
**THE PREVALENCE OF COELIAC
DISEASE IN “AT-RISK” GROUPS IN
THE AUSTRALIAN POPULATION**

By

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"All truths are easy to understand once they are discovered; the point is
to discover them."

Galileo Galilei (1564-1642)

Abstract

Introduction: Coeliac disease (CD) is an immune mediated condition caused by the ingestion of gluten-containing grains in genetically susceptible individuals. Over the last 10 to 15 years a plethora of information on CD has emerged which has enabled us to better understand the genetics, pathogenesis, epidemiology and vast spectra of clinical manifestations of this disease. We now know CD to be a highly protean disease, with a prevalence of approximately 1:300, making it one of the world's most common diseases. Despite this wealth of information CD is still significantly under diagnosed, even in subsets of patients that have been shown to be at increased risk of having the disease.

Aim: To assess the prevalence of CD in couples undergoing In-vitro fertilization, patients with diabetes mellitus type 1 (DM-1), subjects with decreased bone mineral density (BMD) and patients with low trauma fractures.

Methods: Subjects with infertility, osteopaenia/osteoporosis, low trauma fractures and diabetes mellitus type 1 were serologically screened for the presence of coeliac antibodies. Patients in these cohorts found to have biopsy proven CD were enrolled in long-term follow up studies to determine what symptoms the patient had on presentation, and the effect of a gluten free diet (GFD) over a 12 month period.

Results: The prevalence of CD was: 0.25% in subjects undergoing IVF treatment, 3.11% in patients with DM-1, 2.52% in patients with low BMD and 0.55% in patients with low trauma fractures. Twelve month follow up showed that the initiation of a

GFD had many beneficial effects, such as increases in blood iron levels and BMD and resulted in the abrogation of many gastrointestinal symptoms.

Conclusion: CD is a highly prevalent disease. Specific subsets of patients show an increased prevalence of the disease and as such, routine serological screening for coeliac antibodies should be performed in these groups of patients.

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Preface

Except where indicated the work described in this thesis was carried out personally by the author between March 2003 and March 2006.

Presentations arising from this research include:

- Presentation- **Wienholt L**, Hooper MJ, Kean AM, Conigrave A, Loblay R & Williams AJ. **Estimating the Prevalence of Coeliac Disease in Osteodeficient Patients**. The Australian & New Zealand Bone & Mineral Society 2005 Annual Scientific Meeting, Perth, Australia
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The work presented here is original and has not been presented for the purpose of obtaining any other degree.

Louise Anne Wienholt

BSc (Biomedical)

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Abbreviations

BMI- body mass index

BMB- bone mineral density

CD- coeliac disease

DM-1- Diabetes Mellitus Type 1

ELISA - enzyme linked immunosorbent assay

EMA - endomysial antibodies

ESPGAN- European Society of Pediatric Gastroenterology, Hepatology and Nutrition

Gli A- Gliadin IgA

Gli G- Gliadin IgG

GFD - gluten free diet

HLA - human leukocyte antigen

IgA - immunoglobulin A

IgG - immunoglobulin G

IgM - immunoglobulin M

IVF- *invitro* fertilisation

MMP - matrix metalloproteinase

Th-1 – T helper cell type 1

Th-2 – T helper cell type 2

TNF α – tumour necrosis factor α

tTG – tissue transglutaminase

WHO- The World Health Organistaion

CHAPTER

1

INTRODUCTION AND AIMS

Definition

Coeliac disease (CD) is an enteropathy characterised by a T cell mediated inflammation of the upper small intestine resulting from the ingestion of gluten-containing grains in genetically susceptible individuals. Also known as coeliac sprue and gluten-sensitive enteropathy, CD results in malabsorption from partial or total villous atrophy of the small bowel which usually improves with the implementation of a gluten free diet.

History

Human beings did not ingest grains until approximately 10 000 years ago, when the first cultivation of grain and cereal began in areas now covering modern Turkey, Iraq and Iran (Rostami K 2004).

Cultivation initially consisted of non-gluten containing grains such as rice, however the expansion of farming civilizations throughout Europe brought with it the previously unknown grains of wheat, barley, rye and oats, as well as the knowledge of how to use these grains to make flour. These grains were selectively bred and propagated throughout time to yield the best flour, which subsequently increased the gluten component (Feldman M 1981), culminating in the grains we ingest today.

The first descriptions of CD go as far back as the second century AD when a Roman physician termed the disease “koiliakos”, a Greek word meaning suffering in the bowels (Rostami K 2004). In modern times the first medical description of CD was published in London in 1888 when Samuel Gee described a disease affecting

predominantly children with a type of “chronic indigestion”, resulting in a variety of gastrointestinal complaints (Gee S 1888). However, it was not until the 1950’s that Dickey, a Dutch pediatrician, linked these symptoms to gluten-containing grains after a cereal shortage during the Second World War (Dickey W 1950).

Grains

The ingestion of gluten has been shown to be the environmental stimulus and essential factor required for the pathogenesis of CD. Gluten, a protein derived from wheat, is a heterogeneous mixture of gliadins and glutenins (Koning F 2003) and is closely related to proteins found in cereals from the Triticeae family which includes barley and rye (Hogberg 2004) (figure 1).

GRAMINEAE (POACEAE)
THE GRASS FAMILY

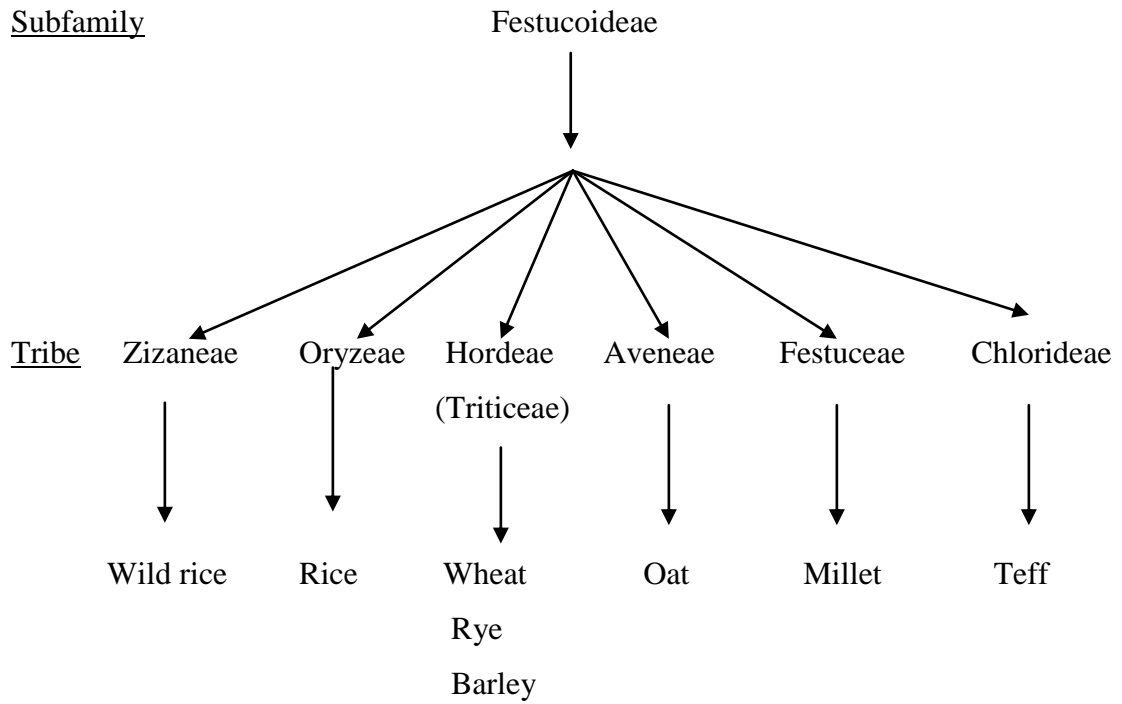


Figure 1: Taxonomic classification of Festucoideae subfamily from (Kasarda)

The immune response of CD is directed against the alcohol soluble fraction of gluten, known as gliadin in wheat, hordein in barley and secalin in rye (Fasano A 2001; Barr GD 1998).

A number of non Triticeae cereals have been identified as being safe for coeliac consumption, including rice and millet, however the toxicity of oats in CD patients has been debated for years. In the earliest work associating CD with gluten, it was recommended that wheat, rye, barley and oats be completely excluded from the diet in order to obtain a complete recovery (Dickey W 1950). This traditional approach to

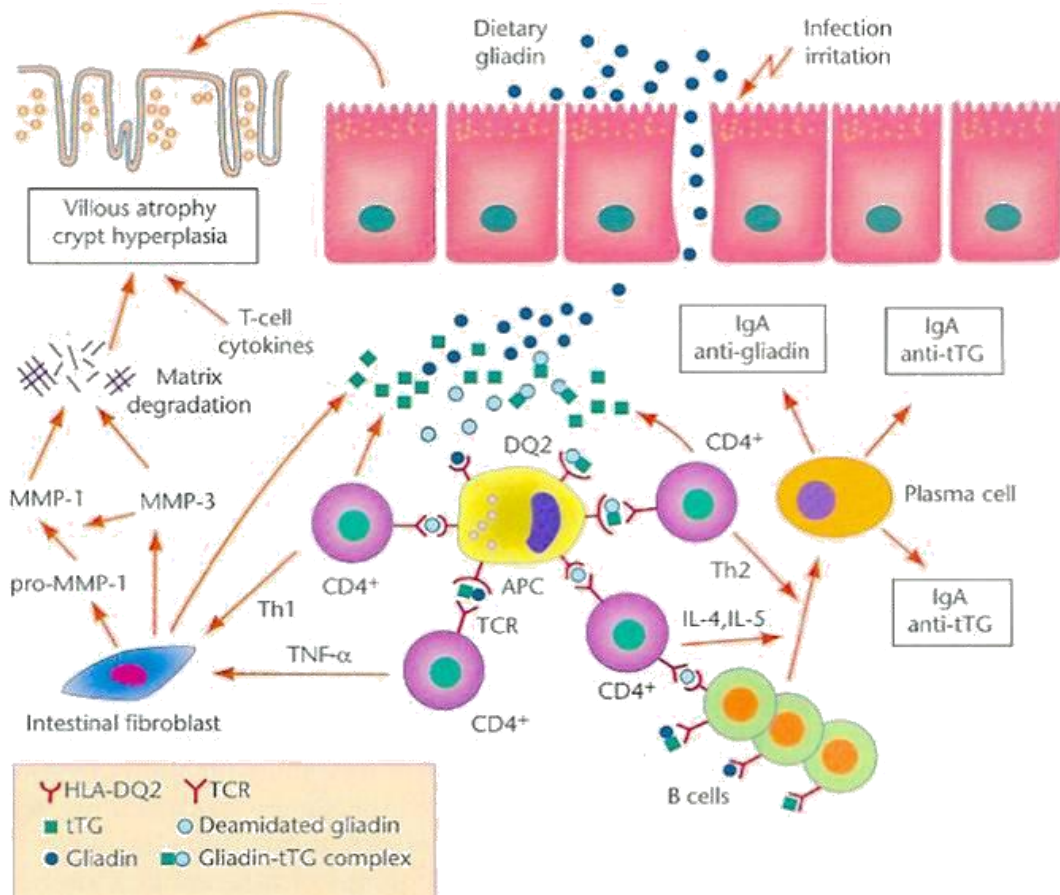
the dietary management of CD has been followed until recently when the value of excluding oats was questioned. Recent studies have shown that significantly large amounts of oats are well tolerated in most adults and children with CD without any nutritional, morphological or serological adverse effect (Storsrud S 2003; Hogberg 2004). It is likely that the perceived “toxicity” of oats stems from the fact that there is considerable scope for contamination with gluten-containing grains, as many oat products are harvested, milled and produced in close proximity to wheat, barley and/or rye. It remains important to ensure that oats are completely free of these contaminants to be safe for consumption by CD patients.

Pathogenesis and Genetic Associations

CD is a genetically complex multifactorial disease, which is evident from both the spectrum of clinical manifestations and the age of onset. Some individuals once exposed to gluten will display gastrointestinal symptoms within weeks or months, while others will take years to exhibit any clinical manifestations, or remain apparently asymptomatic. This spectrum is reflective of both genetic and environmental factors associated with CD. The initiation of the disease occurs through a poorly understood mechanism of gluten peptide crossing the intestinal barrier. It is hypothesized that mechanisms such as infection, injury and other inflammatory responses may cause increased epithelia permeability. Once across the intestinal barrier, the gliadin peptide component of gluten remains in an immunogenic state, resulting in an immune response and an up-regulation of peptides and proteins normally involved in controlling gut permeability (Fasano A 2001), allowing the

facilitation of further gliadin absorption. Recent studies have targeted a number of proteins including ZO-1, zonulin and occludin as playing key roles in increasing intestinal permeability in CD patients (Pizzuti D 2004). The gliadin component that initiates the inflammatory response to gluten has been identified as a 33-mer peptide found in wheat, rye and barley which is resistant to enzymatic breakdown by brush border proteases (Shan L 2002). Once absorbed into the lamina propria this peptide is then exposed to the ubiquitous, predominantly cytoplasmic enzyme tissue transglutaminase (tTG) (Mowat A.M 2003). This enzyme catalyses deamidation of the peptide to form glutamic acid and ammonia (Koning F 2005). This modification greatly enhances the potential for the peptide to be bound to MHC class II molecules, however is not necessarily essential for T cell activation (Dewar D 2004). The modified peptide is then presented to CD4+ T cells in association with human leukocyte antigen (HLA) class II molecules, expressed on antigen presenting cells (such as macrophages and dendritic cells), stimulating a T cell mediated immune response (Figure 2 from Mowat A.M 2003).

tumour necrosis factor α (TNF α) which is a powerful inducer of matrix metalloproteinase (MMP) expression and activator of intestinal fibroblasts (Schuppan D 1998) ultimately leading to the lymphocytic infiltration of the small bowel lamina propria and matrix breakdown leading to villous atrophy (figure 3). The Th-2 response results in the activation of B-cells and the production of "bystander" immunoglobulin G (IgG) and immunoglobulin A (IgA) antibodies against B cell epitopes on gliadin, transglutaminase and other detectable autoantigens (Schuppan D 1998). These autoantibodies inhibit epithelial cell differentiation and increase epithelial cell proliferation, ultimately leading to crypt hyperplasia (Halttunen T 1999). These autoantigens have also been implicated in many of the extra-intestinal manifestations of CD (Korponay-Szabo I.R 2004) (figure 3).



rob Crimi

Figure 3: T cell response to ingested gluten, showing T cell activation through T cell receptors (TCR) recognising gliadin peptides presented by HLA-DQ2 on antigen presenting cells (APC). This elicits a Th-1 and Th-2 response with release of cytokines. Th-1 cytokines induce intestinal fibroblasts to release MMP 1 and 3 that degrade fibular collagen, matrix glycoproteins and proteoglycans, resulting in villous atrophy. A Th-2 response promotes B-cell maturation and expansion of plasma cells that produce antibodies against gliadin, tTG and gliadin-tTG complexes (Schuppan D 1998).

Genetic predisposition has a strong influence on the pathogenesis of this disease with upwards of 90% of CD patients carrying the HLA-DQ2 heterodimer on their HLA class II cells (Agrawal S 2000; Karell K 2003), while the remainder usually express HLA-DQ8 (Dewar D 2004). In contrast only 25-30% of the general population has

the HLA-DQ2 allele (Dewar D 2004). The HLA-DQ2 heterodimer is encoded by the DQA1*501 and DQB1*02 alleles which can exist in the cis or trans position (Karell K 2002), while the HLA-DQ8 is encoded by the DQA1*03 and DQB1*0302 alleles. While HLA-DQ2 predisposes individuals to be at-risk for developing CD, it plays no role in determining the clinical symptoms or time of onset of the disease (Mustalahti K 2002), suggesting that other genetic and environmental factors must influence the vast array of individual disease expression.

In addition to HLA-DQ2/ DQ8 there have been a numerous other genetic studies linking CD to other loci including HLA-DR53 (Clot F 1999) , the CELIAC# region on chromosome 2q33 (Amundsen S.S 2004), gene regions on chromosome 9p21-13 and 6q25.3 (van Belzen M.J 2004), regions 2q23-32 and 6p (Rioux J.D 2004), 18q, 3p and 5p (Neuhausen S.L 2002) and 5q31-33 (Babron M.C 2003).

Considering this strong genetic component it is not surprising that relatives of CD patients have a particularly high prevalence of the disease (Fasano A 2001; Hervonen 2002; Fasano A 2003). Studies have shown as high as 21.3% of siblings, 14.7% of offspring, 17.2 % of first-degree relatives and 19.5% of second degree relatives of CD patients are also affected (Book L 2003).

While genetics clearly play a critical role in the pathogenesis of this disease, there must also be significant environmental factors considering concordance amongst identical twins is only 60 to 70% (Feighery C 1999).

Epidemiology

Over the last 2 decades the reported prevalence of CD has exponentially increased, aided by the introduction of serological screening methods. In the 1980's CD was thought to occur in 1 in 1000 at the highest (Feighery C 1999), however it is now evident that CD is far more common.

Once thought to have wide geographical variation (Askling 2002), areas such as the United States of America where CD was once thought to be a rarity have now been shown to have an exceedingly common, but neglected coeliac population (Fasano A 2001; Fasano A 2003).

The disease has been shown to have a female predominance (Hoffenberg E.J 2003), with a twofold greater risk in females compared with males (Ivarsson A 2003; Ciclitira P.J 2002).

