone (Hardman et al. 1997, Reunala et al. 1998).

By contrast, Lundin and colleagues have shown in a clinical challenge study involving 19 adult coeliac disease patients who consumed 50 g oats daily for 12 weeks, that even pure oats can cause villous atrophy and dermatitis, but in only one patient (Lundin et al. 2003). A study by Arentz-Hansen and group established that four out of nine adult coeliac disease patients using oats had clinical symptoms. They showed that three of these four patients have avenin-reactive mucosal T-cells which can cause mucosal inflammation, as did two patients in the study without clinical symptoms (Arentz-Hansen et al. 2004). A more recent study has indicated that oats, similarly to rye and barley, initiates a T-cell response in patients with coeliac disease (Kilmartin et al. 2006). The authors concluded that this does not necessarily mean that oats should be considered toxic, since the majority of studies have shown the opposite. The studies in question are summarized in Table 10.

Table 10. Studies on the safety of oats in coeliac disease

	Studies indicating that oats are safe for patients with coeliac disease				**Studies indicating that oats are not safe for patients with coeliac disease**				
Author	**Study design**	**No of patients**	**Study period**	**Method**	**Study**	**Study design**	**No of patients**	**Study period**	**Method**
Janatuinen et al. (1995)	Prospective, randomized	45	6-12 m	Small bowel biopsy Laboratory measures	Dicke et al. (1953)	Open challenge	1/1	4 w	Faecal fat
Srinivasan et al. (1996)	Open challenge	10	3 m	Small bowel biopsy AGA, EmA	Moulton (1959)	Open challenge	2/4	52d	Faecal fat
Reunala et al. (1998)	Open challenge	11	6 m	Skin and small bowel biopsy AGA, EmA	Baker and Read (1976)	Open challenge	3/12	14-100d	Xylose test
Hardman et al. (1997)	Open challenge	10	3 m	Skin and small bowel biopsy AGA, ARA, EmA	Lundin et al. (2003)	Open challenge	1/19	12 w	Small bowel biopsy
Srinivasan et al. (1999)	Open challenge	10	3 m	Lactase expression in small bowel biopsy	Arentz-Hansen et al. (2004)	Open challenge	5/9		Small bowel biopsy

Janatuinen et al. (2000)	Prospective, randomized	45	6-12 m	Small bowel biopsy AGA, ARA
Hoffenberg et al. (2000)	Open challenge	10	6 m	Small bowel biopsy tTG-ab
Picarelli et al. (2001)	Open challenge	13		EmA
Kilmartin et al. (2003)	Open challenge	8		
Janatuinen et al. (2002)	Cross sectional	23	5 y	Small bowel biopsy AGA, ARA, EmA
Störsrud et al. (2003)	Follow-up	15	2 y	
Högberg et al. (2004)	Prospective, randomized Double blind	42	1 y	Small bowel biopsy AGA, EmA, tTG-ab
Kemppainen et al. (2007)	Follow-up	12	5y	Small bowel biopsy (immunohistochemistry)

Aim of the study

GFD is the only treatment for coeliac disease. The diet should be as strict as possible. During recent years, it has been debated, whether oats can be tolerated by coeliac disease patients, and whether patients benefit from a GFD, especially as to how it affects on quality of life. Similarly, cereal contamination of gluten-free products has been a major topic for many years. Wheat-starch-based gluten-free products are often considered contaminated although they have been widely used in Northern Europe. Oats and wheat-starch-based gluten-free products are not permitted to coeliac subjects in many countries. The diagnosis of coeliac disease has become challenging, in that in many cases the small bowel mucosal lesion is subtle or borderline when the diagnosis is made early. The demonstration of gluten dependency is important in cases where the histology is equivocal.

Specific aims

1. To study how oats affect dietary treatment and quality of life in coeliac disease (**I**) and how patients with coeliac disease have adopted oats nationwide (**II**).
2. To assess the safety of a wheat-starch-based GFD in the treatment of coeliac disease (**III**)
3. To evaluate the role of gluten challenge and GFD in the diagnosis and treatment of early developing coeliac disease (**IV**)

Patients

Patients and controls in the present study are shown in Table 11.

Table 11. Patients and control subjects in studies **I-IV.** CD= coeliac disease

Study	Design	Patients	n	Controls	n
I	Randomized	Treated CD, oats challenge	23	Treated CD, non-oats	16
II	Cross sectional	Coeliac disease patients	710	-	-
III	Randomized	Newly detected CD patients placed on wheat starch based GFD	28	Newly detected CD patients placed on naturally GFD	29
IV	Randomized	Patients under suspicion CD, gluten challenge	21	Patients under suspicion of CD, GFD	20
				Patients with newly detected CD	18

Study **I** involved adult coeliac disease patients who had been on a gluten-free diet without oats. Mucosal recovery had been evident in each patient. The patients were randomized

either to use 50 g oats daily and adhere to an otherwise GFD or to continue their current diet without oats. The study period was one year.

In study **II** an inquiry was sent to 1000 randomly selected members of the Finnish Coeliac Disease Society, which has altogether 14,000 members. It was asked whether the respondents had adopted oats, and how they appreciated the use of oats in their daily GFD.

Study **III** comprised 57 newly detected adult biopsy-proven coeliac disease patients between April 1998 and February 2000. The patients were randomized to wheat-starch-based or naturally GFD; the study period was one year.

From 1997 to 2000 general phycisicians were asked to send patients with a suspicion of coeliac disease to Tampere University Hospital where altogether 577 patients were evaluated for suspicion of coeliac disease; 126 patients were found to have the disease. Of the remainder, 68 were found to have an increased density of γδ +IELs (>5 cells/mm) without villous atrophy and crypt hyperplasia. These patients were invited to participate in the study, and altogether 41 patients were randomized to use either extra gluten or GFD (**IV**). The follow-up time was 6 months, whereafter the patients were allowed to switch their diet on a voluntary basis from GFD to normal, or vice versa, and were examined again after six months. Newly detected 18 coeliac disease patients who had to a adhered strict GFD served as controls.

Methods

Small bowel biopsy

Morphometrical studies

Small bowel specimens were taken by upper gastrointestinal endoscopy using normal biopsy forceps from the distal part of the duodenum at baseline and after follow-up in studies **I, III** and **IV;** three to five specimens were processed and stained with haematoxylin-eosin and studied under light microscopy. Morphometric analysis, including Vh/CrD (**I, III-IV**) and the ECH (**III**), was made in well-orientated biopsy samples as previously described (Kuitunen et al. 1982). Poorly oriented sections were discarded; when necessary, the samples were dissected again until they were of good quality.

Immunohistochemical studies

Frozen samples had been regularly taken in study **IV**. In studies **I** and **III** in the first biopsy had some cases been taken at other endoscopy units where frozen samples had not been in use routinely. When feasible, immunohistochemical staining was performed at baseline and after follow-up. Two small bowel biopsy specimens were taken and freshly embedded in optimal temperature compound (Tissue-Tec, Miles Inc, Elkhart, IN, USA), snapfrozen in liquid nitrogen and stored at -70 C. Immunohistochemical studies were carried out on 5µm-thick frozen sections. CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, Calif., USA), αβ+ IELs with monoclonal

βF1 antibody (Endogen, Woburn, Mass., USA) and γδ+ IELs with TCRγ antibody (Endogen). IELs were counted with a x 100 flat-field light microscope objective in randomly selected surface epithelium; at least 30 fields with an epithelial length of 1,6 mm were counted, and density of IELs was expressed as the number of cells per millimetre of epithelium (Savilahti et al. 1992, Arranz et al. 1994).

In study **IV** TG2-related extracellular IgA deposits were measured in all available frozen small-bowel mucosal sections before and after dietary intervention, using direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (DAKO AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate-buffered saline (PBS), pH 7.4. Subepithelial IgA deposits are located along the surface and crypt basement membranes and around mucosal vessels in coeliac disease, while IgA is normally found inside plasma cells and epithelial cells (Korponay-Szabo et al. 2004). Two investigators graded coeliac disease type IgA deposits from 0 to 3 according to their intensity along basement membranes in the villous crypt area without knowledge of disease history or intervention.

Gastrointestinal symptoms and quality of life

Quality of life was assessed using the PGWB questionnaire (Dimenäs et al. 1993) (**I, III**). Gastrointestinal symptoms were measured by the GSRS (Svedlund et al. 1988) (**I**, **III)**. The use and effect of oats were inquired with a questionnaire in study **II** (Table 12.)

Table 12. Items of the questionnaire sent to members of coeliac disease society in Finland

Have you ever taken oat-containing products in GFD?
Are you currently using oats?
If not, what is the reason for avoiding oats?
Have you developed any symptoms when consuming oat products?
How do you appreciate oats: taste, cost, healthiness, diversity of diet and availability of oat-containing products?

Dietary evaluation

The dietician provided advice on GFD with or without oats (**I**), on natural or wheat-starch-based GFD **(III),** and how to maintain extra gluten intake or a GFD (**IV**). A detailed dietary analysis was made and a history of occasional or regular consumption of gluten-containing products (**I;III,IV**) or oats and fibre consumption (**I**) was evaluated by means of interview and a 4-day record of food intake. In **(IV)** the gluten challenge group was advised to eat at least 15 g extra gluten per day.

Antibodies

IgA-class endomysial antibodies were determined by an indirect immunofluorescence method using human umbilical cord as substrate (Sulkanen et al. 1998a); a dilution of 1:≥5 was considered positive (**I, III, IV**).

The determinations of IgA-class tTG were carried out by ELISA. Three different methods were employed. In study **III** (Inova Diagnostic, San Diego, CA, USA) a unit value of ≥20 being considered positive (Sulkanen et al. 1998b), in studies **I** (Celikey, Pharmacia, Uppsala, Sweden) and **IV** (Celikey, Pharmacia Diagnostic, GmbH, Freiburg, Germany) a unit value ≥5 was positive.

HLA-typing

The Olerup SSP DQB low resolution kit (Olerup SSP AB, Saltsjöbaden , Sweden) was used in study **IV** to evaluate HLA DQB1* allele groups. This method determines HLA DQ2, DQ4, DQ5, DQ6, DQ7, DQ8 and DQ9 allele groups.

Demonstration of gluten dependency in early developing coeliac disease

The aim here was to assess the role of GFD or gluten challenge in the diagnosis of early developing coeliac disease, where mucosal deterioration is not yet evident. To show clinical evidence for this dependency, a new score was built comprising information on symptoms, mucosal integrity, mucosal inflammation, coeliac antibodies and risk factors for the development of coeliac disease (**IV**).

The baseline score comprised four items: abdominal symptoms, family history of coeliac disease, associated autoimmune conditions and serology. The intervention score comprised three items: changes especially in small-bowel mucosal villous-crypt architecture and inflammation, abdominal symptoms and serology. The principal aim of this study was to demonstrate gluten dependency in the study patients.

This clinical score was compared to TG2-related extracellular IgA deposits, in order to show whether this new method for coeliac disease brings out a relationship with clinical gluten dependency in early developing coeliac disease.

Laboratory investigations

Blood haemoglobin, serum iron, erythrocyte folic acid (**I, III**), serum calcium and serum vitamin B12 (**III**) were measured using routine laboratory methods.

Bone mineral density

In study **III** the BMD in the lumbar spine and the left femoral neck was evaluated by dual-energy X-ray absorptiometry (Norland XR26, Norland Corp, Fort Atkinson, WI, USA) and data were expressed as T scores with references to data on sex-matched young individuals.

Statistics

The data were analysed using analysis of variance for repeated measures. Equality of baseline was tested by t test, which was also applied in the analysis of biopsy samples. Dependence between numerical variables was studied using a correlation coefficient. Logarithm transformation was applied by reason of skewed distributions. The data characteristics are displayed crude, using mean, median and standard deviations.

Computation was carried out using Statistica for Windows (Version6.0) software. The level of significance was set equal at 0.05 and *P* values are presented exact. The required number of participants was determined to achieve a statistical power of 0.80 at a significance level of 0.05; differences of < 0.5 for the villous height to crypt depth ratio, ≥7/100 CD3+ and αβ+ cells, 3/mm for γδ+ cells and an increase ≥0.5 for the GSRS was assessed as clinical significant (**I**).

Cross-tabulations were carried out by x^2 test (**II**).

Quantitative data were expressed as means and 95% confidence intervals (CI). A two-tailed t-test was used to compare the laboratory values between the groups. Cross-tabulations were carried out by Fisher´s exact test. The required number of participants was determined to achieve a statistical power of 0.90 at a significance level of 0.05; differences of < 0.5 for the villous height to crypt depth ratio, ≥7/100 enterocytes for IEL or CD3+ cells, 3/mm for γδ+ cells and an increase ≥0.5 for the GSRS were considered to be clinical relevant (**III**). The data were given as means with 95% CI (**IV**).

Ethical considerations

The protocols of studies were approved by the ethical committee of Tampere University Hospital. Informed consent was obtained from all participants (**I,III,IV**). The Finnish Coeliac Society distributed the questionnaires to the members of the Society; the data were analysed anonymously **(II)**.

Summary of results

Oats in the gluten-free diet

Small-bowel biopsy

At baseline the average Vh/CrD ratio was 2.1 in the oat group and 1.8 in the non-oat-group. Immunohistochemical stainings had been made in 14 out of 39 cases. The mean densities of CD3+ IELs were 47.8 in the oat and 39.6 in the non-oat group, αβ+ cells 27.0 and 25.6, γδ+ cells 14.9 and 12.5, respectively. The differences were not statistically significant.

A follow-up biopsy was taken from 18 patients in the oat and 13 in the non-oat group: altogether eight patients refused. There were no differences between the study groups in small-bowel mucosal morphology after the intervention: Vh/CrD was 2.5 in the oat group and 2.4 in the non-oat group. The densities of IELs were statistically higher in the oat group: CD3+ IELs 44.6 and 26.7, αβ+ cells 29.8 and 19.9, and γδ+ cells 11.3 and 5.3, respectively (Figure 1).

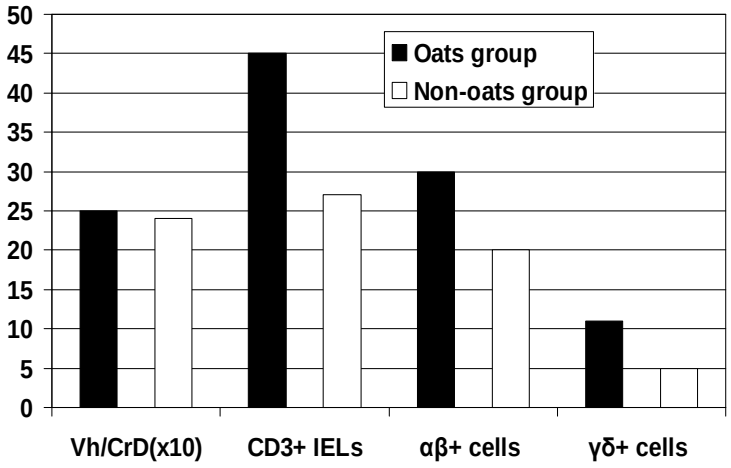

Figure 1. Mucosal findings in coeliac disease after 1 year of follow-up

Symptoms and quality of life

The patients using oats developed more gastrointestinal symptoms than those on the traditional GFD after one year's intervention when evaluated by GSRS, the scores being 2.07 and 1.60, respectively. The experience of diarrhoea was statistically significantly more severe in the oat group, 2.00, than in the non-oat group, 1.33; the constipation score was also higher in the oat group, albeit not significantly. There was no significant correlation between gastrointestinal symptoms and IELs. Three patients decided to discontinue oats because of gastrointestinal pain. For two of them a control biopsy was available, showing mild atrophy; Vh/CrD ratios were 1.7 and 2.0, but there were no changes compared to baseline.

