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Evaluation of the Results of Active Diagnostics and Treatment of Coeliac Disease in a High-Prevalence Area

A Special Focus on Dietary Compliance, Quality of Life and Associated Complications

■

ACADEMIC DISSERTATION
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To My Family

Contents

List of Original Publications

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

I Viljamaa M, Collin P, Huhtala H, Sievänen H, Mäki M, Kaukinen K (2005). Is coeliac disease screening in risk groups justified? Fourteen-year follow-up with special focus on compliance and quality of life. Aliment Pharmacol Ther 22:317-24. (Reprinted with permission of Blackwell Publishing.)

II Viljamaa M, Kaukinen K, Pukkala E, Hervonen K, Reunala T, Collin P (2006). Malignancies and mortality in patients with coeliac disease and dermatitis herpetiformis: 30-year, population-based study. Dig Liver Dis 38: 374-80. (Reprinted with permission of Editrice Gastroenterologica Italiana S.r.l.)

III Viljamaa M, Kaukinen K, Huhtala H, Kyrönpalo S, Rasmussen M, Collin P (2005). Coeliac disease, autoimmune diseases and gluten exposure. Scand J Gastroenterol 40:437-43. (Reprinted with permission from Taylor & Francis, www.tandf.no/sgas.)

IV Hakanen M, Kaukinen K, Salmi J, Collin P (2001). Clinical and subclinical thyroid disease in adult coeliac disease. Dig Dis Sci 46:2631-5. (Reprinted with permission of Springer Science and Business Media.)

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 antibodies
ESPGAN	European Society of Paediatric Gastroenterology and Nutrition
GFD	Gluten-free diet
GSRS	Gastrointestinal Symptom Rating Scale
HLA	Human leukocyte antigen
IBQ	Illness behaviour questionnaire
IEL	Intraepithelial lymphocyte
IgA and IgG	Immunoglobulin A and G
IL	Interleukin
NHL	Non-Hodgkin lymphoma
PGWB	Psychological General Well-Being
SF-36	Short Form 36 Health Survey
SIR	Standardized incidence ratio
SMR	Standardized mortality ratio
T4v	Free thyroxin
TG	Thyreoglobulin antibodies
TPO	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
tTG	Tissue transglutaminase
tTG-Ab	Tissue transglutaminase antibody
TyMS	Thyroid microsomal antibodies
UK	United Kingdom
WHO	World Health Organisation

Abstract

The clinical spectrum of coeliac disease is wide: patients may suffer from malabsorption and evince various gastrointestinal or extraintestinal symptoms, or be totally asymptomatic. Osteoporosis and lymphoma are the most well-known complications of coeliac disease and many autoimmune diseases are more common in patients with coeliac disease than in non-coeliac populations.

The diagnostics of coeliac disease have been actively pursued in the Tampere University Hospital area ever since 1980, and patients with mild symptoms and coeliac disease risk groups have also been diagnosed and treated. Many patients are asymptomatic, but there are no long-term studies on compliance and quality of life in this group.

The aim of the study was to evaluate the dietary compliance, quality of life and coeliac disease complications after long-term follow-up in the area, where the clinical prevalence of the disorder is high, up to 0.3 per cent of the local population.

Dietary compliance was evaluated in 703 coeliac disease patients by questionnaire and patient records and in 53 screen-detected and 44 symptom-detected treated coeliac disease patients by means of interview, 4-day food record and coeliac disease serology. According to these studies, as many as 77 to 83% of the patients adhered to strict gluten-free diet, which is a better result than in many other countries.

Quality of life was measured by Psychological General Well Being questionnaire in 53 screen-detected and 44 symptom-detected, long-term treated coeliac disease patients and was comparable to that in 110 non-coeliac controls. Also gastrointestinal symptoms (measured by Gastrointestinal Symptom Rating Scale questionnaire) were similar in treated coeliac patients and in non-coeliac controls. Quality of life was further evaluated in screen-detected treated patients by Short Form 36 Health Survey questionnaire and the results compared to a Finnish population sample of 2,060 persons. According to the results, mental health was significantly better in screen-detected patients than in the general population. In other items, there were similar trend which showed no significant differences.

Bone mineral density (measured by Dual Energy X-ray Absorptiometry method) in 53 screen-detected long-term treated coeliac disease patients did not differ from the population in general, whereas bone mineral density in 44 symptom-detected patients was lower. The occurrence of fractures was inquired after, but there was no difference between the groups.

The occurrence of autoimmune diseases was studied in 703 treated coeliac disease patients by questionnaire and hospital records and the results compared to that in 299 non-coeliac patients undergoing gastroscopy. The duration of gluten exposure in relation to occurrence of autoimmune diseases in coeliac disease patients was evaluated in logistic regression analysis. Patients with coeliac disease had significantly more autoimmune diseases than controls. The duration of gluten exposure had no promotive effect on the occurrence of autoimmune diseases. Further, the prevalence of clinical and subclinical autoimmune thyroid diseases was studied in 79 treated coeliac disease patients and in 184 non-coeliac controls by measuring serum thyroid hormones and antibodies and by thyroid ultrasound examination. Hypothyreosis and subclinical autoimmune thyroid diseases were significantly more common in patients with coeliac disease than in controls. Coeliac disease patients were prone to atrophic thyroiditis. A hypoechoid pattern in ultrasound examination was even more common in those coeliac patients, whose coeliac disease was diagnosed later in life. This finding suggests that the late diagnosis of coeliac disease predisposes to the later development of manifest autoimmune thyroid disease.

The risk of cancer and mortality in 781 patients with coeliac disease and in 366 with dermatitis herpetiformis were compared to the age- and sex-matched Finnish population. In contrast to many earlier studies, the total cancer risk was not increased. The risk of non-Hodgkin lymphoma was 3-fold in patients with coeliac disease and 6-fold in patients with dermatitis herpetiformis, this risk being fairly similar to that observed in recent studies. The mortality rate was slightly but significantly increased in coeliac disease patients. Mortality in patients with dermatitis herpetiformis was significantly decreased the reason for this remaining unclear.

This study showed that in a high prevalence area of coeliac disease where the diagnostic approach has been active, the majority of the patients are under gluten-free diet. The diagnosis of patients with mild symptoms and patients in coeliac disease risk groups seems justified in that dietary treatment does not worsen the quality of life of these patients. Also the risk of osteoporosis seems to be lower when coeliac disease is diagnosed at an early stage. Although osteoporosis was more common in patients with symptom-detected coeliac disease they did not experience more fractures than screen-detected patients. Fracture risk warrants further study in larger patient series. A gluten-free diet did not seem to affect the occurrence of autoimmune diseases. Interestingly, cancer risk in the patients with coeliac disease was not increased to that in the population in this high-prevalence area but in accordance to earlier studies, the risk of non-Hodgkin lymphoma was increased. In addition, the mortality risk in coeliac disease was lower than in many studies from other countries, where the clinical prevalence of coeliac disease is lower.

Tiivistelmä

Keliakian taudinkuva vaihtelee suuresti: potilaat voivat kärsiä merkittävistä imeytymishäiriöistä ja puutosoireista, suoliston ja sen ulkopuolisista oireista tai olla täysin oireettomia. Osteoporoosi ja lymfooma ovat keliakian parhaiten tunnettuja komplikaatioita. Myös monet autoimmuunitaudit ovat näillä potilailla muuta väestöä yleisempiä.

Tampereen yliopistollisen sairaalan alueella on diagnosoitu ja hoidettu keliakiaa aktiivisesti myös vähäoireisilta ja keliakian riskiryhmiin kuuluvilta potilailta jo 1980-luvulta lähtien. Monet potilaat ovat keliakian suhteen oireettomia, mutta näiden potilaiden elämänlaadusta tai hoitomyöntyvyydestä gluteenittomaan dieettiin ei ole pitkäaikaista tutkimustietoa.

Väitöskirjatyön tarkoituksena oli selvittää keliakiapotilaiden hoitomyöntyvyyttä, elämänlaatua ja komplikaatioita pitkän seuranta-ajan jälkeen alueella, jossa keliakian diagnostiikka on ollut tehostettua ja diagnosoituja potilaita onkin enemmän kuin monella muulla alueella.

Gluteenittoman dieetin hoitomyöntyvyyttä tutkittiin 703 keliakiapotilaalta kyselyn ja sairauskertomustietojen perusteella. Lisäksi tutkittiin 53 riskiryhmien seulonnassa todetun ja 44 oireiden perusteella todetun keliakiapotilaan hoitomyöntyvyys haastattelun ja 4-päiväisen ruokapäiväkirjan avulla sekä määrittämällä keliakian vasta-aineet. Näiden tutkimusten perusteella niinkin moni kuin 77- 83 % potilaista noudatti tarkkaa gluteenitonta ruokavaliota, mikä on parempi tulos kuin monissa muissa maissa tehdyissä tutkimuksissa.

Elämänlaatu (Psychological General Well Being-kyselyllä mitattuna) oli yli 10 vuoden dieettihoidon jälkeen sekä 53:lla seulonnan että 44:llä oireiden perusteella todetulla keliakiapotilaalla yhtä hyvä kuin 110:llä ei-keliaakikoilla. Myöskään vatsaoireiden määrässä (Gastrointestinal Symptom Rating Scale-kyselyllä mitattuna) ei ollut eroja edellä mainittujen ryhmien välillä. Seulonnan perusteella todettujen potilaiden elämänlaatua verrattiin myös 2060 henkilöstä koostuvan suomalaisen väestöotoksen elämänlaatuun Short Form 36 Health Survey-kyselyllä. Sen mukaan seulonnassa todetuilla keliakiapotilailla elämänlaatu oli psyykkisen terveyden osalta merkittävästi parempi.

Luuntiheys (Dual Energy X-ray Absorptiometry-menetelmällä mitattuna) 53 seulonnan perusteella todetulla ja pitkään hoidetulla keliakiapotilaalla ei eronnut merkittävästi väestöstä, kun taas oireiden perusteella todetuilla 44 potilaalla se oli väestön keskimääräistä tasoa merkittävästi huonompi. Murtumien määrä kysyttiin erikseen mutta potilasryhmien välillä ei ollut eroja murtumien suhteen.

Autoimmuunitautien esiintyvyys selvitettiin 703 hoidetulla keliakiapotilaalla kyselyn ja sairauskertomustietojen avulla ja verrattiin 299 terveyskeskuksen

mahantähystyksessä käyneeseen potilaaseen, joilla ei todettu keliakiaa. Gluteenialtistuksen keston vaikutusta autoimmuunitautien esiintyvyyteen keliakiapotilailla selvitettiin logistisessa regressioanalyysissä. Keliakiapotilailla esiintyi merkittävästi enemmän autoimmuunisairauksia kontrolleihin verrattuna. Iän myötä autoimmuunitautien riski näytti kasvavan mutta gluteenialtistuksen pituudella ei näyttänyt olevan selvää yhteyttä autoimmuunitautien esiintyvyyteen. Kliinisten ja subkliinisten kilpirauhassairauksien esiintyvyys selvitettiin lisäksi 79 hoidetulla keliakiapotilaalla ja 184 kontrollipotilaalla, joilla ei todettu keliakiaa määrittämällä kilpirauhashormonit, -vasta-aineet ja tekemällä kilpirauhasen ultraäänitutkimus. Kilpirauhasen vajaatoimintaa sekä subkliinisiä kilpirauhassairauksia todettiin keliakiapotilailla merkittävästi kontrolleja enemmän. Keliakiapotilailla oli ultraäänitutkimuksessa merkittävästi kontrolleja useammin todettavissa atrofiseen kilpirauhastulehdukseen sopiva lyödös. Lisäksi ultraäänitutkimuksessa todettiin vähäkaikuisuutta useammin niillä potilailla, joilla keliakia oli diagnosoitu ja gluteeniton dieetti aloitettu vanhempana. Tämä tulos viittaa siihen, että keliakian myöhäinen diagnoosi lisää riskiä sairastua kliiniseen autoimmuuni-kilpirauhassairauteen.

Syöpäriskiä ja kuolleisuutta tutkittiin sekä 781 keliakiapotilaalla että 366 ihokeliakiapotilaalla ja tuloksia verrattiin ikä- ja sukupuolivakioituun suomalaisväestöön. Vastoin moniin aikaisempiin tutkimuksiin verrattuna potilaiden kokonaissyöpäriski ei ollut suurentunut. Non-Hodgkinin lymfooman riski oli keliakiapotilailla 3-kertainen ja ihokeliakiapotilailla 6-kertainen väestöön verrattuna, riskin ollessa samankaltainen muihin viimeaikaisiin tutkimuksiin verrattuna. Kokonaiskuolleisuus oli keliakiapotilailla lievästi mutta merkittävästi suurentunut väestöön verrattuna, kun taas ihokeliakiapotilaiden kuolleisuus oli merkittävästi väestöä alhaisempi, syyn tälle jäädessä avoimeksi.

Väitöskirjatutkimuksen löydösten mukaan alueella, jossa keliakian kliininen esiintyvyys on suuri johtuen aktiivisesta diagnostiikasta, potilaat noudattavat hyvin gluteenitonta ruokavaliota. Diagnostiikka vaikuttaa kannattavalta, koska myös seulonnan perusteella todetut potilaat noudattavat gluteenitonta ruokavaliohoitoa, eikä hoito huononna heidän elämänlaatuaan vaikka he ovat keliakian suhteen usein varsin vähäoireisia tai jopa oireettomia. Myös osteoporoosia näyttää esiintyvän vähemmän kun keliakia todetaan varhaisessa vaiheessa. Vaikkakin osteoporoosia esiintyi enemmän oireiden perusteella todetuilla keliakiapotilailla kuin seulonnan perusteella todetuilla potilailla, murtumia ei ollut tapahtunut enempää. Murtumariskin arviointi edellyttääkin tutkimuksia suuremmilla potilasaineistoilla ja pidempää seuranta-aikaa. Autoimmuunitautien esiintyvyyteen tässä aineistossa ei gluteenittomalla dieetillä näyttänyt olevan vaikutusta. Keliakiapotilaiden kokonaissyöpäriski ei ollut suurentunut päinvastoin kuin monissa muissa maissa tehdyissä tutkimuksissa, mutta non-Hodgkinin lymfooman riski oli aikaisempien tutkimusten tapaan kohonnut. Kokonaiskuolleisuus oli keliakiapotilailla alhaisempi kuin monissa muissa maissa tehdyissä tutkimuksissa, joissa keliakian kliininen esiintyvyys on pienempi ja keliakian taudinkuva siten usein vaikeampi.

Introduction

Coeliac disease is an autoimmune disorder in which permanent intolerance to dietary gluten causes small-bowel mucosal damage in genetically predisposed individuals. The treatment is a gluten-free diet (GFD), which eliminates the damaging agent and results in mucosal recovery (Walker-Smith et al. 1990). Classical features include gastrointestinal symptoms and malabsorption. However, during recent decades it has been realised that the clinical picture of coeliac disease is wide; many patients have only mild gastrointestinal symptoms and patients may also suffer from extraintestinal symptoms or be totally asymptomatic (Mäki and Collin 1997).

The fact that coeliac disease is associated with certain genetic features, that is Human leukocyte antigen (HLA) -DQ2 or DQ8, increases the risk of the condition in first-degree relatives of the patients up to 10 times that in the general population (Auricchio et al. 1988, Mäki et al. 1991). In addition, patients with other autoimmune diseases such as type-1 diabetes mellitus and autoimmune thyroid diseases run an increased risk of coeliac disease (Mäki et al. 1984, Collin et al. 1994b).

When only patients with classical symptoms were diagnosed, the prevalence of coeliac disease was approximately 1:1000. Upper gastrointestinal endoscopy is still needed to confirm the diagnosis of coeliac disease but with the development of coeliac disease-specific autoantibody tests in the diagnostics, it has become easier to detect many asymptomatic patients and patients with mild symptoms in coeliac disease risk-groups. In fact, the number of detected cases has increased in many countries. Population-based screening studies have shown that coeliac disease is a very common condition, affecting up to a one per cent of the population (Mäki et al. 2003, Bingley et al. 2004). However, population screening is not recommended at present in view of the uncertain cost-benefits.

Many screen-detected coeliac disease patients are asymptomatic or have only mild symptoms. Treatment with GFD requires particular care from the patients and dietary compliance has been only 50-80% in the majority of the studies and the compliance in asymptomatic patients has been suggested to be even lower (Fabiani et al. 2000). Quality of life is important in evaluating the benefits of treatment, in both screen-detected and symptom-detected patients.

Untreated coeliac disease may predispose to complications such as osteopenia and osteoporosis, which affect as many as 50% of untreated patients (Kemppainen et al. 1999). Treatment of coeliac disease with GFD usually

increases bone mineral density (BMD) and thus the beneficial effects of the diet are not confined to the small-bowel mucosa. The treatment may in fact also reduce the risk of the most severe complication, non-Hodgkin lymphoma (NHL), which has been reported to be one cause of the increased mortality rate in patients with coeliac disease (Corrao et al. 2001). It has been suggested that GFD might also prevent the development of other autoimmune diseases (Ventura et al. 1999).

Recognizing the wide clinical spectrum of coeliac disease, our diagnostic approach has been augmented ever since the 1980s. By serological screening we have detected coeliac disease in many patients with atypical features, coeliac disease in family members and in patients with other autoimmune diseases. The clinical prevalence of coeliac disease is thus high in our area, up to 0.3 per cent of the population. Our policy has been to treat all patients similarly by GFD, irrespective of the symptoms at the outset.

There is, however, lack of information concerning the dietary compliance, quality of life and BMD in patients with mild or even asymptomatic coeliac disease after long-term treatment with GFD. In addition, occurrence of autoimmune diseases, risk of malignancies and mortality rate are not known in the area under research where the clinical spectrum of coeliac disease is well represented and the number of detected cases is high. The purpose of the study was to evaluate the dietary compliance, quality of life and occurrence of the above-mentioned complications of coeliac disease and, thus, assess the suitability of the current policy in diagnostics and treatment.

Review of the literature

1. Definition of coeliac disease

Coeliac disease is a permanent intolerance to dietary protein gluten present in wheat, barley and rye. Samuel Gee (1888) was the first who described the typical features of coeliac disease but it took over 60 years before gluten was recognized to be the harmful agent (Dicke 1950). In genetically predisposed individuals gluten induces typical small-bowel mucosal damage with villous shortening, crypt hyperplasia and inflammation, often causing a variety of gastrointestinal symptoms. This coeliac-type mucosal lesion was first reported by Paulley in 1954. Despite the typical small-bowel mucosal lesion, the disease does not affect the gastrointestinal tract alone but is rather a systemic disorder, its best known extraintestinal manifestation being the skin. This skin disease is called dermatitis herpetiformis (van der Meer 1969). The symptoms and small-bowel mucosal damage recover on a GFD.

2. Diagnosis

2.1 Serology

Non-invasive serologic tests are increasingly used as the first step in the diagnostics of coeliac disease. Gluten induces the production of coeliac-specific immunoglobulin (Ig)A-class antibodies, which become detectable in the circulation. The presence of these antibodies is highly indicative of coeliac disease and warrants a diagnostic small-bowel mucosal biopsy.

The most toxic part of wheat gluten causing immunological reactions in the gut is gliadin. Antigliadin antibodies (AGA), usually determined by enzyme-linked immunosorbent assay (ELISA) (O'Farrelly et al. 1983), are common in untreated coeliac disease. The sensitivity of AGA in the diagnostics of coeliac disease has varied between 54-100% and specificity between 47-100%, but in most studies sensitivity and specificity have remained under 90% and the diagnostic value of this test has thus lost its significance (Hill 2005).

Antireticulin antibodies (ARA) directed against reticulin fibres in connective tissues were the first discovered by Seah and colleagues (1971). The specificity of the ARA test was considered to be laboratory-dependent and thus

endomysium antibodies (EmA), which are easier to interpret (Chorzelski et al. 1983), replaced ARA in serological screening for coeliac disease. Both of these antibodies are measured by immunofluorescent method, using rat tissues as a substrate in detecting ARA and monkey oesophagus, subsequently human umbilical cord (Ladinser et al. 1994), in detecting EmA. The high sensitivity of 86 to 100% and specificity of 90 to 100% (Hill 2005) have resulted in the wide use of EmA when coeliac disease is suspected. However, as an immunofluorescent test EmA is laborious and observer-dependent, and other tests have been sought.

In 1997, tissue transglutaminase (tTG) was identified as an autoantigen in coeliac disease (Dieterich et al. 1997). TTG is a calcium-dependent protein cross-linking enzyme widely distributed in human organs. It evinces bioactivity in both intra- and extracellular spaces (Aeschlimann et al. 2000). Human recombinant tTG is used as a substrate in the measurement of tTG-antibodies (tTG-Ab) by ELISA, which is easier to carry out compared to the immunofluorescent method. The sensitivity and specificity of the tTG-Ab test have been 90-100% and 96-100%, respectively (Bonamico et al. 2001 , Fabiani et al. 2001 , Carroccio et al. 2002 , Collin et al. 2005). Patients with selective IgA-deficiency run an increased risk for coeliac disease, in which case IgG-class EmA and tTG-Ab should be measured (Sulkanen et al. 1998 , Korponay-Szabo et al. 2003).

2.2 Small-bowel biopsy

Gastroscopy is required for patients with positive antibodies, as these patients have a high risk for coeliac disease-type small-bowel mucosal damage, including villous atrophy and crypt hyperplasia. The diagnostic criteria for coeliac disease are based on this typical mucosal lesion (Walker-Smith et al. 1990). In the absence of antibodies when clinical suspicion of coeliac disease is high, upper gastrointestinal endoscopy with small-bowel biopsy specimens is needed as a first step to make the possible diagnosis because a minority of patients do not have circulating antibodies. During a GFD clinical recovery is achieved with a concomitant decrease in antibody titers. Especially in asymptomatic patients with negative coeliac disease-antibodies, small-bowel mucosal recovery is the only way to follow the response to GFD.

2.3 Diagnosis of dermatitis herpetiformis

Dermatitis herpetiformis is an extraintestinal manifestation of coeliac disease nowadays sometimes called ‘skin coeliac disease’. The diagnosis of the condition is based on the demonstration of pathognomic granular IgA deposits in the dermal papillae of uninvolved perilesional skin by direct immunofluoresence

examination (van der Meer 1969, Zone et al. 1996). In addition to rash, small-bowel mucosal damage consistent with coeliac disease occurs in 70 to 80% of dermatitis herpetiformis patients and the rest evince coeliac disease-type mucosal inflammation (Marks et al. 1966 , Reunala et al. 1984).

