MORBIDITY ASSOCIATED WITH COELIAC DISEASE

Dr Nina Ruth Lewis
BMedSci, BM BS, MRCP, MSc

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Abstract

Background

Historically considered a rare disorder, it is now appreciated that coeliac disease is a major health problem affecting 1% of the population. The ability to screen for coeliac disease non-invasively and on a large-scale with the development of highly sensitive and specific serological tests has helped crystallise the coeliac iceberg of contemporary disease. Clinically overt coeliac disease only makes up the tip of this iceberg and accounts for only the minority of cases of coeliac disease. The majority of coeliacs in comparison have few obvious symptoms despite the presence of the enteropathy, have atypical symptoms or have physiological derangements such as iron deficiency anaemia.

Recent population-based studies have provided more robust estimates of risks traditionally associated with clinically overt coeliac disease such as mortality, malignancy and fracture. However other morbidity and perhaps potential benefits associated with the spectrum that is contemporary coeliac disease and the effect of treatment need clarification. The benefits and possible harm of detection and treatment of coeliac disease in otherwise asymptomatic, healthy people or those presenting with non-classic features or mild enteropathy disease is also not clear.

The rate of diagnosis of coeliac disease in developed countries has increased dramatically since the introduction of serological tests without an obvious environmental precipitant.

The principal aspect of this thesis is to examine the vascular, hepatic and psychosocial profile in people with contemporary coeliac disease to clarify the morbidity and perhaps potential benefits associated with contemporary coeliac disease. The physiological derangements and morbidity of mild versus severe enteropathy coeliac disease; and coeliacs presenting with classic disease, silent disease or gastrointestinal symptoms will be compared. The benefits and possible harm of detecting contemporary coeliac disease will also be explored by examining the effect of treatment with a gluten-free diet. Reported possession of conventional breast cancer risk factors by female coeliacs will be examined and compared to those possessed by the general population to further explore potential explanations for the apparent 50% reduced risk of breast cancer in women with coeliac disease. The socio-economic distribution of people with incident coeliac disease will also be examined to improve our understanding of the aetiology of the disorder in a further part to the thesis.

Objectives

1. To describe the relationship between degree of enteropathy and physiological derangement, clinical features in incident coeliac disease
2. To examine the incidence of clinically diagnosed coeliac disease by socio-economic status
3. To quantify the impact of diagnosed coeliac disease on the risk of:
    - hypertransaminasaemia
    - hypercholesterolaemia
4. To estimate the vascular risk profile at diagnosis of coeliac disease and quantify any change following treatment with a gluten-free diet

5. To estimate the quality of life at diagnosis of coeliac disease and observe any change following exposure to a gluten-free diet
6. To describe the breast cancer risk profile in women with coeliac disease and compare to that of the general population

Methods

To examine objectives 1, 2 and 3 I generated a historical cohort of people who had been diagnosed with coeliac disease at Nottingham and Sheffield. Dietetic, histopathology, immunology, clinical coding and outpatient records were used to retrospectively identify incident cases of coeliac disease at Nottingham University Hospital and Royal Hallamshire Hospital, Sheffield. I identified 1008 adults with incident coeliac disease between 1st January 2000 and 31st December 2006 at these centres that made up this historical cohort. Demographic, clinical, histological and laboratory data were collected on these identified incident cases of coeliac disease through systematic collection.

Using a longitudinal, observational cohort study design, objectives 3 and 4 were examined. Consecutive cases of incident coeliac disease were identified at Derby Nottingham, Sheffield study centres using clinical alerts and records; dietetic alerts and records; histopathology and immunology databases. Extensive efforts were made to identify all incident adults with coeliac disease at these three centres to help generate an unselected, large and contemporary cohort. Data was systematically collected on the vascular risk profile and health-related quality of life in adults newly diagnosed with coeliac disease and any change following treatment with a gluten-free diet determined.

Objective 5 was studied in a cross-sectional, questionnaire-based survey where the reported possession of conventional breast cancer risk factors by female coeliacs were systematically collected and compared to those possessed by the general population. Female coeliacs that were members of Coeliac UK (population-based cohort) and identified female coeliacs that have attended between 1st January 2000 – 31st December 2006 Nottingham University Hospital, Nottingham; Royal Hallamshire Hospital, Sheffield; or Derby Hospitals NHS Foundation Trust for management of their coeliac disease (historical hospital-based cohort) formed the study population. Female coeliacs with either incident or prevalent coeliac disease were identified using clinical alerts and records; dietetic alerts and records; histopathology and immunology databases. Coeliac UK, the principal national society for people with coeliac disease, has over 70,000 registered members from which we selected a random sample of 9000 women from those women identified as being over the age of 35 years who on their membership information had registered a current UK postal address and they had reported that they have coeliac disease.

Findings

Coeliacs with mild enteropathy have few biochemical deficiencies at diagnosis of coeliac disease and therefore show no important biochemical improvements following treatment with a gluten-free diet in comparison to those with severe enteropathy coeliac disease. Approximately one-third of coeliacs with mild enteropathy coeliac disease had negative EMA serology at diagnosis and had significantly lower tTG values in comparison to those with severe enteropathy coeliac disease. Diarrhoea was the most common symptom reported in adults being diagnosed with mild enteropathy coeliac disease and more common than that observed in severe enteropathy. Iron

deficiency anaemia was much less common in mild enteropathy compared to severe enteropathy.

There was a strong, independent graded association between the incidence rate of new diagnoses of coeliac disease and socio-economic status with the rate twice as high in adults from affluent areas compared with that in adults living in poorer areas. Socioeconomic status was not associated with features of more severe coeliac disease.

Hypertransaminasaemia was uncommon (<2%) in newly diagnosed adults with coeliac disease and in those patients with an abnormal test 86% normalised following a year of treatment with a gluten-free diet. The presence of elevated transaminases in incident coeliac disease was associated independently with clinical features of malabsorption and more severe histological features of intestinal inflammation on duodenal biopsy.

At diagnosis coeliacs have much lower total cholesterol levels than the general population with the observed reduction greater in men (21%) than in women (9%) with no increase in total cholesterol observed on treatment with a gluten-free diet. Furthermore, HDL cholesterol showed a small but statistically significant increase following treatment.

The observed vascular risk profile in our study suggests both protective and adverse associations of coeliac disease. The lower mean levels of total cholesterol, LDL cholesterol, fibrinogen; the higher likelihood of being from more affluent social class; and the small but significant rise in HDL cholesterol and reduction in blood pressure

amongst coeliacs presenting with gastrointestinal symptoms observed following treatment with a gluten-free diet suggests coeliacs have favourable vascular risk profile features in comparison to the general population. However, the higher likelihood of having abdominal truncal obesity amongst incident coeliacs that only worsens following treatment with a gluten-free diet together with the higher proportion of measured systolic hypertension amongst male coeliacs suggests that there are also potentially adverse vascular risk profile features associated with coeliac disease.

Though incident coeliacs with silent disease reported no change in their quality of life prior to diagnosis of coeliac disease, silent coeliacs were as likely to have villous atrophy and physiological derangement to those coeliacs presenting with symptoms or with classic features of coeliac disease. The quality of life reported by coeliacs presenting with silent disease, classic disease and with gastrointestinal symptoms was worse than that observed in the general population. A year's treatment with a gluten-free diet resulted in coeliacs having similar or in some components better quality of life than that observed in the general population. The rate of change of quality of life was similar amongst those coeliacs with silent, classic or symptomatic disease.

The breast cancer risk profile suggests both protective and adverse associations of coeliac disease. The higher proportion of women being parous, having their first full-term pregnancy before 30 years and breastfeeding in addition to the younger mean age at menopause suggests women with coeliac disease have favourable breast cancer risk profile features in comparison to the general population. However, the higher likelihood of being Caucasian and of affluent social class together with higher

proportion having early menarche and irregular menstrual cycles suggests there are also potentially adverse breast cancer risk profile features associated with coeliac disease.

Conclusions

Persons with mild enteropathy disease have few physiological derangements at diagnosis of coeliac disease and show no important biochemical change following treatment with a gluten-free diet in comparison to those with severe enteropathy coeliac disease. The prevalence of hypertransaminasaemia is lower than previously reported which may be reflective of differences in study design or contemporary coeliac disease involves a milder spectrum of disease. The observed vascular and breast cancer risk profile suggests both protective and adverse associations of coeliac disease and on treatment with a gluten-free diet results in an attenuation or indeed reversal of the vascular risk profile in some co-variates. Silent coeliac disease is associated with a reduction with quality in life which improves like in symptomatic and classic disease with treatment with a gluten-free diet. Incident coeliac disease is associated with more affluent social class.

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Chapter one: What is coeliac disease?

1.1. Overview

Previously regarded as a rare disorder, we now appreciate coeliac disease is common affecting 1% of the general population though the morbidity and perhaps potential benefits associated with contemporary disease needs clarification. The benefits and possible harm of treatment of contemporary coeliac disease in otherwise asymptomatic, healthy people or those presenting with non-classic features is not clear. The rate of diagnosis of coeliac disease in developed countries has increased dramatically since the introduction of serological tests without an obvious environmental precipitant.

One aim of this thesis is to further explore the morbidity of contemporary coeliac disease by examining the risk of liver disease, hypercholesterolaemia and physiological derangement in a historical cohort of adults with clinically diagnosed coeliac disease. The effect of treatment of coeliac disease on identified hepatic and vascular morbidity will be explored. Clinical and physiological derangements of mild versus severe enteropathy coeliac disease will be contrasted using this historical cohort. The socio-economic distribution of people with incident coeliac disease will also be examined in this historical cohort to improve our understanding of the aetiology of the disorder. The health-related quality of life in an unselected, representative and contemporary cohort of people with incident coeliac disease presenting with gastrointestinal symptoms, classic or silent disease will be compared and also the effect of treatment of the disease will be examined in a longitudinal study. This longitudinal study will also examine the possible vascular benefits of having coeliac disease and to estimate the effect of treatment upon the vascular risk

profile. In a cross-sectional, questionnaire-based survey, reported possession of conventional breast cancer risk factors by female coeliacs are examined and compared to those possessed by the general population to further explore potential explanations for the apparent 50% reduced risk of breast cancer in women with coeliac disease.

To understand the rationale for these studies this introductory chapter 'What is coeliac disease?' describes how coeliac disease is defined and diagnosed, and the aetiology and clinical manifestations of the disease. The introduction will also describe what is already known and what is not known about the occurrence and the impact of both undetected and clinically diagnosed coeliac disease. The aetiology of breast cancer in the general population and how this may differ in women with coeliac disease is discussed. This section will end with objectives of the thesis.

1.2. Definition

Coeliac disease is regarded by most as an immune-mediated disease of the small intestinal mucosa that results from exposure to dietary gluten in genetically susceptible individuals [1]. It is characterised by a chronic inflammatory state of the small intestinal mucosa that heals when gluten-containing foods are excluded from the diet and returns when gluten-containing foods are reintroduced.

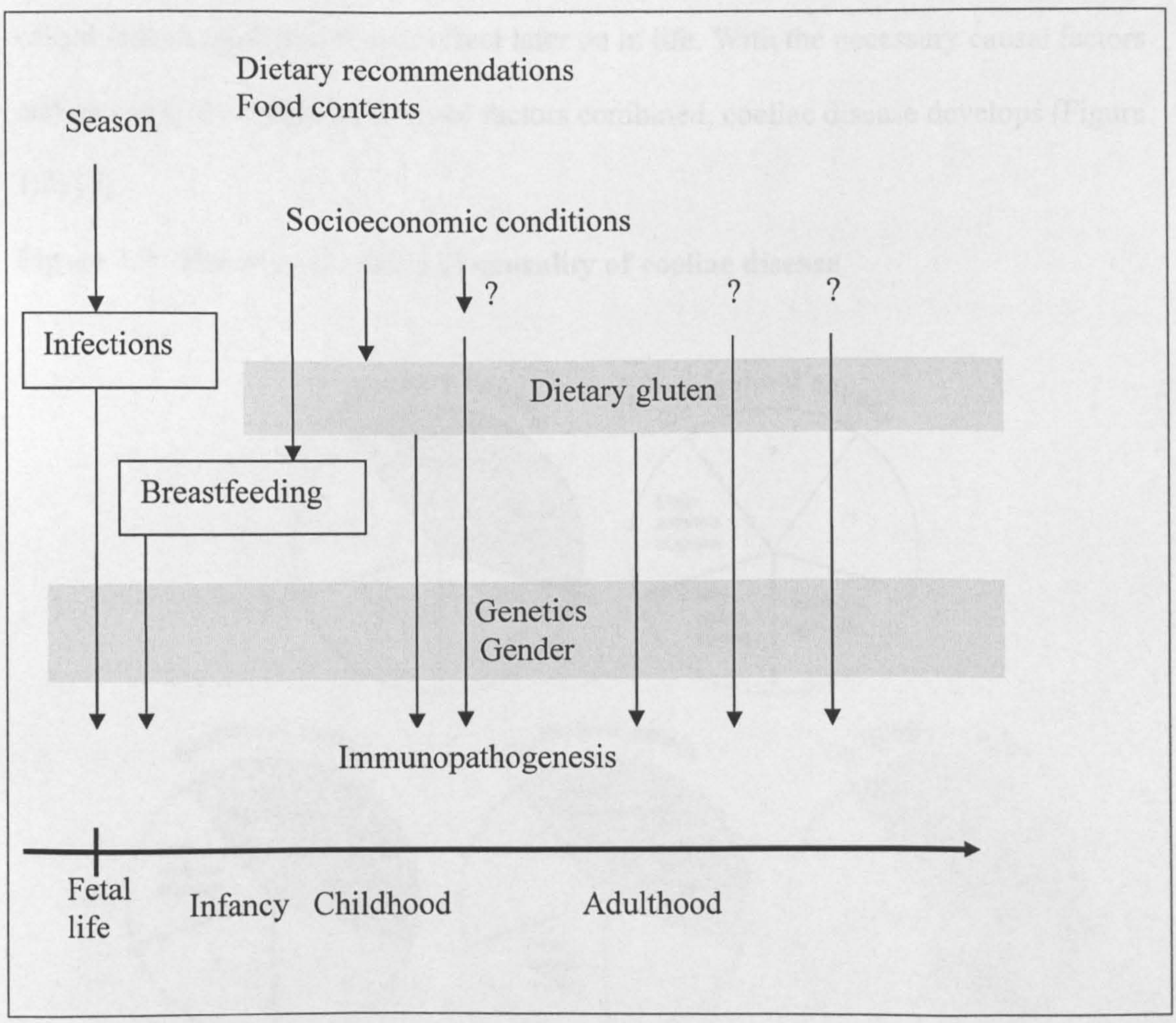
1.3. Aetiology

Coeliac disease is most likely to have a multi-factorial aetiology with interactions between dietary gluten, immune, genetic, and environmental factors conferring either increased or reduced disease risk [1]. Throughout life, possibly even during fetal life, an individual's genetic predisposition interacts with the environment by means of

continuous and varying exposures, and jointly shapes the immunological response to dietary gluten. In most individuals, oral tolerance develops to gluten and prevails throughout life. However if tolerance fails to develop or is later broken down, then the gluten may act as a 'dangerous' foreign antigen with the resulting development of coeliac disease. Coeliac disease could then be viewed as a failure of oral tolerance to gluten.

With particular emphasis on environmental exposures, a simplified and hypothetical model of the multifactorial aetiology of coeliac disease [2] is shown in this figure:

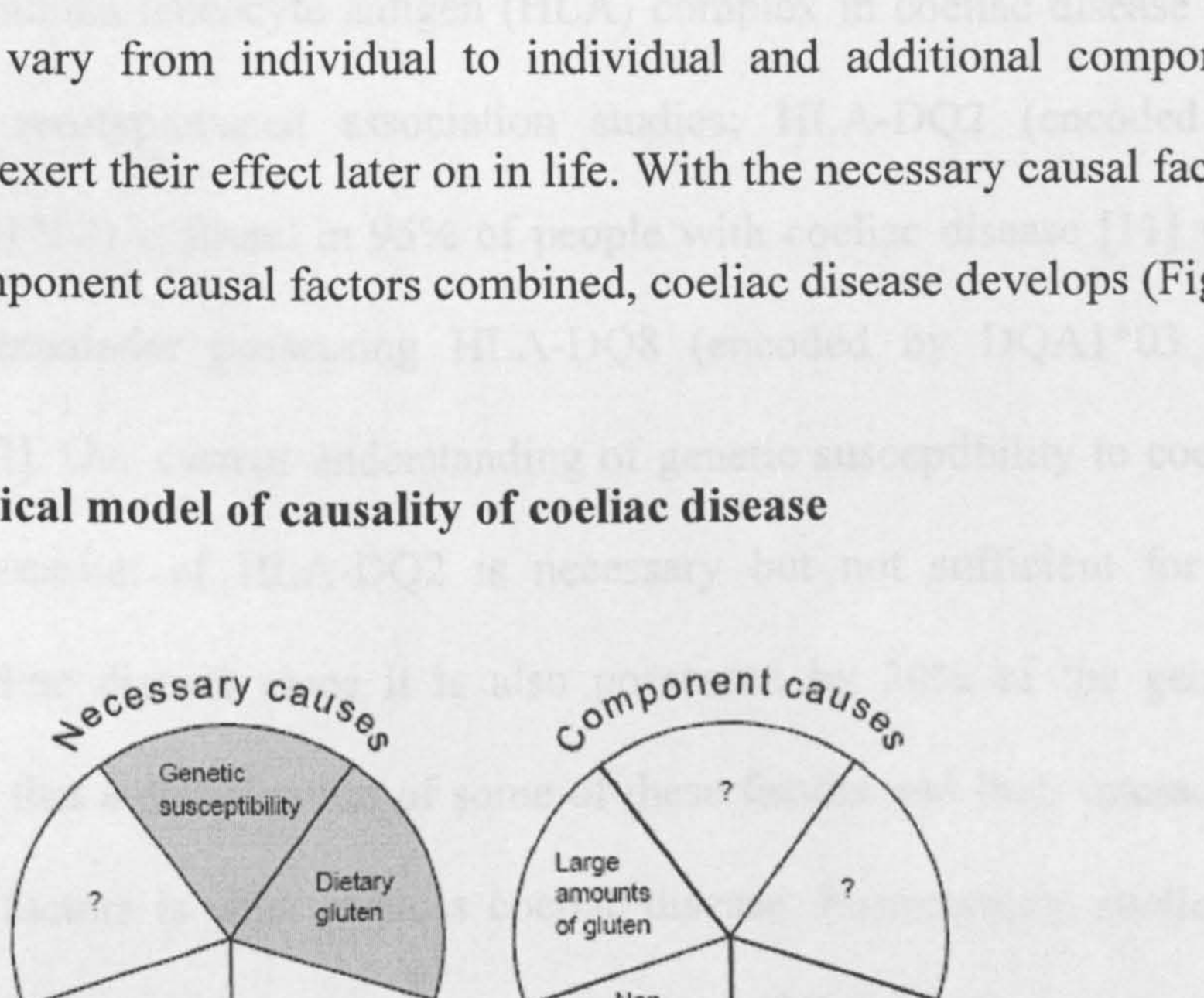
Figure 1.1: Proposed causal model of multifactorial aetiology of coeliac disease



In this multifactorial model there are directly causal factors, defined depending on their role in disease development as either necessary or contributing factors [3]. There are also structural, component and associated causal factors.

A genetic susceptibility and the presence of dietary gluten are considered to be necessary causal factors as without these factors coeliac disease will not develop [4]. However, component causal factors also contribute such as whether breastfeeding is still ongoing during the period in which gluten is introduced into the diet, the amount of gluten ingested during this period [5], and whether or not the infant has repeated infectious insults early in life [6, 7]. However, the combination of these component causal factors may vary from individual to individual and additional component causal factors might exert their effect later on in life. With the necessary causal factors and one or more component causal factors combined, coeliac disease develops (Figure 1.2) [3].

Figure 1.2: Theoretical model of causality of coeliac disease



This model implies that the coeliac disease onset can be avoided by excluding a necessary cause such as excluding dietary gluten or excluding one or more of the component causes such as the consumption of large amounts of gluten.

1.3.1. Necessary causal factors

1.3.1.1. Genetic susceptibility

A genetic susceptibility is considered to be a necessary causal factor for developing coeliac disease [4]. First degree relatives of people with coeliac disease have a 10% risk of developing the condition [8-10] which is ten times higher than that of the general population. The concordance rate in monozygotic twins is 70% [9, 10]. Involvement of the human leucocyte antigen (HLA) complex in coeliac disease was first suggested by serotype-based association studies; HLA-DQ2 (encoded by DQA1*05 and DQB1*02) is found in 95% of people with coeliac disease [11] with nearly all of the remainder possessing HLA-DQ8 (encoded by DQA1*03 and DQB1*0302) [11, 12]. Our current understanding of genetic susceptibility to coeliac disease implies possession of HLA-DQ2 is necessary but not sufficient for the development of coeliac disease since it is also possessed by 30% of the general population, suggests that a combination of some of these factors and their interaction with environmental factors is what induces coeliac disease. Furthermore, studies in siblings and identical twins suggest that the contribution of HLA genes to the genetic component of coeliac disease is less than 50% [13]. Several non-HLA genes including six regions (CCR3, IL12A, IL18RAP, RGS1, SH2B3 (nsSNP rs3184504) and TAGAP) that control immune responses involved in coeliac disease pathogenesis have recently been identified [14].

1.3.1.2. Gluten

Probably one of the most important steps in coeliac disease history occurred when the Dutch paediatrician, Willem Karel Dicke, demonstrated that the malabsorption in coeliac disease was 'elicited or aggravated by certain types of flour, especially wheat and rye flours' [15]. Nowadays, it is well established that coeliac disease is strictly dependent on exposure to wheat gluten and related proteins in rye and barley. Dietary gluten (such as the 33-amino acid peptide sequence PFPQPQLPY PQPQLPYPQ PYPQPQLPY rich in proline and glutamine derived from α-gliadin) is necessary both to initiate and to maintain the disease process [16]. This response to dietary gluten is mediated by both the adaptive and innate immune systems.

Gliadin peptides, derived from partial digestion of dietary gluten, pass through the epithelium of the small intestinal mucosa where they become bound to the HLA-DQ2 (or HLA-DQ8) receptor situated on the surface of antigen-presenting cells. Native gliadin has relatively poor affinity for the HLA-DQ2 (or HLA-DQ8) receptor so undergoes modification by means of deamidation to enhance its affinity [17]. This deamidation is catalysed by tissue transglutaminase [17, 18]. Deamidation also results in a greatly enhanced T cell response [17, 18]. The deamidated gliadin, with its enhanced affinity, becomes bound to the HLA-DQ2 (or HLA-DQ8) receptor on the antigen-presenting cell, and is then presented to sensitised mucosal CD4+ T cells resulting in both the production of proinflammatory cytokines [4, 19] that cause characteristic tissue damage such as villous atrophy and the activation and expansion of B cells that produce antibodies to gliadin.

Gliadin peptides also activate an innate immune response in the intestinal epithelium that is characterized by increased expression of interleukin-15 by enterocytes,

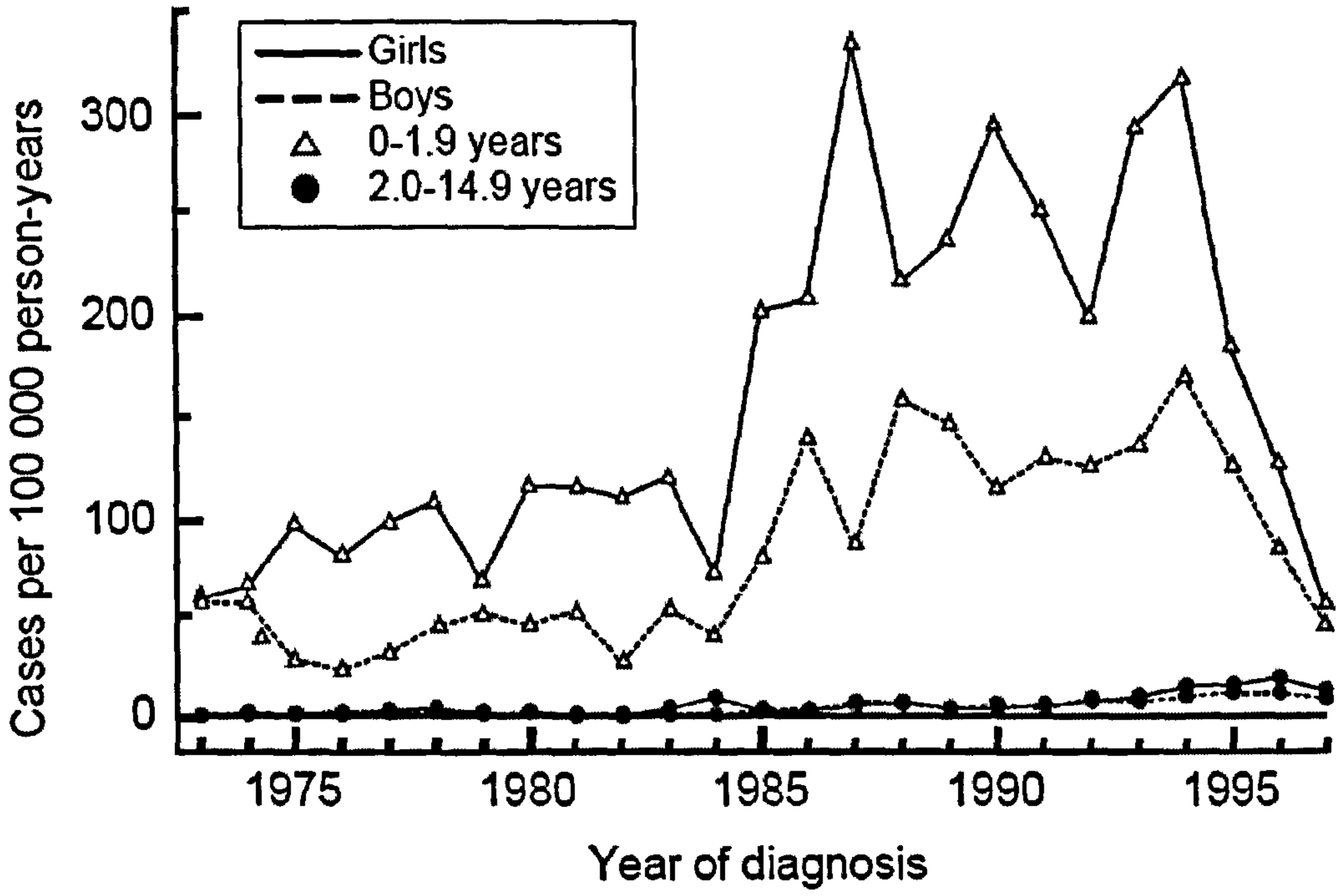
resulting in the activation of intraepithelial lymphocytes expressing the activating receptor NK-G2D, a natural-killer-cell marker [20]. These activated cells become cytotoxic and kill enterocytes with surface expression of major-histocompatibility-complex class I chain related A (MIC-A) which is a cell-surface antigen induced by stress, such as an infection [21, 22].

1.3.2. Component causal factors

1.3.2.1. Breastfeeding and infant feeding practices

It was suggested as early as the 1950s that breast-feeding could delay the onset of coeliac disease, and possibly also reduce the overall risk [23]. In the 1970s there were reports from England, Ireland and Scotland that there appeared to be a decline in the incidence of childhood coeliac disease [24-26]. Changes in infant feeding practices (including breastfeeding for at least two weeks and preferably for four months, avoidance of solids before the age of four months, use of infant formulas, avoidance of cereals being added to the milk in a bottle feed) in Britain were suggested as a possible explanation for the decline [27]. However there was no change in incidence in Sweden with similar infant feeding recommendations [28]. In a series of population-based studies carried out in Sweden during the 1980s and 1990s (an era of high breastfeeding and late gluten introduction) examining national breastfeeding practices and infant feeding on incidence of childhood coeliac disease, Ivarsson et al observed a rise in incidence of coeliac disease that was preceded by an increase in the amount of gluten consumed [7]. Though the increased use of antigliadin antibodies during the 1980s to screen for coeliac disease probably contributed to the rise in incidence, the majority of the diagnosed cases were identified because of symptoms [29].

Figure 1.3: Incidence of childhood coeliac disease in Sweden



The risk for coeliac disease was reduced when gluten-containing foods were introduced into the diet while the infant was still breast-fed [adjusted odds ratio (OR) 0.59; 95% CI: 0.42, 0.83], an effect even more pronounced in infants who continued to be breast-fed even longer [OR 0.36; 95% CI 0.26, 0.51] [5].

The dose of dietary gluten and hence antigen ingested may influence whether or not oral tolerance develops [30]; a larger consumption of wheat gluten was reported for healthy infants in Sweden and Italy as compared to Finland, Denmark and Estonia with higher incidence of coeliac disease reported in Sweden and Italy [31, 32]. In an incident case-referent study introduction of gluten-containing foods in large amounts, as compared to small or medium amounts, was observed to be an independent risk factor for coeliac disease development [adjusted OR 1.5, 95% CI 1.1–2.1] [5]. However, age of the infant at introduction of gluten-containing food was not an independent risk factor, nor was the type of food used as the source of gluten, i.e.

solid foods or follow-on formula. It is, however, not clear whether there is a direct dose-response effect or a threshold effect. Furthermore, it seems likely that the amount of gluten tolerated varies with the genetic predisposition of the individual and other environmental exposures.

Two Italian case-control studies examined infant feeding practices in relation to the risk of developing childhood coeliac disease [33, 34]. Feeding practices in 216 children with coeliac disease with compared with those used for their siblings. Siblings eating gluten within the first two months of life had a slightly greater risk of developing coeliac disease than those who were started on gluten from age of 3 months [34]. A two-fold increase in risk for developing coeliac disease was observed when gluten had been introduced before the end of the second month of life on examining feeding practices in 201 children with coeliac disease and comparing them with 1949 non-coeliac and unrelated children though reporting was based on parents' recall and possibly subject to reporting bias [33].

Infections could also potentially contribute to the aetiology of coeliac disease. This was proposed initially because sequence similarities were identified between proteins of gluten and proteins produced during adenovirus infections [35]. Observational data suggested that children who experienced three or more infectious episodes before six months of age had an increased risk for coeliac disease before two years of age (adjusted OR 1.4, 95% CI 1.0–1.9) [6, 7]. The risk for coeliac disease increased further, in addition to having many infections, the child was also introduced to gluten in large amounts, as compared to small and medium amounts [6, 7]. This association held even when episodes of gastroenteritis were excluded, and after adjustments for

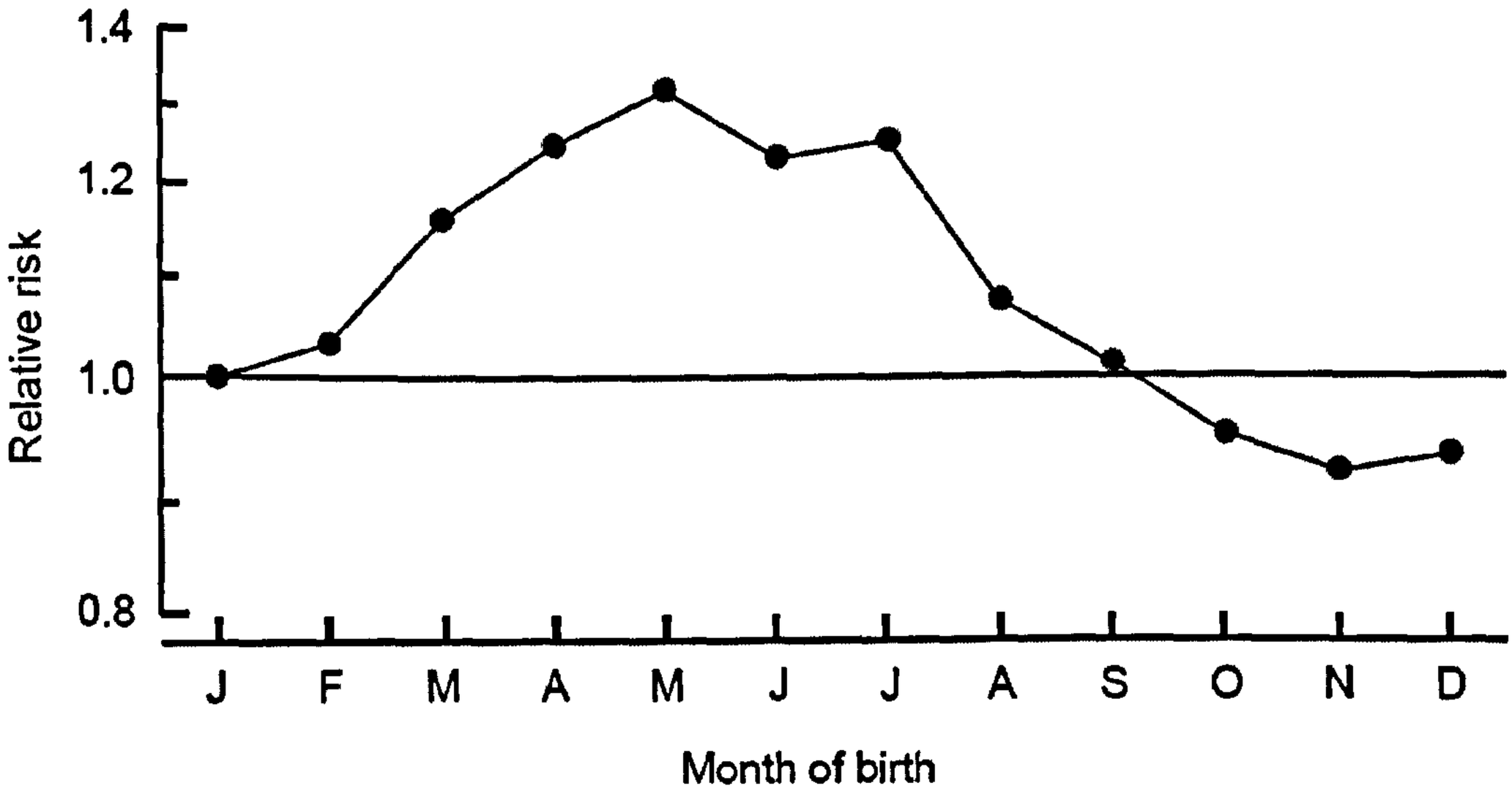
differences in infant feeding patterns and family socio-economic group had been made [6, 7]. Changes in gut permeability caused by infection thereby facilitating the entry of toxic gluten may also have a role [36-38].

1.3.3. Associated factors

1.3.3.1. Early life processes

The intrauterine environment might influence the risk for later coeliac disease since low birth weight and a neonatal infection diagnosis were both associated with an increased risk for coeliac disease [39]. Seasonality in month of birth has been demonstrated for Swedish coeliac disease children [40].

Figure 1.4: Relative risk for coeliac disease in children below two years of age by month of birth from 1973 to 1997



A temporal relationship indicates that this might be due to a casual effect of infections during foetal life and/or an interaction between infections and introduction of gluten into the diet. This is in accord with findings in case-referent studies of an increased risk for coeliac disease associated both with a neonatal infection diagnosis [39] and

repeated infectious episodes early in life [6, 7]. However, non-infectious exposures with a seasonal pattern need to be explored.

1.3.3.2. Socio-economic status

An almost linear gradient has been observed between the prevalence of a number of chronic diseases and socio-economic status; at each more affluent level of socio-economic status, prevalence of chronic diseases such as osteoarthritis, hypertension, ischaemic heart disease; and autoimmune diseases such as rheumatoid arthritis, lupus decreases [41-43]. In addition to morbidity and mortality, a gradient between risk factors for disease and socio-economic status also has been demonstrated. For example, rates of smoking, cholesterol titres and prevalence of sedentary lifestyle are lower the higher one goes on the socio-economic hierarchy and these also occur in a gradient relationship [43-46]. It is unclear the nature of the relationship between socio-economic status and disease. It could be that socio-economic status influences health status, labelled 'social causation'[47]. Alternatively health status contributes to socio-economic status through a 'social drift' [48]. Although there is some reciprocal influence of socio-economic status and health [49], data is more suggestive for a social causation rather than a social drift explanation for the association between socio-economic status and health [50-53]. Furthermore, although some childhood diseases are sufficiently debilitating that childhood health may determine educational attainment and later socio-economic status, these are sufficiently rare that they are unlikely to account for the significant socio-economic association later in life in general populations.

Maternal socio-economic status has been associated with subsequent development of coeliac disease in their infants [54] though the relationship between socio-economic status and incidence of adult coeliac disease is not known. In a population screening study, people with undetected coeliac disease showed a trend towards more affluent socio-economic status in comparison to general population controls although this was not significant at the 5% level (chi-squared test for trend $p = 0.09$) [55]. Although some cases of coeliac disease may be sufficiently debilitating that health may determine socio-economic status i.e. social drift, perhaps the more plausible pathway to explain any possible association between socio-economic status and coeliac disease is through exposure to different environments and adaptations to these environments in a social causation model. One aspect of the environment with health consequences is the differential exposure to antigens such as gluten in the instance of coeliac disease. The association between low birth weight and increased risk for coeliac disease may be in part due to social factors such as impaired poverty-related maternal health status causing lower physiological reserves, quality and quantity of healthcare, housing, diet, increased exposure to toxic antigens and differential risk of infectious disease [56-60]. Socio-economic factors surrounding infant feeding such as breastfeeding practices [61] could be part of the causal model of the multifactorial aetiology of coeliac disease. The environment also shapes health behaviours. For example, it has been suggested that access, uptake and utilisation of health services is lower in people in deprived areas [62-64] that could affect rate of clinical diagnosis of coeliac disease. The combination of individual characteristics and the environmental demands and constraints will affect the likelihood of enacting health-related behaviours. For example, low income neighbourhoods have more alcohol outlets and

together with the socio-economic gradient with smoking may affect the risk of coeliac disease [65, 66].

1.3.3.3. Cigarette smoking

Like ulcerative colitis, coeliac disease appears to be associated with non-smoking though it is unclear as to whether this is a causal association [67-69] or a reflection of the socio-economic association of coeliac disease. Although these case-control studies have observed some inverse relationship between current smoking and diagnosed coeliac disease the strength of the association has varied, probably due to the inconsistent reporting of smoking status amongst the selected control populations in comparison with the coeliac cohorts where the current smoking proportion was approximately 40% and due to the small sample sizes. Undetected coeliac disease was associated with 60% reduced risk of current smoking (OR 0.34; 95% CI 0.14, 0.85) in comparison to general population controls [55].

1.4. Pathogenesis of coeliac disease

The mechanisms of the intestinal immune-mediated response is not completely clear but the pathogenesis of coeliac disease is thought to involve a complex interplay of immunological factors including tissue transglutaminase, intra-epithelial lymphocytes, cytotoxic T-cells, adaptive and innate immune responses and autoimmunity.

1.4.1. Tissue transglutaminase as autoantigen

Tissue transglutaminase (tTG) has emerged as an essential player in the pathogenesis of coeliac disease, generating the antigenic epitopes present in α-gliadin [16]. As alluded above in the aetiology of coeliac disease, tTG is the essential 'pivot' in the

pathogenesis of coeliac disease because it catalyses the deamidation of native gliadin, thereby enhancing gliadin's affinity for the HLA-DQ2 receptor on the antigen presenting cell [17, 18]. Deamidation of gliadin also results in a greatly enhanced cytotoxic T-cell response [17, 18]. tTG is also the target or autoantigen of the humoral immune response, with autoantibodies against tTG pathognomonic of coeliac disease [70]. Normally stored intracellularly, tTG is released during cellular wounding such as that brought on by stress and cross-links several matrix proteins stabilizing the connective tissue scaffold on which the cells rest [71-73]. Activated when intracellular, tTG reacts with several structural and functional proteins setting the stage for apoptosis. tTG also acts as a regulator of CD8+ T cell migration and is a controller of the early non-adaptive innate phases of coeliac disease [73].

1.4.2. Intra-epithelial lymphocytes

The importance of intra-epithelial lymphocytes in the pathogenesis of coeliac disease was first recognised by Ferguson et al [74]. In particular it has now been recognized that the proportion of γ/δ intraepithelial T lymphocytes are increased in people with coeliac disease and recognize bacterial non-peptide antigens and unprocessed stress-related proteins. Two important such stress-related proteins that are expressed on intestinal epithelial cells by interferon-γ are MICA and MICB which resemble major histocompatibility class I genes [75]. MICA and MICB gene expression is regulated by promoter heat-shock elements similar to heat-shock protein 70 [75]. Once activated γ/δ intraepithelial T lymphocytes secrete cytokines that attract and stimulate cells of the innate immune response but also modulate the adaptive immune response by secreting IL-4 which dampers Th1 activity in favour of Th2 reactivity. Such actions suggest γ/δ intraepithelial T lymphocytes protect the intestinal mucosa from

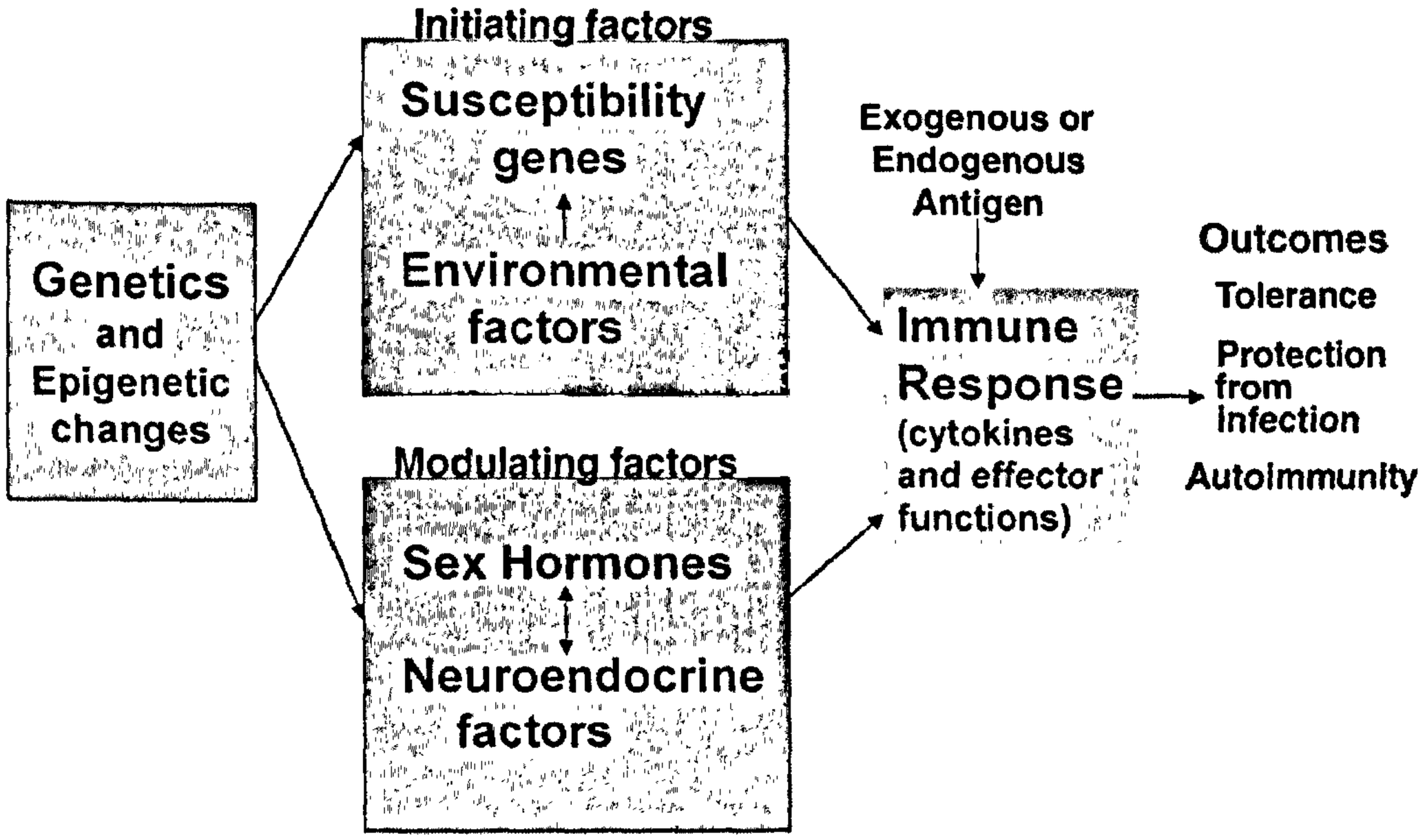
chronic exposure to damaging agents such as dietary gluten in gluten-intolerant individuals [75, 76].