The average PGWB score in study **I** was 103.8 in the oat-consuming group and 105.4 in the traditional gluten-free group before the intervention and after the study period scores were 98.8 and 101.3, respectively; the differences were not statistically significant.

Intake of oats

Altogether 710 (521 female, 189 male; mean age 52; range 5-84 years) members of the Finnish Coeliac Society responded to the inquiry. Before the first nationwide recommendation for the use of oats (in 1997), the diagnosis was made in 397, and thereafter in 295 members.

In the nationwide inquiry the majority of patients (70%) (**II**) were currently using oats. They felt that oats diversified the diet (90%). The patients appreciated the taste (80%), the ease of using oat products (91%) and the low cost (82%) (Figure 2.). Patients with coeliac disease consumed oats more often (73%) than patients with dermatitis herpetiformis (55%).

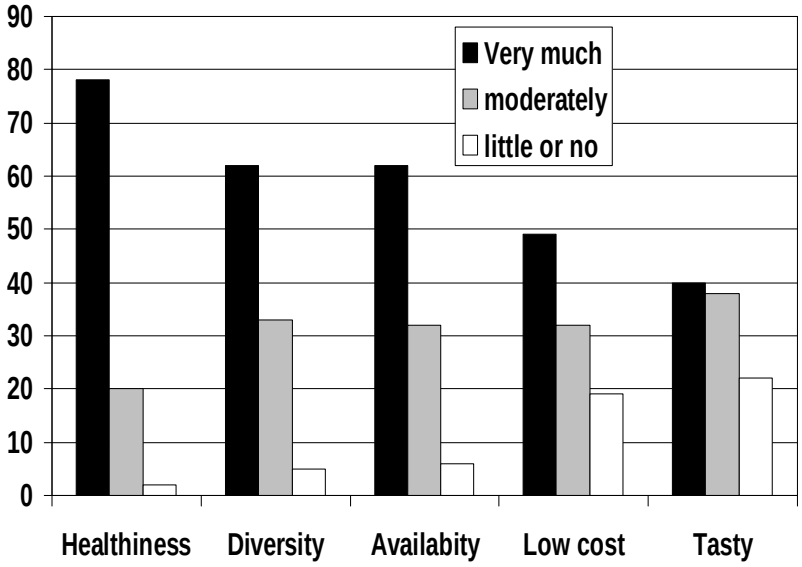

Figure 2. How patients appreciate (%) the use of oats in the gluten-free diet according to nationwide inquiry

Ten per cent (71) of coeliac disease patients did not continue to use oats on account of gastrointestinal symptoms (bloating, diarrhoea); 19% (27) of patients with dermatitis herpetiformis had ceased to use oats, 19 of them experiening skin symptoms. Altogether 16% (111) patients had never used oats. These patients were concerned at possible contamination and adverse effects; nevertheless 43 of them were planning to use oats in the future.

Antibodies and laboratory values

There were no significant differences in serum EmA and tTG antibodies between the study groups (**I**) before and after the study period. In the oat group (**I**) two patients were positive for EmA and tTG-ab and another two only for tTG-ab at randomization, but antibodies decreased in three patients during the study period.

At the time of enrolment and after one year's study period there were no significant differences in blood haemoglobin, serum iron and erythrocyte folic acid levels between the study groups (**I**).

Dietary analysis

All patients adhered to a strict GFD during study period. In study **I** the average daily oat consumption was 30 grams. Neither groups showed significant increase in daily fibre consumption.

Wheat starch-based gluten-free products

Small-bowel biopsy

At baseline in study **III** Vh/CrD and density of intra-epithelial lymphocytes did not differ between the study groups. The groups were statistically equal in terms of small-bowel mucosal architecture after one year follow-up, Vh/CrD was 2.0 in the wheat-starch group (group I) and 1.9 in the naturally gluten-free group (II), αβ+ IELs 21 and 22 and γδ+ 8.9 and 9.4, respectively (Figure 3).

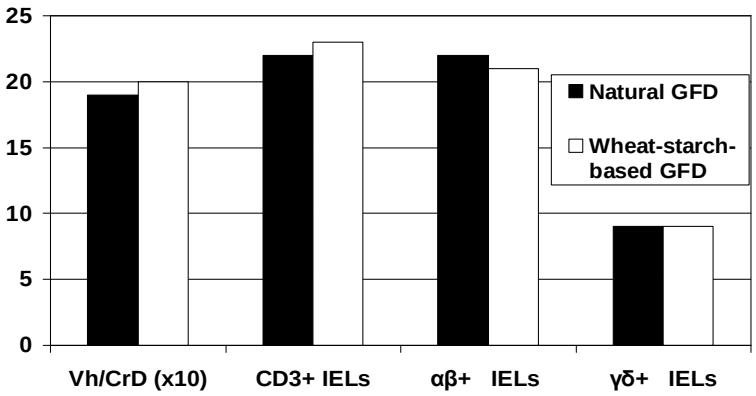

Figure 3. Histological findings between study groups after one year treatment

Quality of life

Gastrointestinal symptoms were alleviated similarly patients in groups I and II. At the beginning of the study the mean GSRS score in group I was 2.7 and in group II 2.7. After one year's treatment the GSRS scores were 1.8 and 1.8, respectively. PGWB scores also improved, before treatment 89.7 in group I and 95.6 in group II, and after intervention 107.3 and 109.9, respectively (difference not significant) (**III**).

Antibodies and laboratory values

There were no differences in serum EmA and tTG antibodies between the study groups, neither at baseline or the after study period. At the time of enrolment and after the one-year study period there were no significant differences between the study groups in blood haemoglobin, serum iron, serum calcium, serum vitamin B12 and erythrocyte folic acid levels (**III**).

Dietary analysis

As noted all patients adhered to a strict GFD during the study period. There were no statistically significant differences in the mean consumption of gluten-free flours between the groups (**III**).

Bone mineral density

BMD did not differ between the study groups at baseline or after the one-year follow-up (**III**).

Patients with a suspicion of coeliac disease

Small-bowel biopsy

Before the dietary intervention both groups showed equal small-bowel morphology, the mean Vh/CrD ratios being 3.6 in the gluten challenge group and 3.4 in the gluten-free group, αβ+ cells 24 and 23, γδ+ cells 10.8 and 11.3, respectively (**IV**).

Intervention score

Before the intervention, the clinical scores did not differ between the study groups. After 6 months` dietary intervention, altogether 11 out of 41 patients had a total clinical score at the level of treated classical coeliac disease patients. These 11 patients were considered to be clinically gluten sensitive. All 11 had coeliac disease-type HLA, 10 had DQ 2 and one DQ8 (**IV**).

Dietary analysis

All patients were consuming a gluten-containing diet at the time of enrolment. All 20 patients in the gluten-free group adhered to a strict GFD during the study period; one of the 21 patients in the gluten challenge group discontinued the challenge on account of symptoms.

Transglutaminase-2-specific small-bowel mucosal IgA deposits

Coeliac disease-type IgA deposits were detectable in 10 out of 11 patients considered gluten-sensitive by clinical score. All six classical coeliac disease patients had this type of IgA deposits. None of the HLA DQ2- and DQ8-negative patients showed IgA deposits typical of coeliac disease.

Discussion

Oats

The present series **(I)** constitutes the first randomized study to evaluate quality of life in coeliac disease patients using oats as a part of their GFD. As expected (Table 10), it was again evident that the majority of coeliac disease patients can use oats, and that the increment is also well tolerated. In acord with this, the questionnaire study (**II**) showed that the majority of coeliac disease patients were indeed currently consuming oats. They felt that oats diversified their GFD, and overall considered oats to comprise an important part of the GFD; they also appreciated the taste, good availability and low cost.

Oats had no effects on the quality of life when evaluated by PGWB, but the questionnaire may be too crude to assess minor changes in general well-being in dietary issues. By contrast, in the present randomized study **(I)** patients taking oats developed gastrointestinal symptoms more often than those using traditional GFD. In the experiencing of diarrhoea there was statistically significant difference to the adverse for oats, as was also in constipation score, albeit not so significantly. The finding is again in agreement with the results of the questionnaire study, where 10.1% of coeliac disease and 18.9% of dermatitis herpetiformis patients experienced gastrointestinal symptoms, and therefore discontinued to take oats.

There are reports of mucosal deterioration after the ingestion of oats (Lundin et al. 2003). In the present controlled study the Vh/CrD was similar in both groups after the study period. There was, however, an increase in IELs in the oat group. There were three drop-

outs owing to gastrointestinal symptoms in this group; a control biopsy was taken in two of these cases, showing incomplete mucosal recovery, similar to that at baseline. Do these findings constitute reason to discourage the use of oats in coeliac disease? On the other hand, in a study by Störsrud and group, patients with coeliac disease used large amounts of oats, up to 100 grams daily, without any harmful effect (Störsrud et al. 2003). It should be noted that, the main purpose of the present study was to assess the quality of life and not histology, which was carried out on voluntary basis. Participation was possible without consent to small intestinal biopsy.

It is not excluded that oats can cause small-bowel mucosal inflammation or even deterioration in some sensitive coeliac disease or dermatitis herpetiformis patients (Lundin et al. 2003, Arentz-Hansen et al. 2004). The drop-outs in study **II** may also bespeak such a possibility. However, for instance temporary skin manifestations may appear in dermatitis herpetiformis even in patients on a traditional strict diet (Reunala et al. 1998).

Nevertheless, the conclusion is, in line with the studies set out in Table 10, that the majority of patients with coeliac disease tolerate oats well, and they feel that oats is an essential part of the diet. One must nevertheless be aware that some individuals may develop symptoms. This is not usually associated with mucosal deterioration, even though it is not out of the question that even this may occur in very rare cases. There is no evidence that the intolerance to oats in some subjects is specific for coeliac disease or avenin indigestion; rather it may reflect intolerance in the population in general.

The contamination of oat products with wheat and other cereals cannot be excluded either in clinical practice, or in clinical studies. The fear of contamination was also the most

common reason for avoiding oats among coeliac patients. Oat production should naturally be free of contamination from field to market. The reliable measurement of for instance wheat and barley contamination in oat products on the market is not yet free of problems.

Wheat starch

A strict life-long GFD is effective and constitutes the only treatment in coeliac disease. It has been debated in the Codex Commission for several years whether the diet should be totally gluten-free, or could it contain trace amounts of gluten. Such gluten contamination is possible in wheat-starch-based products which have been rendered gluten-free industrially. On the other hand, a diet completely devoid of gluten is unrealistic. As seen in Table 9, all previous studies have been cross-sectional, open challenge or uncontrolled. This first randomized study (**III**) showed that the mucosal recovery in patients on a wheat-starch-based GFD was similar to that in patients on a naturally GFD. Of note, wheat-starch-based GFD did not retard mucosal recovery, which occurred as fast as in patients on a naturally GFD. This study has comprises a contribution to previous Codex Statement (Codex-Alimentarius-Commission 2003, Codex-Alimentarius-Commission 2006).

In accord, earlier evidence suggests that wheat-starch-based products are safe. They have been for more than 30 years on the market in Finland, and more than 90 % of coeliac disease patients consume these products. Mucosal recovery has been good in both children and adults (Kaukinen et al. 1999). Malignant complications are rare, and the prognosis of patients does not differ from that in the population in general (Collin et al.

1994). Compliance with diet has been the better than in many other countries (Table 8); whether this is due to better availability of products on the market remains to be seen. Methods such as Vh/CrD to assess the mucosal damage may be relatively crude. In this randomized study **(III)** the focus was also on minor mucosal changes in long-term treatment. The density of αβ+ IELs has been shown to decrease on a strict GFD and again increase during gluten challenge (Savilahti et al. 1990, Kutlu et al. 1993). In the present series (**III**) the density of these cells decreased equally in both groups during dietary treatment. A similar decrease was evident in γδ+ IELs.

It was not possible to measure the exact amount of gluten in the everyday diet in this long-term controlled study. The methods for gluten analysis were crude at the time of the study. It has since been shown that gluten contamination was present in both types of diet (Collin et al. 2004), but more commonly in wheat-starch-based gluten-free products. Conlusion to be drawn from the present study was that the current wheat-starch-based GFD is safe.

Other non-randomized studies also indicate, that trace amounts of gluten in gluten-free products are not toxic or harmful (Table 9). The longest follow-up study is that carried out by a group under Ejderham; patients had taken wheat-starch-based gluten-free products for 10 years on average, and mucosal recovery was complete (Ejderhamn et al. 1988).

Catassi and associates in their short-term study evaluated that challenge with 100-500 mg gliadin caused a decrease in mean Vh/CrD,; it is however to be noted that the villous recovery was not perfect initially (Catassi et al. 1993). Table 9 indicates that wheat contamination of less than 50 mg is well tolerated, whereas grams of gluten induce at

least mucosal inflammation. Randomized microchallenge studies are still needed and are in fact ongoing.

Both diets were well tolerated. The quality of life was equal between the study groups, and as good as in coeliac patients treated with wheat-starch in the long term, or in the population in general (Lohiniemi et al. 2000). Two patients in the naturally gluten-free and one in the wheat-starch-based GFD group discontinued the study. However, there was no evidence that they might be excessive sensitive to wheat starch.

Gluten challenge or gluten-free diet in the diagnosis

The prospective study (**IV**) showed that some patients with suspected coeliac disease not fulfilling the current diagnostic criteria with small-bowel villous atrophy and crypt hyperplasia (Walker-Smith et al. 1990) are gluten sensitive. Earlier in the diagnosis criteria gluten dependency has was essential. In the widened spectrum, this is actually to avoid over diagnosis. It was possible to recognize these patients with gluten dependency clinically by gluten challenge or gluten-free treatment. Altogether 11 out of 41 patients were gluten-dependent according to the clinical score, and all of them had HLA DQ2 or DQ8.

There was other evidence that these patients had early developing or latent coeliac disease. Staining of TG-2 specific IgA deposits in small-bowel mucosa has been described as a reliable diagnostic tool for coeliac disease. These deposits are even predictive of forthcoming overt coeliac disease (Korponay-Szabo et al. 2004, Salmi et al. 2006a). In the present study, IgA deposits detected clinically gluten-dependent patients with probable early developing coeliac disease: the high clinical score and TG-2 specific

small-bowel mucosal IgA deposits found almost completely the same patients, who, again, had coeliac-type HLA DQ.

The concept of latent coeliac disease, where mucosal deterioration has been proved to occur later in life, has been well recognized (Weinstein 1974, Mäki et al. 1990, Mäki et al. 1991a, Holm et al. 1992, Ferguson et al. 1993, Corazza et al. 1996a). The problem is that the diagnosis is retrospective and can be settled only after villous atrophy has developed, and some of the subjects in question may benefit from gluten-free treatment before atrophy is evident (Kaukinen et al. 2001). Early developing coeliac disease would be a more descriptive designation for this condition (Salmi et al. 2006a). On the other hand, a life-long diet is not easy to maintain, and therefore the diagnosis of the condition should be based on definitive evidence. There is no reliable single method to detect early-stage coeliac disease. Mucosal inflammation, positive serology, and, as shown in this study (**IV**), clinical gluten dependence and tTG-specific IgA deposits form the basis on which the diagnosis can be verified in borderline cases. This is important to recognize, since a widespread use of serolocigal screenings tests will yield many cases with these antibodies without villous atrophy. Villous atrophy with crypt hyperplasia will no longer to be the only standard in the diagnosis of coeliac disease. The diagnostic criteria should be widened to take account expression of genetic gluten intolerance.