Initially thought to be strongly associated with Caucasians, there have been numerous reports of a high prevalence of CD in Indian, South African and other Asian countries (Butterworth J.R 2005). Additionally in Middle Eastern countries where wheat cultivation originated, and still constitutes a major part of the staple diet, studies are being conducted to establish the prevalence of CD. In countries such as Iran and Israel the prevalence of the disease is close to 1:150 (0.67%), which is even more common than the Australia population where CD has been shown to affect 1 in 251 (0.3%) (Hovell 2001), a prevalence similar to many European countries as well as America. With reports of high prevalence in Indians, Pakistanis, Blacks, Arabs, Sudanese, Cubans, Mexicans, Brazilians and Asians (Lebenthal E 2002), it is clear that CD is now emerging as a pandemic global problem.

Using screening data, Fasano et al (Fasano A 2001) determined that CD has a world wide prevalence of approximately 1:300, however clinical diagnosis remained at about 1:3000, meaning that currently 9 out of every 10 people with CD remain undiagnosed. This information has led to the creation of “The Coeliac iceberg” model (see figure 4). The prevalence of CD can be seen as the iceberg as a whole with the majority of CD patients being “submerged” or undiagnosed, with only the minority of clinically diagnosed patients, most of which exhibit the classical symptoms of the disease, representing the visible peak. It can also be surmised that the “water-line” (the ratio of diagnosed to undiagnosed cases) is affected by such issues as the awareness of the disease, understanding of the protean nature of the disease and the availability of diagnostic tools.

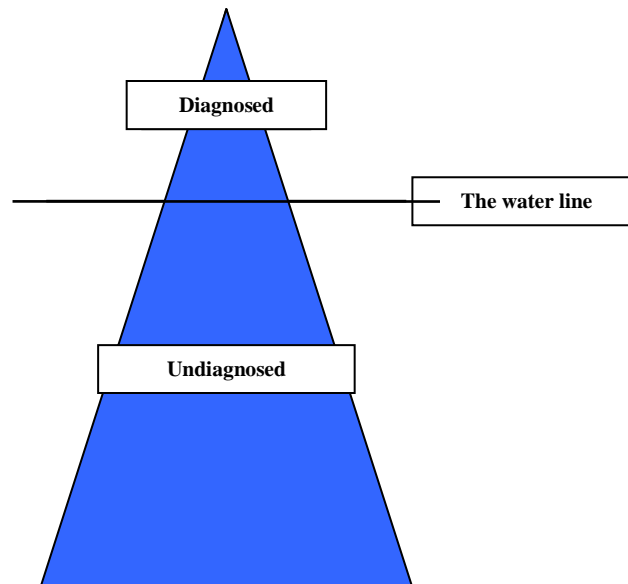


Figure 4- The CD iceberg model. Modified from Fasano (2001)

Determination of the true prevalence of CD has undoubtedly been made possible by the introduction of serological screening methods which are minimally invasive, cost effective and readily applied to mass populations. Utilising these methods, numerous studies have shown CD to be one of the world's most common lifelong diseases.

Diagnosis

The original diagnostic criteria for CD involved 3 small bowel biopsies. The initial biopsy was performed when there was clinical suspicion of CD, looking for the classical abnormal morphology associated with the disease. The second biopsy was performed after the commencement of a gluten free diet (GFD) in order to show histological recovery of the mucosa. The third biopsy was performed after a gluten challenge to demonstrate the return of mucosal abnormality. Since the introduction of sensitive and specific serological tests, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGAN) have amended the consensus criteria for a definite diagnosis of CD. A biopsy demonstrating the typical manifestations of CD with clinical and/or histological recovery after the commencement of a GFD is now considered sufficient evidence for a definite diagnosis of CD (Collin P 2002; Farrell R.K 2001), without a formal gluten challenge.

Serological screening

The diagnosis of CD was traditionally reliant on the presentation of gastrointestinal symptoms, and a subsequent small bowel biopsy showing typical histological changes that improved after withdrawal of gluten from the diet.

This style of diagnosis has changed dramatically over the last 30 years with the development of sensitive and specific serological assays for the detection of antibodies associated with CD.

Total IgA

Serological screening methods for CD are predominantly immunoglobulin A (IgA) class assays, as the vast majority of CD patients produce IgA auto-antibodies.

Patients with IgA deficiency are however not resistant to the development of CD and can develop immunoglobulin G (IgG) coeliac antibodies.

Two to three percent of biopsy proven CD patients are IgA deficient, with positive IgG antibodies (Green P.H 2003), additionally IgA deficiency is 10 to 15 times more common in patients with CD than the normal population (Kumar V 2002).

Additionally children under the age of 2 who have not yet developed IgA may also develop IgG coeliac antibodies.

As such it is important to know the subject's IgA status to ensure effective serological testing for CD.

Anti-reticulin Antibodies

Anti-reticulin antibodies were the first described autoantibody associated with CD. Detected using polycovalent conjugate immunofluorescence on rat liver, kidney and stomach, anti-reticulin antibodies includes 5 subtypes, only 3 of which are related to CD (Damoiseaux JGMC 2002). This assay is highly reliant on subjective assessment, and polycovalent testing means that interfering IgG and IgM antibodies may mask reticulin staining. The sensitivity of the assay is quite low (53 to 92%), but the specificity is close to 100% (Damoiseaux JGMC 2002). This assay has now been replaced by more sensitive, less subjective methods of antibody testing.

Gliadin Antibodies

Like reticulin antibodies, anti-gliadin IgA and IgG antibodies were initially detected using an indirect immunofluorescence method, however they are now detected using an enzyme linked immunosorbent assay (ELISA) method.

Since the development of this assay there has been widespread controversy regarding both the sensitivity and specificity of this test. Modern data has shown this assay has a sensitivity of 52-100% and 57-96%, and a specificity of 84-100% and 74-92% for gliadin IgA and IgG respectively (Wong RC 2003). Low sensitivity is predominantly due to the fact that anti-gliadin antibodies can yield false positive results in the presence of other gastrointestinal disorders such as lactose intolerance and parasite infections (Fasano A 2001).

Whilst gliadin antibodies have frequently been replaced by the more sensitive and specific endomysial and tissue transglutaminase antibodies, there has been recent interest in the development of modified gliadin peptide substrates which has shown

greatly enhanced sensitivity and specificity (Schwertz E 2004). Subsequently this assay may once again become a widely used as a diagnostic tool.

Endomysial Antibodies

The discovery of endomysial antibodies (EMA) was based on the knowledge of the reticulin antibodies and thus is also detected using indirect immunofluorescence, usually on monkey oesophagus substrate. This assay has been shown to have higher sensitivity and specificity than gliadin antibodies, with a sensitivity of 84-100%, and a specificity of 94-100% (Wong RC 2003).

Despite the generally high sensitivity and specificity of this assay there are a number of limitations including the reliance of the assay on subjective operator assessment and thus interpretive resulting. In addition there is also the ethical consideration of using monkey smooth muscle (Baudon JJ 2004), as well as the qualitative nature of the assay which limits the usefulness of follow-up testing. Additionally the majority of commercially available kits for EMA can only detect IgA antibodies and thus the assay is not useful for children under the age of 2 years or in IgA deficient patients. EMA kits which are dual for IgA and IgG are usually associated with many problems due to non-specific IgG binding.

Tissue Transglutaminase

Tissue transglutaminase (tTG) has recently been discovered to be the autoantigen responsible for the EMA pattern (Dieterich W 1998) which has resulted in the development of commercially available IgA tTG ELISA kits for its detection (McPherson 2001). Initially these kits were made using guinea pig tissue that was

cheaper than EMA testing, however they were significantly less sensitive (Dieterich W 1998; Hill I.D 2003). Guinea pig substrate has now been replaced by a human recombinant substrate, which has a diagnostic accuracy similar to that of EMA, sensitivity 66-100% including guinea pig tTG, 91-97% using human antigen and a specificity of 75-100% (Wong R.C 2003; Van Meensel B 2004).

ELISA based testing means that tTG lacks many of the technical difficulties associated with EMAs, with the additional benefit of a quantitative resulting (Dieterich W 1998; Bürgin-Wolff A 2002; Trevisiol C 2002; Miller A 1999; Trevisiol C 2002; West J 2002), meaning tTG may be the best method for monitoring dietary compliance to a GFD (Reeves G.E.M 2000), however this area still needs further investigation.

Despite the perceived benefits of this assay, there has been reports of false positive tTG results in patients with other autoimmune and connective tissue disorders, especially arthritis (Picarelli A 2003; Bizzaro N 2003) and primary biliary cirrhosis (Bizzaro N 2003) as well as reports of low titre false positive results (Kotze L.M 2003; Lock RJ 1999) which may be interference from high levels of monoclonal and polyclonal IgA (Hill 2004).

Like EMA, the tTG test is usually against IgA antibodies, and may obtain false negative results in IgA deficient subjects. In these cases there are immunoglobulin G (IgG) tTG ELISAs available that accurately test for the presence of IgG antibodies (Cataldo F 2000).

On the basis of all markers of CD it is apparent that the best method of serological screening may be a combination of serological tests (Shamir R 2002; Russo P.A 1999).

Other Testing Methods

Smooth muscle antibodies that are specific to f-actin (anti-actin IgA antibodies) have also been described in patients with CD, as well as in numerous other diseases including autoimmune hepatitis, liver disease, viral hepatitis and other connective tissue diseases (Chretien-Leprince P 2005). Despite the apparent low specificity of these antibodies, they have been shown to be highly predictive of the severity of intestinal damage in untreated coeliacs (Granito A 2004). Despite limited application as a diagnostic test, anti-actin antibodies may be useful as a serological means of monitoring histological recovery of CD patients on a GFD.

In addition to serological markers of disease there has been a push in recent years to create a more mobile form of testing for CD that could be administered at sites such as a general practice with instantaneous results. Human tTG dot blots which require one drop of whole blood and give an instantaneous result (Fasano A 2001; Baldas V 2000) and methods of Sorbitol H₂-Breath Tests (Tursi A 2002) have been reported, however considering the need for specialized interpretation of results, as well as ensuring adequate quality control in testing, supplementary forms of analysis are still prospective.

HLA Studies

As discussed, 90-95% of patients with CD carry the HLA-DQ2 heterodimer, encoded by a gene located on the short arm of chromosome 6 (van Heel D.A 2005). This has led to the relatively new development of typing for this heterodimer as a predictor of the likelihood of CD. The merits of this testing are yet to be fully explored,

considering 30-35% of the normal population have this heterodimer without disease, it is likely that this test is only useful as a negative indicator of disease.

Despite this, there are situations in which the HLA status of the patient could be useful for refining diagnosis, including equivocal small bowel biopsies, and patients considered to be at risk of developing CD, such as first degree relatives.

The methodology of HLA typing as a whole has traditionally been difficult due to the large potential for polymorphic variation in individuals. A number of methods are used for the detection of specific HLA regions, the most common of which is a polymerase chain reaction (PCR) based method, however new advances such as the use of capillary-based genetic analysers (Turner D.M 1999) may improve the reliability and reproducibility of this testing.

Despite its limited use as a diagnostic tool, understanding the role and structure of HLA-DQ2, HLA-DQ8 and the pathogenic region of gluten has enabled this junction and interaction to be targeted as an area for future antigen specific immunotherapy (Kim C.Y 2004; Anderson R.P 2000).

Small Bowel Biopsy

In 1969 the small bowel biopsy was introduced as the gold standard for the diagnosis of CD (Meuwisse 1970). Despite the development of serological screening methods the histological examination of a small bowel biopsy from the duodenum and upper jejunum regions remains the benchmark for CD diagnosis (Fasano A 2001). A small bowel biopsy should be performed in all cases of positive coeliac serology or if clinical suspicion of the disease is high, even if serology is negative (Fasano A 2001).

For the diagnosis of CD, a small biopsy (usually obtained by an endoscopy method) is taken from multiple sites, mounted on slides and examined for features including villous atrophy, crypt hyperplasia and increased numbers of intraepithelial lymphocytes, which are usually graded according to the Marsh classifications. Marsh was the first to fully describe and categorize the histological changes associated with progressive CD (Marsh M 1992). Marsh classifications range from the first signs of histological change described as 'preinfiltrative mucosa' with slightly increased intraepithelial lymphocytes (stage 0), to lamina propria infiltration with lymphocytes (stage 1), crypt hyperplasia (stage 2), villous atrophy (stage 3), and finally total mucosal atrophy (stage 4). Marsh classifications have now been broadened to include more descriptive subcategories, especially for Marsh stage 3 (Oberhuber G 1999). Examples of a typical histological change versus a normal control are shown in figure 5, and changes according to Marsh classifications shown in figure 6.

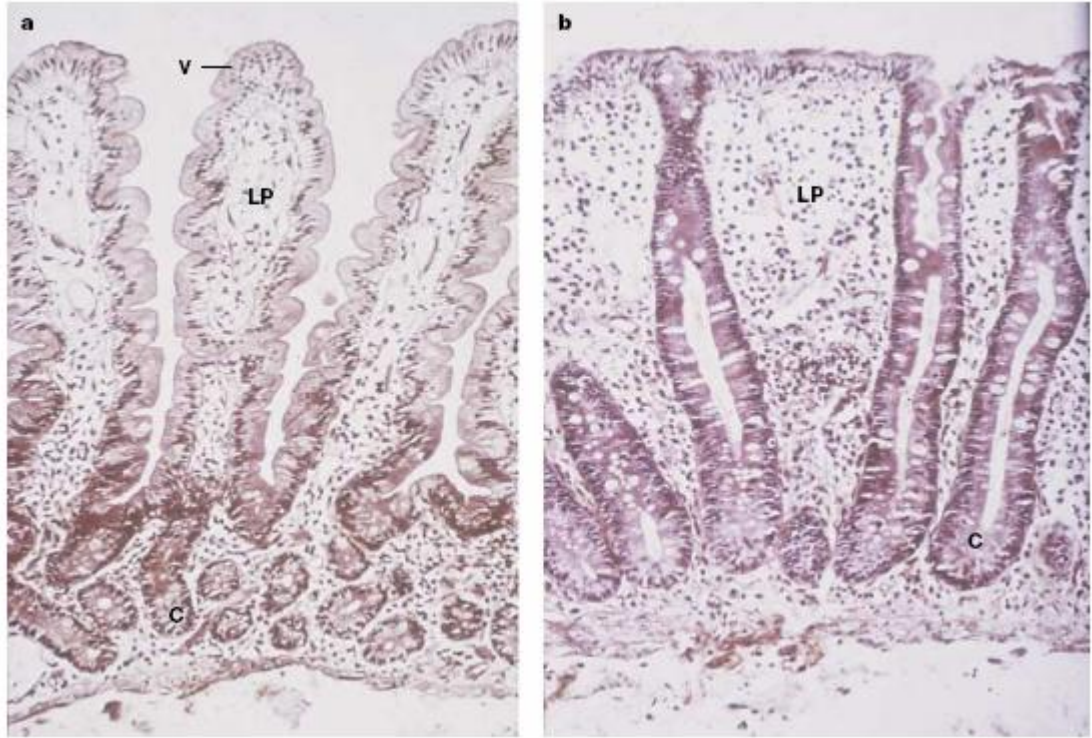


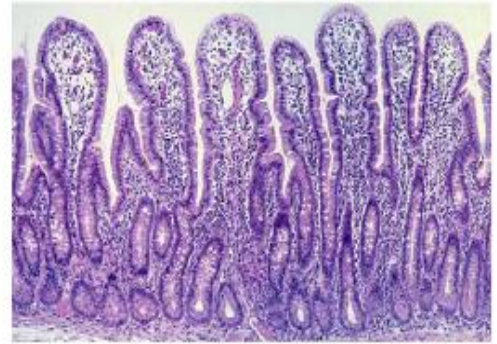
Figure 5: **a**-Normal small intestine

b- Small intestine of Coeliac Patient

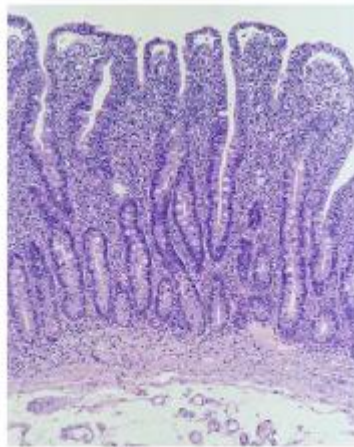
Showing changes in villous (**V**) and crypts (**C**) in the lamina propria (**LP**) and well as lymphocyte infiltration (from Sollid et al, 2002 (Sollid LM 2002)).



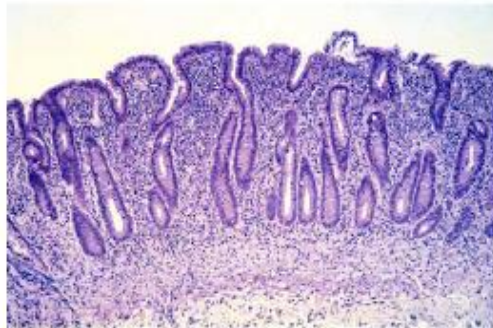
Marsh I: lymphocytic enteritis



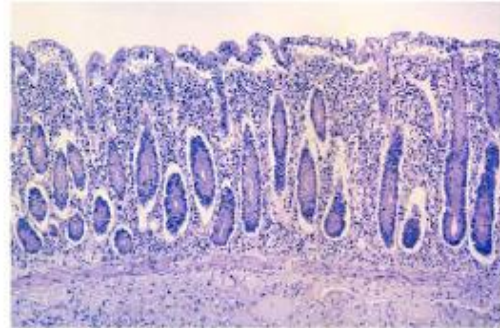
Marsh II: lymphocytic enteritis with crypt hyperplasia



Marsh III A: partial villous atrophy



Marsh III B: subtotal villous atrophy



Marsh III C: total villous atrophy

Figure 6: Histology showing the spectrum of intestinal damage according to Marsh classifications.

Modified from Green et al (Green P.H.R 2005).

Despite a biopsy being the gold standard in diagnosis, there have been reports of non coeliac patients with morphology consistent with CD. These patients show no improvement on a GFD and have a normal repeat biopsy in the continued presence of gluten (Goldstein N.S 2004). These subjects are very uncommon and are thought to have some form of viral induced immune response.