3. Clinical features

3.1 Classical coeliac disease

Coeliac disease was earlier diagnosed mainly on the basis of classical symptoms including symptoms originating from the gastrointestinal tract: diarrhoea, abdominal pain, flatulence, steatorrhoea, weight loss and malabsorption. The disease was diagnosed primarily in infants but gradually it was observed also to affect older children and adults. A shift toward milder symptoms was shown in the early 1980s (Logan et al. 1983). Also age at the diagnosis of coeliac disease has continuously increased (Collin et al. in press).

3.2 Atypical coeliac disease

Research in recent decades has shown that coeliac disease can manifest itself with symptoms other than those of gastrointestinal origin. Neurological symptoms such as peripheral neuropathy (Luostarinen et al. 2003), migraine (Gabrielli et al. 2003), depression and ataxia (Pellecchia et al. 1999 , Burk et al. 2001 , Luostarinen et al. 2001b) or epilepsy (Gobbi et al. 1990 , Luostarinen et al. 2001a) have occurred concomitantly with coeliac disease. Dental enamel defects and recurrent aphtous stomatitis have been reported to be the single presenting symptoms of coeliac disease as well as arthralgia and arthritis of unknown origin (Collin and Mäki 1994). As many as 30% of patients with coeliac disease have had transient elevation of liver enzymes (Farre et al. 2002) and unspecific hypertransaminasaemia (Volta et al. 1998a); even severe liver failure (Kaukinen et al. 2002) may be the main symptom of coeliac disease. There are also cases with infertility as the first sign of the disease (Collin et al. 1996b , Meloni et al. 1999) and problems during pregnancy are more common in coeliac patients on a gluten-containing diet than in those on GFD (Norgard et al. 1999 , Martinelli et al. 2000).

3.3 Silent coeliac disease

The existence of a silent form of coeliac disease came to light in serologic screening studies, where population and patients at increased risk of coeliac disease, e.g. first-degree relatives of patients with coeliac disease and patients having various autoimmune diseases, have been screened with antibodies (Hovell et al. 2001 , Volta et al. 2001a , Mäki et al. 2003). Despite the occurrence of coeliac disease-antibodies and typical gluten-dependent small-bowel mucosal damage these patients lack gastrointestinal and extraintestinal symptoms.

3.3.1 Relatives of patients with coeliac disease

The risk of coeliac disease runs in families; approximately 10% of first-degree relatives of coeliac disease patients have the disease. This has been shown already in 1970s when small-bowel biopsy has been performed in the relatives of the patients (Mylotte et al. 1974, Stokes et al. 1976). Since serology has been used as a first step method for detecting undiagnosed coeliac disease, several other studies has repeated similar results (Auricchio et al. 1988 , Mäki et al. 1991 , Korponay-Szabo et al. 1998). In monozygotic twins, concordance of coeliac disease is as high as 80% (Hervonen et al. 2000).

3.3.2 Patients with other autoimmune diseases

The prevalence of coeliac disease is increased in patients suffering from other autoimmune diseases. The most thoroughly investigated aspect is the association between coeliac disease and type-1 diabetes mellitus, autoimmune thyroid diseases and Sjögren's syndrome, the prevalence of coeliac disease being approximately 5-fold that in the general population (Table 1). Similarly, the occurrence of autoimmune diseases in patients with coeliac disease has been found to be increased (Table 2).

In addition, miscellaneous associations have been reported concomitantly with coeliac disease. Even if at least some have turned out to be fortuitous since the high prevalence of coeliac disease came to be understood, there are certain rare diseases where the association seems real: in studies by O'Leary and colleagues (2002) and Myhre and colleagues (2003), the prevalence of coeliac disease in patients with Addison's disease was 12.5% and 7.9%, respectively. A recent study of 187 patients with autoimmune myocarditis showed that 13 (6.9%) of them had positive tTG-Ab, 9 (4.4%) patients had both tTG-Ab and EmA positive and all of them had small-bowel villous atrophy consistent with coeliac disease (Frustaci et al. 2002). The prevalence of coeliac disease in patients with autoimmune hepatitis and primary biliary cirrhosis has also been

increased (Dickey et al. 1997 , Volta et al. 1998b). In 168 patients with IgA-nephropathy, coeliac disease was found in 3.6% (Collin et al. 2002b). In a study by Meini and associates, (1996) 8% of 65 patients with selective IgA-deficiency had coeliac disease. There are case reports of autoimmune hypopituitarism associated with coeliac disease (Collin et al. 2001) as well as of autoimmune skin disease alopecia areata (Corazza et al. 1995 , Volta et al. 1997).

The early diagnosis of autoimmune diseases is challenging. These conditions develop gradually and the subclinical period may last years and the early signs may be minor. Most of the associations between coeliac disease and various autoimmune diseases have been shown with clinical diseases, but less is known of the occurrence of subclinical autoimmune diseases.

Table 1. *The prevalence of coeliac disease (CD) in patients with type-1 diabetes mellitus, with autoimmune thyroid diseases and with Sjögren's syndrome.*

Author (year)	*Country*	*Patient series*	*Screening method*	*Biopsy proven CD; %*
Type-1 diabetes mellitus				
Mäki et al. (1984)	Finland	215 children	ARA	2.3
Collin et al. (1989)	Finland	195 adults	ARA	4.1
Saukkonen et al. (1996)	Finland	767 children	AGA, ARA	2.4
Sjöberg et al. (1998)	Sweden	848 adults	AGA, EmA	2.6
Aktay et al. (2001)	US	218 children	EmA	4.6
Crone et al. (2003)	Austria	157 children and adolescents	EmA	5.1 10.2*
Cerutti et al. (2004)	Italy	4,322 children	AGA, EmA	6.8
Contreas et al. (2004)	Italy	357 adults	EmA	7.0
Bouguerra et al. (2005)	Tunisia	348 adults	EmA, tTG-Ab	4.0*
Autoimmune thyroid diseases				
Collin et al. (1994b)	Finland	83 adults	ARA, EmA	4.8
Larizza et al. (2001)	Italy	90 children and adolescents	EmA	7.8
Volta et al. (2001)	Italy	220 adults	EmA, tTG-Ab	3.2
Ch'ng et al. (2005)	UK	111 adults	AGA, EmA, tTG-Ab	4.5
Sjögren's syndrome				
Iltanen et al. (1999)	Finland	34 adults	AGA, EmA	14.7
Luft et al. (2003)	Canada	50 adults	tTG-Ab	12* 10
Szodoray et al. (2004)	Hungary	111 adults	AGA, EmA, tTG-Ab	5.4* 4.5

* Prevalence on the basis of positive serology

ARA= antireticulin antibodies, AGA= antigliadin antibodies,

EmA=endomysium antibodies, tTG-Ab= tissue transglutaminase antibodies

Table 2. *The prevalence of type-1 diabetes mellitus (Type-1 DM), autoimmune thyroid diseases (AITD) and Sjögren's syndrome in patients with coeliac disease (CD) and in non-coeliac controls*

Author; (year)	*Country*	*CD patients*	*Controls*	*Prevalence of*								
				Type 1 DM; %			*AITD; %*			*Sjögren's syndrome; %*		
				CD	Controls	p-value	CD	Controls	p-value	CD	Controls	p-value
Collin et al. (1994a)	Finland	335 adults	335 age- and sex-matched non-coeliac patients	5.4	1.5	0.0094	5.4	2.7	0.11	3.3	0.3	0.0059
Ventura et al. (1999)	Italy	909 children and adolescents	1,268 healthy students	3.9	0.0	<0.001	1.2	0.7	0.16	not known	not known	-
Bottaro et al. (1999)	Italy	1026 children and adults	-	7.4	-	-	0.4	-	-	not known	not known	-
Sategna-Guidetti et al. (2001)	Italy	422 adults	605 students/ medical staff/ their relatives	3.8	0.3	<0.001	13.5	2.0	<0.001	not known	not known	-
Ansaldi et al. (2003)	Italy	343 children	230 paediatric patients	not known	not known	-	26.2*	10.0	0.001	not known	not known	-

*includes also euthyroid AITD

Autoimmune diseases associated with coeliac disease, especially type-1 diabetes mellitus, have often been diagnosed prior to diagnosis of coeliac disease (Cronin et al. 1997). It is possible that coeliac disease has existed, but been undiagnosed because of mild symptoms. However, this has led to the hypothesis that a GDF might protect from the development of other autoimmune diseases. In 1999, Ventura and associates (1999) reported that the prevalence of autoimmune diseases was only 5.1% in coeliac disease patients diagnosed and treated under the age of 2 years and did not differ significantly from healthy controls, where the prevalence was 2.8%. By contrast, the prevalence of autoimmune diseases was 23.6% in coeliac patients diagnosed at ages over 10 years, indirectly suggesting that early treatment with GFD might protect from the development of other autoimmune diseases in coeliac disease.

Supporting this hypothesis, Toscano and colleagues (2000) showed that thyroid-related autoantibodies were found in 53% of 19 untreated coeliac disease adolescents but in only 20% of 25 treated patients after 8 years of follow-up. A group under Bonamico (1997) showed that thyroid or diabetes-related antibodies were positive in 47% of 19 untreated adolescents, whereas 13% of 23 treated patients were antibody-positive. In addition, Ventura and associates (2000) showed in 90 adolescents that the prevalence of thyroid-related antibodies decreased from 14% at diagnosis to 2% after two years on GFD and diabetes-related antibodies from 12% to zero. In controls, the prevalence of these antibodies was 1 and 4 per cent, respectively.

In contrast, Di Mario and colleagues (1992) found no significant differences in the prevalence of diabetes-related antibodies between 15 untreated and 15 treated coeliac children, where the titers were positive in 27% and 20%, respectively. Da Rosa Utiyama and group (2001) similarly found thyroid microsomal antibodies positive in 16% of 32 untreated and 17% of 24 treated patients. Sategna-Guidetti and associates (2000) investigated the effect of one year of GFD on the occurrence of thyroid function in 128 adult patients with coeliac disease. Generally, dietary compliance was poor in patients without thyroid function improvement. On the other hand, impairment in thyroid function occurred in 5 out of 91 patients with initially normal function; 4 of the patients followed a strict GFD.

4. Epidemiology

Patients with diagnosed coeliac disease represent a minority of the whole coeliac disease population. Earlier the prevalence of the disease was estimated to be 1 per 1000 individuals (Greco et al. 1992). Even though the wide clinical spectrum of the disease has come to be understood, coeliac disease is still considerably underdiagnosed (Table 3). When patients with only classical symptoms are diagnosed, the prevalence of coeliac disease has varied between 1:6000 to 1:1000 even recently (Schweizer et al. 2004 , Edlinger-Horvat et al. 2005).

Diagnosis of patients with atypical symptoms and patients at high risk of coeliac disease has raised the prevalence of the disease up to 0.3% (Collin et al. 1997) in certain areas, which approaches the figures in population screening studies (Table 3). The prevalence figures for coeliac disease in population screening studies have been very similar in Western populations, i.e. in Europe (West et al. 2003b), the United States (Fasano et al. 2003), Australia (Hovell et al. 2001), the Middle-East (Shahbazkhani et al. 2003) and South America (Pratesi et al. 2003). Although coeliac disease is thought to be rare in Africa and among Afroamericans, the highest prevalence of 5.6% has been reported in Saharawi children in North Africa (Catassi et al. 1999).

Table 3. *Recent studies of the prevalence of recognized coeliac disease (CD) before population screening and the true prevalence after screening*

Author (year)	*Country*	*Study population*	*Prevalence of recognized CD*	*Prevalence of biopsy-proven CD after screening*
Kolho et al. (1998)	Finland	1,070 adults	none	1:97 * 1:133
Mäki et al. (2003)	Finland	3,654 children	1:365	1:67 * 1:99
Pratesi et al. (2003)	Brazil	4,405 children and adults	none	1:275 * 1:294
West et al. (2003b)	UK	7,550 adults	1:328	1:68 *
Bingley et al. (2004)	UK	5,470 children	1:1368	1:100 *
Schweizer et al. (2004)	Netherlands	50,760 adults	1:6345	1:286 * †
Edlinger-Horvat et al. (2005)	Austria	7,660 adolescent males	1:1094	1:295 *

* Prevalence on a basis of positive serology

† 1,432 of 50,760 individuals were screened

5. Pathogenesis

Predisposing genetics and dietary gluten are prerequisite to the development of coeliac disease. No other triggering environmental factors have been proved to cause coeliac disease. In wheat the damaging prolamin is called gliadin; in rye it is secalin and in barley hordein, which are also toxic for coeliac disease patients

(Anand et al. 1978). There is certain evidence that age at first exposure and the amount of gluten in the diet affect the manifestation of the disease (Ivarsson et al. 2000).

Sensitivity to gluten seems to differ between patients. In a few studies patients have been reported to tolerate even 5g of gluten daily without villous atrophy (Kumar et al. 1988 , Montgomery et al. 1988). After gluten challenge, some coeliac disease patients have consumed gluten for over ten years before relapse has occurred (Mäki et al. 1989 , Hogberg et al. 1997). On the other hand, small-bowel mucosal damage has been reported with 0.1-1g daily usage of gluten (Mayer et al. 1991 , Catassi et al. 1993 , Troncone et al. 1995). For comparison, healthy individuals consume about 13g gluten per day (van Overbeek et al. 1997).

Coeliac disease is strongly related to HLA class II molecules, of which the HLA-DQ2 heterodimer exists in up to 90% of coeliac disease patients. This heterodimer is encoded by the DQA1*05 and DQB1*02 alleles which are located either in a single chromosome in the DR3 haplotype or in two separate chromosomes in the DR5/DR7 haplotypes (Sollid et al. 1989). HLA-DQ2-negative patients seem to carry the HLA-DQ8 heterodimer encoded by the DQA1*03 and DQB1*0302 alleles, which are located in the DR4 haplotype, or they may have only one of the alleles DQA1*05 and DQB1*02 coding DQ2 (Spurkland et al. 1992 , Karell et al. 2003). Less than 1 % of coeliac disease patients carry neither of these alleles (Polvi et al. 1998). The fact that 30-40 % of the general population carry the HLA-DQ2 or HLA-DQ8 heterodimer on the DR3 or DR4 haplotype (Polvi et al. 1996) would suggest existence of some other genes or factors predisposing to coeliac disease.

In fact, numerous candidate regions outside the HLA region have been suspected as other genes predisposing to coeliac disease, but there is considerable variability between different studies and populations (King et al. 2000 , Naluai et al. 2001 , Woolley et al. 2002). An interesting candidate gene recently found in a Dutch population is MYO9B on chromosome 19p13.1 (Monsuur et al. 2005). Other candidate genes are encoded on chromosomes 2q11-13, 5q31-33, 11p11, 15q11-13 and 6p12, to mention a few. In addition to genes, adenovirus infection has been suggested to have a role in the development of coeliac disease (Kagnoff et al. 1987).

The immune responses in coeliac disease include both activation of the innate (e.g. macrophages, monocytes and dendritic cells) and the adaptive immune system. HLA-DQ restricted, gluten-specific T-cells are presented in the coeliac disease lesion in the small-intestinal mucosa (Lundin et al. 1993) and are a part of the adaptive immune response together with B-cells. The immunodominant peptides of gliadin are known to cause activation of lamina propria T-cells (Arentz-Hansen et al. 2002). The non-immunodominant peptide p31-43 of gliadin has been demonstrated to be the highly toxic part of gliadin (Maiuri et al. 1996) and this peptide was also able to stimulate an innate immune response in an in vitro organ-culture model. The peptide induced a rapid expression of interleukin-15 (IL-15), which may play a central role in the gluten-induced

immune responses (Maiuri et al. 2003). Interestingly, p31-43 simultaneously enhanced the stimulatory capacity of T-cell immunodominant epitopes of gliadin p57-68 and p62-75, this resulting in activation of lamina propria T-cells (Maiuri et al. 2003).

Tissue transglutaminase was identified as a coeliac disease autoantigen in 1997 (Dieterich et al. 1997). Its crosslink to gliadin increases the immunogenicity of gliadin, and the capability to deamidate glutamine residues to negatively charged glutamic acid further enhances the binding of deamidated gluten peptides to the peptide-binding groove of HLA-DQ2 or –DQ8 on antigen-presenting cells such as dendritic cells (Molberg et al. 2000). Processed gliadin, deamidated gliadin or tTG-gliadin complex is presented to T-helper (Th) cells, which are activated to induce Th1 and Th2-type reactions. Th1 cells release proinflammatory cytokines, most notably interferon-gamma (Nilsen et al. 1995) and tumour necrosis factor-alpha. These stimulate the production of matrix metalloproteinase by fibroblasts, leading to matrix breakdown and mucosal destruction (Schuppan 2000).

The Th2-type reaction induces a humoral response and activates B-cells to produce tTG-Ab. These antibodies are produced in the intestinal mucosa (Marzari et al. 2001) and are deposited extracellularly in the small-bowel mucosa already in early stages of coeliac disease before gluten-induced villous atrophy has developed (Korponay-Szabo et al. 2004 , Kaukinen et al. 2005). An in vitro three-dimensional cell-culture model has shown that tTG-Ab was capable of preventing transforming growth factor beta-mediated epithelial cell differentiation (Halttunen et al. 1999). This finding gave grounds for the hypothesis that tTG-Ab might also contribute to the pathogenesis of coeliac disease apart from T-cell lymphocytes.

6. Treatment

The treatment of coeliac disease and dermatitis herpetiformis is a life-long GFD, which means a permanent elimination of wheat, rye and barley from the diet. The toxic prolamins in these cereals are called gliadins in wheat, secalins in rye and hordeins in barley. Avenins, the prolamins in oats, are tolerated by most patients having coeliac disease or dermatitis herpetiformis. Recovery of the small-bowel mucosa is achieved similarly in patients consuming oats and those not (Janatuinen et al. 1995). Nonetheless, some patients get abdominal symptoms from oats and prefer not to use it (Peräaho et al. 2004b). In addition, there are case reports where dietary oat in a coeliac patient has induced a small-bowel mucosal lesion typical for coeliac disease (Lundin et al. 2003).

During GFD, clinical improvement is often achieved within weeks but may even take years, particularly in adult patients. Autoantibodies normalize gradually during the following months to a year if the diet is strict, and recovery of small-bowel mucosal inflammation and villous atrophy usually requires at

least one year (Kaukinen et al. 1999 , Dickey et al. 2000 , Burgin-Wolff et al. 2002). In dermatitis herpetiformis the itching rash responds slowly to GFD and patients therefore need additional treatment with the anti-inflammatory drug dapsone (Reunala et al. 1977 , Garioch et al. 1994).

There are case reports and non-randomized studies where the beneficial effect of GFD has been achieved in other conditions associated with coeliac disease. Normalization of elevated liver enzymes (Farre et al. 2002) and reversion of hepatic failure (Kaukinen et al. 2002) have been shown to occur during GFD. Curione and associates (2002) showed an improvement in echocardiographic parameters and cardiological features in two coeliac patients with idiopathic dilated cardiomyopathy after 28 month on a strict GFD, whereas no improvement occurred in a patient with poor dietary compliance. GFD may also initiate hair growth in coeliac patients with alopecia areata (Barbato et al. 1998). In some cases, diagnosis and treatment of coeliac disease have resulted in the reversal of infertility (McCann et al. 1988). There are also reports where the diet has improved the metabolic control of type-1 diabetes mellitus (Iafusco et al. 1998 , Sanchez-Albisua et al. 2005). A favourable outcome with GFD has also been reached in occasional cases in patients with cerebellar ataxia, neuropathy and headache (Kaplan et al. 1988 , Pellecchia et al. 1999 , Hadjivassiliou et al. 2001).

There are two types of GFD; naturally gluten-free or wheat starch-based diet. These gluten-free flours may contain trace amounts of gluten (Collin et al. 2004). In cross-sectional studies there has been no difference in small-bowel mucosal morphology and inflammation in patients using a long-term naturally gluten-free and wheat starch-based diet (Kaukinen et al. 1999 , Selby et al. 1999b). This finding has also been shown in a prospective randomized study in newly detected coeliac disease patients (Peräaho et al. 2003). There is no consensus regarding the safety threshold for dietary gluten in patients with coeliac disease, but it has been suggested that consumption of 10 to 50mg of gluten per day might be safe (Collin et al. 2004).

7. Dietary compliance

A gluten-free diet is a cornerstone in the treatment of coeliac disease, but is often difficult to follow strictly. This has been shown in many dietary compliance studies were 17 to 81% of the patients have adhered to a strict GFD (Table 4).

According to earlier studies, different factors influence the maintenance of strict dietary treatment. Dietary compliance has been better in patients with longer duration of coeliac disease (Hogberg et al. 2003 , De Rosa et al. 2004). Female gender especially in adolescents (Ljungman et al. 1993 , Greco et al. 1997), good knowledge of coeliac disease (Ljungman et al. 1993) and better education and success at school (Ciacci et al. 2002a) have also been associated

with better adherence to GFD. In a study by Högberg and colleagues (2003), patients with coeliac disease diagnosed before 4 years of age had better dietary compliance than those diagnosed after 4 years of age. Ciacci and associates (2003), on the other hand, showed better compliance in patients with coeliac disease diagnosed at over 20 years of age than in those diagnosed earlier. In another Italian study of 100 patients, no correlation between adherence to GFD and age at the diagnosis of coeliac disease, diagnostic delay, length of gluten withdrawal or type of clinical presentation was found (Fera et al. 2003).

Most common reasons for non-compliance have been identified as various social restrictions such as difficulties in ordering in restaurants, uneasiness and embarrassment at sharing a table and not wishing to be different from others (Kumar et al. 1988 , Ljungman et al. 1993 , Ciacci et al. 2003). Feelings of anger about coeliac disease also result in lower dietary compliance (Ciacci et al. 2002b). Most of the patients in the studies in question have been diagnosed on the basis of clinical symptoms of coeliac disease, but few have focused on patients with screen-detected coeliac disease, which is often asymptomatic or evince only mild symptoms. Again in Italy, (Fabiani et al. 2000) only 23% of 22 adolescents with screen-detected coeliac disease were on a strict GFD after 5 years of follow-up, compared to 68% of those 22 patients with symptom-detected disease. By contrast, in Finland 19 patients with screen-detected coeliac disease showed 95% adherence to GFD after 1 year's treatment (Mustalahti et al. 2002). Johnston and associates (2004) showed that 3 out of 14 patients with screen-detected coeliac disease had positive serology after 1-year GFD. Taken together, studies on dietary compliance in screen-detected coeliac disease have been carried out with small patient materials and short-term follow-up. The issue remais unclear and calls for further examination.