Summary and conclusions

A body of evidence, including 10 years´ clinical experiences, shows that oats are safe even in the long term. The two oat studies (**I,II**) showed that patients with coeliac disease accepted oats as an essential part of their GFD. They appreciated the taste, easy utilization and low cost. The patients further felt that oats diversified their everyday GFD. Oats was not detrimental to quality of life as measured by PGWB. Patients on oats experienced more gastrointestinal symptoms, diarrhoea and also constipation, than those on a traditional GFD. There were indications that oat avenin may have been responsible for this. In addition, there was also an increase in the density of inflammatory cells in the small bowel mucosa. This has not hitherto been shown in randomized studies, and this study was not mainly focused on histological changes. There still remains a possibility that some individuals are sensitive to the oat prolamin avenin. The findings altogether do no support the conception that all coeliac patients should avoid oats, but these facts should be considered when coeliac disease patients start to take oats, or develop symptoms afterwards.

This first randomized study (**III**) showed that wheat-starch-based gluten-free products in treatment of coeliac disease are safe and well tolerated a matter which has been debatable for many years. The products did not maintain inflammation or mucosal damage, even when minor mucosal changes were investigated: mucosal recovery occurred as rapidly as by naturally GFD. There was no evidence that some patients might be particularly gluten sensitive, i.e. sustain mucosal atrophy or inflammation by taking these products.

Randomized prospective microchallenge studies are needed to estimate the safe threshold in milligrams for contamination in gluten-free products.

Patients with a suspicion of coeliac disease without villous atrophy can be recognized by various methods which support each other (**IV**). The demonstration of gluten dependence, by GFD or gluten challenge, was possible in borderline cases. The results were verified by examining TG-2-specific small-bowel mucosal IgA deposits in clinically gluten sensitive patients despite the absence of villous atrophy. The patients also had coeliac-type HLA DQ, and were very likely suffering from early developing coeliac disease. Judging from, on these findings it would appear to be time to extend the coeliac disease diagnosis from traditional villous atrophy to include genetic gluten dependency with or without atrophy.

Acknowledgements

This study was carried out at the Department of Gastroenterology and Alimentary Surgery, at the Tampere University Hospital.

I wish to express my profound gratitude to:

My supervisor Docent Pekka Collin, MD, the Head of the Department of Gastroenterology and Alimentary Tract Surgery. He gave me an opportunity to join the group and aroused my interest in the world of coeliac disease and science. He always had time to advise and help me, without his helpful attitude and excellent sense of humour this study would never have been completed. ”Peace and profound respect”;

Docent Martti Färkkilä, MD and Docent Markku Heikkinen, MD, the reviewers of this thesis, for their constructive criticism on the manuscript;

Docent Isto Nordback, MD, the Director of the Division of Gastroenterology Surgery and Oncology and Docent Juhani Sand, MD, the Head of the Department of Gastroenterology and Alimentary Tract Surgery, for their support during the final stages of this study;

Professor Markku Mäki, MD, for providing working facilities in his laboratory. His constant encouragement and enthusiastic attitude have been indispensable to this study;

My co-authors Tuula Halttunen, PhD, Tanja Kaartinen, Leila Kekkonen, MA, Ilma Korbonay-Szabo, MD, Susanna Lohiniemi, MA, Sanna Miettinen, MsC, Kirsi Mustalahti, MD, Tuula Nuutinen, Kaija Paasikivi, Docent Jukka Partanen, MD, Docent Harri Sievänen, MD, Nanna Vuolteenaho and Nina Woolley for their valuable help during the study. Especially, co-author Docent Katri Kaukinen,MD is acknowledged for her constructive criticism during the study project, and she showed me the way to Pekka`s room;

Docent Anna-Liisa Karvonen, MD and Docent Pekka Pikkarainen, MD, who guided me in to the difficult world of gastroenterology. It has been a priviledge to work with these experienced gastroenterologists;

All members of Coeliac Disease Study Group for their technical advise and support. Their contribution was essential in completing this study. At the same time I acknowledge the secretary of Coeliac Disease Study Group Mrs Kaija Kaskela;

Emeritus Professor Jarmo Visakorpi, for his fundamental work for coeliac disease research in Tampere;

All coeliac disease patients for participating in this study;

My colleagues and nursing staff at the Department of Gastroenterology and Alimentary Tract Surgery, for their positive attitude towards my work. I also thank colleagues and nurses in Jyväskylä Central Hospital and in the Department of Internal Medicine, University Hospital Tampere;

Mr. Robert MacGilleon, MA, for his excellent work in revising the English text of the manuscripts and thesis;

My friends, especially members of OOK and ryhmä rämä, for being my friends;

Tuula Tyrväinen, MD, who has literally shared with me the joys and sorrows of everydaylife. She has also continously reminded me of more important things of life. Our little girl Saana, the sunshine of my life, has helped me to remember what is more important in life than work and Coeliac Disease. Samu, son of Tuula, is also acknowledged for his skillful technical assistance;

This work was financially supported by the Research Fund of the Finnish Coeliac Society, the Medical Research Fund of Tampere University Hospital and Research Fund of Middle Finland Central Hospital;

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Markku Peräaho
Tampere 24.2.2007

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ORIGINAL PUBLICATIONS

ORIGINAL ARTICLE

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Effect of an Oats-Containing Gluten-free Diet on Symptoms and Quality of Life in Coeliac Disease. A Randomized Study

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Peräaho M, Kaukinen K, Mustalahti K, Vuolteenaho N, Mäki M, Laippala P, Collin P. Effect of an oats-containing gluten-free diet on symptoms and quality of life in coeliac disease. A randomized study. Scand J Gastroenterol 2004;39:27–31.

Background: Evidence suggests the acceptability of oats in a gluten-free diet in coeliac disease. We investigated the impact of an oats-containing diet on quality of life and gastrointestinal symptoms. **Methods:** Thirty-nine coeliac disease patients on a gluten-free diet were randomized to take either 50 g of oats-containing gluten-free products daily or to continue without oats for 1 year. Quality of life was assessed using the Psychological General Well-Being questionnaire and gastrointestinal symptoms using the Gastrointestinal Symptom Rating Scale. Small-bowel mucosal villous architecture, CD3+, $\alpha\beta$+, $\gamma\delta$+ intraepithelial lymphocytes, serum endomysial and tissue transglutaminase antibodies were investigated. **Results:** Twenty-three subjects were randomized to the oats-containing diet and 16 to the traditional gluten-free diet. All adhered strictly to their respective diet. Quality of life did not differ between the groups. In general, there were more gastrointestinal symptoms in the oats-consuming group. Patients taking oats suffered significantly more often from diarrhoea, but there was a simultaneous trend towards a more severe average constipation symptom score. The villous structure did not differ between the groups, but the density of intraepithelial lymphocytes was slightly but significantly higher in the oats group. The severity of symptoms was not dependent on the degree of inflammation. Antibody levels did not increase during the study period. **Conclusion:** The oats-containing gluten-free diet caused more intestinal symptoms than the traditional diet. Mucosal integrity was not disturbed, but more inflammation was evident in the oats group. Oats provide an alternative in the gluten-free diet, but coeliac patients should be aware of the possible increase in intestinal symptoms.

Key words: Coeliac disease; oats; quality of life

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There is a body of evidence indicating that patients with coeliac disease can include oats in their diet. An oats-containing gluten-free diet does not hamper recovery from small-bowel mucosal damage, even long-term use (1–4). Recently, however, the safety of oats has been called into question (5, 6). Clearly, some coeliac disease patients cannot tolerate oats, even though no changes have been observed in morphometrical or serological tests (7). An important issue in the treatment of coeliac disease is quality of life (8). Oats diversify the gluten-free diet for the patient, but the impact on quality of life remains by and large obscure. The aim of this randomized controlled study was to compare an oats-containing gluten-free diet to the traditional one, focusing especially on general well-being and gastrointestinal symptoms in coeliac disease patients in clinical and histological remission. Simultaneously, the effect of an oats-containing gluten-free diet on the small-intestinal mucosa was evaluated.

Materials and Methods

Patients

The study cohort comprised 39 patients with biopsy-proven coeliac disease at the Dept. of Medicine of Tampere University Hospital; all had been on a gluten-free diet without oats. Definite, though not necessarily complete, mucosal recovery was evident in all. The patients were randomized either to take 50 g of oats-containing gluten-free products daily or to continue their current diet without oats. Randomization was carried out using random-number tables (9). Each patient entered the trial before the random treatment assignment was revealed. The follow-up time was 1 year.

Dietary assessment

A detailed dietary analysis and a history of occasional or

*Pekka Laippala deceased after submitting this manuscript.

DOI 10.1080/00365520310007783

regular consumption of gluten-containing products, oats and fibre were assessed by means of an interview and a 4-day record of food intake 0, 6 and 12 months after entering the study.

Gastrointestinal symptoms and quality of life

Quality of life was assessed using the Psychological General Well-Being (PGWB) questionnaire (10, 11), which has been developed to measure emotional states reflecting a sense of subjective well-being. The PGWB index includes 22 items for 6 states: anxiety, depressed mood, self-control, positive well-being, general health and vitality. The questionnaire yielded a total score—the lower the score the more evident were problems in well-being. The Short-Form Health Survey (SF-36) questionnaire has also been used in other studies (8, 12, 13) to evaluate psychological well-being, but here we considered that oats would not have any impact on this questionnaire, which measures for instance physical functioning, general health and vitality.

Gastrointestinal symptoms were evaluated by total score on the Gastrointestinal Symptom Rating Scale (GSRS) (11), which is also well validated in coeliac disease (12, 13). This comprised altogether 15 items in 5 subdimensions describing: (i) abdominal pain (abdominal pain, nausea and vomiting), (ii) gastro-oesophageal reflux (heart burn, acid regurgitation), (iii) indigestion (borborygmus, abdominal distension, eructation, increased flatus), (iv) diarrhoea (increased passage of stools, loose stools, urgent need for defecation), and (v) constipation (decreased passage of stools, hard stools, feeling of incomplete evacuation). Each item was graded from 1 to 7; the higher the score the more gastrointestinal symptoms; the average total and the subdimension scores were counted. Quality of life was evaluated before and at the end of the study.

Small-bowel biopsy

A small-intestinal biopsy was taken during a gluten-free diet from all enrolled patients, and a further biopsy was taken by endoscopy at the end of the study. Small-bowel biopsy specimens were taken from the distal part of the duodenum; three to five specimens were processed and stained with haematoxylin-eosin and studied under light microscopy. Morphometric analysis, including villous height and crypt depth ratio (Vh/CrD), was made in well-oriented biopsy samples as described previously (14). Poorly oriented sections were discarded; when necessary, the samples were dissected repeatedly until they were of good quality.

In some cases the first biopsy had been taken in other endoscopy units, where frozen samples had not been taken routinely; these were still available in 14 out of 39 cases. Immunohistochemical stainings were made of these 14 and of all follow-up samples. Two small-bowel biopsy specimens were freshly embedded in optimal temperature compound (OCT, Tissue-Tec, Miles Inc, Elkhart, Ind., USA), snap-frozen in liquid nitrogen and stored at $-70\,^{\circ}C$. Immunohistochemical studies were carried out on 5-μm-thick frozen sections. $\alpha\beta+$ IELs were stained with monoclonal βF1 antibody (Endogen, Woburn, Mass., USA) and $\gamma\delta+$ IELs with TcR$\gamma\delta$ (Endogen). IELs were counted with a $\times 100$ flat-field light microscope objective in randomly selected surface epithelium; at least 30 fields of 1.6 mm epithelial length were counted and the density of IELs expressed as cells/millimetre of epithelium (15, 16).

Serology and chemical analysis

Serum IgA class endomysial antibodies (EmA) were determined using an indirect immunofluorescence method with human umbilical cord as substrate (17); a dilution $1{:}\geq 5$ was considered positive. Serum IgA-class tissue transglutaminase antibodies (tTg-ab) were investigated by enzyme-linked immunosorbent assay (ELISA) (Celikey; Pharmacia, Uppsala, Sweden), a unit value (U) ≥ 5 being positive (18). Blood haemoglobin, serum iron and erythrocyte folic acid concentrations were measured using routine laboratory methods.

Statistics

The data were analysed using analysis of variance for repeated measures. Equality of the baseline was tested with a *t* test, which was also applied in the analysis of biopsy samples. Dependence between numerical variables was studied using a correlation coefficient. Logarithm transformation was applied because of skewed distributions. The data characteristics are displayed crude, using mean, median and standard deviations. Computation was carried out using Statistica for Windows (Version 6.0) software. The level of significance was set equal to 0.05 and the *P* values are presented exact. The required number of participants was determined to achieve the statistical power of 0.80 at a significance level of 0.05; a difference of <0.5 in Vh/CrD, 7 or more in CD3+ and $\alpha\beta+$ cells, 3 in $\gamma\delta+$ cells and a score of 0.5 or more in GSRS, was considered clinically relevant.

Ethical considerations

The study protocol was approved by the Ethics Committee of Tampere University Hospital. All subjects gave informed consent.

Results

Gluten-free diet

Twenty-three coeliac disease patients were randomized to the oats-containing diet and 16 to the traditional gluten-free diet (Table I). The groups were similar with respect to age and gender. All adhered to a strict gluten-free diet during the study period. The average daily fibre consumption was similar at the time of enrolment, and neither group showed significant alterations in average daily fibre consumption; the average daily consumption of oats in the relevant group was 30 g (Table I), which was less than allowed in the protocol.

Table I. Coeliac patients on remission, randomized to oats-containing and traditional gluten-free diet for 12 months

	Oats	No oats
Patients (male)	23 (6)	16 (4)
Median age (range)	48 (25–69)	46 (22–65)
Time on gluten-free diet, months; median (range)	34 (13–81)	27 (12–48)
Strict dietary compliance	23	16
Interrupted the study (*n*)	3	0
Median (range) daily oat consumption in grams		
Before the study	0	0
At 6 months	35 (0*–60)	0
At 12 months	28 (0*–70)	0
Median (range) daily fibre consumption in grams		
Before the study	12 (7–25)	17 (7–28)
At 6 months	15 (10–32)	16 (9–21)
At 12 months	15 (11–32)	17 (8–23)

*One patient at 6 months and another at 12 months did not report consumption of oats in their 4-day food record.

Table III. Mucosal and laboratory findings in coeliac patients after 1 year of follow-up

	Oats group	Non-oats group	*P* value
Villous height/crypt depth ratio (mean)	2.5	2.4	NS
CD3+ IELs*	44.6 (22.7)	26.7 (21.0)	0.039
$\alpha\beta$+ cells	29.8 (18.8)	19.9 (20.3)	0.141
$\gamma\delta$+ cells	11.3 (6.1)	5.3 (6.2)	0.05
Haemoglobin g/L (120–180) (mean)	130	134	NS
Erythrocyte folate nmol/L (320–900) mean	540	489	NS
Serum iron µmol/L (6–35) (mean)	16.4	17.7	NS

NS = not significant.
*Intraepithelial lymphocytes/millimetre of epithelium.