Treatment

Permanent dietary withdrawal of all products containing wheat, barley and rye, known as a gluten free diet (GFD) is the only treatment currently available for CD.

While there are studies currently under way to develop new and less restrictive therapeutic options such as oral therapies to enhance the degradation of gluten in the gut making it less pathogenic, as well as methods of interfering with T cell mediated responses to gluten and modifying cytokine release (Sollid L.M 2005).

Methods of genetic wheat modification to reduce the toxicity of gluten have also been studied (Pogna 2002), however as a number of proteins (include those from barley and rye) have been identified as having immunogenic potential, this may not prove to be a viable option.

Long term compliance to a GFD should be monitored both serologically and histologically for improvement, however recovery is often slow and mucosal damage may take months or even years to fully recover (Feighery C 1999).

The importance of a GFD in CD patients has been well validated, not only as an effective means of reducing symptoms (Murray 2004) but also in terms of improved quality of life and reduced morbidity (Usai P 2002). There are many determining

factors that correlate with a patient's level of compliance to a GFD and subsequently their clinical improvement. Some of these factors include Coeliac Society membership, knowledge of food labeling, access to gluten-free food products and physician and dietetic care and follow up (Butterworth J.R 2004).

There are a small number of patients who appear to be unresponsive to a gluten free diet, termed refractory CD. The most common cause of the continued perpetuation of symptoms is likely to be continued gluten ingestion, which can be voluntary or inadvertent (Fasano A 2001). Other causes must however be considered including other dietary intolerances, lymphoma and small intestinal bacterial overgrowth.

There is also debate as to the "safe" level of gluten that a patient may ingest before there is a physiological effect and whether a low gluten as apposed to no gluten diet may be sufficient enough to prevent symptoms (Fasano A 2001). It appears that this along with many other aspects of CD is related to individual response.

The spectrum of Coeliac Disease

Classical CD

CD was traditionally thought to only occur in children displaying gastrointestinal symptoms. While gastrointestinal symptoms such as abdominal pain, bloating, diarrhoea, abdominal distension, vomiting and constipation still present in clinically overt cases, these symptoms are absent in the majority of CD patients. Additionally it is now evident that this disease is not confined to the pediatric population, with an increasing number of patients diagnosed in adulthood and at least 20% of cases being diagnosed in patients over the age of 60 years (Farrell R.J 2002).

The classical presentation of CD is most abundant in the pediatric population, typically in infants less than two years of age who present with gastrointestinal symptoms together with “failure to thrive” (Feighery C 1999; Fasano A 2001). Malabsorption symptoms are also associated with the classical presentation of CD, with iron deficiency anaemia, hypoalbuminaemia, hypocalcaemia and other vitamin deficiencies reported (Fasano A 2001).

Silent/latent CD

As the awareness of atypical symptoms of CD grows, other groups of CD patients are also being recognized, such as patients deemed to have silent or latent CD. Silent CD relates to patients who have both coeliac antibodies as well as biopsy proven disease in the absence of symptoms. While once considered a rarity it is now evident that these patients may in fact represent a large proportion of CD patients (Tommasini A 2004), being 7 to 15 times more common than symptomatic CD (Cerf-Bensussan N 2003) and accounting for the huge number of patients who fall into the “submerged” region of the “coeliac iceberg”. This group of patients are perhaps the most medically challenging to treat of all CD patients. Often their diagnosis is an incidental finding, and in the absence of symptoms the merit of the induction of a GFD is questionable and often burdensome to patients who have no incentive to adhere to a strict GFD, and as such the compliance rate to a GFD in this group of patients is extremely low (Fabiani E 2000).

There is speculation that these patients are not truly asymptomatic but rather affected by subtle ailments such as fatigue, behavioural disturbances and reduced bone mineral densities (Fasano A 2001). In long term follow up studies, many patients who

appeared asymptomatic reported some form of improvement after commencing a GFD (Fabiani E 2000).

Latent or potential CD refers to patients who have coeliac antibodies on serological screening, but have a normal intestinal biopsy. Treatment is often confined to monitoring the patient for the development of gastrointestinal involvement. These patients have been the focus of much recent research with emphasis on whether they will ultimately develop clinical CD. Evidence to support development to biopsy positive disease includes the fact that a large proportion of relatives of biopsy proven CD patients have coeliac antibodies but not the histological change associated with CD (Collin P 2002). Additionally some patients with CD antibodies and normal biopsies have submicroscopic abnormalities of microvilli which are only detectable using electron microscopy (Sbarbati A 2003). Other reasons for serological-histological discrepancies include: drugs such as corticosteroids which may normalize gut appearance, sampling/analysis issues, self imposed gluten free diet and a false positive serological result (Reeves G.E.M 2004).

There are also individuals who have gluten allergy in juxtaposition to CD. Rather than an IgA mediated disease, gluten allergy is an IgE mediated condition, with the absence of gastrointestinal histology associated with CD. There is often confusion between the two disorders especially in the absence of comprehensive medical diagnosis.

Associated Disorders and Complications

Despite being a disease of the gastrointestinal tract, there is continuing and mounting evidence that presentation of CD is far more protean and complex than previously considered, with upwards of 50% of patients now being diagnosed without classical symptoms (Fasano A 2001). Serological studies as well as an abundance of case reports have linked CD with a plethora of other diseases and conditions, and in some instances completely asymptomatic disease. While the prevalence of CD continues to grow it can be surmised that many associations may simply be chance findings or ascertainment bias, however many large modern studies have shown that there appears to be a real interaction between CD and other ailments separate to the classical presentation, some of which are explored below.

Dermatitis herpetiformis

Dermatitis herpetiformis is a blistering skin condition considered to be a variant form of CD. In some patients skin involvement appears to be the only manifestation of the disease, with no evidence of associated antibodies and a normal bowel biopsy (Smecuol E 2005), however this appears to be associated with the severity of the skin condition. In severe cases of dermatitis herpetiformis coeliac antibodies and a small bowel biopsy consistent with CD were found in almost 100% of patients (Fasano A 2001). In this condition gastrointestinal symptoms are unusual, with the main feature being a rash most commonly on the knees, elbow and buttocks (Fasano A 2001). Both the skin condition and mucosal damage clear after the commencement of a gluten free diet.

Cancer

The reported incidence of various cancers amongst patients with CD is highly varied, with reported rates of malignancy as high as 21% in untreated CD (Holmes GK 1976), however this figure is highly dependant on the type of neoplasm as well as the method of patient recruitment.

The mostly commonly associated cancer is T-cell small bowel lymphoma, however numerous case reports and small studies can be found linking CD patients with jejunal adenocarcinoma (Amodeo C 2002), small bowel adenoma (Rampertab SD 2003) and small bowel adenocarcinoma (Rampertab SD 2003).

In one of the largest studies to date Askling et al (Askling 2002) demonstrated that adult patients alone have a slightly elevated risk (1.3 fold) for malignant lymphomas and carcinomas of the small intestine, large intestine, oropharyngeal, oesophageus, hepatobiliary and pancreas. This has been correlated in a number of other studies that have also found almost no increase in the risk of lymphoma in CD patients (Card T.R 2004; Farre C 2004).

A study by Catassi et al (2002) found a 3.1 fold increase in the incidence of non Hodgkin's lymphoma (especially gut lymphomas) in untreated CD patients compared with the general population. A Spanish study showed that CD is not a risk factor for lymphoma (Farre C 2004), however it has been established that morphology consistent with CD may be missed in the presence of lymphoma, reducing the apparent prevalence (Green P.H 2004).

The apparent decrease in malignancy rates seen in more recent studies may be reflective of the introduction of serological screening methods, meaning patients are being diagnosed earlier, before severe gastrointestinal symptoms commence which

may reduce the likelihood of cancer developing. It appears that patients with severe symptoms that require hospitalization are the most likely to develop cancer (Asking 2002).

While there is still much debate as to the exact cancer risk in CD, it is evident that subjects adhering to a strict gluten free diet have a mortality rate similar to that of the general population (Logan R.F 1990).

Neurological

It is believed that neurological disturbances in CD patients result from immune mediated cerebella damage (Ghezzi A 1997), and has been termed gluten ataxia (Farrell R.K 2001). Ataxia is the most commonly reported neurological manifestation of CD and may present as the only symptom (Farrell R.K 2001). The prevalence of CD in idiopathic cerebella ataxia has been reported to be as high as 12.5%, 20 times the expected incidence (Pellecchia M.T 1999). Similarly up to 16% of patients with neurological illness of an unknown cause were found to have raised coeliac antibodies (Hadjivassiliou M 1996), however this study was based on small patient numbers, without confirmatory biopsies. Other studies have failed to replicate this association (Combarros O 2000).

Another commonly associated neurological aberration reported with CD is epilepsy (Luostarinen 2000), with the prevalence of CD as high as 1 in 44 in patients attending a seizure clinic (Cronin C.C 1998), however a recent large study using tTG and EMA antibodies found no increase of CD in patients with primary generalized epilepsy (Ranua J 2005).

CD has also been associated with autism, however recent large studies show no significant increase in the prevalence of CD in autistic children (Black C 2002). Numerous other neurological symptoms have also been linked to CD, mostly via published case reports. These case associations are wide spread and include a vast spectrum of symptoms and diseases such as: Noonan syndrome (Amoroso A 2003), white brain matter abnormalities (Hadjivassiliou M 2001), increases in axonal neuropathies (Luostarinen L 2003), depression (Ciacci C 1998), Ramasy Hunt Syndrome (Chinnery P.F 1997) and migraine (Gabrielli M 2003).

In addition to this there are also a number of case reports linking schizophrenia and CD (De Santis A 1997), however any real association appears unlikely with no increased prevalence of CD in schizophrenic patients in controlled studies (Peleg R 2004).

Due to small population studies, and the often broad diagnosis of many neurological anomalies, it remains unclear whether there is a true association between CD and neurological disease.

Other Gastrointestinal Disorders

In addition to the classical gastrointestinal symptoms characteristic of CD there have been numerous associations and presentations of CD with other gastrointestinal manifestations. Impaired gallbladder, stomach and small bowel motility have all been described in CD patients, all with links to decreased secretion of intestinal hormones or reduced sensitivity to these hormones (Fraquelli M 2003). Anecdotal case reports of intestinal obstruction (Koklu S 2004) have also been published.

Additionally there are numerous reports of other gastrointestinal diseases being misdiagnosed in the place of CD including inflammatory bowel disease and irritable bowel syndrome (Cash B.D 2002). Studies have shown that up to 11.4% of patients diagnosed with irritable bowel syndrome actually have CD (Shahbazkhani B 2003), all of which improve after the initiation of a gluten free diet. Clinical diagnosis of these two diseases can often be difficult due to the fact that irritable bowel syndrome and CD have many common symptoms, and often have similar diagnostic findings such as anti-saccharomyces cerevisiae antibodies (ASCA) and perinuclear cytoplasmic antibodies (pANCA), which are also features of inflammatory bowel disease (Damoiseaux J.G.M.C 2002). Subsequently serological screening for CD should be performed in all patients diagnosed with irritable or inflammatory bowel syndromes.

Other gastrointestinal disorders such as parasite infection may also present with symptoms resembling CD and have been shown to be associated with false positive serology, especially gliadin antibodies as is the case with the parasite *Helicobacter pylori* (Fasano A 2001).

Diabetes Mellitus Type 1

Type 1 diabetes (DM-1) is a chronic immune disorder with varying degrees of insulin deficiency resulting from an immune mediated destruction of pancreatic cells (Lazarotto F 2003). Numerous studies have been completed to assess the prevalence of CD in populations of patients with DM-1, with results showing wide geographical variation. The highest rate of CD in DM-1 patients has been reported in West Algeria, where 16.4% of children and adolescents were found to be CD positive (Ashabani A

2003) and the lowest, 2.4% in Iranian children (Shahbazkhani B 2004). Most studies have found rates of CD in DM-1 children to be from 0.97 to 10.3% (Holmes 2001; Barera G 2002; Hanukoglu A 2003; Ashabani A 2003; Spiekerkoetter U 2002). The rate of CD in children with DM-1 in the Australian population has been shown to be 8.4% (Doolan A 2005). DM-1 adults have been shown to have a prevalence of 1.3 to 6.4% (Holmes 2001).

The relationship between CD and DM-1 has been widely examined. As with CD, DM-1 is highly related to the HLA-DQ2 heterodimer (Holmes 2001; Doolan A 2005), implying a susceptibility to both diseases. Studies assessing the prevalence of CD in relatives of probands with DM-1, who do not have DM-1 themselves have found first-degree relatives have a prevalence of CD between 3.8 and 6% (Sumnik Z 2005; Hanukoglu A 2003), which is indicative of the genetic association between the two diseases. Patients with CD and DM-1 have also been shown to be at risk for other autoimmune disorders such as auto immune thyroid disease (Kaukinen K 1999; Not T 2001).

It is unusual for DM-1 patients with CD to have classical gastrointestinal symptoms (Hanukoglu A 2003; Barera G 2002), and more commonly they appear to be asymptomatic. Despite this many patients appear to have beneficial effects especially in relation to glycaemic control after the initiation of a gluten free diet (Mohn A 2001), however there is also the possibility of excessive weight gain which should be monitored closely (Saukkonen T 2002). The introduction of a GFD in this group of patients is often problematic for a number of reasons. Patients within this group will usually already be on restrictive diets as a diabetic control measures, and are often

unwilling to restrict their diets further. Additionally they are often asymptomatic and as such have limited motivation to maintain a GFD.

DM-1 patients appear to be more protean in the development of the CD with up to 40% of patients developing CD, years after the development of DM-1 (Barera G 2002), and at least 90% of patients being diagnosed with DM-1 before being diagnosed with CD (Pocecco M 1995). As such it is especially important to screen this subset of patients for CD on a regular basis.

Infertility

There has been much published and often conflicting data on the prevalence of CD as an underlying cause of infertility as well as a cause of pre and post natal complications. It has been shown that up to 50% of women with untreated CD experience miscarriage or an unfavorable outcome of pregnancy (Martinelli P 2000). In one study, 845 women were screened for CD, with 12 of the 845 (1.42%) having biopsy proven disease (Martinelli P 2000). Of these 12 pregnancies, 3 babies died within the first week of life and 5 had small gestational age newborns.

Ciacci et al (Ciacci C 1996) compared CD women on a gluten free diet and untreated CD women and found that the relative risk of abortion was 8.9 times higher in untreated CD women compared to treated, the risk of low birth weight was 5.84 times higher and the duration of breast feeding was 2.54 shorter in untreated mothers.

In the majority of studies the prevalence of CD in women with unexplained infertility has been shown to be higher than the general population, ranging from 2.1% to 4.1% (Kolho K.L 1999; Collin P 1996; Meloni G.F 1999), however this difference is generally not significant. In contrast, one large study of 5055 pregnant women

showed the prevalence of CD was 1:80, but found no evidence of unfavorable outcome of pregnancy in these women (Greco L 2004)

A history of miscarriage has been shown to be more common in women with untreated CD than in control groups (Martinelli P 2000), as is the rate of still births (Sher K.S 1996).

In addition to pregnancy issues, untreated CD in women can also lead to delayed menarche, amenorrhea and early menopause (Rostami K 2001; Stazi A.V 2000), as well as chronic pelvic pain, dysmenorrhea and deep dyspareunia (Porpora M.G 2002). Despite all these manifestations, once on a GFD, women with CD have no increased incidence of miscarriage or low birth weight babies compared to the general population (Ciacci C 1996).

Increased prevalence of sexual dysfunction and possible infertility in males with untreated CD has also been shown. Untreated male coeliacs have been shown to have an increased rate of impotence (Farthing M.J 1983), as well as impaired hypothalamic-pituitary regulation of gonadal function (Farthing M.J 1983).

The interaction between CD and reproductive anomalies is not well described, but it is hypothesized that the main interaction between the two is likely to be malabsorption of trace elements, most importantly zinc (Rostami K 2001), which is required for DNA synthesis, cell division, protein synthesis and immune responses.

Bone Disorders

A number of endocrinological disorders have been described in association with CD the most common of which is bone disease. As absorption of calcium occurs predominantly in the duodenum and jejunum, it is not surprising that calcium

absorption is impaired in coeliac patients. Reduced calcium absorption subsequently leads to increased parathormone secretion which in turn results in increased bone turn over and cortical bone loss (Scott E.M 2000). It is possible to surmise that CD patients, especially those untreated, have an increased risk of reduced bone mineral density (BMD). Bone mineral density is the most fundamental and best studied parameter of bone structure and strength. Studies have shown that between 40 to 70% of adult coeliacs have a BMD greater than 1 standard deviation below the general population (Thomason K 2003; Kemppainen T 1999), a trend which is similar in untreated CD children who have significantly lower BMD values compared with treated CD children, and normal control groups (Barera G 2004; Kavak U.S 2003; Tau C 2006). In contrast to these results the prevalence of CD in patients with reduced BMD has shown a wide range of results.

Some published data (Gonzalez D 2002; Mather K.J 2003) have shown no increase in the prevalence of CD in patients with decreased BMD, however the majority of studies have shown results ranging from 1.8% to 9.4% (Drummond F.J 2003), at least 9 time the expected prevalence. It is interesting to note in the vast majority of these studies there were many low positive antibody results which did not correlate with histological change consistent with CD, and may be reflective of screening in elderly patients with high polyclonal or monoclonal IgA.

Consequently to a decreased BMD, the risk of fracture is estimated to be equivalent to a doubling of risk for every standard deviation decrease in BMD. Thus far this link between increased risk of fracture in CD patients has not been established. One study compared the risk of fracture in CD patients to controls and discovered no increased risk of fracture in the CD group compared to a control group, even though there was a

decrease in BMD in the CD group (however the median BMD was still within normal limits) (Thomason K 2003). It is worth noting that in this study there was no measure of dietary compliance or if patients had active disease. Judging from the patient selection criteria many of the patients were enrolled from gastroenterologists and thus were likely to have been fully assessed and compliant to a GFD. It has been shown that a patient's BMD significantly increases after the commencement of a GFD (Valdimarsson T 1996; Kemppainen T 1999; Barera G 2004; Tau C 2006) which would diminish the risk of fracture (Arden N. K 2003) and may be illustrative of the results obtained in this study.