Table 4. *Dietary compliance in coeliac disease*

Author (year)	*Country*	*Coeliac disease study population*	*Methods used*	*Duration of gluten-free diet; years*	*Strict gluten-free diet; %*	*Partial gluten-free diet; %*	*No gluten-free diet; %*
Dissanayake et al. (1974)	UK	38 adults	Interview	2	47	34	19
Colaco et al. (1987)	Italy	37 children	Interview	15	43	30	27
Kumar et al. (1988)	UK	102 children	Interview, histology	1	56	35	9
Mayer et al. (1991)	Italy	123 adolescents	Questionnaire, interview, serology	10	65	24	11
Troncone et al. (1995)	Italy	23 adolescents	Interview, serology, histology	>10	17	52	30
Ljungman et al. (1993)	Sweden	47 adolescents	Questionnaire	>10	81	13	6
Greco et al. (1997)	Italy	306 adolescents	Questionnaire	not mentioned	73	15	12
Hallert et al. (1998)	Sweden	89 adults	Interview, histology	10	78	12	10
Kaukinen et al. (1999)	Finland	52 children and adults	Interview, 4-day food record, serology, histology	8	88	12	-
Ciacci et al. (2002a)	Italy	390	Interview, histology	7	42	32	24
Lohiniemi et al. (2000)	Finland	58 adults	Interview, 4-day food	10	94	6	-

			record				
Usai et al. (2002)	Italy	68 adults	Interview, serology	>2	59	38	3
Fera et al. (2003)	Italy	100 adults	Interview, serology, histology	9	49	48	3
Högberg et al. (2003)	Sweden	29 adults	Questionnaire, serology	20	59	41	-
Ciacci et al. (2003)	Italy	581 adults	Self-evaluation	8	74	22	4
Ciacci et al. (2005)	Italy	110 adults with childhood diagnosis	Self-evaluation	16	59	not mentioned	not mentiond

8. Quality of life

Interest in health-related quality of life has increased in recent years. Quality of life can be defined as an individual's general sense of well-being and overall satisfaction with life (Naughton et al. 2003). Health-related quality of life includes the areas of physical function, somatic sensation, psychological state and social interactions which are affected by one's health status. The purpose in using health-related quality of life measures is to assess the effects of a condition and/or its therapies on a person's health. Various valid and reliable instruments are available for use in research investigations. Generic instruments can be used in many populations and assess at least the physical, social and emotional dimensions of life. Condition-specific instruments assess the impact of for example a specific disease or condition on health-related quality of life, but may include generic measurements. Dimension-specific instruments assess a single dimension or aspect of the quality of life, for example depression (Naughton et al. 2003).

Untreated coeliac disease is associated with several psychological disorders, especially depression and anxiety. Several mechanisms such as reduced brain monoamine metabolism and regional cerebral hypoperfucion in the brain have been described as possible factors underlying this association (Hallert et al. 1983 , Addolorato et al. 2004b). On the other hand, it has been hypothesized that psychiatric disorders are related to a decreased sensation of general well-being, and secondary to difficult adjustment to a chronic disease (Addolorato et al. 1996).

In recent years, a number of long-term cross-sectional studies on the quality of life in treated coeliac disease have been carried out, but they have focused mainly on symptom-detected disease (Table 5). The results vary considerably depending on the study population and country.

In Sweden, treated adolescents did not differ from a control population in the items of self-esteem and subjective health (Ljungman et al. 1993). On the other hand, quality of life was significantly decreased after 10 years of treatment in coeliac women compared to male patients or healthy controls, but female patients also suffered more from gastrointestinal symptoms. A negative correlation between gastrointestinal symptoms and subjective well-being was found in both female and male patients (Hallert et al. 1998).

A study of 58 long-term treated coeliac disease patients in Finland showed that quality of life and rate of gastrointestinal symptoms were comparable to those in non-coeliac controls (Lohiniemi et al. 2000). Mustalahti and colleagues (2002) reported that quality of life improved after one year on a GFD even more in 19 screen-detected patients with coeliac disease than in those with symptom-detected disease. In addition, quality of life did not differ from that in healthy controls.

In Italy, in contrast, more depressive symptoms were found in treated coeliacs than in controls (Ciacci et al. 1998 , Fera et al. 2003). Anxiety has been shown to decrease during GFD while depressive symptoms have persisted (Addolorato et al. 2001), although there are case reports of depression improving on a GFD (Corvaglia et al. 1999). Psychological support counselling has a positive effect not only on depression but also on dietary compliance (Addolorato et al. 2004a). A group under Usai (2002) showed that poorer quality of life in coeliac disease patients compared to controls was restricted to those who were non-compliant to GFD. A study of 581 coeliac disease patients who were members in a coeliac society in Italy showed no gender differences in anxiety or depression scores and these scores were not correlated to age at diagnosis. However, patients with childhood diagnosis of coeliac disease felt significantly happier than those with adulthood diagnosis (Ciacci et al. 2003).

In Ireland, patients with coeliac disease reflected after 28 year of follow-up a quality life comparable to that in the general population (O'Leary et al. 2004). In the United Kingdom (UK), general health and vitality improved significantly after 1 year of dietary restriction in typical coeliac disease patients, but no improvement was found in screen-detected patients. However, the quality of life in screen-detected patients did not differ from that in healthy controls (Johnston et al. 2004).

Table 5. *Long-term cross-sectional studies on quality of life in patients with coeliac disease (CD) compared to non-coeliac controls*

Author (year)	*Country*	*CD study population*	*Method(s) used*	*Duration of gluten-free diet; years*	*Quality of life compared to non-coeliac controls*
Hallert et al. (1998)	Sweden	89 adults; 83 symptom-detected and 6 screen-detected	SF-36, GSRS	10	Lower in female patients, no differences in males
Lohiniemi et al. (2000)	Finland	58 symptom-detected adults	PGWB, GSRS	10	No differences
Usai et al. (2002)	Italy	66 symptom-detected adults	SF-36	>2	Lower; restricted to non-compliant patients
Fera et al. (2003)	Italy	100 adults; 41 classical, 43 atypical and 16 asymptomatic	SF-36, IBQ, SCID, M-SDS, STAI	9	More depression and anxiety in CD. No correlation to dietary compliance
O'Leary et al. (2004)	Ireland	50 adults with childhood diagnosis of CD	SF-36	28	No differences

SF-36=Short Form 36 Health Survey, GSRS=Gastrointestinal Symptom Rating Scale, PGWB=Psychological General Well Being, IBQ=Illness Behaviour Questionnaire, SCID=Structured Clinical Interview for DSM, M-SDS=modified version of the Zung Self-Rating Depression Scale, STAI=State and Trait Anxiety Inventory

9. Bone mineral density

Bone mineral density is usually measured by dual energy X-ray absorptiometry (DEXA), which gives a two-dimensional, areal picture. According to World Health Organisation criteria (WHO), the diagnostics of osteoporosis is based on the lumbar and femoral neck BMD measurements. The values are compared to those of young healthy adults of the same sex and the deviation of the individual

value is expressed as standard deviations, referred to as the T-score. A T-score equal to or less than -1 but more than -2.5 is defined as osteopenia, a score less than -2.5 as osteoporosis (WHO study group 1994). Bone mineral density can also be defined by Z-score, which is expressed as the standard deviation of the individual value from the age- and sex-matched population.

BMD is often decreased in untreated coeliac disease and osteopenia and osteoporosis are thus particularly frequent findings. This has been shown in patients with classical, subclinical or silent coeliac disease (Mazure et al. 1994 , Corazza et al. 1996 , Valdimarsson et al. 1996). Mustalahti and associates (1999) reported even lower BMD in 19 asymptomatic patients with coeliac disease than in 21 initially symptomatic patients. In an Italian study (Corazza et al. 1996), 14 patients with untreated subclinical coeliac disease had significantly lower BMD than healthy controls. It is not known whether BMD is decreased in patients with subclinical coeliac disease after long-term dietary treatment. Interestingly, there are also reports of low BMD in patients with early developing coeliac disease, when overt villous atrophy has not yet developed (Kaukinen et al. 2001).

Osteoporosis increases the risk of fractures, and in coeliac disease, an increased risk has been reported in some studies; the relative risk has varied according to different studies from 1.3 to 5.2 (Vazquez et al. 2000, West et al. 2003a, Moreno et al. 2004). In the study of Thomason et al. (2003) the risk was at population level. Greater risk has been associated with clinically more severe coeliac disease, long delay in the diagnosis of coeliac disease and low dietary compliance (Vazquez et al. 2000 , West et al. 2003a , Moreno et al. 2004).

Pathological mechanisms of disturbances in bone mineral metabolism in coeliac disease are poorly understood but are probably multifactorial. One of the leading factors is reduced calcium absorption due to villous atrophy, which may be augmented by vitamin D malabsorption (Valdimarsson et al. 1994 , Molteni et al. 1995 , Corazza et al. 1996). The decreased intestinal absorption of calcium results in a secondary hyperparathyroidism, where bone resorption exceeds bone formation (Corazza et al. 1996); serum calcium levels remain normal despite inadequate intestinal absorption. Low BMD is more common in coeliac disease patients with a high serum parathormone level (Selby et al. 1999a). However, a normal parathormone level does not exclude decreased BMD in coeliac patients.

It has been suggested that immunological factors may contribute to bone mineral metabolism. Mucosal inflammation induces the production of cytokines and serum high IL-6 has been associated with a decreased BMD (Fornari et al. 1998). Loss of bone mineral content is probably not the only cause of bone fragility in coeliac disease; reported changes have also been in the three-dimensional structure of the bones in coeliac disease patients (Ferretti et al. 2003). The coeliac disease autoantigen tTG belongs to a family of multifunctional cross-linking enzymes which also stabilize tissues, and tTG is also an important factor as a part of bone mineralization. It has been hypothesized that tTG-Ab in coeliac disease might affect the function of tTG in bone (Sugai et al. 2002).

GFD is important in maintaining adequate BMD in coeliac disease patients and in most cases GFD even improves BMD (Table 6). Bone metabolism in untreated coeliac disease children has been reported to be low, but dietary treatment has resulted in improvement in bone metabolism (Mora et al. 1999) and after 5 years' treatment no differences were found between coeliac disease children and non-coeliac controls (Mora et al. 2001). The most marked improvement in BMD in coeliac disease patients is seen during the first year of GFD (Bai et al. 1997). Patients with childhood coeliac disease diagnosis reach normal BMD in adulthood if they follow the diet adequately (Mora et al. 1999). In adults, on the other hand, the recovery is not always complete but nonetheless often obvious (Valdimarsson et al. 1996).

Table 6. *Effect of gluten-free diet on bone mineral density (BMD) in patients with coeliac disease (CD).*

Author (year)	*CD patients*	*Duration of GFD; years*	*BMD in untreated coeliac disease*	*BMD on a gluten-free diet; change from baseline*
McFarlane et al. (1996)	21 adults	1	BMD (mean) spine: 81% of controls, femoral neck: 84% of controls, both significantly lower than in controls	BMD (absolute increase) spine +6.6% (95% CI +3.1 to 10.1%) and femoral neck +5.5% (95% CI +2.8 to 8.1%)
Corazza et al. (1996)	16 adults; 8 classical CD 8 subclinical CD	1	Z-score (mean) spine -2.6 in classical CD, -1.3 in subclinical CD	Z-score (mean) increased significantly: + 8.4% in classical CD, + 8.5% in subclinical CD
Valdimarsson et al. (1996)	63 adults	1	Z-score (median) spine -0.63, femoral neck -0.54	BMD (median) spine + 3% ($p<0.001$), femoral neck + 2% ($p<0.001$)
Ciacci et al. (1997)	41 adults	1	Reduced	BMD (mean) spine +14.0% ($p<0.001$), femoral neck +10.4 % ($p=0.002$)
Smecuol et al. (1997)	25 adults	3	Bone mass reduction -15% from mean normal values	Increased significantly ($p<0.002$)
Scotta et al. (1997)	66 children	1 and 2	Decreased more in those with diagnosis of coeliac disease later in life	Better recovery in those with 2 years on a GFD than in those with less than 1 year ($p=0.022$)
Mora et al. (1998)	44 children and adolescents	1.4	BMD in spine and total body significantly lower than in 177 healthy controls, ($p=0.015$ and $p=0.0001$, respectively)	BMD (mean) spine + 9.1%, total body + 6.0%

Kemppainen et al. (1999)	28 adults	5	Decreased, at baseline 41% had Z-score <-1 at spine and 39-50% had Z-score <-1 at proximal femur	Female: BMD Spine +2.5%, femoral neck +0.9%, (not significant) Male: Spine +2,2%, femoral neck +2,4% (not significant)
Mustalahti et al. (1999)	19 asymptomatic adults	1	T-score (median) spine -1.9, femoral neck -0.9	Significant increase in BMD in 8 out of 19 patients
Sategna-Guidetti et al. (2000)	86 adults	1	Z-score (mean) spine -1.5, femoral neck -0.9	Z-score (mean) spine + 0.5 ($p<0.0001$), femoral neck +0.2 ($p=<0.0009$)
Valdimarsson et al. (2000)	105 adults	1-3	Age-adjusted BMD reduced ($p<0.001$)	BMD increased significantly ($p<0.001$). Increase was faster in patients with secondary hyperparathyroidism, but those without it reached better BMD
Mora et al. (2001)	19 children	1 and 4	BMD in spine and total body significantly lower than in 211 healthy controls ($p=0.03$ and $p=0.04$, respectively)	BMD (mean) Spine +7.3% / 1 year, total body +4.3% / 1 year, Spine +21.1% /4 years, total body 13.2% / 4 years, not different from that of controls
Carbone et al. (2003)	48 adolescents	4	BMD (mean) significantly lower than in 30 healthy controls (90.1% of this, $p<0.001$)	BMD (mean) +6.7% (no more significant difference compared to controls)
Barera et al. (2004)	22 children	1	BMC (mean) in spine and total skeleton significantly lower than in 428 healthy controls ($p=0.032$ and $p=0.03$, respectively)	BMC (mean) spine + 22.8%, total skeleton +20.5% (no more significant difference compared to controls)
Tau et al. (2006)	24 children	1	Z-score (mean) spine -1.36	Z-score (mean) spine -0.23, The increase was significant ($p<0.001$)

BMC=bone mineral content, GFD=gluten-free diet

10. Malignancies

The most severe complication of coeliac disease is non-Hodgkin lymphoma (NHL), the first reports of this association dating from 1937 (Fairley et al. 1937). Coeliac disease was in fact formerly considered a premalignant condition (Gough et al. 1962). Further studies showed that lymphoma and other malignant conditions were common in coeliac disease, causing increased mortality in these patients (Holmes et al. 1976 , Brandt et al. 1978 , Selby et al. 1979). From the 1980s, the possible protective effect of GFD in the development of NHL has been studied. Nielsen and associates (1985) could not demonstrate a significant difference in the number of malignancies between patients on normal diet or GFD. A group under Leonard (1983) showed in dermatitis herpetiformis a relative risk of malignancies of 3.1 in patients on normal diet but only 1.0 in patients on GFD. In addition, Holmes and colleagues (1989) showed that the risk of malignancies was not increased after 5 years of GFD compared to that in the general population, but remained elevated in those using gluten. The results, together with increasingly effective diagnostics and prevalence of coeliac disease, have aroused new interest in re-estimating the cancer risk in coeliac disease in many countries.

The mechanisms of cancer development in coeliac disease are poorly understood. Sometimes coeliac disease does not respond to adequate dietary treatment, a condition called refractory coeliac disease. There is an abnormal T-cell population in the epithelium, characterized by a low ratio of CD8+/CD3+ cells and rearrangement of T-cell receptor γ-gene. This is known to be associated with an increased risk of the most widely known cancer associated with coeliac disease, enteropathy-associated T-cell lymphoma (EATL), and refractory coeliac disease can thus be suggested as a premalignant condition (Cellier et al. 2000 , Farstad et al. 2002).

Chronic inflammation has been held to predispose to cancer, but the mechanisms involved are unclear. It has been speculated that increased intestinal permeability to potential environmental carcinogens, immune surveillance problems, release of proinflammatory cytokines and nutritional deficiencies may also be responsible for the development of malignant conditions (Green et al. 2002). It seems that a number of carcinogenic mutations are required for cancer to develop.

10.1 Non-Hodgkin lymphoma

Twenty to 30 years ago NHL was diagnosed in approximately 5% of all patients with coeliac disease. However, later studies have shown much lower prevalence figures (Table 7) and the relative risk of NHL has varied between 2.7 and 42.7 (Table 7). Patients with dermatitis herpetiformis also run an increased risk of NHL. EATL has been the most common form of NHL in coeliac disease patients, but it has been shown that the risk of B-cell lymphoma seems also to be increased, especially in patients with dermatitis herpetiformis (Sigurgeirsson et al. 1994 , Hervonen et al. 2005). For comparison, a recent screening study showed that the prevalence of coeliac disease in patients with NHL was 1.2%, which is same as in many population screening studies (Mearin et al. 2006).

The risk of NHL seems to decrease with time from the diagnosis of coeliac disease: Askling and colleagues found a relative risk of 20 for lymphoma within 2 years from diagnosis. After 5 years of follow-up, the risk had decreased to 3.8. The relative risk also decreased over successive calendar periods from 12 in the 1970s to 3.4 in the 1990s (Askling et al. 2002). A group under Green (2003) found the relative risk to be 9.1 for NHL in the beginning, decreasing to 6.2 during a mean 6 ± 11 years of follow-up. In a study by Card and associates (2004), the relative risk of EATL within 2 years from the coeliac disease diagnosis was 359 compared to general population, but after 2 years of follow-up 41.

Coeliac disease-associated lymphoma is often diagnosed before or simultaneously with coeliac disease (Holmes et al. 1989 , Card et al. 2004) or it develops within a few years after the diagnosis of coeliac disease (Howdle et al. 2003). NHL occurring after the diagnosis of coeliac disease is often associated with poor dietary compliance (Selby et al. 1979 , Hervonen et al. 2005) and patients with lymphoma are usually diagnosed in old age and the diagnostic delay might have been long (Brandt et al. 1978 , Selby et al. 1979 , Freeman 2004). A recent study showed that silent coeliac disease was rare in patients with NHL, suggesting that the risk of NHL in patients suffering from silent coeliac disease is not a major concern (Mearin et al. 2006).

Table 7. *Occurrence of non-Hodgkin lymphoma (NHL) in patients with coeliac disease (CD) or dermatitis herpetiformis (DH)*

Author (year)	*Country*	*Study population; n*	*Study period (patient- years of follow-up, if available)*	*n (%) lymphomas*	*SIR of lymphoma (95% CI)*	*Comments*
Holmes et al. (1976)	UK	210 CD	1941-1974	14 (6.7)	-	13 NHL, 1 Hodgkin's disease
Brandt et al. (1978)	Sweden	74 CD	1965-1977	5 (6.8)	-	5 NHL, located in small-bowel. Diagnosis of NHL was made 0.5 to 6 years after the diagnosis of CD. All the study patients had classical CD.
Selby et al. (1979)	Australia	93 CD	1959-1978	4 (4.3)	-	3 NHL, 1 histiocytic medullary reticulosis All the study patients had classical CD. Dietary compliance was low.
O'Driscoll et al. (1982)	Ireland	198 CD	1969-1981	10 (5.1)	-	7 NHL, 2 histiosytic lymphomas, 1 unclassified
Nielsen et al. (1985)	Denmark	100 CD	1964-1982	3 (3.0)	-	
Midhagen et al.	Sweden	139 CD	1976-1986	1 (0.7)	-	7 CD patients diagnosed prior to 1976

(1988)						
Holmes et al. (1989)	UK	210 CD	1941-1985	9 (4.3)	42.7	All NHL, SIR 16.7 in patients adhering to strict GFD and 77.8 in those not
Collin et al. (1996a)	Finland	383 CD 305 DH	1970-1992 (3,107 CD) (3,029 DH)	1 (0.3) 4 (1.3)	2.7 (0.1-14.8) CD 10.3 (2.8-26.3) DH	All NHL
Lewis et al. (1996)	UK and Finland	768 DH	1969-1993 (4,918)	8 (1.6)	3810	All lymphoma cases developed in patients with normal diet or GFD less than 5 years
Askling et al. (2002)	Sweden	11,019 CD* 1,354 DH*	1964-1995 (97,236 CD) (14,451 DH)	38 (0.3) 6 (0.4)	6.3 (4.2-125) CD 1.9 (0.7-4.0) DH	All NHL SIR 3.8 after 5 years of follow-up in CD
Green et al. (2003)	US	381 CD	1981-2000 (1,977)	9 (2.4)	6.2 (2.9-14)	All NHL
Freeman et al. (2004)	Canada	214 CD	1982-2002	18 (8.4)	-	14 NHL in patients with diagnosis of CD later than age 60 years
West et al. (2004a)	UK	4,732 CD	1987-2002 (18,923)	23 (0.5)	4.3 (2.4-7.7)	Lymphoproliferative malignancies, the risk decreased after 1 year of follow-up (SIR 3.4)
Card et al. (2004)	UK	637 CD	1978-2001 (5,684)	9 (1.4)	20.1 (6.8-48.9) within 2 years from the diagnosis of CD	After 2 years from the diagnosis of CD SIR 5.8. All NHL, 5 cases within 2 years and 4 cases after 2 years from the diagnosis of CD

*hospital inpatients, SIR=standardized incidence ratio, CI=confidence interval, GFD= gluten-free diet

10.2 Gastrointestinal cancers

The first reports of other gastrointestinal tract cancers such as oesophageal and pharyngeal cancer associated with coeliac disease are from the 1960s and 70s (Harris et al. 1967 , Holmes et al. 1976 , Selby et al. 1979) but the studies in question were too small to estimate the relative risk of cancer.