1.4.3. Autoimmunity

Clinically diagnosed coeliac disease is associated with a number of autoimmune diseases though it is possible that in many instances ascertainment bias may be contributing to the associations reported. Nonetheless, recent screening studies have shown an increased prevalence of coeliac disease in autoimmune disorders including type 1 diabetes [77], thyrotoxicosis [78, 79], primary biliary cirrhosis [80] and Sjogren's syndrome [79].

The aetiology of autoimmune disease is multi-factorial where a complex interplay between intrinsic (genetic predisposition and epigenetic changes) and extrinsic (environmental factors) triggers contribute to disease pathogenesis and progression [81].

Figure 1.5: A model for the multifactorial aetiology of autoimmune disease



The intrinsic abnormalities are complicated, with diverse genetic polymorphisms described in different ethnic groups, suggestive of a mosaic of autoimmunity where the immunologic disarray might not be the same for each individual [81]. Comparative analysis of the position of allelic loci within the major histocompatibility complex for type 1 diabetes with candidate allelic loci from other autoimmune diseases such as coeliac disease show considerable overlap consistent with a hypothesis that like, the major histocompatibility complex, some of these loci involve common susceptibility genes or biochemical pathways that are central to normal immune function [81]. These genes or pathways may contribute to immune dysregulation shown to be present in different autoimmune diseases, possibly before the onset of overt clinical symptoms [82]. For example, IDDM1 candidate allelic loci identified for type 1 diabetes located on chromosome 6q21 is found at the exact same position as loci for coeliac disease (marker HLA-DQ2) [83], multiple sclerosis [84], Crohn's disease [85] and lupus [86]. Whilst the end-stage phenotype of a specific

autoimmune disease may be clinically distinct and / or organ specific, the aetiology of many autoimmune diseases may involve shared processes of immune regulation [87]. It is likely that basic pathways affecting pro-inflammatory : anti-inflammatory cytokine ratios, apoptosis, complex antibody regulation, effector T-cell populations and hormonal control of the immune system are involved in these related diseases [87]. Evidence suggestive of viruses encoding molecular mimics that behave as a microbial trigger for autoimmune disease include adenovirus in coeliac disease [35] as described above, enteroviruses in type 1 diabetes [88] and campylobacter jejuni in Guillain-Barre syndrome [89, 90] has also been raised.

Though limited by ascertainment bias but on the other hand perhaps underdiagnosed if testing for coeliac disease using serological tests based on IgA antibody presence, a 10-fold increase in risk of coeliac disease has been observed in people with IgA deficiency [91]. Released at luminal surfaces, IgA serves a variety of functions to protect the vast surface area occupied by the mucosal surfaces such as the gastrointestinal tract, representing the first line of defence against invading antigens through agglutination thereby preventing penetration of the mucosa. It is possible that IgA deficiency leads to insufficient exclusion of food antigens allowing gluten to penetrate the intestinal mucosa resulting in immune complex deposition and thereby facilitate coeliac disease development [91]. The absence of IgA on mucosal surfaces could facilitate the absorption of environmental antigens that may cross-react with self-antigens. Abnormalities in T-cell regulation observed in IgA deficiency could also be a responsible factor for both IgA deficiency and autoimmunity [92].

It is not clear whether the duration of gluten exposure or withdrawal of gluten through treatment of coeliac disease protects against the development of other autoimmune diseases [93, 94].

1.4.3.1. Autoimmunity and gender

Gender bias is characteristic of autoimmune disease [95]. In some of the more common autoimmune disorders such as Sjogren's syndrome, lupus, Hashimoto's thyroiditis, Grave's disease, scleroderma, representing a spectrum of diseases, the patient population is reported to be >80% women [96]. The female predominance of adult coeliac disease is also relatively well described where in general twice as many women are diagnosed as men [97-99].

Such female preponderance for abnormal autoimmune function is largely unexplained. Immune reactivity is reported to be more enhanced in females than in males [100] with higher IgM (but not IgG) concentrations in women than in men [101]. Lower CD4+ counts and lower CD4/CD8 T-cell ratio in men relative to women has also been reported [102]. However, the preponderance of the lupus in young women of childbearing age (female to male ratio of 9:1), the tendency for lupus flares during pregnancy with remissions after menopause suggest that female sex hormones are crucial regulators of lupus activity in this autoimmune disease [103]. This is further supported by observations that prolactin (through induction of follicular zone self-reactive B cells) and oestrogen (through survival and activation of marginal zone autoreactive B cells) are immunostimulators that affect maturation and selection of autoreactive B cells as well as autoantibody secretion in molecular studies on lupus [104]. The effect of prolactin and oestrogen may be based on their capacity to allow

autoreactive B cells to escape the normal mechanisms of tolerance and mature to fully functional antibody-secreting B cells that can cause clinically apparent lupus [104].

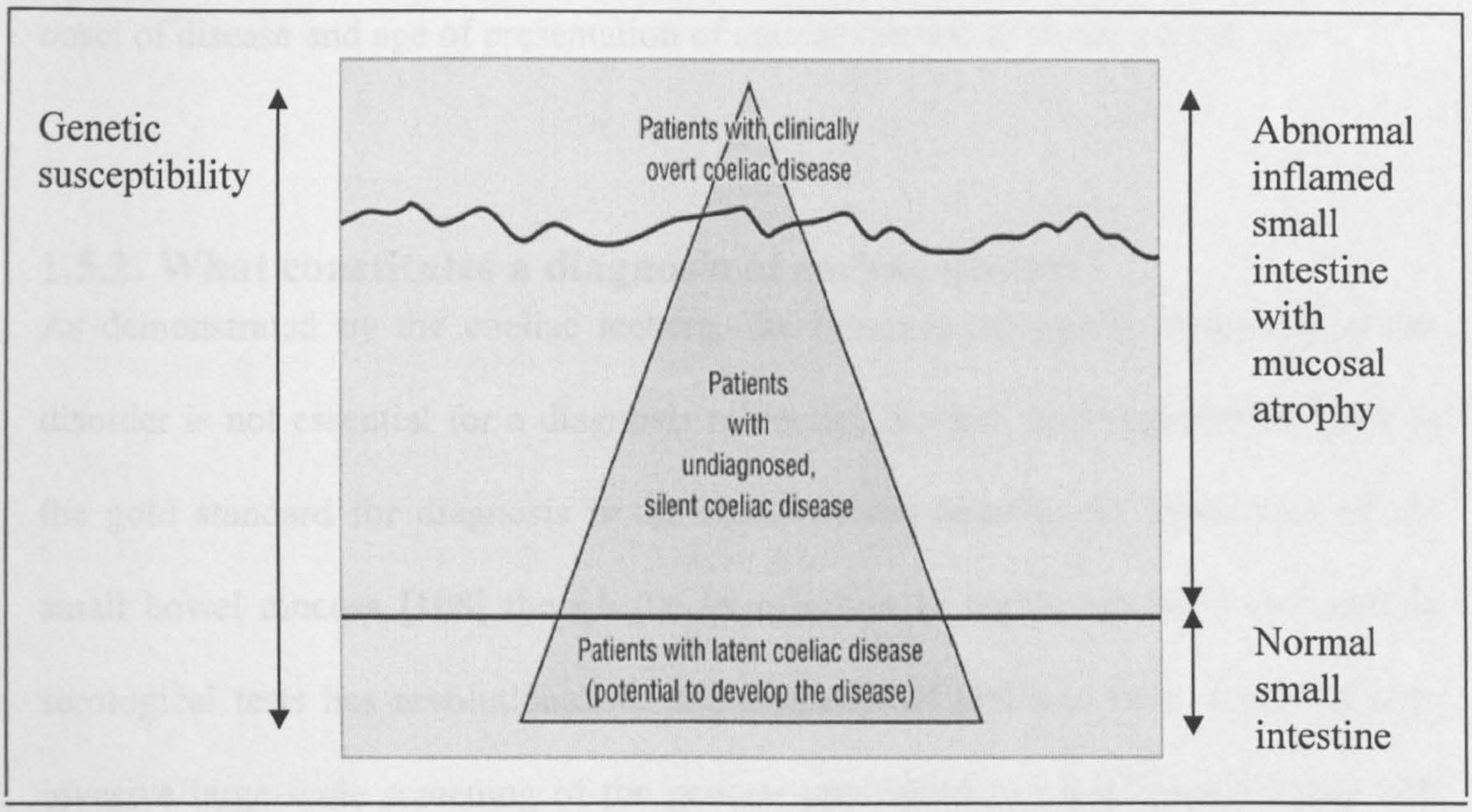
The role of sex hormones and the preponderance of women in coeliac disease have been far less evaluated in comparison to the studies in lupus. One could speculate that the challenges of menstruation and pregnancy may act to accentuate anaemia and exacerbate any effects of coeliac disease in women. The health-seeking behaviour of women relative to men with differential symptom reporting may contribute to the apparent gender difference in clinically diagnosed coeliac disease [98]. Further work on the impact of gender upon contemporary coeliac disease such as mode of presentation is required.

1.5. Presentation of coeliac disease

1.5.1. The coeliac iceberg

The coeliac iceberg concept has often been used to demonstrate the clinical variability of coeliac disease and helps to understand its systemic nature [105-107].

Figure 1.6: The coeliac iceberg



The whole iceberg represents people with a genetic susceptibility for coeliac disease; however the majority are still healthy. The highest stratum of the iceberg (above the waterline) describes patients with typical symptoms of coeliac disease such as weight loss and diarrhoea. These symptomatic patients are usually healthcare seeking and present to gastrointestinal services where the diagnosis may be made. These clinically overt cases make up the tip of the iceberg, accounting for the minority of cases of coeliac disease. In contrast, the majority of people with coeliac disease are undiagnosed and hidden below the waterline. Those not diagnosed with coeliac disease may have overt symptoms though not yet been diagnosed. People with 'silent' coeliac disease have characteristic small bowel morphological changes though in the absence of typical symptoms of the disorder. Silent coeliacs may have atypical symptoms, no overt symptoms and due to the malabsorption caused by the enteropathy may have physiological derangements such as iron deficiency anaemia or osteoporosis. People with 'latent' coeliac disease have a genetic susceptibility to developing the disease though have normal small bowel morphology. Timing and

quality of gluten load combined with environmental triggers may be factors in the onset of disease and age of presentation of coeliac disease in these individuals.

1.5.2. What constitutes a diagnosis of coeliac disease?

As demonstrated by the coeliac iceberg, the presence of typical symptoms of the disorder is not essential for a diagnosis of coeliac disease. Still regarded by most as the gold standard for diagnosis is the characteristic histological appearance of the small bowel mucosa [108] though the introduction of highly sensitive and specific serological tests has revolutionalised the diagnosis of celiac disease, allowing non-invasive large-scale screening of the general population to identify individuals with "silent" coeliac disease who account for the majority of coeliac disease beneath the waterline of the iceberg model.

1.5.2.1. Enteropathy

The small intestinal mucosa from a patient with untreated coeliac disease has a characteristic histological appearance, classified by Marsh in the early 1990s [108]. The coeliac histopathological picture is described as a continuum from normal villous architecture with intraepithelial lymphocytosis, through partial villous atrophy to total villous atrophy [108] with the mucosal lesion classed into five types:

Type 0: preinfiltrative lesion
Mucosa appears normal.

Type 1: infiltrative lesion
Mucosal architecture is normal but there is an increased number of intra-epithelial lymphocytes.

Type 2: hyperplastic lesion
In addition to the infiltration of the mucosa with intra-epithelial lymphocytes, there is an increase in crypt depth without any reduction in villous height.

Type 3: destructive lesion
The mucosa is flat with reduction in normal villous height to crypt depth ratio (villous atrophy which may be partial, subtotal or total) with crypt hyperplasia and intraepithelial lymphocytosis.

Type 4: hypoplastic lesion
There is deposition of collagen in the mucosa and submucosa which is unresponsive to gluten withdrawal and is associated with the development of malignant complications such as lymphomatous transformation.

Small intestinal mucosal biopsies are usually taken at endoscopy (usually 4 – 6 specimens) from the proximal small intestine, usually from the second part of the duodenum [109].

1.5.2.2. Serological tests

Serological tests have assumed greater importance as a screening investigation since histology of small intestinal mucosa, though regarded by most as the gold standard, is inconvenient, expensive, unpleasant and not without risk [110] and has permitted non-invasive and large-scale screening of the general population to identify individuals with "silent" celiac disease. The first reliable screening test was the endomysial antibody (EMA) devised by Chorzelski et al in 1983 [111]. A systematic review of published studies in 2000 calculated the pooled EMA sensitivity and specificity to be 94% and 98% respectively [112]. However there are problems with the EMA test despite its impressive performance – the interpretation of the indirect immunofluorescence assay is subjective, is labour intensive and one commonly used

substrate (monkey oesophagus) is from an endangered species. With the identification of tTG as the autoantigen recognised by EMA, tests for detecting antibodies to tTG was soon devised using a quantitative enzyme-linked immunosorbent assay which is objective and lends itself to automation. A systematic review of published studies I performed (published peer-reviewed manuscript can be found in section 9.1) in 2006 calculated the tTG performance to be comparable to that of EMA [113]. Following the appreciation of the importance of gliadin deamidation catalysed by tTG in the immunopathogenesis of coeliac disease, diagnostic tests based on antibodies to deamidated gliadin peptides have been developed. The performance of the deamidated gliadin peptides antibody test was compared with tTG in a meta-analysis of published studies which I performed (published peer-review manuscript can be found in section 9.2) [114]. Though both tests performed well, tTG with its greater sensitivity and specificity suggests that it should remain the preferred serological test for diagnosing and or excluding coeliac disease [114].

Assessment of serology performance in coeliac disease from published studies however is fraught with difficulty and much of the information required is often missing from reports. The biggest problem in assessing sensitivity of the serological tests is ascertainment bias since most patients now are selected for biopsy because they have positive serology and the resultant sensitivity is likely to be falsely high. Specificity can also be affected by ascertainment bias if a prerequisite for inclusion is negative serology (specificity will be falsely high), or if the controls are taken from patients who are thought to have coeliac disease (partly on the basis of positive serology) but turn out to have normal biopsies (specificity will be falsely low). There are several other factors which are important in assessing the validity of published

studies. Small bowel histology should be used as the gold standard and it is important that the result of histology is given in the published studies because serology depends on the severity of the small bowel mucosal abnormality [115-117]. Criteria for histological normality must be given in the published studies since some authors include Marsh 1 lesions (just an increase in intraepithelial lymphocytes) among their controls whereas others consider this an indication that the patient has mild coeliac disease. Because there may be an age-related serology response the mean age and range should be given or, at the very least, it should be stated whether the patients are adults or children. Finally, the choice of controls may be important. It has been argued that the best control group is a group who are suspected of having the condition being studied but, after investigation, turn out not to have that condition [118]. However, some studies use healthy volunteers and others choose patients with other diseases which might be associated with autoimmunity.

Taking into the difficulties with coeliac serology, small bowel histology should remain the gold standard to diagnose coeliac disease; it not only provides a baseline reference for response to treatment but also allows the detection of coeliacs who would not be detected with reliance on serology alone – perhaps one in ten coeliacs with villous atrophy. On balance, coeliac serology should be used to exclude coeliac disease if the pre-test probability (i.e. proportion of people with coeliac disease in the population being tested) is low. Conversely, small bowel biopsies should be performed in those patients in whom coeliac disease is still suspected even if coeliac serological tests are negative [119, 120].

1.5.3. Presentation of clinically diagnosed coeliac disease

Previously, when the diagnosis of coeliac disease was based exclusively on clinical observations, almost all patients were reported to present with clinically overt "classic" malabsorptive symptoms of diarrhoea and weight loss [121-123]. Later, when small intestinal biopsies became readily available [124-127], it was apparent clinically diagnosed coeliacs presented with an array of both gastrointestinal and non-gastrointestinal symptoms. A number of different modes of presentation of clinically diagnosed coeliac disease have been reported though associations observed may be due to ascertainment (Table 1.1).

Table 1.1: The many faces of clinically diagnosed coeliac disease in published studies

Classic presentation	Diarrhoea [121, 122] Weight loss [121, 122]
Gastrointestinal features	Abdominal pain [128] Heartburn [128] Bloating [129] Aphthous ulcers [129] Glossitis and stomatitis [129] Anorexia and vomiting [130]
Extraintestinal features	Iron-deficiency anaemia [128] Folate-deficiency anaemia [128] Osteoporosis [131] Hypertransaminasaemia [132] Arthralgia [130] Myopathy [130] Neuropathy [133] Ataxia [133] Epilepsy [133] Infertility [134] Delayed puberty [134] Recurrent miscarriages [134] Depression [135] Alopecia [136] Teeth enamel defects [137]
Associated conditions	Type 1 diabetes mellitus [77] Dermatitis herpetiformis [138] Autoimmune thyroid disease [139] IgA deficiency [91] Sjogren's syndrome [140] Primary biliary cirrhosis [141]

1.5.3.1. Temporal changes in mode of presentation

Since coeliac disease was first described the manifestation of clinically diagnosed disease appears to be changing, with increasing numbers of coeliacs being diagnosed as a result of anaemia and or non-malabsorptive symptoms [97, 128, 142-144] with temporal trends in the presenting features, age at diagnosis and gender distribution have been observed in a number of retrospective and historical case series, cohorts and a patient support group survey (Table 1.2).

Table 1.2: Studies observing mode of presentation of clinically diagnosed coeliac disease

Study	20th century period	N	Study design	N (%) diarrhoea	N (%) anaemia	N (%) weight loss	N (%) abdominal pain	Mean age at diagnosis in women/men (y)	N (%) female
Croese [145]	69 – 78	70	Retrospective case series	34 (49)		7 (10)			
Swinson [129]	70 – 79	88	Retrospective case series		20 (23)			44 / 50	64 (73)
Logan [128]	60 – 64 75 – 79	38 102	Retrospective cohort	41 (40)	30 (79) 34 (33)			49 / 49 42 / 48	26/47 (68) 97/138 (70)
Boyd [146]	69 – 83	50	Retrospective case series	37 (74)		25 (50)	21 (42)		
Pare [147]	66 – 75	52	Retrospective case series	32 (62)		40 (72)	31 (59)		
Bode [148]	71 – 92	50	Retrospective case series	28 (56)	11 (22)	22 (44)	32 (64)	44 /27	37 (74)
Sanders [143]	90 – 2000	264	Retrospective case series					45 /43	178 (67)
West	75 – 79 80 – 84 85 – 89 90 – 94 95 – 99	35 55 70 141 266	Prospective cohort	25 (71) 33 (60) 45 (64) 64 (45) 97 (37)	1 (3) 4 (7) 1 (1) 19 (14) 44 (17)				25 (71) 34 (62) 47 (67) 108 (77) 180 (68)
Lo [144]	81 – 2002	227	Retrospective case series	118 (52)	16 (7)	13 (5)	9 (4)		142 (63)
Murray [99]	50 – 69 70 – 89 90 - 99	8 18 35	Retrospective cohort	8 (100) 12 (67) 17 (49)	4 (50) 4 (22) 13 (37)	5 (63) 10 (56) 18 (51)	5 (63) 10 (56) 18 (51)		6 (75) 11 (61) 26 (74)
Zipser [149]	2001	134	Patient group survey	79 (52)	84 (63)	74 (55)	103 (77)	44 / 48	109 (80)
Jones [150]	2005	32	Retrospective case series	12 (38)	21 (66)	9 (32)	13 (41)		21 (67)

The Lothian cohort of people with coeliac disease was one such historical and retrospective cohort, originally set up in 1979 through an attempt to identify all cases of coeliac disease diagnosed in the Lothian region of Scotland [25, 128]. Cases were identified by examination of records of gastrointestinal units of all the hospitals in the Lothian region including the Edinburgh Royal Infirmary, Western General Hospital and Royal Hospital for Sick Children up to December 1981; The Scottish Hospital Inpatient Statistics between 1961 – 1977; the existing regional histopathology records in the three adult and one paediatric pathology laboratories between 1958 and 1980; a postal survey of all the general practitioners in the Lothian region in 1979; and the local branch of the Coeliac Society [25, 128]. Comparing features at presentation through the four quinquennia spanning 1960 – 1979, Logan et al observed 21% coeliacs presented with classic symptoms of malabsorption in 1975 – 1979 in comparison to 63% in the 1960s [25, 128]. The only other cohort of sufficient sample size and of comparable design is that of West et al who observed similar patterns in adults diagnosed with coeliac disease in Southern Derbyshire where data was collected prospectively by a single investigator using a standard proforma. Probably due to differences in study design, study populations, symptom classification and data collection it is difficult to infer the relative proportions of classic, silent to symptomatic modes of presentation.

There are several further limitations to the available data observing the mode of presentation of coeliac disease. Many of the retrospective case series relied on data being collected from medical case records which may be incomplete or the records lost and are therefore exposed to information bias in comparison to the systematic collection used in the prospective cohort by West et al. Identification of all cases of

coeliac disease may not be possible, particularly with earlier non-electronic sources used to trace people with coeliac disease in retrospective case series resulting in selection bias. Further selection bias probably occurred in the patient support group survey with only 134 of 1032 respondents completing the full survey and only 1032 respondents participating in the survey despite recruitment to a nationwide support group over an eight year period. It is likely that the non-responders differ from those who respond. Ability of people to recall information regarding previous consultations and symptoms and test results leading to diagnosis is likely to be inaccurate resulting in recall bias. The sample sizes in many of the studies are low with differences in mode of presentation over temporal periods may not be due to chance but are inadequately powered to demonstrate an effect.

One explanation for any changes in presentation could be that the natural history of coeliac disease is changing, perhaps in response to changes in environmental exposures such as infant feeding practices or smoking. A more likely explanation is that the ability to diagnose coeliac disease has improved in both quality and accessibility over the past twenty years with the development of both highly sensitive and specific non-invasive serological tests and increasing use of endoscopic biopsy techniques rather than the traditional and more unpleasant Crosby capsule biopsy. It is likely that a broader spectrum of people is being investigated for coeliac disease and consequently being diagnosed. There is no real data observing the contemporary mode of presentation of contemporary / 21st century coeliac disease which perhaps includes a different spectrum of individuals with the development of better diagnostic tests and greater test accessibility.

1.5.3.2. Age at clinical diagnosis of coeliac disease

Age at clinical manifestation of coeliac disease has classically been reported as a bimodal distribution with the first peak in early childhood between 9 and 24 months and the second peak between the third and fourth decade [1]. However later studies suggest an adult predominance [1, 99, 148, 149] with dramatic reductions in childhood incidence in some [24, 151] but not all studies [152]. It has been suggested that environmental factors might affect the incidence of coeliac disease in specific birth cohorts such as differences in infant feeding practices or relatively high gluten content in the infant diet [5, 7, 153]. The implications of coeliac disease being diagnosed in childhood rather than in adulthood are only beginning to be unravelled [154]. In the Lothian coeliac disease cohort, all-cause mortality more than 5 years after diagnosis was increased threefold in children (SMR 3.32, 95% CI 2.05–5.07) compared with only a 38% increase in adults (SMR 1.38, 95% CI 1.16–1.63) [154]. This excess mortality in children was primarily attributed to an increased risk of death from accidents, suicide, and violence (seven deaths, SMR 3.22, 95% CI 1.29–6.63), cancer (five deaths, SMR 3.72, 95% CI 1.21–8.67), and cerebrovascular disease (two deaths, SMR 10.03, 95% CI 1.21–36.00) [154]. Further work using large, longitudinal coeliac cohorts observing the implications of the timing of coeliac disease diagnosis over the course of the human lifecycle is required.

1.6. Occurrence of coeliac disease

1.6.1. Prevalence of undetected coeliac disease

Several serological screening studies from Europe, South America, Australasia and America have shown that approximately 0.5 – 1.0% of these developed country populations may have undetected coeliac disease (Table 1.3) [155-160]. There is variation in reported prevalence estimates of coeliac disease in screening studies which may be due to screening strategy used (such as choice of coeliac serological test) and population screened (such as adult blood donors versus general population). Apart from the American study, all of the adult blood donor studies confirmed the presence of coeliac disease following positive serological result with small bowel histology. Two of the general population-based screening studies used populations recruited for the World Health Organisation Monitoring of trends and determinants in cardiovascular disease (MONICA) project [155, 156] but not all people with positive serology had coeliac disease confirmed on small bowel histology. In the MONICA project, samples of a country's general population were randomly selected from population registers stratified by age and by sex. The adult blood donor studies show in general lower prevalence of coeliac disease than that observed in the general population studies which may reflect exclusion of coeliacs with anaemia due to transfusion services only allowing safe donation of blood by donors having minimum haemoglobin levels [161].

Table 1.3: Estimates of prevalence of undetected adult coeliac disease

Country		Mean or median* age	Proportion female (%)	Population screened	Serological test used	Cases	Number screened	Prevalence (%) [95%CI]
Ireland [156]	1997	Range 15 – 65	?	General population	AGA and if AGA-+ EMA	15	1823	0.82 [0.46, 1.35]
Italy [162]	1997	44	53	General population	EMA	4	2237	0.18 [0.05, 0.46]
Sweden, [163]	1999	41	35	Adult blood donors	AGA and if AGA-+ EMA	4	1970	0.20 [0.06, 0.52]
Italy [164]	1999	35	25	Adult blood donors	EMA	10	4000	0.25 [0.12, 0.46]
Holland [165]	1999	?	?	Adult blood donors	EMA	3	1000	0.30 [0.06, 0.87]
Sweden [155]	1999	50	50	General population	EMA	10	1894	0.53 [0.25, 0.97]
Brazil [166]	2000	33*	13	Adult blood donors	AGA and if AGA-+ EMA	3	2045	0.15 [0.03, 0.43]
Spain [158]	2000	45	55	General population	EMA	3	1170	0.26 [0.05, 0.75]
America [167]	2001	39	48	Adult blood donors	EMA	8	2000	0.40 [0.17, 0.79]
Italy [159]	2001	Range 12 – 65	52	General population	EMA	17	3843	0.49 [0.28, 0.78]
Argentina [168]	2001	29*	50	General population	AGA and if AGA-+ EMA	12	2000	0.60 [0.31, 1.05]
Australia [169]	2001	Range 20 – 79	50	General population	EMA	7	3011	0.23 [0.09, 0.48]
England [170]	2003	59	59	General population	EMA	87	7055	1.2 [0.9, 1.4]

1.6.2. Prevalence of clinically diagnosed coeliac disease

Estimates of the prevalence of clinically diagnosed coeliac disease range from 0.05% - 0.27% [171-174] with variation likely to be differences in case ascertainment reflecting some local interest in coeliac disease.

1.6.3. Incidence of adult coeliac disease

The rate of diagnosis of adult coeliac disease has risen dramatically in most areas of the world where there is data available to monitor such trends [172, 173, 175, 176]. Although the rate of diagnosis does not completely represent incidence with the method of case ascertainment changing with the advent of increasingly sensitive serological markers, coeliac disease is being more commonly recognised. The estimated incidence per 1000 population has increased from 0.061 in the years 1987 – 1991, 0.088 in the years 1992 – 1996, 0.195 in the years 1997 – 2001 and 0.169 in the years 2002 – 2006 in Derby [174]. Despite an active case-finding strategy adopted by some centres, the incidence and clinically diagnosed prevalence estimates of coeliac disease suggest that there is a substantial gap between the number of adults with clinically diagnosed and undetected coeliac disease. The ratio of undetected coeliacs disease to symptomatic disease is reported to be approximately 8:1 at present [174].

1.7. Impact of clinically diagnosed coeliac disease

1.7.1. Metabolic bone disease

1.7.1.1. Risk of osteoporosis and osteopenia in untreated coeliac disease

Earlier reports on bone involvement in coeliac disease were principally based on clinical and biochemical findings and probably on a highly selected group of adult coeliacs with severe disease [177-179]. With the introduction of single and dual x-ray absorptiometry (DEXA) providing more precise quantitative bone data in the 1980s, reduced bone mineral density (BMD) in adults with untreated coeliac disease was observed in published studies (Table 1.4) which have been subject to a recent systematic review that I performed [180].

Table 1.4: Reduced BMD in adults with untreated coeliac disease in published studies

Study	Country	Number of patients	DEXA site	Mean Z score	Median Z score	Mean T score	%osteoporosis	% osteopenia
Mazure et al [181]	Argentina	28	Lumbar Total skeleton	All -1.98 Asymptomatic -1.05 Symptomatic -2.37 All -2.16 Asymptomatic -1.54 Symptomatic -2.41				
Gonzalez et al [182]	Argentina	20	Spine Total skeleton	-2.5 -2.9				
Mautalen et al [183]	Argentina	14	Spine Total skeleton	-1.3 -1.5				
Bai et al [184]	Argentina	25	Spine Total skeleton	-1.9 -2.2				
Fornari et al [185]	Argentina	16	Spine Total skeleton		-1.6 -2.4			
Vasquez et al [186]	Argentina	51	Spine Total skeleton	-1.3 -2.0				
Corazza et al [187]	Italy	14 silent 10 classic	Spine Spine	-1.3 -2.6			0 80	
Corazza et al [188]	Italy	20	Spine Femur		-2.0 -2.0			
Di Stefano et al [189]	Italy	16	Spine Femur	-1.70 -1.93				
Di Stefano et al [190]	Italy	21 silent 18 classic	Spine Femur	-2.5 classic -1.1 silent -2.5 classic -1.2 silent				
Sategna-Guidetti et al [191]	Italy	86	Spine Femur	-1.5 -1.8		-1.7 -1.4	26	40

Study	Country	Number of patients	DEXA site	Mean Z score	Median Z score	Mean T score	%osteoporosis	% osteopenia
Walters et al [192]	England	10	Spine Femur	-1.85 women -0.95 men -0.89 women -0.95 men				
Lewis et al [193]	England	43	Spine Femur	-0.26 0.22 women -1.16 men -0.36 classic 0.13 silent 0.04 0.22 women -1.04 men 0 classic 0.15 silent			14 7	40 36
Valdimarsson et al [194]	Sweden	63	Spine Femur		-0.63 -0.57 women -0.78 men -0.54 -0.48 women -0.95 men			
Valdimarsson et al [195]	Sweden	29	Spine Total hip	-1.12 -1.23				
Valdimarsson et al [196]	Sweden	105	Spine Total hip Forearm	-0.72 -0.79 -0.88				
Kemppainen et al [197]	Finland	28	Spine Femur				33 0	49 54
Meyer et al [198]	USA	31	Spine Femoral neck Radius				34 27 36	38 44 32

There are limitations to these published studies. Many of the studies are small with only 1 of the 18 studies having a sample size above 100 patients with untreated coeliac disease. Most of the studies are based on observations from specialty coeliac disease clinics and may not reflect the true risk. Only 4 of the studies included a control group. However, these published studies suggest that there is a moderate reduction of BMD in untreated coeliac disease with weighted mean Z scores at the lumbar spine and hip of -1.3 (95% CI -1.4, -1.2) and -1.1 (95%CI -1.2, -1.0) [180]. It is also not clear whether coeliac patients have the same major risk factors for osteopenia or osteoporosis such as female gender [180] or if degree of intestinal inflammation contributes to reduced BMD. Classic or symptomatic disease was associated with reduced BMD in some but not all studies, raising implications about screening for coeliac disease should asymptomatic disease be associated with reduced BMD [170].

Osteoporosis is a major public health problem because of its potentially severe consequences for both the patient and the health care system if it leads to fracture [199]; osteoporotic fractures are associated with pain, disability and up to 30% mortality at 1 year in addition to an estimated monetary cost in the UK of £940 million yearly [200-202]. Although osteoporosis is just one of many factors predisposing to fracture, with the appreciation that the risk of sustaining an osteoporotic fracture doubles with each standard deviation decrease in BMD [203], determining the true risk of reduced BMD in an unselected and contemporary cohort of people with coeliac disease and identifying which coeliacs are at particular risk of reduced BMD is important. Such risk identification would then be of benefit such as in the rationalising referrals for BMD assessment rather adopting the policy of BMD

assessment in all newly diagnosed coeliacs [204] particularly in the wake of recent studies suggesting the risk of fractures, is at most, only slightly increased in people with coeliac disease (risk ratio 1.42; 95%CI 1.17, 1.65) [180].

1.7.1.2. Effects of gluten-free diet on bone mineral density

Observational studies have suggested that a gluten-free diet improves bone density in people with symptomatic coeliac disease. Valdimarsson observed a median 3% (interquartile range 1 – 7) increase in BMD at the lumbar spine in 62 coeliacs following 12 months treatment with a gluten-free diet [194] whereas McFarlane observed a 6.6% (95% confidence interval 3.1, 10.1) absolute increase in the lumbar spine over an identical time period of treatment in 21 coeliacs [205]. Similar observations regarding improvement in BMD with treatment with a gluten-free diet were reported in other studies observing change over longer periods of follow-up [184, 191, 196, 206-208]. Despite the improvement in bone mass with treatment with a gluten-free diet, bone mass still appears to be reduced in comparison to age- and sex-matched general population controls [194, 205] at one year with no studies evaluating whether the observed reduced BMD in coeliac disease is completely reversible by evaluating BMD changes in coeliacs and age- and sex-matched controls over a longer follow-up period. A separate but related question is whether people with silent / asymptomatic coeliac disease gain any improvement in their bone mass if initiated on a gluten-free diet, again raising the issue of whether or not we should be actively screening otherwise healthy people for coeliac disease.

1.7.2. Liver disease

A number of liver conditions have been reported to be associated with coeliac disease. For example, recent population-based data using the General Practice Research Database and Swedish In-Patient Registry observed a four-fold increased risk of having an autoimmune liver disease such as primary biliary cirrhosis in people with coeliac disease in comparison to general population controls [209, 210]. However several case series report the most common hepatic injury to affect people with untreated coeliac disease is of an isolated hypertransaminasaemia, observed to affect over 40% of adults newly diagnosed with coeliac disease (Table 1.5).

Table 1.5: Prevalence of elevated transaminases in untreated coeliac disease

Study	Number of incident adult coeliacs	Proportion with abnormal ALT at diagnosis	Complete normalisation of ALT with GFD
Hagender *et al* 1977 [211]	53	55%	
Jacobsen *et al* 1990[212]	132	47%	
Dickey *et al* 1995 [212]	129	15%	87%
Bardella *et al* 1995 [213]	158	42%	95%
Novacek *et al* 1999 [214]	178	40%	96%

ALT alanine transferase; GFD gluten-free diet

Coined "gluten" or "coeliac hepatitis" this hepatic injury is reputed to be characterised by absence of serum auto-antibodies (other than endomysial and tissue transglutaminase antibodies), elevated transaminases and the presence of mild lobular and portal tract inflammation that is reversible on treatment with a gluten-free diet [215]. Given the relatively high prevalence of coeliac hepatitis observed in the

reported case series and the potential of the coeliac hepatitis to progress to liver fibrosis, cirrhosis and end-stage liver failure, some groups have recommended a vigorous search for liver disease in people newly diagnosed with coeliac disease [216-218]. To help determine if the liver work-up is warranted in modern coeliac disease, the prevalence of hypertransaminasaemia in an unselected, large and contemporary population of people newly diagnosed with coeliac disease is required.

1.7.3. Symptoms and quality of life

Above the waterline of the coeliac iceberg are usually symptomatic patients whom are usually healthcare seeking because of the overt symptoms where the diagnosis is made and treatment introduced. In contrast, the majority of people with coeliac disease are undiagnosed and silent – either due to absence of symptoms; presence of atypical symptoms resulting in coeliac disease going unrecognized for many years [219, 220]. Predicting who will benefit from being diagnosed with coeliac disease is a fundamental question. It appears from available albeit limited studies, people with symptomatic coeliac disease not only reap an improvement in their quality of life but suffering from gastrointestinal symptoms like diarrhoea are alleviated with a gluten-free diet [221]. We do not know whether there should be a case for population screening for coeliac disease in otherwise asymptomatic, healthy people as it is unclear from a quality of life perspective whether this gets better with a gluten-free diet. A further fear is whether detection of silent coeliac disease will force otherwise healthy people to change their eating habits, which in turn may worsen their quality of life and be less willing to adhere to a strict gluten-free diet.

Only a few studies have explored health-related quality of life measures with a longitudinal design such as in 35 coeliacs using Zung self-rated depression scale [222]

and in 40 coeliacs using the Psychological Well-Being Questionnaire [223]. However both of these quality of life assessment tools are generic instruments that are complex to administer and to score, prone to missing important clinical change and are a source of bias due to coeliac disease itself through containing items evaluating gastroenterological symptoms of depression such as decreased appetite, weight loss, change in bowel habit [224].

Only two further longitudinal studies evaluating change in quality of life with exposure to a gluten-free diet in incident coeliac disease has been performed [225, 226], both of which used the Short-Form (SF-36) questionnaire which is both validated and reproducible for use in both the general population and in disease states with data from representative population samples giving a general population comparison [227]. Both studies looked at quality of life measured by SF-36 in silent and symptomatic coeliacs and compared these with healthy controls [225, 226]. Johnston et al observed that 14 silent coeliacs had not different quality of life in comparison to healthy controls [225] whereas Nachman et al observed 35 silent coeliacs had significantly worse off quality of life in comparison to their control group [226]. Both groups observed that symptomatic coeliacs had lower quality of life in comparison to controls [225, 226]. On exposure to one year on a gluten-free diet, Johnston et al observed that symptomatic but not the silent coeliacs reaped a significant improvement in their quality of life [225] using EMA positivity to assess for dietary compliance. Due to large losses to follow-up (over three-quarters of the study population) no meaningful interpretation could be made of the silent coeliac patients change in quality of life with treatment though a gluten-free diet was of significant benefit in the symptomatic group [226]. The small sample size, the

selection bias associated with the choice of healthy controls, exclusion of cases were further limitations to the studies [225, 226]. A number of historical cross-sectional studies have also been performed on the quality of life of clinically diagnosed prevalent coeliacs established on a gluten-free diet [228-235] suggesting that health and psychological well-being is poor in prevalent coeliac disease with a high frequency of depression and anxiety.

A large prospective longitudinal study assessing change in quality of life using validated assessment tools in a contemporary cohort of unselected coeliac disease is clearly required.

1.7.4. Body composition and anthropometrics

Other than the observational data on bone mineral density in selected coeliacs, there are few studies that have observed the effect of coeliac disease upon body composition and anthropometrics. A historical study based on a small number of people with classic coeliac disease were observed to have lower weight, height, body mass index, fat mass and lean mass in comparison to controls which increased significantly with twelve months treatment with a gluten-free diet [236]. Height, bone mineral content, arm muscle area, triceps skin fold, fat area index were significantly lower in 23 children newly diagnosed with coeliac disease in comparison to age- and sex-matched controls [237]. Treatment with twelve months of a gluten-free diet resulted in a significant improvement in these measures though the improvement in height did not reach control levels [237].

With the broader spectrum of people being investigated for coeliac disease and consequently being diagnosed, prospective longitudinal studies are required to determine the impact of coeliac disease upon body composition and anthropometrics as well as quality of life and the effect of treating diagnosed coeliac disease in a contemporary cohort.

1.7.5. Cardiovascular disease

1.7.5.1. Impact and aetiology of cardiovascular disease in the general population

Worldwide, ischaemic heart disease is the leading cause of death and loss of disability-adjusted life years and is expected to remain the leading cause of mortality and morbidity in the western world well into the 21st century [238, 239]. The lifetime risk of having a cardiovascular event at 40 years of age is 49% in men (95% CI 45.8 – 51.3) and 32% in women (95% CI 29.2 – 34.2) [240].

Established high-risk demographic factors for vascular disease are advancing age (relative risk 8 for 60 versus 40 year olds), male sex (relative risk 2 – 5 men versus women), lower socioeconomic status (relative risk 3 for social class V versus social class I) and immigrants born in the Indian subcontinent (standardised mortality ratio 146 (95% CI 136, 156) relative to England and Wales standard rate) [241-244].

There are a number of modifiable aetiological factors associated with vascular disease. Cigarette smoking is associated with a nearly two-fold increased risk of vascular disease with the risk increasing with the number of cigarettes smoked [245]. Smoking accounted for 28% of male and 26% of female all-cause vascular deaths aged 35 to 69 [246]. Smoking cessation results in reduced risk of vascular disease

(relative risk reduction 0.64; 95%CI 0.58, 0.71 for those smokers that quit versus those who continued smoking) [247].

Increasing systolic and diastolic blood pressure is associated with increasing risk of vascular disease (relative risk 2 for developing vascular disease in 8 years with systolic blood pressure >/ 160 mmHg) [241]. A sustained reduction of 5 mmHg blood pressure over a 5-year period reduced coronary artery events by 25% and strokes by 30% in patients with ischaemic heart disease in five years [248].

Obesity is associated with increase in death from vascular disease (standardised mortality ratio 136 of BMI >/27 kg/m^2 versus <22.4 kg/m^2) [249]. People with central obesity are at increased risk of vascular disease in comparison to those of similar BMI but with peripheral adiposity [250]. Waist: hip circumference of >/ 0.91 was associated with nearly a threefold (relative risk 2.69 [95%CI 1.36, 5.31) compared to <0.91) increased risk of coronary artery disease events whilst a waist circumference > *95 centimetres was associated with a twofold increased risk (relative risk 2.02 [95%CI 1.17, 3.48] compared to <83.5 cm) in men during mean 10 year follow-up [251].* Overweight men as defined by BMI 25 – 30 kg/m^2 were also observed to have a twofold (relative risk 2.09 [95%CI 1.24, 3.53) to BMI < 24 kg/m^2) increased risk of coronary artery disease in this study [251].

There is a strong, independent and graded relationship between total cholesterol and risk of ischaemic heart disease with a reduction in total cholesterol of 0.6 mmol/L associated with 25 – 30% reduction in the risk of mortality from ischaemic heart disease in people aged 55 – 64 years [252]. The higher the LDL cholesterol level, the

higher the risk of vascular disease [253]. Reduction in LDL cholesterol by 1.3 mmol/L resulted in reduction in risk of non-fatal acute coronary syndrome or death from vascular disease by a third (risk reduction 31%; 95%CI 17, 43) in middle-aged men with hypercholesterolemia (non-fasting cholesterol 6.5 mmol/L or more) and no history of myocardial infarction [254]. HDL cholesterol is independently and inversely associated with the development of vascular disease where a rise in HDL cholesterol by 0.02 mmol/L results in a 3% reduced risk [255, 256]. Elevated levels of triglycerides are associated with increased risk of coronary artery disease events though it is not clear whether it is the triglyceride levels or the associated changes in the lipoprotein metabolism as well as with other vascular disease risk factors such as insulin resistance, obesity and lowered levels of HDL cholesterol that are responsible [257, 258].

Fibrinogen is independently associated with vascular disease risk (risk ratio 1.8 [95%CI 1.6, 2.0] of 0.35 g/L versus 0.25 g/L] [259] though it is not clear whether the increased risk observed in elevated levels of CRP are causal (odds ratio 2.13 [95%CI 1.38, 3.28] for 2.4 v 0.9 mg/L) [260].

Diabetes mellitus is associated with an excessive risk of vascular disease events with risks higher in women than in men (relative risk 3.5; 95%CI 2.7, 4.5 in female diabetics to those without diabetes) [261].

1.7.5.2. Impact of cardiovascular disease in coeliac disease

Like in the general population, vascular disease is the most important single cause of mortality in coeliac disease, accounting for 40% of all deaths [262]. However, the possibility that coeliac disease might afford some protection from vascular disease

was first raised by Whorwell et al in 1976 who observed a 40% reduction in ischaemic heart disease mortality in people with diagnosed coeliac disease [263] and further supported by studies in Italy and Scotland [264, 265]. Any reduction in vascular-related mortality in coeliac disease needs consideration particularly as it might outweigh larger increases in mortality from less common conditions and any benefit might be reduced by treatment with a gluten-free diet.