Even though the majority of CD patients with low BMDs will improve after the commencement of a GFD, hypocalcaemia (low serum calcium) can persist even when morphology has completely returned to normal (Agrama M. T 2002). This is due to the fact that calcium is preferentially deposited in bone and the re-establishment of normal calcium levels may take years.

Secondary hyperparathyroidism is also a possibility in patients with long term calcium malabsorption and may further exacerbate low BMD (Farrell R.K 2001; Bernstein C.N 2003; Selby P.L 1999), as well as upregulate renal hydroxylase activity which ultimately leads to faster vitamin D metabolism and thus vitamin D deficiency (Walters J.R 2003).

In addition to malabsorption, recent studies have looked at the presence of bone specific antibodies. Increased autoimmunity has been widely studied in CD patients and thus an increase in bone specific antibodies would be in keeping with these findings. Sugai et al (2002) showed that 51.5% of CD patients had antibodies against intra and extracellular bone structures, which may exacerbate bone turn over and loss.

Case Associations

In addition to the aforementioned associations, numerous other studies and case reports have described other conditions in association with CD especially other autoimmune disorders. These associations include: Hereditary Angioneurotic Edema (Farkas H 2002), idiopathic dilated cardiomyopathy (Curione M 2002; Prati D 2002) Hashimoto's thyroiditis / autoimmune thyroiditis (Agrama M.T 2002; Ravaglia G 2003; Volta U 2002), autoimmune cholangitis (Marignani M 2002; Volta U 2002), psoriasis (Addolorato G 2003; Woo W.K 2004), Fabry disease (Tumer L 2004), non-alcoholic fatty liver disease (Bardella M.T 2004), chronic granulomatous disease (Hartl D 2204), primary biliary cirrhosis (Kumar P 2002; Floreani A 2002), nodular prurigo (Delfino M 2002), cavitating mesenteric adenopathy (Huppert B.J 2004), oral ulcers (Biel K 2000), Williams syndrome (Giannotti A 2001), Down's syndrome (Gale L 1997), Addisons disease (Myhre A.G 2003), ulcerative colitis (Wurm P 2003), idiopathic thrombocytopenic purpura (Williams S.F 2003), thrombocytosis (Carroccio A 2002) and rickets (Jain 2002).

CD in practice

Over the last decade there has been progressive recognition that CD is a common disorder presenting with a wide spectra of symptoms, making diagnosis on clinical presentation alone seemingly impossible. Despite the introduction of serological screening methods it appears there is still a chronic neglect to screen for CD as well as a lack of understanding of the significance of a positive result.

There is much conflict within the scientific and medical communities as to the subgroups of patients who should be routinely screened for CD, and even suggestions of whole population screening for the disease (Fasano A 2003; Tommasini A 2004; Young E.H 2004; Mearin M.L 2005). While there are clearly merits to this approach there are still many uncertainties, primarily the age that screening should be initiated, and the benefit of treating CD in patients with no manifestations of the disease.

It is evident that there is also a lack of understanding of the significance of positive CD serology. In European countries where awareness of CD is relatively high, as many as 82% of patients with positive coeliac serology were not offered a confirmatory biopsy (Sinclair D 2004), a result which is especially indicative of general practitioners ordering CD related serology (Pearce C.B 2002). While many consensus statements relating to CD screening have been published, it is clear that there is still a lack of information filtering to the medical community, especially frontline general practitioners who have primary care of many CD patients, the majority of which would be considered to have atypical or seeming asymptomatic disease.

Clearly there is much need for multidisciplinary reform and education with regards to the prevalence, presentation, diagnostic tools, confirmatory screening, referral and long term management of CD.

Aims

To establish the prevalence of coeliac disease in patients deemed to be ‘at-risk’ of developing the disease including;

1. Patients with infertility
2. Patients with Diabetes Mellitus Type 1
3. Patients with decreased bone mineral density
4. Patients with low trauma fractures

To follow patients with biopsy proven CD to establish the effect of a gluten-free diet on symptoms.

CHAPTER

2

METHODS

Patient recruitment

Invitro Fertilisation

From March 2004 to July 2005 all patients undergoing *invitro* fertilisation (IVF) at the Fertility unit at Royal Prince Alfred Hospital, Sydney were serologically screened for tissue transglutaminase antibodies (tTG) and total immunoglobulin A (IgA) as part of their pre IVF protocol. All assays were performed at the Department of Clinical Immunology, Royal Prince Alfred Hospital. Patients with undetectable or low IgA levels were also screened using an IgG/IgA endomysial (EMA) method.

Diabetes Mellitus Type-1

From March 2003 to May 2005 all patients attending the Diabetes Clinic at the Department of Endocrinology, Royal Prince Alfred Hospital, Sydney were screened for tTG antibodies or EMA antibodies as part of their routine serological testing. Assays were performed at either the Department of Clinical Immunology, Royal Prince Alfred Hospital or at a private pathology provider. When performed at a private pathology provider, total IgA results were not available.

Patients with Decreased Bone Mineral Density

From March 2003 to May 2005 all patients with a t score of greater than -1 at either the total femoral head or vertebrae L2-4 on bone mineral density (BMD) at the endocrinology departments at Concord Hospital and Royal Prince Alfred Hospital, Sydney, were serologically screened for tTG antibodies or EMA antibodies and total IgA at the Department of Clinical Immunology, Royal Prince Alfred Hospital.

Patients with Low Trauma Fractures

From March 2003 to May 2005 patients presenting to Royal Prince Alfred Hospital, Sydney with ‘a fracture caused by injury that would be insufficient to fracture normal bone’ as defined by the World Health Organisation (usually regarded as a fracture sustained with no trauma or from a height of standing or less) were serologically screened for tTG antibodies or EMA antibodies as well as Gliadin IgA and IgG antibodies (Gli A/Gli G) and total IgA. All assays were performed at the Department of Clinical Immunology, Royal Prince Alfred Hospital. In addition a BMD was also performed on each patient.

Serological methods

Total Immunoglobulin A (IgA)

Total IgA was measured using serum samples on the automated IMAGE Immunochemistry System (Beckman Coulter, Sydney, Australia) by rate nephelometry and reported in grams per liter (g/L)

Normal range 0.60-3.96 g/L (adults).

Gliadin Antibodies

Gliadin IgA and IgG (Gli A/Gli G) antibodies were measured in human serum using the QUANTA Lite Gliadin IgA and QUANTA Lite Gliadin IgG enzyme linked immunosorbent assay (ELISA) (INNOVA Diagnostics Inc, San Diego, CA, USA) and performed on the automated DYNEX DSX system (DYNEX technologies,

Chantilly, Virginia , USA). This involved a semi-quantitative indirect enzyme immunoassay, using microwells coated with purified gliadin. Patient serum was incubated in the well and anti-gliadin antibodies if present, bound to the gliadin solid phase. After washing, anti-human IgG or IgA conjugated with horseradish peroxidase was added. At the end of the second incubation, unbound conjugate was removed by washing and enzyme substrate is added. Colour developed in proportion to the amount of anti-gliadin antibodies present. The reaction was stopped and the absorbance measured at 450 nm and reported in units. Normal value <20 units, 20-30 units weak positive, >30 units moderate to strong positive.

Endomysial Antibodies

Endomysial antibodies (EMA) were detected using dilute human serum via indirect immunofluorescence on monkey smooth muscle (IMMCO diagnostics, Buffalo, New York, USA) using dual IgA / IgG conjugate. As per manufacturer's instructions serum was diluted at 1:10 for the initial screen. Samples positive at 1:10 were then serially diluted at 1:40 and 1:160. Normal range <10.

Tissue transglutaminase

As with Gliadin antibodies, Tissue transglutaminase antibodies (tTG) (Genesis Diagnostics, Cambridgeshire, United Kingdom) were detected via an ELISA method on the automated DYNEX DSX system (DYNEX technologies) according to the manufacture's instructions. Serum samples were incubated in microtitre wells coated with human recombinant tissue transglutaminase. Antibodies to tTG, if present, bound to the plate and unbound material was removed by washing. The

antigen/antibody complexes were then detected by a rabbit anti-human IgA horse-radish peroxidase conjugated antibody, which produced a colour change when incubated with TMB substrate. The colour intensity of the patient well was compared to a standard curve and result reported in units per millilitre (U/mL). Normal range <7 U/mL.

Bone Mineral Density

Bone mineral density (BMD) measurements were made with a dual-energy X-ray absorptiometers (DEXA) either the Lunar Prodigy (GE healthcare, Madison, Wisconsin, USA) or Hologic-4000 (Hologic Inc, Waltham, Massachusetts, USA). Measurements were made of the left or right femoral head (hip) and at the L2-L4 vertebrae level (lumbar spine), unless degenerative changes at either site were present. Data was analysed using manufacturer specific software (version 5.00.211 for the Lunar Prodigy and version 8.26 for the Hologic 4000). A t and z score was reported for each patient.

According to The World Health Organisation (WHO) definitions (WHO Study Group 1994) a t score is the deviation of the individual value from the normal mean value of young adults of the same sex expressed in number of standard deviations. A z score is the deviation of an individuals measured value from the age and sex matched normal mean, also expressed in number of standard deviations.

A t score of between -1 and -2.5 was consistent with osteopaenia, while scores of greater than -2.5 were diagnostic of osteoporosis, in accordance with World Health Organisation (WHO) definitions (WHO Study Group 1994).

Small Bowel Biopsy

Patients with serology suggestive of CD were asked to undergo a confirmatory biopsy at the Gastroenterology Department at Royal Prince Alfred Hospital. Patients with equivocal serology (EMA =10, tTG 7-12 IU/ml) were individually assessed to determine the appropriateness of undergoing a biopsy. Patients who were not biopsied were asked to be retested for coeliac antibodies in one year's time.

For patients who consented to biopsy, multiple small bowel biopsies from the distal duodenum were obtained during upper gastrointestinal endoscopy. Biopsy samples were then orientated on glass slides, fixed with formalin, stained with haematoxylin-eosin and examined under light microscopy. Findings of villous atrophy, crypt hyperplasia and increased intraepithelial lymphocytes were considered to be consistent with CD. Patients with raised serology who did not undergo biopsy were excluded from further analysis.

Body Mass Index

Body mass index (BMI) was calculated for each subject with low BMD and all subjects enrolled in follow up studies. It was calculated by weight in kilograms divided by height in meters squared ($\text{kg}/(\text{height})^2$).

Follow up

Subjects with small bowel biopsies consistent with CD were asked to consent to long term follow up studies to assess if gastrointestinal symptoms were present at diagnosis, monitor adherence to a gluten free diet and assess the effect of gluten free diet (GFD) on initial presenting symptoms. Subjects were assessed within 1 month of

biopsy, at 3 months, 6 months and 12 months. Specialist dietitian Dr Kim Faulkner-Hogg at the Allergy Unit, Royal Prince Alfred Hospital educated patients, and assessed compliance to the GFD. Each subject kept a food diary over a period of 1 week, every two months for 1 year, and also filled in a questionnaire regarding the brand of product ingested in the food diary. Additionally patients kept a log of all instances where they were aware of ingesting gluten containing products. This log was replaced every two months over the year period. From these diaries the level of gluten intake was estimated.

Dr Robert Loblay, Director of the allergy Unit at Royal Prince Alfred Hospital clinically assessed patients.

Consent forms, questionnaires and food diaries can be found in the appendix.

Follow up serology

Subjects enrolled in follow up studies were monitored using serology including:

Blood Iron Levels

Assessed in all consented patients. This was performed on the Hitachi 917 (Roche Diagnostics, Castle Hill, NSW, AUSTRALIA) using whole blood. In this assay iron was separated from transferrin by means of guanidinium chloride in weakly acidic pH then reduced with ascorbic acid. This then formed a colored complex with Ferrozine which was spectrophotometrically assayed at 546nm.

Normal range 10-38 umol/L.

Calcium

Performed on the Hitachi 917 (Roche Diagnostics), using serum samples. Calcium forms a violet complex with o-cresolphthalein complexone in alkaline medium. This complex was then detected at 546nm. The intensity of the final reaction colour was proportional to the amount of calcium in the specimen.

Normal range 2.15-2.55 mmol/L.

25-hydroxy Vitamin D

Serum 25-hydroxy vitamin D levels were measured by radioimmunoassay (Immuno Diagnostic Systems, Boldon, UK).

Normal range 31-107 nmol/L.

HbA1c

HbA1c was performed on whole blood by high performance liquid chromatography on the automated Bio-Rad Variant 11 (Bio-Rad) according to manufactures instructions.

Normal range 4-6%.

Folate

Folate was performed on the DXI automated immunoassay (Beckman Coulter) according to manufacture's instructions, using serum.

Normal range 7-25 nmol/L.

Statistics

Data was analysed using z test for variables including t score, z score and age while Fishers exact test was used for sex distribution. Statistical software used was Analyse-it Software version 1.73 (Analyse-it Software Ltd, Leeds, England, UK)

Ethical considerations

This study was performed in accordance with South Western Sydney Ethical committee (Royal Prince Alfred Zone) approval.

CHAPTER

3



RESULTS

Summary of results

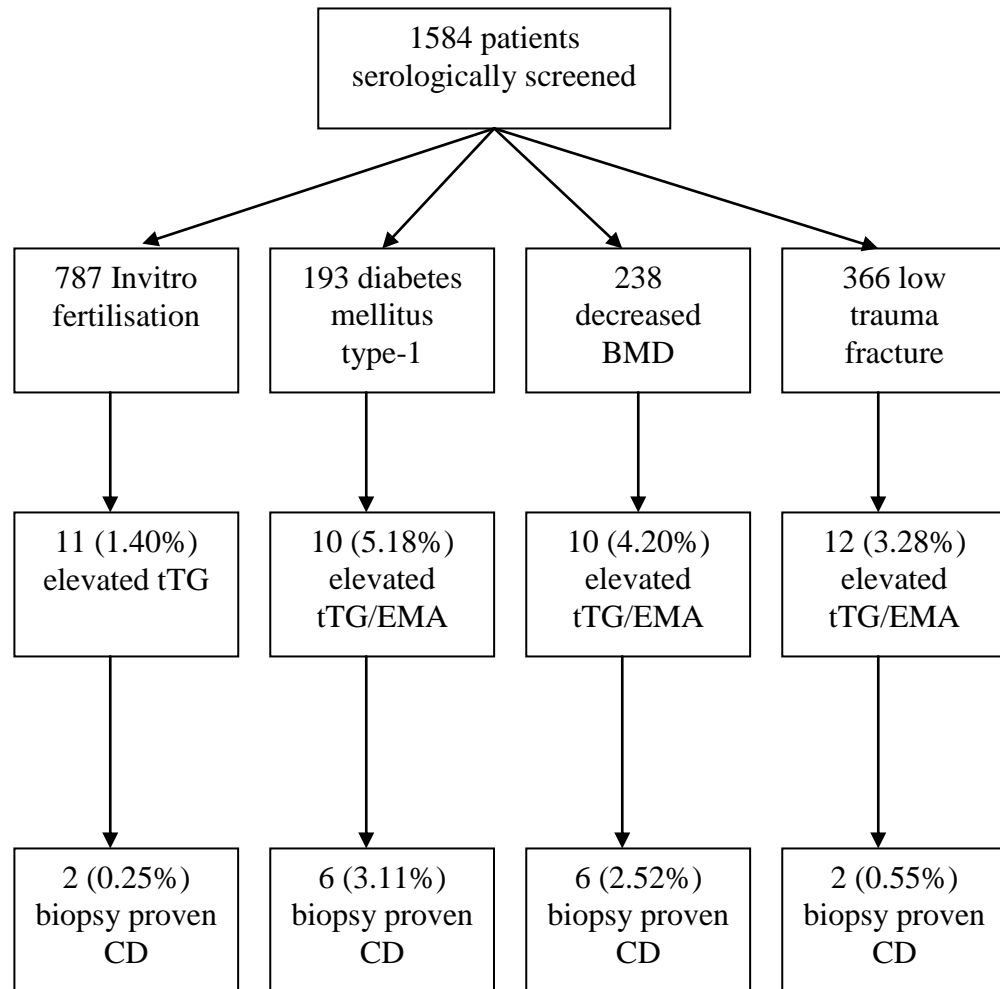


Figure 9- Summary of total subjects screened, division of groups screened and prevalence of serologically positive and biopsy proven subjects.

Invitro Fertilisation

787 IVF patients were screened for CD

- 411 females / 376 males
- Age range 21-63 years. Mean age 35.7 years. Median age 35 years.
- Female age range 21-53. Mean age 34.1 years. Median age 34 years.
- 376 couples and 35 women undergoing IVF with donor sperm.

2/787 (0.25%) subjects were IgA deficient (<0.08g/L). Both were negative for EMA IgG.

11 of 787 (1.4%) patients had elevated tTG levels (table 1).

Table 1: Summary of positive serology in IVF patients.

Sex	Age (years)	tTG (U/mL)	IgA (g/L)	Comment
M	36	8	6.91 (H)	No biopsy. tTG to be repeated in 1 year.
F	41	9	3.45	Normal biopsy
M	37	9	2.8	No biopsy. tTG to be repeated in 1 year.
M	38	10	1.57	No biopsy. tTG to be repeated in 1 year.
F	33	11	1.55	No biopsy. tTG repeated after 1 year. Increased to 13 . Biopsy pending.
F	36	12	2.42	No biopsy. tTG to be repeated in 1 year.
F	40	14	2.67	Normal biopsy
F	41	15	2.92	Normal biopsy
M	31	16	4.07	Patient refused biopsy
F	34	21	2.23	Biopsy consistent with CD
F	31	>100	3.34	Biopsy consistent with CD

In total 2/787 (0.25%) of people with infertility biopsy proven CD or 2/411 females undergoing IVF (0.5%).