Quality of life

The average PGWB score was 103.8 (standard deviation (*s*) = 11.4) in the oats-consuming group and 105.4 (17.2) in the traditional gluten-free diet group at the time of randomization. After 1 year the scores were 98.8 (20.0) and 101.3 (16.1), respectively. The differences were not statistically significant. The average GSRS scores (total and subdimensions) did not differ statistically significantly between the two groups at the time of enrolment. At the end, there was a trend towards a higher GSRS total score in the oats group, and the symptoms of diarrhoea were statistically significantly more severe in this group at the end of the study; despite this, the constipation score also increased similarly in both groups (Table II). Symptoms of indigestion were alleviated in both groups, but more effectively in patients taking oats. Reflux score increased similarly in both groups.

Small-bowel biopsy

Small-bowel biopsy specimens had been taken from the patients approximately 1 year after they had adopted a gluten-free diet; this biopsy was the routine control to ensure histological remission on a gluten-free diet. In this biopsy, the average V/CrD ratio was 2.1 in patients randomized to oats and 1.8 in those randomized to omit oats; immunohistochemical stainings were available in 6 patients in the oats-consuming group and in 8 in the non-oats group; the mean densities of CD3+ IELs were 47.8 and 39.6, $\alpha\beta$+ cells 27.0 and 25.6, and $\gamma\delta$+ cells 14.9 and 12.5, respectively.

Eighteen patients in the oats group and 13 in the traditional gluten-free group consented to a biopsy at the end of the study; altogether 8 refused. There were no differences between the two groups in small-bowel mucosal morphology, whereas the density of intraepithelial lymphocytes (IELs) was statistically significantly higher in the oats group (Table III). The increase in GSRS was not associated with the increase in density of IELs: in fact there was a trend towards a negative correlation, though not statistically significant.

Dropouts in the oats group

Three patients in the oats group decided to discontinue after developing gastrointestinal pain and abdominal distension. A control biopsy taken in two cases showed incomplete

Table II. Gastrointestinal symptom rating scale (GSRS) total score and subdimensions in coeliac patients randomised to take oats containing and oat-free gluten-free diet

	Oats				No oats				*P* values, analysis of variance		
	At the beginning		At the end		At the beginning		At the end				
Symptom	Mean (*s*)	Median	Mean (*s*)	Median	Mean (*s*)	Median	Mean (*s*)	Median	Group	Period	Interaction
Total score	1.86 (0.55)	1.80	2.00 (0.50)	2.07	2.08 (0.77)	2.00	1.94 (0.70)	1.60	0.917	0.876	0.094
Diarrhoea	1.59 (0.51)	1.33	2.03 (0.74)	2.00	1.90 (0.88)	1.67	1.69 (0.91)	1.33	0.645	0.540	0.010
Indigestion	2.27 (0.66)	2.13	2.06 (0.59)	2.00	2.81 (1.17)	2.63	2.13 (1.14)	1.63	0.597	0.002	0.051
Constipation	1.79 (1.03)	1.33	2.24 (0.70)	2.33	1.88 (1.22)	1.50	2.23 (1.23)	1.67	0.785	0.010	0.297
Abdominal pain	1.85 (0.73)	1.67	1.56 (0.39)	1.33	1.85 (0.57)	2.00	1.83 (0.58)	1.83	0.307	0.267	0.297
Reflux	1.75 (1.07)	1.50	2.07 (0.92)	2.00	1.63 (1.00)	1.00	1.81 (0.87)	1.50	0.432	0.051	0.781

s = standard deviation.

Table IV. Patients who discontinued oats consumption because of symptoms: IgA antiendomysial (EmA) and antitissue-transglutaminase (tTg-ab) antibodies, small-bowel morphology and intraepithelial lymphocytes at the time of refusal

Discontinued	EmA	TTg-ab	V/CrD	CD3+ cells	$\alpha\beta$+ cells	$\gamma\delta$+ cells
Female, 35 years	0	1.1	Not done	Not done	Not done	Not done
Female, 52 years	0	1.0	2.0	46	19	20.5
Female, 46 years	0	1.9	1.7	42	32	5.6

V/CrD = villous height/crypt depth ratio.

recovery; the serological tests were normal in all three (Table IV).

Laboratory analysis

There were no statistical differences between the study groups in blood haemoglobin, serum iron, folic acid or antibody levels (Table III). Two patients were positive for EmA and tTg-ab, and two for tTg-ab only at the time of enrolment in the oats group. During follow-up, the antibody levels decreased in three (Table V). These patients had no symptoms; a control biopsy was taken from one patient showing incomplete mucosal recovery.

Discussion

Evidence suggests that oats are not harmful for coeliac disease patients (2–4, 7). Recent in vitro studies have indicated that oat prolamine avenin does not stimulate endomysial antibody (19) or inflammatory cytokine production (20). This notwithstanding, the adoption of oats products in a gluten-free diet is discouraged in the United States and in many countries elsewhere (21). Fear of contamination is certainly the most common reason, but there is still concern over the toxicity of oats. Recently, Lundin et al. (5) described a patient who developed symptoms and villous atrophy after ingesting oats.

Quality of life has become an important question in coeliac disease. Studies in Sweden have shown quality of life to be impaired in well-treated coeliac disease patients, especially women (8, 12). In the question of whether or not to recommend oats for patients with coeliac disease, also quality of life must be considered.

In this randomized study, oats had no effect on quality of life as measured by the PGWB questionnaire. However, patients taking oats developed more gastrointestinal symptoms than those on the traditional gluten-free diet. Not surprisingly, an increase in diarrhoea score was most evident, but not the sole one. It is notable that the constipation score also increased. The appearance of symptoms could not be explained by an increased use of fibre: average daily fibre consumption was by and large the same in both groups throughout the study (Table I). The patients consumed oats 30 g on average, which was less than stated in the protocol. We believe that for many patients it is difficult to follow a diet that contains 50 g of oats every day.

The villous structure recovered equally in both groups, but IELs were increased in the oats group. This slight increase mainly in $\gamma\delta$+ IELs does not in our opinion mean that oats should be abandoned in the coeliac diet. First, the density of IELs was relatively mild, comparable to what we have seen in treated coeliac patients in general. Second, we saw no correlation between the symptoms and intraepithelial lymphocytosis. Third, no other previous controlled studies have demonstrated any harmful effects from an oats-containing gluten-free diet (2–4, 7). Nevertheless, it is possible that avenin may cause a limited inflammatory reaction in the gut, as Lundin et al. (5) suggest.

Wheat contamination of oat products was of course possible in the present study. However, the proposal for a new codex standard for gluten-free products, 200 ppm, was exceeded in only 1 out of 20 commercial oat products available and tested in Finland (Finnish Coeliac Disease Society, unpublished observations).

Even though this was a randomized study, there were some differences between the two groups. Mucosal shortening was initially more evident in patients randomized to the oats group, which also comprised all initially antibody-positive subjects. On the other hand, the relatively long study period with thorough dietary surveillance probably compensated for this difference.

We conclude that oats can be included in the coeliac diet. However, patients and physicians should be aware that abdominal complaints may even be asseverated by an oats-

Table V. Antibody-positive patients in the study: Serology (IgA antiendomysial; EmA, and antitissue-transglutaminase; tTg-ab antibodies), histology and symptoms score before and at the end of the study

	EmA before	EmA after	TTg-ab Before	TTg-ab after	Histology before	Histology after	GSRS total
Male, 60 years	0	0	9.8	9.9	2.3	1.2	1.7
Male, 42 years	0	0	8.3	2.9	1.5	ND	2.3
Female, 43 years	200	50	6.8	6.1	1.5	ND	2.2
Female, 33 years	5	5	8.6	4.5	1.7	ND	1.1

GSRS = gastrointestinal symptom rating scale.

containing gluten-free diet, even though villous damage is unlikely to ensue.

Acknowledgements

This study was supported by the Medical Research Fund of Tampere University Hospital, the Central Hospital of Jyväskylä and the Yrjö Jahnsson Foundation.

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Received 17 June 2003
Accepted 14 October 2003

Perspectives in Practice

Oats Can Diversify a Gluten-Free Diet in Celiac Disease and Dermatitis Herpetiformis

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ABSTRACT

Finnish celiac disease and dermatitis herpetiformis patients have used oat-containing gluten-free diets since 1997. The aim of this study was to evaluate how the use of oats has been adopted. The use of oats and the effect of oats on symptoms and quality of life were investigated in 1,000 randomly selected members of the Celiac Society. Altogether, 710 patients responded: 423 (73%) with celiac disease and 70 (55%) with dermatitis herpetiformis were currently consuming oats. Patients appreciated the taste, the ease of use, and the low costs; 94% believed that oats diversified the gluten-free diet; 15% of celiac disease and 28% of dermatitis herpetiformis patients had stopped eating oats. The most common reasons for avoiding oats were fear of adverse effects or contamination. There is a market demand for oats, and celiac societies and dietitians should make efforts to promote the development of products free of wheat contamination.

J Am Diet Assoc. 2004;104:1148-1150.

Celiac disease is a chronic inflammatory disorder of the small intestine resulting from the ingestion of gluten found in wheat, barley, and rye (1). The cornerstone in the treatment of the disease is lifelong strict adherence to a gluten-free diet. A body of evidence shows that most patients with celiac disease and dermatitis herpetiformis can consume moderate amounts of uncontaminated oats without harmful effects on the intestinal mucosa, even in long-term use (2-7). Despite this, the adoption of oat products in the gluten-free diet is discouraged in the United States and in many countries elsewhere (8,9), the major concern being whether oat products are free of wheat contamination. The possible toxicity of oat avenin has also been implicated (10), but all controlled clinical studies have so far demonstrated its safety.

The prevalence of celiac disease in the United States seems to be similar to that in many European countries, which is 0.5% to 1.0% (11). Assuming that even a half of sufferers are detected, we can estimate that 1 million US citizens should adhere to a gluten-free diet. Provided that oat-containing gluten-free products constitute an essential part of the celiac diet, the industry should proceed to the development of oat products free of wheat contamination.

In 1997, the scientific advisory board of the Finnish Coeliac Disease Society issued a statement whereby oat-containing gluten-free-products were permissible for adults with celiac disease. The statement was extended in 1998 to concern patients with dermatitis herpetiformis and to children in 2000. The content of contaminating gliadin in commercial oat products had previously been analysed: 29 samples had gliadin levels below 28 mg/kg and only one organic cultured oat product was clearly wheat-contaminated, containing 390 mg/kg of gliadin (12); by comparison, one proposal for the threshold for residual gluten in gluten-free products has been set at 200 mg/kg (13). The aim of this study was to establish how patients with celiac disease adopted the use of oats nationwide during this period and whether they feel that the oat products diversify their gluten-free diet.

SUBJECTS AND METHODS

The inquiry involved 1,000 randomly selected celiac disease and dermatitis herpetiformis patients out of 14,000 members of the Finnish Celiac Society: 710 (521 female; 189 male; median age, 52; range, 5 to 84 years) patients responded. The questionnaire comprised 7 items asking (a) whether patients had tried oats; (b) are currently using oats; (c) whether those with newly detected celiac disease are more willing to eat oats than patients who had adhered to the diet for a long time; (d) how long and regularly patients had consumed oats; (e) had they developed any symptoms; (f) whether they feel that oats diversifies the diet; and (g) if not using oats, the reason for this. Cross tabulations were carried out by χ^2 test.

RESULTS

Altogether, 494 (70%) out of 710 patients were currently eating an oat-containing gluten-free diet, 75% of them

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0002-8223/04/10407-0007$30.00/0

doi: 10.1016/j.jada.2004.04.025

Table. Current use of oat-containing gluten-free diets in different subgroups in 710 patients with celiac disease or dermatitis herpetiformis

	Number of patients[a]	Number of Patients Currently Eating Oats		*P* value; χ^2 test
		n	%	
Sex				
Female	521	365	70	.71
Male	189	129	69	
Disease				
Celiac disease	581	423	73	<.001
Dermatitis herpetiformis	127	70	55	
Age group				
Adults ≥18 y	647	449	69	.045
Children <18 y	56	45	80	
Time of diagnosis				
Before 1997[b]	397	246	62	.001
In 1997 or later	295	236	80	

[a]Data were missing in some questionnaires.
[b]The first nationwide recommendation was given in 1997.

daily or two to three times in a week. Fifteen percent had never tried to eat oats. In subgroup analysis, patients with celiac disease consumed oats more often than those with dermatitis herpetiformis; the use of oats was also more frequent in children than in adults and in those whose diagnosis was made after the nationwide recommendation (Table).

Of all patients, 71 (10.1%) with celiac disease and 27 (18.9%) with dermatitis herpetiformis had stopped using oats, patients with celiac disease mainly because of bloating and diarrhea; 19 patients with dermatitis herpetiformis had experienced some skin symptoms. Of the 111 patients who had never used oats in their otherwise gluten-free diet, 43 were going to try these products in the future. The most common reason for avoiding oats was the fear of contamination and adverse effects. The majority (94%) of the 494 patients currently consuming oats felt that oats diversified the gluten-free diet and considered oats to constitute an important part of the diet; 80% appreciated the taste, 91% the ease of using the oat products, and 82% the low costs (Figure).

DISCUSSION

In the present survey, the majority, altogether 70% of celiac disease and dermatitis herpetiformis patients, were consuming oats in their gluten-free diet and tolerated it well. Fear of adverse effects and contamination was the most common reason for avoiding oats, but, on the other hand, one third of those who had never taken oats were willing to try in the future.

Some individuals developed symptoms that, according to the current literature, were unlikely to be due to avenin toxicity (3,4,6). The symptoms were, in general, mild and resulted only rarely in withdrawal of oat products. The most frequent adverse effect was gastrointestinal symptoms, which had also been shown in a study from Sweden, no correlation between symptoms and intestinal mucosal architecture or inflammation being evident (6).

The occurrence of rash was relatively high, but, on the other hand, dermatitis herpetiformis patients on a traditional gluten-free diet developed rash as frequently as those avoiding oats, and, again, no differences were seen with respect to intestinal mucosal inflammation (3).

One important issue in celiac disease is the quality of life, and Hallert and colleagues (14,15) have shown this to be reduced even in well-treated celiac patients, especially in women. In the present study, almost all (94%) celiac patients currently eating oats felt that oats diversified their gluten-free diet and was in many respects beneficial. Oats might moderate the adherence to an otherwise gluten-free diet; in the present survey, all responding patients were on a gluten-free diet, and, in general, dietary compliance in Finland is good (88%) (16). Compliance with a gluten-free diet is not always so high, with only 17% to 65% of celiac disease patients adhering strictly (17-19).

Currently, oats are, in general, used in only a few countries, for instance, in Finland and the United Kingdom. By contrast, the adoption of oat products in a gluten-free diet is discouraged in the United States (9). This study shows, however, that oats constitute an important element in the treatment of celiac disease and dermatitis herpetiformis. Celiac societies and dietitians should therefore make efforts to promote the development of oat products free of wheat contamination.