In recent studies, an increased overall risk of gastrointestinal cancer has been reported: In a study of West and associates (2004a) where the total follow-up was 18,923 patient-years, the relative risk was 3.3 for gastrointestinal cancers in 4,732 coeliac disease patients during the first year of follow-up after coeliac disease diagnosis. After one year from the diagnosis, the risk was decreased, being 1.65. Card and group (2004) reported that the relative risk of gastrointestinal cancers was 4.2 within 2 years from the diagnosis of coeliac disease (during 1,612 patient-years of follow-up). Thereafter the risk was only 1.6 (during 5,684 patient-years of follow-up).

The relative risk of oropharyngeal cancer was 2.3 in a study by Askling and associates (2002), involving 11,019 hospitalized patients with coeliac disease. It is noteworthy that this study did not represent the whole coeliac disease population, the patients being hospital inpatients, which might have resulted in an overpresentation of more severe coeliac disease cases. The relative risk of stomach cancer in this study was not increased, being 0.9, but the risk of oesophageal cancer was increased, being 4.2. In a study of 381 patients in the United States (Green et al. 2003), 3 oesophageal cancers developed, giving a relative risk of 12.

Askling and group (2002) also showed an increased risk of small-bowel adenocarcinoma (relative risk 10), whereas Green and colleagues (2003) reported a relative risk of 34. All of these cancers had developed before or simultaneously with coeliac disease and no new cases were found during the mean 6 years of follow-up. Card and associates (2004) followed up 637 coeliac disease patients for 5,684 person-years and found that the relative risk of small-bowel adenocarcinoma was 60 within 2 years from the diagnosis of coeliac disease. Thereafter, no new cases were found. In a Canadian study of 214 coeliac disease patients, 3 cases of small-bowel adenocarcinoma developed during the follow-up. Coeliac disease was diagnosed in these patients at the age of 48, 60 and 89 years (Freeman 2004).

The relative risk of colon cancer has been in two recent studies 1.9 (Askling et al. 2002) and 0.8 (Green et al. 2003).

The risk of gastrointestinal cancers in 1,354 patients with dermatitis herpetiformis was assessed in a recent study by Askling and group (2002) and no increased risk was found.

Although the above-mentioned studies are suggestive of a protective effect of dietary treatment in the development of gastrointestinal cancers in coeliac

disease, considering that the risk has decreased during GFD treatment, the follow-up of the patients has been relatively short to make any definite conclusions of the protective effect of GFD. In addition, Howdle and colleagues (2003) found that most patients with coeliac disease who later developed small-bowel adecocarcinoma had adhered to a strict GFD.

10.3 Other malignant diseases

Askling's group (2002) was the first to report an increased risk of primary liver cancer (SIR 2.7; 95% CI 1.3-4.7) in 11,019 patients with coeliac disease. Also carcinoma of the pancreas was modestly increased; SIR 1.9; 95% CI 0.9-3.6. No such findings were made in 1,354 patients with dermatitis herpetiformis. A 5-fold risk of melanoma in 381 coeliac disease patients was found in a study by Green and colleagues (2003). Such a finding has not been reported in other studies.

The risk of breast cancer has been found reduced in two recent studies. Askling's group (2002) found a marked decrease (SIR 0.3; 95 % CI 0.1-0.5) in 11,019 coeliac disease patients, but the risk was not decreased in patients with dermatitis herpetiformis (SIR 0.9; 95% CI 0.4-1.6). In the UK, the risk of breast cancer was markedly reduced, SIR 0.3; 0.2-0.6 (West et al. 2004a). Also Card and associates (2004) showed a slightly decreased risk of breast cancer 2 years after the diagnosis of coeliac disease (SIR 0.6; 95 % CI 0.1-1.7), but the risk was at population level prior to that (SIR 1.3; 95% CI 0.2-4.5). No difference compared to the general population was found by Green's group (2003) in the United States (SIR 1.2; 95% CI 0.2-7.2). The reasons for the lower rate have been speculated to be linked with lower body weight in patients with coeliac disease and with lower smoking rates (Snook et al. 1996 , West et al. 2003b).

Lung cancer is also reported to occur less often in coeliac disease than in the population at large, the most marked decrease being shown by West and colleagues (2004a) (SIR 0.4; 95% CI 0.1-1.0). This difference may also be related to lower smoking rates in patients with coeliac disease (Snook et al. 1996 , West et al. 2003b). A modest decrease was found in the United States (SIR 0.8; 95% CI 0.1-7.2) (Green et al. 2003). In Sweden the risk was at population level in both patients with coeliac disease (SIR 1.0; 95% 0.5-1.7) and dermatitis herpetiformis (SIR 0.9; 95% CI 0.4-1.8) (Askling et al. 2002). In 637 patients with coeliac disease in the UK, a modest but only non-significant increase was found (SIR 1.5; 95% CI 0.6-3.3) (Card et al. 2004).

There are only a few case reports of coeliac disease-associated cancer in children. In a review of 22 cancers in children with coeliac disease, more intestinal lymphomas (n=5) and thyroid carcinomas (n=3) were found than expected (Schweizer et al. 2001).

11. Mortality

Logan and associates (1989) were the first to investigate the relative risk of mortality in a larger series of patients with coeliac disease in 1989. The mortality rate among 653 coeliac disease patients after 8,823 patient-years of follow-up was two-fold that in the general population. The increased risk was restricted to patients with adulthood diagnosis of coeliac disease, whereas in patients with coeliac disease detected in childhood, no increased mortality rate was found suggesting that early diagnosis of coeliac disease might be important. The rate also decreased during follow-up. Deaths due to malignant diseases were 3 times more common than expected and NHL accounted for most of the excess. Mortality due to ischaemic heart diseases or cerebrovascular diseases did not differ from that in the population in general.

Corrao and associates (2001) found in 1072 patients with coeliac disease a 70-fold increase in mortality attributable to NHL, which was the main reason for the slight excess of two-fold mortality. The risk in this study was not increased in patients with silent coeliac disease. Increased mortality was also found in 10,032 hospitalized patients in Sweden (Peters et al. 2003) and in 228 adults patients with coeliac disease in Italy (Cottone et al. 1999), 2.0 and 3.8 respectively; mortality due to NHL was increased even more. A long diagnostic delay, severe clinical manifestations and poor dietary compliance seem to be involved in the excess mortality rate among coeliac patients, whereas in cases where the diagnosis has been made early enough, the risk seems to be lower or even negligible (Collin et al. 1994a , Corrao et al. 2001 , Peters et al. 2003). West and colleagues (2004a) reported in a population-based study that the overall mortality ratio in 4,732 coeliac disease patients was 1.3 compared to that in the 23,609 controls. After 1 year follow-up the ratio was 1.2. A reduction in mortality rates during follow-up has also been observed in other studies (Table 8).

Table 8. *Mortality in patients with coeliac disease (CD) and dermatitis herpetiformis (DH).*

Author, year	*Country*	*Study population*	*Patient-years of follow-up*	*SMR (95% CI)*	*Mortality during follow-up, any specific*
Logan et al. (1989)	UK	653 CD	8823	1.9 (1.5-2.2)	SMR 1.5 within 5 years from the diagnosis of CD
Swerdlow et al. (1993)	UK	152 DH	2288	0.9 (0.6-1.2)	SMR 0.5 (0.2-1.2) in those with GFD and 1.0 (0.7-1.4) in those without GFD
Collin et al.(1994a)	Finland	335 CD adults	-	-	5-year survival rate 87% in male, 99% in female patients
Collin et al.(1996a)	Finland	383 CD adults 305 DH adults	3107 CD 3029 DH	-	5-year relative survival rate 94% in male, 99% in female CD patients. 5-year relative survival rate 101% in male, 101% in female DH patients
Cottone et al.(1999)	Italy	228 CD adults	1349	3.8 (1.9-6.7)	SMR 5.8 (2.5-11.5) within 4 years from the diagnosis of CD
Corrao et al. (2001)	Italy	1072 CD adults	6444	2.0 (1.5-2.7)	SMR 0.98 (0.96-0.99) 3 years after the diagnosis of CD
Peters et al. (2003)	Sweden	10032 CD, hospitalized patients	81182	2.0 (1.8-2.1)	SMR 1.7 (1.5-2.0) after 10 years of follow-up
West et al. (2004a)	UK	4372 CD, general practice research database	18923	1.3 (1.1-1.5)	SMR 1.2 (1.0-1.4) 1 year after the diagnosis of CD

SMR=standardized mortality ratio, CI=confidence interval, GFD=gluten-free diet

Aims of the present study

Coeliac disease has been actively diagnosed since the 1980s in the Tampere University Hospital area. Even mild gastrointestinal symptoms have aroused suspicion of coeliac disease and many patients have been diagnosed as a result of risk-group screening, or by routine duodenal biopsy taken during gastroscopy. This has led to a finding of many patients with mild or even asymptomatic coeliac disease, and to a high clinical prevalence of coeliac disease in the area. However, there are no long-term studies on patients with mild or asymptomatic coeliac disease. The aim of this study was to evaluate patients' dietary compliance, quality of life and coeliac disease-associated complications in Tampere University Hospital area, as the clinical spectrum of coeliac disease here is well represented and the number of detected cases high.

Specific aims were to ascertain:

1) the dietary compliance (I,III), quality of life (I) and bone mineral density (I) in patients with coeliac disease after long-term treatment with a gluten-free diet when the diagnosis had been made on the basis of risk-group screening or typical symptoms

2) the occurrence of clinical (III,IV) and subclinical autoimmune diseases in the treated patients with coeliac disease, and the influence of duration of gluten exposure on the presence of coeliac disease-associated autoimmune diseases (III,IV)

3) the occurrence of malignant diseases and mortality (II)

Patients

Coeliac disease patients (I-IV)

All the patients in the series (Table 9) were investigated at the outpatient clinics of the departments of internal medicine or paediatrics in the Tampere University Hospital area, where the clinical prevalence of coeliac disease is relatively high (Collin et al. 1997 , Collin et al. in press). The diagnosis of coeliac disease was established between 1963 and 2000 and in each case was based on the diagnostic criteria of the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) (Walker-Smith et al. 1990).

Dietary compliance, quality of life and BMD were evaluated in both patients with screen-detected and symptom-detected long-term treated coeliac disease. Altogether, there were 100 patients with screen-detected coeliac disease **(I)**. The reasons for screening were: first-degree relative of coeliac disease patient, autoimmune diseases, rheumatoid diseases and joint pain, atopy and aphtous stomatitis, neurological diseases and infertility. Of these 100 patients, 9 had moved away from the area and 10 were deceased. Finally, 81 patients were invited to participate, of whom fifty-three consented and 28 refused. The results were compared to forty-four randomly selected long-term treated coeliac disease patients diagnosed on the basis of typical symptoms (abdominal complaints or malabsorption) **(I)**.

The study of malignant diseases and mortality in coeliac disease included all consecutive patients with coeliac disease, resulting in a total of 781 patients **(II)**. Malignant diseases and mortality were also investigated in 366 patients with dermatitis herpetiformis. The diagnosis of dermatitis herpetiformis was based on the demonstration of pathognomic granular IgA deposits in the dermal papillae of uninvolved perilesional skin by direct immunofluoresence examination (van der Meer 1969, Zone et al. 1996).

The occurrence of autoimmune diseases was investigated in 703 **(III)** consecutive coeliac disease patients, the cohort comprising both adults and children. In addition, both clinical and subclinical thyroid diseases were studied in 79 **(IV)** coeliac disease patients matched for age and sex with hospital outpatient controls, without prior knowledge of clinical manifestation of coeliac disease. The effect of GFD on the occurrence of autoimmune diseases was evaluated in both groups.

Controls (I-IV)

Quality of life in 53 screen-detected and 44 symptom-detected patients with coeliac disease was compared to that in 54 consecutive patients with untreated coeliac disease and 110 persons without known coeliac disease, and finally to the respective data on a Finnish population of 2060 subjects **(I)**.

The age- and sex-matched Finnish general population was used as the reference in evaluation of the risk of malignant diseases and mortality in both patients with coeliac disease and dermatitis herpetiformis **(II)**.

The occurrence of autoimmune diseases was compared to that in a group of 299 consecutive patients suffering from heartburn or dyspepsia and undergoing upper gastrointestinal endoscopy at primary care; they had no suspicion of coeliac disease, and the condition was excluded by small-bowel biopsy in each case **(III)**. The occurrence of thyroid diseases was compared to that in 184 hospital outpatients. Hypertension was the reason for consultation in 47 control subjects and other cardiovascular symptoms in 38; 47 were investigated due to gastrointestinal symptoms not related to coeliac suspicion, 13 had anaemia without malabsorption; eight suffered from neurological and eight from renal symptoms, 23 had miscellaneous reasons for examination. None suffered from coeliac disease or dermatitis herpetiformis nor had been investigated due to endocrine disorders; coeliac disease had been excluded by small-bowel biopsy in all controls with gastrointestinal symptoms or anaemia **(IV)**, Table 9.

Table 9. *Coeliac disease (CD) patients and controls in studies I-IV*

Study number and design	*Coeliac patients*	*n (f %)*	*Median age; range*	*Controls*	*n (f %)*	*Median age; range*
I Dietary compliance, quality of life and bone mineral density in treated coeliac disease	Screen-detected treated CD	53 (53)	51; 20-75	Untreated CD	54 (74)	48; 20-72
				Non-coeliac controls	110 (81)	48; 23-87
	Symptom-detected treated CD	44 (77)	50; 29-74			
				Finnish population sample	2060 (58)	49 ± 17*
II Malignancies and mortality in coeliac disease	CD outpatients	781 (68)	53; 9-89	General population		
	Patients with dermatitis herpetiformis	366 (48)	57; 12-93			
III Autoimmune diseases in coeliac disease	CD outpatients	703 (68)	47; 2-88	Primary care patients undergoing gastroscopy	299 (67)	58; 14-86
IV Thyroid diseases in coeliac disease	CD outpatients	79 (65)	48; 20-75	Hospital outpatients	184 (65)	44; 16-81

* mean age with standard deviation, median age not available

f % = percentage of females

Methods

Evaluation of dietary compliance (I,III)

In study **I,** dietary adherence was assessed in interview by one experienced dietitian, and patients were also asked to complete a 4-day food record. GFD was graded as follows: (i) strict, (ii) gluten less often than once per month, (iii) gluten 1 to 4 times per month, (iv) weekly gluten intake and (v) normal gluten-containing diet; grades (ii) and (iii) were considered fairly strict in further analysis. Serologic tests were employed to further monitor disease activity: serum IgA class EmA were determined using an indirect immunofluoresence method with human umbilical cord as substrate (Sulkanen et al. 1998), and serum IgA class tTg-Ab by ELISA (Celikey; Pharmacia, Uppsala, Sweden); a dilution 1: $\geq$ 5 and a unit value $\geq$ 5 were considered positive.

In study **III,** adherence to the GFD was evaluated from the questionnaire and patient records. The diet was classified as strict (no dietary lapses), partial (regular dietary lapses at least weekly) or normal gluten-containing.

Quality of life and gastrointestinal symptoms (I)

Quality of life and gastrointestinal symptoms were self-evaluated by questionnaires widely applied in coeliac disease with proven validity and reliability.

The Psychological General Well-Being (PGWB) questionnaire (Dimenäs et al. 1996 , Peto et al. 2001) contained 22 items comprising six sub-dimensions: anxiety, depression, well-being, self-control, general health and vitality, scoring being based on a 6-grade Likert scale, higher scores indicating better psychological well-being.

The SF-36 health survey questionnaire was used to assess health-related quality of life (McHorney et al. 1994 , Hallert et al. 1998 , O'Leary et al. 2004). The raw scores on all 36 items are re-scored from 0 to 100, higher scores indicating better health and quality of life (Aalto et al. 1999). Items are then divided into eight sub-dimensions: physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems, and mental health.

Gastrointestinal symptoms were evaluated using the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire (Svedlund et al. 1988 , Revicki et al. 1998 , Mustalahti et al. 2002). This comprises 15 items covering five major gastrointestinal symptoms: diarrhoea, indigestion, constipation, abdominal pain and gastrooesophageal reflux. Rating is based on a 7-grade Likert scale and higher scores indicate more severe gastrointestinal symptoms.

Bone mineral density (I)

BMD in the lumbar spine and left femoral neck was measured by DEXA (Norland XR 26, Norland Corp, Fort Atkinson, Wisconsin) according to standard procedures; the in vivo precision of these measurements is about 1% (Sievänen et al. 1996). BMD values were expressed as standard deviation scores, which compare individual BMD values to the mean BMD of sex-matched young adults (T-score) or to the mean BMD of the age- and sex-matched population (Z-score). WHO considers T-scores above -1.0 normal, between -1.0 and -2.4 ostopenia and equal to or under -2.5 osteoporosis (WHO study group 1994). The history of fractures was inquired after during consultation.

Assessment of manifest autoimmune diseases (III)

The occurrence of autoimmune disorders was assessed by reviewing patient records in all patients with coeliac disease and by a structured questionnaire mailed to the patients, of whom sixty-one per cent responded. All concomitant diseases and the time of diagnosis were inquired after. The following diseases were asked separately: type-1 diabetes mellitus, autoimmune thyroid diseases comprising hypothyroidism and hyperthyroidism, and Addison's disease and primary Sjögren's syndrome. It was then checked from patient records that the diagnosis of autoimmune disease had been properly made. The current diagnostic criteria were applied in all diseases (Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee 1980, Tan et al. 1982 , Fox et al. 1986 , Arnett et al. 1988 , Fries et al. 1990 , Bertolino and Freeberg 1993 , Christophers et al. 1993 , Loriaux and Mc Donald 1995 , Costabel and Hunninghake 1999). Where a patient failed to return the questionnaire, the data were based on patient records only provided reliable information was available. In the case of controls medical history and structured questionnaire were taken at the time of endoscopy; further information was obtained from patient records **(III)**.

Assessment of clinical and subclinical thyroid diseases (IV)

The diagnosis of autoimmune hypothyroidism was based on reduced thyroid function (low free thyroxin (T4v) level and high thyroid-stimulating hormone (TSH) level in sera), the presence of antithyroid antibodies and the finding of diffuse or nodular goiter or thyroid atrophy in ultrasound. Graves' disease comprised clinical and chemical hyperthyroidism (high T4v, low TSH) and the exclusion of toxic nodules **(IV)**.

Thyroid disease was considered subclinical when positive thyroid antibodies and a grade 3 hypoechoid pattern in ultrasound examination was found but thyroid function was still normal **(IV)**.

Thyroid ultrasonography examination

Thyroid ultrasonography examination was performed with a real-time Aloka instrument (Aloka Co. LTD, Tokyo, Japan) using a 50 MHz transducer, without prior knowledge of thyroid function, and in all cases by the same investigator. The volume of the thyroid gland was measured by ultrasonography applying a special formula: width * length * thickness * 0.48 for each lobe (Brunn 1981). Thyroid echogenicity was classified as homogeneous (grade 1), mild or at most moderate (grade 2) and marked hypoechogenicity (grade 3), the last being suggestive of autoimmune thyroiditis. (Gutengust et al. 1989). The nodules were recorded separately and thyroid needle aspiration cytology was carried out when appropriate. Non-toxic goiter and thyroid cancer were classified according to clinical criteria **(IV)**.

Laboratory examinations

Laboratory examinations determined in the hospital laboratory covered serum TSH (reference value 0.40-6.0 mU/L), T4v (9.0-19.0 pmol/L) and circulating antibodies against thyroid microsomal (TyMs, <1:400), peroxidase (TPO, <1:100), thyreoglobulin (TG, <1:100) (Soppi et al. 1990).

Evaluation of duration of gluten exposure (III, IV)

End-points for actual gluten exposure time **(III)** were specified as follows: (i) to commencement of strict GFD; (ii) to the diagnosis of an autoimmune disorder in cases where autoimmune diseases occurred before adoption of a strict GFD; (iii)

to the end of follow-up in cases where patients did not adhere to a strict GFD and autoimmune disease did not develop. When there was no recovery in the control biopsy or the antibodies remained positive, this was considered equal to gluten exposure, even if the patients reported maintaining a strict GFD.

In study **IV**, gluten exposure time was considered to be the same as the age at which coeliac disease was diagnosed, because the occurrence of subclinical thyroid diseases was not known in advance.

Occurrence of malignant diseases and mortality (II)

Malignant diseases were identified by linking the personal identification codes with records from the nationwide database of the Finnish Cancer Registry, which includes more than 99% of incident cases diagnosed in Finland since 1953 (Teppo et al. 1994). Causes of death were identified from the files of Statistics Finland.

Follow-up commenced January 1st, 1971 for those patients whose diagnosis had been established before 1970; in others since the year of diagnosis of coeliac disease. The end points were 31st December 2002 or the date of emigration or death.

Statistical analyses (I-IV)

The data between study populations were compared to each other using Fisher's exact test, chi square test **(I,III,IV),** two-tailed t-test or Mann Whitney U-test **(IV)** when appropriate. A two-tailed p-value ≤ 0.05 was considered statistically significant **(I,III,IV)**.

The data in study **I** were described using means with 95% confidence intervals or medians with range and lower and upper quartiles.

The expected numbers of malignant diseases and deaths were calculated by multiplying the observed person-years of follow-up by the incidence rate of each malignant disease and mortality rate in the respective sex and age-matched Finnish population in the respective calendar period. Standardized incidence ratio (SIR) was used to measure the relative risk of cancer; that is, the ratio of observed-to-expected cancers. Subgroup analysis was also made for different cancers, choosing cancers earlier reported to have an increased or decreased risk in patients with coeliac disease or dermatitis herpetiformis. The analyses were stratified according to the time since diagnosis. Similarly, a standardized mortality ratio (SMR) was used to assess the risk of death (dos Santos Silva 1999).

Exact 95% confidence intervals (CI) were computed by assuming that the observed numbers of cancers and deaths followed Poisson distribution **(II)** and by Wilson's method as recommended by Altman (Altman et al.) **(III)**.

The incidences of autoimmune diseases were determined by calculating the ratio of the number of new autoimmune cases arising in the study populations during the follow-up time to the total person-years at risk during that time. Incidence figures were given per 10 000 person-years. For calculations Stata 7.0 statistical software (Stata Corporation, Tx, USA) was used **(III)**.