Recent studies have found some evidence of decreased cardiovascular morbidity suggesting that people with coeliac disease appear to have a favourable vascular risk profile. Coeliac disease appears to be associated with non-smoking although it is unclear whether this is a causal association [266-268]. In a cross-sectional population screening study people with positive endomysial antibodies had an 8% (0.5 mmol/L; $p < 0.01$) reduction in mean serum cholesterol and a 2.4 mmHg ($p < 0.05$) lower diastolic blood pressure in comparison to negative controls [170]. People with treated coeliac disease are reported to be less likely to have a diagnosis of hypercholesterolaemia (odds ratio 0.58 (95% CI 0.47 – 0.72)) or hypertension (odds ratio 0.68 (95% CI 0.60 – 0.76) and a lower reported antihypertensive medication use in comparison to age- and sex-matched general population controls [269].

Despite this apparent favourable cardiovascular risk profile and reduced risk of vascular-related mortality in comparison to the general population, not all studies have observed coeliac disease having a protective effect upon cardiovascular disease events. A Swedish hospital-based cohort study of 13,358 people with coeliac disease observed people with coeliac disease were at increased risk of myocardial infarction (hazard ratio 1.27 (95% CI 1.09 – 1.48)) and angina pectoris (hazard ration 1.46 (95%

CI 1.25 – 1.70)) [270]. In contrast, no differences were observed in the risk of neither myocardial infarction (hazard ratio 0.85 (95% CI 0.63 – 1.13)) nor stroke (hazard ratio 1.29 (95% CI 0.98 – 1.70)) in people with treated coeliac disease in comparison to general population controls in a population-based cohort study [269]. Furthermore, 367 coeliac patients identified by presence of positive coeliac serology and or characteristic change on small bowel histology had no increased risk of cardiovascular disease events such as myocardial infarction (unadjusted hazard ratio 1.10; 95%CI 0.62, 1.92) in comparison to 5537 seronegative controls [271]. Reasons for the observed lack of protection against cardiovascular disease events in diagnosed coeliac disease are unclear but possible explanations include an altering, attenuating effect on the vascular risk profile by treatment with a gluten-free diet or that other [272] processes particular to people with coeliac disease mediate the increased risk. For example, a low grade systemic inflammation causing accelerated atherosclerosis, adverse HDL-cholesterol and LDL-cholesterol profiles; anaemia; folate deficiency; homocysteine; weight changes; or some other reason [273-281]. The relationship between coeliac disease and vascular risk profile is clearly complex and requires unravelling. The effects of treatment with a gluten-free diet should also be determined before any screening programme for coeliac disease instituted.

1.7.6. Malignancy

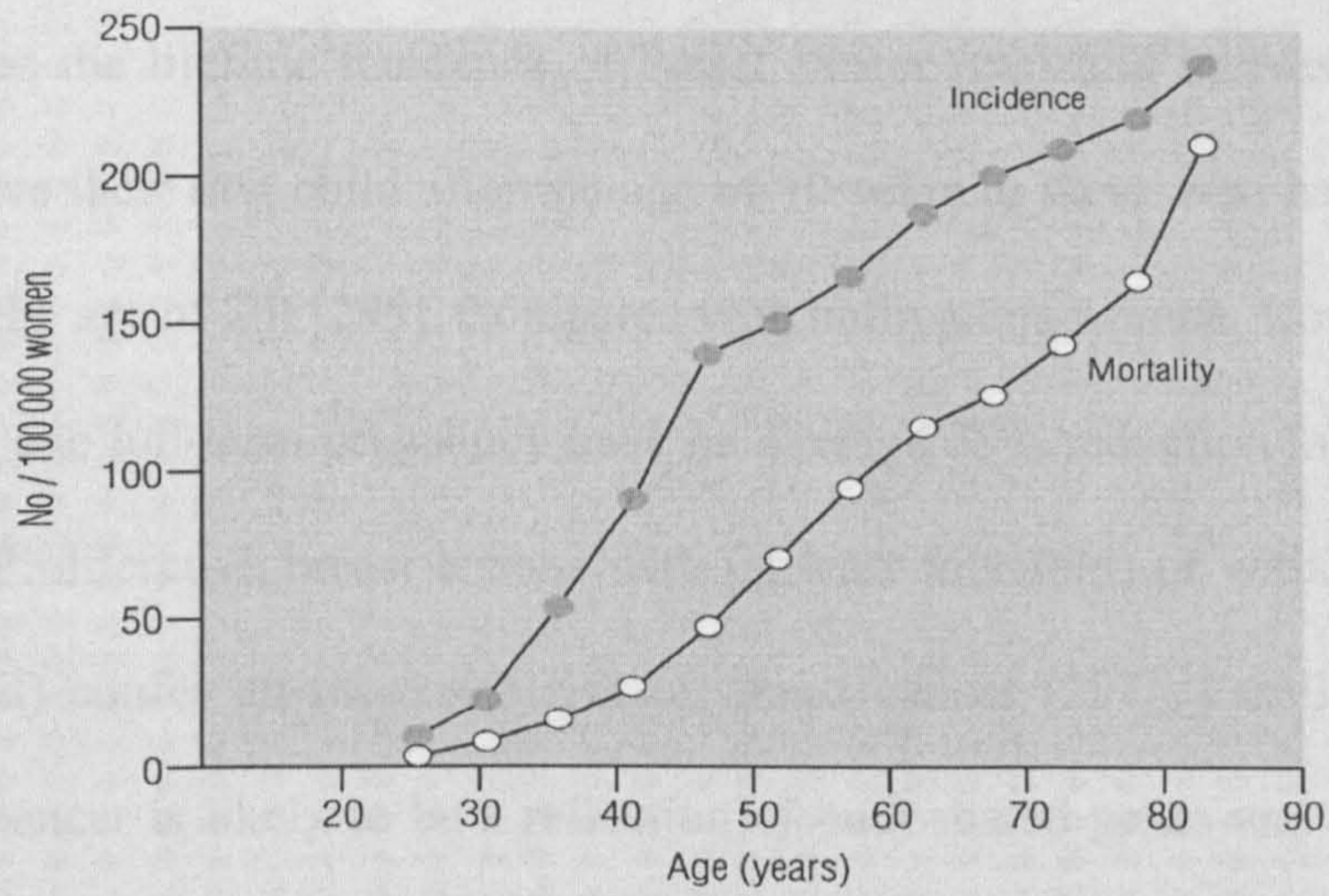
Diagnosed coeliac disease has been traditionally linked with greatly increased risks of lymphoproliferative malignancies [262, 264, 265, 282-287]. Early studies have been limited in their ability to provide precise estimates of the risks of cancer experienced by people with coeliac disease in comparison with the general population by their small sample size, selected nature from specialist coeliac centres and ability to adjust for potential confounders. In addition, when people are first investigated for coeliac disease the likelihood of detecting an occult or overt malignancy may be increased or conversely coeliac disease may be more likely to be detected during the investigation for cancer. The excess risk of gastrointestinal malignancy is likely, in part, to be contributed by the more detailed investigation of gastrointestinal symptoms particularly at presentation of coeliac disease. Furthermore findings from these studies probably do not reflect the risks in contemporary coeliac disease. More recent data from Sweden based on cases of coeliac disease obtained from their hospital in-patient register have suggested more modest though still increased risks of malignancy in people with coeliac disease [262, 283]. Although these studies were based on large numbers of people with coeliac disease, these studies are dependent on hospital admission of the index case for ascertainment and it is therefore possible that this may have led to an overestimate of the risks. Other recent data from a large population-based cohort study using the General Practice Research Database in the United Kingdom observed similar modest increases in the risks but still found that people with coeliac disease were at excess risk of certain malignancies [282]. Strengths of this study included validation of coeliac disease diagnosis and restriction analyses including assessment for ascertainment bias performed. However, with visit rate to general practitioners more frequent than in the general population, there is also the

possibility of differences in ascertainment of some malignancies as a result of opportunistic or systematic screening [282].

1.7.6.1. Impact and aetiology of breast cancer in the general population

Breast cancer is the commonest malignancy in women, comprises 18% of all female cancers and is the commonest cause of cancer death among women worldwide [288]. Incidence rates are high in more developed countries; in the United Kingdom where the age standardised incidence and mortality is the highest in the world, the cumulative incidence among women aged 55 years is 2.7% [289]. The incidence of breast cancer increases rapidly with age (relative risk > 10), doubling every 10 years during the reproductive years until the menopause, when the rate of increase slows dramatically (Figure 1.7) [289].

Figure 1.7: Age-specific incidence and mortality of breast cancer in the UK [290]



It is a disease mostly, though not exclusively, of women. Established high-risk demographic factors for breast cancer are advancing age (relative risk > 10), Caucasian race (relative risk >5 to Asians) and women of higher socioeconomic status (relative risk 2 for social classes I and II to other classes) [289, 291, 292]. Many of the other established aetiological factors of breast cancer are linked to oestrogens with hormonal factors playing a key role. Oestrogen stimulates the mitosis of breast epithelial cells, and this mitogenic effect may be augmented by progesterone [293]. Factors extending the relative exposure of the breasts to high concentrations of oestrogen (and perhaps progesterone) are associated with an increased risk of breast cancer such as early onset of menarche and later onset of menopause [293]. Such exposures, in addition to being relatively unmodifiable, are highly prevalent amongst women of the developed world [294]. Women who start menstruating early in life (relative risk 3 for age at menarche before age 11 years) or who have a late menopause (relative risk 2 for menopause after age 54 years) have an increased risk of developing breast cancer. The magnitude of this effect is similar whether menopause occurs naturally or as a result of bilateral oopherectomy [289]. Late age at first birth increases the lifetime incidence of breast cancer (two-fold increased risk in women who have their first child after the age of 30 years to those who have their first child before the age of 20) [295]. Compared with nulliparous women, women who have had at least one full-term pregnancy have on average 25% reduction in breast cancer risk [296]. Proliferative breast lesions with (at least four-fold) or without atypia (at least two-fold) confer an increased risk of breast cancer [297]. Familial aggregation of breast cancer is likely to be a reflection of both shared genes and to shared physical environments and lifestyles. A woman's risk of breast cancer is two or more times greater if she has a first degree relative who developed the disease before the age of

50 years with the younger the relative when she developed breast cancer the greater the risk [298]. Breastfeeding (pooled odds ratio 0.84; 95%CI 0.78, 0.91) [299], regular menstrual cycles (relative risk 0.76; 95%CI 0.62, 0.94 for <1 year to onset of regular menstrual cycles) [300] and severe caloric restriction caused by anorexia nervosa [301] have observed to have a protective effect upon breast cancer risk. Current use of oral contraceptives (relative risk 1.24) and use of hormone replacement therapy for more than 10 years (relative risk 1.34) are also aetiological factors [289, 302]. Post-menopausal obesity, increasing adult height, high alcohol intake, high intake of saturated fat and folate are also associated with increased breast cancer risk (relative risk 1.3 – 1.5) [303-309]. Severe caloric restriction caused by anorexia nervosa prior to age 40 years was observed to be associated with 50% lower incidence of breast cancer [301].

Population attributable risk (PAR) estimates suggest that age at first birth at >29 years, nulliparity, menarche before the age of 14 years, family history of breast cancer in 1st degree relative and history of benign breast disease account for the largest fraction of breast cancer cases in published studies (e.g. PAR 29.5% for age at first birth > 29 years and nulliparity in white women in United States ; 95%CI 5.6, 53.3) [292, 310].

The early life aetiological model for breast cancer emphasises early life events and conditions as determinants of breast cancer risk and summarises the distinct epidemiological characteristics of the disease on the basis of three major components [311-316]. The first major component of this model is that the likelihood of breast cancer occurrence depends on the number of mammary tissue-specific stem cells,

which is determined in early life processes. In early and later life, grow-enhancing mammotrophic hormones affects the replication rate of mammary tissue specific stem cells, the likelihood of retention of cells with spontaneous somatic mutations as well as the rate of expansion of initiated clones in the second component of the model. In the third component, pregnancy conveys long-term protection through differentiation of a large fraction of the mammary tissue-specific stem cells. The established breast cancer risk factors may be categorized into the different components of this model (Table 1.6)[317]:

Table 1.6: Grouping of breast cancer risk factors according to the general principles of carcinogenesis and the postulated pathogenic process

General principles of carcinogenesis	Number of mammary tissue specific stem cells	Growth enhancing mammotrophic hormones	Terminal differentiation
Age Ionising radiation Family history Specific genes	Gland mass Atypical hyperplasia Gender Birth weight Growth in early life Height Ethnic group	Gender Age at menarche Age at menopause Type of menopause Oral contraceptives Hormone replacement Pregnancy timing Postmenopausal obesity Ethanol intake Physical activity Adult life diet	Age at 1st term pregnancy Parity Lactation

Understanding the determinants underlying recognized risk factors and study of other factors that may confer risk or protection may help to advance our understanding of breast cancer aetiology and to aid in devising strategies for prevention.

1.7.6.2. Impact and aetiology of breast cancer in coeliac disease

Several studies have suggested coeliac disease is associated with a reduced risk of breast cancer (Table 1.7).

Table 1.7: Reduced risk of breast cancer in coeliac disease

Authors	Coeliac disease study population	Reduced risk of breast cancer in comparison to general population
Logan et al [265]	Population-based cohort in Edinburgh	SMR 0/1.52
Askling et al [283]	Swedish in-patient registry	SIR 0.3 (0.1 – 0.5)
West et al [282]	Population-based cohort in UK	Hazard ratio 0.24 (0.10 – 0.60)
Card et al [287]	Population-based cohort in Derby	SIR 0.59 (0.12 – 1.73)
Silano et al [318]	Hospital-based cohort in Italy	SIR 0.2 (0.04 – 0.62)
Solaymani-Dodaran et al [319]	Population-based cohort in Edinburgh	SMR 0/3.10

SMR standardised mortality ratio; SIR standardised incidence ratio

Figures in brackets refer to 95% confidence interval

Reasons for the apparent reduced risk of breast cancer in coeliac disease are unclear. Later age at menarche and earlier onset of menopause has been observed in women with coeliac disease in studies using small, selected populations [320-323] though a more recent and population-based study observed female coeliacs had similar fertility to that of the female general population though female coeliacs tended to have their babies at an older age [324]. Short stature, low body mass, caloric restriction, fat and folate deficiencies associated with coeliac disease [170, 221, 325] may also be implicated in the apparent reduced risk of breast cancer in women with coeliac disease. It may be the caloric reduction as a consequence of the intestinal inflammation and subsequent malabsorption that has an effect on breast cell growth and development; alternatively the reduced expression of oncogenes, levels of epidermal growth factor and insulin-like growth factor observed in caloric restriction

may be implicated [326-328]. The timing of the energy restriction may also be relevant. Energy restriction may be critical during early life and prior to first pregnancy when mammary tissue is particularly susceptible to carcinogenic processes [329, 330]. This hypothesis is supported by the observation that greater height, which although is genetically influenced is reflective of nutritional status and hence caloric intake during growth, is associated with an increased risk of breast cancer [331].

With breast cancer in the general population associated with reproductive, hormonal and anthropometric exposures, comparing the possession of these risk factors in women with coeliac disease to women without coeliac disease may clarify as to whether reproductive, hormonal and or anthropometric exposures contribute to the reduced risk of breast cancer in coeliac disease.

1.7.7. Mortality

Various studies have observed that diagnosed coeliac disease still confers a 1.3 – 2-fold increased risk in all-cause mortality compared with the general population [262, 265, 282, 332-334]. It is unclear how much of this excess risk is related to coeliac disease itself and how much the increase might be only indirectly related through associated conditions. There has also been speculation that the duration of gluten exposure prior to diagnosis has long-term adverse effects and therefore contributes to mortality [334] though a recent study observed children diagnosed with coeliac disease had a three-fold increased risk of long-term mortality in marked contrast to the experience of adulthood diagnosed coeliac disease where there was a modest increase in mortality [319] refuting this speculation.

Dermatitis herpetiformis, which forms part of the same spectrum of gluten-sensitive disorders as coeliac disease [335], has recently been observed to be not associated with an excess risk of mortality in comparison to the general population [336]. Differences in intestinal inflammation, reputed to be milder in dermatitis herpetiformis [337], or some other reason may be responsible for the difference in risk in comparison to that observed with coeliac disease.

Data using the Swedish In-Patient Registry observed people (n = 3719) with latent coeliac disease, defined in the study as having positive coeliac serology up to 180 days before duodenal biopsy was performed demonstrating normal mucosa, had excess mortality in comparison to those people without latent coeliac disease (1.7 per 1000 person years; hazard ratio 1.35, 95%CI 1.14, 1.58) [338]. People with positive coeliac serology drawn as part of a population-based screening study in Finland had no excess mortality in comparison to serology-negative controls [339] in contrast to

the 4-fold increased mortality risk in 14 serology-positive military recruits compared to seronegative controls (hazard ratio 3.9; 95%CI 2.0, 7.5) [340].

1.8. Summary

The prevalence of coeliac disease in the general population is 1% though only the minority of cases and a broader spectrum of people are clinically diagnosed with the disorder. Fracture risk, malignancy and mortality associated with clinically diagnosed coeliac disease have recently been described in large and contemporary population-based studies though other morbidity associated with clinically diagnosed coeliac disease and effect of treatment needs clarification. The benefits and possible harm of early detection and treatment of coeliac disease in otherwise asymptomatic, healthy people or those presenting with non-classic features is not clear. Understanding the determinants underlying recognized morbidity associated with coeliac disease and other factors that may confer risk or protection may help to advance our understanding of disease aetiology such as vascular disease, breast cancer and to aid in devising strategies for prevention. The rate of diagnosis of coeliac disease in developed countries has increased dramatically since the introduction of serological tests without an obvious environmental precipitant with further work needed to improve our understanding of the aetiology of the disorder.

1.9. Objectives of thesis

The aim of the thesis is to achieve the following objectives:

1. To describe the relationship between degree of enteropathy and physiological derangement, clinical features in incident coeliac disease
2. To examine the incidence of clinically diagnosed coeliac disease by socio-economic status
3. To quantify the impact of diagnosed coeliac disease on the risk of:
    - hypertransaminasaemia
    - hypercholesterolaemia
4. To estimate the vascular risk profile at diagnosis of coeliac disease and quantify any change following treatment with a gluten-free diet
5. To estimate the quality of life at diagnosis of coeliac disease and observe any change following exposure to a gluten-free diet
6. To describe the breast cancer risk profile in women with coeliac disease and compare to that of the general population

1.10. Outline of thesis

The outline of the thesis with aims and contents of each chapter is given below:

Chapter 2: Study design

- Introduce historical, prospective and cross-sectional study cohorts used in the studies of the thesis

Chapter 3: Historical cohort studies in incident adult coeliac disease

- Describe relationship between degree of enteropathy and physiological derangement, clinical features
- Examine the risk of hypertransaminasaemia and effect of treatment
- Examine incidence of diagnosed coeliac disease and socio-economic status
- Estimate cholesterol profile and effect of treatment

Chapter 4: Prospective, longitudinal studies in incident adult coeliac disease

- Quantify impact of coeliac disease on health-related quality of life and vascular risk profile and change with treatment

Chapter 5: Cross-sectional survey of women with coeliac disease

- Estimate breast cancer risk profile in women with coeliac disease and compare with general population

Chapter two: Description of cohorts used in thesis

2.1. Introduction

There are three different populations of adults with coeliac disease used in this thesis:

1. Historical, contemporary incident cohort (chapter 3)
2. Prospective, longitudinal incident cohort (chapter 4)
3. Cross-sectional survey of incident and prevalent female coeliacs (chapter 5)

This chapter will describe how the cohorts used in this thesis were generated. The chapter also describes the demographic, clinical, histological and laboratory features of the historical cohort used in the thesis.

2.1.1. Study design

For the series of studies described in chapters 3, 4 and 5 I needed to identify adults with coeliac disease. The cohorts would need to be a representative sample of the coeliac disease population so that my results would be applicable to the coeliac population as a whole. People acquire a diagnosis of coeliac disease based on the results of a small bowel biopsy (requiring histopathologists) and or positive coeliac serology (performed by immunologists); and should have contact with healthcare teams (usually gastroenterologists, dietitians) for explanation of the diagnosis made and treatment recommended. Confirmation of these pathways and any others taken by a person with coeliac disease would be needed, thereby identifying the likely coeliac disease population from which recruitment to the study (and an assessment of how representative study sample is) could take place. Furthermore, the likely numbers of people being diagnosed with coeliac disease by a centre each year would help guide the number of centres needed and that could be practically involved to run the study within time constraints (such as that dictated by study funding). It would be useful to know when coeliacs had contact with health services (such as attending outpatient

clinic appointment, endoscopy investigation) to help coincide routine clinical care with collection of data for studies, thereby minimising hassle to the study participant with the view of maximising recruitment and continued compliance with the study. Though involving a number of centres to help achieve sample size aim, the different centres were likely to approach clinical care of people with coeliac disease differently such as the frequency and timing of outpatient clinics with gastroenterologists and or dietitians which would need to be explored and incorporated into study design.

2.1.2. Possible choice of centres to involve in study

Being based at the University of Nottingham for my PhD student training, it was logical to have Nottingham University Hospital that serves a population of about 640 000 people as a study centre. Derbyshire Hospitals NHS Foundation Trust also forms part of the mid-Trent hospital network serving a population of approximately 600 000 people and had repeatedly been a source of collaboration on previous coeliac disease projects with my PhD supervisors and was therefore chosen as another centre for the study. A research collaboration was developed between Joe West and David Sanders between the Nottingham and Sheffield centres. Serving a local population of around 550 000 people, the Royal Hallamshire Hospital thereby became the third centre for the study.

2.1.3. Evolution of historical coeliac cohorts

With Nottingham, Derby and Sheffield hospitals confirmed as centres for the studies, I had to work out how to identify people being newly diagnosed with coeliac disease and how to get to meet these incident coeliac cases at each centre so that I could invite them to participate in the longitudinal studies of chapter 4. If I determined how incident coeliacs currently journeyed through each centre, I believed I could develop

systems in which to identify who was being diagnosed with coeliac disease and where I could meet them. For this I would need to firstly establish who had recently been diagnosed with coeliac disease and then determine what interactions (such as contact with an outpatient clinic) these people had had in the centre concerned. My plan was to identify all the people who were diagnosed with coeliac disease in the three centres between 2000 – 2006. How I worked out the journeys taken by people being newly diagnosed with coeliac disease at each centre is described in section 2.2.

From performing extensive searches of who had been diagnosed with coeliac disease at each centre, I generated historical cohorts for Nottingham and Sheffield centres which included systematic collection of demographic, clinical, histological and laboratory data. These historical cohorts not only helped me develop systems of how to recruit incident coeliacs for the prospective studies of chapter 4 but the historical cohorts formed the study populations for exploring mild enteropathy disease; socioeconomic status; and liver and vascular morbidity associated with coeliac disease (chapter 3). Women with coeliac disease identified in these searches also formed the study population for the cross-sectional survey on breast cancer risk profile (chapter 5).

2.2. Journeys taken by people newly diagnosed with coeliac disease

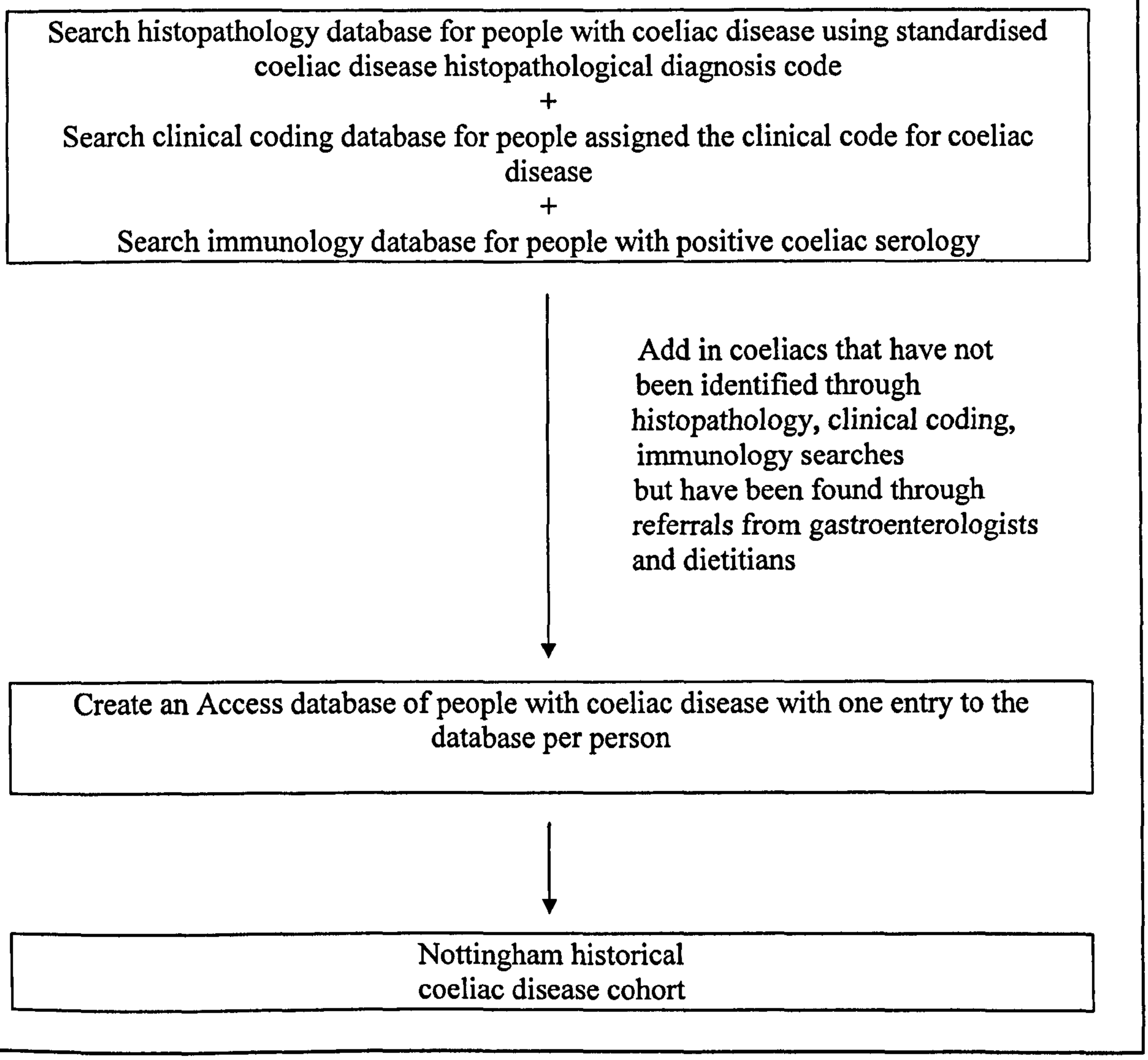
This section will describe how I identified who was being diagnosed with coeliac disease at each of the study centres and what interactions (such as outpatient clinics) these coeliacs had with the centre in question. A summary flow diagram summarising how the historical cohort was constructed and how I identified adults newly diagnosed with coeliac disease at the Nottingham and Sheffield centres is first given.

2.2.1. Nottingham University Hospital, Nottingham

2.2.1.1. Coeliac disease cohort at Nottingham University Hospital creating the Nottingham historical cohort: a summary

Combining histological data on coeliacs identified from histopathology, clinical coding and immunology searches with clinical information (such as symptoms at presentation) obtained from general practitioner and hospital letters, endoscopy reports; coeliac serology results; and laboratory data the Nottingham historical cohort was constructed:

Figure 2.8: Creation of historical coeliac disease cohort at Nottingham University Hospital



2.2.1.2. Incident coeliac disease cohort at Nottingham University Hospital: a summary

Monthly updates from histopathology, clinical coding and immunology databases were used to generate a list of people with coeliac disease. This list was cross-referenced with clinical information obtained from general practitioner and hospital letters to determine those that had been newly diagnosed with coeliac disease. The subsequent hospital attendances of these people newly diagnosed with coeliac disease was determined in order for me to go and discuss participation in the prospective studies.

Figure 2.9: Flow diagram of method of identifying people with newly diagnosed coeliac disease at Nottingham University Hospital to recruit for prospective vascular risk profile study

Search histopathology database each month of study for people with coeliac disease using standardised coeliac disease histopathological diagnosis code

+

Search clinical coding database each month of study for people assigned the clinical code for coeliac disease

+

Search immunology database each month of study for people with positive coeliac serology

↓

Add in coeliacs that have not been identified through histopathology, clinical coding, immunology searches but have been found through referrals from gastroenterologists and dietitians

↓

Create an Access database of all people newly diagnosed with coeliac disease during the study period

↓

Use the patient administration system, secretaries, dietitians to determine date, time and place when these incident coeliacs are attending the hospital for appointments

↓

Attend hospital appointments of people newly diagnosed with coeliac disease and invite them to participate in prospective study

↓

Obtain informed consent

↓

Newly diagnosed coeliacs become study participants for prospective study

2.2.1.3. Histopathology records

I assumed the majority of people would acquire a diagnosis of coeliac disease on the basis of small bowel histological appearances regardless of the events leading up to having the small bowel biopsy being taken. There is a single histopathology computerised database on which all tissue samples taken at the hospital that are submitted for histological examination are logged under an unique laboratory number. The referring consultant is included in the information logged with the tissue specimens which may be a gastroenterologist (indicated by their initials) but not necessarily so with people presenting to other specialists (such as to the Haematology or Respiratory Consultant). The range of specialties of referring consultants highlighted the variety of routes through which people could come through to get diagnosed with coeliac disease by means of a small bowel biopsy. Standardised SNOMED (Systemized Nomenclature of Medicine) codes are assigned to the histopathological diagnosis of the tissue specimens. The computerised histopathological database can be searched for specific diagnoses by entering the standardised code of interest such as 'T6430D62180' which uniquely specifies coeliac disease. Following liaison with Dr Philip Kaye, a consultant histopathologist with subspecialty interest in gastrointestinal disease, a list of all people having tissue specimens assigned the code for coeliac disease between 1st January 2000 and 31st December 2006 was generated from the histopathology database. An excerpt of the list generated is shown below:

Figure 2.10: Excerpt of list of people possessing code for coeliac disease from histopathology database

Lab number	Consultant	Surname*	Forename*	Date of birth	SNOMED code
03109205	KT	Xx	Xx	11/12/1952	T6430D62180
03109605	PAJ	Xx	Xx	11/04/1966	T6430D62180
03110054	WPG	Xx	Xx	10/04/1955	T6430D62180
03110822	ROM	Xx	Xx	04/12/1929	T6430D62180
03111028	RGL	Xx	Xx	16/04/1937	T6430D62180
03111328	KT	Xx	Xx	13/04/1960	T6430D62180
03111828	JTM	Xx	Xx	21/09/1963	T6430D62180

*Excerpt anonymous but the real list generated that I used isn't anonymous

The list of all people having tissue specimens assigned the code for coeliac disease between 1st January 2000 and 31st December 2006 generated from the histopathology database generated 983 unique laboratory numbers though some people appeared on the list on more than one occasion i.e. had further small bowel biopsies taken on a subsequent date where again coeliac disease was demonstrated. Some people on the histopathology list were children i.e. aged under 18 years and so not relevant to my studies.

Using this list I entered each unique laboratory number into the histopathology computerised database to bring up the linked histopathology report and read through each person's small bowel histology result. I used a number of ways to determine whether the small bowel biopsy was taken to diagnose coeliac disease or whether the small bowel biopsy was taken in someone who had already been diagnosed with coeliac disease.

Features suggestive of the small bowel biopsy being taken to newly diagnose a person with coeliac disease included:

- clinical information submitted with the small bowel specimen (such as 'diarrhoea – rule out coeliac disease', 'anaemia and positive endomysial antibody – confirm diagnosis of coeliac disease)
- clinical information obtained around the time the small bowel specimen was taken from general practitioner referral letters to the hospital (such as 'please see this person who has developed diarrhoea')
- clinical information obtained around the time the small bowel specimen was taken from hospital outpatient clinic and in-patient discharge letters to the general practitioner (such as 'this person was diagnosed with coeliac disease following presentation with anaemia which was further investigated with endoscopy where duodenal biopsies showed coeliac disease')
- no previous small bowel specimens demonstrating coeliac disease

On the basis of these features, the following definition was used to define incident coeliac disease:

Definition 1: Incident coeliac disease and date of coeliac disease diagnosis
A Consultant Gastroenterologist using the information available at the time made a diagnosis of coeliac disease. This decision was presumed to have been arrived at by a combination of suggestive clinical symptoms, characteristic small bowel pathology or abnormal coeliac serology around the time the diagnosis was made. The date at which they made the diagnosis as recorded in the medical notes or the date of the abnormal small bowel biopsy, whichever was earliest, was the diagnosis date.

.

Definition 2: Time period of inclusion of incident coeliacs within historical cohort
Adults newly diagnosed with coeliac disease on a date falling within 1st January 2000 – 31st December 2006.

Features suggestive of the small bowel specimen being taken in people with previously diagnosed coeliac disease was derived from clinical information such as 'coeliac – monitor response to treatment' or 'coeliac with persistent symptoms query cause' either submitted with the specimen or contained on hospital letters. An attempt was made to establish the original date of diagnosis of incident coeliac disease in these people with previously diagnosed disease through looking through the histopathology database and hospital records. If the date of diagnosis of incident coeliac disease was between 1st January 2000 – 31st December 2006 then these people were included within the incident cohort.

Definition 3: People with prevalent coeliac disease within historical cohort
A Consultant Gastroenterologist using the information available at the time made a diagnosis of coeliac disease. This decision was presumed to have been arrived at by a combination of suggestive clinical symptoms, characteristic small bowel pathology or abnormal coeliac serology around the time the diagnosis was made. The date at which they made the diagnosis as recorded in the medical notes or the date of the abnormal small bowel biopsy, whichever was earliest, was the diagnosis date with the diagnosis date prior to 1st January 2000.

It soon became evident that the histopathology database was of considerable help in identifying who had been diagnosed with coeliac disease on the grounds of the appearances of the small bowel specimens at Nottingham University Hospital over the defined time period. Each month I received a list of all people that had small bowel specimens taken that demonstrated coeliac disease. Using the techniques described above to determine whether these specimens were taken to newly diagnose a person with coeliac disease I was able to establish who was being newly diagnosed with coeliac disease. I would then work out using the hospital patient administration system when and where these people newly diagnosed with coeliac disease were attending outpatient clinics (such as to discuss the newly made diagnosis of coeliac disease) and or meeting the dietitian (such as to discuss how to switch to a gluten-free diet). This mapping out of hospital attendances was fundamental to the prospective studies of chapter 4 – I needed to attend these key events to meet newly diagnosed coeliacs and use the opportunity to invite them to participate in the longitudinal studies on vascular risk profile and quality of life.

2.2.1.4. Immunology records

Like with the histopathology department, there is a single immunology database on which all serum samples taken whether at the hospital or in the community that are submitted for coeliac serology testing are logged under an unique laboratory number. Like with the histopathology specimens, the referring consultant is included in the information logged with the serum sample which may be a gastroenterologist or from another specialty, again highlighting the variety of specialties potentially diagnosing coeliac disease.

Definition 4: Coeliac serology status
Coeliac serology is reported as 'positive', 'weak positive' or 'negative' for the EMA test and a quantitative titre given for the tTG test with values above 4.0 U/L for the assay used considered as a positive test result. The tTG test is also banded by 300 U/L as maximum value reported.

If a small bowel biopsy was not taken despite extensive efforts to do so (such as due to patient refusal, patient unable to tolerate endoscopy) and the person had clinical features suggestive of coeliac disease and had positive coeliac serology with symptoms and serology improving with a gluten-free diet then they would also acquire a diagnosis of coeliac disease by the reviewing gastroenterologist.

Definition 5: Histology unproven but serology positive coeliac disease
If a small bowel biopsy was not taken but there were clinical features suggestive of coeliac disease and coeliac serology was positive which both improved with treatment with a gluten-free diet then the person was assumed to have histology unproven but serology positive coeliac disease. The date of diagnosis of histology unproven but serology positive coeliac disease was the date when the coeliac serology was performed.

Each week a paper printout of all coeliac serology tests performed with their results is generated and stored by the Immunology department. Following liaison with Dr Liz McDermott, a consultant immunologist, I was allowed access to these weekly paper results. Searching for people with positive coeliac serology over a six year period (between 1st January 2000 and 31st December 2006 like with the small bowel specimen histopathology search) using this paper printout was fairly laborious and prone to error, scanning through the results of hundreds of tests performed each week attempting to identify those with positive coeliac serology. However, consulting the coeliac serology printouts was an useful adjunct to the histopathology search for identifying people newly diagnosed with coeliac disease for the prospective studies.

Like with the histopathology search, I used a number of ways to determine whether the coeliac serology was being performed to diagnose coeliac disease or whether it was being used to monitor prevalent coeliac disease. Using the same approach as with the histopathology search, features differentiating positive coeliac serology due to a new diagnosis of coeliac disease from prevalent coeliac disease included clinical information supplied with the serum request or from hospital and general practitioner

letters at around the time the serology testing was performed such as 'diarrhoea – rule out coeliac disease'.

The date of coeliac disease diagnosis assigned on histopathological grounds of when coeliac disease was first detected on small bowel specimens was cross-referenced to the date when (if serological testing was performed) a person had positive coeliac disease serology. If the date of positive serology preceded the date where small bowel biopsies were performed showing characteristic histological changes, the date of diagnosis remained as the date on which small bowel biopsies were performed as per definition I have used of incident coeliac disease. Looking through coeliac serology test results proved to be an useful addition to the histopathology search.

2.2.1.5. Clinical coding records

Clinical coding refers to the "translation of medical terminology as written by the medical professional to describe a patient's symptoms, diagnosis, treatment or reason for seeking medical attention into a coded format" which is nationally and internationally recognised [341]. There are two coding systems used by NHS hospitals: ICD10 (International Classification of Diseases 10th Revision) and OPCS4 (The Office of Population Censuses and Surveys 4th Revision) [341]. ICD10 has been devised by the World Health Organisation and its codes are widely used internationally. ICD10 codes cover all reasons for patients admissions to hospital [341]. OPSC4 in comparison covers all operative procedures and interventions that patients have undergone during their hospital stay and codes are only used in the UK [341]. Clinical codes are used to support many functions within a hospital trust both

clinically (such as clinical governance) and numerically (such as commissioning, health trends) [341].

Following liaison with Brenda Brown, Clinical Coding Manager, I obtained a list of all people, identified by name, age and hospital number that had a clinical code for coeliac disease between 1st January 2000 and 31st December 2006. An excerpt of the clinical coding list electronically generated is shown below:

Figure 2.11: Excerpt of list of people possessing clinical code for coeliac disease

```
REPORT:              PATIENT DETAIL LISTING - ACTIVITY BASED
GROUPED BY:          Age Groups (10 Year Bands) - current
FILTERED BY:         From 01 January 2000 to 31 December 2006 inclusive
                     Problem 1: COELIAC DISEASE
                     OR
                     Problem 2: COELIAC DISEASE

SORTED BY AGE GROUPS (10 YEAR BANDS) - CURRENT
```

1. AGE GROUPS (10 YEAR BANDS) - CURRENT: 000 010

1.1.
XX* Hospital number Sxxxxxx**

1.2.
XX* Hospital number Sxxxxxx**

1.3.
XX* Hospital number Sxxxxxx**

*Name and **hospital number made anonymous in this excerpt but the real list generated isn't anonymous

The list of all people assigned the code for coeliac disease between 1st January 2000 and 31st December 2006 generated from the clinical coding database generated 780 unique names and hospital numbers. The data was presented in 10 year age brackets so included children with coeliac disease aged 10 years or younger and a couple of centenarians!

In 1998, an in-house electronic system of all gastroenterology consultations, endoscopies and nurse specialist services was introduced at Nottingham City Hospital with "live" data collection of all referrals and interventions involving people with

coeliac disease performed by Dr Kathy Teahon, Consultant Gastroenterologist, based on the clinical coding described above. I was allowed access to this database which listed 458 adults with a code for coeliac disease, identified by name and hospital number. An excerpt of the Nottingham City Hospital in-house electronic database of adults with a code for coeliac disease is shown below:

Figure 2.12: Excerpt of list of people possessing clinical code for coeliac disease in Nottingham City Hospital in-house electronic system

Surname	Forename	Patient number	Diagnosis1
XX*	XX*	XX**	K900
XX*	XX*	XX**	K900
XX*	XX*	XX**	K900

Like with the histopathology and coeliac serology searches I needed to determine whether the clinical code assigned to the person was a newly acquired code i.e. the person had been newly diagnosed with coeliac disease or whether the person was known to have coeliac disease but acquired a further clinical code for coeliac disease such as through attending a follow-up dietetic appointment or having a repeat small bowel biopsy to monitor response to treatment. Using the histopathology, coeliac serology data and information contained on endoscopy reports, general practitioner and hospital letters as described above I was able to assign date of diagnosis of coeliac disease to each individual contained on the list.

Clinical coding enabled identification of those who had been diagnosed with coeliac disease on the grounds of hospital attendances, procedures and interventions where coeliac disease code was acquired at Nottingham University Hospital over the defined time period. However, there was the possibility that some people newly diagnosed with coeliac disease may not appear on the clinical coding list during the period of

recruitment for the prospective studies. Each month I received a list of all people that had acquired a clinical code for coeliac disease. Using the techniques described above to determine whether the code was given to someone newly diagnosed with coeliac disease or someone with prevalent disease, I was able to establish who was being newly diagnosed with coeliac disease. I would then work out using the hospital patient administration system when and where these people newly diagnosed with coeliac disease were attending outpatient clinics (such as to discuss the newly made diagnosis of coeliac disease) and or meeting the dietitian (such as to discuss how to switch to a gluten-free diet). This mapping out of hospital attendances was fundamental to the prospective studies of chapter 4 – I needed to attend these key events to meet newly diagnosed coeliacs and use the opportunity to invite them to participate in my study.

2.2.1.6. Outpatient clinic set-up

Nottingham University Hospital has a single gastroenterology and endoscopy department, encompassing subspecialty interests such as luminal gastroenterology, hepatology and nutrition. However there was no specific coeliac disease gastroenterology clinic. People were being diagnosed with coeliac disease by a number of different gastroenterologists as well as surgeons and other non-gastroenterological medical physicians. Using histopathological, serological and clinical coding searches described above, I was able to flag up who was being newly diagnosed with coeliac disease and what hospital appointments (such as dietetic) these people were due to attend so that I could exploit these opportunities to meet these incident coeliacs and invite them to participate in my prospective studies.

In addition, I emailed all gastroenterology consultants to invite them to forward me any referrals they received or contact they had with people with coeliac disease. The minority of gastroenterologists wished to continue reviewing people with coeliac disease so I attended their outpatient clinics. Any referrals I arranged to see in Professor Logan's outpatient clinic on Tuesday mornings which coincided with when Karen Columbell, senior dietitian with a special interest in coeliac disease, who held her dietetic appointments to see people with coeliac disease (newly diagnosed as well as monitoring those with established disease) referred by any consultant, whether physician or surgeon, within the hospital. Karen and I developed a system whereby people newly diagnosed with coeliac disease would be seen at an 'one-stop' coeliac clinic – I would explain the diagnosis and implications of having coeliac disease and then once clinical care had been delivered, invite the person to participate in my prospective studies. If the person agreed to participate then following informed consent I would collect the necessary study data before passing over to Karen who would explain how to take a gluten-free diet. We would then arrange to review the person at the 'one-stop' clinic together for monitoring response to treatment as well as to repeat study data collection.

The majority of people taking part in my prospective studies were followed up in this one-stop coeliac clinic. For the patient there was continued contact with a doctor if needed as well as a dietitian (many gastroenterologists discharged patients to dietetic care) using the opportunity to ask clinically-based questions about their condition as well as have blood tests to monitor (and highlight need for treatment such as for anaemia) coeliac disease. Karen felt there was readily available medical advice when and if needed as well as accountability for reviewing normal and abnormal blood test

results. This simplified the process of meeting or following up patients who were recruits for the prospective study.