CONCLUSIONS

We conclude that, provided that safe oat products are available, the majority of celiac disease and dermatitis herpetiformis patients prefer to consume oats; it is well tolerated and patients believe that this increment diversifies the diet. There would appear to be a market demand for uncontaminated oat products, and celiac socie-

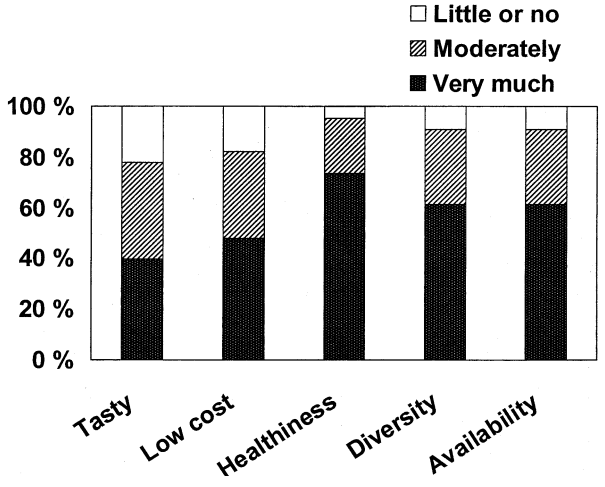

Figure. Reasons why celiac disease patients say they include oats in their gluten-free diet.

ties and dietitians should make efforts to promote the development of oat products free of wheat contamination.

Supported by the Research Fund of the Finnish Coeliac Society and the Medical Research Fund of Tampere University Hospital.

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Aliment Pharmacol Ther 2003; 17: 587–594. doi: 10.1046/j.0269-2813.2003.01425.x

Wheat-starch-based gluten-free products in the treatment of newly detected coeliac disease: prospective and randomized study

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Accepted for publication 1 October 2002

SUMMARY

Background: The safety of wheat-starch-based gluten-free products in the treatment of coeliac disease is debatable. Prospective studies are lacking.

Aim: To compare the clinical, histological and serological response to a wheat-starch-based or natural gluten-free diet in patients with newly detected coeliac disease.

Methods: Fifty-seven consecutive adults with untreated coeliac disease were randomized to a wheat-starch-based or natural gluten-free diet. Clinical response, small bowel mucosal morphology, CD3+, αβ+ and γδ+ intra-epithelial lymphocytes, mucosal human leucocyte antigen-DR expression and serum endomysial, transglutaminase and gliadin antibodies were investigated before and 12 months after the introduction of the gluten-free diet. Quality of life measurements were performed by standardized questionnaires and the bone mineral density was analysed.

Results: In both groups, abdominal symptoms were alleviated equally by a strict diet. There were no differences between the groups in mucosal morphology, the density of intra-epithelial lymphocytes, serum antibodies, bone mineral density or quality of life tests at the end of the study. Four patients on a natural gluten-free diet and two on a wheat-starch-based gluten-free diet had dietary lapses; as a result, inadequate mucosal, serological and clinical recovery was observed.

Conclusions: The dietary response to a wheat-starch-based gluten-free diet was as good as that to a natural gluten-free diet in patients with newly detected coeliac disease.

INTRODUCTION

A gluten-free diet affords clinical and small bowel mucosal recovery in coeliac disease. Patients who maintain a normal gluten-containing diet or have frequent dietary transgressions run an increased risk of osteoporosis[1–3] and possibly of intestinal lymphoma.[4, 5] However, it remains unproven whether trace amounts of gluten are detrimental, provided that the diet is, on the whole, strict.

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Industrially purified wheat-starch-based gluten-free products meeting the current Codex Alimentarius Standard are allowed to contain up to 0.05 g nitrogen per 100 g of food product on a dry matter basis;[6] therefore, these products may contain residual gluten up to 40–60 mg per 100 g of dry matter.[7, 8]

Theoretically, small amounts of gluten may be harmful in coeliac disease and, indeed, such products have evoked abdominal symptoms in an open challenge study.[9] On the other hand, according to some studies, wheat-starch-based gluten-free flours are well tolerated and cause no small bowel mucosal deterioration.[10–13] In some challenge studies, small bowel mucosal inflammation has appeared after the ingestion of 5 mg to 5 g

of gluten,[14, 15] whereas, in others, no significant mucosal changes have been observed.[16–18] Due to this discrepancy, controversy persists as to the safety of wheat-starch-based gluten-free products: these have been used for over 30 years in the UK and Scandinavia, but their consumption is discouraged in the USA.[19]

All previous investigations concerning wheat-starch-based gluten-free products have been either open, short-term challenge studies[9, 16, 17] or cross-sectional trials.[10, 11, 13] In the event that wheat-starch-based gluten-free flours are harmful, one would expect mucosal recovery to be slower and inflammation to be sustained longer when these are used instead of natural gluten-free products. This prompted us to compare, in a randomized 1-year prospective study, the histological response to wheat-starch-based gluten-free and natural gluten-free diets in a group of patients with newly detected coeliac disease. We especially focused on minor mucosal inflammatory changes, and the clinical response to the diets was also evaluated.

PATIENTS AND METHODS

Subjects

Altogether, 65 adults were diagnosed as having coeliac disease at the Department of Medicine, Tampere University Hospital, between April 1998 and February 2000; eight refused to participate in the study. The remaining 57 were randomized to receive a wheat-starch-based or natural gluten-free diet. The randomization was carried out using random-number tables with permuted blocks.[20] Each patient entered the trial before random treatment assignment was revealed. The diagnosis of all coeliac patients was based on severe partial or subtotal small bowel villous atrophy with crypt hyperplasia. The symptoms or signs leading to the diagnosis of coeliac disease, duration of symptoms, immunosuppressive medication, family history of coeliac disease and associated conditions were recorded. Clinical, histological and serological studies were carried out before and after the adoption of the gluten-free diet; the follow-up time was 1 year.

Fifty-nine adults who underwent upper gastrointestinal endoscopic examination due to indigestion or heartburn served as controls for histological analysis; all were consuming gluten and had no relatives with coeliac disease.

Dietary assessment

The dietitian provided advice on natural or wheat-starch-based gluten-free diets at the start of the study. A detailed dietary analysis was taken and a history of occasional or regular consumption of gluten-containing products was assessed by means of an interview and a 4-day record of food intake 3 and 9 months after adopting the diet. The dietitian evaluated the daily consumption of gluten-free flours (either natural or wheat-starch-based) in grams.

Small bowel biopsy

Small bowel biopsy specimens were taken by upper gastrointestinal endoscopy from the distal part of the duodenum at baseline and 12 months after commencing a gluten-free diet. Three to five biopsy specimens were processed and stained with haematoxylin–eosin and studied under light microscopy. Morphometric analysis, including the villous height to crypt depth ratio, enterocyte cell height and the density of intra-epithelial lymphocytes per 100 enterocytes, was performed for well-oriented biopsy samples, as described previously.[17, 21] Poorly oriented sections were discarded; when necessary, the samples were dissected again until they were of good quality.

Frozen samples had not been taken routinely at the first endoscopy, but were still available in 20 of the 57 patients with newly detected coeliac disease. Immunohistochemical staining was performed in these 20 samples, and in all follow-up and control biopsy specimens. Two small bowel biopsy specimens were taken and freshly embedded in optimal temperature compound (Tissue-Tec, Miles Inc, Elkhart, IN, USA), snap frozen in liquid nitrogen and stored at -70 °C. Immunohistochemical studies were carried out on 5-μm-thick frozen sections. The αβ+ intra-epithelial lymphocytes were stained with monoclonal βF1 antibody (Endogen, Woburn, MA, USA) and the γδ+ intra-epithelial lymphocytes with TcRγδ (Endogen). Intra-epithelial lymphocytes were counted with a $\times 100$ flat-field light microscope objective in randomly selected surface epithelium; at least 30 fields with an epithelial length of 1.6 mm were counted, and the density of intra-epithelial lymphocytes was expressed as the number of cells per millimetre of epithelium.[22, 23] Mucosal human leucocyte antigen (HLA)-DR expression was detected with monoclonal HLA-DR antibody

(Becton Dickinson, San Jose, CA, USA) at a dilution of 1 : 1000. HLA-DR expression was considered to be enhanced (positive) when it was strong in the villous epithelium or expression was seen in the crypts; negative expression in the crypts and only slight to moderate staining in the villous epithelium was considered to be normal (negative).[24]

In our laboratory, the correlation coefficients for intra-observer variation for αβ+ and γδ+ intra-epithelial lymphocytes were 0.85 and 0.98, respectively, and those for inter-observer variation were 0.82 and 0.98, respectively. The intra-observer estimates of the enhanced up-regulation of small bowel HLA-DR expression were similar in 86% and the inter-observer estimates in 91%. In this study, all specimens were evaluated by the same investigator, who had no previous knowledge of the disease history or laboratory findings.

Serology and chemical analysis

Serum immunoglobulin A (IgA) class endomysial antibodies were determined by an indirect immunofluorescence method using human umbilical cord as a substrate;[25] a dilution of 1 : ≥ 5 was considered to be positive. Serum IgA class tissue transglutaminase antibodies were investigated by enzyme-linked immunoabsorbent assay (ELISA) (Inova Diagnostics, San Diego, CA, USA), a unit value (U) of ≥20 being considered to be positive.[26] Serum IgA class gliadin antibodies were measured by ELISA; the lower limit for positivity was 0.2 ELISA units per millilitre.[27] Blood haemoglobin, serum iron, serum calcium, serum vitamin B_{12} and erythrocyte folic acid concentrations were measured using routine laboratory methods.

Clinical evaluation and bone mineral density

Gastrointestinal symptoms were evaluated by total score on the Gastrointestinal Symptom Rating Scale. This comprises 15 items describing abdominal pain (such as colicky pain, undefined pain), gastro-oesophageal reflux (epigastric pain, heartburn, acid regurgitation), indigestion (borborygmus, abdominal distension), diarrhoea and constipation (loose stools, hard stools, urgent need for defecation).[28, 29] The quality of life was assessed by the Psychological General Well-Being Questionnaire.[28, 29] The Psychological General Well-Being Index measures subjective well-being. It includes 22 items (anxiety, depressed mood, positive well-being, self-control, health and vitality); the total score gives a maximum value of 132 and a minimum of 22. The higher the score, the better the well-being. The body mass index was calculated using the formula: weight/height^2 (kg/m^2). The bone mineral density in

Table 1. Baseline characteristics of patients with newly diagnosed coeliac disease randomized to receive a natural or wheat-starch-based gluten-free diet (GFD)

	Natural GFD (n = 29)	Wheat-starch-based GFD (n = 28)
Female/male	23/6	22/6
Median age (years) (range)	47 (24–68)	44 (22–69)
Symptoms or signs leading to the diagnosis of coeliac disease, *n*		
Abdominal symptoms	18	21
Anaemia	5	2
Arthritis/arthralgia, rash, dermatitis	3	1
Screening in risk groups*	3	4
Median duration of symptoms (years) (range)	2 (0–30)	2 (0–20)
Other diseases, *n*		
Autoimmune thyroid disease	6	4
Primary Sjögren's syndrome	2	1
IgA glomerulonephritis	1	1
Diabetes mellitus type I	1	0
Sarcoidosis	0	1
Collagenous colitis	0	1
Immunosuppressive medication, *n*	0	0
Family history of coeliac disease, *n*	13	10

IgA, immunoglobulin A.
* First-degree relatives of patients with coeliac disease, diabetes mellitus, autoimmune thyroid disorders, Sjögren's syndrome, osteoporosis.

the lumbar spine and the left femoral neck was measured by dual-energy X-ray absorptiometry (Norland XR26, Norland Corp, Fort Atkinson, WI, USA). The bone mineral density data were expressed as *T* scores with reference to data on sex-matched young individuals.

Statistics

Quantitative data were expressed as means and 95% confidence intervals (CI). A two-tailed *t*-test was used to compare the laboratory values between the groups. Cross-tabulations were carried out by Fisher's exact test. The required number of participants was determined to achieve a statistical power of 0.90 at a significance level of 0.05; differences of < 0.5 for the villous height to crypt depth ratio, $\geq 7/100$ enterocytes for intra-epithelial lymphocytes or CD3+ cells, 3/mm for γδ+ cells and ≥ 0.5 for the Gastrointestinal Symptom Rating Scale were considered to be clinically relevant.

Ethical considerations

The study protocol was approved by the Ethical Committee of Tampere University Hospital. All subjects gave informed consent.

RESULTS

Twenty-nine patients with newly detected coeliac disease were randomized to receive a natural gluten-free diet (group I) and 28 to receive a wheat-starch-based gluten-free diet (group II). The groups were similar with respect to age, gender and symptoms (Table 1). Two patients in group I decided to discontinue the study after 2 months, as they found the diet too convoluted. In addition, four patients in group I and two in group II did not follow a strict gluten-free diet. Thus, 23 in group I and 26 in group II completed the study with a proper diet. The mean consumption of gluten-free flours after 3 months of follow-up was 79 g/day (range, 20–186 g/day) in group I and 82 g/day (35–184 g/day) in group II; after 9 months, the corresponding values were 77 g/day (20–186 g/day) and 81 g/day (37–173 g/day), respectively.

The villous height to crypt depth ratio and enterocyte cell height increased and the density of intra-epithelial lymphocytes decreased equally in the groups on natural and wheat-starch-based gluten-free diets (Figure 1). On

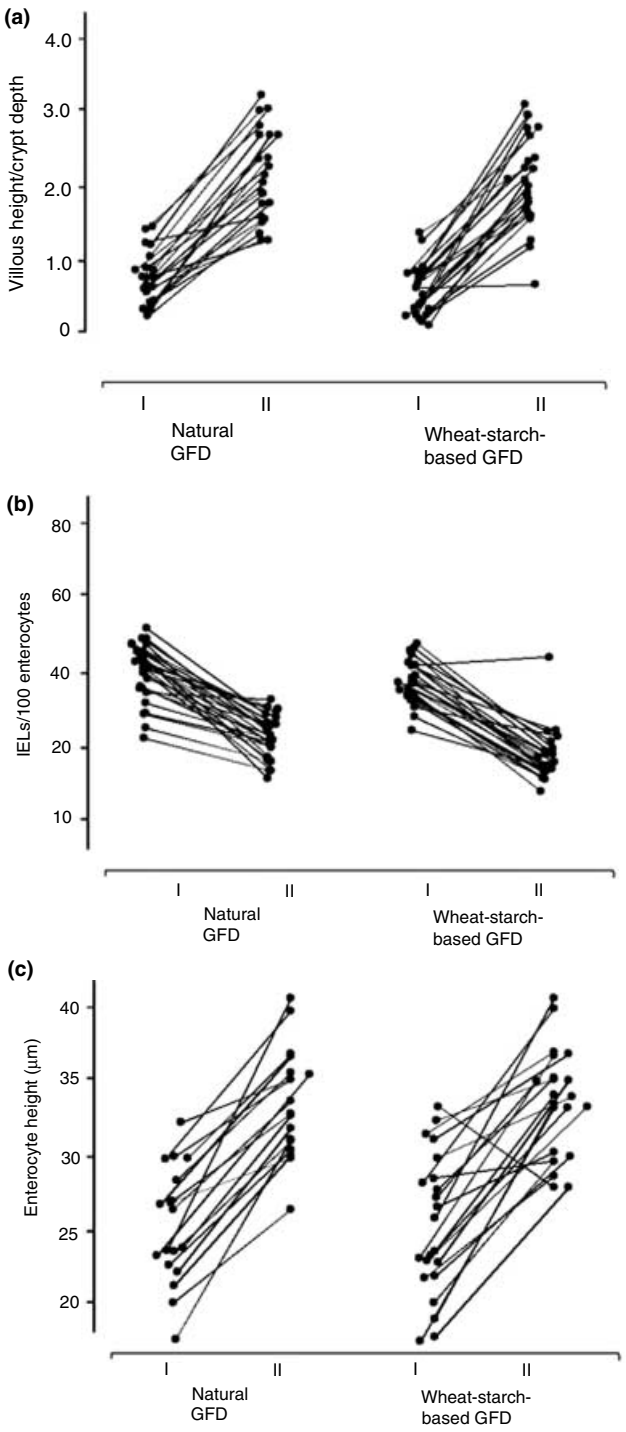

Figure 1. Villous height to crypt depth ratio (a), density of intra-epithelial lymphocytes (IELs) (b) and enterocyte height (c) before (I) and after (II) 1 year of treatment in coeliac patients randomized to receive a natural or wheat-starch-based gluten-free diet (GFD). The differences before and after a gluten-free diet were statistically significant ($P < 0.01$), whereas the differences between treatment groups were not significant.

a gluten-free diet, small bowel mucosal αβ+ and γδ+ intra-epithelial lymphocytes and mucosal HLA-DR expression did not differ between the two study groups (Table 2). The density of γδ+ intra-epithelial lymphocytes and epithelial HLA-DR expression remained elevated in both groups compared with the values in non-coeliac controls. There were no significant differences in serum endomysial antibody, tissue transglutaminase antibody and gliadin antibody positivity, blood haemoglobin, serum iron, serum calcium, serum vitamin B_{12} and erythrocyte folic acid levels between the study groups (Table 3).