The effect of sex, age at end of follow-up, age at diagnosis of coeliac disease, actual gluten exposure time and diagnostic delay on the presence of autoimmune diseases (=dependent variable) were analyzed in logistic regression analysis using univariate and multivariate approach. Multivariate analysis was applied using the forward stepwise method to take into account confounding variables. The analyses were also made separately for children and adults. A probability of 0.05 was used for stepwise entry of variables and 0.10 for removal of variables **(III)**.

The impact of coeliac disease, age and sex on thyroid volume and echoid pattern was assessed in multivariate analysis. In addition, in the coeliac group the age at diagnosis, which was virtually the same as the age at adoption of a GFD, was taken as a predictor. When analyzing the impact of coeliac disease, sex and age on thyroid volume and echoic pattern, analysis of covariance was used; coeliac disease and sex were taken into the model as factors and age as a covariate. In view of skewed distributions, logarithmic transformation was adopted for outcome variables. The computation was carried out using Statistica/win (version '98) software **(IV)**.

Ethical considerations (I-IV)

The study protocols were approved by the ethical committee of Tampere University Hospital. Informed consent was obtained from all participants.

Summary of results

Dietary compliance (I, III)

Seventy-seven to 83% of the patients adhered to a strict GFD (**I,III**) (Table 10). Strictness of GFD did not differ between patients with screen-detected and symptom-detected coeliac disease, p=0.48. In study **I**, EmA and tTG-Ab were positive in all five patients who admitted consuming gluten more than once a week, in one screen-detected patient who consumed gluten less than once per month and in one symptom-detected patient who consumed gluten 1-4 times per month. One screen-detected patient announced to consume a strict GFD but had positive EmA and tTG-Ab. In treated patients with screen-detected and symptom-detected coeliac disease, the dietary compliance was not associated with age at diagnosis of coeliac disease, follow-up time, age at time of the study, family history of coeliac disease, gastrointestinal symptoms or quality of life. Dietary compliance as determined from hospital records before enrolment to the study was strict in 43 (81%) and partial in 8 (15%) of the patients. Two (4%) of the patients continued on normal gluten-containing diet.

Altogether, 28 patients with screen-detected coeliac disease refused to participate in study **I** (not included in Table 10). According to hospital records, 16 (70%) out of 23 non-participating patients were adhering to a strict GFD, 4 (17%) used partial GFD and 3 (13%) were on a normal diet; there were no data on five.

Table 10. *Adherence to a gluten-free diet (GFD) in patients with coeliac disease (CD)*

	Screen-detected CD patients n=53	*Symptom-detected CD patients n=44*	*Outpatient register of coeliac disease patients n= 703*
Median follow-up after the diagnosis of CD; years (range)	14 (5-21)	10 (9-20)	9 (0-36)
Strict GFD; % (n)	83 (44)	77 (34)	78 (546)
Partial GFD; % (n)	14 (7)	23 (10)	12 (83)
Gluten <1/month	8 (4)	2 (1)	not specified
Gluten 1-4/month	6 (3)	14 (6)	not specified
Gluten >1/week	0 (0)	7 (3)	not specified
Normal gluten-containing diet;% (n)	4 (2)	0 (0)	6 (45)
Not known; % (n)	0	0	4 (29)

n= number of patients

Quality of life and gastrointestinal symptoms (I)

Quality of life measurements (PGWB) in screen-detected long-term treated patients did not differ from those in symptom-detected long-term treated patients or in non-coeliac controls (Table 11); there were no differences between men and women. In the respective sub-dimensions of PGWB, screen-detected and symptom-detected treated patients did not differ from the general population (data not shown). Quality of life was not correlated to the length of follow-up. In SF-36, the mean value for the mental health sub-dimension was significantly better in screen-detected treated coeliac disease patients than in the general population; in other items there was a similar but non-significant tendency (Table 12).

The GSRS total score was lower, indicating fewer gastrointestinal symptoms in treated screen-detected than in symptom-detected coeliac disease patients or in non-coeliac controls, but the difference was not statistically significant (Table 13). In the different sub-dimensions of GSRS, there were no significant differences between screen-detected and symptom-detected treated patients or the general population (data not shown).

Untreated coeliac disease patients had more gastrointestinal symptoms and had a lower quality of life than subjects in other groups on average.

Table 11. *Quality of life by scores on Psychological General Well-Being (PGWB) questionnaire in coeliac disease (CD) and controls.*

Study group	*PGWB total scores †*		
	Median	*25% Quartiles*	*Range*
Screen-detected treated CD patients; n=53	108	98; 117	76-132
Symptom-detected treated CD patients; n=44	103	96; 115	49-129
*Untreated CD patients; n=54**	98	87; 106	63-129
Non-coeliac controls; n=110	107	100; 114	65-126

* Statistically lower scores than in screen-detected CD patients (p=0.004) and non-coeliac controls (p=0.004)

† Higher values indicate better quality of life

n = number of subjects

Table 12. *Quality of life by scores on SF-36 Health Survey Questionnaire†*

	Patients with screen-detected treated coeliac disease n=53		*Controls, Finnish study population n=2060*	
	Mean	*95% confidence interval*	*Mean*	*95% confidence interval*
Physical functioning	87	82 - 92	85	84 - 86
Role limitations due to physical problems	84	75 - 92	75	73 - 76
Social functioning	87	81 - 92	82	81 - 83
Bodily pain	81	76 - 86	76	75 - 77
General mental health *	79	75 - 83	74	73 - 75
Role limitations due to emotional problems	81	72 - 90	75	73 - 77
Vitality	67	62 - 72	64	63 - 65
General health perceptions	67	61 - 72	65	64 - 66

* Statistically significant difference

† Higher scores indicate better health-related quality of life, except for role limitations due to physical or emotional problems

n = number of subjects

Table 13. *Gastrointestinal symptoms in coeliac disease (CD) patients and controls in Gastrointestinal Symptom Rating Scale (GSRS) questionnaire*

Study group	*GSRS scores †*		
	Median	*25% Quartiles*	*Range*
Screen-detected treated CD patients; n=53	1.5	1.3; 1.9	1.1 - 3.0
Symptom-detected treated CD patients; n=44	1.9	1.5; 2.2	1.0 - 3.7
Untreated CD patients; n=54 *	2.6	2.0; 3.1	1.1 - 4.9
Non-coeliac controls; n=110	1.7	1.4; 2.3	1.0 - 4.2

* Statistically higher scores than in any other group, $p \leq 0.001$ in all comparisons

† Higher scores indicate more gastrointestinal symptoms

n = number of subjects

Bone mineral density (I)

After long-term treatment, screen-detected coeliac disease patients tended to have somewhat higher BMD than patients with symptom-detected treated coeliac disease (Table 14), but the difference was not statistically significant. Osteoporosis was found in 12% (6/52) of screen-detected and 27% (12/44) of symptom-detected treated patients (p=0.05), whereas 42% (22/53) and 45% (20/44) had osteopenia, respectively (p=0.76). The occurrence of fractures did not differ between the groups: 32% (17/53) of screen-detected and 25% (11/44) of symptom-detected treated patients had sustained one or more fractures (p=0.44).

Table 14. *Bone mineral density as Z-scores with 95% confidence intervals (CI) in patients with screen-detected and symptom-detected coeliac disease (CD) after long-term gluten-free dietary treatment.*

	Z-score spine (95% CI)	*Z-score femur (95% CI)*	*T-score spine (95% CI)*	*T-score femur (95% CI)*
Screen-detected CD	-0.1 (-0.5 to 0.2)	0.2 (-0.1 to 0.5)	-0.8 (-1.2 to -0.4)	-0.5 (-0.9 to -0.2)
Symptom-detected CD	-0.6 (-1.0 to -0.3)	-0.2 (-0.4 to 0.1)	-1.5 (-1.9 to -1.1)	-1.1 (-1.4 to -0.8)

Autoimmune diseases (III, IV)

Clinical diseases

Autoimmune diseases were found in altogether 21.8% (153/703) of individuals with coeliac disease and in 10.7% (32/299) of controls ($p<0.001$). In the coeliac group **(III)**, 31% of autoimmune diseases were detected subsequent to the diagnosis of coeliac disease; of these patients, 83% had adhered to a strict GFD after a mean 10.7 years of treatment, compared to whole series of 703 patients where 78% adhered to a strict diet (Table 10) after a mean of 10.5 years of GFD.

The incidence rates per 10, 000 patient-years for all autoimmune disorders were significantly higher in coeliac disease patients than in controls **(III)**. When different autoimmune conditions were observed, a significant difference was seen for type-1 diabetes mellitus and Sjögren's syndrome; the same trend was seen throughout in other autoimmune conditions, though not statistically significantly (Table 15).

Altogether, 13.9% (11/79) of coeliac disease patients and 2.1% (4/184) of controls had manifest thyroid disease: 3.8% (3/79) of the patients with coeliac disease and 0.5% (1/184) of the controls had Graves' disease ($p=0.15$), whereas autoimmune hypothyroidism was found in 10.1% (8/79) and 1.6% (3/184), respectively ($p=0.005$) **(IV)**.

Table 15. *Incidence of autoimmune diseases in patients with coeliac disease (CD) and non-coeliac controls (Non-CD).*

	Incidence per 10 000 person-years			
	CD	*95% CI*	*Non-CD*	*95% CI*
*All **	52.2	44.6-61.1	19.7	13.9-27.9
*Type-1 diabetes mellitus**	12.6	9.0-17.2	0.6	0.1-4.3
Autoimmune thyroid disease	17.6	13.5-22.9	12.8	8.3-19.6
Hypothyroidism	11.4	8.2-15.8	9.0	5.4-15.0
Hyperthyroidism	6.0	3.8-9.5	3.6	1.6-8.0
*Sjögren's syndrome **	7.3	4.8-11.0	0.6	0.1-4.2
Rheumatoid arthritis	5.4	3.4-8.7	4.8	2.4-9.7
Psoriasis	4.1	2.4-7.1	0.6	0.1-4.2
Sarcoidosis	3.5	1.9-6.3	0.0	0.0-2.2
IgA-glomerulonephritis	1.9	0.9-4.2	0.0	0.0-2.2
Sacroiliitis	1.6	0.7-3.8	0.6	0.1-4.3
Systemic lupus erythematosus	1.3	0.5-3.4	0.0	0.0-2.2
Scleroderma	0.9	0.3-2.9	0.0	0.0-2.2
Alopecia areata	0.9	0.3-2.9	0.0	0.0-2.2
Addison's disease	0.6	0.2-2.5	0.0	0.0-2.2
Vasculitis	0.3	0.0-2.2	0.0	0.0-2.2

* Statistically significant difference between coeliac disease patients and controls.
CI=confidence intervals

Subclinical thyroid diseases

Patients with coeliac disease had significantly more often a subclinical thyroid disease than controls; 8 (10.1%) and 6 (3.3%), respectively (p=0.048) **(IV)**. The presence of thyroid antibodies did not differ significantly between coeliac disease patients and controls, but a hypoechoid pattern of the thyroid gland was seen more often in patients with coeliac disease (Table 16). Positive thyroid antibodies were associated with a severe hypoechoid finding more often in coeliac disease patients than in controls (Table 17). The volume of the thyroid gland was significantly smaller in patients with coeliac disease (mean 8.34 mL ± 4.51) than in controls (10.35 mL ± 5.86), p=0.007. Thyroid nodules were found in 16 (20%) patients with coeliac disease and in 26 (14%) controls, (p=0.29). Cysts were found significantly more often in patients with coeliac disease than in controls, in 11 (14%) and 9 (5%), respectively, (p=0.02).

Table 16. *Prevalence of positive thyroid antibodies and hypoechogenicity of thyroid gland in ultrasound examination in patients with coeliac disease and in controls.*

Parameter	Coeliac disease patients n=79	Controls n=184	p-value
Serum antithyroid antibodies positive	n (%)	n (%)	
S-TPO-Ab	9 (11.4)	9 (5.1)	ns
S-TyMS-Ab	14 (17.7)	28 (15.7)	ns
S-TG-Ab	7 (8.8)	9 (5.1)	ns
Thyroid echogenicity grading			
Grade 2 hypoechogenicity	31 (39.2)	34 (18.5)	0.0005
Grade 3 hypoechogenicity	27 (34.2)	44 (23.9)	ns
Grade 2 or 3 hypoechogenicity	58 (73.4)	78 (42.4)	<0.0001

ns= not significant, S-TPO-Ab=thyroid peroxidase antibodies, S-TyMS-Ab=thyroid microsomal antibodies, S-TG-Ab=thyreoglobulin antibodies, n=number of subjects, p-value ≤ 0.05 was considered statistically significant

Table 17. *Relationship between presence of thyroid antibodies and severe hypoechogenicity*

	Number of patients with thyroid hypoechogenicity grade 3	
Titre	Coeliac disease patients	Controls
S-TPO-Ab > 100	8/9 (89%)	4/9 (44%)*
S-TPO-Ab < 100	21/70 (30%)	40/169 (24%)
S-TyMs-Ab > 400	13/14 (93%)	10/28 (36%)†
S-TyMs-Ab < 400	14/65 (22%)	34/150 (23%)
S-TG-Ab > 100	3/7 (43%)	3/9 (33%)
S-TG-Ab < 100	24/72 (33%)	41/169 (24%)

* p=0.006

† p=0.0004

S-TPO-Ab=thyroid peroxidase antibodies, S-TyMS-Ab=thyroid microsomal antibodies, S-TG-Ab=thyreoglobulin antibodies

Duration of gluten exposure and autoimmunity

In the univariate logistic regression model **(III)** covering the whole coeliac population, age at end of follow-up and age at diagnosis of coeliac disease increased the odds ratio for developing autoimmune disease by one per cent per year. By contrast, actual gluten exposure time seemed to reduce it. Gender had no significant effect on the probability of autoimmune disease. In controls, neither gender nor age at end of follow-up influenced the probability of autoimmune disease. A similar trend was seen within different age groups (Table 18).

In initial multivariate logistic regression analysis, information on diagnostic delay (that is the time between the beginning of symptoms and the diagnosis of coeliac disease) in coeliac disease patients was available in 439 out of 703 cases, and the delay did not influence the probability of having an autoimmune disease. The final multivariate logistic analysis was therefore also carried out without the diagnostic delay item, which made it possible to include all 703 patients. In final multivariate analysis, age at end of follow-up was the strongest indicator of the presence of autoimmune disease in coeliac subjects (Table 19). Age at diagnosis of coeliac disease and female gender also increased the probability, but actual gluten exposure time seemed to reduce it.

In study **IV**, there were no associations between TPO or TyMS and the duration of GFD: 20% (4/20) of the patients with GFD four years or more had positive antibodies, whereas 17% (10/59) of those with GFD less than four years were positive for these antibodies. This difference was not statistically significant. Age at diagnosis of coeliac disease influenced the echoid pattern: grade 2 and 3 hypoechogenicity were more common in patients whose coeliac disease was diagnosed later in life than in younger patients. Thyroid hypoechogenicity was not dependent on age at the time of the study, neither was gender.

Table 18. *Univariate logistic regression analysis of different variables as predictors of the presence of autoimmune disease in coeliac disease (CD) patients and in non-coeliac controls.*

	CD diagnosis under 10 years of age n=96		CD diagnosis between 10 and 20 years of age n=68		CD diagnosis over 20 years of age n=539		CD total n= 703		Non-coeliac controls, n=299	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age at end of follow-up	1.04 (0.84-1.29)	0.699	0.87 (0.69-1.10)	0.247	1.00 (0.99-1.02)	0.712	1.01 (1.00-1.02)	0.005	1.02 (1.00-1.10)	0.060
Age at diagnosis of CD	1.05 (0.97-1.1)	0.206	0.96 (0.86-1.06)	0.413	1.01 (1.00-1.02)	0.220	1.01 (1.00-1.02)	0.027	-	-
Actual gluten exposure time	0.84 (0.69-1.02)	0.071	0.59 (0.43-0.82)	0.002	0.96 (0.95-0.98)	<0.001	0.99 (0.98-1.00)	0.021	-	-
Gender (male reference =1)	1.10 (0.32-3.70)	0.876	1.72 (0.49-6.25)	0.392	1.19 (0.76-1.89)	0.439	1.25 (0.84-1.85)	0.204	2.33 (0.86-5.88)	0.084

OR= odds ratio, CI= confidence interval
n= number of subjects

Table 19. *Multivariate logistic regression analysis, in which the effect of different variables was evaluated as predictive of the presence of autoimmune disease.*

	Coeliac disease patients n=703		
	Odds ratio	95% confidence interval	p-value
Age at end of follow-up	1.06	1.02-1.10	0.001
Actual gluten exposure time	0.39	0.27-0.56	<0.001
Age at diagnosis of coeliac disease	2.45	1.70-3.52	<0.001
Gender (male reference=1)	1.83	1.05-3.18	0.033

n= number of subjects

Malignant diseases (II)

Interestingly, the total risk of malignancies in patients with coeliac disease did not differ from that in the population in general (Table 20). No cancers were diagnosed in coeliac disease children. There was however a significant three-fold risk of NHL in coeliac disease, and six-fold in dermatitis herpetiformis. Patients with dermatitis herpetiformis with NHL were on average younger than those with coeliac disease, and six had other NHL than EATL (Tables 21 and 22). The diagnosis of dermatitis herpetiformis had been established in all patients with subsequent NHL before 1982.

Non-Hodgkin lymphoma

Five patients with coeliac disease had NHL (SIR 3.2; 95% CI 1.0-7.5), all of small-bowel origin; four were T-cell and one of B-cell lymphomas (Table 21). Coeliac disease was diagnosed at ages over 50 in each case. NHL was diagnosed almost simultaneously with coeliac disease in two cases; in the rest, the time from the diagnosis of coeliac disease to the diagnosis of NHL was 3 to 8 years and the patients had followed a strict GFD for 2 to 4 years.

Seven NHLs were diagnosed in patients with dermatitis herpetiformis (SIR 6.0; 2.4-12.4). Only one originated from small-bowel and was histologically T-cell type (Table 22). Patients were 16 to 64 years old at the time of their dermatitis herpetiformis diagnosis, and the time from this to the diagnosis of lymphoma varied between 2 to 32 years. The patients had followed a strict GFD 0 to 12 years, but 71 per cent of them had prevously had some dietary lapses.

Table 20. *Observed and expected number (n) of cancers in patients with coeliac disease and dermatitis herpetiformis with standardized incidence ratio (SIR) of malignant diseases and 95% confidence intervals (CI).*

Site of malignant disease	*Coeliac disease*			*Dermatitis herpetiformis*		
	Observed, n	*Expected, n*	*SIR (95% CI)*	*Observed, n*	*Expected, n*	*SIR (95% CI)*
All cancers	49	41.9	1.2 (0.9-1.5)	20	20.7	1.0 (0.6-1.5)
Non-Hodgkin's lymphoma	5	1.6	3.2 (1.0-7.5)*	7	1.2	6.0 (2.4-12.4)*
Hodgkin's lymphoma	0	0.3	0.0 (0.0-14.4)	0	0.2	0.0 (0.0-21.6)
Digestive system	10	8.0	1.3 (0.6-2.3)	6	6.6	0.9 (0.3-2.0)
Oesophagus	0	0.3	0.0 (0.0-10.7)	0	0.3	0.0 (0.0-11.7)
Stomach	2	1.2	1.2 (0.2-4.5)	3	2.1	2.1 (0.4-6.3)
Small-bowel cancer	0	0.1	0.0 (0.0-80.1)	0	0.04	0.0 (0.0-104.0)
Colon and rectum	4	3.7	1.1 (0.3-2.8)	2	2.9	0.7 (0.1-2.5)
Lung and trachea	2	3.4	0.6 (0.1-2.1)	1	3.5	0.3 (0.0-1.6)
Breast	9	10.2	0.9 (0.4-1.7)	5	4.71	1.1 (0.3-2.5)

* The risk of non-Hodgkin's lymphoma significantly increased

Table 21. *Characteristics of patients with coeliac disease (CD) and non-Hodgkin's lymphoma (NHL)*

n:o	*Gender*	*Diagnosis of CD; year (age)*	*Diagnosis of NHL; year (age)*	*Time from the diagnosis of CD to diagnosis of NHL; years*	*Organ involved*	*Histology of NHL*	*Adherence to gluten-free diet*	*Year and cause of death*
1	female	1987 (58)	1995 (66)	8	Ileum	EATL	1987-1990 no diet, 1991-1995 strict	1995; NHL
2	male	1989 (65)	1992 (68)	3	Jejunum	EATL	1989-1990 no diet, 1991-1992 strict	1997; pulmonary embolism
3	male	1990 (51)	1996 (57)	6	Ileum	EATL	1990-1995 no diet, 1995-1996 strict	1996; NHL
4	female	1994 (53)	1995 (54)	1	Jejunum	DLBCL	1994-1995 strict	1999; pneumonia
5	male	1997 (73)	1997 (73)	0	Jejunum	EATL	1997-strict	2003; NHL

EATL=enteropathy-associated T-cell lymphoma, DLBCL= diffuse large B-cell lymphoma

Table 22. *Characteristics of patients with dermatitis herpetiformis (DH) and non-Hodgkin's lymphoma (NHL).*

n:o	*Gender*	*Diagnosis of DH; year (age)*	*Diagnosis of NHL; year (age)*	*Time from the diagnosis of DH to diagnosis of NHL; years*	*Organ involved*	*Histology of NHL*	*Adherence to gluten-free diet*	*Villous atrophy*	*Year and cause of death*
1	male	1967 (16)	1999 (48)	32	Stomach	DLBCL	1964-1988 no diet, 1988-1999 strict	yes	alive
2	male	1971 (58)	1992 (79)	21	Penis	DLBCL	1971-1975 no diet, 1976-1979 partial, 1980-1992 strict	yes	1995; unknown
3	male	1972 (30)	1979 (37)	7	Mesenterium, colon	Diffuce	1972-1975 no diet, 1975-1979 strict	yes	alive
4	male	1973 (47)	1976 (50)	3	Ileum	EATL	1973-1976 partial	yes	1976; NHL
5	female	1978 (64)	1983 (69)	5	Mesenterium, cervical lymph node	DLBCL	1978-1983 strict	no	1983; NHL
6	female	1978 (40)	1980 (42)	2	Inguinal lymph node	FL	1978-1980 strict	yes	alive
7	female	1981 (31)	1996 (46)	15	Mesenterium, ovario	DLBCL	1982-1996 partial	no	2003; NHL

EATL=enteropathy-associated T-cell lymphoma, DLBCL= diffuse large B-cell lymphoma, FL= follicular lymphoma

Mortality (II)

The overall mortality rate was significantly higher in patients with coeliac disease (SMR 1.26; 95% CI 1.00-1.55) than in the population. Within the first five years, SMR was 1.42 and thereafter 1.18. The increased risk of mortality was seen only in males and no longer after five years of follow-up. In patients with dermatitis herpetiformis, mortality was significantly lower than expected (SMR 0.52; 0.36-0.72) and there were no gender differences (Table 23).