2.2.2. Royal Hallamshire Hospital, Sheffield

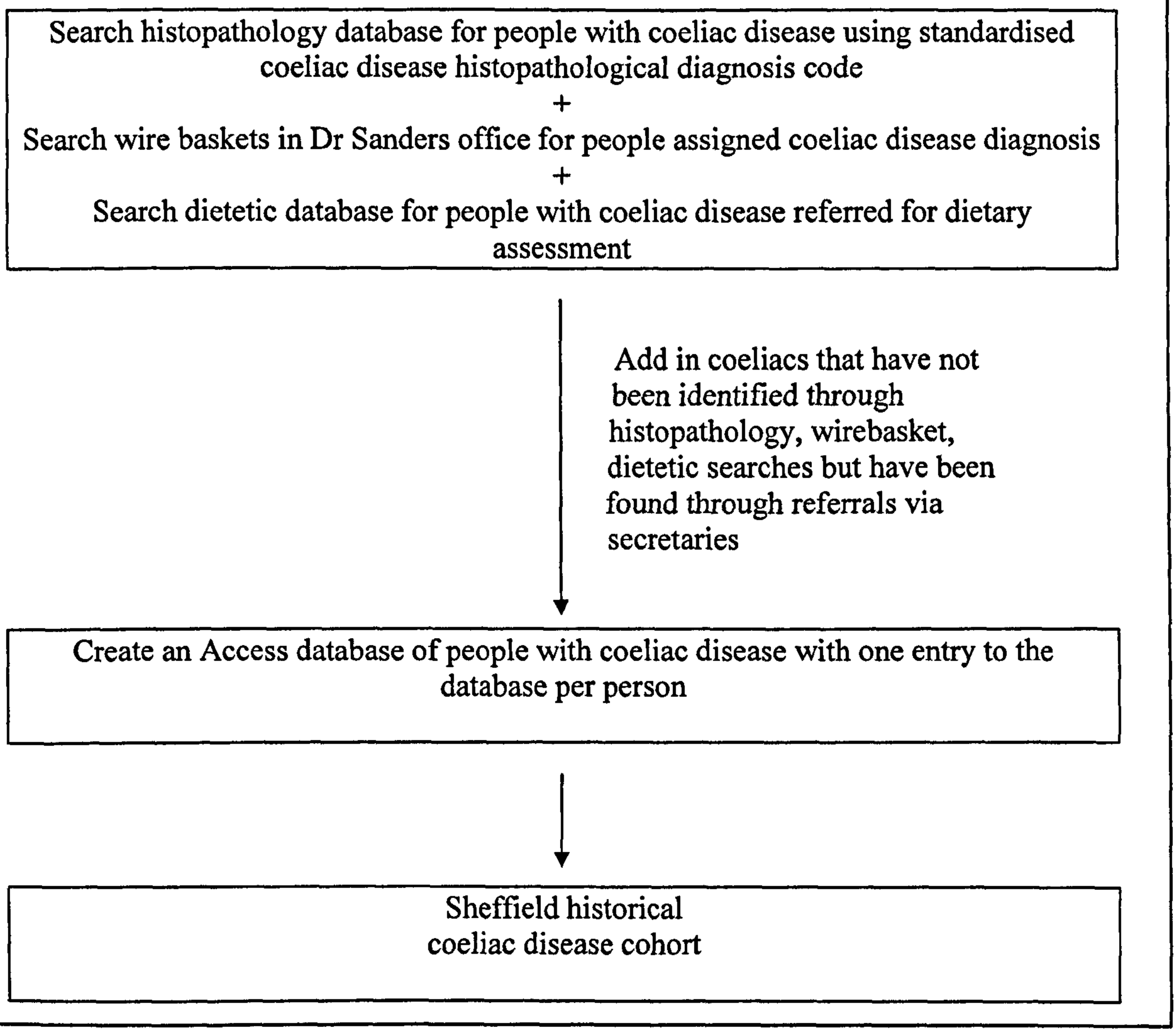
Like Nottingham University Hospital, the Royal Hallamshire Hospital has a single gastroenterology and endoscopy department though in comparison it has a .gastroenterology consultant with a special interest in coeliac disease, Dr David Sanders. Dr Sanders has held a dedicated outpatient clinic for people with coeliac disease on Thursday afternoon since 2002. Adults suspected as having coeliac disease were referred to this clinic by general practitioners, gastroenterologists and other specialists from within the Royal Hallamshire Hospital and from the other hospital of the same trust, the Northern General Hospital. The clinic was also attended by adults that were having a review of their coeliac disease. People attending this clinic could therefore be in different stages of their coeliac disease journey – some people were waiting for the diagnosis of coeliac disease to be confirmed and at this clinic confirmatory tests such as small bowel biopsies would be arranged; some had coeliac disease diagnosed though were attending clinic to be informed of this and counselled on the need for treatment; some had coeliac disease diagnosed an earlier point in time either at the Hallamshire or some other hospital and were being monitored at this clinic.

A summary flow diagram summarising how the historical cohort was constructed and how I identified adults newly diagnosed with coeliac disease at the Sheffield centre is first given.

2.2.2.1. Coeliac disease at the Royal Hallamshire Hospital creating the Sheffield historical cohort: a summary

Combining histological data on coeliacs identified from histopathology, dietetic, wire basket searches with clinical information (such as symptoms at presentation) obtained from general practitioner and hospital letters, endoscopy reports; coeliac serology results; and laboratory data the Sheffield historical cohort was constructed:

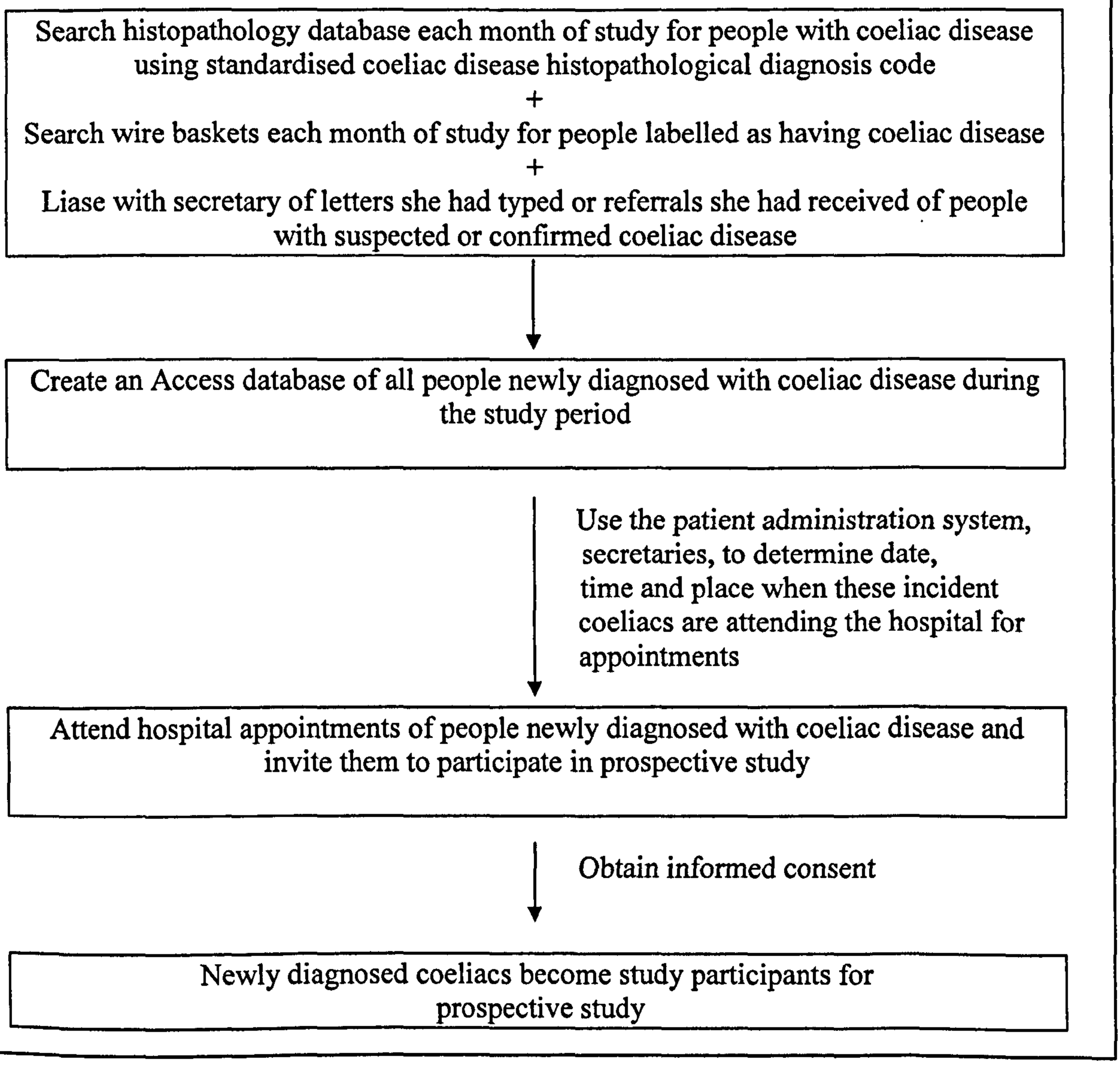
Figure 2.13: Creation of historical coeliac disease cohort at Royal Hallamshire Hospital



2.2.2.2. Incident coeliac disease at Royal Hallamshire Hospital: a summary

Monthly updates from histopathology database, wirebasket searches, and secretarial alerts were used to generate a list of people with coeliac disease. This list was cross-referenced with clinical information obtained from general practitioner and hospital letters to determine those that had been newly diagnosed with coeliac disease. The subsequent coeliac disease clinic appointments of these people newly diagnosed with coeliac disease was determined in order for me to go and discuss participation in the prospective studies.

Figure 2.14: Identification of people with newly diagnosed coeliac disease at Royal Hallamshire Hospital to recruit for prospective studies



2.2.2.3. Histopathology records

From experiences at Nottingham University Hospital in identifying who had coeliac disease, I first set about obtaining a list of people who had small bowel biopsies demonstrating coeliac disease via the histopathology department. Like at Nottingham University Hospital, there were consultant histopathologists with a special interest in gastrointestinal diseases. Dr Simon Cross was a consultant histopathologist at the Hallamshire who has a special interest in coeliac disease. Following discussion with Dr Cross and the Histopathology Manager, Louise Dunk, it was clear that small bowel histology would be routinely reported by Dr Cross but in his absence by other histopathologists also. However all small bowel specimens suggestive of or demonstrating coeliac disease would be ultimately reviewed by Dr Cross who would then grade the degree of intestinal inflammation according to modified Marsh criteria [342] if coeliac disease related enteropathy was present. I acquired the list of all people having a small bowel biopsy demonstrating coeliac disease from 1st January 1997 – 31st December 2006. An excerpt of this list is shown below:

Figure 2.15: Excerpt of list of people possessing code for coeliac disease from histopathology database

Date of biopsy	Pathology specimen number	Code of specimen site	Specimen site	Histopathology diagnosis code	Name	Date of birth	Hospital number	Source of referral
25/10/2004	PH011365V/04	T-58400	Jejunum	D5-47100	Xx	Xx	Xx	MOPD
26/10/2004	LH025113T/04	T-58200	Duodenum	D5-47100	Xx	Xx	Xx	MOPD
27/10/2004	LH025265K/04	T-58200	Duodenum	D5-47100	Xx	Xx	Xx	ENDO
27/10/2004	LH025289K/04	T-58200	Duodenum	D5-47100	Xx	Xx	Xx	ENDO
02/11/2004	LH025650T/04	T-58200	Duodenum	D5-47100	Xx	Xx	Xx	ENDO
03/11/2004	LH025782E/04	T-58200	Duodenum	D5-47100	Xx	Xx	Xx	MTH

Just as at Nottingham University Hospital, each tissue samples taken at the Hallamshire that is submitted for histological examination are logged under an unique pathology specimen number. The source of referral is included in the information

logged with the tissue specimens such as endoscopy ('ENDO'), outpatients ('MOPD') despite the small bowel specimen presumably being taken in the endoscopy suite and pr operating theatres ('MTH') perhaps for a person requiring a general anaesthetic rather than local anaesthetic and sedation for the endoscopy. Despite the wide variety of routes to get to the stage of having small bowel specimens being taken, they all were analysed by the histopathology department.

The list of all people having tissue specimens assigned the code for coeliac disease between 1st January 2000 and 31st December 2006 generated from the histopathology database generated 663 unique laboratory numbers though some people appeared on the list on more than one occasion i.e. had further small bowel biopsies taken on a subsequent date where again coeliac disease was demonstrated. Some people on the histopathology list were children i.e. aged under 18 years.

Using this list I entered each unique laboratory number into the histopathology computerised database to bring up the linked histopathology report, reading through each person's small bowel histology result. I used identical techniques to those I used at Nottingham University Hospital described above to identify whether the small bowel histology was taken to diagnose someone with coeliac disease or whether the small bowel biopsy was taken in someone who had already been diagnosed with coeliac disease and then work backwards in time to determine the date of diagnosis.

Combining this histological data with clinical information (such as symptoms at presentation) obtained from general practitioner and hospital letters, endoscopy

reports; coeliac serology results; and laboratory data the Sheffield historical cohort was constructed.

During the period of recruitment for the prospective studies of chapter 4, I regularly received a list of all people that had small bowel specimens taken that demonstrated coeliac disease. Using the techniques described above to determine whether these specimens were taken to newly diagnose a person with coeliac disease I was able to establish who was being newly diagnosed with coeliac disease.

2.2.2.4. Outpatient clinic set-up

Adults with coeliac disease and attending the dedicated coeliac clinic at the Royal Hallamshire Hospital were systematically collected by Dr Sanders through copying each of the clinical letters written on each person to himself. An example of a letter sent from Dr Sanders to the general practitioner or other healthcare professional is demonstrated below:

Figure 2.16: Example of letter sent from coeliac disease clinic

GASTROENTEROLOGY & LIVER UNIT

Gastroenterology:
Dr A J Lobo MD FRCP
Tel: 0114 2712353 / 2712832

Dr M E McAlindon BMed Sci DM FRCP
Tel: 0114 2261180

Dr D S Sanders MD FRCP FACG
Tel: 0114 2261179

Dr D P Hurlstone MD (Dist) MRCP
Tel: 0114 2268706

Fax: 0114 2712692

Hepatology:
Dr D C Gleeson MD BSc FRCP
Tel: 0114 2713652

Dr M A Karajeh MRCP
Tel: (0114) 2268746

Royal Hallamshire Hospital
Glossop Road
Sheffield
S10 2JF

Tel: 0114 271 1900
Fax: 0114 271 1901

DSS/MH/[redacted]

Clinic 30 July 08
Typed 14 July 08

Dr P A Bradbury
Jordanthorpe Health Centre
1 Dyche Close
Sheffield
S8 8DJ

Dear Dr Bradbury

Re: [redacted]

Problem:
1. Coeliac disease?
2. EMA positive, IgA and IgG gliadin positive – checked in primary care
3. Bilateral hernia 2006/7
4. Mother died of stomach cancer at the age of 69.
5. Fulfils ROME II criteria for IBS (symptoms ongoing for at least 10 years)/also has anaemia intermittently since childhood.

Thanks for referring this 43 year old gentleman who may well have coeliac disease. I have discussed these issues with him but currently he is not on a gluten free diet and we have not confirmed the diagnosis with duodenal biopsy.

Plan is as follows:
1. I have checked FBC, ESR, U&Es, LFTs, glucose, CRP, calcium, TFTs, immunoglobulins, antigliadin antibodies, EMA, TTG, vitamin B12, folate, ferritin, HDL cholesterol ratio, thyroid autoantibodies and HLA typing.
2. I will review these then arrange for a gastroscopy with duodenal biopsy.
3. At the time of his endoscopy we can arrange follow up.

Current medication: Nil

Best wishes.

Yours sincerely

Dr D S Sanders
Consultant Physician & Gastroenterologist
Honorary Reader in Gastroenterology, University of Sheffield

Cc Dr Marios Hadjivassiliou, Consultant Neurologist, L Floor, RHH

For your interest, anti-GAD Ria/Elisa

These letters would be stored in wire baskets on the shelves in Dr Sanders' office and filed under specifically named wire baskets. For example, people with refractory coeliac disease would be filed under 'refractory coeliac disease'; people with irritable bowel symptoms and coeliac disease would be filed under 'IBS coeliacs' and so on. Here is a photograph of what the filing system is like:

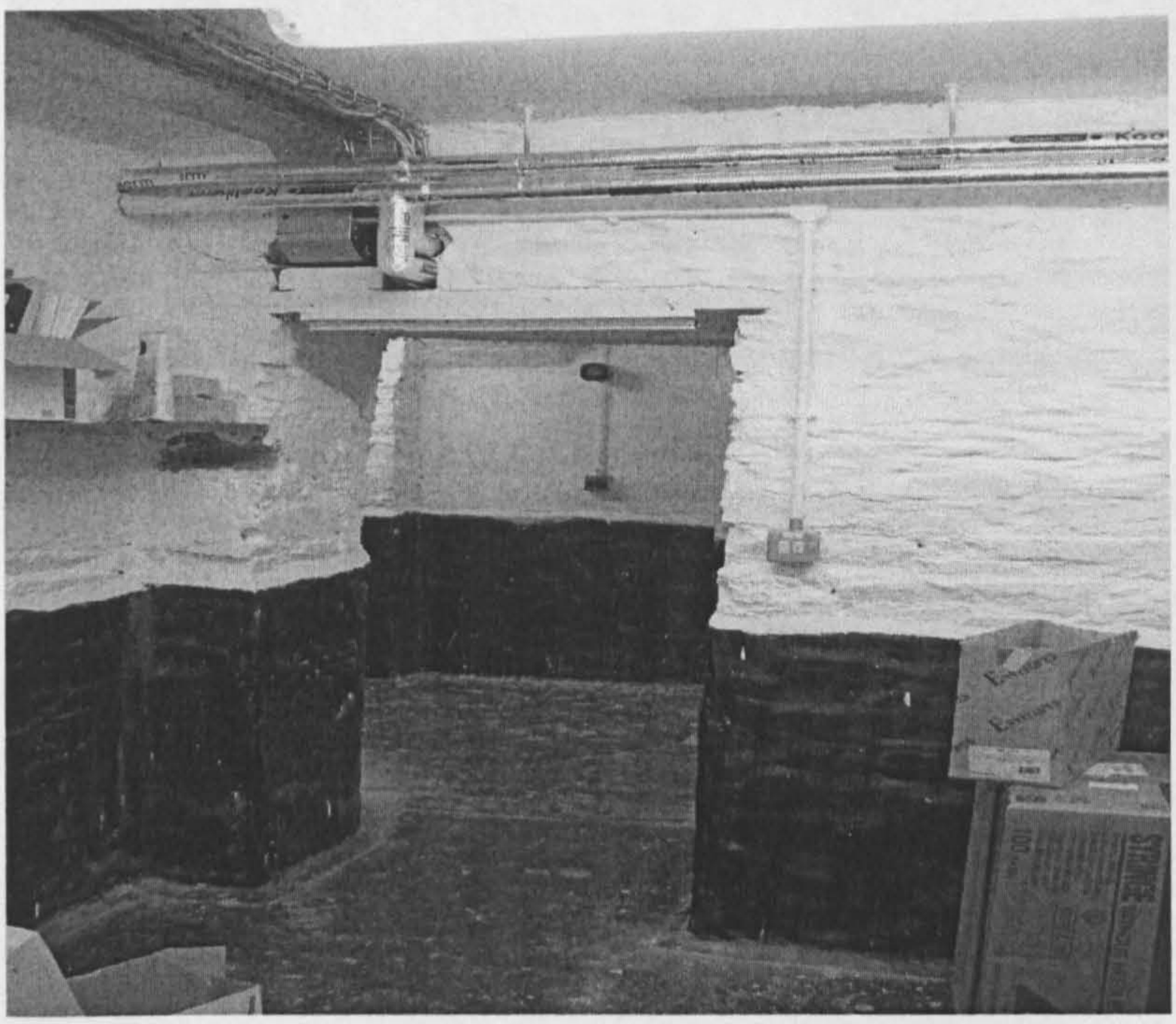
Figure 2.17: Wire baskets filing away coeliacs attending the Hallamshire coeliac clinic

With dictated clinic letters being typed up by Dr Sanders secretary, Deborah French, and then printed out to be put in the patient's case notes and in a particular wire basket, a system was set up where Deborah would copy me into letters of patients with newly diagnosed coeliac disease that Dr Sanders or a member of his team had seen.

2.2.2.5. Dietetic case notes

Following referral to the dietitian from the Hallamshire coeliac clinic, the patient would be seen by a dietitian at the Northern General Hospital. There was no specific dietitian or dietetic team that would review referred coeliac patients nor was there a dedicated time or clinic. The Hallamshire referral letter and replying Northern General dietetic letter were stored in the department of dietetics based at the Hallamshire hospital. It was here in the notes stores of the dietetic department where these observations are kept.

Figure 2.18: Entrance to the notes stores of the dietetic department at the Royal Hallamshire Hospital



Unfortunately, the dietetic notes were paper-based and had to be searched through manually by hand. All people regardless of diagnosis who had contact with the dietitian had notes stored in this basement.

Figure 2.19: Boxes containing case notes of all patients regardless of diagnosis having contact with a dietitian

The filing system was alphabetically by surname and then according to the date when they last had contact with a dietitian as shown in this close-up:

Figure 2.20: Alphabetical filing system of dietetic records

So if a patient was diagnosed with coeliac disease on the basis of characteristic small bowel histology say in September 2004 and then seen reviewed in the outpatient clinic in October 2004 by the gastroenterologist to discuss the diagnosis and the need for treatment with a gluten-free diet, this person would then be referred to the dietitian. Trying to find this particular patient's dietetic case notes would involve looking in the year 2004 boxes by alphabetical name as well as in the 2005 boxes as the patient may not get seen by the dietitian until 2005 due to dietetic clinic time pressures. If this patient was then reviewed again by the dietitian, provided this appointment took place in 2005 then the same place where the index appointment occurred would be fine. However if the patient was given a 12 month review then their dietetic case notes would be moved out of the 2005 box and transferred to the year 2006 box despite being diagnosed with coeliac disease in 2004. If the patient seen by the dietetic died then their dietetic case notes would be removed from the 'alive patient' year boxes

and put into a box of all people seen by the dietitians in that particular year that had died that year. For example, the '2005 R.I.P.' box contained patients that had contact with the dietitians in 2005 but subsequently died in 2005. The deceased patient boxes were not alphabetically filed.

I had to manually trawl through each box of case notes to determine which patients contained in each box were coeliacs. I primarily used the 'reason for referral' information contained on the dietetic referral card that was pinned to the front of each set of case notes as a screen to prompt detailed reading of those case notes. For example, if the reason for referral information given on a set of case notes was 'nutritional assessment – dysphagia post-stroke' then I would reject this set of case notes due to no compelling features to suggest coeliac disease and move on to the next set. However if coeliac disease was suggested in the reason for referral information such as 'coeliac – for GFD (gluten-free diet)' or even 'GFD' I would then review the entire set of dietetic case notes to confirm or refute whether this person had coeliac disease. This is because not all people referred to dietitians for treatment with a gluten-free diet had coeliac disease – I picked up a handful of patients with no evidence of coeliac disease that had irritable bowel syndrome that were referred for a gluten-free diet to try and help their symptoms.

From observations based on manually going through each set of dietetic case notes and reviewing the journey each person with coeliac disease had through the system, it became apparent that most patients had one appointment with the dietitian which occurred at diagnosis of coeliac disease where details of how to take a gluten-free diet were given. The patient was then discharged from further dietetic review unless specifically requested by Dr Sanders involving a further formal referral to dietetics to

re-review the patient. Linking this information in with the pattern of coeliac outpatient clinic attendances and hospital letters generated from the clinic attendance, it was Dr Sanders that provided follow-up and monitoring of the condition. The pattern and frequency of coeliac outpatient clinic attendances was typically around the time of diagnosis of coeliac disease, then three months later, then every twelve months thereafter.

2.2.3. Derbyshire Royal Infirmary

Compared to the processes described above at Nottingham University Hospital and at the Hallamshire, identifying how people were diagnosed with coeliac disease and passed through the hospital system at Derby Royal Infirmary was much more straight forward.

Derbyshire Royal Infirmary and Derby General City Hospital form the Derbyshire Foundation NHS Trust. This trust has a single gastroenterology department with Dr Geoff Holmes, one of the consultant gastroenterologists, having a clinical and research interest in coeliac disease. Dr Holmes had developed a system over several decades where people with coeliac disease within the trust would be seen in his Friday afternoon coeliac disease clinic. This clinic Dr Holmes held with Fiona Moor, chief dietitian with a special interest in coeliac disease. As much as it was possible, people with suspected or confirmed coeliac disease were streamlined to Dr Holmes and his coeliac disease clinic. For example, if general practitioners suspected someone had coeliac disease, it would be Dr Holmes that the referral was addressed to. If the suspected or prevalent coeliac disease referral was addressed to 'dear gastroenterologist' then the patient would be directed to the attention of Dr Holmes. If one of the other gastroenterologists diagnosed coeliac disease in their outpatient clinic

or on the hospital ward, then referral letters (or at least the clinic letters or discharge notes would be copied to Dr Holmes) would be addressed to Dr Holmes for Dr Holmes to continue the care of this patient with coeliac disease.

In addition to the clinical activity alerts, Dr Holmes would receive printouts each month from Dr Peter Hill, consultant biochemist, of all the people having positive coeliac serology results. Dr Holmes would also be informed on a monthly basis of any people with small bowel specimens demonstrating coeliac disease by Dr David Semerero, consultant histopathologist. With each of these alerts, Dr Holmes would add 'new' coeliacs to his list of people having coeliac disease managed and or diagnosed at Derby. Dr Holmes kept this list in two different modes. He would add each new person to the list in a dedicated notebook locked away in his office. He would note their demographic details, date of diagnostic small bowel biopsy and its laboratory reference number as well as the result of the coeliac serology test in this book. Each coeliac would be given an unique reference called a 'coeliac number'. An excerpt of what the notebook looked like is shown below:

Figure 2.21: Excerpt of Dr Holmes notebook list of people with coeliac disease at Derby

Coeliac number	Name*	Hospital number*	Date of birth	Biopsy date	Biopsy reference	EMA result
1214	XX	xx-xx-xx	02/07/1957	27/10/2004	19322/04	++
1215	XX	xx-xx-xx	03/05/1966	15/02/2005	2752/05	+
1217	XX	xx-xx-xx	22/06/1951	26/06/2001	10886/01	+
1218	XX	xx-xx-xx	29/05/1966	22/10/2004	19080/04	+

*Anonymous for the purpose of this thesis but names were used in the notebook

The same information as well as a variety of clinical and laboratory data would be entered into an Access database for each coeliac again using the coeliac number as the

unique reference identity. This cohort of Derby coeliacs has been used as the study population for many studies on coeliac disease by Dr Holmes [287, 343].

Through Dr Holmes, I was able to determine the number of people being newly diagnosed with coeliac disease in Derby. I was also able to meet these people being newly diagnosed with coeliac disease by attending the coeliac disease outpatient clinic and invite them to participate in my prospective studies.

2.2.4. Study population for breast cancer risk profile study

To estimate the breast cancer risk profile in women with coeliac disease and compare it to that of the general population, we elected to systematically collect data on reported conventional breast cancer risk factors in women with coeliac disease using a questionnaire-based survey in a cross-sectional study design. The study population had to be women that had coeliac disease and were alive in order to be able to participate in the study such as completing the questionnaire. There were two principal groups of women with coeliac disease that comprised the study population for the breast cancer risk profile study:

1. Women with incident and prevalent coeliac disease of the Nottingham, Sheffield and Derby historical cohorts
2. Women with coeliac disease who were members of the Coeliac UK patient charity organisation

2.2.4.1. Women with incident and prevalent coeliac disease of the Nottingham, Sheffield and Derby historical cohorts

From performing extensive searches of who had been diagnosed with coeliac disease at each centre between 1st January 2000 – 31st December 2006 as described in sections 2.2.1. and 2.2.2., I generated historical cohorts for Nottingham and Sheffield centres of people with incident and prevalent coeliac disease, adding in demographic, clinical, histological and laboratory data through systematic collection. Women with incident and prevalent coeliac disease in these historical cohorts and in the prospectively collected Derbyshire coeliac cohort were invited to participate in the breast cancer risk profile study and form the study population in association with the Coeliac UK members. The methods of invitation and recruitment to the study are described further in Chapter 5.

2.2.4.1. Women with coeliac disease who were members of the Coeliac UK patient charity organisation

Coeliac UK is the principal national society for people with coeliac disease offering invaluable dietary guidelines and represents the largest population-based cohort of people with coeliac disease in the United Kingdom. It has over 70,000 registered members of which 26,238 are women aged between 45 and 75 years [Lawrence Munday, Coeliac UK, October 2006]. As a patient support group, it is likely that there are members of Coeliac UK that have coeliac disease. However, family of affected coeliacs (such as mothers of affected children, wives of affected husbands) that do not have coeliac disease are also members of Coeliac UK. People with functional bowel disturbances whose symptoms are helped with dietary exclusion such as wheat though do not have coeliac disease could also be members of Coeliac UK to utilise the dietary support offered by the organisation. The use of the Coeliac UK database to identify potential study participants for the breast cancer risk profile study would have to

involve screening questions to ensure the member is female and has coeliac disease as described further in Chapter 5. The methods for random selection of female Coeliac UK members, invitation and recruitment to the study are also described in Chapter 5.

2.3. Summary of different cohorts used in the studies

There are three different populations of adults with coeliac disease used in this thesis:

1. Historical, contemporary incident cohort (chapter 3)

2. Prospective, longitudinal incident cohort (chapter 4)

3. Cross-sectional survey of incident and prevalent female coeliacs (chapter 5)

Chapter 3: Historical cohort studies in incident coeliac disease

- Describe relationship between degree of enteropathy and physiological derangement, clinical features
- Examine the risk of hypertransaminasaemia and effect of treatment
- Examine incidence of diagnosed coeliac disease and socio-economic status
- Estimate cholesterol profile and effect of treatment

Using Nottingham and Sheffield historical cohorts

Chapter 4: Prospective, longitudinal studies in incident coeliac disease

- Quantify impact of coeliac disease on health-related quality of life and vascular risk profile and change with treatment

Recruiting people with newly diagnosed coeliac disease at Nottingham, Sheffield and Derby

Chapter 5: Cross-sectional survey of women with coeliac disease

- Estimate breast cancer risk profile in women with coeliac disease and compare with general population

Using Nottingham, Sheffield and Derby historical cohort; female members of Coeliac UK with coeliac disease

2.4. Definitions of presenting features used within cohorts

As described in section 2.2., histopathology, immunology, clinical coding, dietetic and other databases were searched to identify people with coeliac disease. Features that led to the person being investigated and diagnosed with coeliac disease were systematically collected using information contained in:

- general practitioner's or referring doctor's referral letter
- replying gastroenterologist letter
- dietetic case notes
- clinical information supplied with the small bowel biopsy or coeliac serology request
- laboratory data such as the presence of iron deficiency anaemia

Below are descriptions of how I have defined the presenting features of coeliacs used within the studies of this thesis.

2.4.1. Mode of presentation

Definition 6: Iron deficiency anaemia
Presence of serum ferritin, haemoglobin and mean cell volume below the lowest limit of normal range for the hospital laboratory concerned. Further corroborated by report of "iron deficiency anaemia" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. In the absence of available laboratory data, report of "iron deficiency anaemia" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 7: B12 deficiency
Presence of serum B12 below the lowest limit of normal range for the hospital laboratory concerned. Further corroborated by report of "B12 deficiency" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. In the absence of available laboratory data, report of "B12 deficiency" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 8: Folate deficiency
Presence of serum or red cell folate below the lowest limit of normal range for the hospital laboratory concerned. Further corroborated by report of "folate deficiency" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. In the absence of available laboratory data, report of "folate deficiency" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 9: Diarrhoea
Presence of loose, mushy or watery stools and or increase in frequency of bowel movements in comparison to normal stool frequency. In the absence of documentation of stool consistency and or frequency in the referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist, report of "diarrhoea" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 10: Weight loss
Reduction in weight in comparison to normal or baseline weight as evidenced by decrease in measured weight, reduction in clothes size. In the absence of documentation of weight change (e.g. "5 kilogram weight loss") in the referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist, report of "weight loss" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 11: Malabsorption
Presence of weight loss; diarrhoea; and B12, folate and or iron deficiency anaemia.

Definition 12: Constipation
Presence of hard and or dry stools; and or infrequent bowel movements; and or straining to pass stool. In the absence of documentation of stool consistency and or frequency in the referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist, report of "constipation" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 13: Irritable bowel syndrome (IBS) symptoms
Episodes of recurrent abdominal pain (which may be described as abdominal cramps, bloating, discomfort, flatulence) with change in bowel habit (which may be diarrhoea, constipation or alternating between diarrhoea and constipation). Onset of abdominal pain is related to a change in bowel frequency and consistency of stool and the pain is relieved by bowel movement. In the absence of documentation of abdominal pain, stool consistency and or frequency in the referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist, report of "IBS symptoms", "functional bowel disturbance" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 14: Hypertransaminasaemia
Presence of serum transaminases (ALT and or AST) above the highest limit of normal range for the hospital laboratory concerned. Further corroborated by report of "abnormal or deranged liver chemistries" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. In the absence of available laboratory data, report of "deranged or abnormal liver chemistries" in referring or replying letter, clinical information supplied with small bowel biopsy and or coeliac serology request.

Definition 15: Osteoporosis
Presence of reduced bone mineral density with T score $\leq$-2.5 [344].

2.4.2. Marsh grading of duodenal biopsy

Definition 16: Marsh 0
Normal histological appearance of small bowel mucosa.

Definition 17: Marsh 1
Mucosal architecture is normal but there is an increased number of intra-epithelial lymphocytes (more than 40 intra-epithelial lymphocytes per 100 enterocytes).

Definition 18: Marsh 2
In addition to the infiltration of the mucosa with intra-epithelial lymphocytes, there is an increase in crypt depth without any reduction in villous height.

Definition 19: Marsh 3a
The mucosa is flat with partial reduction in normal villous height to crypt depth ratio with crypt hyperplasia and intraepithelial lymphocytosis. Also referred to as partial villous atrophy.

Definition 20: Marsh 3b
The mucosa is flat with subtotal reduction in normal villous height to crypt depth ratio with crypt hyperplasia and intraepithelial lymphocytosis. Also referred to as subtotal villous atrophy.

Definition 21: Marsh 3c
The mucosa is flat with total reduction in normal villous height to crypt depth ratio with crypt hyperplasia and intraepithelial lymphocytosis. Also referred to as total villous atrophy.

Definition 22: Unspecified degree of villous atrophy
The mucosa is flat with unspecified degree of reduction in normal villous height to crypt depth ratio with crypt hyperplasia and intraepithelial lymphocytosis. Also referred to as villous atrophy.

2.4.4. Coeliac serology status

This is defined in definition 4 in section 2.2.1.2.

2.4.5. Co-morbidity present at diagnosis of coeliac disease

Co-morbid conditions present at diagnosis at coeliac disease were also systematically collected including those considered as inactive such as appendicectomy.

Definition 23: Graves disease
Presence of thyroid-associated ophthalmopathy (such as exophthalmos) and hyperthyroidism (overproduction of thyroid hormones T3 and T4 which can be measured in the serum). Detection of thyroid-stimulating autoantibodies serologically are supportive of the presence of Graves disease. In the absence of available laboratory data, report of "Graves disease" in referring or replying letter.

Definition 24: Hypothyroidism
Underproduction of thyroid hormones T3 and T4 as measured in the serum as suggested by higher levels than normal of thyroid-stimulating hormone (TSH). In the absence of available laboratory data, report of "hypothyroidism" in referring or replying letter.

Definition 25: Type 1 diabetes
Lack of endogenous insulin production by pancreas resulting in fasting venous blood glucose more than 6.1 mmol/L or more than 10.0 mmol/L at 2 hours post glucose load. Presence of anti-islet autoantibodies, lack of insulin resistance as determined by a glucose tolerance test and absence of C-peptide in serum are supportive of presence of type 1 diabetes. In the absence of available laboratory data, report of "IDDM" or "type 1 diabetes mellitus" in referring or replying letter.

Definition 26: Hypertension
Blood pressure consistently above 140 / 90 mmHg (130 / 80 mmHg in people with diabetes mellitus). In the absence of available clinical data, report of "hypertension" in referring or replying letter.

Definition 27: Hypercholesterolaemia

Fasting serum LDL (low density lipoprotein) above 4.5 mmol/L; the patient receiving regular statin therapy may further support the diagnosis. In the absence of available laboratory data, report of "hypercholesterolaemia" in referring or replying letter.

Definition 28: Ischaemic heart disease (IHD)
IHD includes the clinical syndromes of myocardial infarction and angina pectoris. The diagnosis of myocardial infarction is based on conventional grounds of cardiac symptoms, elevated cardiac enzymes with or without electrocardiograph (ECG) changes. Angina pectoris diagnosis may be presumed on the basis of typical exertional chest pain relieved by drugs such as glyceryl trinitrate and further supported by confirmatory results of an exercise tolerance test or coronary angiography.

Definition 29: Stroke disease
Clinical syndrome characterised by sudden onset of focal neurological deficit with diagnosis may be further supported with radiological such as computed tomography imaging.

2.5. Description of historical cohorts

Collecting the Nottingham and Sheffield historical cohorts not only helped me understand how to develop systems of recruiting a representative population of incident coeliacs for the prospective studies but they formed contemporary cohorts in which there was systematic collection of data that could be used for:

- exploring morbidity associated with coeliac disease

  e.g. prevalence of hypertransaminasaemia in incident coeliac disease

- reference study population (from the same geographical area, contemporary in time) with which to compare the prospective cohort to

  e.g. proportion of coeliacs presenting with iron deficiency anaemia and marsh

  3b histological changes similar in historical and prospective cohorts?

This section 2.5. describes the demographic, clinical, histological and laboratory features of the Nottingham and Sheffield historical cohorts.

2.5.1. Methods

2.5.1.1. Study design

Historical cohort study.

2.5.1.2. Study cohort

Adults with incident coeliac disease diagnosed at Nottingham University Hospital (Nottingham historical cohort) and Royal Hallamshire Hospital (Sheffield historical cohort) between 1st January 2000 - 31st December 2006 and identified as described in sections 2.2.1. and 2.2.2. respectively.

2.5.1.3. Outcome measures

- Number of adults per year with incident coeliac disease identified at Nottingham and at Sheffield
- Demographic features of adults with incident coeliac disease (age, sex, ethnicity)
- Proportion of adults newly diagnosed with coeliac disease presenting with diarrhoea, anaemia, weight loss, constipation, abdominal pain, IBS symptoms
- Distribution of Marsh grading and coeliac serology status in adults newly diagnosed with coeliac disease
- Prevalence of previously diagnosed autoimmune and vascular disease in adults newly diagnosed with coeliac disease

2.5.1.4. Outcome ascertainment

Systematic data collection of demographic, clinical, histological and laboratory data present at diagnosis of coeliac disease was performed as described in sections 2.2.1. and 2.2.2. respectively. Modes of presentation were defined as described in section 2.4. Comparisons to the population structures of Nottingham and Sheffield general populations such as ethnicity were made using data from the Office of National Statistics [341, 342].

2.5.1.5. Statistical analysis

Descriptive analyses with calculation of mean and median were performed. Comparisons between binary variables were performed using Chi-squared tests and between continuous data using unpaired t-tests.

We considered a p-value of 0.05 to represent statistical significant in all tests. All analyses were performed using Stata 9.2 [TexCorp].

2.5.1.6. Ethical approval

Advice was sought from local ethics committees (Nottingham 1 and South Sheffield) with Nottingham University Hospital (reference ID 290) and Royal Hallamshire Hospital (reference 04/63) giving audit approval under service evaluation guidelines.

2.5.2. Results: Nottingham historical cohort

2.5.2.1. Number of identified adults newly diagnosed with coeliac disease

588 adults with incident coeliac disease between 1st January 2000 and 31st December 2006 were identified. The number of adults newly diagnosed each year is shown in the following table.

Table 2.8: Number of adults newly diagnosed with coeliac disease at Nottingham University Hospital

Year	Number of new diagnoses of coeliac disease in adults
2000	57
2001	60
2002	79
2003	91
2004	102
2005	101
2006	98

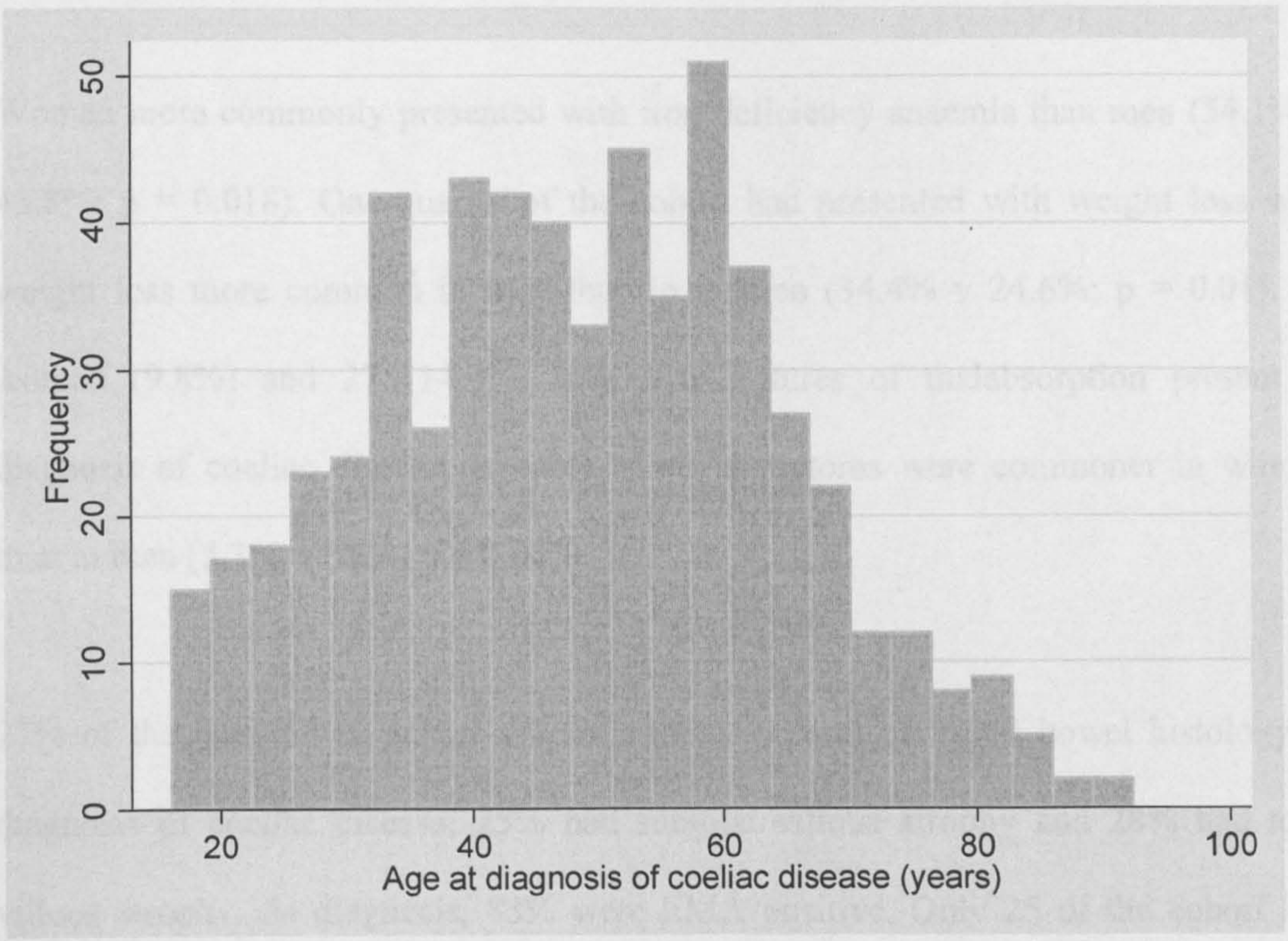
Of the 588 coeliacs identified during the study period, 501 were identified through searches of histopathological records. These 501 coeliacs identified through histopathological searches were also found to have entries within the clinical coding databases for coeliac disease. A further 87 coeliacs not identified through histopathological searches were found through searching of clinical coding databases.