Gastrointestinal symptoms were alleviated similarly in patients receiving a natural gluten-free diet or a wheat-starch-based gluten-free diet (Figure 2). Mean Gastrointestinal Symptom Rating Scale scores were 2.7 (95% CI, 2.4–3.0) in group I and 2.7 (2.4–2.9) in group II before treatment, and 1.8 (1.7–2.0) and 1.8 (1.6–2.0), respectively, at the end of the study. Psychological General Well-Being scores were 95.6 (90.4–100.8) in group I and 89.7 (83.4–95.4) in group II before treatment, and 109.9 (105.4–114.3) and 107.3 (103.2–111.4), respectively, at the end of the study (Figure 2). The body mass index was 24.8 in group I before treatment and 24.8 after treatment; in group II, the corresponding values were 26.5 and 26.8, respectively. At baseline and after 1 year on a gluten-free diet, there were no significant differences in bone mineral density (Table 4).

A slower response to treatment was not dependent on the flour intake in compliant patients (data not shown). Of the six non-compliant patients, two of three had villous atrophy at the end of the study; three patients refused biopsy, but had elevated antibody levels; five had anaemia.

DISCUSSION

We have shown, for the first time, in a randomized prospective study that a natural gluten-free diet and a

Table 2. Small bowel mucosal intra-epithelial lymphocytes (IELs) and mucosal human leucocyte antigen (HLA) expression in coeliac disease patients adhering to a natural or wheat-starch-based gluten-free diet (GFD) for 1 year and in non-coeliac control subjects

		Coeliac disease patients		
	Before treatment ($n = 20$)	Natural GFD ($n = 23$)	Wheat-starch-based GFD ($n = 26$)	Non-coeliac controls ($n = 59$)
αβ+ IELs (cells/mm), mean (95% CI)	45 (38–53)*	22 (18–26)	21 (16–26)	22 (18–25)
γδ+ IELs (cells/mm), mean (95% CI)	13.2 (10.9–15.5)*	8.9 (6.7–11.1)*	9.4 (7.0–11.8)*	2.3 (1.6–3.1)
Enhanced HLA-DR expression (*n*)	15 (75%)*	15 (60%)*	16 (59%)*	23 (39%)

*$P < 0.05$ compared to non-coeliac controls.

Table 3. Laboratory investigations before and after diet in coeliac disease patients randomized to receive a natural or wheat-starch-based gluten-free diet (GFD)

		Natural GFD ($n = 23$)		Wheat-starch-based GFD ($n = 26$)	
	Reference	Before GFD	On GFD	Before GFD	On GFD
Haemoglobin (g/dL), mean (range)	12–18	12.6 (10.9–14.4)	12.7 (11.1–14.5)	12.9 (9.8–15.3)	13.3 (10.8–14.9)
Serum calcium (mmol/L), mean (range)	2.15–2.60	2.3 (2.12–2.45)	2.3 (2.13–2.51)	2.3 (2.08–2.54)	2.3 (2.2–2.6)
Serum vitamin B_{12} (pmol/L), mean (range)	170–640	301 (166–597)	363 (191–661)	271 (148–441)	316 (127–602)
Erythrocyte folic acid (nmol/L), mean (range)	320–900	398 (138–847)	499 (293–1050)	433 (120–632)	585 (163–1200)
Serum iron (μmol/L), mean (range)	6–35	16.8 (3.6–46.2)	19.0 (7.0–31.1)	14.6 (3.3–22.8)	17.4 (6.2–29.4)
Serum AGA, number of abnormal levels	≤ 0.2 EU/L	14/23 (61%)	2/23 (9%)	18/26 (70%)	4/26 (15%)
Serum EmA, number of abnormal levels	> 1 : 5	18/23 (78%)	1/23 (4%)	17/26 (65%)	2/26 (8%)
Serum tTg-ab, number of abnormal levels	≤ 20 U/L	22/23 (96%)	5/23 (22%)	23/26 (88%)	3/26 (12%)

AGA, gliadin antibodies; EmA endomysial antibodies; tTg-ab, tissue transglutaminase antibodies.
The differences between study groups were not statistically significant.

wheat-starch-based gluten-free diet produce a similar histological and clinical recovery in patients with newly detected coeliac disease. Full mucosal recovery may take more than 1 year,[30] and the density of intra-epithelial lymphocytes has been considered to be the most sensitive gluten-induced intestinal mucosal change in coeliac disease.[15, 18, 31, 32] In particular, the density of αβ+ intra-epithelial lymphocytes decreases when a gluten-free diet is adhered to, and increases again during gluten challenge.[33, 34] It is therefore important that the densities of αβ+ intra-epithelial lymphocytes were similar in both groups. The γδ+ intra-epithelial lymphocytes remained at a high level, but decreased from baseline, which has been shown to occur previously by dietary treatment.[35] Although mucosal recovery was not complete in all patients, all histological findings indicated that there were no differences in the healing rate between the study groups after 1 year on a gluten-free diet.

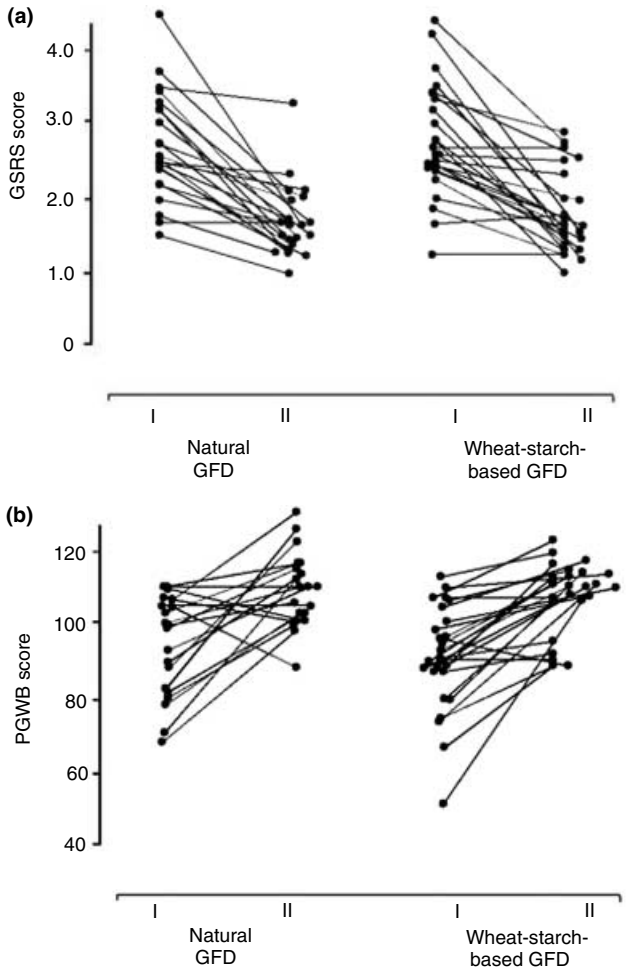

Figure 2. Gastrointestinal symptoms (a) and general well-being (b) before (I) and after (II) 1 year of treatment in coeliac patients randomized to receive a natural or wheat-starch-based gluten-free diet (GFD). A higher score on the Gastrointestinal Symptom Rating Scale (GSRS) indicates more intestinal symptoms, and a higher score on the Psychological General Well-Being (PGWB) scale indicates a better quality of life. The differences before and after a gluten-free diet were statistically significant ($P < 0.01$), whereas the differences between treatment groups were not significant.

In this study, wheat-starch-based gluten-free products were well tolerated. This is in contrast with the open challenge study by Chartrand *et al.*, where these products evoked more abdominal symptoms.[9] In a study by Faulkner-Hogg *et al.*, abdominal symptoms were alleviated when a wheat-starch-containing gluten-free diet was changed to a natural gluten-free diet.[36] However, 36% of the patients had small bowel villous atrophy and there were no significant changes in mucosal morphology after switching to a natural gluten-free diet. It should be noted that neither of these two studies was randomized.[9, 36]

Due to the wide variety of products available, it was virtually impossible to evaluate the exact amounts of gluten consumed in the two diets during 1 year. We therefore assumed that the natural gluten-free diet contained no gluten, but it is possible that these products may also be gluten contaminated. This study does not answer the question of whether minor amounts of gluten produce inflammation, but it shows that wheat-starch-based gluten-free products are as safe and well tolerated as natural ones.

Repetitive dietary transgressions resulted in incomplete small bowel mucosal recovery, and this may clearly

Table 4. Bone mineral density (*T* score with 95% confidence intervals) before and after 1 year of treatment in patients randomized to receive a natural or wheat-starch-based gluten-free diet (GFD)

	Lumbar spine		Femoral neck	
Diet group	Before GFD	After GFD	Before GFD	After GFD
Natural GFD ($n = 23$)	− 1.27; − 0.91, − 1.64	− 0.72; − 0.29, − 1.14	− 0.86; − 0.48, − 1.23	− 0.85; − 0.52, − 1.17
Wheat-starch-based GFD ($n = 26$)	− 1.01; − 0.58, − 1.45	− 0.86; − 0.34, − 1.38	− 0.43; − 0.12, − 0.74	− 0.44; − 0.13, − 0.76

expose patients to health risks. It thus appears that the overall compliance with diet is much more important than the trace amounts of gluten possibly present in wheat-starch-based gluten-free products.

ACKNOWLEDGEMENTS

This study was supported by grants from the Yrjö Jahnsson Foundation and the Medical Research Fund of Tampere University Hospital.

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Scandinavian Journal of Gastroenterology, 2005; 40: 564–572

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ORIGINAL ARTICLE

Small-bowel mucosal transglutaminase 2-specific IgA deposits in coeliac disease without villous atrophy: A prospective and randomized clinical study

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Abstract

Objective. In coeliac disease, autoantibodies directed against transglutaminase 2 are produced in small-bowel mucosa, and they have been found to be deposited extracellularly. The aim of this study was to investigate whether such mucosal IgA deposits are important in the diagnostic work-up of early-stage coeliac disease without small-bowel mucosal villous atrophy. ***Material and methods***. Forty-one adults suspected of coeliac disease owing to increased density of mucosal $\gamma\delta+$ intraepithelial lymphocytes but normal villous morphology were randomized to gluten challenge or a gluten-free diet for 6 months. Clinically and histologically verified gluten dependency was compared with existence of small-bowel mucosal transglutaminase 2-specific extracellular IgA deposits and (coeliac disease-type) HLA DQ2 and DQ8; 34 non-coeliac subjects and 18 patients with classical coeliac disease served as controls. ***Results***. Of the 41 patients, 5 in the challenge group and 6 in the gluten-free diet group were clinically gluten sensitive; all 11 had HLA DQ2 or DQ8. Ten of these 11 patients showed transglutaminase 2-targeted mucosal IgA deposits, which were dependent on gluten consumption. Minimal IgA deposits were seen in only 3 out of 30 patients with suspected coeliac disease without any clinically detected gluten dependency. The deposits were found in all classical coeliac patients and in none of the non-coeliac control subjects. ***Conclusions***. Clinically pertinent coeliac disease exists despite normal small-bowel mucosal villous architecture. Mucosal transglutaminase 2-specific IgA deposits can be utilized in detecting such patients with genetic gluten intolerance.

Key Words: *Coeliac disease, IgA-deposit, intraepitehlial lymphocytes, latency, transglutaminase antibodies*

Introduction

The current diagnostic criteria for coeliac disease require small-bowel mucosal villous atrophy that recovers on a gluten-free diet [1]. Clearly, the spectrum of the disease is wider: the mucosal damage develops gradually from inflammation to crypt hyperplasia and finally to villous atrophy [2]. The mucosal inflammation is unspecific, and can be found in a variety of disorders [3,4]; therefore, it is difficult to tell whether minor mucosal changes are due to early development of coeliac disease. Increased density of $\gamma\delta$ T-cell-receptor-bearing intraepithelial lymphocytes (IELs) is considered to be typical for coeliac disease [5]. These cells have been found in the early stage of the disease, even before the development of villous atrophy [6–8], but unfortunately also in conditions other than coeliac disease [9]. In some patients evincing normal small-bowel mucosal villous morphology positive serum endomysial (EmA) [10–12] or jejunal fluid coeliac disease-associated antibodies (IgA- and IgM-class gliadin and tissue transglutaminase antibodies) [13,14] have predicted impending coeliac disease. Nonetheless, the concept of early coeliac disease is poorly understood.

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(Received 9 February 2005; accepted 27 February 2005)

ISSN 0036-5521 print/ISSN 1502-7708 online
DOI: 10.1080/00365520510023422

The process of mucosal deterioration may take years or even decades [15,16] and a long follow-up without treatment may sometimes be harmful. Symptoms are not related solely to villous atrophy, and many proven early coeliac disease cases have suffered from gluten-dependent gastrointestinal symptoms even before the villous atrophy has developed [7,11,15]. Cooper et al. [17] reported diarrhoea to be alleviated by a gluten-free diet in 9 adults without villous atrophy. In a study by Arranz & Ferguson [13], gastrointestinal symptoms resolved on a gluten-free diet in 5 adults who had positive jejunal fluid coeliac disease-associated antibodies, high counts of IELs, but normal intestinal villi. We showed that 10 adults with only minor small-bowel mucosal changes and increased densities of $\gamma\delta+$ IELs experienced clinical, serological and histological recovery on a gluten-free diet. Eight of them suffered from osteopenia or osteoporosis, thus warranting treatment before classical villous atrophy became evident [18].

Coeliac disease autoantibodies against tissue transglutaminase (TG2) are produced in the intestinal mucosa [19] and the antibodies can deposit on extracellular TG2 in the small-bowel mucosa even when not measurable in serum [20]. Our aim was to investigate whether extracellular IgA deposits in the gut could be important in the diagnostic work-up of early coeliac disease, when small-bowel mucosal histology is not yet diagnostic. First, we conducted a prospective randomized study to uncover clinically early-stage coeliac disease. Secondly, small-bowel mucosal TG2-specific IgA deposits were determined, and the results were tallied with the presence of clinically detected gluten sensitivity and coeliac disease-type HLA. All the patients had suspected coeliac disease but were excluded for the disease because of normal villous morphology.