In subanalysis, significantly increased mortality in patients with coeliac disease was found in ischaemic heart diseases, malignant neoplasms of lymphoproliferative or haematopoietic diseases and non-malignant diseases of the digestive system, while mortality due to cerebrovascular diseases was decreased. In contrast to coeliac disease, patients with dermatitis herpetiformis had an overall disease mortality rate significantly decreased and the trend was seen in almost all categories, albeit not statistically significantly (Table 24).

Table 23. *Overall mortality rate in patients with coeliac disease (CD) and dermatitis herpetiformis (DH).*

	Patient-years of follow-up after the diagnosis of CD	*Observed deaths*	*Expected deaths*	*SMR*	*95% confidence intervals*
Total follow-up period					
CD all	10956	85	67.6	1.26	1.00-1.55*
men	2400	40	28.0	1.43	1.02-1.94*
women	7556	45	39.6	1.14	0.83-1.51
DH all	6289	34	65.2	0.52	0.36-0.72*
men	3271	20	37.2	0.54	0.33-0.83*
women	3017	14	28.0	0.50	0.27-0.83*
Mortality rate in the first 5 years of follow-up					
CD all	3728	31	21.9	1.42	0.96-2.01
men	1174	20	11.9	1.68	1.03-2.59*
women	2554	11	10.0	1.10	0.55-1.97
DH all	1774	7	14.0	0.50	0.20-1.02
men	927	4	8.3	0.48	0.13-1.23
women	847	3	5.7	0.52	0.11-1.53
Mortality rate after 5 years of follow-up					
CD all	7228	54	45.7	1.18	0.89-1.54
men	2225	20	16.1	1.24	0.76-1.91
women	5002	34	29.7	1.15	0.79-1.60
DH all	4514	27	51.2	0.53	0.35-0.76*
men	2344	16	28.9	0.55	0.32-0.90*
women	2170	11	22.3	0.49	0.25-0.88*

* p=statistically significant difference compared to general population

SMR= standardized mortality ratio

Table 24. *Disease-specific mortality rates in patients with coeliac disease and dermatitis herpetiformis.*

	Coeliac disease; n=781		*Dermatitis herpetiformis; n=366*	
Cause of death	*O/E*	*SMR (95% CI)*	*O/E*	*SMR (95% CI)*
All diseases	75/60.9	1.2 (1.0-1.5)	33/59.1	0.6 (0.4-0.8)*
Ischaemic heart diseases	27/16.9	1.6 (1.1-2.3)*	13/18.1	0.7 (0.4-1.2)
Cerebrovascular diseases	1/6.7	0.2 (0.0-0.8)*	3/6.5	0.5 (0.1-1.4)
Other heart diseases	5/2.3	2.1 (0.7-5.0)	3/2.5	1.2 (0.3-3.6)
Malignant neoplasms	17/17.8	1.0 (0.6-1.5)	9/14.6	0.6 (0.3-1.2)
Lymphoproliferative/ haematopoietic diseases	7/1.7	4.1 (1.7-8.5)*	3/1.4	2.2 (0.5-6.4)
Malignant neoplasms of stomach	2/1.2	1.7 (0.2-6.2)	2/1.1	1.9 (0.2-6.8)
Malignant neoplasms of respiratory tract	1/3.0	0.3 (0.0-1.9)	1/3.2	0.3 (0.0-1.7)
Non-malignant diseases of digestive system	6/1.7	3.6 (1.3-7.8)*	0/1.6	0.0 (0.0-2.3)
Endocrine diseases	1/1.0	1.0 (0.0-5.7)	0/0.8	0.0 (0.0-4.5)
Dementia	3/2.2	1.3 (0.3-3.9)	2/2.7	0.7 (0.1-2.7)
Other neurological diseases	2/1.2	1.7 (0.2-5.9)	0/0.9	0.0 (0.0-3.8)
Infectious diseases	0/0.6	0.0 (0.0-6.8)	0/0.5	0.0 (0.0-7.5)
Respiratory diseases	7/4.1	1.7 (0.7-3.6)	1/4.5	0.2 (0.0-1.2)
Urinary diseases	1/0.6	1.7 (0.0-9.4)	0/0.6	0.0 (0.0-6.1)
Other diseases (non-alcohol-related)	3/0.9	3.4 (0.7-10.1)	0/0.6	0.0 (0.0-6.3)
Diseases associated with alcohol misuse	1/2.3	0.4 (0.0-2.5)	0/2.2	0.0 (0.0-1.6)

*Statistically significant difference compared to general population

0=observed, E=expected, SMR=standardized mortality ratio, CI=confidence interval

n = number of subjects

Discussion

Dietary compliance

Gluten-free diet is at present the only known treatment for coeliac disease, but is often difficult to follow-up even when symptomatic patients are in question. Application of serologic screening tests has increasingly revealed many asymptomatic patients or patients with minor symptoms (Fasano 2003), but no long-term studies on dietary compliance in these patients have hitherto been carried out.

This study showed an excellent dietary compliance in patients with coeliac disease: 77 to 83% followed a strict GFD **(I, III)**, which is better than in many other studies (Table 4). It is of note that also patients with screen-detected coeliac disease showed good dietary compliance. In fact, no significant differences were found between patients with symptom- or screen-detected coeliac disease; over 90% of these patients adhered to strict or fairly strict diet after a median 10 and 14 years of GFD **(I)**. The results are in contrast to earlier reports from other countries: in an Italian study only 23% of 22 screen-detected adolescents followed a strict diet after 5 year follow-up (Fabiani et al. 2000). In addition, in Ireland only 25 (50%) out of 50 coeliac disease patients with childhood diagnosis adhered to a strict diet 28 years after diagnosis, the reason in 20 of them being that they did not experience any symptoms after gluten intake (O'Leary et al. 2004).

The difference between compliance in the present and earlier studies might depend on the high level of awareness of coeliac disease in Finland. The disorder is common, affecting up to 0.5% of the population (Collin et al. in press). The availability of gluten-free products is rather good in food markets, and in restaurants gluten-free products are often separately labelled, making ordering easy, which may not be the case in other countries. It has been shown that the consumption of gluten-containing foods plays an important role in social aggregation (Addolorato et al. 2001 , Hallert et al. 2003).

Patients in Finland (as in other Scandinavian countries, Ireland and the UK) may decide whether to use a naturally gluten-free or wheat-starch-based diet, which may increase the diversity of the diet, and thus make it easier to follow a strict GFD. For example in Sweden, dietary compliance has been good (Table 4). In addition, the possibility to use oats has also enhanced the diversity of the diet (Peräaho et al. 2004a). However, some patients get intestinal symptoms from

oats and thus do not tolerate it (Peräaho et al. 2004b). Even intestinal damage has been reported to have developed when consuming oats (Lundin et al. 2003).

Coeliac disease patients with depression have been shown to have more dietary lapses than those without it. In these patients, psychological counselling via improvement of depression has resulted in better adherence to GFD (Addolorato et al. 2004a). The psychological well-being in the present series **(I)** was in general good both in screen-detected and symptom-detected patients and comparable to that in Finnish population in general, which may also have contributed to better dietary compliance.

Twenty-eight out of 100 screen-detected coeliac disease patients in study **I** refused to participate. Estimated from hospital records, compliance was similar between participating and non-participating screen-detected patients. Furthermore, in participants the compliance judged from hospital records correlated well to what was eventually found at follow-up. It is thus probable that those patients who did not participate would not have been less compliant than those who consented.

Quality of life and gastrointestinal symptoms

It is noteworthy that the quality of life in treated screen-detected coeliac disease patients remained comparable to that in the treated symptom-detected coeliac disease and non-coeliac control groups. Two earlier studies have shown that quality of life in screen-detected patients was improved (Mustalahti et al. 2002) or remained stable and similar compared to healthy controls (Johnston et al. 2004). The present study showed that quality of life remains also good after long-term GFD treatment.

Previous long-term and controlled studies on treated coeliac disease patients have been restricted mainly to patients who have had classical symptoms of coeliac disease at the time of the diagnosis, and if there has been a minority of patients with screen-detected coeliac disease, the analysis has not been classified on the basis of the clinical picture. In general, the quality of life has been reported to be poorer in treated coeliac disease patients than in non-coeliac controls (Table 5).

Social restrictions and difficulties in adjustment to a chronic disease have been suggested to explain the poorer quality of life in treated coeliac disease (Hallert et al. 1998 , Addolorato et al. 2001 , Fera et al. 2003). However, Usai and associates (2002) showed that quality of life did not differ significantly between adult symptom-detected coeliac disease patients adhering to a strict GFD and non-coeliac controls, whereas coeliac disease patients consuming gluten had significantly lower scores in mental health, social functioning and vitality subdimensions of the SF-36 questionnaire. Hallert and colleagues (1998) reported more gastrointestinal symptoms and lower quality of life in women with long-term treated symptom-detected coeliac disease than in men with coeliac

disease or non-coeliac controls. We found no gender differences, and the treated coeliac disease patients did not experience more gastrointestinal symptoms than non-coeliac controls.

The patients in the present study did not experience more depressive symptoms than non-coeliac controls. On the contrary, patients with screen-detected coeliac disease had significantly better scores in mental health subdimension of SF-36 questionnaire than the general population. There are earlier case reports where depressive symptoms have improved after GFD and anxiety symptoms have alleviated (but not depression) after one year of a GFD (Corvaglia et al 1999, Addolorato et al 2001). In fact, early treatment with GFD might be of benefit in protection from the development from long-term depressive symptoms. A recent Finnish study of 9 adolescents showed low free tryptophan concentrations in untreated patients with coeliac disease and depression, suggesting that serotonergic dysfunction may play a role in the vulnerability to depression among untreated patients. During 6 months GFD tryptophan increased, with a subsequent decrease in depressive symptoms (Pynnönen et al. 2005).

Bone mineral density

The present study was the first to evaluate the BMD in screen-detected coeliac disease after long-term gluten-free dietary treatment. BMD in screen-detected treated patients did not differ significantly from that in the age- and sex-matched population **(I)**. The finding that patients with symptom-detected treated coeliac disease had lower BMD than age- and sex-matched population after long-term treatment suggests that BMD might already have been lower at the diagnosis of coeliac disease; the bone recovery in these patients had not perhaps succeeded as well as it did in the patients with screen-detected treated coeliac disease.

Earlier studies have focused mainly on clinically symptomatic coeliac disease, and in only two studies screen-detected patients have been considered: Mustalahti and associates (1999) showed that lumbar T-score in 19 asymptomatic patients with coeliac disease was -1.9 compared to -1.1 in 21 initially symptomatic coeliac disease patients. During one year GFD, 8 out of 19 asymptomatic patients significantly increased their BMD. In two patients not following a strict GFD, BMD decreased during the follow-up. In a study by a group under Corazza (1996), BMD increased similarly 8% in 8 patients with subclinical and 8 with classical coeliac disease during one year GFD.

Despite the better BMD in patients with screen-detected treated coeliac disease, the occurrence of fractures did not differ between the groups. However, analysis of fracture risk would demand a larger study population to draw any conclusions regarding the fracture risk in patients with screen-detected coeliac disease.

Occurrence of autoimmune diseases and and their relation to gluten-free diet

The present study showed a higher prevalence of manifest autoimmune diseases in coeliac disease patients compared to controls, as reported elsewhere (Collin et al. 2002a). The present controlled trial also demonstrated that subclinical thyroid diseases are found in treated coeliac disease patients when actively sought, and that they are more frequent among coeliac patients than in the non-coeliac control population. Previous studies have mainly concentrated on newly diagnosed coeliac disease cases, children or adolescents, or have not included non-coeliac control groups (Counsell et al. 1994 , Toscano et al. 2000 , Sategna-Guidetti et al. 2001). It is of note that although serum antithyroid antibody titers did not differ between coeliac disease patients and controls, even then coeliac patients were more prone to have a hypoechoid pattern in thyroid ultrasound examination, which is known to precede manifest thyroid disease (Marcocci et al. 1991).

In 1999 a group under Ventura (1999) reported in children that older age at diagnosis of coeliac disease (and thus longer duration of gluten exposure) was related to a greater prevalence of autoimmune diseases, indirectly suggesting that GFD might prevent the development of other autoimmune diseases. However, the evidence concerning the possible effect of GFD on autoimmunity in coeliac disease is contradictory. Furthermore, the studies were mainly limited to children or adolescents (see page 22). Sblattero and colleagues (2006) recently reported a gluten-dependent immunogical response in two cases: intestinal tTG-Ab disappeared during 12 months' GFD in a 35-year-old man with HLA-DQ2-positivity and type-1 diabetes, with a simultaneous improvement in diabetic microalbuminuria. In a 2-year-old HLA-DQ2-positive boy the intestinal tTG-Ab and islet cell antibodies also decreased during GFD.

The present study showed that patients with a coeliac disease diagnosis later in life yielded a hypoechoid finding in thyroid ultrasound examination more often than controls. This finding supports the hypothesis that duration of gluten exposure might have some influence on thyroid function, especially as the age of the patients at the time of the study had no significant effect on echoid pattern. In other words, it seemed that GFD might prevent from the development of subclinical autoimmune thyroid diseases. It is also notable that thyroid ultrasound examination is a more sensitive marker of autoimmune thyroiditis than serum antithyroid antibodies (Gutengust et al. 1989). However, duration of GFD seemed to have no significant effect on the appearance of clinical thyroid diseases.

In the large series of 703 coeliac patients, age at the time of the study was the strongest factor increasing the probability of autoimmune disease. In this series, the time of the diagnosis of autoimmune disease and dietary compliance were taken into account when gluten exposure time was evaluated. One year of gluten exposure time surprisingly reduced the risk of autoimmune disease by

approximately by one per cent (Odds ratio 0.99). Age at diagnosis of coeliac disease, on the other hand, increased the risk by one per cent per year (Odds ratio 1.01) in the whole series, but this was not seen in patients with childhood or adulthood diagnosis when comparing the results separately in univariate analyses. It is of note that 31% of autoimmune diseases were diagnosed after the diagnosis of coeliac disease. Since these 31% had adhered to a strict diet equally to all coeliac patients, the finding would indirectly indicate that GFD seems not to prevent the development of autoimmune diseases. As in a study of 422 adult coeliac disease patients by Sategna-Guidetti and colleagues (2001), the prevalence of manifest autoimmune diseases in the current study did not seem to depend on the duration of gluten exposure, in contrast to Venturas' hypothesis based on childhood diagnosis of coeliac disease.

There are, however, several limitations when connections between autoimmune diseases and gluten exposure are evaluated, as prospective controlled long-term studies are impossible to carry out: gluten exposure time may be overestimated because many autoimmune diseases may be subclinical for years. Similarly, coeliac disease may have been unrecognized for years before the diagnosis. There is also strong dependency between actual gluten exposure time and age at diagnosis of coeliac disease, because the end of follow-up in actual gluten exposure time and age at diagnosis of coeliac disease was often same. This strengthens their implications in multivariate logistic regression analysis. It is also impossible to estimate the amount of ingested gluten in a cross-sectional study, but the amount of ingested gluten in undetected coeliac disease probably equals that in the general population (van Overbeek et al. 1997). The presence of coeliac disease may also increase the physicians' alertness to other underlying autoimmune diseases and vice versa.

Risk of malignant diseases

The overall cancer risk in patients with coeliac disease and dermatitis herpetiformis in the present study remained at the population level. However, the risk of NHL was increased 3-fold in patients with coeliac disease, and in dermatitis herpetiformis the risk was even 6-fold. These figures are in line with recent studies where the relative risk of NHL has varied between 2.7 and 10.3 (Table 7). NHL in both patients with coeliac disease and dermatitis herpetiformis was associated with late diagnosis of coeliac disease or dermatitis herpetiformis and/or poor dietary compliance. Only one dermatitis herpetiformis patient was diagnosed during adolescence, but he continued gluten consumption for 24 years after the diagnosis of dermatitis herpetiformis, which might have predisposed to the development of NHL. The reason why patients with dermatitis herpetiformis developed lymphomas other than EATL, including B-cell lymphomas, is unexplained. The less severe small-bowel mucosal damage in dermatitis

herpetiformis may be the reason for the difference. Nevertheless, NHL no longer appeared in dermatitis herpetiformis patients whose diagnosis was made recently. It is of interest that NHL in patients with dermatitis herpetiformis developed only in those whose diagnosis was made in 1981 or earlier. GFD became a treatment of choice in dermatitis herpetiformis at the end of the 1970s and beginning of the 1980s (Reunala et al. 1977, Fry et al. 1982). Before these patients were treated mainly with dapsone, and the long-term gluten intake may have contributed to the development of these lymphomas.

In contrast to some recent reports (Askling et al. 2002 , Green et al. 2003 , Card et al. 2004 , West et al. 2004a), the occurrence of gastrointestinal cancers other than EATL was not increased in the present study. The earlier reports may be biased in that the association may be seen only in series where mainly coeliac disease with classical symptoms was detected.

Further studies, including the smoking habits of the patients, are needed to confirm that the risk of lung cancer is reduced in coeliac disease and dermatitis herpetiformis, as seen here (SIR 0.6 in coeliac disease and 0.3 in dermatitis herpetiformis). Even though this did not reach statistical significance, the observation is nonetheless of note, because the same finding (SIR 0.4; 95% CI 0.1-1.2 compared to the general population) was made in the UK (West et al. 2004a). The current study did not concentrate on smoking habits of the patients, but it has been reported that patients with coeliac disease and dermatitis herpetiformis smoke less than people in general (Snook et al. 1996 , Lear et al. 1997 , West et al. 2003b). This might explain the lower risk of lung cancer.

There is some epidemiological evidence that GFD might protect from the development of malignant diseases in coeliac disease, since the risk of malignancies has decreased during consecutive years of follow-up (Table 7). In addition, a recent study of 17 children with coeliac disease showed that on a diet the increased frequency of chromosome aberrations in peripheral blood lymphocytes was significantly reduced during 24 months gluten-free. This genomic instability, which may be involved in the development of cancer in coeliac disease patients, seemed to be a secondary and reversible phenomenon, being cured by GFD (Kolacek et al. 2004).

The present study showed that in a high-prevalence area of coeliac disease, where the diagnostics has been augmented and the majority of patients are under GFD treatment **(I, III)** the overall and intestinal cancer risks were not increased. The risk of NHL was associated only with those with continued gluten consumption and to a late diagnosis of coeliac disease. This may speak in favour of the early diagnosis of coeliac disease. In addition, no cancers were diagnosed in children; a similar finding has been made in a Swedish study where patients with childhood diagnosis of coeliac disease did not evince an increased risk of malignancies (Askling et al. 2002).

The overall risk of malignant diseases in coeliac disease seems negligible when patients follow a GFD. However, the risk of malignant diseases in undetected coeliac disease remains unclear. It is also noteworthy that malignancies have developed while patients are on a strict GFD, and not all

patients on a normal diet get cancers. For ethical reasons there is no place for randomized studies to investigate the effect of GFD in the development of malignancies in coeliac disease. Since no other factors except GFD have been found to be protective against the development of malignant conditions in coeliac disease, a strict GFD is also recommendable in this respect.

Mortality

The mortality rate was increased in male patients with coeliac disease, while in females it was at population level. Overall, the mortality rate was somewhat lower than shown in other studies (Table 8). Augmented diagnostics, including the diagnosis of patients with mild symptoms and risk-group screening, may have contributed to better prognosis because it has resulted in a high clinical prevalence and thus a great proportion of the patients are diagnosed and treated. In addition, dietary compliance has been better than in many other countries (Table 4).

Surprisingly, in contrast to coeliac disease, the mortality rate in dermatitis herpetiformis was significantly reduced (SMR 0.52). This low rate was seen in almost all diseases, though not statistically significantly. A similar weaker trend was already seen in 1996 in patients with dermatitis herpetiformis in Finland (Collin et al. 1996a). The lower mortality rate in patients with dermatitis herpetiformis is a subject to further studies.

The mortality rate due to lymphoproliferative diseases was higher in coeliac disease than in dermatitis herpetiformis, possibly because the predominant NHL in coeliac disease was EATL, which is known to have a poor prognosis (Gale et al. 2000). Nevertheless, mortality due to lymphoproliferative malignancies in coeliac disease in the present study (SMR 4.1) was lower than earlier reported: SMR for NHL was 11.4 in Sweden (Peters et al. 2003) and for lymphoproliferative diseases 31 in the UK (Logan et al. 1989).

There was a conspicuous difference between coeliac disease and dermatitis herpetiformis in mortality due to ischaemic heart diseases (SMR 1.6 and 0.7, respectively). In previous studies, mortality due to ischaemic heart diseases was decreased in a study by Whorwell and colleagues (1976) (SMR 0.45) in patients with coeliac disease, but over-representation of mortality due to lymphoma (SMR 25.0) may have influenced the results. Swerdlow and colleagues (1993) reported similar results in mortality due to ischaemic heart diseases in patients with dermatitis herpetiformis (SMR 0.37; 95% CI 0.12-0.86). In addition, West and associates (2004b) showed the relative risk of myocardial infarction to be decreased in 3,590 coeliac disease patients (SIR 0.85; 95% CI 0.63-1.13) . In contrast, mortality due to ischaemic heart diseases was increased (SIR 1.5; 95% CI 1.3-1.8) in a Swedish study of 10,032 hospitalized patients (Peters et al. 2003). The fat intake has been reported to be higher in Finnish coeliac disease

patients than in healthy controls (Kemppainen et al. 1995), which might have some effect on the results in the present series, but we have no data on patients with dermatitis herpetiformis. It has been shown that in untreated coeliac disease serum cholesterol absorption is decreased and cholesterol synthesis increased, GFD causing opposite effects. However, plasma total and low density lipoprotein cholesterol level have not been changed significantly after treatment (Vuoristo and Miettinen 1982, Vuoristo et al. 1993). In fact, serum cholesterol levels have been relatively low in both patients with coeliac disease and dermatitis herpetiformis, even when they have adhered to a GFD (Lear et al. 1997 , West et al. 2003b). In coeliac disease, folic acid malabsorption entails high levels of plasma homocysteine, which again may increase the risk of cardiovascular diseases (Hallert et al. 2002). This mucosal damage and consequently folic acid malabsorption might be milder in dermatitis herpetiformis. Altogether, the above-mentioned issues are speculative and the reasons behind the increased mortality rate in patients with coeliac disease compared to the general population and patients with dermatitis herpetiformis remain unclear.