Serology results of 65 of 588 coeliacs identified in histopathological and clinical coding databases could not be found through searches of immunological databases.

2.5.2.2. Demography of cohort

There were approximately twice as many women (n = 399) as men (192) in the cohort. The age at diagnosis appeared to be broadly normally distributed.

Figure 2.22: Histogram of age at diagnosis of coeliac disease in Nottingham historical cohort



The mean age at diagnosis was 48.4 (SD 15.9) years; the median age at diagnosis was 48.3 (IQR 36.9 – 59.9) years. Women were diagnosed with coeliac disease at an earlier age (46.9 (SD 16.2) years) than men (48.4 (SD 14.8) years; p = 0.002).

Whilst a lesser proportion of the cohort were Caucasian (n = 559, 94.6%) in comparison to the Nottinghamshire general population (97.2%; p = 0.0001) [345], the

proportion of Asians within the coeliac cohort were greater than that of the Nottinghamshire general population (5.4% v 1.1%; p = 0.00001). There were no coeliacs from black ethnic groups in comparison to the 0.5% prevalence in the Nottinghamshire general population.

2.5.2.3. Presenting features of cohort

Iron deficiency anaemia and diarrhoea were the most common presenting features affecting over one-third of the cohort (Table 2.9).

Women more commonly presented with iron deficiency anaemia than men (54.1% v 43.8%; p = 0.018). One-quarter of the cohort had presented with weight loss with weight loss more common in men than in women (34.4% v 24.6%; p = 0.01). 39 women (9.8%) and 27 (14.1%) men had features of malabsorption present at diagnosis of coeliac disease. Irritable bowel symptoms were commoner in women than in men (5.3% v 1.6%; p = 0.03).

27% of the cohort had partial villous atrophy present on small bowel histology at diagnosis of coeliac disease; 25% had subtotal villous atrophy and 28% had total villous atrophy. At diagnosis, 83% were EMA positive. Only 25 of the cohort had tTG measured at diagnosis of coeliac disease with introduction of the test late 2006.

45.2% (n = 267) of the cohort had ferritin values below 22 μg/L, the lowest limit of the normal range. The median ferritin was significantly lower in females (8 (IQR 4.9 – 23) μg/L) than in males (12 (IQR 6.2 – 34) μg/L); p = 0.01. 13.1% (n = 77) had B12

and 16.2% (n = 95) had folate values below the lowest limit of the normal ranges, respectively.

Table 2.9: Presenting features of the Nottingham historical cohort

	Female (n = 399)	Male (n = 192)	All coeliacs (n = 588)
Mode of presentation (%)*			
Iron deficiency anaemia	216 (54.1)	84 (43.8)	300 (51.0)
B12 deficiency	48 (12.0)	29 (15.1)	77 (13.1)
Folate deficiency	66 (16.5)	29 (15.1)	95 (16.2)
Diarrhoea	156 (39.1)	85 (44.3)	241 (40.9)
Weight loss	98 (24.6)	66 (34.4)	164 (27.9)
Constipation	13 (3.3)	2 (1.0)	15 (2.6)
IBS symptoms	21 (5.3)	3 (1.6)	24 (4.1)
Abdominal pain	38 (9.5)	24 (12.5)	62 (10.5)
Marsh grading (%)			
0	2 (0.5)	0	2 (0.3)
1	16 (4.0)	4 (2.1)	20 (3.4)
2	2 (0.5)	1 (0.5)	3 (0.5)
3a	106 (26.6)	55 (28.7)	161 (27.4)
3b	97 (24.3)	50 (26.0)	147 (25.0)
3c	110 (27.6)	56 (29.2)	166 (28.2)
Unspecified degree of VA	4 (1.0)	1 (0.5)	5 (0.9)
Not done or missing data	62 (15.5)	25 (13.0)	87 (14.8)
EMA status at diagnosis (%)			
Positive	334 (83.7)	158 (82.3)	492 (83.7)
Weak positive	4 (1.0)	0	4 (0.7)
Negative	9 (2.3)	14 (7.3)	23 (3.9)
IgA deficiency	2 (0.5)	0	2 (0.3)
Not done or missing data	45 (11.3)	20 (10.4)	65 (11.1)

* Patients may have possessed one or more of these presentations

2.5.2.4. Co-morbidity of the cohort

A selection of autoimmune and vascular co-morbid conditions are displayed in the following table.

Table 2.10: Prevalence of previously diagnosed co-morbid conditions in the Nottingham historical cohort

Co-morbidity	Female (n = 288)	Male (n = 124)	p-value
Autoimmune (%)			
Graves disease	5 (1.3)	3 (1.6)	0.76
Hypothyroidism	27 (6.8)	5 (2.6)	0.03
Type 1 diabetes	7 (1.8)	4 (2.1)	0.78
Rheumatoid arthritis	2 (0.5)	1 (0.5)	0.98
Vascular (%)			
Hypertension	31 (7.8)	26 (13.5)	0.03
Hypercholesterolaemia	3 (0.8)	7 (3.7)	0.01
IHD	4 (1.0)	13 (6.8)	0.0001
Type 2 diabetes	2 (0.5)	4 (2.1)	0.07
Stroke disease	14 (3.5)	9 (4.7)	0.49

Hypothyroidism was the most common autoimmune disease previously diagnosed in the incident cohort of people with coeliac disease, affecting 5% of the cohort. Autoimmune diseases appeared to have a female gender predominance in the coeliac cohort similar to ratios observed in the general population [346]. Diagnosed vascular disease appeared to have male gender predominance such as that observed in the general population [347].

2.5.3. Results: Sheffield historical cohort

2.5.3.1. Number of identified adults newly diagnosed with coeliac disease

I identified 412 people with incident coeliac disease between 1st January 2000 and 31st December 2006 with the number of adults diagnosed each year shown in the following table.

Table 2.11: Number of identified adults newly diagnosed with coeliac disease at Royal Hallamshire Hospital

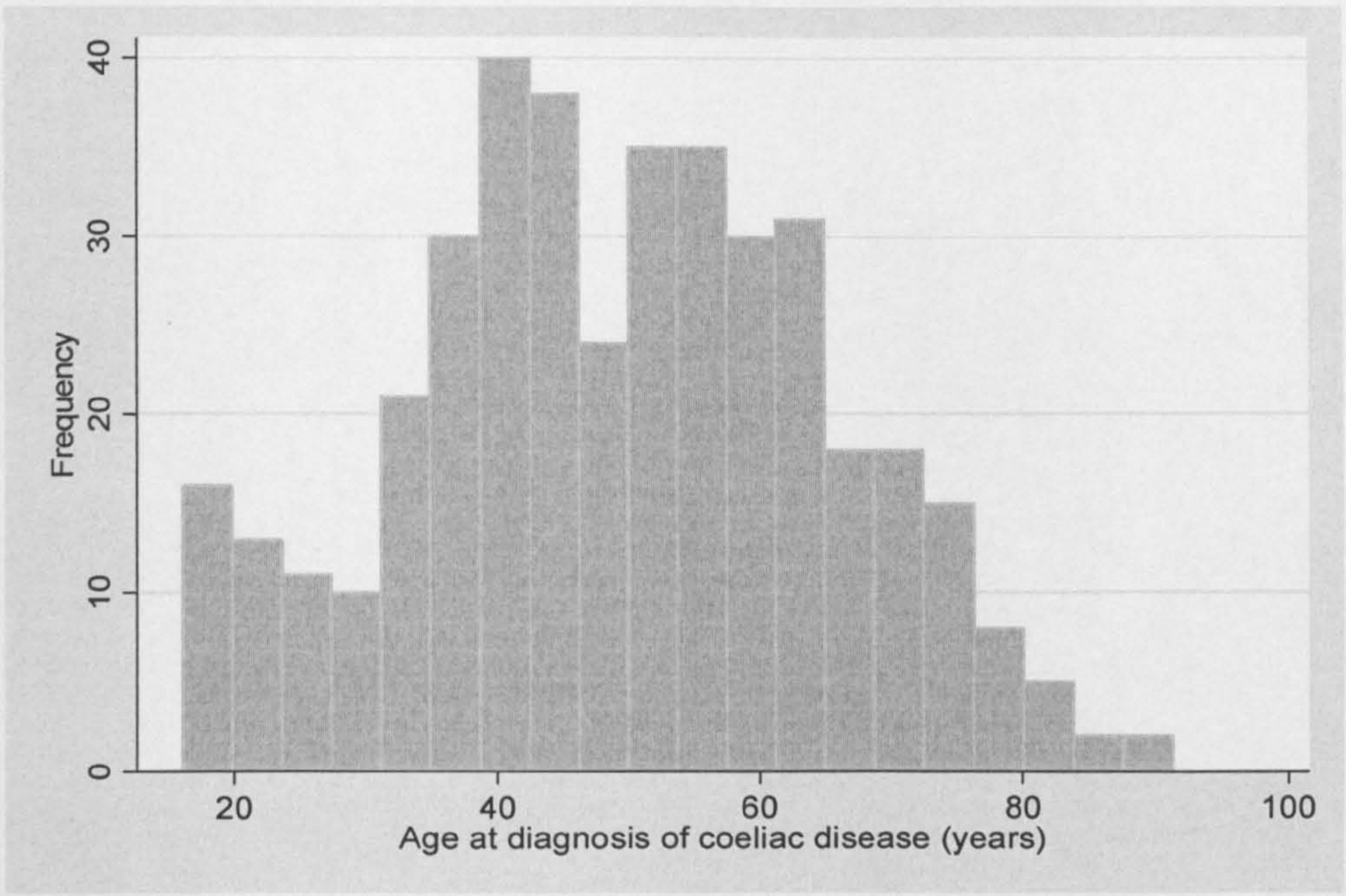
Year	Number of new diagnoses of coeliac disease in adults
2000	44
2001	42
2002	43
2003	45
2004	62
2005	96
2006	80

Of the 412 coeliacs identified during the study period, 383 of the 412 coeliacs were identified through searches of histopathological records. A further 24 coeliacs not identified through histopathological searches were found through searching of wire baskets in Dr Sanders' office of letters. Another 5 coeliacs not identified through histopathological searches were found through searching of dietetic records. Serology results of 34 coeliacs could not be found through searches of immunological databases. These 34 coeliacs without serology results had entries in histopathology databases as well as being assigned a diagnosis of coeliac disease in letters in Dr Sanders' wire baskets as well as in dietetic records.

2.5.3.2. Demography of cohort

There were approximately twice as many women (n = 288) as men (n = 124) newly diagnosed with coeliac disease in the cohort. The age at diagnosis appeared to be broadly normally distributed. The mean age at diagnosis was 48.9 (SD 16.4) years; the median age at diagnosis was 48.8 (IQR 38.2 – 61.1) years. Women were diagnosed with coeliac disease at an earlier age (47.8 (SD 15.8) years) than men (51.6 (SD 17.5) years; p = 0.03).

Figure 2.23: Histogram of age at diagnosis of coeliac disease in Sheffield historical cohort



A greater proportion of the cohort were Caucasian (n = 391, 94.5%) in comparison to the Sheffield general population (91.2%; p = 0.02). The proportion of Asians within the coeliac cohort were similar to that of the Sheffield general population (5.6% v 4.6%; p = 0.35). There were no coeliacs from black ethnic groups in comparison to the 1.8% prevalence in the Sheffield general population.

2.5.3.4. Presenting features

Iron deficiency anaemia and diarrhoea were the most common presenting features affecting over one-third of the cohort.

Table 2.12: Presenting features of the Sheffield historical cohort

	Female (n = 288)	Male (n = 124)	All (n = 412)
Presentation mode* (%)			
Iron deficiency anaemia	125 (43.4)	36 (29.0)	161 (39.1)
B12 deficiency	15 (5.2)	6 (4.8)	21 (5.1)
Folate deficiency	16 (5.6)	10 (8.1)	26 (6.3)
Diarrhoea	112 (38.9)	50 (40.3)	162 (39.3)
Weight loss	55 (19.1)	17 (13.7)	72 (17.5)
Constipation	8 (2.8)	2 (1.6)	10 (2.4)
IBS symptoms	45 (15.6)	13 (10.5)	58 (14.1)
Abdominal pain	47 (16.3)	14 (11.3)	61 (14.8)
Marsh grading (%)			
0	1	3 (2.4)	4 (1.0)
1	8 (2.8)	4 (2.4)	12 (2.9)
2	6 (2.1)	8 (6.5)	14 (3.4)
3a	86 (29.9)	33 (26.6)	119 (28.9)
3b	104 (36.1)	35 (28.2)	139 (33.7)
3c	56 (19.4)	26 (20.9)	82 (19.9)
Unspecified degree of VA	9 (3.1)	4 (2.4)	13 (3.2)
Not done or missing data	18 (6.3)	11 (8.9)	29 (7.0)
Median tTG (IQR) iu	171 (36 – 300)	241.5 (41 – 300)	197 (36 – 300)
Not done (%)	107 (37.2)	30 (24.2)	137 (33.3)
EMA status (%)			
Positive	220 (76.4)	97 (78.2)	317 (76.9)
Weak positive	8 (2.8)	3 (2.4)	11 (2.7)
Negative	30 (10.4)	16 (12.9)	46 (11.2)
IgA deficiency	1	3 (2.4)	4 (1.0)
Not done or missing data	29 (10.1)	5 (4.0)	34 (8.3)

*Patients may have possessed one or more of these presentations

Women more commonly presented with iron deficiency anaemia than men (43.4% v 29.0%; p = 0.008). Nearly one-fifth of the cohort had presented with weight loss. 16 women (5.6%) and 4 (3.2%) men had features of malabsorption present at diagnosis of coeliac disease. Abdominal pain and irritable bowel symptoms were also common gastrointestinal symptoms possessed by the coeliac cohort at diagnosis.

29% of the cohort had partial villous atrophy present on small bowel histology at diagnosis of coeliac disease; 34% had subtotal villous atrophy and 20% had total

villous atrophy. At diagnosis, the median tTG was 197 (IQR 36 – 300) iu and 80% were EMA positive.

40.0% (n = 165) of the cohort had ferritin values below 22 μg/L, the lowest limit of the normal range. The median ferritin was significantly lower in females (14.5 (IQR 5.9 – 45.5) μg/L) than in males (27.5 (IQR 8.5 – 89.5) μg/L); p = 0.004. 5.1% (n = 21) had B12 and 6.3% (n = 26) had folate values below the lowest limit of the normal ranges, respectively.

2.5.3.4. Co-morbid conditions diagnosed before coeliac disease

A selection of autoimmune and vascular co-morbid conditions are displayed in the following table.

Table 2.13: Prevalence of previously diagnosed co-morbid conditions in the Sheffield historical cohort

Co-morbidity	Female (n = 288)	Male (n = 124)	p-value
Autoimmune (%)			
Graves disease	7 (2.4)	1 (0.8)	0.28
Hypothyroidism	36 (12.5)	7 (5.6)	0.04
Type 1 diabetes	12 (4.2)	4 (3.2)	0.66
Rheumatoid arthritis	5 (1.7)	1 (0.8)	0.48
Vascular (%)			
Hypertension	33 (11.5)	15 (12.1)	0.83
Hypercholesterolaemia	11 (3.8)	10 (8.1)	0.07
IHD	14 (4.9)	13 (10.5)	0.03
Type 2 diabetes	7 (2.4)	4 (3.2)	0.63
Stroke disease	2 (0.7)	5 (4.0)	0.02

Hypothyroidism was the most common autoimmune disease previously diagnosed in the incident cohort of people with coeliac disease, affecting 10% of the cohort. Autoimmune diseases appeared to have a female gender predominance in the coeliac cohort similar to ratios observed in the general population [346]. Diagnosed vascular

disease appeared to have male gender predominance such as that observed in the general population [347].

2.6. Discussion

2.6.1. Principal findings

Clinically diagnosed coeliac disease is more common in women than in men and mean age at diagnosis in women is younger in comparison to men. Iron deficiency anaemia and diarrhoea were the most common modes of presentation of clinically diagnosed coeliac disease affecting over one-third of the cohort. Approximately 5% clinically diagnosed coeliacs have no villous atrophy changes affecting their small bowel and approximately 80% of clinically diagnosed coeliacs have positive coeliac serology at diagnosis.

2.6.2. Merits and limitations

This is the largest study describing the systematic, routine collection of demographic, clinical, histological and laboratory data in a contemporary, unselected and population-based cohort of adults newly diagnosed with coeliac disease. While one cannot be certain that every patient with coeliac disease has been identified in both hospitals during the study period there have been extensive efforts made to do so. On balance it is unlikely that the omission of the few patients that will have inevitably been missed will have led to a substantial underestimate of the mode of presentation of clinically diagnosed coeliac disease.

Presence of symptoms such as diarrhoea, constipation were usually obtained by review of medical notes such as those made by the reviewing gastroenterologist. The

clinical mode of presentation of coeliac disease is thus dependent on the recording of the presence or absence of these symptoms in the medical notes. If a symptom is not documented such as abdominal pain it may not mean that the person presenting with coeliac disease does not have it. More formal and standardized approaches such as the use of a structured questionnaire would limit such measurement bias.

There are also limitations specific to the search strategies used to identify people with coeliac disease which are described further below.

2.6.2.1. Histopathology searches

The histopathology database was of considerable help in identifying who had been diagnosed with coeliac disease on the grounds of the appearances of the small bowel specimens. However, there were also a number of reasons why all people newly diagnosed with coeliac disease over the same defined time period did not appear on the histopathology database generated list. Some people may not have had a small bowel biopsy taken to diagnose coeliac disease – either due to refusal to have the procedure done, ill health preventing the procedure being formed with the diagnosis of coeliac disease made on the grounds of symptoms and positive coeliac serology alone, or that alternative specimens were taken such as immunofluorescence of skin biopsies in people with dermatitis herpetiformis and labelled to have coeliac disease without small bowel biopsy confirmation. People may have had small bowel biopsies taken at another hospital (whether NHS or private) but were receiving treatment for coeliac disease at either Nottingham University Hospital or the Royal Hallamshire Hospital. People may have had small bowel specimens that were interpretated as being normal (i.e. Marsh 0 lesion) either because the person was taking a gluten-free

diet at the time of sampling and the small bowel had recovered or that small bowel histology was normal because the person had Marsh 0 lesions with positive coeliac serology and symptoms interpreted by the reviewing clinician as being consistent with a diagnosis of active rather than latent coeliac disease. For these reasons I felt use of histopathology searches alone was not a foolproof system to capture all people newly diagnosed with coeliac disease for the prospective studies nor to generate the historical cohort and that additional searches had to be employed to capture all coeliacs.

2.6.2.2. Immunology serology results

Looking through coeliac serology test results proved to be an useful addition to the histopathology search. However, there were a number of limitations to using coeliac serology test results that would prevent this system being used in isolation. Small bowel histology is regarded as the gold standard to diagnose coeliac disease and national guidelines recommend that small bowel biopsies should be taken in people in whom coeliac disease is suspected [348, 349]. Furthermore, small bowel histology identifies coeliacs that could not be detected with reliance on serology alone [350, 351]. Indeed 20 coeliacs had negative coeliac serology per 100 tested in our historical cohort. This may be due to the performance of the serology test with previous studies suggesting that IgA-tTG tests are less sensitive with milder degrees of histological abnormality [352, 353]. People with IgA deficiency have a ten-fold increased risk of having coeliac disease in comparison to people without IgA deficiency so would not have positive coeliac serology.

2.6.2.3. Clinical coding

Despite being within the catchment area of either Nottingham University Hospital or the Royal Hallamshire Hospital, people may have had gastroenterology appointments and endoscopic procedures performed at another hospital. However, provided there was a referral for dietetic review, these people would end up acquiring a code for coeliac disease as they would receive treatment for coeliac disease at Nottingham University Hospital from NHS registered dietitians with no local dietitians holding a private license to practice (personal communication, Sandra Evison Head of Dietetics, Nottingham University Hospital). There also may be delays in coding hospital attendances, procedures and interventions due to delay in medical case notes being returned from gastroenterologists, endoscopists, dietitians to the clinical coders.

2.6.2.4. Dietetic records

There were a number of limitations to using the dietetic notes stores as a means of identifying people with coeliac disease. If people did not attend their dietetic appointment or were not referred to the dietitian for whatever reason then they would not have an 'entry' in the note stores. If the dietetic notes were currently being used then they would be found in an outpatient clinic department or on a secretary's desk and not in the notes stores. Though the case notes were meant to be filed alphabetically, this was not strictly adhered to. However by manually going through each set of case notes in turn hopefully this issue would be overcome.

2.6.3. Comparison with other studies

Previous studies, largely based on less recent and smaller case series, have observed a much higher prevalence of weight loss and diarrhoea in incident coeliac disease (section 1.5.3.1.). The marked differences between these older studies and our own are probably partly explained by the era in which they were carried out. These older

studies were carried out prior to the advent of serological tests for EMA and tTG and therefore do not represent contemporary practice of identification and diagnosis of coeliac disease in the same way that our studies do. A fall in proportion of coeliacs presenting with overt symptoms such as diarrhoea though a rise in those presenting with anaemia over successive quinquennia in the study by West et al in Derbyshire [343] is suggestive of a fall in the clinical severity of the disease; this may reflect a greater awareness of the disease with improved diagnostic methods with a greater part of the coeliac iceberg tip being exposed. Another explanation for the difference is the manner in which we identified our coeliac population. For example, the use of a patient support group to estimate the proportion of coeliacs that had presented with diarrhoea and anaemia is likely to be limited by recall and selection bias.

Our observation that 80% of people newly diagnosed with coeliac disease have positive coeliac serology may be reflective of the broader histological spectrum in our cohort with over 10% of the study population having 'mild' (marsh 0, 1 or 2) coeliac disease changes.

2.6.4. Summary

Clinically diagnosed coeliac disease is more common in women than in men and mean age at diagnosis in women is younger in comparison to men though the possible explanations for these observations are unclear. The lower prevalence of clinically overt symptoms in comparison to previous studies may be due to differences in study design but may also reflect identification of people with disease below the waterline of the coeliac iceberg with the introduction of improved diagnostic methods. Approximately 5% clinically diagnosed coeliacs have no villous atrophy changes affecting their small bowel though the benefits and possible harm of early detection of mild enteropathy coeliac disease is not clear.

Chapter three: Historical cohort studies in incident coeliac disease

Using the Nottingham and Sheffield historical coeliac disease cohorts the aim of this thesis chapter is to:

- describe relationship between degree of enteropathy and physiological derangement, clinical features in incident coeliac disease
- examine the risk of hypertransaminasaemia in incident coeliac disease and effect of treatment
- examine incidence of clinically diagnosed coeliac disease by socio-economic status
- estimate cholesterol profile in incident coeliac disease and effect of treatment

3.1. Mild enteropathy (Marsh 1 and 2) coeliac disease: a comparison with more severe disease using a population based cohort

3.1.1. Introduction

While a recent randomized trial reported some benefits from gluten withdrawal in adults found to have mild enteropathy (Marsh 1, 2) coeliac disease [354], the benefits and possible harm of early detection of mild enteropathy coeliac disease is not clear and only limited data exist on the occurrence and clinical features at diagnosis of this group. The aim of this study is to compare the clinical and laboratory features of people presenting with mild (Marsh 1, 2) versus severe (Marsh 3) enteropathy coeliac disease.

3.1.2. Methods

3.1.2.1. Study design

Historical cohort study.

3.1.2.2. Study population

Adults with incident coeliac disease diagnosed at Nottingham University Hospital (Nottingham historical cohort) and Royal Hallamshire Hospital (Sheffield historical cohort) were studied. Dietetic, histopathology, immunology, clinical coding and outpatient records were used to retrospectively identify incident cases of coeliac disease as described in sections 2.2.1. and 2.2.2., respectively.

Serum haemoglobin (g/L), mean cell volume (fl), ferritin (μg/L), folate (μg/L), B12 (ng/L), ESR (mm/hr), CRP (mg/L), leucocyte count (10^9/L), albumin (g/L), platelet

count (10^9/L) were routinely measured on newly diagnosed adult cases of coeliac disease attending Nottingham University Hospital and Royal Hallamshire Hospital between 1st January 2000 and 31st December 2006 at both diagnosis of coeliac disease and in the majority following approximately 12 months treatment with a gluten-free diet. Demographic, clinical, histological and serological data in addition to these laboratory co-variates was systematically collected, as described in sections 2.2.1. and 2.2.2., respectively. The modes of presentation of coeliac disease and coeliac serological status at diagnosis of coeliac disease was determined and defined as described in section 2.4.

3.1.2.3. Outcome measures

- Presenting:
    - demographic characteristics (proportion female, mean age at diagnosis);
    - clinical features (proportion presenting with classic, gastro-intestinal, extra-intestinal and associated conditions);
    - laboratory values (mean haemoglobin, ferritin, folate, B12, albumin, alanine transferase, ESR, platelet count);
    - coeliac serological status (proportion EMA positive, median tTG titre)

in adults newly diagnosed with mild enteropathy (Marsh 1, 2) coeliac disease were compared to those adults with severe (Marsh 3) enteropathy coeliac disease.

- Change in the mean value between diagnosis and following 12 months treatment with a gluten-free diet the laboratory values of haemoglobin, ferritin, folate, B12, albumin, ESR, platelet count in coeliacs with mild (Marsh 1, 2) versus severe (Marsh 3) enteropathy coeliac disease.

3.1.2.4. Statistical analysis

Descriptive analyses with calculation of mean and median where appropriate were performed. Comparisons between mild versus severe enteropathy disease for binary variables were performed using Chi-squared tests and for continuous data using unpaired t-tests. Comparisons between mild and severe enteropathy disease for binary dependent variables were performed using logistic regression where odds ratios and their 95% confidence interval were computed in univariate and multivariate analyses where age and sex were adjusted for. Paired values such as the mean haemoglobin value at diagnosis and the mean haemoglobin following treatment were compared using paired t-tests.

We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed using Stata SE 9.2 [TexCorp].

3.1.2.5. Ethical approval

Advice was sought from local ethics committees (Nottingham 1 and South Sheffield) with Nottingham University Hospital (reference ID 290) and Royal Hallamshire Hospital (reference 04/63) giving audit approval under service evaluation guidelines.

3.1.3. Results

3.1.3.1. Demography of cohort

We identified 775 adults newly diagnosed with coeliac disease during the study period of whom 51 (7%) had mild (Marsh 1,2) enteropathy. There was no difference in the gender proportion between mild (women n = 36; 70.6%) and severe (women n = 496; 68.5%). There was no difference in age at diagnosis of coeliac disease between mild and severe enteropathy groups (47.8 (SD 16.8) versus 49.7 (SD 16.0) years; p = 0.41).

3.1.3.2. Presenting features

Diarrhoea was the most common presenting feature in mild enteropathy coeliac disease affecting over half of this group (Table 3.14). Coeliacs with severe enteropathy had a 10-fold increased risk (OR 10.1; 95%CI 3.6, 28.4) of presenting with iron deficiency anaemia in comparison to those with mild enteropathy (proportion affected 46.3% versus 7.8%, respectively). Though it did not reach statistical significance, people with mild enteropathy coeliac disease appeared to have less severe modes of presentation with lower proportions presenting with weight loss, malabsorption, B12 and or folate deficiency in comparison to those coeliacs with severe enteropathy coeliac disease. There was no difference in odds of presenting with gastrointestinal symptoms (such as irritable bowel like symptoms, constipation) or associated conditions such as dermatitis herpetiformis between mild and severe enteropathy coeliac disease.

Table 3.14: Presenting clinical features in mild versus severe enteropathy coeliac disease

	Mild enteropathy disease (n = 51)	Severe enteropathy disease (n = 724)	Odds ratio [95% CI]	Adjusted* odds ratio [95% CI]
Classic features				
Diarrhoea	28 (54.9%)	275 (38.0%)	0.5 [0.3, 0.9]	0.5 [0.3, 0.9]
Weight loss	7 (13.7%)	158 (21.8%)	1.8 [0.8, 4.0]	1.7 [0.7, 3.8]
Malabsorption	2 (3.9%)	65 (9.0%)	2.4 [0.6, 10.2]	2.2 [0.5, 9.5]
Gastrointestinal symptoms				
Abdominal pain	3 (5.9%)	58 (8.0)	1.4 [0.4, 4.6]	1.4 [0.4, 4.7]
Constipation	2 (3.9%)	17 (2.4%)	0.6 [0.1, 2.6]	0.7 [0.1, 3.0]
IBS symptoms	6 (11.8%)	66 (9.1%)	0.8 [0.3, 1.8]	0.8 [0.3, 1.9]
Extra-intestinal features				
Iron deficiency anaemia	4 (7.8%)	335 (46.3%)	10.1 [3.6, 28.4]	10.4 [3.7, 29.1]
Folate deficiency	2 (3.9%)	70 (9.7%)	2.6 [0.6, 11.0]	2.5 [0.6, 10.5]
B12 deficiency	1 (1.9%)	64 (8.8%)	4.8 [0.7, 35.7]	4.9 [0.7, 35.8]
Associated conditions				
Dermatitis herpetiformis	2 (3.9%)	20 (2.8%)	0.7 [0.2, 3.1]	0.7 [0.2, 3.1]
Type 1 diabetes	3 (5.9%)	20 (2.8%)	0.5 [0.1, 1.6]	0.5 [0.1, 1.6]

*Adjusted for age and for sex

3.1.3.3. Coeliac serological status at diagnosis of coeliac disease

2 of the 51 (4%) coeliacs with mild enteropathy and 12 of the 724 (2%) coeliacs with severe enteropathy did not have an EMA result at diagnosis of coeliac disease. The proportion of coeliacs that were EMA positive was lower in mild enteropathy (71.4% versus 92.4% in severe enteropathy; p = 0.0001) disease.

Only 26 (51%) coeliacs with mild enteropathy disease and 200 (28%) coeliacs with severe enteropathy disease had tTG measured at diagnosis. However the median tTG (iu) was also statistically significantly lower in mild enteropathy (18.5 [IQR 6 – 300] versus 210.5 [IQR 42.5 – 300] in severe enteropathy; p = 0.0001).

3.1.3.4. Laboratory profile at diagnosis of coeliac disease

The mean values of serum haemoglobin, ferritin, folate, B12, and albumin in people newly diagnosed with mild enteropathy coeliac disease were within normal laboratory range (Table 3.15). These values were also higher than in those patients with severe enteropathy coeliac disease.

Table 3.15: Haematological and biochemical profile in mild versus severe enteropathy incident coeliac disease

Mean value	N	At diagnosis of coeliac disease [SD]	Mean difference [95%CI]
Hb g/dL			
Mild enteropathy	44	13.9 [1.6]	
Severe enteropathy	662	12.3 [2.3]	-1.6 [-2.3, -0.9]
MCV fl			
Mild enteropathy	44	90.4 [4.7]	
Severe enteropathy	651	86.5 [13.7]	-3.9 [-7.9, -0.1]
Platelets 10^9/L			
Mild enteropathy	44	286.7 [58.6]	
Severe enteropathy	650	327.1 [110.0]	40.3 [7.5, 73.2]
ESR ml/hr			
Mild enteropathy	32	12.5 [13.6]	
Severe enteropathy	394	15.3 [15.4]	2.8 [-2.8, 8.3]
Ferritin µg/L			
Mild enteropathy	31	67.7 [53.2]	
Severe enteropathy	516	35.9 [70.5]	-31.9 [-57.2, -6.5]
B12 ng/L			
Mild enteropathy	32	415.9 [205.0]	
Severe enteropathy	492	389.2 [581.8]	-26.7 [-229.7, 176.3]
Folate µg/L			
Mild enteropathy	32	10.7 [5.9]	
Severe enteropathy	494	8.7 [6.1]	-2.0 [-4.7, 0.8]
ALT u/L			
Mild enteropathy	19	29.6 [14.9]	
Severe enteropathy	391	34.1 [22.8]	4.5 [-5.9, 14.9]
Albumin g/L			
Mild enteropathy	44	41.0 [3.1]	
Severe enteropathy	645	38.8 [4.6]	-2.2 [-3.6, -0.8]

3.1.3.4. Laboratory profile following 12 months gluten-free diet

Follow-up values were obtained after a mean of 12.8 (SD 1.9) months on a gluten-free diet. The majority of these coeliacs were considered by gastroenterologist and dietitian to adhere strictly to a gluten-free diet. The proportion of coeliacs that remained EMA positive was 25.5% (compared to 71.4% at diagnosis) in mild enteropathy and 14.0% (compared to 92.4% at diagnosis) in severe enteropathy coeliac disease.

The haematological and biochemical laboratory measures did not change on exposure to treatment with a gluten-free diet in mild enteropathy disease while the expected beneficial improvements were observed in severe enteropathy disease (Table 3.16).

Table 3.16: Change in laboratory values on treatment in mild enteropathy coeliac disease

Mean value	N	At diagnosis of coeliac disease [SD]	After 12 months of GFD [SD]	Mean difference [95% CI]	p-value
Hb g/dL					
Mild enteropathy	39	13.87 [1.72]	13.53 [1.38]	-0.34 [-1.13, 0.45]	0.384
Severe enteropathy	571	12.32 [2.24]	13.48 [1.43]	1.15 [0.94, 1.37]	0.00001
MCV fl					
Mild enteropathy	39	90.02 [4.77]	90.21 [8.52]	0.19 [-2.98, 3.36]	0.904
Severe enteropathy	559	86.67 [14.09]	89.71 [7.14]	3.04 [1.73, 4.34]	0.00001
Platelets 10^9/L					
Mild enteropathy	39	285.15 [60.04]	281.56 [87.62]	-3.59 [-27.31, 20.13]	0.761
Severe enteropathy	574	328.13 [112.89]	298.99 [81.61]	-29.14 [-50.61, -7.66]	0.0081
ESR ml/hr					
Mild enteropathy	23	12.43 [15.33]	13.65 [9.26]	1.22 [-6.89, 9.32]	0.758
Severe enteropathy	142	16.10 [13.98]	11.10 [10.06]	-5.00 [-7.81, -2.19]	0.0006
Ferritin µg/L					
Mild enteropathy	24	68.92 [49.20]	98.83 [130.34]	29.92 [-34.45, 94.28]	0.346
Severe enteropathy	262	34.79 [58.26]	59.55 [107.25]	24.75 [9.75, 39.75]	0.0013
B12 ng/L					
Mild enteropathy	26	384.15 [124.20]	562.34 [458.98]	178.18 [-19.28, 375.65]	0.075
Severe enteropathy	249	432.74 [789.72]	453.85 [417.68]	21.12 [-85.03, 127.26]	0.696
Folate µg/L					
Mild enteropathy	32	8.89 [5.69]	9.64 [5.02]	0.75 [-3.96, 5.46]	0.734
Severe enteropathy	250	8.80 [6.14]	11.83 [6.62[	3.03 [0.88, 5.18]	0.006
Albumin g/L					
Mild enteropathy	38	40.84 [3.18]	39.39 [4.57]	-1.45 [-3.34, 0.44]	0.129
Severe enteropathy	546	38.82 [4.66]	39.59 [3.75]	0.75 [0.27, 1.24]	0.0025

3.1.4. Discussion

3.1.4.1. Principal findings

Coeliacs with mild enteropathy have few biochemical deficiencies at diagnosis of coeliac disease and therefore show no important biochemical improvements following treatment with a gluten-free diet in comparison to those with severe enteropathy coeliac disease. Diarrhoea was the most common symptom reported in adults being diagnosed with mild enteropathy coeliac disease and more common than that observed in severe enteropathy. Iron deficiency anaemia was much less common in mild enteropathy compared to severe enteropathy. Approximately one-third of coeliacs with mild enteropathy coeliac disease had negative EMA serology at diagnosis and had significantly lower tTG values in comparison to those with severe enteropathy coeliac disease.

3.1.4.2. Merits and limitations

This is a large prospective study describing the systematic routine collection of enteropathy profile in an unselected and population-based cohort of adults newly diagnosed with coeliac disease. While we cannot be certain that we have identified every patient diagnosed with coeliac disease in both Nottingham and Sheffield hospitals during the study period we have made extensive efforts to do so and thus it is unlikely that omission of the few patients inevitably missed will have led to a significant change of enteropathy spectrum observed.

Small bowel histology has likely to have identified coeliacs that could not be detected with reliance on coeliac serology alone [350, 351]. Indeed 29 coeliacs in our study with mild enteropathy coeliac disease had negative coeliac serology per 100 tested.

Presence of overt symptoms such as diarrhoea probably led to the person seeking medical attention where investigation of the gastrointestinal tract resulted in the diagnosis of coeliac disease being made. In the absence of overt symptoms or in the presence of atypical symptoms in a person with negative coeliac serology, further investigations such as a small bowel biopsy may not have felt warranted. There is a possibility of further misclassification bias, where people were labelled with a diagnosis of coeliac disease that did not have the condition. For example, those people with negative coeliac serology and mild enteropathy changes may not have coeliac disease but some other cause for their histological changes and symptoms such as Helicobacter pylori infection or gallstones. Often however the reviewing gastroenterologist proposed other information in support of their diagnosis of coeliac disease in clinical notes such as HLA-DQ2 genotyping and or unequivocal improvement in symptoms to gluten withdrawal to minimise this misclassification bias.

3.1.4.3. Comparison with other studies

Case reports and small case series have suggested that people with mild enteropathy coeliac disease may suffer from abdominal pain [355, 356], weight loss [355, 356], diarrhoea [355, 356], anaemia [355, 357], fatigue [356] and osteopenia [358] though are limited in their small sample size and selected observations from coeliac disease specialty clinics rather than on unselected and population-based contemporary cohort of people with coeliac disease.

In a population-based screening study, adults with undetected coeliac disease as defined by positive EMA serology were found to have an increased risk of mild

anaemia and osteoporosis though they did not regard themselves as being unwell in comparison to serological negative controls [170]. However neither the presumed cases of coeliac disease or the serological negative controls had small bowel histology so it is unclear what the proportion of cases of coeliac disease that had mild enteropathy histological changes present.

Recent study in Finland based on 23 adults newly diagnosed with mild (Marsh 1 and 2) enteropathy coeliac disease and 47 adults newly diagnosed with severe (Marsh 3) enteropathy coeliac disease similarly observed that there was no difference in gender proportion or age at diagnosis between mild and severe enteropathy groups [354]. Lower tTG titres were also present in mild enteropathy disease with 19 of the 23 coeliacs having tTG titres less than 20 iu [354]. Mean values of haemoglobin, albumin, calcium, iron, folate and B12 were also noted to be normal at diagnosis of coeliac disease in coeliacs with mild enteropathy disease [354] with no significant change in those coeliacs randomised to receive treatment with a gluten-free diet or in those randomised to continue on a gluten-containing diet. Clinical symptoms in people with mild and severe enteropathy coeliac disease were reported to be "alleviated" on treatment with a gluten-free diet [354]. However approximately one-third (3/23 with mild enteropathy; 17/47 with severe enteropathy changes) of the newly diagnosed coeliacs had no or only occasional symptoms present at diagnosis of coeliac disease with no change in level of symptoms observed in 3 of the 23 coeliacs with mild enteropathy disease on treatment [354]. On balance with the semi-quantitative, non-validated methods used to determine clinical symptoms any meaningful observations and or comparisons are limited and a larger study using validated and quantitative methods is required.

The prevalence of folate and B12 deficiency in our cohort overall appears lower than in previous studies. In coeliacs (n = 50) diagnosed in the early 1990s, 49% had low folate and 11% were B12 deficient [359]. B12 deficiency has been reported to be as high as 41% in another historical case series [360]. The marked differences between these older studies and our own are probably explained by the era in which they were carried out: the previous studies were carried out prior to the advent of EMA and tTG serological testing and do not represent contemporary practice of identification and diagnosis of coeliac disease in the same way that our study does. The apparent lower proportions of iron, B12 and or folate deficiency, presence of malabsorptive symptoms in coeliacs with mild enteropathy disease in comparison to those with severe enteropathy disease may also reflect that earlier published studies were based on coeliacs with severe enteropathy disease.

3.1.4.4. Possible benefits and risks of having mild enteropathy coeliac disease

In the absence of biochemical derangement at diagnosis of coeliac disease together with no meaningful improvement upon treatment with a gluten-free diet observed in this study and that of Kurppa et al [354] one could propose there are few physiological risks with mild enteropathy coeliac disease. Though it is unclear if changes occur with treatment, a reduction in cholesterol concentration with each unit increase in tTG titre in people with undetected coeliac disease may suggest of further physiological benefit of coeliac disease [170]. Over half of the coeliacs with mild enteropathy disease had diarrhoea though our study is limited in that we are unable to quantify the severity of this and other symptoms or its change with treatment with a gluten-free diet.

3.1.4.5. Degree of intestinal inflammation and severity of malabsorption

With the proximal duodenum being the site of maximal absorption of iron [361], it would follow that iron deficiency is more likely in individuals with duodenal villous atrophy than in those without villous atrophy, reflecting the malabsorption of iron.

3.1.4.5. Summary

Coeliacs with mild enteropathy have few biochemical deficiencies at diagnosis of coeliac disease and thus show no important biochemical improvements following treatment with a gluten-free diet. Severe enteropathy disease appeared to have less biochemical deficiencies in comparison to previously reported studies with almost all deficiencies correcting following 1 year's treatment with a gluten-free diet. Diarrhoea is more common and iron deficiency anaemia is less common in mild enteropathy compared to severe enteropathy. Approximately one-third of coeliacs with mild enteropathy coeliac disease had negative EMA serology at diagnosis and had significantly lower tTG values in comparison to those with severe enteropathy coeliac disease.

3.2. Prevalence and consequence of hypertransaminasaemia in a contemporary cohort of adults with incident coeliac disease: how common is it and does it matter?

3.2.1. Introduction

Coeliac disease is known to be associated with several autoimmune liver diseases [209, 210]. The most common hepatic abnormality found in people with untreated coeliac disease is of an isolated hypertransaminasaemia, observed to affect over 40% of adults newly diagnosed with coeliac disease [211-214, 362]. However, the majority of these studies were carried out prior to the widespread use of anti-endomysial antibody (EMA) or anti-tissue transglutaminase antibody (tTG) tests so represent coeliac diagnosed in a different era to current practice. In addition, these studies mostly involved small, hospital-based case series and were likely to have concerned patients with more serious disease or other medical conditions being investigated at the time. Therefore the estimates of the prevalence of hypertransaminasaemia may poorly reflect the prevalence in contemporary coeliac disease.

The observed hypertransaminasaemia has been coined "gluten" or "coeliac hepatitis" [363]. The hepatic injury is reputed to be characterised by absence of serum auto-antibodies (other than endomysial and tissue transglutaminase antibodies), elevated transaminases and the presence of mild lobular and portal tract inflammation, steatosis that is reversible on treatment with a gluten-free diet. Given the relatively high prevalence of coeliac hepatitis observed in the reported case series and the potential of the coeliac hepatitis to progress to fibrosis, cirrhosis and end-stage liver failure [364, 365], some groups have recommended a vigorous search for liver disease in people newly diagnosed with coeliac disease [363-365]. To help determine if the liver work-up is warranted in contemporary coeliac disease, we have assessed the prevalence of

hypertransaminasaemia in an unselected, large and contemporary population of people newly diagnosed with coeliac disease.

3.2.2. Methods

3.2.2.1. Study design

Historical cohort study.

3.2.2.2. Study population

Adults with incident coeliac disease diagnosed at Nottingham University Hospital (Nottingham historical cohort) and Royal Hallamshire Hospital (Sheffield historical cohort) were studied. Dietetic, histopathology, immunology, clinical coding and outpatient records were used to retrospectively identify incident cases of coeliac disease as described in sections 2.2.1. and 2.2.2., respectively.