Material and methods

Patients and study design

Between January 1997 and December 2000, 577 adults came under suspicion of coeliac disease in the Department of Gastroenterology and Alimentary Tract Surgery of Tampere University Hospital; small-bowel mucosal morphology and density of $\gamma\delta+$ IELs were determined in each case. A total of 126 patients fulfilled the histologic criteria for coeliac disease; that is, partial or subtotal villous atrophy with crypt hyperplasia and increased density of IELs [1]. The study group comprised the 68 patients evincing an increased density of $\gamma\delta+$ IELs but normal villous morphology, being thus excluded for coeliac disease. Twenty-seven patients refused to participate in the study; the remaining 41 were randomized either to undergo an extra gluten challenge (GC group) or to start a gluten-free diet (GFD group) (Figure 1). Each patient entered the trial before random treatment assignment was revealed. Randomization was carried out using random number tables with permuted blocks [21]. Clinical, histological and serological studies were carried out before and after 6 months of dietary intervention. Thereafter, patients found to have gluten-sensitive disease were asked to switch from a gluten-containing to a gluten-free diet, or from a gluten-free to a gluten-containing diet on a voluntary basis (Figure 1). A second follow-up evaluation was then made after 6 to 12 months. Patients suffering from dermatitis herpetiformis were excluded.

A similar follow-up evaluation was done in 18 patients with classical coeliac disease. All adopted the gluten-free diet since gluten challenge was considered unethical. Histological data were further compared with those on 34 consecutive patients suffering from dyspepsia, upper abdominal pain or heartburn without coeliac disease suspicion (Figure 1).

Dietary assessment

At the beginning of the study all patients and controls were consuming a normal gluten-containing diet. Advice was given on how to maintain extra gluten intake or a gluten-free diet. Patients in the GC group were encouraged to eat at least 15 g extra gluten per day (5 slices of bread). A dietitian interviewed the participants and the analysis was completed with a 4-day record of food intake.

Clinical evaluation of gluten-dependency

To evaluate clinical gluten-dependency, a new clinical scoring system was developed on the basis of the clinical outcome and small-bowel histology at baseline and after dietary interventions. The scoring system was decided before its application in the

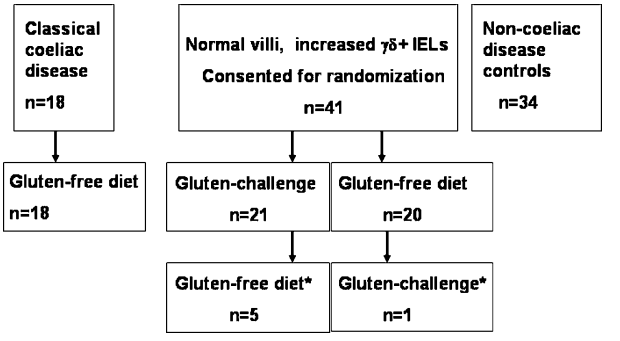

Figure 1. Flow chart of the study. *Gluten-sensitive patients were asked to switch diet on a voluntary basis.

present study. The selection of different items was based on indicators known to increase the likelihood of coeliac disease (Table I; [22,23]). The baseline score comprised four items: abdominal symptoms compatible with coeliac disease, family history of coeliac disease, associated autoimmune conditions and serology. The intervention score was based on changes in abdominal symptoms, small-bowel mucosal villous-crypt architecture and inflammation, and serology. When the change in a clinical parameter was the opposite of that expected in coeliac disease after dietary intervention (challenge or gluten-free diet), the scores were negative. The total score was the sum of baseline and intervention scores. Total scores as high as those observed in classical coeliac disease (in response to a gluten-free diet) were considered equal to clinical gluten sensitivity.

Table I. Scoring system for clinical gluten-dependency.

Variable	Score
Baseline score	
Abdominal symptoms	
Typical coeliac disease-like abdominal symptoms (diarrhoea, loose stools, abdominal distension, flatulence)	2
Atypical symptoms (abdominal pain, indigestion)	1
No symptoms	0
Family history of coeliac disease	
First-degree relatives with coeliac disease	2
Distant relatives with coeliac disease	1
No relatives with coeliac disease	0
Associated autoimmune conditions	
Definite associations (IDDM, AIT, Sjögren's syndrome, IgA-nephropathy)*	2
Possible associations (sarcoidosis, inflammatory bowel disease)*	1
No associated conditions	0
Positive coeliac disease serology	
Positive endomysial and transglutaminase antibodies	2
Positive endomysial or transglutaminase antibodies	1
Both negative	0
Intervention score**	
Change in villous height/crypt depth ratio	
≥0.5 decrease†/increase‡	1
<0.5 decrease†/increase‡	0
Change in the density of CD3+ IELs‡	
>100% increase†/>50% decrease‡	2
30–100% increase†/30–50% decrease‡	1
<30 increase†/<30% decrease‡	0
Change in the density of αβ+IELs	
>100% increase†/>50% decrease‡	2
30–100% increase†/30–50% decrease‡	1
<30 increase†/<30% decrease‡	0
Change in the density of γδ+IELs	
>100% increase†/>50% decrease‡	2
30–100% increase†/30–50% decrease‡	1
<30 increase†/<30% decrease‡	0
Change in coeliac disease serology	
Both endomysial and transglutaminase antibody titres increase†/decrease‡	2
Either endomysial or transglutaminase antibody titres increase†/decrease‡	1
No change in antibodies†,‡	0
Change in the total score of the Gastrointestinal Symptom Rating Scale questionnaire§	
>1.0 increase†/decrease‡	2
0.5–1.0 increase†/decrease‡	1
<0.5 increase†/decrease‡	0

Abbreviations: IDDM = insulin-dependent diabetes mellitus; AIT = autoimmune thyroid disease; IELs = intraepithelial lymphocytes.

*According to data from [22]. **When the change in clinical parameter was opposite to that expected in dietary intervention (challenge or gluten-free diet) the scores were negative.

†Change after gluten challenge. ‡Change after gluten-free diet.

§According to data from [23].

Small-bowel mucosal TG2-specific IgA deposits

TG2-related extracellular IgA deposits were investigated before and after dietary intervention in all available frozen small-bowel mucosal sections. Altogether, 6 unfixed, 5 μm-thick frozen sections were examined in each patient by direct immunofluorescence using fluorescein isothiocyanate-labelled rabbit antibody against human IgA (DAKO AS, Glostrup, Denmark) at a dilution of 1:40 in phosphate buffered saline (PBS), pH 7.4. According to the preliminary results, IgA is normally detected only inside plasma and epithelial cells, whereas in coeliac disease subepithelial IgA deposits are found along the surface and crypt basement membranes and around mucosal vessels, corresponding to the intestinal localization of TG2 [20]. These coeliac disease-type IgA deposits were graded from 0 to 3 according to their intensity along basement membranes in the villous-crypt area. The evaluation was carried out blindly by two investigators who had no knowledge of the disease history or results of intervention, and who were found to be in full agreement regarding their respective results. To confirm whether coeliac-type IgA deposits co-localize with TG2, sections from 8 patients before and after dietary intervention were double-stained for human IgA (as above, green) and for TG2 (red) using monoclonal mouse antibodies against TG2 (CUB7402; NeoMarkers, Fremont, Calif., USA) followed by rhodamine-conjugated anti-mouse immunoglobulin antibodies (DAKO), both diluted 1:200 in PBS.

Small-bowel mucosal morphology

Small-bowel mucosal biopsies were taken by endoscopy from the distal part of the duodenum. Four biopsy specimens were processed and stained with haematoxylin-eosin and studied under light microscopy. Morphometric analysis covering villous height

and crypt depth ratio (Vh/CrD) was performed in well-oriented biopsy samples, as previously described [24]. Poorly oriented sections were not accepted: the samples were re-dissected until they were of good quality.

For immunohistochemical stainings, two small-bowel biopsy specimens freshly embedded in optimal cutting temperature (OCT, Tissue-Tek; Miles Inc., Elkhart, Ind., USA) compound, snap-frozen in liquid nitrogen and stored at -70°C until used. Immunohistochemical studies using the avidin-biotin immunoperoxidase system were carried out on 5 μm-thick frozen sections. CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, Calif., USA), αβ+ IELs with monoclonal βF1 antibody (Endogen, Woburn, Mass., USA) and γδ+IELs with TCRγ antibody (Endogen). The numbers of positively stained IELs were counted with a ×100 flat-field light microscope objective from randomly selected surface epithelium; at least 30 fields along the epithelium were counted and the density of IELs expressed as cells/millimetre epithelium as described in detail elsewhere [25,26].

Serology and HLA typing

Serum IgA-class EmA values were investigated by an indirect immunofluorescence method using human umbilical cord as substrate; [27] a dilution of 1: ≥5 was considered positive. Serum IgA-class TG2-ab values were determined by enzyme-linked immunosorbent assay (Celikey®; Pharmacia Diagnostics, GmbH, Freiburg, Germany) using human recombinant TG2 as antigen, a unit value (U) ≥5 being considered positive [28].

HLA DQB1* allele groups were determined using the Olerup SSP DQ low resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden). This method determines HLA DQ2, DQ4, DQ5, DQ6, DQ7, DQ8 and DQ9 allele groups.

Statistics

The data were given as means with 95% confidence intervals (CI).

Ethical considerations

The study protocol was approved by the Ethics Committee of Tampere University Hospital. All subjects gave written informed consent.

Results

Baseline characteristics

Twenty-one patients with a normal small-bowel mucosal villous morphology were randomized to gluten challenge (GC group) and 20 to a gluten-free diet (GFD group); the groups were similar with respect to age, gender and symptoms. The clinical history did not differ between the GC- and GFD groups or the classical coeliac disease group (Table II).

At baseline there were no significant differences in small-bowel mucosal villous morphology or density of IELs, and serum EmA and TG2-ab values were at equal levels between the GC- and GFD groups. In coeliac disease Vh/CrD was significantly lower, densities of CD3+ and αβ+ IELs were higher, and coeliac disease serology was more often positive than in the GC- and GFD groups. Compared with the histology in non-coeliac controls, only γδ+IELs were, as expected, higher in the GC- and GFD groups (Table III).

Table II. Characteristics of patients with suspected coeliac disease before randomization to the gluten challenge (GC) or gluten-free diet (GFD).groups. Data from control patients having classical coeliac disease and non-coeliac control subjects are given as comparison.

	Study group			
	GC group ($n=21$)	GFD group ($n=20$)	Classical coeliac disease ($n=18$)	Non-coeliac controls ($n=34$)
Female/male	18/3	16/4	15/3	21/13
Median age (range), years	45 (19–70)	43 (19–61)	47 (22–68)	52 (22–72)
Median duration of symptoms (range), years	5 (0–13)	5 (0.5–20)	2 (0–10)	No
Family history of coeliac disease, *n*	3	6	7	0
Original symptoms or signs leading to suspicion of coeliac disease, *n*				
Abdominal symptoms*	17	18	11	No
Anaemia and malabsorption	2	1	1	No
Screening in risk groups**	2	1	6	No

*Diarrhoea, loose stools, abdominal distension, flatulence. **Autoimmune thyroid disease, Sjögren's syndrome, arthritis, osteoporosis.

Table III. Baseline data on small-bowel mucosal findings and serum endomysial and transglutaminase 2-antibodies in patients with suspected coeliac disease randomized to gluten challenge (GC) or gluten-free diet (GFD) groups. Data from control patients having classical coeliac disease and non-coeliac control subjects are given as comparison.

	Study group			
	GC group ($n=21$)	GFD group ($n=20$)	Classical coeliac disease ($n=18$)	Non-coeliac controls ($n=34$)
Villous height/crypt depth ratio, mean (95% CI)	3.6 (3.3–3.9)	3.4 (3.2–3.6)	0.6 (0.4–0.8)	3.5 (3.3–3.7)
γδ+IELs (cells/mm), mean (95% CI)	10.8 (7.5–14.1)	11.3 (8.3–14.3)	13.2 (10.6–15.8)	3.8 (2.0–5.6)
CD3+IELs (cells/mm), mean (95% CI)	40 (32–48)	35 (25–45)	58 (51–65)	33 (28–38)
αβ+IELs (cells/mm), mean (95% CI)	24 (18–30)	23 (16–30)	44 (36–52)	25 (21–29)
Serum EmA, number of abnormal levels, *n* (%)	4 (19%)	2 (10%)	16 (89%)	0 (0%)
Serum TG2-ab, number of abnormal levels, *n* (%)	5 (24%)	5 (24%)	17 (94%)	0 (0%)

Abbreviations: CI = confidence interval; IEL = intraepithelial lymphocytes; EmA = endomysial antibodies; TG2-ab = tissue transglutaminase antibodies.

Dietary intervention and detection of gluten-sensitive patients

Before dietary intervention, all patients and controls were consuming a gluten-containing diet. Twenty out of 21 patients in the GC group completed the 6 months' study with a proper gluten-containing diet; in one case the follow-up examination was carried out after 3 months because the subject had to discontinue gluten challenge owing to aggravated symptoms. All 20 patients in the GFD group adhered to the diet throughout the 6-months study period, as did all 18 with coeliac disease.

When the clinical scoring system (Table I) was applied, the baseline scores did not differ between the study groups and were equal to those in classical coeliac disease (Figure 2). After dietary intervention, the total scores of 11 patients increased to levels as high as those in patients with classical coeliac disease (scores ranging from 8 to 14); 5 in the GC group and 6 in the GFD group. All total score-positive patients had coeliac disease-type HLA: 10 patients had HLA DQ2 and 1 patient had HLA DQ8. Ten patients in the study group had no HLA DQ2 or DQ8; none of them showed any increase in clinical total score after dietary intervention (Figure 2). Sixteen out of 18 classical coeliac disease patients had HLA DQ2, 1 patient had HLA DQ8, and in 1 patient the typing was unsuccessful for technical reasons.

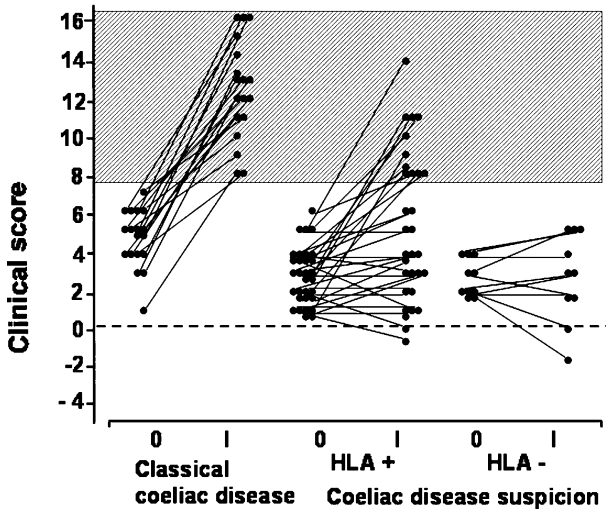

Figure 2. Baseline and total scores after 6 months' gluten intervention in patients with classical coeliac disease and in patients suspected of coeliac disease but with normal villous morphology; the study group is shown according to the presence of HLA DQ2 or DQ8 (HLA+), and absence of both (HLA−). The shaded area depicts the range of total score values in patients with manifest coeliac disease. 0 = baseline score; I = total score.