Summary and Conclusions

This study showed adherence to gluten-free diet to have been very good also in patients with initially asymptomatic or mild coeliac disease after long-term follow-up. However, it has to be taken into account that in earlier studies results have varied considerably depending on the country concerned and this finding can thus not to be generalized to other countries.

One important finding was the good quality of life in patients observing a strict gluten-free diet, and this study was the first to show this also in screen-detected patients with good dietary compliance. Screening of coeliac disease risk-groups might not be justified if it worsens the patients' general well-being. In the current study, only patients with untreated coeliac disease evinced a lower quality of life when compared to all other groups. This further confirms the importance of early detection of patients with undiagnosed coeliac disease.

The finding that bone mineral density in screen-detected long-term treated coeliac disease patients was comparable to the age- and sex-matched population supports the early diagnosis and treatment of coeliac disease, especially when the density in symptom-detected patients did not reach the levels in the age- and sex-matched population after long-term gluten-free dietary treatment. However, the frequency of fractures was similar in patients with screen- and symptom-detected coeliac disease.

The increased prevalence of manifest autoimmune diseases was predictable, and the prevalence of subclinical thyroid diseases was also high despite adequate dietary treatment. The question whether gluten-free diet protects from the development of autoimmune diseases yielded somewhat conflicting results. Patients who had been diagnosed and treated for coeliac disease earlier in life showed less evidence of subclinical thyroid diseases. For manifest autoimmune diseases no such finding was made and in this series it seemed that gluten-free diet does not play a significant role in the development of manifest autoimmune diseases.

The overall cancer risk in the coeliac population was at general population level. In addition, no increased risk of gastrointestinal cancers was detected, as has been the case in earlier studies where mainly symptomatic coeliac disease has been detected. The good dietary compliance in the patients in general might have effected on the results, since no other reasons apart from gluten-free diet have been found to protect from malignant conditions. In addition, there is no proof of increased risk of malignancies in screen-detected coeliac disease. Despite the high clinical prevalence of coeliac disease and the fact that patients generally show good dietary compliance, the occurrence of non-Hodgkin

lymphoma seemed to be increased. It is of note that lymphoma was diagnosed only in coeliac disease patients whose diagnosis of coeliac disease was made at the age of 51 or older and/or dietary compliance was poor. Interestingly, the risk of lung cancer seemed to be reduced, but this needs further confirmation and also information on the smoking habits of the patients.

The mortality rate in patients with coeliac disease was slightly increased during the first years of follow-up but lower than in other studies. Surprisingly, patients with dermatitis herpetiformis had significantly lower mortality rates. The reasons underlying this finding remained obscure and warrant further studies.

There is an ongoing discussion whether coeliac disease should be screened in the population or in risk-groups. According this thesis, this diagnostic approach, where coeliac disease patients with typical, atypical and mild symptoms or totally asymptomatic patients are diagnosed, seems reasonable, since even patients with screen-detected coeliac disease are compliant with dietary treatment and despite this (or because of it) the quality of life is not impaired. Gluten-free diet appears to be beneficial in preventing osteopenia or osteoporosis in screen-detected coeliac disease, even though the occurrence of fractures was not decreased compared to symptom-detected patients. The fracture risk should be studied in larger series before firm conclusions can be drawn. Early diagnosis and treatment might also have influenced the lower cancer rates and better prognosis than in many other countries where the clinical prevalence is lower. Patients with coeliac disease run a significantly increased risk of both manifest and subclinical thyroid diseases. Therefore, thyroid function is recommended to be examined in all coeliac disease patients.

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Is coeliac disease screening in risk groups justified? A fourteen-year follow-up with special focus on compliance and quality of life

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SUMMARY

Background: The benefits of serologic screening for coeliac disease in asymptomatic individuals are debatable.
Aim: To investigate dietary compliance, quality of life and bone mineral density after long-term treatment in coeliac disease patients found by screening in risk groups.
Methods: The study comprised 53 consecutive screen-detected coeliac patients diagnosed 14 years (median) ago. Dietary compliance was assessed by interview, 4-day food record and serology. Quality of life was evaluated by the Psychological General Well-Being and SF-36 questionnaires, gastrointestinal symptoms by the Gastrointestinal Symptom Rating Scale and bone mineral density by dual-energy x-ray absorptiometry. Comparisons were made to 44 symptom-detected-treated coeliac patients, 110 non-coeliac subjects and the general population.
Results: A total of 96% of screen-detected and 93% of symptom-detected coeliac patients adhered to a strict or fairly strict gluten-free diet. In screen-detected patients, quality of life and gastrointestinal symptoms were similar to those in symptom-detected patients or non-coeliac controls and bone mineral density was similar to that in the general population.
Conclusions: Long-term dietary compliance in screen-detected patients was good. Quality of life and bone mineral density were comparable with those in non-coeliac subjects and the general population. Active screening in coeliac disease risk groups seems to be reasonable rather than harmful.

INTRODUCTION

Coeliac disease patients suffering from classical symptoms evidently benefit from a gluten-free diet. Abdominal complaints are alleviated or disappear, and in the long run, the risks of complications such as malabsorption, osteoporosis and small-bowel lymphoma are reduced.

There is evidence that coeliac disease affects up to 1% of the general population.[1, 2] Serologic screening studies have further shown that many patients suffer only minimal if any symptoms. The prevalence of coeliac disease thus remains underestimated, when only patients with classical coeliac disease are recognized.[3–5] Mass-screening for coeliac disease might be the ideal method to identify as many patients as possible. However, evidence that a large majority of patients with apparently asymptomatic coeliac disease would benefit from diagnosis and treatment is sparse. In this respect, the impact of gluten-free dietary treatment on quality of life in coeliac patients without apparent symptoms is of utmost importance. Dietary restrictions may in fact increase the burden of illness and reduce quality of life,[6, 7] and it is conceivable that this might be aggregated in initially asymptomatic patients. Good dietary compliance and improvement in quality of life have been observed in screen-detected

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coeliac disease patients after 1 year of follow-up,[8] but the long-term outcome is obscure. Similarly, long-term dietary compliance in screen-detected patients is uncertain. In a small series in Italy, only a minority of screen-detected adolescents were compliant to diet after 5 years of follow-up.[9] It is thus futile to detect asymptomatic coeliac patients who do not eventually adhere to a gluten-free diet.

The rationale for coeliac disease screening has been discussed[10–12] and so far, no evidence to support population screening has been found. Further studies on the long-term quality of life and dietary compliance in screen-detected patients have seen to be warranted.

Our centre has since 1983 screened for coeliac disease in risk groups such as first-degree relatives of coeliac patients and patients with autoimmune diseases; all detected subjects have been advised to start a gluten-free diet. The aim of this study was to investigate dietary compliance, quality of life, physical well-being and bone mineral density (BMD) in these screen-detected coeliac patients after a median of 14 (range 5–21) years from commencement of gluten-free diet. Comparisons were made to long-term-treated symptom-detected patients, untreated coeliac patients, non-coeliac control subjects and the population in general.

MATERIALS AND METHODS

Patients

The study comprised 100 consecutive patients with screen-detected, biopsy-proven coeliac disease diagnosed between 1983 and 1998 in the Tampere University Hospital area; nine had moved away from the area and 10 were deceased. Mucosal recovery was demonstrated by small bowel biopsy after gluten-withdrawal. Finally, 81 patients were invited to participate. Information on clinical history and dietary compliance was evaluated from hospital records in all 81 before the study. Fifty-three patients consented to participate (women 53%; median age 51 years; range 20–75) and 28 refused (women 36%; median age 42 years; range 18–81).

Comparisons were made to three different control groups. The first comprised 44 randomly selected symptom-detected, biopsy-proven coeliac disease patients (women 77%; median age 50 years; range 29–74), diagnosed between 1984 and 1994 upon abdominal complaints or malabsorption. The second group comprised 54 consecutive untreated coeliac disease patients (women 74%; median age 48 years; range 20–72) and the third 110 persons without known coeliac disease (women 81%, median age 48 years, range 23–87). In addition, results on a standard Finnish population of 2060 subjects (women 58%, mean age 49 ± 17 years, median not known) were available for quality of life measurement by SF-36 health survey questionnaire.[13]

Assessment of dietary compliance

Dietary adherence was assessed in interview by an experienced dietitian, and patients were also asked to complete a 4-day food record. Gluten-free diet was graded as follows: (i) strict; (ii) gluten less often than once per month; (iii) gluten 1–4 times per month; (iv) weekly gluten intake; and (v) normal gluten-containing diet; grades (ii) and (iii) were considered fairly strict in further analysis. Serologic tests were employed to further monitor disease activity: serum IgA class endomysial antibodies (EmA) were determined using an indirect immunofluoresence method with human umbilical cord as substrate,[14] and serum IgA class tissue transglutaminase antibodies (tTg-Ab) by enzyme-linked immunosorbent assay (ELISA) (Celikey; Pharmacia, Uppsala, Sweden); a dilution 1: ≥5 and a unit value ≥5 considered positive, respectively.

Quality of life and gastrointestinal symptoms

Quality of life and gastrointestinal symptoms were evaluated by questionnaires widely used in coeliac disease and with proven validity and reliability. The Psychological General Well-Being (PGWB) questionnaire[15] was used to assess quality of life. It contained 22 items comprising six sub-dimensions: anxiety, depression, well-being, self-control, general health and vitality, scoring being based on a 6-grade Likert scale, higher scores indicating better psychological well-being.

The SF-36 health survey questionnaire was used to assess health-related quality of life.[6,16,17] It was available for screen-detected patients and a Finnish population sample. The raw scores on all 36 items are re-scored from 0 to 100, higher scores indicating better health and quality of life. Items were then divided into eight sub-dimensions: physical function-

ing, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems, and mental health.

Gastrointestinal symptoms were evaluated using the Gastrointestinal Symptom Rating Scale questionnaire (GSRS).[8, 18, 19] This comprises 15 items covering five major gastrointestinal symptoms: diarrhoea, indigestion, constipation, abdominal pain and reflux. Rating is based on a 7-grade Likert scale and higher scores indicate more severe gastrointestinal symptoms.

Clinical evaluation

Body mass index (BMI) was computed as weight/height^2 (kg/m^2). BMI values <18.5 were considered underweight, 18.5–24.9 normal, 25.0–29.9 overweight and >30.0 obese.

Blood haemoglobin (reference values: men 13.0–18.0 g/dL, women 12.0–16.0 g/dL), vitamin B12 (reference values: 170–640 pmol/L) and ionized calcium (reference values: 1.20–1.35 mmol/L) were measured by routine laboratory methods.

Bone mineral density and fractures

Bone mineral density in the lumbar spine and left femoral neck was measured by dual-energy X-ray absorptiometry (Norland XR 26, Norland Corp, Fort Atkinson, WI, USA) according to our standard procedures; the *in vivo* precision of these measurements is about 1%.[20] BMD values were expressed as standard deviation scores, which compare individual BMD values to the mean BMD of sex-matched young adults (T-score) or to the mean BMD age- and sex-matched population (Z score). According to World Health Organisation criteria, T-scores above −1.0 were considered normal, those between −1.0 and −2.4 ostopenia and those equal to or under −2.5 osteoporosis.[21] In addition, the history of fractures was inquired.

Statistics

The data were described using means with 95% confidence intervals or medians with range and lower and upper quartiles. Differences between frequencies were tested using chi-squared test or Fisher's exact test, when needed. A two-tailed P-value ≤ 0.05 was considered significant.

Ethical issues

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

RESULTS

Demographic data on participating coeliac disease patients

The median follow-up after commencement of gluten-free diet was 14 years (range 5–21) in screen-detected and 10 years (range 9–20) in symptom-detected coeliac disease patients. The median age at diagnosis the disease was 39 years both in screen-detected (range 5–67) and in symptom-detected (range 20–64) long-term-treated patients. Nine of the screen-detected patients were diagnosed under the age of 18 years. The indications for screening are depicted in Table 1, the most common being a family history of coeliac disease.

Dietary compliance

After long-term treatment, 96% of screen-detected patients and 93% of symptom-detected patients had adhered to a strict of fairly strict gluten-free diet, $P = 0.66$ (Table 2). Endomysial and tTg-Ab were positive in all those who admitted consuming gluten more than once a week. Two patients had ingested a normal gluten-containing diet, both in the screen-detected group. One subject claimed to have maintained a strict gluten-free diet but had positive antibodies.

Dietary compliance was not associated with the age at diagnosis of coeliac disease, follow-up time, age at time

Table 1. Reasons for screening in 53 patients with screen-detected long-term-treated coeliac disease

Reason for screening	Screen-detected patients *n* (%)
First-degree relative of coeliac disease patient	22 (41.5)
Autoimmune diseases*	10 (18.9)
Rheumatoid diseases and joint pain	10 (18.9)
Atopy and aphtous stomatitis	8 (15.1)
Neurological diseases†	2 (3.8)
Infertility	1 (1.9)

* Type 1 diabetes mellitus, Basedow's disease, IgA glomerulonephritis.
† Ataxia and paresthesiae.

Table 2. Dietary compliance in long-term-treated screen-detected and symptom-detected coeliac disease patients

	Screen-detected patients ($n = 53$)		Symptom-detected patients ($n = 44$)	
	n (%)	EmA and tTg-Ab positivity, n	n (%)	EmA and tTg-Ab positivity, n
Strict gluten-free diet*	44 (83)	1	34 (77)	–
Minor dietary lapses				
Gluten less than once per month	4 (8)	1	1 (2)	–
Gluten 1–4 times per month	3 (6)	–	6 (14)	1
Major dietary lapses				
Gluten several times per week	0 (0)	–	3 (7)	3
Normal gluten-containing diet	2 (4)	2	0 (0)	–

* Strictness of gluten-free diet did not differ significantly between the groups, $P = 0.48$.
EmA = endomysial antibody.
tTg-Ab = tissue transglutaminase antibody.

of study, family history of coeliac disease, gastrointestinal symptoms or quality of life in treated screen-detected or symptom-detected patients.

Quality of life and gastrointestinal symptoms

Quality of life (PGWB) in screen-detected long-term-treated patients did not differ from that in either symptom-detected patients or non-coeliac controls (Figure 1); there were no differences between men and women. In different sub-dimensions of PGWB, screen- and symptom-detected-treated patients did not differ from the general population. Quality of life was not correlated to the length of follow-up (data not shown). In SF-36, the mean value for the mental health sub-dimension was significantly better in screen-detected-treated coeliac disease patients than in the general population; in other items, there was a similar non-significant tendency (Figure 2).

The GSRS total score was lower, indicating fewer gastrointestinal symptoms in treated screen-detected than in symptom-detected coeliac disease patients or in non-coeliac controls, but the difference was not statistically significant (Figure 3).

Untreated coeliac disease patients had more gastrointestinal symptoms and lower quality of life than subjects in other groups on average.

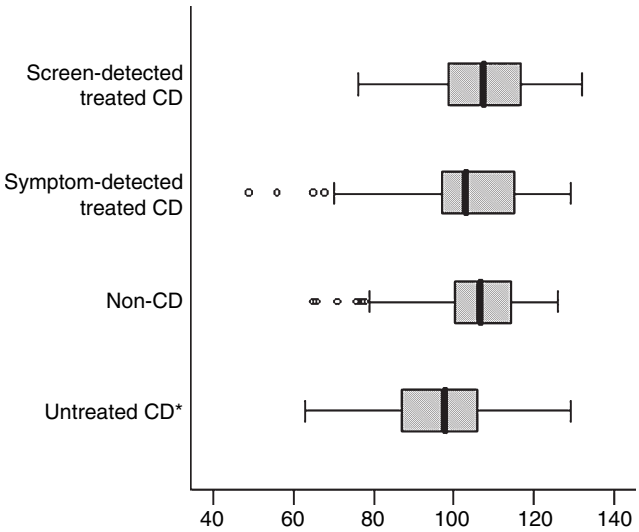

Figure 1. Psychological General Well-Being total scores in patients with screen-detected-treated coeliac disease ($n = 53$), in patients with symptom-detected-treated coeliac disease ($n = 44$), in non-coeliac control group ($n = 110$) and in patients with untreated coeliac disease ($n = 54$); median; lower and upper quartiles; range. * Statistically lower scores in untreated coeliac disease indicating lower quality of life than in treated screen-detected coeliac disease ($P = 0.004$) or non-coeliac control group ($P = 0.004$).

Clinical evaluation

Mean BMI in treated screen-detected coeliac disease patients was 26.6 (range 18.6–46.1) and in symptom-detected 24.6 (range 18.6–34.1). Ten (19%) screen-detected and six (14%) symptom-detected patients were obese ($P = 0.46$). Laboratory results on screen- and symptom-detected patients did not differ significantly: in screen-detected patients, mean blood haemoglobin was 14.6 (95% CI, 14.2–15.0) g/dL in men and 13.2 (95% CI, 12.9–13.5) g/dL in women, and in symptom-detected patients, 15.1 (95% CI, 14.4–15.7) g/dL and 12.8 (95% CI, 12.5–13.2) g/dL,

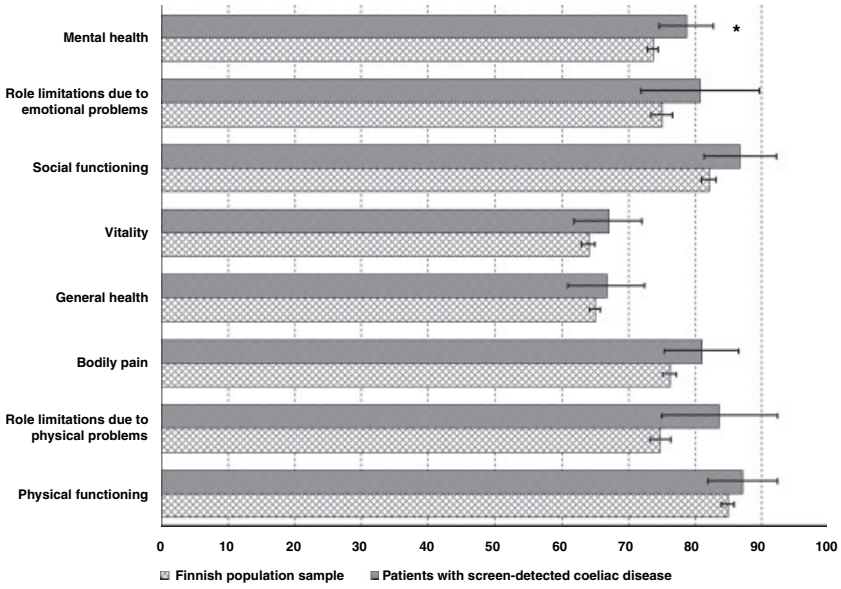

Figure 2. SF-36 scores in patients with screen-detected-treated coeliac disease ($n = 53$) and in a Finnish population sample ($n = 2060$). The scores are given crude and 95% confidence intervals (segment of lines). * Statistically higher score in screen-detected coeliac disease patients than in the population sample, indicating better health-related quality of life.

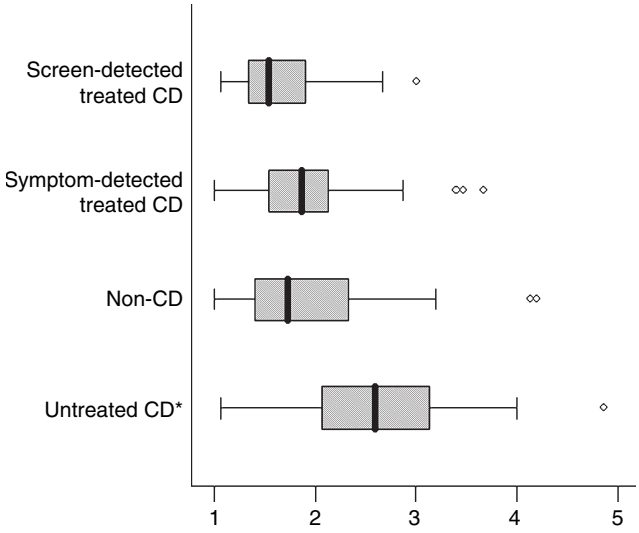

Figure 3. Gastrointestinal Symptom Rating Scale total scores in patients with screen-detected treated coeliac disease ($n = 53$), in patients with symptom-detected-treated coeliac disease ($n = 44$), in non-coeliac control group ($n = 110$) and in patients with untreated coeliac disease ($n = 54$); median; lower and upper quartiles; range. * Statistically higher scores in untreated coeliac disease indicating more gastrointestinal symptoms than in any other group ($P \leq 0.001$ in all comparisons).

respectively; mean vitamin B12 values were 376 (95% CI, 336–415) pmol/L and 456 (95% CI, 380–531) pmol/L, and serum ionized calcium 1.26 (95% CI, 1.25–1.27) mmol/L and 1.25 (95% CI, 1.23–1.26) mmol/L, respectively.

Bone status

Screen-detected-treated patients had somewhat higher Z scores than symptom-detected-treated coeliac disease patients: the mean Z score in the lumbar spine was −0.1 (95% CI, 0.2 to −0.5) and −0.6 (95% CI, −0.3 to −1.0), and in the femoral neck 0.2 (95% CI, 0.5 to −0.1) and −0.2 (95% CI, 0.1 to −0.4), respectively. Osteoporosis was found in 6 (12%) out of 52 screen-detected and 12 (27%) out of 44 symptom-detected-treated patients ($P = 0.05$), whereas 22 (42%) and 20 (45%) had osteopenia, respectively ($P = 0.76$). Seventeen out of 53 (32%) screen-detected and 11 out of 44 (25%) symptom-detected-treated patients had sustained one or more fractures ($P = 0.44$).