IVF Follow-up Studies

One biopsy proven CD enrolled in long term follow-up studies (table 2).

Patient – M.K.

Table 2 - Subject M.K, 34 year old female with idiopathic infertility followed for 11 months

	Initial consultation	1 month	6 month	12 month
Symptoms	Infertility, flatulence, diarrhoea and constipation		Gastrointestinal symptoms are less frequent	No gastro symptoms
tTG (normal <7 U/mL)	21 U/mL	40 IU/mL	16 IU/mL	7 IU/mL
Biopsy result	Partial villous atrophy		Partial villous atrophy	Results pending
BMI	22.3		21.2	Results pending
Serum folate (normal 7-25 nmol/L)	17.2 nmol/L	25.5 nmol/L	>45.3 nmol/L	
Blood iron level (normal 10-38 umol/L)	9 umol/L	17 umol/L	14 umol/L	14 umol/L
Comment	Subject decided to withhold on IVF treatment and initiate a gluten free diet.	Patient still ingesting large amounts of gluten (2 month food diary shows 19 gluten containing products ingested). Diet was reviewed	Gluten intake reduced (6 gluten containing products ingested over 2 months) Patient still not pregnant and opted to return to IVF treatment.	Subject had not conceived, opted to return to IVF treatment.

Diabetes Mellitus Type-1

193 patients diagnosed with diabetes mellitus type 1 (DM-1) were serologically screened for CD.

- 96 female / 97 males
- Age range 13-70 years. Mean 35 years. Median 33 years.

10 (5.2%) had elevated serology (table 3)

Table 3: Summary of positive serology in DM-1 group

Sex	Age (years)	tTG (U/mL)	EMA (titre)	Biopsy
M	38	8		Normal biopsy. Repeat tTG in 1 year
F	28	19	80	Biopsy consistent with CD
M	68	13	10	Biopsy consistent with CD
F	24	17	10	Normal biopsy
M	33		>160	Biopsy consistent with CD
F	43		>160	Pt refused biopsy
M	35	>100		Pt refused biopsy
F	41	>100		Biopsy consistent with CD
M	38	>100	>160	Biopsy consistent with CD
M	39	>100	>160	Biopsy consistent with CD

In total 6/193 (3.1%) biopsy proven coeliac patients.

In addition 2 patients were IgA deficient (<0.08 g/L), both were negative for Gliadin IgG and EMA IgG antibodies.

DM-1 Follow-up Studies

One patient enrolled in long term follow up studies (table 4).

Patient-C.M

Table 4 - Subject C.M, 41 year old female with diabetes mellitus type 1 followed for 12 months.

	Initial consultation	1 month	6 month	12 month
Symptoms	Intermittent mild gastrointestinal pain and mild fatigue		Pain and fatigue reduced	No pain, reduced fatigue
tTG (normal <7 U/mL)	>100 U/mL	>100 U/mL	44 U/mL	30 U/mL
Biopsy result	Total villous atrophy		No significant abnormalities	Results pending
BMI	24.8		25.7	26.1
Blood iron level (normal 10-38 umol/L)	6 umol/L	3 umol/L	13 umol/L	24 umol/L
HbA1c (normal 4.1-6%)	7.5% (H)		7.3% (H)	7.3% (H)
Comment		Food diary consistent with no detectable levels of gluten in diet.	Food diary consistent with no detectable levels of gluten in diet.	Food diary consistent with no detectable levels of gluten in diet.

Subjects with Decreased Bone Mineral Density

In total 238 patients with low bone mineral density (BMD) serologically screened for CD

- 198 (83%) females / 40 (17%) males
- Age range 18-91 years. Mean age 61.4 years. Median age 64 years.
- 121 (51%) osteopaenic / 117 (49%) osteoporotic

10/238 (4.2%) of patients with elevated serology (table 5)

Table 5: Summary of positive serology in subjects with decreased BMD

Sex	Age (years)	tTG (U/mL)	EMA (titre)	IgA (g/L)	T score L2-4	Z score L2-4	T score hip	Z score hip	BMI	Biopsy
F	56	6	160	0.86	-2.8	-1.7	-1.2	-0.4	24.2	EMA <10 tTG 4 (normal) on repeat
F	58	8		3.66	-1.3	-0.8	-0.3	0.1	33.8	Normal Biopsy. tTG 5 (normal) on repeat
F	43	9		1.51	-0.6	-0.7	-1.6	-1.5	26.5	tTG 2 (normal) on repeat
F	71	11		3.07	-2.1	-0.1	-1.5	0.2	28.5	Normal biopsy
M	78	16		1.89	-2	-0.6	-2.3	-0.7	21.2	Biopsy consistent with CD
F	75	34		2.04	3.9	6	-2.7	-0.8	23.6	Refused biopsy
F	69	>100	160	2.45	-3.4	-1.9	-1.6	-0.4	27.4	Biopsy consistent with CD
M	40		160	2.27	0.2	-0.1	-2.5	-2.2	31	Biopsy consistent with CD
M	48	>100		3.03	-2.6	-1.9	-2.2	-1.3	19.9	Biopsy consistent with CD

Table 5 con't

Sex	Age (years)	tTG (U/mL)	EMA (titre)	IgA (g/L)	T score L2-4	Z score L2-4	T score hip	Z score hip	BMI	Biopsy
F	81		>160	1.94	-1.84	1	-3.5	-1.1	21.6	Refused biopsy, but commenced GFD

In addition there were also 2 patients with known CD on a GFD

Table 6: Additional biopsy proven CD subjects with decreased BMD

Sex	Age (years)	tTG (U/mL)	EMA (titre)	IgA (g/L)	T score L2-4	Z score L2-4	T score hip	Z score hip	BMI	Biopsy
F	73	1	<10	3.16	-2.7	-0.5	-2.1	-0.2	20.7	Biopsy consistent with CD 5 years prior
M	64		<10	2.32	-3.5	-2.1	-2.6	-1.7	28.2	Biopsy consistent with CD 10 years prior

In total 6/238 (2.5%) of low BMD patients had biopsy proven CD

Graph of lumbar spine and total hip t and z scores of all patients with decreased BMD

may be found in the appendix.

Statistical Analysis of Subjects with Decreased BMD

Statistical difference between patients with biopsy proven CD and decreased BMD,

and non CD subjects with decreased BMD were not significant in;

- Age (p=0.90)
- t score lumbar spine (L2-L4) (p=0.79)
- z score lumbar spine (L2-L4) (p=0.71)
- t score total femoral head (hip) (p=0.21)
- z score total femoral head (hip) (p=0.19)

Statistical difference was significant in sex ratio between the two groups (p=0.01)

Subjects with Decreased BMD Follow-up Studies

Two patients enrolled in long term follow up (table 7 and 8)

Patient -R.R

Table 7 - Subject R.R, 78 year old male with a history of osteopaenia and auto immune haemolytic anemia treated with prednisone. Followed for 12 months.

	Initial consultation	1 month	6 month	12 month
Symptoms	Osteopaenia autoimmune haemolytic anemia. No gastrointestinal symptoms	No change. Felt no benefit of being on a GFD	No change. Felt no benefit of being on a GFD	Osteoporosis, autoimmune haemolytic anemia.
tTG (normal <7 IU/ml)	16 IU/ml	63 IU/ml	36 IU/ml	40 IU/ml
Biopsy result	Partial villous atrophy		Partial villous atrophy	Partial villous atrophy
BMI	20.2			19.4
Blood iron level (normal 10-38 umol/L)	17 umol/L		19 umol/L	18 umol/L
Calcium (normal 10-38 umol/L)	2.28 mmol/L		2.25 mmol/L	2.19 mmol/L
25-hydroxy vitamin D (normal 31-107 nmol/L)	62 nmol/L		95 nmol/L	83 nmol/L
Bone mineral density (BMD)	<u>Spine (L2-4):</u> t score = -2 z score = -0.6 <u>Hip:</u> t score = -2.3 z score = -0.7			<u>Spine (L2-4):</u> t score = -2 z score = -0.3 <u>Hip:</u> t score = -2.7 z score = -0.9
Comment	Dietary review show high gluten intake. Diet reviewed.	Dietary review show high gluten intake. Patient admitted to a minimum of 2 beers per day.		Gluten intake was still high. Subject possibly abandoning GFD.

Subject- C.C

Table 8 - Subject C.C, 48 year old male with osteoporosis and paget’s disease.

Followed for 12 months.

	Initial consultation	1 month	6 month	12 month
Symptoms	Osteoporosis, bloating, abdominal pain, flatulence and fatigue		Less gastrointestinal complaints and fatigue	Osteopaenia. Less severe and frequent gastrointestinal complaints. Reduced fatigue
tTG (normal <7 IU/ml)	>100 IU/ml	75 IU/ml	>100 IU/ml	15 IU/ml
Biopsy result	Chronic inflammation and moderate villous atrophy		Partial villous atrophy	Declined biopsy
BMI	20.05		21.3	21.7
Blood iron level (normal 10-38 umol/L)	6 umol/L		17 umol/L	17 umol/L
Calcium (normal 10-38 umol/L)	2.23 mmol/L		2.40 mmol/L	2.43 mmol/L
25-hydroxy vitamin D (normal 31-107 nmol/L)	44 nmol/L		71 nmol/L	79 nmol/L
Bone mineral density (BMD)	<u>Spine (L2-4):</u> t score = -2.6 z score = -1.9 <u>Hip:</u> t score = -2.2 z score = -1.3			<u>Spine (L2-4):</u> t score = -1.5 z score = -0.8 <u>Hip:</u> t score = -1.7 z score = -1
Comment		No detectable level of gluten in food diary	No detectable level of gluten in food diary	No detectable level of gluten in food diary

Subjects with Low Trauma Fractures

366 patients admitted to hospital with low trauma fractures were serologically screened for CD.

- 274 (75%) females / 92 (25%) males
- Age range 24-94 years. Mean age 66.5 years. Median age 67 years.

390 fractures were identified (23 patients with multiple fractures).

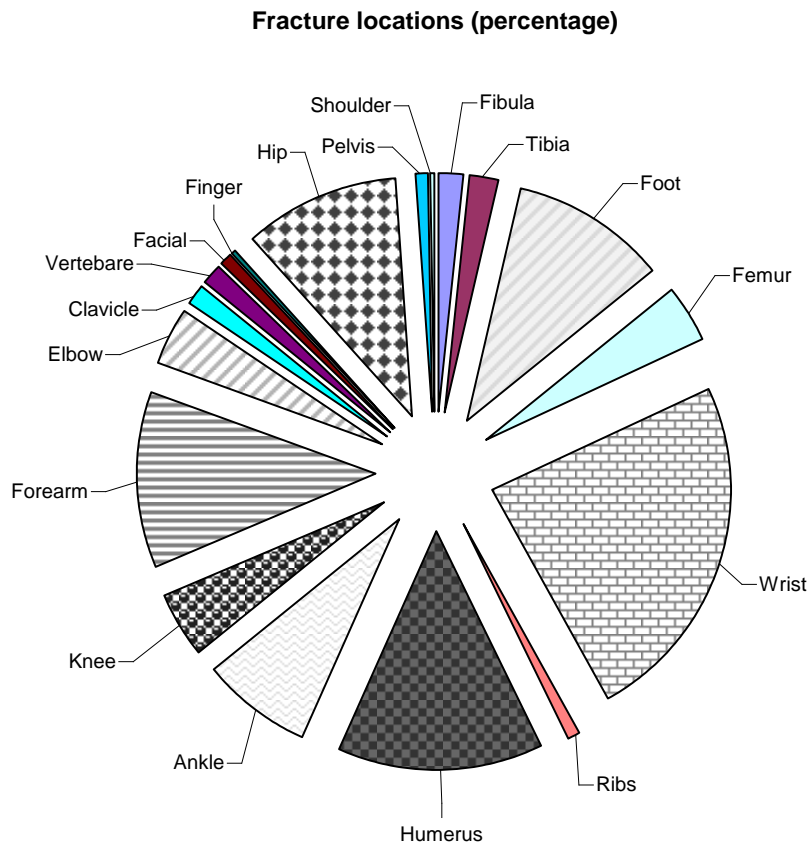


Figure 9- Fracture locations as a percentage for all patients with low trauma fractures

In total 12/366 (3.3%) had elevated coeliac serology (table 9).

Table 9: Summary of positive serology for patients with low trauma fractures

Sex	Age (years)	IgA (g/L)	tTG (U/mL)	Gli A	Gli G	T L2-4	Z L2-4	T hip	Z hip	Fract site	Comment
F	71	5.79	8	<20	<20	-3	-1	-3.3	-1.1	Wrist	Repeat tTG in 1 year
F	83	5.36	8	<20	<20	-4	-2	-1.6	1.3	Forearm	Repeat tTG in 1 year
M	72	3.86	8	<20	<20	-2	-1	0.7	1.6	Wrist	Repeat tTG in 1 year
M	37	13	8	51	31	-2.3	-1.8	-1	-0.5	Wrist	tTG repeated. Now 10. Biopsy pending
F	84	3.45	8	<20	<20	-2	0.9	-3.1	-0.1	Hip	Repeat tTG in 1 year
F	57	2.42	9	<20	<20	-1		-2.6		Foot	Repeat tTG in 1 year
F	88	7.03	10	<20	<20	-3.7	-1.5	0.6	2.8	Forearm	Repeat tTG in 1 year
F	82	2	10	NA	NA	-3	-1	-3.5	-1	Ankle	Normal biopsy. tTG 6 (normal) after 1 year
F	90	1.27	14	<20	<20	-4	-1	-3	-0.5	Forearm	Unable to biopsy
F	75	2.06	15	63	61	-1	0.2	-4	-2	Hum and foot	Biopsy consistent with CD
M	62	5.64	30	<20	23	0.9	1.2	-1.6	-1.7	Forearm	Equivocal biopsy. Repeat in 1 year
F	51	3.2	54	39	31	-3	-2	0.7	1.5	Wrist	Biopsy consistent with CD

In total 2/366 (0.55%) biopsy proven CD.

Low Trauma Fractures Follow-up Studies

1 patient consented to follow up study (table 10).

Subject- D.F

Table 10 - Subject D.F, 75 year old female with non-traumatic fracture of the humerus and foot, and osteoporosis. Followed for 12 months.

	Initial consultation	1 month	6 month	12 month
Symptoms	Non-traumatic fracture to left humerus and right metatarsal, osteoporosis and nausea.	Nausea has completely resolved	Nausea and vomiting after ingesting gluten	Nausea has resolved
tTG (normal <7 IU/ml)	15 IU/ml	>100 IU/ml	80 IU/ml	14 IU/ml
Biopsy result	Total villous atrophy		Subtotal villous atrophy	Declined biopsy
BMI	27.5		29.5	28.7
Blood iron level (normal 10-38 umol/L)	35 umol/L		25 umol/L	20 umol/L
Calcium (normal 10-38 umol/L)	2.36 mmol/L		2.52 mmol/L	2.59 mmol/L
25-hydroxy vitamin D (normal 31-107 nmol/L)	22 nmol/L		35 nmol/L	53 nmol/L
Bone mineral density (BMD)	<u>Spine (L2-4):</u> t score = -4 z score = -2 <u>Hip:</u> t score = -1.4 z score = 0.2			<u>Spine (L2-4):</u> t score = -2.9 z score = -1.1 <u>Hip:</u> t score = -0.6 z score = 0.8
Comment		Subject still ingesting small amounts of gluten. Diet reviewed.	Subject admitted to eating gluten containing foods at weddings, birthdays and Christmas	Strict adherence to GFD. No detectable level of gluten in food diary.

CHAPTER

4

DISCUSSION and CONCLUSION

Discussion

The results presented in this study support the conclusion that CD is a highly prevalent, chronically under diagnosed disease that may present with spectrum of manifestations, with onset at any age. 1584 patients were serologically screened for CD with 16 (1.01%) confirmed by biopsy as coeliac patients, giving a prevalence of at least 1:99 in this population with selected clinical conditions. The mean age at diagnosis for this group of patients was 50 years, with a median of 41 years.

Previously undiagnosed CD patients were detected in all groups investigated, and an increased prevalence of the disease in specific subsets of patients including those with decreased bone mineral density (BMD) and diabetes mellitus type 1 (DM-1) was identified.

Despite the high prevalence of CD obtained in this study it is evident that the results presented here represent the minimum prevalence in these groups. As a small bowel biopsy showing histological changes was considered necessary for confirmatory diagnosis of CD in this study it is likely that more patients, especially those with high titre coeliac antibodies who refused biopsy have undiagnosed CD.