Follow-up of gluten-sensitive patients after first intervention

In the GC group, all 5 total score-positive patients started a gluten-free diet after the first intervention (Figure 1); 3 patients consented to a new follow-up small-bowel mucosal biopsy after 1 year on the diet; clinical, serologic and mucosal recovery was evident in all (Figure 3A). In addition, the symptoms were alleviated in 1 patient and serum EmA and tTG-ab became negative; 1 patient refused the follow-up examination.

In the GFD group, 5 total score-positive patients decided to continue on a gluten-free diet. One patient consented to resume a gluten-containing diet; within 6 months her abdominal symptoms were aggravated, serum EmA, TG2-ab became positive and the density of small-bowel mucosal CD3+ IELs increased in normal villi; all these findings suggesting clinical gluten sensitivity.

Transglutaminase-2-specific small-bowel mucosal IgA deposits

In the study groups, small-bowel mucosal frozen sections were available at baseline in 34 patients, and at the follow-up examination in 38 out of 41. Before the dietary intervention, subepithelial coeliac

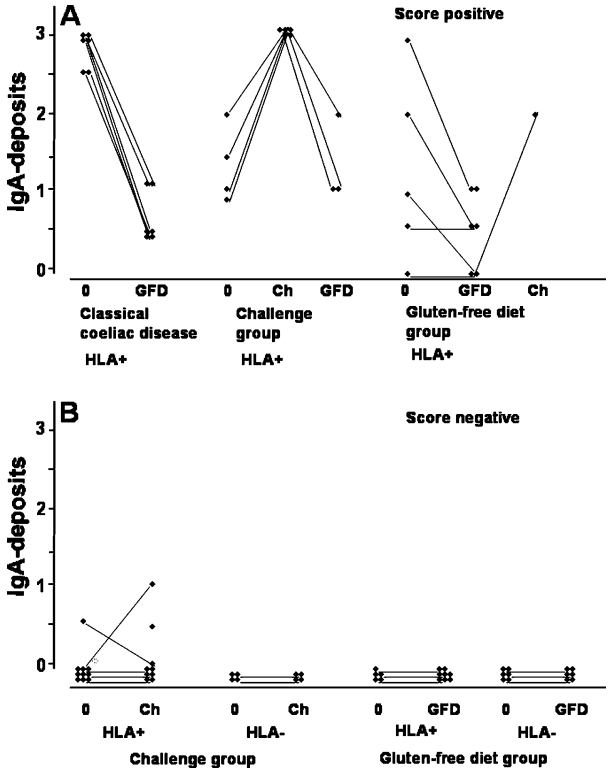

Figure 3. Density of small-bowel mucosal coeliac disease-type IgA deposits in clinical total score- positive (A) and negative (B) patients suspected to have coeliac disease and classical coeliac controls. 0 = at baseline; GFD = after gluten-free diet; Ch = after gluten challenge.

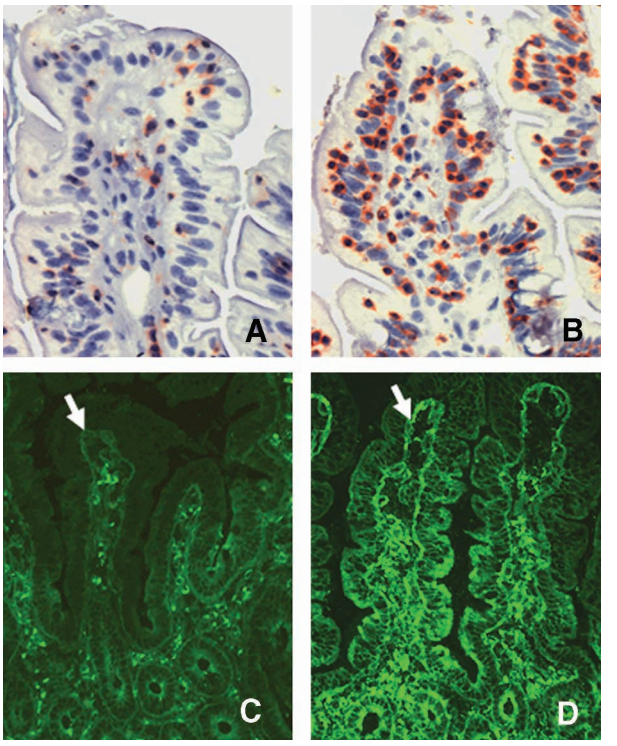

Figure 4. Clinical score-positive patient in the gluten challenge group. At baseline, increased density of CD3+ intraepithelial lymphocytes (A. red-stained cells) in normal villi is found in small-bowel mucosa, together with subepithelial coeliac-type, grade 1, mucosal IgA deposits (C. green, *arrow*). After gluten challenge, small-bowel mucosal inflammation increased (B) and the intensity of IgA deposits became stronger, grade 3 (D). Exposure time in Figure 4C is 2-fold that for Figure 4D.

disease-type IgA deposits were detectable along the surface and crypt basement membranes and around mucosal vessels in all patients except one, who finally proved to be total score-positive and thus clinically gluten sensitive (Figure 3A). In these patients the density of IgA deposits increased upon challenge and again decreased on a gluten-free diet (Figures 4 and 5). Double-colour immunofluorescence staining with TG2 showed the deposited IgA to be co-localized with TG2 (Figure 5D, E and F).

Of the total score-negative patients, minimal small-bowel mucosal IgA deposits were detectable before gluten challenge in 1 patient and after challenge in 2 patients; in the remainder, including all HLA DQ2- and DQ8-negative patients, no deposits were seen (Figure 3B). In the deposit-negative samples, endogenous IgA was detectable inside plasma cells and epithelial cells, implying that none had selective IgA deficiency.

In the classical coeliac disease control patients, all 6 showed intense coeliac disease-type IgA deposits in the small-bowel mucosa when untreated, and the densities of these deposits decreased clearly on a gluten-free diet (Figure 3A). No IgA deposits could be detected in 6 non-coeliac controls.

Comparison of parameters in prediction of clinical gluten dependency

We presupposed that a positive clinical total score would indicate clinical gluten dependency and would be the diagnostic standard. At baseline, IgA deposits were positive in 8 out of 9 patients and false-positive in 1 out of 25 (Figure 3A), giving a sensitivity of 89% and a specificity of 96% for positive IgA deposits for predicting gluten dependency. In these series, the respective figures for positive serum EmA or TG2-ab were 73 and 97%, for small-bowel mucosal inflammation (increased density of CD3+ IELs indicating Marsh 1-lesion [2]) 73 and 70%, and for HLA DQ2 or DQ8 the figures were 100 and 33%, respectively.

Discussion

In a prospective randomized study we showed that in the present series 27% of the patients with suspected coeliac disease and increased density of γδ+IELs were clinically gluten sensitive, even though they did not fulfil the current diagnostic criteria. With the exception of one patient, gluten-dependent

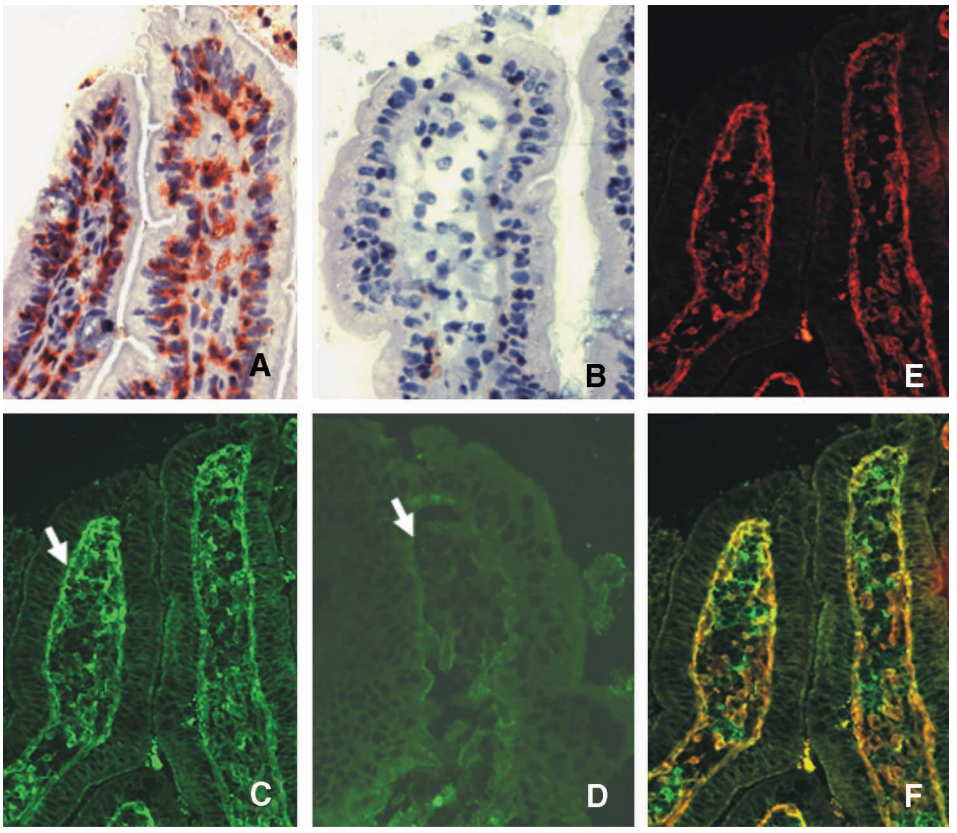

Figure 5. Clinical score-positive patient in the gluten-free diet group. At baseline, increased density of CD3+ intraepithelial lymphocytes (A. red-stained cells) in normal villi is found in small-bowel mucosa; coeliac-type subepithelial grade 3 mucosal IgA deposits are strongly visible (C. green, *arrow*). After gluten-free diet, small-bowel mucosal inflammation (B) and abdominal symptoms improved; faint, grade 1, coeliac-type IgA deposits are seen only in a follow-up biopsy (D). Transglutaminase-2 pattern is shown in baseline biopsy (E. red). Co-localization of the IgA deposits with transglutaminase-2 is indicated in yellow (F). Exposure time in Figure 5D was 2-fold that for Figure 5C.

TG2-specific small-bowel mucosal IgA deposits were able to identify all clinically gluten-sensitive individuals. Furthermore, the new method turned out to be highly specific for coeliac disease.

So far, there has been no reliable method to detect early-stage coeliac disease. An increased density of $\gamma\delta+$ IELs in the small-bowel mucosa, though typical for coeliac disease [5], is not always related to HLA DQ2 and DQ8, and these cells have also been found in other conditions than coeliac disease [8,29,30]. In the present study, 10 (24%) of the 41 patients with an increased density of $\gamma\delta+$ IELs were not carrying HLA DQ2 or DQ8; it is noteworthy that no gluten-sensitive cases were detected among these patients. Thus, an increased density of $\gamma\delta+$ IELs in the small-bowel mucosa *per se* is not a sufficient marker for gluten sensitivity. Small-bowel mucosal intraepithelial lymphocytosis can also occur in conditions unrelated to coeliac disease [3,4]. Similarly, in the present series, intestinal lymphocytosis (Marsh 1-lesion) had only 70% sensitivity and specificity for predicting early development of coeliac disease.

There was no prior serologic selection in our study group; patients were enrolled from a large series based on small-bowel mucosal biopsies. Despite this, serum EmA and TG2-ab turned out to be the specific markers in predicting gluten dependency. Many proven latent coeliac disease cases have indeed had EmA before the development of small-bowel mucosal villous atrophy [7,10–12,31,32]. In a screening study by Fasano et al. [33], only a minority (34%) of EmA-positive patients showed severe small-bowel mucosal atrophy, while most evinced various degrees of partial villous atrophy or only crypt hyperplasia. Furthermore, in a recent screening study involving 3654 schoolchildren, 37 (1%) patients were found to have coeliac disease, whereas 9 had EmA and TG2-ab but normal villous morphology, and thus possibly early coeliac disease [28]. Previous studies and our current reports are thus in contrast with the suggestions that EmA and TG2-ab are not present in mild small-bowel mucosal atrophy but seen only as a consequence of more severe damage [34].

Our results also support the concept that coeliac disease antibodies are deposited in the small-bowel mucosa before mucosal deterioration, and before they are measurable in serum. This is in accordance with the findings that coeliac autoantibodies are produced in the small-bowel mucosa [19] and that mucosal samples from treated coeliac patients produce EmA when challenged with gliadin peptides in *in vitro* organ cultures [35]. IgA- and IgM-class gliadin and transglutaminase antibodies have been found in jejunal fluid in coeliac disease patients and in those suspected of having potential coeliac disease, which also suggests mucosal production of coeliac disease-type antibodies [13,14,36]. Sblattero et al. [37] developed a phage display library method to detect TG2-ab synthesis in small-bowel mucosa. They hypothesized that borderline small-bowel mucosal findings consistent with coeliac disease could be detected using this method. Here, using a simple staining method, we showed that TG2-specific gluten-dependent subepithelial IgA deposits in small-bowel mucosa predicted clinically detected gluten sensitivity and early coeliac disease. In all, these findings suggest that, apart from T-cell-mediated reactions, a humoral response also occurs early in the progression of coeliac disease.

Coeliac disease is strongly associated with the HLA class II extended haplotypes B8-DR3-DQ2, DR5/7-DQ2, and less often with the DR4-DQ8; 95–100% of patients with coeliac disease share one of these haplotypes, compared with 30–40% in the general population [38,39]. In the present study, all 11 gluten-sensitive patients had HLA DQ2 or DQ8, again confirming that we did in fact find latent cases using these methods.

The natural history of HLA DQ2- and DQ8-negative gluten-sensitive patients is poorly understood. It is not known whether these patients have to maintain a life-long gluten-free diet, and whether they are prone to complications known to be associated with HLA DQ2 and DQ8-linked classical coeliac disease. Cooper et al. [17] noted a clinical response to a gluten-free diet in 9 out of 17 patients with incapacitating diarrhoea and normal small-bowel mucosal morphology; the clinical response was seen together with a slight decrease in mucosal IELs. However, the coeliac disease-type HLA B8 haplotype was seen in only 3 of the 9 patients, and the investigators concluded that these patients were not suffering from coeliac disease. In a study by Picarelli et al. [40], 10 EmA-positive patients evincing a normal small-bowel mucosal architecture benefited from gluten withdrawal; all 10 had normal densities of $\gamma\delta+$ IELs but only 2 patients had HLA DQ2, which would indicate that the mild enteropathy in these patients was different from that of classical coeliac disease. In the present study, all 10 HLA DQ2- and DQ8-negative patients showed no response upon dietary intervention. We consider that HLA DQ2 and DQ8-negative cases should be distinguished from genetic gluten intolerance and coeliac disease.

A life-long gluten-free diet is not easy to maintain and therefore the diagnosis of coeliac disease should be based on definitive evidence. Patients with early developing coeliac disease with unequivocal small-bowel mucosal lesion can be found by demonstration of gluten-dependent TG2-specific IgA deposits in the small-bowel mucosa. It is foreseen that the demonstration of small-bowel mucosal villous atrophy with crypt hyperplasia will no longer be the gold standard in the diagnosis of coeliac disease, when the diagnostic criteria are widened to include "genetic gluten intolerance".

Acknowledgements

The present study and the Coeliac Disease Study Group are supported by grants from the Medical Research Fund of Tampere University Hospital, the Foundation of the Friends of the University Children's Hospitals in Finland, the Päivikki and Sakari Sohlberg Foundation, the Finnish Medical Foundation and the Academy of the Finland Research Council for Health.

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