3.2.2.3. Outcome measures

- Proportion of adults with abnormally high ALT at diagnosis of coeliac disease
- Change in the mean value between diagnosis and following 12 months treatment with a gluten-free diet the value of liver chemistries (alkaline phosphatase, alanine transferase, gamma-glutamyl transferase, albumin) in adults with coeliac disease

3.2.2.4. Outcome ascertainment

Serum alkaline phosphatase (U/L), liver transaminase (alanine transferase (U/L) and or aspirate transaminase (U/L), gamma-glutamyl transferase (U/L), bilirubin (μmol/L), ferritin (μg/L), folate (μg/L), ESR (mm/hr), CRP (mg/L), glucose

(mmol/L), leucocyte count (10^9/L), albumin (g/L), platelet count (10^9/L), haemoglobin (g/L) were routinely measured on newly diagnosed adult cases of coeliac disease attending Nottingham University Hospital and Royal Hallamshire Hospital between 1st January 2000 and 31st December 2006 at both diagnosis of coeliac disease and in the majority following approximately 12 months treatment with a gluten-free diet. Demographic, clinical, histological and serological data in addition to these laboratory co-variates was systematically collected, as described in sections 2.2.1. and 2.2.2., respectively. The modes of presentation of coeliac disease and coeliac serological status at diagnosis of coeliac disease was determined and defined as described in section 2.4.

Dietary compliance was subjectively assessed by the gastroenterologist and dietitian. In addition, changes in endomysial antibody positivity, and when later introduced by the laboratories levels of tissue transglutaminase titre, were noted as a surrogate for compliance whilst following a gluten-free diet.

Diagnosis of vitamin D deficiency and osteomalacia was based on characteristic biochemical abnormalities (including elevated parathyroid hormone and low 25 (OH) Vitamin D levels which were only usually measured on patients with elevated alkaline phosphatase).

3.2.2.5. Statistical analysis

Prevalence of abnormal liver profile at diagnosis of coeliac disease was determined by calculating the proportion (%) of coeliacs with a liver test result above the upper limit of normal for the hospital laboratory concerned. With respect to albumin, an abnormal albumin result was defined as any value below the lower limit of normal for the

hospital laboratory concerned; thrombocytopaenia was defined as any platelet count below 150×10^{12}/L.

Univariate logistic regression analysis was performed to determine the association between abnormal liver profile at diagnosis of coeliac disease and all potential explanatory independent covariates. Multivariate logistic regression analyses were performed to determine the odds ratios with 95% confidence intervals of abnormal liver profile at diagnosis of coeliac disease with respect to proxy markers of severity of coeliac disease after adjusting for age at diagnosis, gender and presence of autoimmune liver disease. Degree of intestinal inflammation as defined by Marsh grading (mild (Marsh 0, 1, 2); moderate (Marsh 3a); and severe (Marsh 3b, 3c)) and malabsorption mode of presentation (presence of weight loss and diarrhoea and anaemia) were used as proxy markers of severity of coeliac disease; titres of tTG were not used due to missing data.

Paired t-tests were used to examine changes in serum liver profile from baseline to 12 months following a gluten-free diet.

We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed using Stata SE 9.2 [TexCorp].

3.2.2.6. Ethical approval

Advice was sought from local ethics committees (Nottingham 1 and South Sheffield) with Nottingham University Hospital (reference ID 290) and Royal Hallamshire Hospital (reference 04/63) giving audit approval under service evaluation guidelines.

3.2.3. Results

3.2.3.1. Demography of cohort

We identified 1008 adults newly diagnosed with coeliac disease between 2000 – 2006 of whom 905 had at least one measurement of liver transaminases: 877 had a measurement of alanine transferase, 28 had a measurement of aspartate transaminase at diagnosis. There were nearly twice as many female (n = 610) as male (n = 295) incident cases in the study cohort. The mean age at diagnosis was 50.2 (SD 16.2) years with women (49.1 (SD 15.9) years) being diagnosed at an earlier age than men (52.5 (SD 16.4) years; p = 0.003).

3.2.3.2. Presenting features

Iron deficiency anaemia and diarrhoea were the most common presenting features, affecting over one-third of the cohort. 10% (n = 92) of the cohort presented with malabsorption. 32% had partial villous atrophy; 30% had subtotal villous atrophy and 26% had total villous atrophy. At diagnosis, the median tTG was 203 (IQR 37 – 300; n = 263) and 92% were EMA positive. 449 (49%) of the study cohort had ferritin values below 22 μg/L, the lowest limit of the normal range, whereas 143 (16%) had B12 values below 211 ng/L. 68 (8%) of the study cohort had red cell folate or serum folate values below the lower limit of the normal range of values. 21 (2%) of the cohort had evidence of osteomalacia with vitamin D deficiency at diagnosis of coeliac disease.

The presenting features of those coeliacs presenting with abnormal ALT (n = 82) and normal ALT (n = 775) is shown in the following table.

Table 3.17: Presenting features of incident coeliacs with elevated alanine transferase

Presenting feature	Coeliacs with normal ALT (n = 795)	Coeliacs with abnormal ALT (n = 82)
Sex (%)		
Male	255 (32)	34 (41)
Female	540 (68)	48 (59)
Mean age (SD) years	50.8 (16.3)	46.4 (14.5)
Marsh grading		
0	4 (1)	0
1	29 (4)	1 (1)
2	14 (2)	2 (2)
3a	263 (33)	16 (20)
3b	240 (30)	28 (34)
3c	194 (24)	33 (40)
VA* unspecified	32 (4)	2 (2)
Not done	19 (2)	0
EMA status (%)		
Positive	694 (89)	77 (95)
Weak positive	15 (2)	1 (1)
Negative	64 (8)	3 (4)
Not done	4 (1)	0
Mean Hb (SD) g/L	12.3 (2.3)	12.2 (2.5)
Mean ferritin (SD) µg/L	36.1 (69.7)	38.1 (75.5)
Mean weight (SD) kg	66.3 (18.0)	66.9 (15.4)

*VA degree of villous atrophy unspecified

3.2.3.3. Prevalence of diagnosed hepatobiliary conditions in incident coeliac disease

5% (n = 44) of the cohort had some form of diagnosed hepatobiliary condition at diagnosis of coeliac disease. With respect to autoimmune liver disease, 6 (0.7%) coeliacs had been previously diagnosed with primary biliary cirrhosis, 5 with autoimmune hepatitis and 1 with primary sclerosing cholangitis. 19 coeliacs (2.1%) had been treated for gallstone disease prior to the diagnosis of coeliac disease whilst 8 (0.6%) were known to have alcoholic liver disease.

3.2.3.4. Liver profile at diagnosis of coeliac disease

The mean alanine transferase (ALT) at diagnosis of coeliac disease was 33.5 (SD 49.7; median 26, IQR 20 - 38) U/L with no significant difference between men and women. The mean ALP was 87.5 (SD 53.2; median 76, IQR 60 – 98) U/L (n = 485) and 195.8 (SD 141.9; median 166.5, IQR 115 - 237) U/L (n = 402) in the two different assays used in the study period. 55 (6%) of the cohort had an abnormally low albumin and 12 coeliacs had thrombocytopaenia.

3.2.3.5. Prevalence of abnormal liver profile at diagnosis of coeliac disease

9.4% (n = 82) of our study population had an abnormal ALT value (Table 3.18). The majority with an abnormal ALT at diagnosis of coeliac disease had an ALT value within two times the upper limit of normal range (71 of 82) with only 1.2% (n = 11) having an abnormal ALT above two times the upper limit of the normal range. 12.3% (n = 109) of the cohort had an abnormal ALP. However the majority of incident coeliacs with an abnormal ALP result (94 of the 109) had an ALP value within two times the upper limit of normal range with only 15 (1.7% of cohort) having a abnormal ALP above two times the upper limit of the normal range.

Table 3.18: Prevalence of abnormal ALP and ALT at diagnosis of coeliac disease

	Number with valid test (n = 1008)	Number abnormal	% abnormal [95% CI]
ALP	887 (88%)	109	12.29 [10.29, 14.61]
>1 but <2 x ULN		94	10.60 [8.74, 12.80]
Above 2 x ULN		15	1.69 [1.03, 2.77]
ALT	877 (87%)	82	9.35 [7.60, 11.46]
>1 but <2 x ULN		71	8.10 [6.47, 10.09]
Above 2 x ULN		11	1.25 [0.70, 2.23]

3.2.3.6. Associations of abnormal ALP and ALT at diagnosis of coeliac disease

ALT

Having an abnormal ALT was associated with male gender (OR 1.50 [95% CI 1.02, 2.39]) but not with age at diagnosis nor BMI (Table 3.19). Unsurprisingly, abnormal ALT at diagnosis was associated with previously diagnosed autoimmune liver disease (OR 7.30 [95%CI 2.26, 23.58]). A strong independent graded relationship was observed between the presence of abnormal ALT and the degree of intestinal inflammation. Abnormal ALT was associated with a 2-fold increased odds (OR 2.02 [95%CI 1.10, 3.69]) of having moderate intestinal inflammation (Marsh 3a) and 3-fold (OR 2.70 [95%CI 1.49, 4.87]) increased odds of having severe intestinal inflammation (Marsh 3b, 3c) relative to the baseline category of mild intestinal inflammation (Marsh 0, 1 or 2) after adjusting for age at diagnosis, gender and presence of autoimmune disease. Abnormal ALT was associated with clinically more severe coeliac disease with 7-fold increased risk of presenting with features of malabsorption being present.

Table 3.19: Associations with abnormal ALP and ALT in incident coeliac disease

Exposure	Proportion (n = 887) with abnormal ALP	OR [95% CI] univariate for having abnormal ALP	OR [95% CI] multivariate for having abnormal ALP	Proportion (n = 877) with abnormal ALT	OR [95% CI] univariate for having abnormal ALT	OR [95% CI] multivariate for having abnormal ALT
Gender						
Male	14.0%	1.27 [0.84, 1.92]	1.38 [0.88, 2.17]	11.8%	1.50 [1.02, 2.39]	1.72 [1.06, 2.79]
Female	11.4%	1.00	1.00	8.2%	1.00	1.00
Age quartile (years)						
<38.8	8.7%	1.00	1.00	10.6%	1.00	1.00
38.9 – 50.9	8.6%	0.47 [0.26, 0.83]	0.97 [0.48, 1.97]	9.6%	1.02 [0.59, 1.76]	0.63 [0.33, 1.20]
51.0 – 61.7	12.6%	0.88 [0.55, 1.44]	1.39 [0.72, 2.69]	12.4%	1.52 [0.91, 2.52]	0.56 [0.29, 1.07]
>61.8	19.2%	2.74 [1.79, 4.20]	2.05 [1.09, 3.86]	5.5%	0.48 [0.25, 0.92]	0.24 [0.11, 0.53]
Missing data (n=3)	0%			0%		
BMI group (kg/m^2)						
<18.5	15.0%	1.92 [0.70, 5.25]	1.47 [0.36, 6.04]	4.4%	0.43 [0.06, 3.26]	0.49 [0.04, 6.56]
18.5 – 24.9	20.8%	0.43 [0.19, 1.02]	0.36 [0.10, 1.21]	4.2%	0.39 [0.14, 1.09]	0.82 [0.14, 4.75]
25.0 – 30	6.1%	1.00	1.00	5.1%	1.00	1.00
>30	4.8%	0.35 [0.05, 2.64]	0.33 [0.04, 2.96]	5.0%	0.50 [0.07, 3.81]	0.83 [0.07, 10.09]
Missing data (n=704)	12.9%	1.36 [0.80, 2.32]	0.58 [0.23, 1.47]	10.6%	2.52 [1.19, 5.32]	1.95 [0.45, 8.38]
Autoimmune LD*						
Present	41.7.5%	5.30 [1.65, 16.99]	4.23 [1.20, 14.89]	41.7%	7.30 [2.26, 23.58]	11.65 [2.92, 46.44]
Absent	11.9%	1.00	1.00	8.9%	1.00	1.00
Osteomalacia						
Osteomalacia	81.0%	35.76 [11.78, 108.54]	29.19 [9.32, 91.46]	4.8%	0.48 [0.06, 3.61]	
No osteomalacia	10.6%	1.00	1.00	9.46%	1.00	

Exposure	Proportion (n = 887) with abnormal ALP	OR [95% CI] univariate for having abnormal ALP	OR [95% CI] multivariate for having abnormal ALP	Proportion (n = 877) with abnormal ALT	OR [95% CI] univariate for having abnormal ALT	OR [95% CI] multivariate for having abnormal ALT
Duodenal histology						
Marsh 0, 1, 2	10.08%	1.00	1.00	5.8%	1.00	1.00
Marsh 3a	13.3%	1.37 [0.84, 2.24]	1.16 [0.68, 1.97]	10.5%	1.90 [1.05, 3.43]	2.02 [1.10, 3.69]
Marsh 3b, 3c	15.6%	1.65 [1.11, 2.69]	1.28 [1.03, 2.21]	14.5%	2.77 [1.55, 4.92]	2.70 [1.49, 4.87]
Missing data (n=19)	0%	1.33 [0.52, 3.41]	1.20 [0.45, 3.19]	0.0%	0.72 [0.16, 3.25]	0.71 [0.16, 3.26]
Malabsorption						
No malabsorption	10.4%	1.00	1.00	6.7%	1.00	1.00
Malabsorption	29.2%	3.56 [2.13, 5.92]	3.07 [1.75, 5.38]	32.6%	6.70 [3.97, 11.32]	6.63 [3.76, 11.69]

ALP

Abnormal ALP was not associated with gender, age at diagnosis nor body mass index. However presence of previously diagnosed autoimmune liver disease (OR 5.30 [95%CI 1.65, 16.99]) and the presence of osteomalacia (OR 35.76 [95%CI 11.78, 108.54]) were statistically significantly associated with abnormal ALP at diagnosis of coeliac disease. Those coeliacs with an abnormal ALP were four times as likely to present with features of malabsorption (OR 3.56 [95%CI 2.13, 5.92]). Furthermore a graded relationship was observed between having abnormal ALP and odds of having mild, moderate or severe degrees of histological inflammation. After adjusting for age at diagnosis, gender, presence of autoimmune disease, abnormal ALP was associated with 29-fold increased odds of having a diagnosis of osteomalacia (OR 29.12 [9.32, 91.46]).

3.2.3.7. Change in liver profile following treatment with gluten-free diet

Follow-up liver profile values were obtained after a mean of 12.8 (SD 1.9) months on a gluten-free diet in approximately 80% of individuals in our study (Table 3.20).

Table 3.20: Change in liver chemistries with gluten-free diet

	N	At diagnosis of coeliac disease [SD]	After 12 months of GFD [SD]	Mean difference [95% CI]	p-value
Mean ALP U/L	749	139.11 [115.14]	105.66 [73.66]	-33.46 [-39.58, -27.33]	0.00001
Mean ALT U/L	665	33.55 [53.59]	26.91 [24.76]	-6.65 [-10.30, -2.99]	0.0005
Mean GGT U/L	511	27.91 [36.38]	31.10 [48.06]	3.19 [-0.39, 6.78]	0.08
Mean bilirubin µmol/L	676	10.26 [8.89]	10.73 [11.17]	0.47 [-0.33, 1.27]	0.24
Mean albumin g/L	759	38.77 [4.53]	39.56 [3.87]	0.79 [0.46, 1.11]	0.00001

The vast majority of these patients were considered by the gastroenterologist and dietitian to adhere strictly to a gluten-free diet. At diagnosis 92% were EMA positive while after a year of treatment only 19% remained positive. There were no significant changes in GGT or bilirubin on treatment with a gluten-free diet. However there were statistically significant reductions in ALP (mean difference -33.46 [95%CI -39.58, -27.33] U/L) and ALT (mean difference -6.65 [95%CI -10.30, -2.99] U/L). Only 14 (17.1%) of the 82 coeliacs with abnormal ALT at diagnosis of coeliac disease had persistently abnormal ALT following treatment with a gluten-free diet with the ALT values normalising in the remainder. Of these, 2 had dyslipidaemia, 1 had type 2 diabetes, 1 had primary biliary cirrhosis, 1 had hypothyroidism, 1 was non-compliant with gluten-free diet. The remaining 8 coeliacs only had non-invasive tests for liver disease which were negative.

3.2.4. Discussion

3.2.4.1. Principal findings

We found that in newly diagnosed adults with coeliac disease, clinically important hypertransaminasaemia (i.e. greater than 2 x ULN) was uncommon (<2%). In those patients with an abnormal test of any level 86% normalised following a year of treatment with a gluten-free diet. The presence of elevated transaminases in incident coeliac disease was associated independently with clinical features of malabsorption and more severe histological features of intestinal inflammation on duodenal biopsy. Although relatively uncommon, abnormally high ALP at diagnosis of coeliac disease was strongly associated with a diagnosis of osteomalacia.

3.2.4.2. Merits and limitations

This is the first prospective study describing the systematic, routine collection of biochemical liver profile in a large, unselected and population-based cohort of adults newly diagnosed with coeliac disease. While we cannot be certain that we have identified every patient diagnosed in both hospitals during the period we made extensive efforts to do so. We believe it unlikely that the omission of the few patients that will have inevitably missed will have led to a substantial underestimate of the prevalence of hypertransaminasaemia we have reported. The recent widespread availability of highly sensitive and specific serological tests for coeliac disease has allowed identification and diagnosis of rapidly rising numbers of people with coeliac disease [343, 366, 367]. These people with, relatively, less serious disease, now account for the majority of people diagnosed with coeliac disease [366-368]. The patients in our study were all diagnosed during this era and therefore represent contemporary practice in a manner that has not been reported previously.

3.2.4.3. Comparison with other studies

Previous studies, based on historical and smaller case series, have observed a much higher (40 – 55%) prevalence of hypertransaminasaemia in incident coeliac disease [211, 213, 214, 362]. The marked differences between these older studies and our own are probably partly explained by the era in which they were carried out. The previous studies were carried out uniformly prior to the advent of serological tests for EMA and tTG and therefore do not represent contemporary practice of identification and diagnosis of coeliac disease in the same way that ours does. Emerging evidence suggests that patients diagnosed with coeliac disease more recently have, to some extent, less severe disease [366]. Another explanation for the difference is the manner in which we identified our population. In Jacobsen's case series 17% had some form of malignancy [362] in contrast to our cohort where none of our coeliacs were known to have cancer. In addition, not all of the coeliacs had a liver profile measurement in some studies such as in Hagander et al case series [211] suggesting that there were likely to have been specific reasons for doing so. Our observation that only 9.4% people have hypertransaminasaemia at diagnosis of coeliac disease is similar to the prevalence one study that used serological tests to screen for coeliac disease [212, 214] indicating perhaps that those diagnosed in our region in the year 2000 onwards are more similar to these groups of screen-detected individuals.

Alkaline phosphatase is regarded as an useful initial biochemical test to screen for osteomalacia in the general population although high false positive rates are recognised [369, 370]. The strong association observed between elevated alkaline phosphatase and presence of osteomalacia in our coeliac cohort is consistent with these findings in the general population [369, 370]. Our observed prevalence of elevated alkaline phosphatase in incident coeliac disease is similar to previous studies

investigating metabolic bone disorders in coeliac disease [191, 194, 207] though the prevalence of osteomalacia (2%; n = 21) observed in our study is considerably lower than in these studies [191, 194, 207] suggesting there were specific reasons for measuring different elements of bone profile. Such ascertainment bias is likely to be present in our study too with no routine measurement of parathyroid hormone nor vitamin D levels unless there was a clinical or biochemical suspicion of underlying metabolic bone disease. The observed reduction in alkaline phosphatase with treatment of coeliac disease is in keeping with previous studies [191, 194, 207] and supports the importance of gluten withdrawal in coeliac disease to also help treat any underlying osteomalacia [191, 194, 207]. Normalisation of an isolated elevated alkaline phosphatase with treatment of coeliac disease may also remove the need for further invasive investigations such as a bone biopsy.

3.2.4.4. Possible explanations for hypertransaminasaemia in coeliac disease

With 86% of cases of hypertransaminasaemia in newly diagnosed coeliac disease normalising with a gluten-free diet in keeping with previous studies [212-214], this suggests the mechanism(s) underlying the liver abnormality involves a relationship between gluten intake, intestinal damage and potential hepatic injury. Hypothesising that the liver insult in coeliac disease may be due to increased intestinal permeability resulting from the gluten-related intestinal damage (thereby allowing the entrance of toxins, antigens and pro-inflammatory mediators (such as interleukins and γ-interferon) to the portal circulation with subsequent hepatocyte insult) [371-373] could be supported by our findings of a strong independent graded relationship between the presence of elevated ALT at diagnosis of coeliac disease and the degree of intestinal inflammation. A role for malabsorption-induced 'starvation' state causing

steatosis may be reflected by our observation that elevated ALT is associated with 9-fold increased odds of presenting with features of malabsorption. Chronic intestinal mucosal inflammation may have a role such as that observed in ulcerative colitis [374]. Extracellular deposition of IgA tTG-2 in liver biopsies of people with active coeliac disease [375] may suggest a pathogenic role for the humoral-mediated immune responses in the liver injury observed in coeliac disease.

3.2.4.5. Possible role of vitamin D and calcium malabsorption in bone derangement of coeliac disease

With the observation that elevated alkaline phosphatase in incident coeliac disease was independently associated with clinical features of malabsorption, one could speculate that the vitamin D and calcium malabsorption with the consequent osteomalacia state originates in the same context of a widespread nutritional failure, forming an important pathogenic mechanism of bone derangement in these coeliacs. The resolution of elevated alkaline phosphatase and dramatic increase in serum vitamin D levels in those patients with osteomalacia on gluten withdrawal further supports this speculation and is in keeping with previous observations of a gluten-free diet leading to significant bone mass improvement [191, 194, 207].

3.2.4.6. Summary

In summary, clinically important hypertransaminasaemia is uncommon (<2%) in people newly diagnosed with coeliac disease and any abnormality of ALT or ALP only occurred in around 10% of patients. The majority of these abnormalities normalised following treatment with a gluten-free diet. The lower prevalence observed in comparison to previous studies may be due to differences in study design but may also reflect identification of people with disease below the waterline of the

iceberg of coeliac disease with the introduction of highly sensitive and specific serological screening tests. Our findings suggest that investigations for liver disease should only be initiated in those patients with persistent hypertransaminasaemia or if otherwise indicated.

3.3. Is the diagnosis of coeliac disease associated with socio-economic status? A population-based study

3.3.1. Introduction

The rate of diagnosis of coeliac disease in developed countries has increased dramatically since the introduction of serological tests without an obvious environmental precipitant. Little is known about the socio-economic distribution of coeliac disease; there is some evidence that it is less common in more deprived social groups [170]. The aim of this study is to quantify the incidence of new diagnoses of coeliac disease by socio-economic status in a large, contemporary and population-based cohort.

3.3.2. Methods

3.3.2.1. Study design

Historical cohort study.

3.3.2.2. Study population

Adults with incident coeliac disease diagnosed at Nottingham University Hospital (Nottingham historical cohort) and Royal Hallamshire Hospital (Sheffield historical cohort) between 1st January 2000 and 31st December 2006 were studied. Dietetic, histopathology, immunology, clinical coding and outpatient records were used to retrospectively identify incident cases of coeliac disease as described in sections 2.2.1. and 2.2.2., respectively.

3.3.2.3. Outcome measures

- Incidence rate of coeliac disease (all; by sex; and by age) by quintile of rank of IMD07 score (most deprived, below average, average, above average, least deprived) per 1000 population.

3.3.2.4. Outcome ascertainment

The postcode of residence at diagnosis was routinely collected on newly diagnosed adult cases of coeliac disease attending Nottingham University Hospital and Royal Hallamshire Hospital. This postcode of residence at coeliac disease diagnosis was used to determine the Index of Multiple Deprivation 2007 (IMD07) score and rank. The IMD07 score and rank determined was then used as an indicator of socio-economic status [376].

The IMD07 is a measure of multiple deprivation at the small area level, produced on a statistical geography known as a Lower Layer Super Output Area (LLSOA) that contains approximately 1500 people and 750 households. The IMD07 is a statistically generated output that combines data from a number of indicators, chosen to cover a range of economic, social and housing issues into a single deprivation score for each LLSOA in England [376]. Separate indices at the LLSOA level are provided for each of the seven domains / themes of deprivation: income (9 indicators including proportion on Income Support), employment (5 indicators including employment deprived such as those with forced exclusion from work due to sickness); health deprivation and disability (5 indicators including SMR <65 years, limiting long term illness, low Birthweight); education, skills and training (6 indicators including level of qualifications and education amongst adults); access to housing and services (3 indicators including proportion living in unsatisfactory housing); crime (4 indicators

such as recorded burglary); and living environment (such as proportion of houses without central heating) [376]. The IMD07 deprivation score generated allows all LLSOA to be ranked according to how deprived they are relative to each other [376]. A rank of 1 indicates the most deprived LLSOA and 32,482 the least deprived. The ranks are categorised into quintiles with quintile 1 (rank 1 - 6496) being the most deprived and quintile 5 (rank 25,987 – 32,482) least deprived [376].

The area and boundaries of Nottingham and Sheffield were geographically defined by their super output area [377]. Each LLSOA within the middle super output area of Sheffield is listed as an example in Appendix 7.1.

Demographic, clinical, histological and serological data in addition to these laboratory co-variates was systematically collected, as described in sections 2.2.1. and 2.2.2., respectively. The modes of presentation of coeliac disease and coeliac serological status at diagnosis of coeliac disease was determined and defined as described in section 2.4. The study population was divided into the age bands (16 – 29 years; 30 – 44 years; 45 – 64 years; 65 years and older) as used by the Office of National Statistics [42, 378].

3.3.2.5. Statistical analysis

Incidence rates of coeliac disease by quintile of rank of IMD07 score were calculated using the total adult population for Sheffield and Nottingham derived from the UK 2001 National Census [42, 378]. Multivariate poisson regression analyses were performed to estimate the incidence rate ratio (IRR) of coeliac disease according to

quintile of rank of IMD07 score with respect to proxy markers of severity of coeliac disease after adjusting for age and for sex as a priori confounders.

Degree of intestinal inflammation as defined by Marsh grading (severe (Marsh 3b, 3c) and mild (Marsh 1, 2, 3a) and malabsorption mode of presentation (presence of weight loss and diarrhoea and anaemia) were used as proxy markers of severity of coeliac disease.

We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed using Stata SE 9.2 [TexCorp].

3.3.2.6. Ethical approval

Advice was sought from local ethics committees (Nottingham 1 and South Sheffield) with Nottingham University Hospital (reference ID 290) and Royal Hallamshire Hospital (reference 04/63) giving audit approval under service evaluation guidelines.

3.3.3. Results

3.3.3.1. Demography of cohort

We identified 877 adults newly diagnosed with coeliac disease between 1st January 2000 – 31st December 2006 of whom 837 had postcode at residence at diagnosis available. There were approximately twice as many female (n = 570) as male (n = 267) incident cases in the study cohort. The mean age at diagnosis was 49.6 (SD 16.0) years with women (48.4 (SD 15.9) years) being diagnosed at an earlier age than men (52.1 (SD 15.9) years); p = 0.0016.

3.3.3.1. Presenting features

Iron deficiency anaemia and diarrhoea were the most common presenting features, affecting over one-third of the cohort. 31% had partial villous atrophy; 28% had subtotal villous atrophy and 23% had total villous atrophy. At diagnosis, the median tTG was 163 (IQR 28 - 300; n = 241) and 87% were EMA positive.

3.3.3.2. Incidence of coeliac disease by quintile of rank of IMD07 score

The incidence rate of identified adults with coeliac disease at Nottingham and Sheffield was 0.11 per 1000 population. There was a strong, independent graded association between the incidence rate of coeliac disease and socio-economic status. The incidence rate of coeliac disease was twice as high in coeliacs in the least deprived and most affluent quintile (age- and sex-adjusted incidence rate ratio (IRR) 1.93; 95%CI 1.61, 2.44) as compared to those coeliacs in the most deprived and poorest quintile (Table 3.21).

Table 3.21: Incidence of coeliac disease by quintile of rank of IMD07 score

Quintiles of rank of IMD07 score	N	N total adult exposed population	Rate /1000 population	Crude Incidence Rate Ratio [95% CI]	Adjusted* Incidence Rate Ratio [95% CI]
I Most deprived	160	2173052	0.07	1.00	1.00
II Below average	125	1361311	0.09	1.23 [0.99, 1.58]	1.22 [0.96, 1.53]
III Average	170	1397949	0.13	1.69 [1.36, 2.09]	1.61 [1.30, 2.00]
IV Above average	168	1175846	0.15	1.97 [1.59, 2.44]	1.82 [1.50, 2.30]
V Least deprived	189	1193626	0.17	2.20 [1.79, 2.71]	1.93 [1.61, 2.44]

* adjusted for age and for sex

Socio-economic status in incident coeliac disease was not associated with more severe coeliac disease (Table 3.22).

Table 3.22: No association of severity of coeliac disease with quintile of rank of IMD07 score

Quintiles of rank of IMD07 score	Proportion with Marsh 3c, 3b	OR [95% CI] univariate	Proportion with malabsorption	OR [95% CI] univariate
I Most deprived	54.7%	1.00	7.4%	1.00
II Below average	67.0%	1.68 [1.01, 2.79]	5.6%	0.74 [0.28, 1.94]
III Average	59.0%	1.19 [0.76, 1.87]	11.3%	1.60 [0.76, 3.39]
IV Above average	59.4%	1.21 [0.77, 1.91]	7.5%	1.02 [0.45, 2.30]
V Least deprived	52.1%	0.87 [0.56, 1.34]	7.6%	1.04 [0.47, 2.28]

3.3.3.4. Incidence of coeliac disease by sex

The incidence rate of coeliac disease in women was 0.15 per 1000 population and 0.07 per 1000 population in men. There was a strong, independent association between the incidence rate of coeliac disease and sex with higher rates of coeliac disease in women with respect to men with the effect most marked in the youngest age band (Table 3.23). The incidence rate of coeliac disease was nearly four times as high in female coeliacs in the youngest age band 16 – 29 years (unadjusted incidence rate ratio (IRR) 3.75; 95%CI 2.31, 6.32) as compared to males coeliacs within the same age band (Table 3.10). In comparison the incidence rate of coeliac disease was twice as high in female coeliacs aged 30 – 44 years (unadjusted IRR 2.34; 95%CI 1.78, 3.11) as compared to males within the same age band. Over 65 years of age there was no difference in incidence rate of coeliac disease between sexes.

Table 3.23: Incidence of coeliac disease by sex

Age band	N female coeliacs	N total female exposed population	Rate per 1000 population in women	N male coeliacs	N total male exposed population	Rate per 1000 population in men	Crude IRR [95% CI]
I 16–29 years	77	994042	0.078	22	1064623	0.021	3.75 [2.31, 6.32]
II 30 – 44 years	174	911267	0.191	76	932974	0.082	2.34 [1.78, 3.11]
III 45 – 64 years	228	786191	0.290	108	1015497	0.106	2.72 [2.15, 3.46]
IV 65+ years	91	1010394	0.090	61	586796	0.104	0.86 [0.62, 1.22]

Incidence of coeliac disease was independently associated with presence of iron deficiency anaemia in female coeliacs within the younger age bands though there was no difference in proportions between male and female coeliacs presenting with malabsorption or more severe histological changes on small bowel biopsy (Table 3.24).

Table 3.24: Association of iron deficiency anaemia and severity of coeliac disease with age and sex

Age band	Proportion of women with IDA	Proportion of men with IDA	OR [95% CI]	Proportion of women with Marsh 3c, 3b	Proportion of men with Marsh 3c, 3b	OR [95% CI]	Proportion of women with malabsorpt[n]	Proportion of men with malabsorpt[n]	OR [95% CI]
I 16–29 years	46.8%	18.2%	3.95 [2.08, 7.51]	47.2%	50.0%	0.89 [0.51, 1.56]	3.9%	4.6%	0.84 [0.21, 3.34]
II 30–44 years	50.0%	21.1%	3.74 [2.01, 6.95]	54.3%	59.1%	0.82 [0.47, 1.44]	4.0%	7.9%	0.49 [0.14, 1.67]
III 45–64 years	43.4%	44.4%	0.96 [0.56, 1.65]	63.0%	67.0%	0.84 [0.47, 1.50]	9.7%	13.0%	0.72 [0.30, 1.74]
IV 65+ years	42.9%	45.9%	0.89 [0.51, 1.55]	59.3%	41.1%	2.09 [1.19, 3.67]	7.7%	11.5%	0.64 [0.25, 1.67]

3.3.4. Discussion

3.3.4.1. Principal findings

The estimated incidence of adult coeliac disease in Nottingham and Sheffield in the period 2000 – 2006 was 0.115 per 1000 population. There was a strong, independent graded association between the incidence rate of new diagnoses of coeliac disease and socio-economic status with the rate twice as high in adults from affluent areas compared with that in adults living in poorer areas. Socioeconomic status was not associated with features of more severe coeliac disease. The incident rate of new diagnoses of coeliac was also independently associated with female sex in comparison to men with the effect most marked in the younger adult years but this did not explain the relationship with socioeconomic status.

3.3.4.2. Merits and limitations

This is the first study where there has been systematic and routine collection of socioeconomic status in a large, unselected and population-based cohort of adults newly diagnosed with coeliac disease. While we cannot be certain that we have identified every coeliac diagnosed in all three hospitals during the study period we made extensive efforts to do so. We believe it unlikely that the omission of the few patients that will have inevitably been missed will have led to a substantial under- or overestimate of the relationship between socioeconomic status and coeliac disease we have reported. The recent widespread availability of highly sensitive and specific serological tests for coeliac disease allowing identification and diagnosis of rapidly rising numbers of people with coeliac disease [343, 366, 367] adds to the contemporary nature to our cohort and representing today's coeliac disease.

The IMD07 score is a nationally consistent measure of how deprived an area is by identifying the degree to which people are disadvantaged by factors such as low income, unemployment, lack of education, poor health and crime [376]. Such indicators of social status and material conditions have been demonstrated to be associated with increased risks for health impairment and mortality [379-381]. The IMD07 is formed by pulling together a total of 38 different individual indicators chosen to cover a range of economic, social and housing issues for each small area in England (LLSOA) providing an overall measure of socioeconomic deprivation [376]. Despite the IMD07 score covering aspects of income, education, housing and access to services including health care, these are not direct measures of the quality of health care delivery in the communities [376]. There is also an element of ecological fallacy with the IMD07 score as it indicates that an area has a particular level of deprivation but it is not necessarily the case that specific individuals living in that area are similarly deprived. Furthermore, unmeasured environmental, social, behavioural factors are also beyond these proxy markers of socioeconomic status. The socioeconomic status given by the IMD07 score in this study is based on area of residence at the time of diagnosis of coeliac disease but it may not necessarily represent every coeliac's current or historical socioeconomic group. However, generally people living in the same area have similar levels of deprivation and have found to be robust over time [382]. Another possible source of bias is in the ascertainment of denominator populations. The UK census was criticised for underestimating the size of populations living in inner city areas [383]. However, this under-ascertainment would only act to strength the associations between socioeconomic status and rate of new diagnoses of coeliac disease. Any misclassification bias involved in defining the area and boundaries of Nottingham and

Sheffield geographically by their super output area is likely to be non-differential. It is feasible that coeliacs living within Nottingham or Sheffield could attend hospitals other than those in Nottingham or Sheffield. However in the lack of a regional or national tertiary referral centres for coeliac disease and assuming with the referral access of primary care practitioners for hospitals local to place of residence for routine care of coeliac disease, such bias is minimal.

3.3.4.3. Comparison with other studies

Our estimated incidence of adult coeliac disease of 0.115 per 1000 population is lower but is somewhat in keeping with that estimated in Derby (0.169 per 1000 population) during the same time period of study [174]. The higher estimates observed in Derby may reflect the active case-finding strategy adopted by the author of this study who has prospectively followed identified coeliacs in a weekly-run, dedicated coeliac clinic since 1978 [174]. The higher estimates may also be due to further steps being taken by the author to identify coeliacs such as using membership records of the coeliac society, Coeliac UK, and dermatitis herpetiformis clinic in addition to the steps we also took to identify the numerator population. The denominator population was defined also using Office for National Statistics Census data though it is unclear whether super output areas were the geographical mode to delineate the areas and boundaries.

Our observation that the incidence rate of new diagnoses of coeliac disease is associated with socioeconomic status is new though it was suggested by the non-significant trend observed by West et al where EMA positive adults in a population-based screening study were less likely to be from partly skilled and unskilled occupational positions (OR 0.51 partly skilled and unskilled to professional positions,

95%CI 0.18, 1.43) [170]. Whether these findings are limited by selection bias with volunteers for the study likely to differ in their attitudes, behaviours and health status compared to non-volunteers is unclear. However maternal exposures in infancy suggest direct and indirect evidence for the association of social class with incidence of coeliac disease. With breastfeeding at the time of gluten introduction associated with protection against coeliac disease [384] and breastfeeding associated with less deprived social class [385], this suggests an increased risk of coeliac disease for children whose mothers are from more deprived social classes. Conversely increased risks of coeliac disease for children whose mothers were from manual social classes (assessed by spouse's occupational position) was observed in the Oxford Linkage study [386]. The association between low birth weight and increased risk for coeliac disease [387] may be in part due to social factors such as poverty-related maternal health status causing lower physiological reserves, quality and quantity of healthcare, housing, diet, increased exposure to toxic antigens and differential risk of infectious disease [388-391] and supporting the observations of the Oxford Linkage Study [386]. It is known that people from poorer social classes are less likely to attend for routine health checks at which blood tests are performed [392-394], access healthcare [395, 396] and have different health seeking behaviours [397, 398] in comparison to people from more affluent social classes. Indeed, the incidence of coeliac disease was not associated with more severe coeliac disease. Whether the observed association between incidence rate of coeliac disease and affluence is a reflection of the variation in environmental exposures to aetiological factors or could be accounted for by differences in uptake and utilisation of health services it is unclear but further work to unravel the contribution of social class to the aetiology and incidence of coeliac disease is required.

The female predominance of adult coeliac disease observed in our study is well described where in general twice as many women as men are diagnosed with coeliac disease [172, 173, 399]. This may be a reflection of female preponderance for abnormal autoimmune function with enhanced immune reactivity due to female sex hormones or some other factor [400-403]. With the incidence of coeliac disease independently associated with presence of iron deficiency anaemia in female coeliacs and particularly within the younger age bands, one could speculate challenges of menstruation and pregnancy may act to accentuate anaemia and exacerbate any effects of coeliac disease in women. Women are also more likely to access and attend health checks than men in these childbearing years such as for antenatal care and contraception-related routine health reviews where the opportunity for detecting anaemia is very much greater. The health-seeking behaviour of women relative to men with differential symptom reporting may also contribute to the apparent gender difference in clinically diagnosed coeliac disease [399].

3.3.4.4. Summary

The estimated incidence of adult coeliac disease in Nottingham and Sheffield in the period 2000 – 2006 was 0.115 per 1000 population. The incident rate of coeliac disease is strongly associated with socio-economic status with the rate twice as high in adults from affluent areas compared with that in adults living in poorer areas. Socioeconomic status was not associated with features of more severe coeliac disease though could be a reflection of the variation in environmental exposures to aetiological factors or could be accounted for by differences in uptake and utilisation of health services. The associations of incident rates of coeliac disease with female sex and with iron deficiency anaemia with the effect most marked in the younger

adult years may be explained by the physiological challenges of menstruation and pregnancy acting to accentuate anaemia and exacerbate any effects of coeliac disease in women or due to gender differences in attending health checks, access to healthcare and health-seeking behaviour.

3.4. Cholesterol profile in people with newly diagnosed coeliac disease: a comparison with the general population and changes following treatment

3.4.1. Introduction

Recent studies have suggested that untreated coeliac disease is associated with a low serum cholesterol. Ciacci et al observed 0.7 mmol/L lower total cholesterol in 10 adults with coeliac disease presenting with hypochromic anaemia in comparison to those with hypochromic anaemia due to other causes [272] while West et al found that total cholesterol in people with undetected coeliac disease as assessed by EMA positivity was 0.5 mmol/L lower in comparison to EMA negative general population controls [170]. While these reductions might seem modest it has been calculated that a reduction in total cholesterol of 0.6 mmol/L will result in 25 – 30% reduction in the risk of mortality from ischaemic heart disease in people aged 55 – 64 years [252].

Nevertheless, patients with coeliac disease do not appear to have a decreased risk of ischaemic heart disease or stroke, despite an apparently favourable vascular risk profile such as the low cholesterol, lower levels of hypertension [269] and lower prevalence of smoking [266, 267] observed in people with coeliac disease. Indeed, the evidence on vascular outcomes appears to be conflicting with some studies [270, 404] suggesting an increased risk of ischaemic stroke and heart disease while others no increase in risk [269]. Ludvigsson et al observed a 27% (HR 1.27; 95% CI 1.09 – 1.48) increased risk of myocardial infarction and 35% increased risk of ischaemic stroke (HR 1.35; 95% CI 1.14 – 1.60) in coeliacs using the Swedish in-patient registry whereas West et al observed an adjusted HR 1.90 (95% CI 1.00 – 3.60) in an

endomysial antibody positive cohort versus an antibody negative cohort [170, 270]. In contrast the hazard ratio for myocardial infarction was 0.85 (95% CI 0.63 – 1.13) and for stroke 1.29 (95% CI 0.98 – 1.70) in the study by West et al [269].

Concern has also been raised as to whether treatment of coeliac disease may have an adverse effect on serum cholesterol. For example, Brar et al worryingly observed a 0.5 mmol/L increase in serum cholesterol following treatment of incident coeliac disease [405] though the study is based on a retrospective identification of patients that had happened to have cholesterol profile measured before coeliac disease was diagnosed so was limited by its non-systematic assessment of cholesterol profile. We have therefore prospectively examined cholesterol profiles at diagnosis and after one year of a gluten-free diet in a contemporary cohort of newly diagnosed coeliac disease patients.

3.4.2. Methods

3.4.2.1. Study design

Historical cohort study.

3.4.2.2. Study population

Adults with incident coeliac disease diagnosed at Royal Hallamshire Hospital (Sheffield historical cohort) were studied. Dietetic, histopathology, immunology, clinical coding and outpatient records were used to retrospectively identify incident cases of coeliac disease as described in section 2.2.2.

3.4.2.3. Outcome measures

- Proportion of adults with raised total cholesterol at diagnosis of coeliac disease
- Change in the mean value between diagnosis and following 12 months treatment with a gluten-free diet the value of lipid profile (total cholesterol, HDL cholesterol, total : HDL cholesterol ratio, triglycerides) in adults with coeliac disease

3.4.2.4. Outcome ascertainment

Serum total cholesterol (mmol/L), HDL cholesterol (mmol/L), total cholesterol: HDL cholesterol ratio, triglyceride (mmol/L), ferritin (μg/L), folate (μg/L), ESR (mm/hr), CRP (mg/L), glucose (mmol/L), leucocyte count (10^9/L), albumin (g/L), platelet count (10^9/L), haemoglobin (g/L) were routinely measured on newly diagnosed adult cases of coeliac disease attending Royal Hallamshire Hospital between 1st January 2004 and 31st December 2006 at both diagnosis of coeliac disease and in the majority following approximately 12 months treatment with a gluten-free diet. Demographic, clinical, histological and serological data in addition to these laboratory co-variates was systematically collected, as described in section 2.2.2. The modes of presentation of coeliac disease and coeliac serological status at diagnosis of coeliac disease was determined and defined as described in section 2.4.

3.4.2.5. Statistical analysis

We examined the association between baseline HDL cholesterol and other continuous baseline variables using Spearman's rank correlation coefficients. We examined the association between baseline HDL cholesterol with each of sex, age group and symptoms using student's unpaired t-tests. Paired t-tests were used to examine changes in blood variables from baseline to 12 months following a gluten-free diet.

We explored univariate associations between baseline characteristics and change in HDL cholesterol using correlation coefficients for continuous variables and student's t-tests for categorical variables. Finally, in order to examine the potential effect of a change in the severity of coeliac disease we modelled the change in tTG against change in HDL cholesterol using multiple linear regression. We included any factors that were found to be associated in the univariate model as well as adjusting for *a priori* confounders of age and sex and, to account for potential regression to the mean, for baseline HDL cholesterol measurements.