Invitro fertilisation

Subjects undergoing *invitro* fertilisation (IVF) had the lowest rate of CD, with a rate equivalent to that seen in the general population (1:394 in IVF couples or 1:205 women undergoing IVF, versus 1:251 in the general population). These findings are comparable to other studies such as those by Kolho et al (1999) who also found no increase in the frequency of CD in women with a history of miscarriage or infertility. In contrast a number of other groups have demonstrated CD to be more prevalent in

infertility (Collin P 1996; Kolho K.L 1999) however the majority of these studies have generally focused on female idiopathic infertility. In this study presented here ethical restrictions resulted in the actual diagnosis of each couple being unattainable; however it is probable that infertility in the vast proportion of subjects screened here would not have been idiopathic. Infertility in general can be attributed to sperm anomalies in 25% of cases, ovulation problems in 25%, tubal problems in 20% and endometriosis in 5% of cases (Templeton A 1995), with idiopathic infertility accounting for only 25% of total infertility cases. While the cause of fertility problems may be corrected without the use of IVF, it is likely that the diagnosis of idiopathic causes would account for a similar rate in subjects being treated with IVF. In addition to this, 35 women were undergoing IVF without male partners, suggesting a need only for donor sperm, rather than true infertility. Age related infertility may also account for a significant proportion of women subjects within this group. Women in this subset had a mean and median age of 34 years with an age range extending to 53 years, in contrast to the general population mean age at delivery of 30.5 years (according to the Australian Bureau of Statistics 2003 birth rate), again suggesting the interpretation that the patients in this cohort do not have idiopathic infertility. Considering all of these factors it is likely that the result obtained in this study represents the minimal prevalence of CD in infertile subjects. While it is evident that CD is not significantly increased in subjects undergoing IVF treatment, it is still present in 1 of 205 of women. Considering the large collation of data indicating that untreated CD is highly related to unfavorable outcomes of pregnancy in untreated women (Martinelli P 2000; Sher K.S 1996), the identification and treatment of this disease may still be an important factor in prenatal care. Under

current recommendations by Glibert (2002), prenatal serological assessment in Australia (which forms part of pre IVF protocols) should include screening for: Human Immunodeficiency Virus (HIV) is prevalent in approximately 0.1 per 1000 deliveries (Graves N 2004), with a 20% chance of affecting the newborn (Martinelli P 2000), Rubella prevalent in 0.01 per 1000 deliveries with a 80% chance of neonate involvement (Martinelli P 2000) and Syphilis prevalent in 0.015 per 1000 deliveries with variable neonatal involvement (Martinelli P 2000). Compared to these diseases, subjects undergoing IVF have a prevalence of CD of approximately 5 per 1000 deliveries (assuming all women diagnosed with CD conceived on IVF treatment), with a 30% chance of affecting the newborn (Martinelli P 2000). Thus CD is at least 50 times more prevalent than any other disease on the recommended prenatal screening panel, and 500 times more prevalent than rubella in pregnancy. In light of this, despite CD not being significantly increased in women undergoing IVF, it still occurs at a rate far higher than any other disease currently identified prenatally. This, combined with the fact that CD is relatively simple to treat, and treatment practically obliterates the chance of related fetal anomalies (Ciacci C 1996) strongly supports the view that serological screening for CD should be included in routine prenatal serology, a recommendation that had also been highlighted in other studies (Collin P 1996; Meloni G.F 1999).

The one IVF subject that was followed for an 11 month period had not conceived and intends to resume IVF treatment. Despite this, it is evident that the initiation of a gluten free diet (GFD) resulted in vast improvements in the patient (table 2), which may subsequently lead to better prenatal health. Before treatment the subject had a range of gastrointestinal symptoms together with iron deficiency. Six months post

GFD iron and folate levels have increased while symptoms and tTG levels have decreased, indicating mucosal recovery and increased absorption.

Diabetes Mellitus Type 1

In contrast to the IVF subjects, patients diagnosed with diabetes mellitus type 1 (DM-1) have a significantly increased prevalence of CD, with at least 1 in 32 subjects having both diseases, 7.8 times the incidence of the general population. A finding of increased prevalence is similar to almost all other studies assessing the link between the two diseases (Sanchez-Albisua I 2005; Barera G 2002; Aygun C 2005; Crone J 2003; Ashabani A 2003; Lazzarotto F 2003; Fasano A 2003; Sanchez-Albisua I 2005).

Despite this high prevalence, it is likely that this is still an under estimation of the actual rate of CD in this DM-1 population. Two patients in this group with high titre coeliac antibodies refused biopsy. It is highly likely that both these patients have undiagnosed CD meaning that the true prevalence in this cohort would be closer to 4% or 1 in 24 patients.

Additionally many studies have proven that CD can evolve over a period of years in DM-1 patients (Saukkonen T 2002). Patients in this study were only screened at a single time point it is likely that more subjects will develop coeliac antibodies over time.

The one patient that was followed for 1 year post diagnosis followed the clinical course that has often been described in DM-1 patients diagnosed with CD. Over the 12 month follow up this subject's body mass index (BMI) continued to increase from just within normal range at the time of diagnosis to overweight after 12 months

follow up. BMI increase has been observed in other diabetic patients after the commencement of a GFD, especially children (Saukkonen T 2002; Saadah O.I 2004; Sanchez-Albisua I 2005). This increase in body mass is primarily attributed to increased absorption in the intestine, however many foods that are gluten free also have a high glycaemic index, meaning that subjects are more likely to consume higher amounts of foods and will often eat more frequently to attain glycaemic control. This subject's glycaemic control (as measured by HbA1c) did not change as a result of the GFD; however it has been shown that this is mostly a feature of diabetics who have been diagnosed with CD because of malabsorption and not of subjects detected by routine screening (Holmes G.K.T 2002). One year after the commencement of a GFD the subject felt marked improvement in the general gastrointestinal pain that had been present previously, as well as a reduction in fatigue.

Due to the high prevalence and the perceived benefit of diagnosis it has been recommended that all patients with DM-1 be routinely screened for CD antibodies (Holmes G.K.T 2002).

Decreased Bone Mineral Density

Subjects with decreased bone mineral density also had a higher rate of CD than the general population with a prevalence of at least 1:40, 6.3 times the rate in the general population. While osteopaenia and osteoporosis have been established as long term manifestations of undiagnosed CD patients (Kemppainen T 1999), the prevalence of CD in patients with decreased BMD has been controversial. Studies by Mather et al (2003) and Gonzalez et al (2002) found no significant increase in CD in populations

low BMD, while other studies including those by Lindh et al (1992) and Nuti et al (2001) showed elevated rates of coeliac antibodies in osteoporotic patients, however the majority of patients do not have biopsy proven disease. Two recent studies identified a prevalence of biopsy proven CD in of 9 out of 266 (3.4%) (Stenson W.F 2005) and 11 out of 435 (2.56%) (Fasano A 2003) osteoporotic patients. These figures compare favourably to the results obtained in the study presented here, even though osteopaenic subjects were also included in this cohort of patients.

There appear to be no identifying features that distinguish CD patients from other subjects with decreased BMD, with no significance difference in age ($p=0.90$), BMI ($p=0.89$), or t or z score at either femoral head or vertebrae ($p=0.21, 0.19, 0.79, 0.71$ respectively). The only demographic feature of significance was sex ratio. Four of the 6 (67%) biopsy proven CD patients were male, with a prevalence of 4 in 40 (10%), making the sex bias significant ($p=0.01$). This finding is similar to the only other study that included males, where CD was detected in 2 out of 27 (7.4%) of males with osteoporosis (Stenson W.F 2005).

Examination of the two subjects who consented to follow up highlights the importance of treatment in these patients. Patient C.C (table 8) a 48 year old male diagnosed with CD had symptoms including osteoporosis, gastrointestinal complaints, fatigue, low blood iron levels and a BMI at the lower limit of normal before the commencement of a GFD. After 1 year of compliance to a GFD this subject's BMD had improved from osteoporotic to osteopaenic, gastrointestinal symptoms and fatigue had decreased, blood iron levels were within the normal reference range, the level of tTG antibodies had decreased and the subject's BMI had increased to be within normal limits. In contrast, patient R.R (table 7) a 78 year old

male diagnosed with CD had no gastrointestinal symptoms or fatigue at diagnosis, with iron, calcium and 25-hydroxy vitamin D all within normal ranges. The patient did not strictly adhere to a GFD. Over the subsequent 12 month period the subject's BMD continued to decrease, now falling within the osteoporotic range. Additionally there was also a decline in BMI with the subject now clinically underweight. While it is difficult to validate the benefits of a GFD based only on two patients, the tentative conclusion is supported by an increased in BMD identified in CD patients compliant with a GFD which has been validated in other studies (Stenson W.F 2005; Lindh E 1992; McFarlane X.A 1995; Valdimarsson T 1996; Barera G 2004; Ciacci C 1997; Corazza G.R 1996; Mautalen C 1997). It has also been shown that most CD patients with low BMD return to within normal limits within 5 years on a GFD (Kemppainen T 1999).

In contrast, the two subjects in this cohort who had previously been diagnosed with CD still had BMD scores consistent with osteoporosis, despite the fact that they were adherent to a gluten free diet. Without knowing the baseline BMD of these patients it is impossible to comment on whether these results represent an improvement or stabilization in their BMD, however it can be surmised that noncompliance to a GFD would ultimately reduce BMD further.

The results obtained here suggest that all patients with osteopaenia and osteoporosis should be serologically screened for the presence of CD antibodies, especially males with decreased BMD. According to the Australian Institute of Health and Welfare, the direct cost of osteoporosis in Australia from 2000 to 2001 was estimated at \$221 million dollars (Australian Institute of Health and Welfare 2004). Considering Australia's ageing population this figure is set to increase over the next thirty years.

A reduction in the rate of osteoporosis by 2.52% would have a significant reduction in both the direct and indirect cost of osteoporosis, estimated to be in the billions (Australian Institute of Health and Welfare 2004). It can be surmised that this reduction would offset the relatively small cost of routine serological screening in this group, estimated to be only \$7 per tTG assay per patient.

Subjects with Low Trauma Fractures

In contrast to the low BMD group, subjects admitted to hospital with low trauma fractures did not have a significantly increased prevalence of CD with a rate of 1:183 (0.5%) of biopsy proven disease, despite having a high rate of osteopaenia and osteoporosis. However, this group had the lowest rate of confirmation of positive serology by biopsy, which may account for the observed prevalence. Only 16% of patients with elevated serology had a small bowel biopsy. This is predominantly due to the majority of patients (8 out of 12 (67%)), having very low titre tTG antibodies (tTG \leq 10 U/mL), with only one of these having elevated gliadin antibodies.

Of the remaining patients with tTG results greater than 12 u/mL, a 90 year old female with dementia was deemed too ill to undergo biopsy, one biopsy was equivocal with only a mild infiltrate of plasma cells eosinophils and lymphocytes in the lamina propria and 2 patients had a biopsy consistent with CD. As with subjects with low BMD there appeared to be no distinguishing features in these patients; however due to the low number of biopsy positive patients, reliable statistical analysis was not able to be performed.

One of the two subjects consented to follow up. As with the patients with decreased BMD that were followed, the initiation of a GFD significantly increased BMD. This subjects vertebral t score increased by 1 standard deviation in 12 months, while her total femoral head t score increased by just under 1 standard deviation. Additionally the nausea experienced before the commencement of a GFD completely abated. The patient was overweight at diagnosis, and experienced a further increase in weight over the first 6 months on a GFD, before a slight loss over the subsequent six months.

The association between CD and decreased BMD has been well established. Studies similar to the one presented here have shown no increase in the incidence of fracture in CD patients (Thomason K 2003). In CD, it is likely that prolonged decreased absorption of vitamins and minerals results in decreased bone density (decreased bone quantity as assessed by BMD), but may not alter the bone integrity (bone quality), a possible rationale for the absence of an increased fracture risk. Methods of assessing bone quality have found that the combination of BMD with quantitative measurements of bone quantity and quality (as measured by quantitative computer tomography), can better identify subjects at potential risk of fracture (Dougherty G 1996) and better characterise bone loss. While it has been shown that adults presenting with classical severe gastrointestinal symptoms have decreased bone strength as determined by quantitative computer tomography (Ferretti J 2003), the bone strength of subjects identified in screening studies who often lack gastrointestinal symptoms is yet to be investigated.

Summary

Examination of these groups as a whole highlights a number of issues, in particular the diagnostic utility and limitations of specific serological screening. Of the 1584 patients screened with tTG in this study, 18 (1.13%) had tTG levels of 12 u/mL or less. While the diagnostic significance of these low positive results remains to be determined, it appears that there may be a correlation between total IgA levels and the low level positive tTG results obtained in this study. It is unclear whether total IgA levels are increased as a result of gut involvement in early stages of CD development correlating with the low levels of tTG, or whether low tTG values result from non specific binding of mono or polyclonal IgA in patients with high IgA levels. It is clear this observation needs further study, however it is likely that low titre tTG results may represent non-specific binding. Five of the 7 (71.4%) subjects with high total IgA levels had low positive tTG results (8 to 10 U/mL), while only 2 of 16 (12.5%) patients with tTG results greater than 10 had elevated IgA levels suggesting that increased total IgA is not a disease specific effect. These results indicate it may be beneficial to have an equivocal or low positive range as opposed to a single cut off value (7 U/mL in the case of the tTG assay used in this study) in the interpretation of this result. The tTG assay used in the study reported here has a manufacturer reported sensitivity of 100% and specificity of 97%, however as with many commercial assays these figures do not correlate totally with results obtained in routine diagnostic settings, and highlights the need to validate commercial cutoff values in relation to local patient populations. Through consultation with referring clinicians, gastroenterologists and patients themselves, it was decided that subjects in this study would not undergo a small bowel biopsy when tTG results were equal to or less than

12 U/mL. Of the 18 subjects who had tTG results between 8-12 U/mL, 6 patients insisted on a small bowel biopsy, only 1 of which (17%) showed histology associated with CD. Five of the 18 subjects were reassessed after 1 year: 2 subject's tTG level increased, both of whom have small bowel biopsies pending, while the coeliac serology of 3 patients reverted to normal limits. The significance of low level positive serology, positive serology in absence of histological change and patients with transient positive serology is still unknown. Whether these patients represent false positive results, caused by anomalies such as high IgA or are latent CD patients can only be determined through long term analysis.

Though less utilised in this study, the EMA results obtained highlight the possibility of error in diagnostic testing. A 56 year old female with decreased BMD originally reported a high positive EMA of 1:160. Clearly this result represents a false positive considering the tTG performed on the same collection was within normal limits, and the 3 subsequent EMA and tTG tests over the following 18 month period were all normal. Whether this anomaly denotes incorrect sampling, interference by other auto antibodies (such as smooth muscle), operator error or a typographical mistake it demonstrates that no method of laboratory testing is impervious to error. The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGAN) current guidelines state if there is strong clinical suspicion of CD a small bowel biopsy remains the principal indicator of disease and should be utilised, even in the presence of negative or equivocal serology. Reliance on EMA as a single test has been demonstrated to underestimate the prevalence of CD by 20-25% (Green P.H.R 2005), with similar reports regarding the diagnostic accuracy of tTG in cases of CD with mild histological changes (Tursi A 2003). As all cases of CD in this

study were initially detected via serological screening it is possible that the prevalence of CD in this study may still be an underestimation of the true prevalence. Long term evaluation of 5 patients identified in this study highlights the important and significant benefits of the introduction and compliance with a gluten free diet. Three out of the 5 (60%) subjects followed had low blood iron levels that reverted to within normal range after the commencement of a GFD. Four out of the 5 (80%) had some form of gastrointestinal symptom that abated or reduced after the commencement of a GFD; however the incidence of gastrointestinal symptoms may be biased by the fact that symptomatic patients may have felt more compelled to be part of a study process than those with no apparent symptoms. None of these patients were underweight at the time of diagnosis, however the only patient non-compliant to a GFD was underweight 12 months after diagnosis. Two of the 3 patients with decreased BMD showed a positive increase in BMD results after 1 year on a GFD. The third non-compliant subject demonstrated further reductions in BMD. It is evident that implementation and adherence to a GFD in CD patients has many benefits. Four of the 5 (80%) patients followed with expert medical and dietetic advice were compliant with a strict gluten free diet after a 12 month period, compared with an average compliance of only 17-65% (Mayer M 1991) suggesting that expert referral and follow up may promote better dietary compliance. The one patient (patient R.R) who did not strictly adhere to the diet was the only subject who had no gastrointestinal symptoms, correlating with other data which shows subjects with atypical disease are less compelled to maintain a GFD. This man was also the oldest patient with biopsy proven CD identified in this study and the one who found it the most difficult adapting to the changes imposed by a GFD. A beer and a sandwich at

the bowling club had been part of this man's routine for close to three decades. With a limited choice of alternative foods, and a lack of perceived benefit it is easy to understand why this subject doubted the value of continuing a GFD. This subject had been attending his hospital endocrinology department for close to 10 years, with his worsening BMD linked to prednisone treatment for hemolytic anemia. Had a diagnosis of CD been made at a younger age this patient may have been more conducive to the challenges of a GFD although it is of course not known when his serological abnormalities could initially have been detected.

Conclusion

- CD is a highly prevalent, under-diagnosed illness in the Australian population.
- There was no increase in the prevalence of CD in patients seeking IVF treatment compared to the general population. Despite this, the high general prevalence of the disease as well as the possible negative effects on pregnancy outcome suggests that it is important to identify CD prenatally.
- Subjects with DM-1 have a high prevalence of CD (3.11%). These results suggest that routine screening for coeliac antibodies in DM-1 patients is warranted.
- Subjects with osteopaenia and osteoporosis had a prevalence of CD of 2.52%. Males within this group had a remarkably high prevalence of 10%. These results suggest that patients with decreased BMD should be screened for CD as part of routine serological investigations.

- Despite having decreased BMD, patients with low trauma fractures did not have an increased prevalence of CD (0.55%) suggesting that while bone content is reduced in CD patients, bone structure may be less affected.
- The initiation of a GFD in patients with CD results in the aberration of many symptoms including gastrointestinal complaints, and often has a beneficial effect on presenting symptoms such as decreased BMD.
- The effect of increased poly and monoclonal IgA on tTG testing needs to be evaluated.
- Clinicians including general practitioners need to be educated and made aware of the increased prevalence of CD in specific subsets of patients.

CHAPTER

5



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APPENDIX

PARTICIPANT CONSENT FORM

Research study into the prevalence and effects of coeliac disease in people with insulin dependent diabetes mellitus, osteoporosis, infertility and cardiomyopathy.

I,
[name]

of
[address]

I have read and understood the Information for Participants on the above named research study and have discussed the study with either Dr. Robert Loblay, Dr. Warwick Selby or Kim Faulkner-Hogg.

I have been made aware of the procedures involved in the study, including any known or expected inconvenience, risk, discomfort or potential side effect and of their implications as far as they are currently known by the researchers.