Abdominal symptoms at time of diagnosis of coeliac disease in screen-detected-treated coeliac disease group

Twenty-nine (55%) out of 53 screen-detected patients recalled at the follow-up visit that they had been completely asymptomatic, and 24 (45%) had suffered from minor abdominal symptoms before commencing a gluten-free diet. At follow-up, there was no difference in compliance between these two groups; dieting was strict in 79% and in 88%, respectively ($P = 0.49$).

Screen-detected patients who did not participate

Altogether 28 screen-detected coeliac disease patients refused to participate in this study. Coeliac disease was

found at a median age of 36 years (range 7–81) and the median follow-up time was 4 years (range 0–15). Reasons for screening in these non-participating patients were comparable with those in participants.

According to hospital records, 16 (70%) out of 23 non-participating patients were adhering to a strict gluten-free diet, four (17%) used a partial diet and three (13%) were on a normal diet; there were no data on five. The respective results, based analogously on hospital records before enrolment, were for participating patients 43 (81%), eight (15%) and two (4%), respectively.

DISCUSSION

Application of serologic screening in subjects carrying an increased risk of coeliac disease has revealed many patients with only minor if any symptoms.[22] The ultimate question is whether this approach is of benefit or harm. This study gives new information especially with regard to dietary compliance and quality of life.

Good long-term dietary compliance was achievable also in screen-detected coeliac disease patients, irrespective of whether they had initially suffered symptoms. In comparable series, Fabiani *et al.*[9] found much poorer compliance: only 23% of 22 screen-detected adolescents adhered to a strict gluten-free diet after 5-year follow-up. O'Leary *et al.*[17] showed that only 25 (50%) out of 50 coeliac disease patients with childhood diagnosis adhered to a strict diet 28 years after diagnosis, the reason in 20 being that they did not experience any symptoms after gluten intake. Good dietary compliance in the present study was further supported by serological tests, antibodies being positive in a few patients only (Table 2).

Why are our results in conspicuous contrast to these reports? One reason might be that in Finland awareness of coeliac disease is in general good, and the disease is common; there are at least 22 000 people living with this diagnosis; i.e. about 0.4% of the population.[23] Gluten-free products are fairly readily available in food markets and in restaurants gluten-free products are often separately labelled making ordering easy, which perhaps may not be the case in other countries. Indeed, the consumption of gluten-containing foods plays an important role in social aggregation.[24, 25]

Estimated from hospital records, compliance was similar between participating and non-participating screen-detected patients. Furthermore, in participants the compliance judged from hospital records correlated well to what was eventually found at follow-up. We thus assume that those who did not participate would not have been less compliant than those who consented to participate.

Quality of life in screen-detected-treated coeliac disease patients was comparable with that in symptom-detected-treated coeliac disease patients, non-coeliac controls and the Finnish population sample. The results are in accordance with earlier short-term studies: the quality of life in untreated screen-detected coeliac disease improved[8] or remained stable[26] after 1 year of treatment. In this context, it is also noteworthy that a strict gluten-free diet was not detrimental to the quality of life and our treated patients did not experience more depressive symptoms than people in general. This is in contrast with some other studies for instance in Sweden and in Italy.[6, 25, 27] Depression should be considered, as it has been the main reason for poor compliance in chronic diseases.[28] Addolorato *et al.*[29] showed that psychological support therapy in coeliac patients resulted in the recovery of depression; this again was associated to better dietary compliance. The good compliance and the absence of depressive symptoms in our patients may thus depend on each other. Nevertheless, serologic screening in risk groups and subsequent dieting did not increase the burden of illness, which might well have been possible.[7]

We found that the BMD in screen-detected long-term-treated coeliac disease patients was comparable with that in the population of the same age and gender (Z scores). There is some evidence that in untreated screen-detected patients BMD is decreased, and improves on a gluten-free diet.[30, 31] Our cross-sectional data thus support the conclusion that active serologic screening for coeliac disease and subsequent fairly strict adherence to diet are beneficial in preventing osteopenia or osteoporosis. Another argument for active screening for coeliac disease might be the prevention of small-intestinal malignancies, although there is no direct evidence that asymptomatic patients with untreated coeliac disease are exposed to an increased risk of lymphoma.[32]

It is important to note that the present study concentrated on coeliac patients who were detected by screening in risk groups, where the likelihood of coeliac disease was approximately five times higher than in the population in general.[33] Cost-benefit studies are certainly needed in coeliac screening, whether directed to risk groups or to unselected individuals. For

comparison, however, in irritable bowel disease active screening for coeliac disease would appear to be justified when the likelihood of the condition is about 5% or more.[34]

In conclusion, we found excellent dietary compliance in our screen-detected coeliac patients after long-term treatment. Quality of life was comparable with that in symptom-detected-treated coeliac patients or, despite obvious dietary restrictions, even to that in the population. Against this background, active screening for coeliac disease in risk groups seems to be reasonable.

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ORIGINAL ARTICLE

Coeliac Disease, autoimmune diseases and gluten exposure

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Abstract

Objective. Gluten-free diet treatment has been proposed to prevent the development of autoimmune diseases in coeliac subjects. The aim here was to investigate the occurrence of autoimmune disorders in relation to gluten intake in coeliac patients in a well-defined area. ***Material and methods***. The frequency of autoimmune disorders was evaluated in 703 adults and children with coeliac disease and in 299 controls with normal duodenal histology. Incidence figures were given per 10,000 person-years. In logistic regression analysis, where the prevalence of autoimmune disorders was a dependent variable, the effect of age at end of follow-up, age at diagnosis of coeliac disease, actual gluten exposure time, gender and diagnostic delay were assessed. ***Results***. The prevalence of autoimmune diseases was significantly higher in coeliac subjects than in controls. In logistic regression analysis, age at end of follow-up, age at diagnosis of coeliac disease and female gender increased the risk of autoimmune disorders, whereas actual gluten exposure time reduced the risk; diagnostic delay had no effect. A similar, though not statistically significant, trend was seen in childhood coeliac disease to that in the whole study group. ***Conclusions***. Despite that fact that patients with coeliac disease are at increased risk of various autoimmune conditions, the duration of gluten exposure seems not to be of crucial importance in the development of autoimmune diseases.

Key Words: *Autoimmune disorders, coeliac disease, gluten-free diet*

Introduction

Coeliac disease is a gluten-dependent disorder causing small-bowel mucosal inflammation, villous atrophy and crypt hyperplasia in genetically affected individuals. Its association with other autoimmune diseases has been well established in many cross-sectional studies [1–4].

Ventura et al. [5] were the first to propose that gluten load might be involved in the pathogenesis of autoimmune disorders in coeliac disease; the longer children and adolescents had used a gluten-containing diet before the diagnosis of coeliac disease, the more autoimmune diseases they contracted later in life. Furthermore, the same group showed in an uncontrolled study that serum insulin-related antibodies disappeared and anti-thyroid antibodies decreased in coeliac children during a gluten-free diet [6]. The protective effect of a gluten-free diet has, however, been disputed. Sategna-Guidetti et al. [7,8] reported that gluten withdrawal did not protect against the development of autoimmune diseases in adults. Results presented by Biagi et al. [9] were consistent with those of Sategna-Guidetti et al. Clearly, confirmatory studies of the effect of gluten-free diet are also needed in adults; the issue is important in considering the benefits of population screening in coeliac disease.

During recent decades the recognition of coeliac disease has improved [10] and many of the so-called associated diseases have turned out to be fortuitous. In our area, the clinical prevalence of coeliac disease was 1:300 as recently as 10 years ago, which equals that in some population screening studies [11,12]. The follow-up of our patients has been a long one, and precise information on the occurrence of associated diseases has been available, thus enabling us to calculate the incidences of autoimmune diseases

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in a single centre. The aim of the present study was to establish the frequency of autoimmune diseases in coeliac subjects and to find out how these diseases develop in relation to gluten intake.

Material and methods

Patients and controls

Altogether 703 consecutive biopsy-proven coeliac disease patients were enrolled in the study, the cohort comprising both adults and children. The diagnosis of coeliac disease had been made in Tampere University Hospital between 1963 and 2000. The control group consisted of 299 consecutive patients suffering from heartburn or dyspepsia and undergoing upper gastrointestinal endoscopy at primary care; coeliac disease was not suspected in the controls, and the condition was excluded by small-bowel biopsy in each case. The total follow-up time was 31,746 patient-years in coeliac disease and 16,712 in controls.

Autoimmune diseases

The occurrence of autoimmune disorders was established by reviewing patients' records in all cases. In addition, a structured questionnaire was mailed to the coeliac disease patients. Questions inquired about all possible diseases and the time of diagnosis. The following diseases were elicited separately: insulin-dependent diabetes mellitus (IDDM), autoimmune thyroid diseases comprising autoimmune hypothyroidism and hyperthyroidism, Addison's disease and primary Sjögren's syndrome. The patients' records were again scrutinized to ensure that the diagnosis of autoimmune disease had been made appropriately. The current diagnostic criteria were applied in all diseases [13–21]. Where a patient failed to return the questionnaire, information was based on patient records provided reliable information was available. In the case of controls, medical history and structured questionnaire were taken at the time of endoscopy; further information was obtained from patients' records.

Dietary assessment

In coeliac disease patients, adherence to the gluten-free diet was evaluated from the questionnaire and patients' records. The diet was classified as strict (no dietary lapses), partial (regular dietary lapses at least weekly) or normal gluten-containing diet. Endpoints for actual gluten exposure time were specified as follows: (i) to commencement of strict gluten-free diet; (ii) to the diagnosis of an autoimmune disorder in cases where autoimmune diseases occurred before adoption of a strict gluten-free diet; (iii) to the end of follow-up in cases where patients did not adhere to a strict gluten-free diet and autoimmune disease did not develop. If there was no recovery in control biopsy or the antibodies remained positive, we considered this equal to gluten exposure, even if the patients announced having a strict gluten-free diet.

Statistical analyses

The incidence calculations were based on cases fulfilling the criteria for autoimmune diseases. The incidences were resolved by calculating the ratio of the number of new autoimmune cases arising in the study populations during the follow-up time to the total person-years at risk during that time [22]. Incidence figures were given per 10,000 person-years. For calculations, Stata 7.0 statistical software (Stata Corporation, Tex., USA) was used. Standard error and 95% confidence intervals (CIs) were calculated using Wilson's method as recommended by Altman et al. [23]. Differences between frequencies were tested using Fisher's exact test or the χ^2 test, when needed. A two-tailed p-value ≤ 0.05 was considered significant. In logistic regression analysis, where the presence of autoimmune disorders was set as a dependent variable, the effect of gender, age at end of follow-up, age at diagnosis of coeliac disease, actual gluten exposure time and diagnostic delay, that is the time from the onset of apparent symptoms to the diagnosis of coeliac disease, were analysed using the univariate and multivariate approaches. Multivariate analysis was applied using the forward stepwise method to take confounding variables into account. The analyses were also made separately for children and adults. A probability of 0.05 was used for stepwise entry of variables and 0.10 for removal of variables.

Results

The demographic data on coeliac disease patients and controls are presented in Table I. Sixty-one percent of coeliac disease patients returned the questionnaire. Autoimmune diseases were found in 153 (21.8%) out of 703 individuals with coeliac disease and in 32 (10.7%) out of 299 controls ($p < 0.001$). In the coeliac group, 31% of autoimmune diseases were detected subsequent to the diagnosis of coeliac disease; of these 83% had adhered to a strict gluten-free diet, compared with 78% in the whole series.

The incidence rate per 10,000 patient-years for all autoimmune disorders was significantly higher in coeliac disease patients than in controls. When

Table I. Demographic data on 703 coeliac disease patients and 299 non-coeliac controls.

	Coeliac disease $n=703$	Controls $n=299$
Female (%)	478 (68)	200 (67)
Age at end of follow-up, median (range); years	46.5 (2–88)	58.0 (14–86)
Age at diagnosis of coeliac disease, median (range); years	38.2 (0.3–84)	
Strictness of gluten-free diet; %		
Strict	77.7	–
Partial	11.8	–
Gluten-containing diet	6.4	100.0
Not known	4.2	–

different autoimmune conditions were observed, a significant difference was seen for IDDM and Sjögren's syndrome; the same trend was seen throughout other autoimmune conditions, though not statistically significantly (Table II).

In a univariate logistic regression model concerning the whole study population, age at end of follow-up and age at diagnosis of coeliac disease increased the odds ratio for developing autoimmune disease. Actual gluten exposure seemed to reduce the ratio. Gender or diagnostic delay had no significant effect. In controls, neither gender nor age at end of follow-up influenced the probability of autoimmune disease (Table III). A similar trend was also seen within different age groups (Table IV).

In the initial multivariate logistic regression analysis, information on diagnostic delay in coeliac disease patients was available in 439 out of 703 cases, and the delay did not influence the probability of having an autoimmune disease. The final multivariate logistic analysis was therefore also carried out without diagnostic delay, which made it possible to include all 703 patients.

In the final multivariate analysis, age at end of follow-up was the strongest indicator favouring the presence of autoimmune disease in coeliac subjects (Table V). Age at diagnosis of coeliac disease and female gender also increased the probability. Again, actual gluten exposure time seemed to reduce the probability.

Discussion

The overall prevalence of autoimmune disorders was higher in coeliac subjects than in non-coeliac controls, as has also been shown earlier [4,24–26]. The incidence of autoimmune disorders per 10,000 patient-years was similarly increased, but statistically significantly only in IDDM and Sjögren's syndrome. However, the same trend was evident in virtually each autoimmune disorder (Table II).

Extraintestinal manifestations are common in coeliac disease, and these should be distinguished from associated conditions. We did not include dermatitis herpetiformis in the present study: dermatitis herpetiformis is a gluten-dependent manifestation: it needs gluten to develop and the rash heals when a gluten-free diet is followed [27]. Osteopenia and dental enamel defects are additional examples of extraintestinal manifestations or complications of coeliac disease [28–31]. The autoimmune conditions listed in Table II are, in our opinion, clearly

Table II. The incidence of autoimmune diseases in coeliac disease patients and in non-coeliac controls.

	Incidence per 10,000 person-years			
	Coeliac disease	95% CI	Non-coeliac controls	95% CI
All*	52.2	44.6–61.1	19.7	13.9–27.9
IDDM*	12.6	9–17.2	0.6	0.1–4.3
Autoimmune thyroid disease	17.6	13.5–22.9	12.8	8.3–19.6
Hypothyroidism	11.4	8.2–15.8	9.0	5.4–15.0
Hyperthyroidism	6.0	3.8–9.5	3.6	1.6–8.0
Sjögren's syndrome*	7.3	4.8–11.0	0.6	0.1–4.2
Rheumatoid arthritis	5.4	3.4–8.7	4.8	2.4–9.7
Psoriasis	4.1	2.4–7.1	0.6	0.1–4.2
Sarcoidosis	3.5	1.9–6.3	0.0	0.0–2.2
IgAglomerulonephritis	1.9	0.9–4.2	0.0	0.0–2.2
Sacroiliitis	1.6	0.7–3.8	0.6	0.1–4.3
Systemic lupus erythematosus	1.3	0.5–3.4	0.0	0.0–2.2
Scleroderma	0.9	0.3–2.9	0.0	0.0–2.2
Alopecia areata	0.9	0.3–2.9	0.0	0.0–2.2
Addison's disease	0.6	0.2–2.5	0.0	0.0–2.2
Vasculitis	0.3	0.0–2.2	0.0	0.0–2.2

Abbreviations: CI = confidence interval; IDDM = insulin-dependent diabetes mellitus.
*Statistically significant difference between coeliac disease patients and controls.

Table III. Univariate logistic regression analysis of different variables as predictors of the presence of autoimmune disease in coeliac disease patients and in non-coeliac controls.

	Coeliac disease patients			Non-coeliac controls		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age at end of follow-up	1.01	1.00–1.02	0.005	1.02	1.00–1.10	0.060
Gender (male reference = 1)	1.25	0.84–1.85	0.204	2.33	0.86–5.88	0.084
Actual gluten exposure time	0.99	0.98–1.00	0.021	–	–	–
Age at diagnosis of coeliac disease	1.01	1.00–1.02	0.027	–	–	–
Diagnostic delay	1.00	0.98–1.02	0.813	–	–	–

Abbreviations: OR = odds ratio; CI = confidence interval.

associated conditions and not extraintestinal manifestations of coeliac disease.

An intriguing hypothesis is that the gluten load might be involved in the development of autoimmune diseases in coeliac disease. Apart from Ventura et al. [5,6], other investigators have supported such a hypothesis. Di Mario et al. [32] showed that 27% of children with untreated coeliac disease had anti-insulin antibodies, compared with 20% of treated coeliac children and none of the controls. Similarly, Toscano et al. [33] observed that antibodies against thyroid peroxidase and other endocrine-related autoantibodies were present in 53% of adolescents with untreated coeliac disease and 20% of adolescents with treated coeliac disease; the titres were higher in untreated disease. There are also case reports where a gluten-free diet has induced hair growth in concomitant coeliac disease and alopecia areata [34,35], and where the diet has reversed severe liver dysfunction [36]. A body of evidence shows that immunological mechanisms are implicated in the pathogenesis of coeliac disease [37]. Coeliac disease and many other autoimmune disorders share a common genetic predisposition, i.e. HLA DQ2 or DQ8. In addition, environmental conditions may be important in association with coeliac disease and other autoimmune disorders, gluten being a likely candidate for such a factor, since it is the only antigen known to be involved in the pathogenesis of coeliac disease.

The enzyme tissue transglutaminase is the predominant autoantigen in coeliac disease. Tissue transglutaminase deamidates gliadin peptides and thus facilitates the binding of peptides to the peptic groove of HLA DQ2 molecules in antigen-presenting cells [38]. This again results in increased T-cell activity and a local inflammatory response. The association between gluten exposure and autoimmune disorders might be due to molecular mimicry, whereby gliadin or tissue transglutaminase activates T cells, which are cross-reactive with various organ-specific self-antigens. The production of neo-epitopes might also enhance the development of other autoimmune conditions in coeliac disease [37]; in fact, intermolecular T-cell epitope spreading has been demonstrated in animal models of autoimmune diseases [39].

How does the present study from a single centre elucidate the hypothesis of Ventura et al. [5]? In our study, as many as 31% of the autoimmune diseases developed after the diagnosis of coeliac disease. Since this 31% adhered to a strict gluten-free diet equally to all coeliac cases, our finding would indirectly indicate that a gluten-free diet would not prevent the development of autoimmune conditions. On the contrary, a longer actual gluten exposure time seemed to be associated with a decreased risk of

Table IV. Univariate logistic regression analysis of different variables as predictors of the presence of autoimmune disease in subgroups of coeliac disease patients.

	Patients with coeliac disease diagnosed under 10 years of age		Patients with coeliac disease diagnosed between 10 to 20 years of age		Patients with coeliac disease diagnosed over 20 years of age	
	n = 96		*n* = 68		*n* = 539	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Actual gluten exposure time	0.84 (0.69–1.02)	0.071	0.59 (0.43–0.82)	0.002	0.96 (0.95–0.98)	<0.001
Age at end of follow-up	1.04 (0.84–1.29)	0.699	0.87 (0.69–1.10)	0.247	1.00 (0.99–1.02)	0.712
Age at diagnosis of coeliac disease	1.05 (0.97–1.1)	0.206	0.96 (0.86–1.06)	0.413	1.01 (1.00–1.02)	0.220
Gender (male reference = 1)	1.10 (0.32–3.70)	0.876	1.72 (0.49–6.25)	0.392	1.19 (0.76–1.89)	0.439

Table V. Multivariate logistic regression analysis, in which the effect of different variables has been evaluated as predictive of the presence of autoimmune disease in coeliac disease patients.

	Coeliac disease patients, $n=703$		
	Odds ratio	95% CI	p-value
Age at end of follow-up	1.06	1.02–1.10	0.001
Actual gluten exposure time	0.39	0.27–0.56	<0.001
Age at diagnosis of coeliac disease	2.45	1.70–3.52	<0.001
Gender (male reference = 1)	1.83	1.05–3.18	0.033

Abbreviations: OR = odds ratio; CI = confidence interval.

autoimmune disease. In the whole study population, one year of gluten exposure time decreased the risk of autoimmune disease by approximately 1% (odds ratio of 0.99), provided that the decrease is linear, which is not necessarily the case. Even in childhood, the age at diagnosis of coeliac disease or the time of gluten exposure did not have an effect on the risk of autoimmune diseases.

Actual gluten exposure is difficult to interpret. The exposure time might be overestimated, because many autoimmune diseases may be subclinical for several years. Similarly, coeliac disease may have been unrecognized for years before the diagnosis. There is also a strong dependency between actual gluten exposure time and age at diagnosis of coeliac disease, because the end of gluten exposure is usually equal to age at diagnosis of coeliac disease. This connection strengthens their implications in multivariate analysis. Nevertheless, the low odds ratio for gluten exposure indicates that the exposure is not of major significance in the development of autoimmune diseases in coeliac disease; at least in our population, where an augmented diagnostic approach in risk groups results in the finding of subclinical and mild coeliac disease cases.

Altogether, our study does not support the hypothesis that gluten exposure might be involved in the occurrence of autoimmune conditions in coeliac disease. On the other hand, as no prospective controlled long-term studies have been reported, and will probably never be carried out, there is room for criticism.

First, it is not possible to estimate the amount of ingested gluten in the long-term in a cross-sectional study. There is, however, evidence to show that the daily intake of gluten in undetected coeliac disease does not differ from that in the population in general [40].

Secondly, the present study did not include subclinical autoimmune diseases, which are also more frequent in coeliac disease patients than in non-coeliac controls [41,42]. We do not know the time of onset of the autoimmune condition, and the prior gluten exposure period is hence overestimated when extended to the time of clinical diagnosis. A third potential bias is that the presence of coeliac disease may increase the physicians' alertness to other underlying autoimmune conditions and vice versa.

The gluten exposure time in adults with coeliac disease may have been too long to prevent the development of autoimmune diseases. There may also be differences in the immune system between children and adults [43]. As to the development of autoimmune diseases, we did not find any differences between children and adults when comparing the results in univariate analyses. In addition, the evidence does not support the conception that individuals in whom coeliac disease has been detected in adulthood have had the disease all their lives [43].

To conclude, the results of this study suggest that gluten exposure does not increase the risk of autoimmune diseases in subjects with coeliac, notwithstanding that the patients run an increased risk of various autoimmune conditions.

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