Finally, we carried out a comparison of baseline cholesterol with the findings from the Health Survey for England 2006 (Cardiovascular disease and risk factors in adults) [347], which is representative of the whole population at both national and regional level. In this survey, non-fasting blood samples were collected from a representative sample of the general population (70% of sampled population provided valid blood samples) with total cholesterol measurements obtained on 3618 men and 3850 women. For our comparisons we firstly age-adjusted the mean cholesterol values in our coeliac cohort compared with those in the survey. Secondly we calculated a ratio of observed number of people with total cholesterol greater than 5.0 mmol/L versus expected, standardised for age. We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed using Stata SE 9.2 [TexCorp].

3.4.2.6. Ethical approval

North Staffordshire Local Research Ethics Committee gave ethical approval (reference 06/Q2604/91) to this study in October 2006. Royal Hallamshire Hospital gave research and development approval (reference STH14597) in March 2007.

3.4.3. Results

3.4.3.1. Demography of cohort

We identified 100 people with incident coeliac disease between 2004 – 2006. There were nearly twice as many female (n = 65) as male (n = 35) incident cases in the study cohort. The mean age at diagnosis was 50.6 (SD 15.8) and was similar in men and women (p = 0.45). The cohort was 96% Caucasian compared with 89% for Sheffield as a whole [378]).

3.4.3.2. Presenting features

Iron deficiency anaemia and diarrhoea were the most common presenting features, affecting 30% of the cohort respectively. 28 % had partial villous atrophy; 36% had subtotal villous atrophy and 23% had total villous atrophy. At diagnosis, the median tTG was 194 (range 2 – 300) and 86% (n = 86) were EMA positive. The median weight at diagnosis of coeliac disease was 58 (IQR 50, 67) kilograms in women (n = 39) and 73 (IQR 64, 79) kilograms in men (n = 17). The mean haemoglobin was 12.4 (SD 2.0) g/L in women and 14.0 (SD 1.7) g/L in men at diagnosis of coeliac disease. 49 (49%) of the study cohort had ferritin values below 22 μg/L, the lowest limit of the normal range, whereas 14 (14%) had B12 values below 211 ng/L. 17 (17%) of the study cohort had folate values below 3.4 μg/L, the lowest limit of the normal range of values.

3.4.3.3. Lipid profile at diagnosis of coeliac disease

The mean total cholesterol and mean triglyceride at diagnosis of coeliac disease was 4.84 mmol/L (SD 1.2) and 1.24 (SD 0.8) mmol/L respectively (Table 3.25). Mean HDL cholesterol was 1.36 (SD 0.48) mmol/L; the ratio of total cholesterol to HDL

cholesterol was 4.00 (SD 2.4)). HDL cholesterol at diagnosis of coeliac disease was associated with weight and sex but no other variables.

Table 3.25: Change in lipid profile with gluten-free diet

	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]	p-value
Mean cholesterol mmol/L	100	4.84 (1.18)	4.82 (1.23)	-0.013 [-0.16, 0.14]	0.87
Mean HDL cholesterol mmol/L	92	1.36 (0.48)	1.48 (0.58)	0.12 [0.05, 0.18]	0.0005
Mean total: HDL cholesterol	92	4.00 (2.36)	3.71 (1.82)	-0.28 [-0.09, -0.47]	0.0035
Mean triglyceride mmol/L	95	1.24 (0.78)	1.20 (0.70)	-0.04 [-0.15, 0.07]	0.48
Mean ferritin µg/L	89	71.55 (13.80)	84.21 (18.08)	12.66 [-7.60, 32.92]	0.22
Mean folate µg/L	93	8.99 (0.68)	15.12 (3.98)	6.14 [-1.57, 13.85]	0.12
Mean ESR mm/hr	89	13.40 (1.3)	11.92 (1.14)	-1.48 [-3.23, 0.27]	0.10
Mean CRP mg/L	94	4.35 (0.41)	4.17 (0.55)	-0.18 [-1.21, 0.86]	0.73
Mean glucose mmol/L	89	5.85 (0.27)	5.69 (0.21)	-0.15 [-0.61, 0.31]	0.51
Mean leucocyte count 10^9/L	96	7.20 (0.25)	7.18 (0.23)	-0.02 [-0.37 , 0.42]	0.90
Mean albumin g/L	98	39.51 (0.40)	40.12 (0.38)	0.61 [-1.36, 0.14]	0.11
Mean platelet count 10^9/L	96	307.78 (8.91)	303.15 (9.23)	4.64 [-19.91, 10.64]	0.55

	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]	p-value
Mean haemoglobin g/L	96	12.99 (0.21)	13.64 (0.15)	0.65 [0.29, 1.02]	0.0006
Mean tTG iu (SD)	83	172.2 (123.0)	71.4 (104.7)	-100.84 [-72.63, -129.06]	0.00001

Both men and women with incident coeliac disease had lower age-adjusted mean total cholesterol in comparison to the general population (difference in mean adjusted total cholesterol -1.09 [95% CI -0.97, -1.21] mmol/L; -0.46 [95% CI -0.24, -0.68] mmol/L, respectively). At diagnosis of coeliac disease, men had 21% lower and women had 9% lower mean total cholesterol in comparison to the general population. When we carried out age standardisation (Table 3.26), we found that men with incident coeliac disease were 60% less likely (ratio of observed versus expected 0.40 [95% CI 0.12, 0.68]) to have a total cholesterol of >=5.0 mmol/L when compared with the general population. In women the effect was smaller and not statistically significant (ratio of observed versus expected 0.91 [95% CI 0.61, 1.20]).

Table 3.26: Total cholesterol profile in coeliac disease in comparison to the general population

	Mean cholesterol age- adjusted for study population (mmol/l) [sd]	Reference population mean cholesterol (mmol/l) [sd]	Difference in mean total cholesterol (mmol/L) [95%CI for difference]	Observed number of patients with raised cholesterol*	Expected number of patients with raised cholesterol*	Observed : Expected [95% CI]
Men	4.21 [0.73]	5.30 [1.20]	-1.09 [-0.97, -1.21]	8	19.95	0.40 [0.12, 0.68]
Women	4.94 [1.77]	5.40 [1.24]	-0.46 [-0.24, -0.68]	36	39.65	0.91 [0.61, 1.20]

* Raised cholesterol 5.0 mmol/L or greater

3.4.3.4. Change in weight and haematinic profile with gluten-free diet

The mean values of haemoglobin, mean cell volume and B12 significantly increased with exposure to a gluten-free diet (Table 3.25). There were no substantial changes in folate, ferritin, albumin, ESR, CRP, leucocyte count or platelet counts. There was a 4.22 kilogram increase in mean weight [95% CI 2.90, 5.54] from the baseline mean weight of 66.51 (SD 4.02) kilograms.

3.4.3.5. Change in lipid profile following treatment with gluten-free diet

Follow-up lipid values were obtained after a mean of 12.6 (SD 1.9) months on a gluten-free diet (Table 3.25). There was no change in mean cholesterol or triglyceride level with treatment of incident cases of coeliac disease. However, there was a small and statistically significant increase of 0.12 mmol/L [95% CI 0.05, 0.18] in the mean value of HDL cholesterol with exposure to a gluten-free diet. Furthermore the total cholesterol: HDL cholesterol ratio was reduced by 0.28 [95% CI -0.09, -0.47].

On univariate analyses the changes in HDL cholesterol observed did not vary with demographic variables such as age at diagnosis nor gender. Features suggestive of malabsorption (such as by stratifying the cohort into those having diarrhoea and or weight loss; height, weight, albumin, haemoglobin), systemic inflammation (such as ferritin, ESR, white cell count, platelet count) or more severe coeliac disease (such as by stratifying the cohort into those with subtotal or total villous atrophy, those with higher tTG) were not associated with change in HDL cholesterol.

However when we examined change in tTG, a proxy marker often used in clinical practice to reflect response to treatment in coeliac disease, this was weakly associated

with change in HDL cholesterol on univariate analysis (p = 0.06). After adjusting for age at diagnosis, gender, baseline HDL cholesterol and baseline tTG, change in tTG was independently associated with change in HDL cholesterol (p = 0.03) on treatment of incident coeliac disease with a gluten-free diet on multivariate analyses. Thus for each 50 unit decrease in tTG that occurred on treatment of coeliac disease there was a 0.03 mmol/L increase in HDL cholesterol.

3.4.4. Discussion

3.4.4.1. Principal findings

Our study shows that at diagnosis coeliacs have much lower total cholesterol levels than the general population with the observed reduction greater in men (21%) than in women (9%). In addition to the changes in weight and haemoglobin profile expected when diagnosing and treating people with coeliac disease, our study reassuringly observed no increase in total cholesterol on treatment with a gluten-free diet. Furthermore, HDL cholesterol showed a small but statistically significant increase following treatment. These findings indicate that any increase in risk of ischaemic heart disease or stroke in people with coeliac disease is not due to increases in total cholesterol induced by the diet. On the contrary the lower total cholesterol levels and increases in HDL cholesterol on treatment should afford people with coeliac disease relative protection against ischaemic heart disease and stroke.

3.4.4.2. Merits and limitations

This is the first prospective cohort study where there has been systematic collection of lipid profile in a large and unselected sample of cases of incident coeliac disease. Since serum total cholesterol and HDL cholesterol can both be measured accurately

on a random non-fasting sample [406-408], fasting status or timing of blood collection is unlikely to have had an effect on the values obtained. In the absence of malabsorptive processes and systemic inflammation one would not expect HDL cholesterol to increase as substantially as we have observed simply during a 12 month period. For example, exposure to four years of the Mediterranean dietary pattern did not cause any change in serum levels of total cholesterol nor HDL cholesterol suggesting that change in dietary patterns alone was not responsible for the change in HDL cholesterol observed in our study [409]. Clearly though, if the gluten free diet is markedly different with respect to its effect on cholesterol profile then this could be a potential explanation for our observations but there is no evidence that this is true. Neither the effects of body mass index nor exercise have been shown to alter cholesterol profile substantially over such a short time period [410].

3.4.4.3. Comparison with other studies

Our observation that people with newly diagnosed coeliac disease have a lower total cholesterol compared with the general population is new though it was suggested in the only comparable study of 10 patients, presenting with hypochromic anaemia where no general population comparison was made [272]. The reductions in total cholesterol we have found in diagnosed coeliacs were somewhat greater than those reported by West et al who found total cholesterol levels were 10% lower in endomysial antibody positive people in comparison to endomysial antibody negative general population controls [170].

The finding of no increase in total cholesterol following treatment with a gluten-free diet is in contrast to the only other study to have examined cholesterol profile before

and after treatment. Brar et al observed a 0.5 mmol/L (11%) increase in total cholesterol [405] despite similar proportions of men and women in the cohort, and similar proportions of partial to total villous atrophy present on duodenal biopsy at diagnosis. This is probably a reflection of differences in the study populations. As only a small proportion of their study population (132 out of 700) had a cholesterol measurement there are likely to have been specific reasons for doing so. Conversely, our study was based on all newly diagnosed patients over a 2 year period and is likely to be more representative of celiac disease in general.

Intestinal malabsorption, reduced cholesterogenesis, increased biliary secretion, and or high faecal elimination of cholesterol have all been proposed as mechanisms which might lower total cholesterol in people newly diagnosed with coeliac disease in comparison to the general population [411-413]. However the lack of increase in total cholesterol with treatment of coeliac disease suggests that any mechanism based on intestinal malabsorption is less likely. Conversely there has been no reported increase in risk of cholesterol gallstones or cholecystectomy in people with coeliac disease to support increased biliary secretion as the mechanism involved.

An inverse association has been reported between proxy markers of vascular inflammation (such as CRP, ESR) and HDL cholesterol [39, 274, 414]. HDL cholesterol is regarded as a potent anti-atherogenic mediator having a wide range of anti-oxidative, anti-thrombotic and anti-inflammatory effects [275]. Indeed HDL cholesterol increasing compounds have been shown to attenuate systemic inflammation , vessel wall inflammation as well as reducing risk of ischaemic heart disease events [275]. Anti-inflammatory treatment of active rheumatoid arthritis, an

autoimmune and chronic inflammatory disorder like coeliac disease, has been observed with a reduction in median ESR levels by 36% (23 mm/hour) and an increase in median HDL cholesterol by 9% (0.10 mmol/L) over a 12 month period [414]. With this in mind, perhaps the increase in HDL cholesterol we have observed on treatment of active coeliac disease a proxy marker for reduction in intestinal and or systemic inflammation? We did not however observe any particularly strong associations between the inflammatory markers we measured and cholesterol profile.

3.4.4.4. Summary

People with coeliac disease have lower total cholesterol levels than the general population, with the reduction greater in men (21%) than in women (9%). While we observed no increase in total cholesterol following treatment with a gluten-free diet, there was a small but significant increase in HDL cholesterol. It is unclear why despite an apparently favourable cardiovascular risk profile including also the lower prevalence of smoking and hypertension in people with coeliac disease, coeliac disease has not consistently been shown to be associated with a reduced risk of ischaemic heart disease. This paradox remains unresolved. However, with the calculation that a reduction in total cholesterol of 0.6 mmol/L will result in 25 – 30% reduction in the risk of mortality from ischaemic heart disease, any increase in risk of ischaemic heart disease or stroke in people with coeliac disease is unlikely due to an adverse cholesterol profile either before diagnosis or after treatment with a gluten free diet.

A copy of the published peer-reviewed manuscript may be found in section 9.3.

Chapter four: Longitudinal change in vascular risk profile and quality of life on treating incident coeliac disease with a gluten-free diet

In an unselected and large sample of adults newly diagnosed with coeliac disease at Nottingham, Sheffield and Derby hospitals a longitudinal prospective study was performed with the aim to:

- estimate the vascular risk profile at diagnosis of coeliac disease and quantify any change following treatment with a gluten-free diet
- estimate the quality of life at diagnosis of coeliac disease and observe any change following exposure to a gluten-free diet

4.1. Vascular risk profile at diagnosis of coeliac disease and changes on exposure to a gluten-free diet

4.1.1. Introduction

Vascular disease is the commonest cause of mortality in the developed world, accounting for 40% of all deaths in the general population [415]. As alluded to in section 1.7.5.1., established high-risk demographic factors for vascular disease [241-244] are:

- advancing age (relative risk 8 for 60 versus 40 year olds)
- male sex (relative risk 2 – 5 men versus women)
- lower socioeconomic status (relative risk 3 for social class V versus social class I)
- immigrants born in the Indian subcontinent (standardised mortality ratio 146 (95% CI 136, 156) relative to England and Wales standard rate)

There are a number of modifiable aetiological factors associated with vascular disease as discussed in section 1.7.5.1. including:

- Cigarette smoking (nearly two-fold increased risk of vascular disease with the risk increasing with the number of cigarettes smoked [245])
- Increasing systolic and diastolic blood pressure (sustained reduction of 5 mmHg blood pressure over a 5-year period reduced coronary artery events by 25% and strokes by 30% in patients with ischaemic heart disease in five years [248])
- Obesity (standardised mortality ratio for death from vascular disease 136 of BMI >/27 kg/m^2 versus <22.4 kg/m^2 [249])
- Central obesity (increased risk of vascular disease in comparison to those of similar BMI but with peripheral adiposity [250])

- Raised waist: hip circumference ratio (waist: hip ratio >/ 0.91 was associated with nearly a threefold increased risk of coronary artery disease events compared to <0.91 in men during 10 year follow-up [251])
- Raised waist circumference (> 95 centimetres associated with a twofold increased risk of coronary artery disease events compared to <83.5 cm in men during mean 10 year follow-up [251])
- Raised total cholesterol (reduction in total cholesterol of 0.6 mmol/L associated with 25 – 30% reduction in the risk of mortality from ischaemic heart disease in people aged 55 – 64 years [252])
- Raised LDL cholesterol (reduction in LDL cholesterol by 1.3 mmol/L resulted in reduction in risk of non-fatal acute coronary syndrome or death from vascular disease by a third in middle-aged men with hypercholesterolemia and no history of myocardial infarction [254])
- Low HDL cholesterol (rise in HDL cholesterol by 0.02 mmol/L results in a 3% reduced risk of vascular disease [255, 256])
- Elevated triglycerides [257, 258]
- Raised fibrinogen (risk ratio for vascular disease 1.8 for 0.35 g/L levels versus 0.25 g/L fibrinogen [259])
- Elevated CRP (odds ratio 2.13 for 2.4 v 0.9 mg/L CRP [260])
- Diabetes mellitus (relative risk in female diabetics to those without diabetes for vascular disease events [261])

Vascular disease is also the most common cause of mortality in coeliac disease [262]. However, recent studies have found some evidence of decreased cardiovascular morbidity suggesting that people with coeliac disease may have some favourable

features to their vascular risk profile. We have observed adults with incident coeliac disease have lower total cholesterol levels than the general population which did not change following treatment with a gluten-free diet (study 3.4.). Potentially beneficial increases in HDL cholesterol however were observed following treatment (study 3.4.). As alluded to in section 1.7.5.2. coeliac disease appears to be associated with non-smoking although it is unclear whether this is a causal association [266-268]. In a cross-sectional population screening study people with positive endomysial antibodies had 2.4 mmHg ($p < 0.05$) lower diastolic blood pressure in comparison to negative controls [170] and those with treated coeliac disease are reported to be less likely to have a diagnosis of hypertension (odds ratio 0.68 (95% CI 0.60 – 0.76) and a lower reported antihypertensive medication use in comparison to age- and sex-matched general population controls [269].

Despite this apparent favourable cardiovascular risk profile, population-based studies have not observed coeliac disease having a protective effect upon cardiovascular disease events [269] with even increased risks observed [270]. Reasons for the observed lack of protection against cardiovascular disease events in diagnosed coeliac disease are unclear but one could speculate (persistent) systemic inflammation driven by coeliac disease perpetuates the inflammatory atherosclerotic lesion.

The aim of this longitudinal study is to describe the vascular risk profile in adults newly diagnosed with coeliac disease and to observe any change in the profile following treatment with a gluten-free diet.

4.1.2. Methods

4.1.2.1. Study design

Longitudinal observational study.

4.1.2.2. Study population

Data was collected on the vascular risk profile in adults with incident coeliac disease and following their treatment with a gluten-free diet that had attended Nottingham University Hospital, Nottingham; Royal Hallamshire Hospital, Sheffield; or Derby Hospitals NHS Foundation Trust for management of their coeliac disease. Consecutive cases of incident coeliac disease were identified at Nottingham using clinical alerts and records; dietetic alerts and records; and pathology databases as described in sections 2.2.1.4. and 2.2.1.6. At the Royal Hallamshire Hospital, consecutive incident cases of coeliac disease were identified using clinical and dietetic records as well as pathology and immunology databases as described in section 2.2.2.5. Clinical and dietetic records were used to identify incident coeliacs in Derby as described in section 2.2.3. Extensive efforts were made to identify all incident adults with coeliac disease at these three centres in order that they could be invited to participate in this study.

4.1.2.2.1. Classification of coeliac disease within study population

In view of comparisons being made with respect to the quality of life between different 'glaciers' of the coeliac iceberg in study 4.2., the vascular risk profile was also similarly compared between coeliacs presenting with classic disease, gastrointestinal symptoms and those with silent disease as defined below:

Definition 30: Classic symptoms
Those coeliacs presenting with weight loss and diarrhoea.

Definition 31: Gastrointestinal symptoms
Those coeliacs presenting with gastrointestinal symptoms including diarrhoea but in absence of weight loss (would be included as having 'classic symptoms'), weight loss in the absence of diarrhoea (would be included as having 'classic symptoms'), constipation, IBS syndrome, nausea, bloating, steatorrhoea, acid reflux, heartburn, vomiting, abdominal pain.

Definition 32: Silent coeliac disease
Those coeliacs presenting with no gastrointestinal symptoms; or physiological derangements such as anaemia, osteoporosis, deranged liver chemistries in the absence of gastrointestinal symptoms.

4.1.2.3. Inclusion criteria

Adults with incident coeliac disease.

4.1.2.4. Exclusion criteria

Significant co-morbidity that would prevent the study participants being well enough to take part.

4.1.2.5. Outcome measures

- Proportion of people at diagnosis of coeliac disease that smoke (current, ex- and never) and were ever diagnosed with hypertension, hypercholesterolaemia, hypertriglyceridaemia, diabetes mellitus, coronary artery disease, atrial fibrillation, peripheral vascular disease and or stroke compared to the general population.
- Total cholesterol, HDL cholesterol, total cholesterol: HDL cholesterol ratio, triglycerides, fibrinogen, CRP, HbA1C, weight, systolic and diastolic blood pressure, body mass index, waist circumference, waist: hip circumference at diagnosis of coeliac disease (all coeliacs, coeliacs with classic symptoms, coeliacs with gastrointestinal symptoms, coeliacs with silent disease) and in comparison to the general population
- Proportion of people at diagnosis of coeliac disease that are obese (BMI 30 kg/m^2 or more), raised waist circumference, raised waist: hip circumference ratio compared to the general population
- Change in the mean value between diagnosis of coeliac disease and following 12 months treatment with a gluten-free diet the value of weight, body mass index, pulse rate, blood pressure, waist circumference, waist: hip circumference ratio, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, ESR, CRP, folate, ferritin, HbA1C, glucose, leucocyte count, albumin, platelet count, haemoglobin, haematocrit, fibrinogen, APTT, PT.

Below are descriptions of how I have defined the outcome measures used within this study.

Definition 33: Body mass index (BMI)
Refers to and calculated by determining the ratio of the weight in kilograms divided by the square of the height in metres. Study participants were classified into the following BMI groups according to the WHO and National Institute for Health and Clinical Excellence (NICE) BMI classification [416]: underweight (BMI less than18.5 kg/m^2); normal (BMI 18.5 to less than 25 kg/m^2); overweight (BMI 25 to less than 30 kg/m^2); obese (BMI 30 kg/m^2 or more); morbidly obese (BMI 40 kg/m^2 or more).

Definition 34: Raised waist circumference
Waist circumference that is greater than 102 centimetres in men and greater than 88 centimetres in women in accordance with the definition of abdominal obesity used by NICE [416].

Definition 35: Raised waist: hip circumference ratio
Ratio of waist circumference to hip circumference more than 0.85 in women and more than 0.95 in men in accordance with the definition of abdominal obesity used by NICE [416].

Definition 36: Smoker
Current smoker referred to a person who was currently smoking cigarettes or has stopped smoking in the 12 months preceding date of diagnosis of coeliac disease. An ex-smoker referred to someone who has smoked in the past and has last smoked more than 12 months preceding the date of diagnosis of coeliac disease.

Definition 37: Hypertension
Presence of consistently elevated blood pressure above 140 / 90 mmHg (130 / 80 mmHg in people with diabetes mellitus). Further corroborated by report of "hypertension" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular anti-hypertensive medication also further supported the diagnosis.

Definition 38: Hypercholesterolaemia
Presence of serum total cholesterol above the highest limit of normal range for the hospital laboratory concerned. Further corroborated by report "hypercholesterolaemia" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular statins also further supports the diagnosis.

Definition 39: Hypertriglyceridaemia
Presence of serum triglycerides above the highest limit of normal range for the hospital laboratory concerned. Further corroborated by report "hypertriglyceridaemia" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular fibrates also further supported the diagnosis.

Definition 40: Diabetes mellitus
Presence of fasting venous blood glucose more than 6.1 mmol/L corroborated by report by study participants that they currently had diabetes and or whether the study participants had been told by a doctor that they had diabetes. Further corroborated by report "diabetes mellitus" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular anti-diabetic medication also further supported the diagnosis. For classification purposes, type 1 diabetes was defined as those study participants on insulin therapy alone (no oral anti-diabetic medication) and further corroborated with report of "type 1 diabetes mellitus" or "insulin-dependent diabetes mellitus" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Type 2 diabetes was defined as those study participants taking oral anti-diabetic medication and further corroborated with report of "type 2 diabetes mellitus" or "non-insulin-dependent diabetes mellitus" or "diet controlled diabetes mellitus" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist.

Definition 41: Coronary artery disease
Refers to atherosclerotic disease of the coronary arteries resulting in clinical syndromes of myocardial infarction and angina. Supported by confirmatory cardiac investigations (such as cardiac angiography reports, exercise tolerance tests, serum cardiac enzymes results, stress echocardiography) and or interventions to help treat the condition (thrombolysis, angioplasty, coronary artery bypass grafting). Suggested by report of study participant suffering from angina and or heart attack and the study participants reporting diagnosis had been confirmed by a doctor. Corroborated by report "myocardial infarction" and or "angina pectoris" and or "ischaemic heart disease" and or "coronary artery disease" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular anti-anginal and anti-platelet medication also further supported the diagnosis.

Definition 42: Atrial fibrillation
Refers to the presence of irregularly irregular pulse confirmed electrocardiographically with absence of P waves. Suggested by report of study participant suffering from an irregular heart beat and the study participants reporting diagnosis had been confirmed by a doctor. Corroborated by report "atrial fibrillation" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular anti-arrhythmic and anti-thrombotic medication also further supported the diagnosis.

Definition 43: Peripheral vascular disease
Refers to atherosclerotic disease of the arteries resulting in clinical syndromes of intermittent claudication, ischaemic arterial limb ulcers and aortic aneurysm. Supported by confirmatory investigations (such as angiography reports, radiological imaging demonstrating aneurysmal dilatation, Doppler flow studies) and or interventions to help treat the condition (such as angioplasty, vascular bypass). Suggested by report of study participant suffering from claudication, gangrene, arterial ulcers and or aortic aneurysm with the study participants reporting a doctor had confirmed the diagnosis. Corroborated by report "claudication" and or "aortic aneurysm" and or "peripheral vascular disease" and or "gangrene" and or "arterial ulcers" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular anti-platelet medication also further supported the diagnosis.

Definition 44: Stroke
Refers to atherosclerotic disease of the cerebral arteries resulting in clinical syndromes of acute stroke, chronic stroke disease and transient ischaemic attack. Supported by confirmatory cerebral investigations (such as computed tomography appearances of brain). Suggested by report of study participant suffering from mini-strokes, strokes and the study participants reporting diagnosis had been confirmed by a doctor. Corroborated by report "stroke" and or "transient ischaemic attack" and or "cerebrovascular disease" in referring letter of the general practitioner or referring doctor; and or in the replying letter from the gastroenterologist. Person receiving regular anti-anginal and anti-platelet medication also further supported the diagnosis.

Definition 45: Any cardiovascular condition
Study participants were classified as having any cardiovascular condition if they reported ever having any of the following conditions (as defined above): angina pectoris, myocardial infarction, stroke, atrial fibrillation.

4.1.2.6. Outcome ascertainment

4.1.2.6.1. Anthropometry measurements

All measurements were done while the study participants were without shoes, lightly clothed and had no restrictive underwear [417]. All measurements were done by the same study investigator (NRL). Measurements were taken at the end of respiration while the participant was standing erect, with the arms at the side and the feet together [417]. All hip and waist circumference measurements were taken using the same inelastic tape (SECA circumference measuring tape SE200ST) without compressing the skin and were recorded to the nearest 0.1 cm.

Waist circumference was measured around the smallest circumference between the lowest rib and the iliac crest, or for obese subjects with no natural waist midway between the lowest rib and iliac crest.

Hip circumference was measured horizontally at the level of the greatest lateral extension of the hips.

Weight was measured to the nearest 100g using the same set of digital scales (SECA electronic scale SE888/4).

Height was measured to the nearest 0.1cm using the same free-standing stadiometer (SECA Leicester portable height measure SE001).

4.1.2.6.2. Vital sign measurements

All measurements were done by the same study investigator (NRL).

Blood pressure measurements were performed using an A&D UA-774 non-invasive oscillometric monitor (A&D Instruments, UK). Study participants were seated in a chair with their backs supported and their right arm bared at the level of the heart. After 5 minutes of rest, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with at least a 2 minute interval between measurements. The averages of the 2 readings for both SBP and DBP were used.

Heart rate measurements were performed using an A&D UA-774 non-invasive oscillometric monitor (A&D Instruments, UK). Study participants were seated in a chair with their backs supported and their right arm bared at the level of the heart. After 5 minutes of rest, heart rate was measured twice with at least a 2 minute interval between measurements.

4.1.2.6.3. Serum and plasma measurements

Plasma and serum samples were obtained from blood taken by venepuncture. A Coulter MD18 haematology analyser was used for measurement of full blood counts. Serum ferritin, vitamin B12 and red cell folate were analysed using radioimmunoassay. CRP was measured using ELISA (Kalon Biological, Hants, UK). Serum albumin was determined using standard automated methods. Serum total cholesterol, high density lipoprotein cholesterol and triglyceride concentrations were be measured by colorimetry (RA 1000, Bayer Diagnostics, Basingstoke) and low density lipoprotein cholesterol concentrations calculated by the Friedewald formula.

Glycated haemoglobin assays (HbA1C) used a Biorad Diomat high pressure liquid chromatography analyser.

4.1.2.6.4. Smoking history and vascular disease diagnoses

Smoking history and previous diagnoses of vascular disease were obtained by review of the medical notes and medication history as well as interviewing the study participant by the same investigator (NRL) using a standard questionnaire. The questionnaire examined the previous diagnoses of vascular disease, current medication and smoking histories as well as collected data on proxy markers of socioeconomic status including occupational history, educational and vocational training. The questionnaire used may be found in Appendix 7.2.

4.1.2.7. Assessment of compliance with gluten-free diet

Compliance with the gluten-free diet was assessed clinically and immunologically.

Study participants were asked to rate their compliance with the gluten-free diet using a visual analogue scale (see Appendix 7.3). The visual analogue scale is a 100 mm horizontal line with the two anchors of 'no compliance to diet' and 'strict compliance with diet' [418]. The study participants were asked to mark the scale with a single vertical mark through the 100 mm horizontal visual analogue scale to illustrate their compliance to the gluten-free diet.

Study participants compliance with the gluten-free diet was also assessed by the reviewing dietician and described as 'optimal' (no dietary transgressions), 'fair' (one or two dietary transgressions since last dietary review) or 'poor' (more than two dietary transgressions since last dietary review).

Antiendomysial antibody (EMA) and quantitative titres of human recombinant tissue transglutaminase (tTG) were used as laboratory measures to assess compliance to gluten-free diet [419, 420].

4.1.2.8. Controls

Data from the Health Survey for England 2006 (Cardiovascular disease and risk factors in adults) [347], which is representative of the whole population at both national and regional level, was used as a general population comparator for serum proxy markers of vascular disease (such as total cholesterol) and possession of vascular disease diagnoses. In this survey, a general population sample of 14,400 addresses were randomly selected from the Postcode Address File using a multi-stage sample design to help ensure that households were sampled proportionately across the nine Government Office regions of England. 720 postcode sectors were selected, and 20 addresses selected within each sector between January 2006 – December 2006. Where an address was found to have multiple dwelling units, one was selected at random. Where there were multiple households at a dwelling unit, up to three households were included and if there were more than three then a random selection was made. Each individual within a selected household was eligible for inclusion; at each address, all households, and all persons in them, were eligible for inclusion in the survey. A nurse visit was arranged for all participants who consented. In this survey, interviews were held with 14,142 adults aged 16 and over in 8614 households from the general population. Response to the survey at a household level was 68% of sampled eligible households in the general population. At an individual level, interviews were obtained with 88% of adults within the general population sample.

Adults were asked modules of questions including general health, cardiovascular disease (including the Rose Angina Questionnaire), physical activity, alcohol consumption, smoking, and fruit and vegetable consumption.

Approximately 70% of adults allowed for their height, weight, waist circumference and blood pressure to be measured for the study. Height was measured using a portable stadiometer with a sliding head plate, a base plate and three connecting rods marked with a metric measuring scale. Survey participants were asked to remove shoes. One measurement was taken, with the informant stretching to the maximum height and the head positioned in the Frankfort plane. The reading was recorded to the nearest millimetre. Weight was measured using Soehnle, Seca and Tanita electronic scales with a digital display. Study participants were asked to remove shoes and any bulky clothing. A single measurement was recorded to the nearest 100g. Those who were pregnant, chairbound, or unsteady on their feet were not weighed. The waist was defined as the midpoint between the lower rib and the upper margin of the iliac crest. It was measured using a tape with an insertion buckle at one end. The measurement was taken twice, using the same tape, and was recorded to the nearest even millimetre. Waist circumference was categorised according to NICE guidelines: for men, less than 94cm was low, 94–102cm was high, and more than 102cm was very high; and for women, less than 80cm was low, 80–88cm was high, and more than 88cm was very high. Three blood pressure readings were taken, at one-minute intervals, using an appropriately sized cuff on the right arm, with the informant in a seated position after five minutes' rest using oscillometric automated device, the Omron HEM 907. Systolic (SDP) and diastolic pressures (DBP) were displayed on the Omron from each measurement. The blood pressure variables used in the survey were the means of the second and third measurements obtained from the informants in whom three readings

were successfully obtained, excluding those who had eaten, drunk alcohol, exercised, or smoked in the 30 minutes before the measurement was taken. Survey participants were classified in one of four groups on the basis of their SBP and DBP readings and their current use of antihypertensive medication:

- Normotensive untreated SBP<140 mmHg and DBP<90 mmHg , not currently taking medication specifically prescribed to treat high blood pressure
- Hypertensive controlled SBP<140 mmHg and DBP<90 mmHg, currently taking medication specifically prescribed to treat their high blood pressure
- Hypertensive uncontrolled SBP>140 mmHg or DBP>90 mmHg, currently taking medication specifically prescribed to treat their high blood pressure
- Hypertensive untreated SBP>140 mmHg or DBP>90 mmHg, not currently taking medication specifically prescribed to treat their high blood pressure

The last three categories together were considered as 'hypertensive' in the survey with the threshold of 140/90 mmHg used in accordance with available guidelines on hypertension management [421].

Non-fasting blood samples and spot urine samples were collected from a representative sample of the general population; 74% of men (n = 5076) and 71% (n = 5418) of women of the sampled population provided valid blood samples.

4.1.2.9. Potential confounders

Age, sex, socio-economic class.

Occupational social class and Index of Multiple Deprivation 2007 (IMD07) score were used as measures of socio-economic class with data collected using a standard questionnaire as described in section 4.1.2.6.4. Social class by current or last known

occupation of the incident coeliac was coded according to the Registrar General's classification [422] and grouped into categories: professional (social class I), managerial (social class II), non-manual skilled (social class IIIN), manual skilled (social class IIIM), manual semi-skilled (social class IV), manual unskilled (social class V) [422]. The postcode of residence at enrolment to the study was used to determine the IMD07 score and rank [376] as described in section 3.3.2.2. Data was also collected on indicators of material condition including formal education attained at school, vocational training, and house ownership as other measures of socioeconomic status [423-425].

4.1.2.10. Statistical analysis

Descriptive analyses with calculation of mean and median where appropriate were performed.

Prevalence of:

- previously diagnosed vascular disease (hypertension, angina, myocardial infarction, stroke, diabetes mellitus);
- smoking (current, never, ex-);
- abnormal anthropometric vascular profile (overweight, obese, raised waist circumference, raised waist: hip circumference, hypertension,);
- abnormal serum vascular profile (raised total cholesterol, low HDL cholesterol, raised LDL cholesterol, raised triglycerides, raised CRP, raised blood glucose, raised glycated haemoglobin)

were determined by calculating the proportion (%) of coeliacs that had been previously diagnosed with vascular disease, were smokers or had an anthropometric or serum value above the upper limit of normal as described in the definitions for the measurement or test concerned.

The mean anthropometric and serum values in adults newly diagnosed with coeliac disease were then compared with the findings from the Health Survey for England 2006. We firstly age-adjusted the mean anthropometric and serum values in our coeliac cohort compared with those in the Health Survey for England with men standardised to the male population and women to the female population. We then performed unpaired t-tests to examine for any difference in the mean anthropometric and serum values, standardised for age and sex, between the coeliac cohort and the Health Survey for England cohort.

Secondly we calculated, standardised for age and sex, the ratio of observed number of coeliacs with:

- hypertension (>/ 140 systolic and or >/ 90 diastolic);
- raised waist circumference (>/ 88 cm in women; >/102 cm in men);
- raised waist: hip circumference ratio (>0.85 in women; >0.95 in men);
- overweight or obese body mass index;
- raised total cholesterol (greater than 5.0 mmol/L)
- low HDL cholesterol (\< 1.0 mmol/L);
- raised LDL cholesterol (>/ 3.0 mmol/L);
- raised triglycerides (>/ 1.0 mmol/L);
- raised CRP (>/ 5.0 mg/L);
- raised HbA1C (greater than 7%);

versus expected number of coeliacs. We calculated the number of outcomes of interest (such as number of coeliacs with hypertension) we would expect in the observed coeliac population if it experienced the same age- and sex-specific rates of the general population comparator, the Health Survey for England. The standard error was calculated by dividing observed: expected ratio by the square root of the observed proportion of coeliacs with the outcome of interest ((O/E)/$\sqrt{O}$) from which the 95% confidence interval could be calculated.

Such standardisations were performed to adjust for the confounding effects of differences in the age and sex population structure between the coeliac and Health Survey for England cohorts, allowing the two populations with different demographic characteristics to be compared directly with each other.

Paired t-tests were used to examine changes in anthropometric, vital signs and blood variables from baseline in incident coeliacs to following 12 months treatment with a gluten-free diet.

Logistic regression modelling was performed to examine the factors associated with selected outcome variables, after adjusting for a priori confounders such as age and sex. For example, regression analyses were performed to examine the association between change in total cholesterol (outcome variable) and predictor variables (such as change in tTG coeliac serology titre, presence of villous atrophy). Forward stepwise models were used with a wide range of possible predictor variables tested in each model with any that were significant included in the final multivariate model. The gradient of the increase or decrease of each outcome was modelled, adjusting for confounders, in the multivariate analyses.

We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed using STATA SE 9.2 [TexCorp].

4.1.2.11. Sample size and power

In study 3.4. we observed incident coeliacs (n = 100) had 15% lower mean total cholesterol in comparison to the general population and following 12 months treatment with a gluten-free diet there was a 9% increase in HDL-cholesterol. If we recruited at least 100 incident coeliacs in our study, we anticipated that we would have in excess of 90% power at the 95% level of significance to observe 10% lower mean total cholesterol at diagnosis of coeliac disease and or a 10% increase in HDL-cholesterol following a gluten-free diet. Our sample size aim was to recruit 150

incident coeliacs in order to have sufficient power to detect any change in the other vascular risk profile co-variates following treatment with a gluten-free diet.

4.1.2.12. Ethical approval

North Staffordshire Local Research Ethics Committee gave ethical approval (reference 06/Q2604/91) to this study in October 2006. Nottingham University Hospital gave research and development approval (reference 06GM012) to this study in January 2007. Derby Hospitals NHS Foundation Trust gave research and development approval (reference DHRD/2007/005) in April 2007. Royal Hallamshire Hospital gave research and development approval (reference STH14597) in March 2007.

4.1.3. Results

4.1.3.1. Study population

Following securing ethical approval and then research approval at each of the centres the first study participant was recruited to the study in March 2007 at Nottingham, April 2007 in Sheffield and May 2007 in Derby. 151 adults newly diagnosed with coeliac disease were recruited to participate in the study following informed consent by July 2008 of whom 50 were from Nottingham, 62 from Derby and 39 were from Sheffield study centres (Figure 4.24).

Figure 4.24: Sample of incident coeliacs recruited to study from all incident coeliacs diagnosed during the study period

Queen's Medical Centre	**Derby Royal Infirmary**	**Royal Hallamshire Hospital**
56 incident adult coeliacs diagnosed at centre during study period	76 incident adult coeliacs diagnosed at centre during study period	74 incident adult coeliacs diagnosed at centre during study period
↓	↓	↓
50 (89%) incident adult coeliacs identified through searches	62 (82%) incident adult coeliacs identified through searches	39 (53%) incident adult coeliacs identified through searches
↓	↓	↓
50 of 50 (100%) coeliacs approached agreed to participate in study following informed consent	62 of 62 (100%) coeliacs approached agreed to participate in study following informed consent	39 of 39 (100%) coeliacs approached agreed to participate in study following informed consent

During this time period, 78 incident adult coeliacs were diagnosed at Nottingham University Hospital: 56 at Queen's Medical Centre and 22 at Nottingham City Hospital. 50 of the 56 (89%) coeliacs diagnosed at Queen's Medical Centre, Nottingham University Hospital were approached and all agreed to participate in the study following informed consent. 76 adults were newly diagnosed with coeliac disease at Derbyshire Hospitals of whom 62 (82%) were approached and all agreed to participate in the study following informed consent. Only 39 of the 74 (53%) adults newly diagnosed with coeliac disease at the Royal Hallamshire Hospital during this time period were approached though all 39 agreed to participate in the study following informed consent. No coeliac approached refused to participate in the study.

The proportion of incident coeliacs recruited to the study (n = 151) out of all incident coeliacs diagnosed during the study period at the study centres (n = 206) was 73%. Reasons for not capturing all incident coeliacs diagnosed during the study period included those where the diagnostic work-up and subsequent management for coeliac disease occurred along 'non-conventional routes' with delay in either identifying them as potential recruits as I was unaware they had been diagnosed or that the person had been taking gluten-free diet for more than four weeks (n = 33); non-attendance at hospital clinic following referral for treatment (n = 12).

4.1.3.2. Demography of cohort

151 people newly diagnosed with coeliac disease were included in the study. There were nearly twice as many women (n =94; 62%) as men (n =57) in the study cohort. The mean age at diagnosis was 50.6 (SD 19.1) years. The mean age at diagnosis of coeliac disease was significantly younger (p = 0.0029) in women (46.9 (SD 19.7) years) than in men (56.4 (SD 16.6) years).

4.1.3.3. Presenting features of incident coeliac disease

Iron deficiency anaemia and diarrhoea were the most common presenting features, affecting half of the cohort (Table 4.27). 32% (n = 48) had partial villous atrophy; 31% (n = 47) had subtotal villous atrophy and 23% (n = 34) had total villous atrophy. 12 (8%) coeliacs had mild enteropathy changes on duodenal biopsy with no evidence of villous atrophy. 10 of the 151 incident coeliacs did not have a duodenal biopsy. At diagnosis, the median tTG was 56 (IQR 13 – 164; n = 151) and 94% (n = 142) were EMA positive.

Table 4.27: Presenting features of incident coeliac cohort

Presenting features	Incident coeliac cohort (n = 151)
Classic	
Diarrhoea	74 (49%)
Weight loss	38 (25%)
Malabsorption	8 (5%)
Gastrointestinal	
Abdominal pain	42 (28%)
Constipation	3 (2%)
IBS symptoms	19 (13%)
Heartburn and or reflux	10 (6%)
Extra-intestinal	
Iron deficiency anaemia	78 (52%)
Folate deficiency	8 (5%)
B12 deficiency	5 (3%)
Associated conditions	
Dermatitis herpetiformis	6 (4%)
Type 1 diabetes	5 (3%)

The presenting features of those coeliacs presenting with gastrointestinal symptoms

(n = 85), with classic (n = 22) or silent disease (n = 44) are shown in the following table.

Table 4.28: Presenting features of classic, symptomatic and silent coeliac disease

Presenting feature	Classic disease (n = 22)	Symptomatic disease (n = 85)	Silent disease (n = 44)
Sex (%)			
Male	9 (41)	31 (36)	17 (39)
Female	13 (59)	54 (64)	27 (61)
Mean age (SD) years	56.9 (17.3)	47.8 (19.0)	52.4 (19.5)
Marsh grading			
1	0	3 (4)	3 (7)
2	0	4 (5)	2 (5)
3a	5 (23)	30 (35)	13 (30)
3b	8 (35)	23 (27)	16 (36)
3c	7 (32)	19 (22)	8 (18)
Not done	2 (10)	6 (7)	2 (4)
EMA status (%)			
Positive	17 (77)	70 (82)	39 (89)
Weak positive	2 (9)	10 (12)	4 (9)
Negative	3 (14)	5 (6)	1 (2)
Median tTG [IQR] iu	114.7 [15 – 300]	112.1 [12 – 151]	133.9 [15 – 300]
Mean Hb (SD) g/L	12.6 (1.9)	12.8 (1.7)	11.4 (2.3)
Mean weight (SD) kg	62.0 (13.9)	73.1 (16.5)	70.0 (18.2)

There was no difference in proportion of females presenting with classic, symptomatic or silent coeliac disease. There was no difference in mean age at diagnosis in those coeliacs presenting with symptoms (classic or gastrointestinal) in comparison to those coeliacs presenting with silent coeliac disease. There was no difference in proportion of those coeliacs with villous atrophy or EMA positivity or median tTG in those coeliacs presenting with classic, symptomatic or silent coeliac disease. Coeliacs with classic disease had the lowest presenting weight though this did not significantly differ from that observed in silent or symptomatic disease.