I understand that my participation in this study will allow the researchers to have access to my medical record, and I agree to this.

I freely choose to participate in this study and understand that I can withdraw at any time.

I also understand that the research is strictly confidential.

I hereby agree to participate in this research study.

NAME:

SIGNATURE:

DATE:

NAME OF WITNESS:

SIGNATURE OF WITNESS:

ONE WEEK FOOD DIARY

Name:.....

Date:.....

Please record your USUAL daily diet for one week.

Kim Faulkner-Hogg will instruct you to either:

- bring this record with you to your appointment or
- send it back to her, in the envelope supplied, at 2 monthly intervals with the “*Gluten Intake Diary*”

Food Record diary number.....

Kim Faulkner-Hogg:

Phone: 02 9515 8244

Fax: 02 9519 8420

Email: kim.faulkner-Hogg@email.cs.nsw.gov.au

INSTRUCTIONS

This booklet is to be used to record your daily food intake

1. In the DIETARY INTAKE column, record:
 - the time of your meals and snacks
 - which foods and drinks you had
 - vitamin and mineral supplements

2. In the REMARKS column, record:
 - medications used
 - any additional factors which may have influenced your diet choice (eg. restaurants, home of friends)

Day: Wednesday		Date: 11:2:2004
TIME	REMARKS	DIETARY INTAKE (including vitamin & mineral supplements)
7.00 am	Chinese restaurant	<u>Breakfast:</u> gluten free muesli: rice flakes, rice bran, sunflower seeds, dried fruit & nuts milk gluten free bread, butter & blackberry jam 2 cups of tea/milk/sugar multi-vitamin (1 x Macro M) calcium (Caltrate 600mg)
10.00 am		<u>Morning snack:</u> coffee/milk/sugar banana small block of Cadbury milk chocolate
12.30 pm		<u>Lunch:</u> spring rolls lemon chicken and cashews with vegetables boiled rice lychees and ice-cream lemonade
3.00 pm		<u>Afternoon snack:</u> rice cakes, margarine & honey coffee/milk/sugar
7.30 pm		<u>Dinner:</u> roast beef gluten free gravy roast potato, pumpkin, onion, broccoli pavlova, cream, mango and strawberries glass of white wine
9.00 pm		<u>Evening snack:</u> gluten free chocolate cake tea/milk/sugar

Day:		Date:
TIME	REMARKS	DIETARY INTAKE (including vitamin & mineral supplements)
		<u>Breakfast:</u>
		<u>Morning snack:</u>
		<u>Lunch:</u>
		<u>Afternoon snack:</u>
		<u>Dinner:</u>
		<u>Evening snack:</u>

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Allergy Service, Dept. of Clinical Immunology
Royal Prince Alfred Hospital, Camperdown NSW 2050

Brand Questionnaire

Name: _____

Date: / /

	Frequency						Product / Ingredient names	Manufacturer contact details
	Daily	Few days	Weekly	Monthly	<Monthly	Never		
What brands of ready made bread do you eat? Indicate how frequently you eat them?								
Which bread mixes do you eat? Indicate how frequently you eat them?								
List the flours that you use if you make your own bread. How frequently do you eat this?								
Do you use pizza base pre-mixes? Indicate frequency.								
List the frequency and brands of rice cakes corn cakes & other similar products that you eat.								

	Frequency						Product / Ingredient names	Manufacturer contact details
	Daily	Few days	Weekly	Monthly	<Monthly	Never		
How frequently do you eat crackers? Indicate this for each brand consumed. E.g. rice crackers, rice snacks								
What brand/s of biscuits do you eat? Indicate how frequently/								
What flours do you use when baking biscuits, cakes or muffins or pastries? How frequently would you eat these?								
What brands of ready-made baking goods, cakes, and muffins do you eat?								
Which baking mixes do you use and how frequently?								

	Frequency		
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	Daily	Few days	Weekly	Monthly	<Monthly	Never	Product / Ingredient names	Manufacturer contact details
Which brand/s of cornflour do you use? How frequently?								
Which brands of pasta, spaghetti, lasagne sheets or noodles do you purchase? Indicate how frequently you would have them.								
Which breakfast cereals do you purchase? Indicate how frequently you would have them.								
Indicate which brands of soy milk you use and how frequently.								
Which brands of fresh and powdered custards do you eat? How frequently?								

	Frequency						Product / Ingredient names	Manufacturer contact details
	Daily	Few days	Weekly	Monthly	<Monthly	Never		
Which flours, starches or vinegar's do you use in home-made sauces? Indicate frequency?								
List the commercial sauces (bottled or pre-mixed) you use and their frequency.								
List the stock cubes or commercial liquid or powdered stocks & gravies you would purchase. Indicate how frequently you would use them.								
List the frequency and brand names of the commercial dressings you use.								
Which flours starches or vinegars do you use in home-made dressings? Indicate frequency of use.								

	Frequency	
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	Daily	Few days	Weekly	Monthly	<Monthly	Never	Product / Ingredient names	Manufacturer contact details
List the brands of commercial spreads you use and indicate frequency. E.g. Honey, peanut butter, cheese spread, etc								
Indicate which processed or deli meats you purchase and how frequently they are eaten.								
List the frequency and brands of the commercial, prepared frozen meals that are eaten								
List any 2 or 3 minute rice snacks that are eaten. Indicate frequency.								
List the chocolates that you commonly snack on. Indicate frequency where possible.								

	Frequency						Product / Ingredient names	Manufacturer contact details
	Daily	Few days	Weekly	Monthly	<Monthly	Never		
List other snack items that you consume and indicate frequency. E.g.: crisps, ice-blocks, health bars, lollies.								
List the frequency of taking either wheat or rice based communion wafers.								
List the brands and frequency of the alcoholic beverages that you drink.								
If you eat oats. List the brands and frequency of eating								

Please fill out this section similarly to the example shown here

EATING OUT	Frequency						Food description	You sometimes suspect that small amounts of gluten may be in the.....
	Daily	Few days	Weekly	Monthly	<Monthly	Never		
Restaurant example			√				Thai: boiled rice, satay beef and stir fry veges.	Soy sauce
				√			Pub: Meat, salad & vegetables. Ice-cream	Gravies, sauces or salad dressings
Restaurant								
Take away shops								

When eating out: what do you do if you *feel* something *may* contain gluten?

a). at restaurants:

b). at friends houses:

Please fill out this section similarly to the example shown here.

MEDICATIONS	Frequency						Product name and amount taken	Manufacturer contact details
	Daily	Few days	Weekly	Monthly	<Monthly	Never		
Medications Example:	✓						Oroxine 100mcg/day	Glaxo Smith Kline Ltd: 3 97295100
	✓						Caltrate	Lederle: 5 Gibbon Rd, Baulkham Hills NSW
Medications								
Vitamin and Mineral supplements								

Have you eaten gluten of some sort in the last 3 months? Y / N If so what was it? _____

On what type of occasion would you knowingly eat gluten?

How frequently does this occur? _____

What is it that you are eating? _____

Gluten Intake Diary

NAME: _____

DATE: _____

Please write down in this diary any time over the next 2 months when you

1. have knowingly eaten gluten-containing foods, or
2. if you think you may have accidentally had some gluten
 - record what the food was
 - record how much of the food was eaten
 - record if it was at a restaurant, home or other
 - record any symptoms that may have occurred due to this ingestion
 - *the approximate time of the onset of symptoms after eating*
 - *severity (in brackets), graded 1 - 3:*
 - (1) *Mild* *Aware of the symptom, but it is easily tolerated.*
 - (2) *Moderate* *Enough to cause interference with daily life or usual activities*
 - (3) *Severe* *Incapacitating, with inability to work or to take part in usual activities.*
3. If you have been allowed to eat oats, malt extract or occasionally wheat starch, please record each time you ate products containing these AND how much you ate.

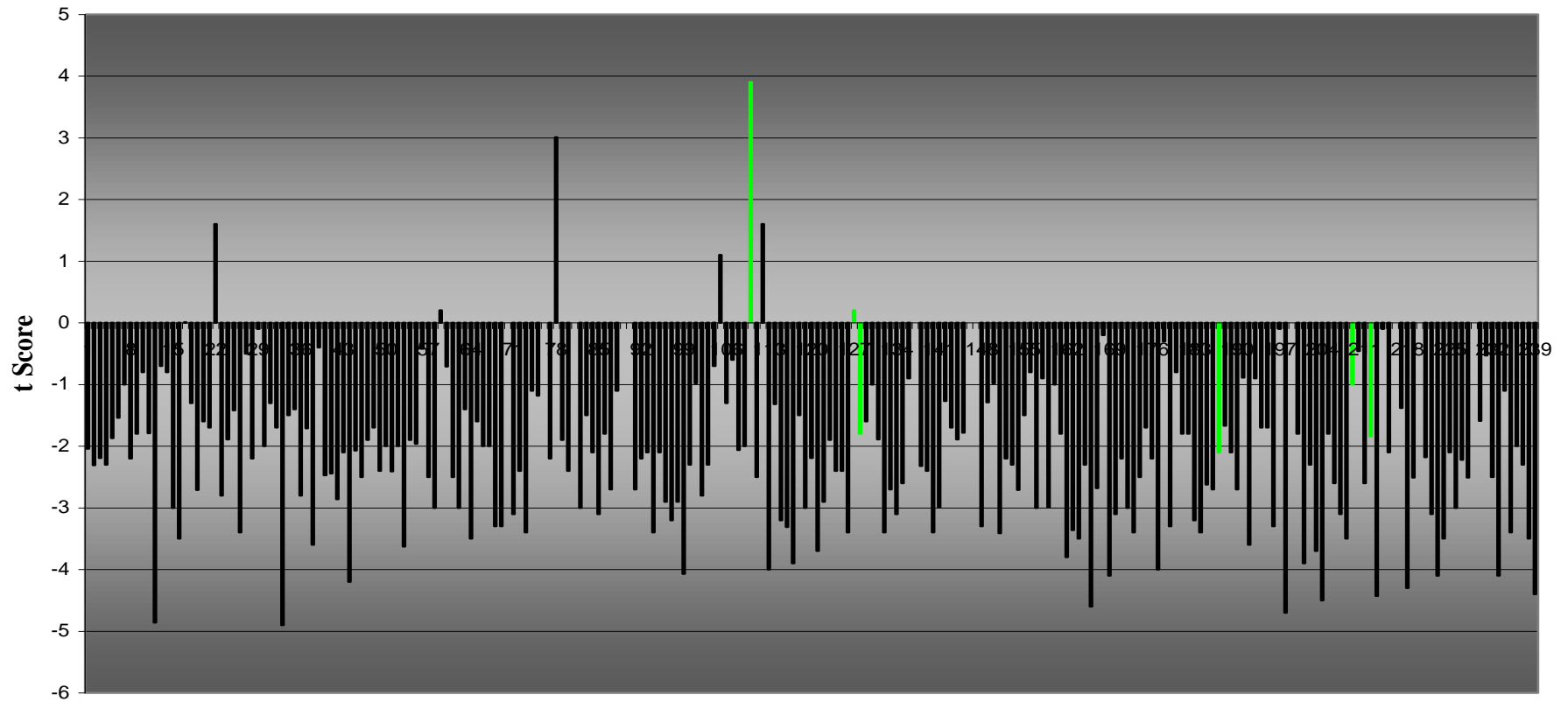
Please keep and complete this diary over the next 2 months. Every 2 months you will receive in the mail a new “*Gluten Intake Diary*” and a “*One Week Food Diary*”. Please complete the “*One Week Food Diary*” and return it to Kim Faulkner-Hogg (in the reply paid envelope) with your completed 2 month “*Gluten Intake Diary*”.

Day & Date	Incidence where gluten was or may have been eaten (Type, amount, where)	Symptom & time of onset
Thursday: Jan 1st 2004	New Years fruit cake, small slice (~50g). Eaten at friends party	No symptoms
Tuesday 4.2.2004	Ate at a restaurant. It should have been a gluten free meal. Chicken stuffed with avocado and camembert; baked potato, beans, carrots, corn. Gelato icecream for dessert.	Felt ill 2 hours later
Thursday 19.2.2004	1 Mc Donalds Mc Oz meal with fries and coke.	5 hours later: pains in the stomach (1).
Wednesday 11.2.2004	1/12 cups of Uncle Toby's traditional oat porridge	No symptoms

Day & Date	Incidence where gluten was or may have been eaten (Type, amount, where)	Symptom & time of onset

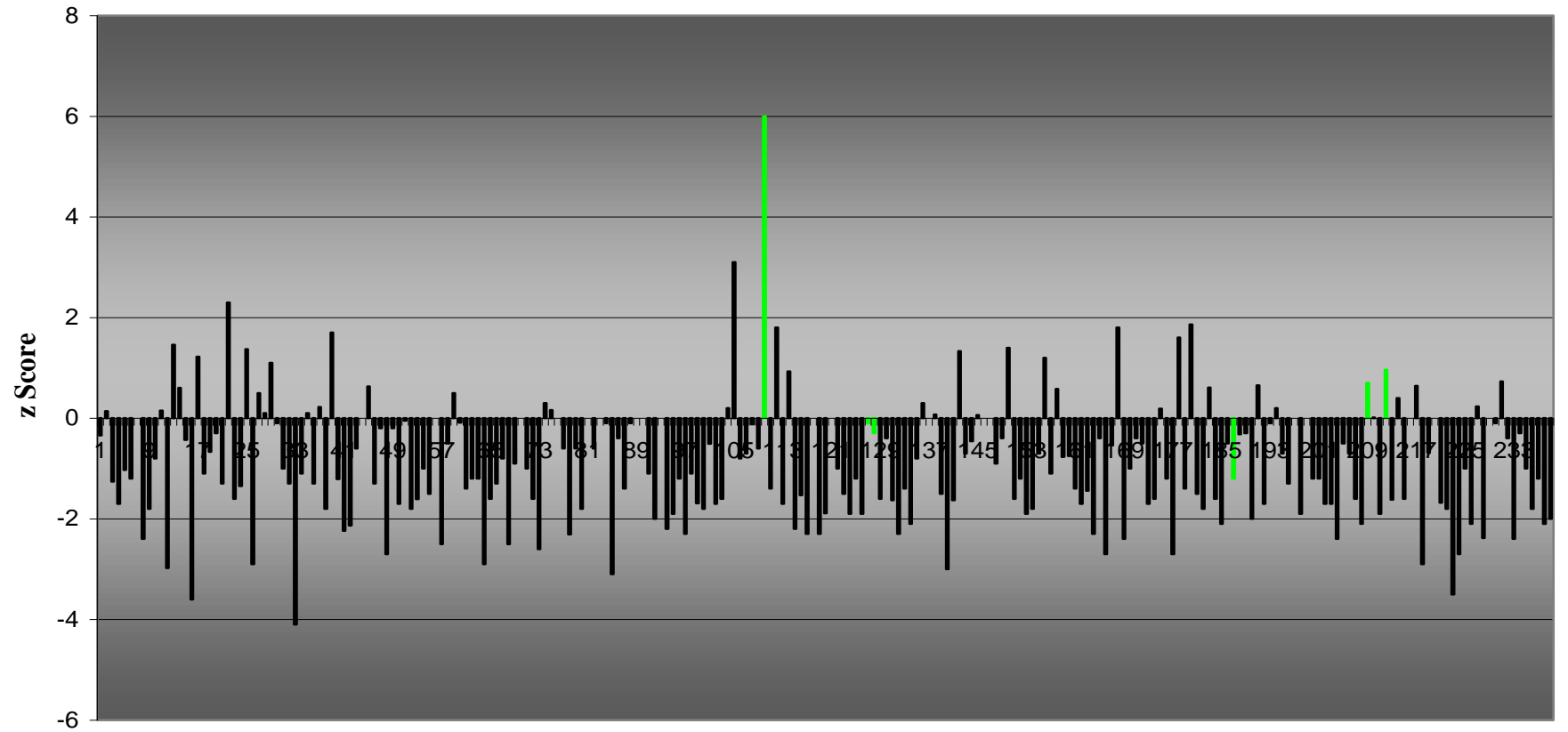
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Royal Prince Alfred Hospital, Camperdown NSW 2050

Lumbar (L2-L4) Spine t Scores of Decreased BMD Subjects



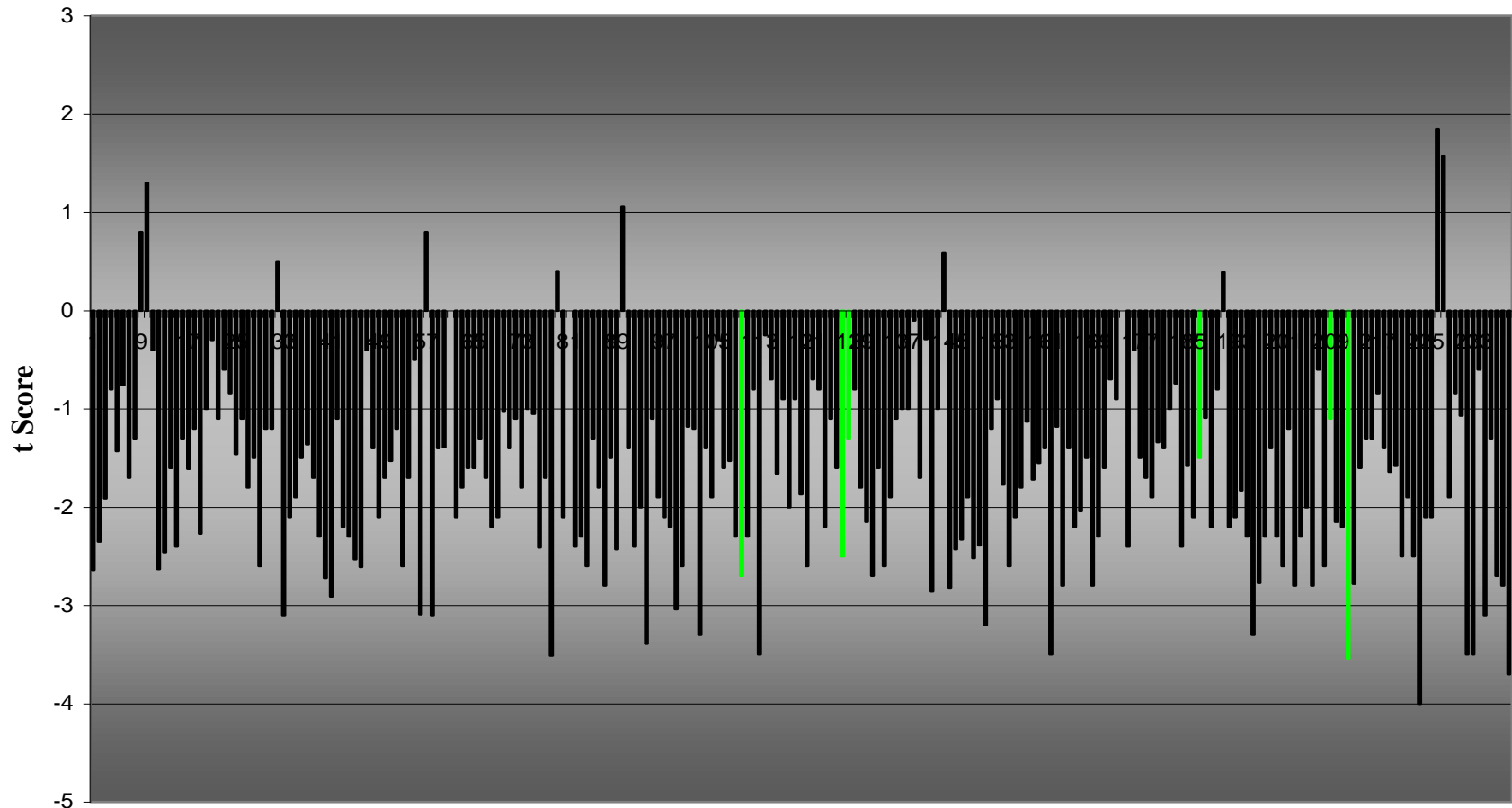
Biopsy proven CD Patients


Lumbar Spine (L2-L4) z Scores of Decreased BMD Subjects



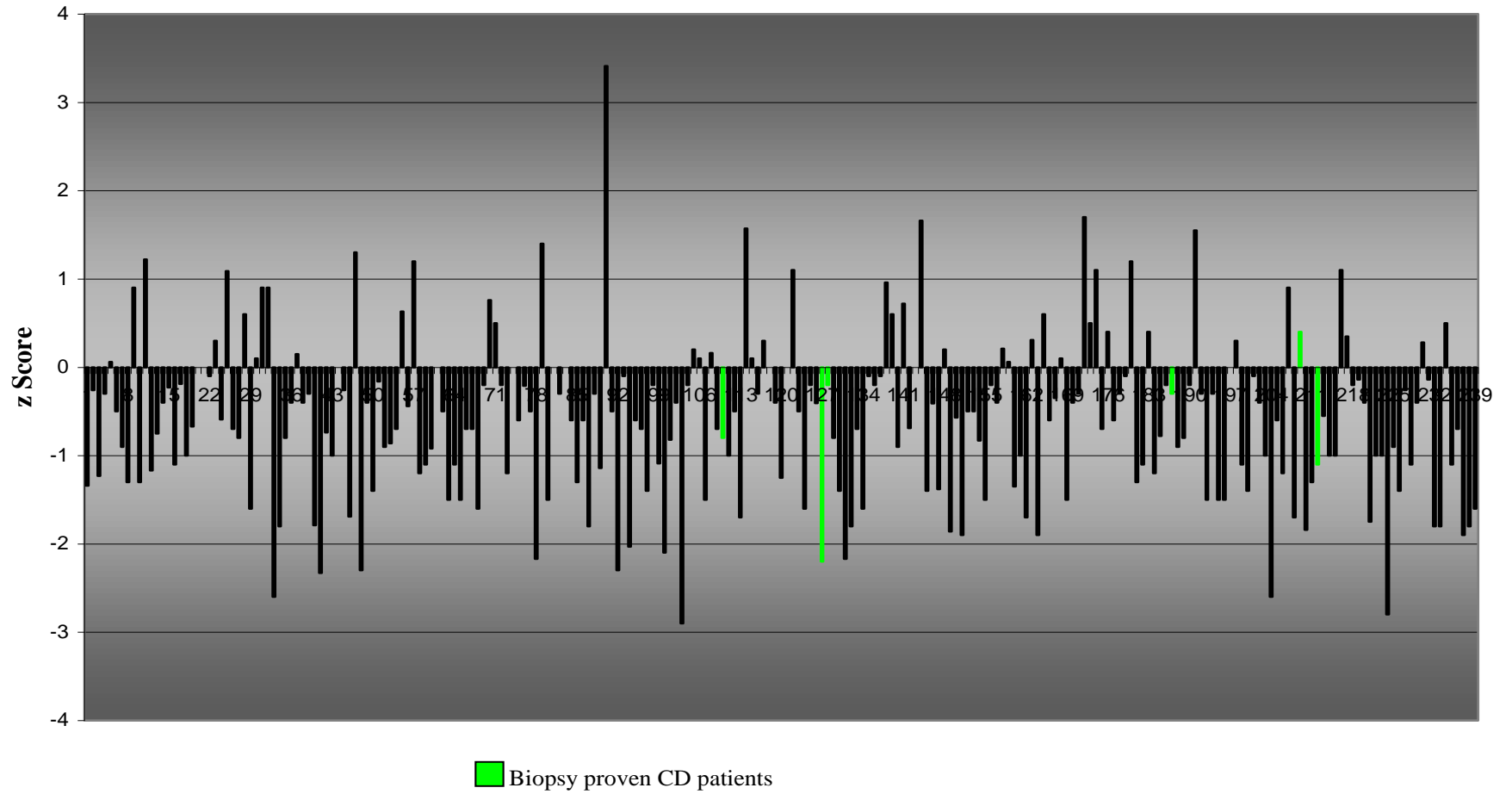
■ Biopsy proven CD patients

Femoral Head (Hip) t Scores of Decreased BMD Subjects

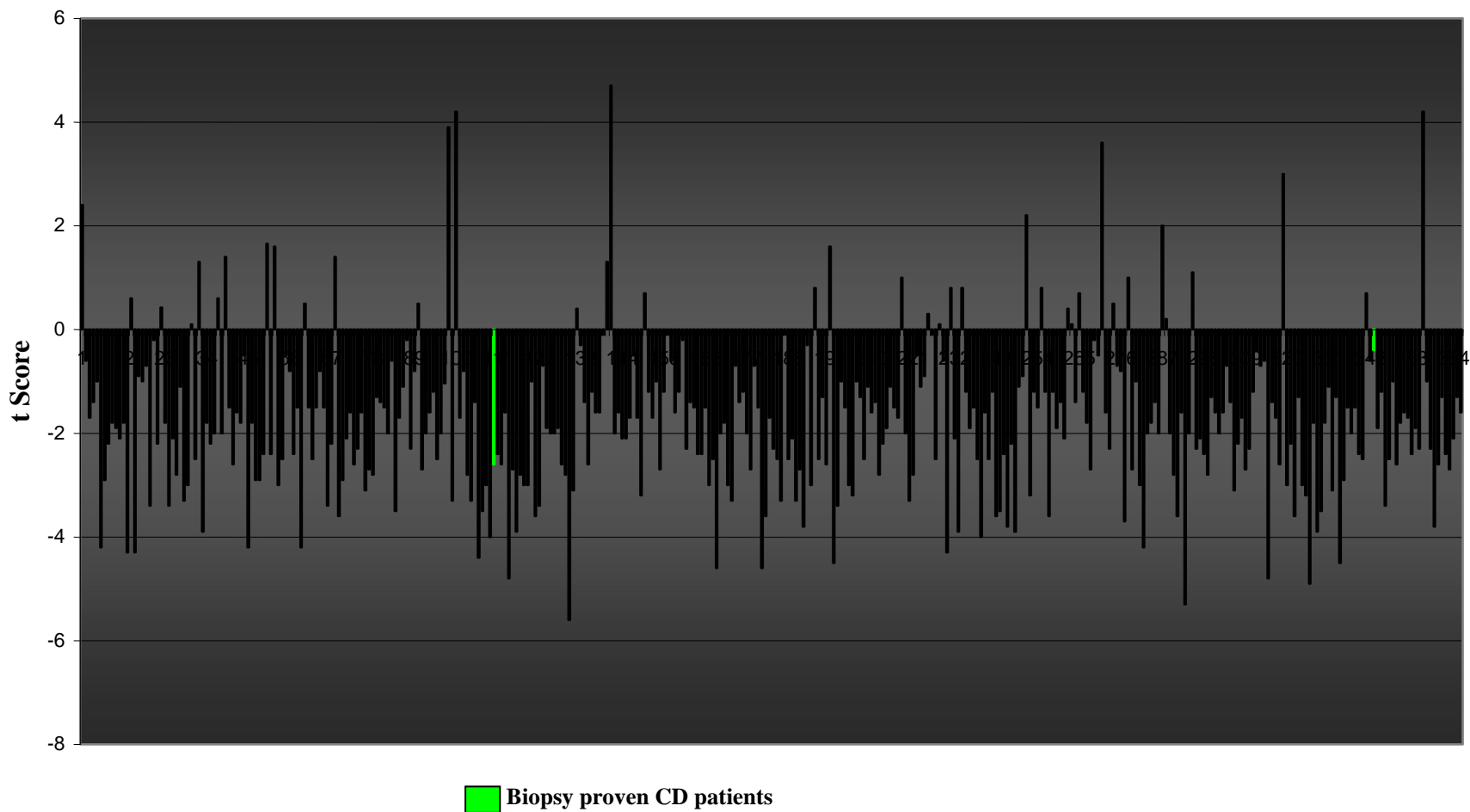


 Biopsy proven CD patients

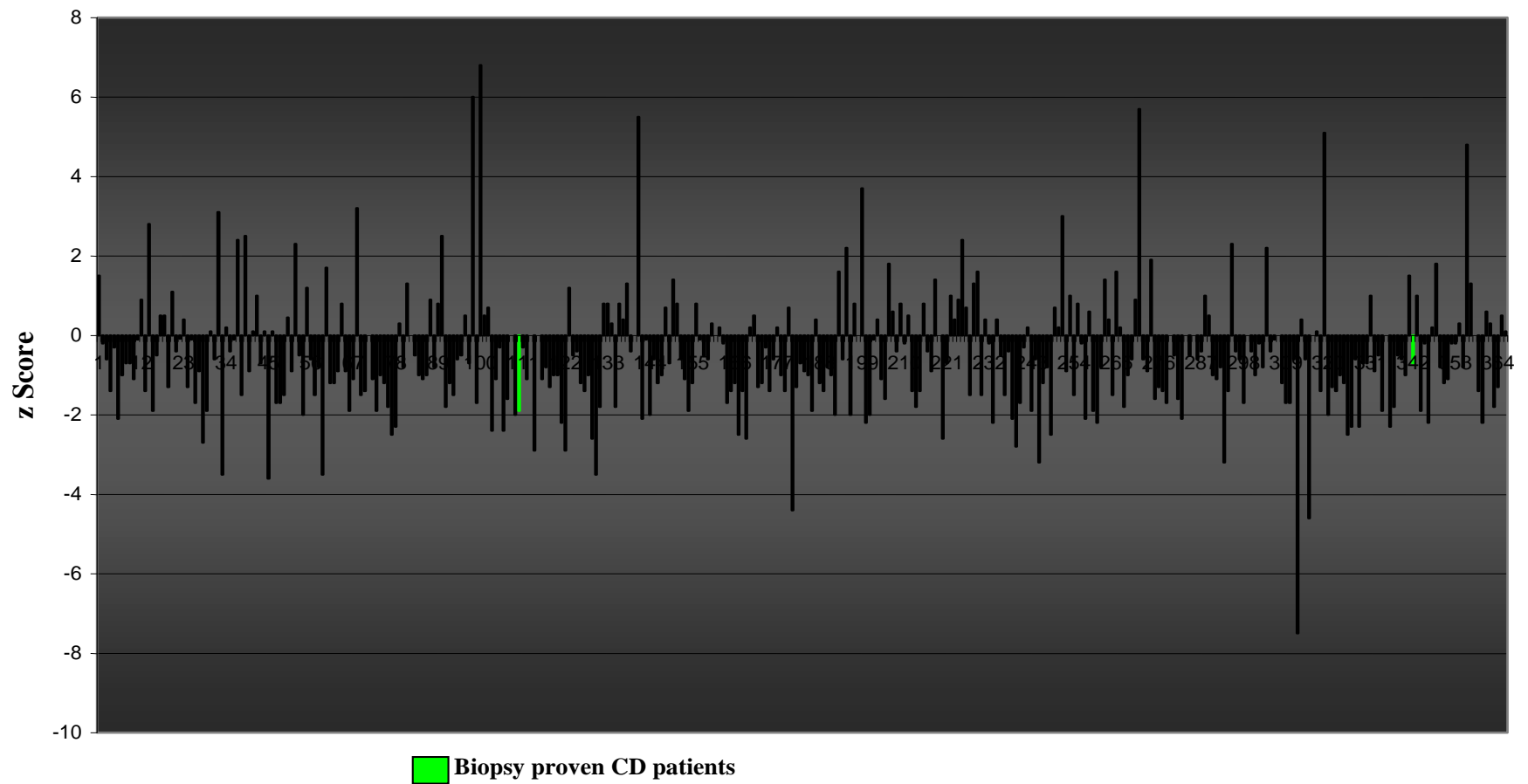
Femoral Head (Hip) z Scores of Decreased BMD Subjects



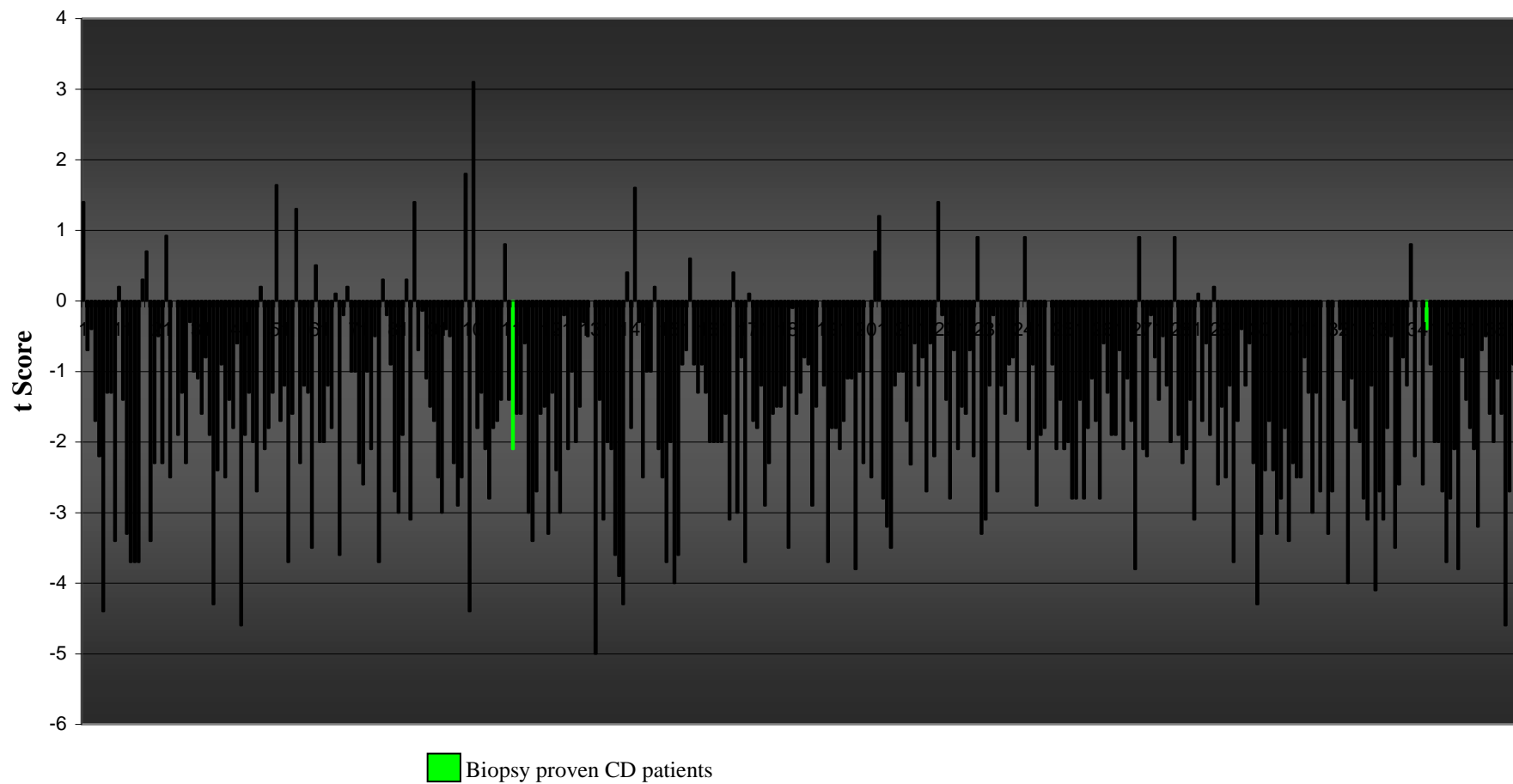
Lumbar Spine (L2-L4) t Score for Subjects with Low Trauma Fractures



Lumbar Spine (L2-L4) z Score for Subjects with Low Trauma Fractures



Femoral Head (hip) t Scores of Subjects with Low Trauma Fractures



Femoral Head (hip) z Scores for Subjects with Low Trauma fractures