4.1.3.4. Prevalence of diagnosed vascular disease in incident coeliac disease

92 (61%) of the incident coeliacs had never smoked. 29 (19%) were ex-smokers, smoking for a mean 24.1 (SD 12.9) pack years. 29 (19%) were current smokers with a mean 22.1 (SD 15.0) pack year history. 23% (n = 13) of men and 17% (n = 16) of women reported as being current smokers at the time coeliac disease was diagnosed. Prevalence of reported current smoking varied by socio-economic status. For both men and women, those in the most deprived and below average quintiles were more than twice as likely to smoke cigarettes as those in the above average and least deprived quintiles (p = 0.03). The proportion of coeliacs that were current smokers or had never smoked was not significantly different from the smoking behaviours reported in the general population (Table 4.29).

Table 4. 29: Prevalence of smoking in adults newly diagnosed with coeliac disease compared to the general population

Smoking history	Observed number in coeliac cohort (n = 151)	Prevalence in general population (%)	Expected number in coeliac cohort	Observed: expected [95% CI]
Current smokers				
Men	13	24.5	14.0	0.93 [0.42, 1.43]
Women	16	22.2	20.9	0.77 [0.38, 1.15]
Never smoked				
Men	26	49	27.9	0.93 [0.57, 1.28]
Women	66	57	53.6	1.23 [0.93, 1.53]

21% (n = 12) of male coeliacs and 8% (n = 7) of female coeliacs reported having been diagnosed with a cardiovascular condition (Table 4.30). The prevalence of coronary artery disease was higher in male coeliacs (n = 8, 14%) than in women (n = 4; 4%)

with coeliac disease. Stroke disease was more than twice as prevalent in male coeliacs (n = 5, 9%) than in female coeliacs (n = 2, 2%).

The prevalence of coronary artery disease did not significantly change with socio-economic status though appeared to higher in more affluent socio-economic classes. For example, 11 of 19 coeliacs reported having been diagnosed with any cardiovascular disease were from the highest two quintiles (above average and least deprived) quintiles of IMD07 rank.

Coeliacs were as likely to have reported to been diagnosed with stroke disease or ischaemic heart disease as to the general population (Table 4.30). Female coeliacs were over 50% less likely (observed: expected 0.47; 95%CI 0.19, 0.75) to have been diagnosed with hypertension in comparison to the general population.

Table 4.30: Prevalence of ever diagnosed vascular disease in incident coeliac disease compared with general population

Ever diagnosed vascular disease	Observed number in coeliac cohort	Prevalence in general population (%)	Expected number in coeliac cohort	Observed: expected [95% CI]
Angina				
Men	8	4.8	2.74	2.90 [0.88, 4.92]
Women	4	3.3	3.10	1.29 [0.03, 2.55]
Myocardial infarction				
Men	4	4.1	2.34	1.71 [0.02, 3.40]
Women	2	1.7	1.60	1.25 [-0.48, 2.98]
Abnormal heart rhythm				
Men	1	2.8	1.60	0.63 [-0.6, 1.86]
Women	2	5.8	5.55	0.36 [-0.14, 0.86]
Stroke				
Men	5	2.4	1.37	3.65 [0.46, 6.84]
Women	2	2.2	2.07	0.97 [-0.37, 2.31]
Hypertension				
Men	17	24.0	13.68	1.24 [0.65, 1.83]
Women	11	25.0	23.50	0.47 [0.19, 0.75]
Type 1 diabetes				
Men	2	0.5	0.29	6.90 [-2.66, 16.46]
Women	3	0.5	0.47	6.38 [-0.84, 13.60]
Type 2 diabetes				
Men	5	5.1	2.91	1.72 [0.21, 3.23]
Women	2	3.7	3.48	0.58 [-0.22, 1.38]
Any coronary artery disease				
Men	8	6.5	3.71	2.16 [0.67, 3.65]
Women	4	4.0	3.76	1.06 [0.02, 2.10]

4.1.3.5. Vascular risk profile at diagnosis of coeliac disease

4.1.3.5.1. Weight and body mass index

The mean weight at diagnosis of coeliac disease was 64.7 (SD 14.3) kg in women and 80.3 (SD 16.6) kg in men (Table 4.31). Mean BMI was lower in women (24.4 kg/m^2, SD 4.85) than in men (26.2 kg/m^2, SD 4.7) with incident coeliac disease (Table 4.31). 56% (n = 32) of men and 36% (n = 34) of women were either overweight or obese at diagnosis of coeliac disease (Table 4.32). A greater proportion of men than women were overweight (42% v 23%; p = 0.00001) though there was no significant difference in the proportion obese (14% v 13%) or underweight (5% v 4%). A greater proportion of women than men were of normal BMI (60%; n = 56 v 39%; n = 22) at diagnosis of coeliac disease (p = 0.01). One female (BMI 44.9 kg/m^2) and one male (BMI 43.3 kg/m^2) coeliac were morbidly obese at diagnosis of coeliac disease. Being overweight or obese at diagnosis of coeliac disease was weakly associated with higher affluence (proportion amongst above average and least deprived quintiles 52% (n = 35) versus average, below average, most deprived quintiles 38% (n = 31); p = 0.08).

4.1.3.5.2. Waist circumference and waist: hip circumference

The mean waist circumference was 93.9 (SD 13.4) cm in men and 81.6 (SD 10.8) cm in women at diagnosis of coeliac disease (Table 4.31). The proportion of coeliacs with a raised waist circumference (Table 4.32) was higher in women than in men (21% v 16%). Raised waist circumference was not associated with quintile of socio-economic rank.

The mean waist: hip circumference was 0.94 (SD 0.1) in men and 0.84 (SD 0.1) in women at diagnosis of coeliac disease. Over half of the incident coeliacs had an unfavourable waist: hip ratio with 52% (n = 78) of the cohort having a raised ratio.

Table 4.31: Vascular anthropometric risk profile in incident coeliac disease compared to the general population

Vascular risk profile co-variate	N	Incident coeliac disease	General population	Mean difference [95% CI]
Mean waist circumference cm (SD)				
Men	57	91.5 (0.2)	96.8 (16.9)	-5.3 [-0.9, -9.7]
Women	94	81.6 (27.7)	86.4 (16.6)	-4.8 [-1.4, -8.2]
Mean waist: hip circumference (SD)				
Men	57	0.9 (0.1)	0.9 (0.1)	0.0 [-0.1, 0.1]
Women	94	0.8 (0.1)	0.8 (0.1)	0.0 [-0.1, 0.1]
Mean height cm (SD)				
Men	57	177.4 (10.7)	174.2 (9.1)	3.2 [0.8, 5.6]
Women	94	162.9 (19.2)	161.4 (6.8)	1.5 [0.1, 2.9]
Mean weight kg (SD)				
Men	57	82.3 (30.1)	82.9 (68.0)	-0.6 [-18.3, 17.1]
Women	94	65.3 (37.7)	69.7 (60.6)	-4.4 [-16.7, 7.9]
Mean BMI kg/m^2 (SD)				
Men	57	25.7 (6.6)	27.2 (5.4)	-1.5 [-2.9, -0.1]
Women	94	24.6 (12.3)	26.8 (6.2)	-2.2 [-0.9, -3.5]

The proportion of coeliacs with a raised waist: hip ratio was similar in men and women (47% v 54%, respectively); p = 0.41 and did not vary with socio-economic status.

4.1.3.5.3. Measured blood pressure and measured hypertension

The mean systolic blood pressure measured at diagnosis of coeliac disease was 132.6 (SD 20.0) mmHg in men and 120.4 (SD 16.7) mmHg in women. The mean diastolic blood pressure was 74.6 (SD 9.5) mmHg in incident male coeliacs and 71.5 (SD 10.1) mmHg in incident female coeliacs. The prevalence of measured hypertension (at least 140 mmHg systolic and or at least 90 mmHg diastolic blood pressure) was 5% (n = 3) in men and 1% (n = 1) in women in the coeliac cohort (Table 4.32).

Table 4.32: Prevalence of obesity and hypertension (diagnosed and measured) in incident coeliac disease compared to the general population

Vascular risk profile co-variate	Observed proportion in general population	Observed number of coeliacs	Expected number of coeliacs	Observed : expected [95%CI]
Hypertension[a]				
Men	31%	33/57	17.67	1.87 [1.22, 2.52]
Women	28%	29/94	26.32	1.10 [0.70, 1.50]
Underweight				
Men	1%	3/57	0.57	5.26 [-0.69, 11.21]
Women	2%	4/94	1.88	2.13 [0.04, 4.22]
Normal				
Men	32%	22/57	18.24	1.21 [0.70, 1.72]
Women	42%	56/94	39.48	1.42 [1.05, 1.79]
Overweight				
Men	43%	24/57	24.51	0.98 [0.59, 1.37]
Women	32%	22/94	30.08	0.73 [0.42, 1.04]
Obese				
Men	23%	8/57	13.11	0.61 [0.19, 1.03]
Women	25%	12/94	23.50	0.51 [0.22, 0.80]
Raised waist circumference				
Men	32%	9/57	18.24	0.49 [0.17, 0.81]
Women	41%	20/94	38.54	0.52 [0.29, 0.75]
Raised waist: hip ratio				
Men	33%	30/57	18.81	1.59 [1.02, 2.16]
Women	30%	43/94	28.20	1.52 [1.07, 1.97]

a Systolic BP >/140 mmHg or diastolic BP >/90 mmHg or taking prescribed medication for hypertension

4.1.3.5.4. Cholesterol profile

The mean levels of total cholesterol as shown in Table 4.33 was 4.2 (SD 1.0) mmol/L for men and 4.7 (SD 1.1) mmol/L for women (mean difference in total cholesterol 0.5 [95%CI 0.1, 0.8] mmol). A higher proportion of female coeliacs (38%; n = 34) had raised total cholesterol (5.0 mmol/L or above) in comparison to male coeliacs (20%; n = 11) at diagnosis of coeliac disease; p = 0.009 (Table 4.34). Total cholesterol and raised total cholesterol was not related to age or socio-economic status.

Table 4.33: Vascular serum risk profile in incident coeliac disease compared to the general population

Vascular risk profile co-variate	N	Incident coeliac disease	General population	Mean difference [95% CI]
Mean cholesterol mmol/L (SD)	57	4.2 (1.3)	5.30 (1.2)	-1.1 [-0.8, -1.4]
Men	94	4.6 (2.3)	5.40 (1.2)	-0.8 [-0.5, -1.1]
Women				
Mean HDL cholesterol mmol/L (SD)	56	1.1 (0.5)	1.3 (0.6)	-0.2 [-0.1, -0.4]
Men	89	1.3 (0.8)	1.6 (0.6)	-0.3 [-0.2, -0.4]
Women				
Mean LDL cholesterol mmol/L(SD)	40	2.3 (0.9)	3.6 (0.9)	-1.3 [-1.0, -1.6]
Men	46	2.9 (0.9)	3.6 (0.9)	-0.7 [-0.4, -1.0]
Women				
Mean triglycerides mmol/L (SD)	47	1.4 (0.8)	1.8 (1.4)	-0.4 [-0.8, 0.1]
Men	62	1.3 (0.7)	1.4 (0.8)	-0.1 [-0.3, 0.1]
Women				
Mean fibrinogen g/L (SD)				
Men	46	2.5 (0.9)	2.8 (0.6)	-0.3 [-0.1, -0.5]
Women	64	3.1 (2.3)	3.1 (0.6)	0.0 [-0.2, 0.2]
Mean CRP mg/L (SD)				
Men	54	3.5 (0.2)	3.1 (7.2)	0.4 [-1.5, 2.3]
Women	87	4.1 (9.5)	3.6 (8.9)	0.5 [-1.4, 2.4]
Mean HbA1C % (SD)				
Men	31	6.0 (0.2)	5.5 (1.2)	0.5 [0.1, 0.9]
Women	30	5.3 (0.2)	5.5 (0.6)	-0.2 [-0.4, 0.1]

Women had higher HDL cholesterol at diagnosis of coeliac disease in comparison to men (1.4 (SD 0.4) versus 1.1 (SD 0.3) mmol/L; mean difference 0.3 mmol/L; 95%CI 0.1, 0.4). Men had significantly higher prevalence of low HDL cholesterol (below 1.0 mmol/L) than women with coeliac disease (29% (n = 16) versus 6% (n = 5); p = 0.0001). Low HDL cholesterol was not related to age or socio-economic status. HDL cholesterol at diagnosis of coeliac disease was related to weight, BMI, waist circumference, waist: hip circumference ratio but not to proxy markers of more severe coeliac disease (such as presence of villous atrophy, malabsorption).

The total cholesterol: HDL cholesterol ratio was 4.0 (SD 1.3) in men and 3.6 (SD 0.9) in women at diagnosis of coeliac disease (mean difference -0.4; 95%CI -0.1, -0.8).

Mean LDL cholesterol was lower in men (2.3 (SD 0.9) mmol/L) than in women (2.8 (SD 0.9) mmol/L); mean difference -0.5 mmol/L; 95%CI -0.1, -0.9. Men had significantly lower prevalence of raised LDL cholesterol (3.0 mmol/L or more) than women with incident coeliac disease. 41% of women and 22% men had raised LDL cholesterol (mean difference in proportion 19%; 95C% 2, 37). LDL cholesterol at diagnosis of coeliac disease was not related to anthropometric measures such as weight, waist: hip circumference nor markers of more severe coeliac disease. Raised LDL cholesterol was not related to socio-economic status.

Triglycerides were significantly higher in incident male coeliacs (1.5 (SD 0.8) mmol/L) than in females (1.2 (SD 0.7) mmol/L); p = 0.02. Triglyceride titres were related to weight, waist circumference, waist: hip circumference, systolic blood pressure, tissue transglutaminase titre, and age but not to proxy markers of deprivation

or more severe coeliac disease. Prevalence of raised triglyceride (1.6 mmol/L or higher) was not significantly different between men (31%; n = 16) and women (22%; n = 19); p = 0.29. Raised triglyceride levels did not vary significantly across quintiles of rank of IMD07 score.

Table 4.34: Prevalence of dyslipidaemia, raised CRP and glycated haemoglobin in incident coeliacs in comparison to the general population

Vascular risk profile co-variate	Observed proportion in general population	Observed number of coeliacs	Expected number of coeliacs	Observed : expected [95%CI]
Raised total cholesterol[b]				
Men	57%	11/57	31.92	0.34 [0.14, 0.54]
Women	61%	34/94	54.29	0.63 [0.42, 0.84]
Low HDL[c]				
Men	9.4%	16/56	5.26	3.04 [1.55, 4.53]
Women	1.8%	5/89	1.60	3.13 [0.39, 5.87]
Raised LDL[d]				
Men	77%	10/45	34.65	0.29 [0.11, 0.47]
Women	74%	24/58	42.92	0.56 [0.34, 0.78]
Raised triglycerides[e]				
Men	41%	16/53	21.73	0.74 [0.38, 1.10]
Women	31%	19/86	26.66	0.71 [0.39, 1.03]
Raised CRP[f]				
Men	19%	11/54	10.26	1.07 [0.44, 1.70]
Women	19%	26/87	16.53	1.57 [0.97, 2.17]
Raised HbA1c[g]				
Men	2.8%	7/28	0.78	8.97 [2.32, 15.62]
Women	2.4%	3/30	0.72	4.17 [-0.55, 8.89]

b Total cholesterol >/5.0 mmol/L; c HDL cholesterol \<1.0 mmol/L; d LDL cholesterol >/ 3.0 mmol/L; e Triglycerides >/ 1.0 mmol/L;
f CRP >/ 5.0 mg/L; g HbA1c >/ 7.0%

4.1.3.5.5. Haemostasis and thrombosis profile

The mean fibrinogen at diagnosis of coeliac disease was significantly higher in women (3.1 (SD 1.3) g/L) than in men (2.6 (SD 0.8) g/L); p = 0.02 as shown in Table 4.33. Fibrinogen was not related to age, anthropometric measures such as weight or waist circumference, blood pressure or cholesterol profile. Fibrinogen was associated with the presence of malabsorption (p = 0.0022) but not other features of severe coeliac disease such as level of tissue transglutaminase or the presence of villous atrophy. Level of fibrinogen was related to current smoking (p = 0.0185), CRP (p = 0.00001) but not HDL cholesterol. Fibrinogen levels were significantly higher in those coeliacs who had been diagnosed previously with vascular disease (mean difference in fibrinogen 0.7 g/L; 95% CI 0.07, 1.3).

Platelet count at diagnosis of coeliac disease was significantly higher in women (325.0 (SD 89.4) x 10^9/L) than in men (279.3 (SD 80.3) 10^9/L and in those coeliacs presenting with iron deficiency anaemia (336.4 v 277.1 10^9/L); p = 0.00001. Total cholesterol (p = 0.0014) and HDL cholesterol (p = 0.03) were the only co-variates associated with platelet count.

The mean APTT at diagnosis of coeliac disease was 26.1 (SD 2.9) seconds. The presence of malabsorption was the only variable associated with APTT with significantly longer APTT when malabsorption was present (mean difference in APTT with the presence of malabsorption 2.0 seconds; 95%CI 0.3, 4.2). The mean prothrombin time at diagnosis of coeliac disease was 10.5 (SD 1.9) seconds. Prothrombin time was associated with HDL cholesterol (p = 0.05) and previously diagnosed vascular disease where prothrombin time was significantly higher than in

coeliacs without diagnosed vascular disease (mean difference 1.2 seconds; 95%CI 0.2, 2.1).

4.1.3.5.6. Inflammatory markers

Mean CRP levels in incident male and female coeliacs were 3.9 (SD 6.0) and 4.5 (SD 5.2) mg/L, respectively (Table 4.33). Presence of diarrhoea and malabsorption was associated with significantly higher CRP levels at diagnosis of coeliac disease. For example, mean difference in CRP with the presence of malabsorption compared to the absence of malabsorption was 8.3 mg/L; 95%CI 3.9, 12.6. The mean CRP was significantly higher in coeliacs of more deprived socio-economic status (mean CRP 4.6 (SD 4.4) in most deprived quintile versus 2.9 (SD 1.5) mg/L in least deprived quintile; p = 0.05) and in those coeliacs with previous diagnosis of stroke disease. Fibrinogen and triglycerides were the only other variables associated with CRP (r = 0.44, p = 0.0001; r = 0.21, p = 0.01 respectively). There was a non-significant higher proportion of women with raised CRP (more than 4.9 mg/L) than men (30% v 20%; p = 0.21). The proportion of coeliacs with raised CRP rose with increasing deprivation with 37% of coeliacs from the most deprived quintile having raised CRP compared to 12% of those from the least deprived quintile (mean difference in proportion 24.8%; 95%CI 6.1, 43.6).

The mean ESR was higher in women at diagnosis of coeliac disease than in men (19.6 (SD 19.8) versus 11.9 (SD 17.5) mm/hr, respectively); p = 0.01. Mean ESR was also associated with age, weight (0.03), waist circumference (p = 0.05), HDL cholesterol (p = 0.05), LDL cholesterol (p = 0.04), presence of anaemia (p = 0.004), tissue transglutaminase (p = 0.008) and fibrinogen (p = 0.0001). 27% (n = 38) of the

incident coeliacs had ESR of 20 mm/hr or more with increasing age the only associated variable ($p = 0.012$).

The mean ferritin in incident coeliac disease was significantly lower in women than in men (mean difference -35.4 μg/L; 95%CI -55.6, -15.2). 54% of women and 42% of men had ferritin values below 15 μg/L. Mean ferritin was associated with haemoglobin and presentation with iron deficiency anaemia ($p = 0.00001$); tissue transglutaminase ($p = 0.001$); triglycerides ($p = 0.002$); and waist circumference, waist: hip ratio, weight and BMI ($p = 0.03$). Mean ferritin was higher with increasing affluence. For example, mean ferritin in most deprived quintile 14.3 (SD 17.1) μg/L versus 40.8 (SD 60.6) μg/L; $p = 0.04$). Only men ($n = 6$, 11%) had raised ferritin (200 μg/L or higher). Mean leucocyte count at diagnosis of coeliac disease was 6.6 (SD 2.2) x 10^9/L with no difference between men and women. Mean leucocyte count was associated with waist circumference, waist: hip ratio, age at diagnosis of coeliac disease and presentation with weight loss.

The mean white cell count was significantly higher in current smokers than in non-smokers (mean difference 1.5 10^9/L; 95%CI 0.7, 2.4). The mean white cell count was significantly lower in more affluent male coeliacs than in more deprived (5.9 (SD 1.5) 10^9/L versus 9.9 (SD 2.5) 10^9/L, respectively; $p = 0.0006$) though there was no association with deprivation and leucocyte count amongst female coeliacs.

4.1.3.5.7. Glycaemic profile

The mean random glucose was significantly higher in men than in women and in those coeliacs who had been previously diagnosed with diabetes mellitus (mean

difference -1.4 mmol/L; 95%CI -2.0, -0.8). Mean random glucose was associated with waist circumference, waist: hip ratio and systolic blood pressure. Four coeliacs had raised random glucose (7 mmol/L or more) at diagnosis of coeliac disease.

The mean HbA1c was 5.8 (SD 1.2) % and was associated with waist: hip ratio and age at diagnosis of coeliac disease. Raised HbA1c (7% or higher) was observed in those coeliacs with previous diagnosis of ischaemic heart disease and diabetes mellitus.

4.1.3.5.8. Anaemia profile

Mean haemoglobin was significantly lower in women (11.9 (SD 1.8) g/L) than in men (13.0 (SD 2.0) g/L). Mean haemoglobin was associated with weight, BMI, waist circumference, waist: hip ratio and ferritin. Current smokers had 1g/L (95%CI of difference 0.2, 1.7) higher haemoglobin than non-smokers. 44% (n = 66) of the incident coeliacs were anaemic (haemoglobin 12.0 g/L or less) with a higher proportion amongst women than men (mean difference in proportion 16.2%; 95%CI 2.0, 32.1). Only 3 of the 151 incident coeliacs had haemoglobin value of 16 g/L or more.

4.1.3.6. Vascular risk profile in incident coeliac disease compared to the general population

4.1.3.6.1. Weight and body mass index

The mean weight of both male and female coeliacs was significantly lighter than that of the general population (Table 4.31). However, there was no difference in the proportions between coeliacs and general populations of being underweight, of normal BMI and of being overweight (Table 4.32). Female coeliacs were less likely to be obese in comparison to the general population (observed: expected 0.51; 95%CI 0.22, 0.80) (Table 4.32).

4.1.3.6.2. Waist circumference and waist: hip circumference

The mean waist circumference of both male and female coeliacs was significantly lower than that of the general population (Table 4.31). For example, mean difference in waist circumference in male coeliacs v males -5.3 cm; 95% -0.9, -9.7. The proportion of coeliacs with a raised waist circumference was also lower than that of the general population (Table 4.32). Female coeliacs were 48% (observed: expected 0.52; 95%CI 0.29, 0.75) less likely and male coeliacs were 51% (observed: expected 0.49; 95%CI 0.17, 0.81) to have a raised waist circumference in comparison to the general population.

Despite the apparent favourable waist circumference, coeliacs had no significantly lower nor different waist: hip circumference to the general population (Table 4.32). Furthermore, both male (observed: expected 1.59; 95%CI 1.02, 2.16) and female (observed: expected 1.52; 95%CI 1.07, 1.97) coeliacs were more likely to have raised waist: hip circumferences in comparison to the general population.

4.1.3.6.3. Blood pressure and hypertension

The prevalence of hypertension was no different in female coeliacs in comparison to the general population (Table 4.32). However, there was almost two-fold increased prevalence of hypertension in male coeliacs in comparison to the general population (observed: expected 1.87; 95%CI 1.22, 2.52).

4.1.3.6.4. Cholesterol profile

The mean levels of total cholesterol were significantly lower in coeliacs compared to that of the general population (Table 4.33). Male coeliacs had -1.1 (95%CI for difference -0.8, -1.4) mmol/L lower total cholesterol and female coeliacs had -0.8 (95%CI for difference -0.5, -1.1) mmol/L lower total cholesterol in comparison to the general population. Male coeliacs were 66% less likely to have raised total cholesterol (observed: expected 0.34; 95%CI 0.14, 0.54) than the general population (Table 4.34). Female coeliacs were 37% (observed: expected 0.63; 95%CI 0.42, 0.84) less likely to have raised total cholesterol than the general population.

Coeliacs had significantly lower HDL cholesterol than that observed in the general population (Table 4.33) with men three times as likely to have low HDL cholesterol (observed: expected 3.04; 95%CI 1.55, 4.53).

Coeliacs had significantly lower LDL cholesterol than that observed in the general population with the effect more marked in men than in women (Table 4.33). For example, male coeliacs had -1.3 (95%CI -1.0, -1.6) mmol/L lower LDL cholesterol than the general population. Male coeliacs had over 70% lower LDL cholesterol than

Table 4.35: Prevalence of dyslipidaemia, raised CRP and glycated haemoglobin in incident coeliacs in comparison to the general population

Vascular risk profile co-variate	Observed proportion in general population	Observed number of coeliacs	Expected number of coeliacs	Observed : expected [95%CI]
Raised total cholesterol[b]				
Men	57%	11/57	31.92	0.34 [0.14, 0.54]
Women	61%	34/94	54.29	0.63 [0.42, 0.84]
Low HDL[c]				
Men	9.4%	16/56	5.26	3.04 [1.55, 4.53]
Women	1.8%	5/89	1.60	3.13 [0.39, 5.87]
Raised LDL[d]				
Men	77%	10/45	34.65	0.29 [0.11, 0.47]
Women	74%	24/58	42.92	0.56 [0.34, 0.78]
Raised triglycerides[e]				
Men	41%	16/53	21.73	0.74 [0.38, 1.10]
Women	31%	19/86	26.66	0.71 [0.39, 1.03]
Raised CRP[f]				
Men	19%	11/54	10.26	1.07 [0.44, 1.70]
Women	19%	26/87	16.53	1.57 [0.97, 2.17]
Raised HbA1c[g]				
Men	2.8%	7/28	0.78	8.97 [2.32, 15.62]
Women	2.4%	3/30	0.72	4.17 [-0.55, 8.89]

b Total cholesterol >/5.0 mmol/L; c HDL cholesterol \<1.0 mmol/L; d LDL cholesterol >/ 3.0 mmol/L; e Triglycerides >/ 1.0 mmol/L;
f CRP >/ 5.0 mg/L; g HbA1c >/ 7.0%

the general population (observed: expected 0.29; 95%CI 0.11, 0.47). In female coeliacs, LDL cholesterol was 44% lower than in the general population (observed: expected 0.56; 95%CI 0.34, 0.78).

There was no difference in the mean triglyceride levels in coeliacs to the general population nor did the proportion of having raised triglycerides differ (Tables 4.33, 4.34).

4.1.3.6.5. Haemostasis and thrombosis profile

Mean fibrinogen was significantly lower in male coeliacs (-0.3 mmol/L; 95%CI for difference -0.1, -0.5) than in the general population. Female coeliacs had similar fibrinogen levels to the general population.

4.1.3.6.6. Inflammatory markers

The mean CRP at diagnosis of coeliac disease was not significantly different from that of the general population (Table 4.33). Furthermore, the proportion of coeliacs with raised CRP did not differ from that of the general population (Table 4.34).

4.1.3.6.7. Glycaemic profile

The mean random glucose was significantly higher in men than in women and in those coeliacs who had been previously diagnosed with diabetes mellitus (mean difference -1.4 mmol/L; 95%CI -2.0, -0.8). Mean random glucose was associated with waist circumference, waist: hip ratio and systolic blood pressure. Four coeliacs had raised random glucose (7 mmol/L or more) at diagnosis of coeliac disease.

The mean HbA1c was 5.8 (SD 1.2)% and was associated with waist: hip ratio and age at diagnosis of coeliac disease. Raised HbA1c (7% or higher) was observed in those coeliacs with previous diagnosis of ischaemic heart disease and diabetes mellitus.

Though the mean HbA1c did not differ between coeliacs and the general population, male coeliacs were nine times as likely to have raised HbA1c in comparison to the general population (observed: expected 8.97; 95%CI 2.32, 15.62).

4.1.3.7. Vascular risk profile at diagnosis of classic, silent and symptomatic coeliac disease

4.1.3.7.1. Weight and body mass index

Coeliacs with classic disease had the lowest presenting weight 62.0 (SD 13.9) kg (Table 4.28) and lowest BMI 21.9 (SD 4.2) kg/m^2 (Table 4.36) in comparison to those presenting with silent disease or with gastrointestinal symptoms. 54% (n = 44) of coeliacs presenting with gastrointestinal symptoms were either overweight or obese at diagnosis of coeliac disease which was three times the proportion of those coeliacs with classic disease being obese or overweight (18%); p = 0.0002. 41% (n = 18) of coeliacs presenting with silent disease were obese or overweight at diagnosis of coeliac disease.

4.1.3.7.2. Waist circumference and waist: hip circumference

The mean waist circumference (Table 4.36) was significantly lower in those coeliacs presenting with classic disease (78.7 (SD 9.3) cm) compared to those with silent (85.8 (SD 12.7) cm) or with gastrointestinal symptoms (88.1 (SD 13.6) cm); p = 0.002. In keeping with the highest mean waist circumference, the proportion of coeliacs presenting with gastrointestinal symptoms with a raised waist circumference (27%) was more than twice that observed with silent disease (11%) and more than five times that observed with classic disease (Table 4.36).

The mean waist: hip circumference ratio did not significantly vary between silent, classic and symptomatic coeliac disease (Table 4.36). However those presenting with silent disease had the highest proportion with an unfavourable waist: hip ratio, affecting two-thirds of silent coeliacs (Table 4.36); p = 0.04.

Table 4.36: Vascular anthropometric risk profile in classic, silent and symptomatic coeliac disease at diagnosis

At diagnosis	Classic disease (n = 22)	Symptomatic disease (n = 85)	Silent disease (n = 44)
Mean BMI kg/m^2 (SD)	21.9 (4.2)	26.0 (4.9)	24.8 (4.6)
BMI group N (%)			
Underweight	4 (18)	1 (1)	2 (5)
Normal	14 (64)	40 (47)	24 (55)
Obese	3 (14)	28 (33)	15 (34)
Overweight	1 (5)	16 (19)	3 (7)
Mean waist circumference cm (SD)	78.8 (9.3)	88.1 (13.6)	85.8 (12.7)
Proportion (%) raised waist	4.5	27.1	11.4
Mean waist : hip ratio (SD)	0.88 (0.07)	0.89 (0.08)	0.87 (0.07)
Proportion (%) raised waist : hip ratio	50.0	47.1	61.4
Mean systolic BP (SD) mmHg	126.0 (23.9)	125.4 (17.2)	123.8 (19.7)
Mean diastolic BP (SD) mmHg	73.8 (12.5)	72.6 (10.2)	72.1 (8.3)
Proportion (%) with hypertension	4.6	3.5	0

4.1.3.7.3. Blood pressure and hypertension

Both the mean systolic and diastolic blood pressure appeared to be the highest in those coeliacs presenting with classic disease (Table 4.36).

4.1.3.7.4. Cholesterol profile

Coeliacs presenting with classic disease appeared to have a more favourable cholesterol profile than those coeliacs presenting with silent or symptomatic disease (Table 4.37). Coeliacs with classic disease had the lowest observed mean total cholesterol (4.39 (SD 1.22) mmol/L), highest mean HDL cholesterol (1.32 (SD 0.43) mmol/L), and lowest mean LDL cholesterol (2.46 (SD 0.90) mmol/L). In comparison, those coeliacs presenting with silent disease appeared to have the least favourable cholesterol profile. Silent coeliacs had the lowest HDL cholesterol (1.26 (SD 0.39) mmol/L), highest LDL cholesterol (2.71 (SD 1.08) mmol/L) and highest proportion with raised triglycerides (33%).

4.1.3.7.5. Haemostasis and thrombosis profile

The mean fibrinogen at diagnosis of coeliac disease was highest in classic disease (3.34 (SD 1.55) g/L) than in symptomatic or silent disease (Table 4.37). Those with silent coeliac disease had the highest mean platelet count at diagnosis (333 (SD 100) x 10^9/L). The mean APTT and prothrombin time did not significantly differ between classic, silent and symptomatic coeliac disease.

4.1.3.7.6. Inflammatory markers

Despite the apparent favourable cholesterol profile, coeliacs presenting with classic disease had the highest mean white cell counts and CRP (Table 4.37) with silent disease having the lowest values.

Table 4.37: Vascular serum risk profile in classic, symptomatic and silent disease

At diagnosis	Classic disease (n = 22)	Symptomatic disease (n = 85)	Silent disease (n = 44)
Mean total cholesterol mmol/L (SD)	4.39 (1.22)	4.58 (1.09)	4.46 (1.10)
Proportion (%) with raised total cholesterol	33.3	32.5	27.3
Mean HDL cholesterol mmol/L (SD)	1.32 (0.43)	1.29 (0.38)	1.26 (0.39)
Proportion (%) low HDL cholesterol	14.3	11.1	22.7
Mean LDL cholesterol mmol/L (SD)	2.46 (0.90)	2.54 (0.88)	2.71 (1.08)
Proportion (%) raised LDL cholesterol	41.2	25.0	43.3
Mean triglycerides mmol/L (SD)	1.25 (0.54)	1.34 (0.81)	1.21 (0.63)
Proportion (%) raised triglycerides	19.0	23.1	32.5
Mean fibrinogen g/L (SD)	3.34 (1.55)	2.87 (1.13)	2.80 (0.89)
Mean CRP mg/L (SD)	6.13 (9.87)	4.46 (5.22)	2.98 (1.70)
Proportion (%) with raised CRP	25.0	29.1	21.4
Mean white cell count x 10^9/L (SD)	6.87 (2.42)	6.94 (2.39)	5.97 (1.46)
Mean platelet count x 10^9/L (SD)	291.0 (79.5)	299.1 (82.9)	333.3 (99.6)
Mean HbA1c % (SD)	6.09 (1.24)	5.56 (0.75)	6.07 (1.62)
Proportion (%) with raised HbA1c	10.0	11.1	28.6

4.1.3.8. Continued participation in study and adherence to gluten-free diet

118 of the initial 151 (78%) recruited study participants with incident coeliac disease completed the full 12 months of the study. Of the 33 study participants that did not complete the full 12 months of their study period:

- 5 were students attending universities away from the study centres with their ongoing coeliac care switched to hospitals local to their university
- 1 study participant returned to her native Poland shortly after coeliac disease was diagnosed (had been resident in Derby for preceding 5 years)
- 1 study participant did not wish to take a GFD following extensive discussions with the gastroenterology team and elected not to return back to the reviewing hospital as he elected not to be treated or reviewed for his coeliac disease
- 1 study participant was an existing nursing home resident where the gastroenterologist elected for further care and monitoring to be performed by her local general practitioner rather than returning to the hospital
- 1 study participant due to rapidly deteriorating cognitive functioning was transferred to a nursing home away from Nottingham near his daughter
- 9 other study participants attended for routine coeliac disease review at three months post-initiation of a GFD where their vascular risk profile was measured for the purpose of this study but did not attend later coeliac disease reviews despite repeated appointments sent by the reviewing centres to alert them of the appointment date
- 4 other study participants attended for routine coeliac review at six months post-initiation of a GFD where their vascular risk profile was measured for the purpose of this study but did not attend later coeliac disease reviews

despite repeated appointments sent by the reviewing centres to alert them of the appointment date

The remaining 11 coeliacs were lost to follow-up after index appointment with the hospital where their baseline vascular risk profile was measured. Repeated appointments were sent to offer alternative follow-up appointment dates but to no avail.

The mean score on the self-rated visual analogue scale of compliance to a GFD at 12 months was 9.7 (SD 1.3) cm (maximum score 10 cm). 91% (n = 106) reported strict adherence to GFD with no dietary transgressions with self-rated visual analogue scale score of 10. Of the 11 coeliacs not complying with a strict GFD, 6 reported good (self-rated visual analogue scale score of >/ 8.0 and \<9.9 cm) adherence to a GFD. The remaining 5 coeliacs reported adherence to GFD less than 50% of the time (self-rated visual analogue scale score of <5.0 cm).

Dietitians assessing the compliance of the study participants with a GFD as being optimal in 91% (n = 106), fair in 5% (n = 6) and poor in remaining 4% (n = 5).

The mean tTG titre fell from 112.5 (SD 156.7) iu at diagnosis of coeliac disease to 12.0 (SD 24.8) following treatment (mean difference -100.5; 95%CI -127.2, -73.7).

94% of the 151 incident coeliacs were EMA positive at diagnosis of coeliac disease.

18% of the 117 coeliacs remained EMA positive following treatment.

4.1.3.9. Change in vascular risk profile following 12 months treatment of coeliac disease with a gluten-free diet

4.1.3.9.1. Weight and body mass index

There was mean increase in weight of 2.7 (95%CI 1.9, 3.6) kg following treatment with a GFD (Table 4.38). The gain in mean weight was twice as much in those coeliacs that had villous atrophy present at diagnosis (mean weight change 2.9 kg; 95%CI 1.9, 3.9) in comparison to those coeliacs with mild enteropathy changes (mean weight change 1.1 kg; 95%CI -0.1, 2.3) though the difference in weight gain between the severe and mild enteropathy did not reach statistical significance. Change in weight was not related to presence of villous atrophy; presentation with weight loss, malabsorption; incident weight; incident tTG or change in tTG titre on regression analyses.

65% (n = 49) of men (mean difference in proportion following treatment to at diagnosis 8.9%; 95%CI -10.7, 28.4) were either overweight or obese following 12 months treatment with a GFD. There was a similar but not significant increase in the proportion of obese and overweight female coeliacs following treatment (mean difference in proportion 11.3%; 95%CI -26.0, 3.5). The two morbidly obese coeliacs at diagnosis of coeliac disease put on further weight following 12 months treatment (mean increase in weight 2.6 kg) causing an increase in their BMI. There was no significant change in the proportion underweight following treatment to that observed at diagnosis of coeliac disease.

Table 4.38: Change in anthropometric vascular risk profile in incident coeliac disease following treatment with a gluten-free diet

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean weight kg (SD)				
Men	40	80.5 (17.8)	83.1 (17.4)	2.5 [1.3, 3.7]
Women	78	65.9 (13.8)	68.7 (14.0)	2.9 [1.7, 4.0]
Mean BMI kg/m^2 (SD)				
Men	40	26.3 (4.8)	27.1 (4.5)	0.8 [0.4, 1.2]
Women	78	24.8 (4.8)	25.8 (5.2)	1.0 [0.6, 1.4]
Mean waist circumference cm (SD)				
Men	39	94.5 (13.3)	96.5 (13.2)	2.1 [1.4, 2.7]
Women	78	82.0 (10.5)	84.4 (11.1)	2.3 [1.6, 3.1]
Mean hip circumference cm (SD)				
Men	39	99.2 (11.1)	100.5 (10.6)	1.3 [0.4, 2.2]
Women	78	97.4 (10.9)	98.5 (11.7)	1.1 [0.4, 1.8]
Mean waist: hip ratio (SD)				
Men	39	0.95 (0.1)	0.96 (0.1)	0.01 [-0.01, 0.02]
Women	78	0.84 (0.1)	0.86 (0.1)	0.01 [-0.01, 0.03]
Mean pulse beats/min (SD)				
Men	39	70.8 (SD 11.9)	68.0 (9.9)	-2.8 [-5.8, 0.2]
Women	78	71.8 (SD 12.5)	68.9 (9.3)	-2.8 [-4.7, -1.0]
Mean systolic BP mmHg (SD)				
Men	39	133.3 (21.0)	129.7 (18.2)	-3.5 [-5.7, -1.3]
Women	78	120.4 (14.3)	119.6 (13.7)	-0.8 [-2.2, 0.7]
Mean diastolic BP mmHg (SD)				
Men	39	74.5 (8.6)	72.8 (8.1)	-1.8 [-4.2, 0.7]
Women	78	71.3 (10.1)	69.9 (10.1)	-1.4 [-3.5, 0.8]

The mean weight and BMI of both male and female coeliacs was no different to that of the general population following 12 months treatment with a GFD (Table 4.39). There was also no difference in the proportions between coeliacs and general populations of being underweight, having normal BMI, of being overweight or obese (Table 4.40).

4.1.3.9.2. Waist circumference and waist: hip circumference

There was a mean increase in waist circumference by 2.3 cm (95%CI 1.7, 2.8) following 12 months treatment with a GFD (Table 4.38) with no difference in the gain between men and women. Change in waist circumference was not related to clinical or histological presenting features of coeliac disease; or incident or change in anthropometric profile on regression analyses.

The mean waist circumference in male coeliacs following exposure to a GFD continued to be lower than that observed in the general population (mean difference in waist circumference -8.4 cm; 95%CI -13.7, -3.1) though there was no difference observed between female coeliacs and the general population. However the proportion of coeliacs with a raised waist circumference rose with treatment with coeliacs as likely as the general population to have a raised waist circumference (Table 4.40).

The mean waist: hip ratio increased following treatment with a GFD with a greater change in women than in men (mean difference in waist: hip between men and women 0.12; 95%CI 0.09, 0.14). The change in waist: hip ratio was significantly greater in those coeliacs with mild enteropathy (marsh 1 and 2 changes) present at diagnosis of coeliac disease in comparison to those with villous atrophy. The mean change in waist: hip ratio was 0.05; 95%CI 0.03, 0.10 in those with mild enteropathy

Table 4.39: Vascular anthropometric risk profile in treated coeliacs compared to the general population

Vascular risk profile co-variate	N	Treated coeliac disease	General population	Mean difference [95% CI]
Mean waist circumference cm (SD)				
Men	39	88.94 (0.19)	96.8 (16.9)	-7.9 [-13.2, -2.6]
Women	78	84.07 (23.78)	86.4 (16.6)	-2.3 [-6.1, 1.4]
Mean waist: hip circumference (SD)				
Men	39	0.94 (0.06)	0.9 (0.1)	0.04 [0.01, 0.07]
Women	78	0.86 (0.13)	0.8 (0.1)	0.06 [0.04, 0.08]
Mean BMI kg/m^2 (SD)				
Men	40	27.15 (4.50)	27.2 (5.4)	-0.05 [-1.72, 1.62]
Women	78	25.82 (5.19)	26.8 (6.2)	-0.98 [-2.36, 0.40]

Table 4.40: Prevalence of hypertension and obesity in coeliac disease following GFD

Vascular risk profile co-variate	Observed proportion in general population	Observed number of coeliacs	Expected number of coeliacs	Observed : expected [95%CI]
Hypertension				
Men	31%	19/39	12.09	1.57 [0.86, 2.27]
Women	28%	14/78	21.84	0.64 [0.30, 0.98]
Overweight				
Men	43%	19/40	17.20	1.10 [0.60, 1.60]
Women	32%	23/78	24.96	0.92 [0.54, 1.30]
Obese				
Men	23%	7/40	9.20	0.76 [0.20, 1.32]
Women	25%	14/78	19.50	0.72 [0.34, 1.09]
Raised waist circumference				
Men	32%	10/38	12.16	0.82 [0.31, 1.33]
Women	41%	23/78	31.98	
Raised waist: hip ratio				
Men	33%	23/39	12.87	1.79 [1.03, 2.52]
Women	30%	42/78	23.40	1.79 [1.25, 2.33]

changes. There was no association of change in waist: hip ratio with tTG titre or any other variates.

Despite at diagnosis of coeliac disease coeliacs having no different waist: hip circumference to the general population, following 12 months treatment with a GFD, coeliacs had significantly higher waist: hip ratio than the general population (Table 4.40). For example, the mean difference in waist: hip ratio in male coeliacs to the general population was 0.04; 95%CI 0.01, 0.07. Furthermore, coeliacs were 80% more likely to have raised waist: hip circumferences in comparison to the general population (observed raised waist: hip in male coeliacs versus general population 1.79; 95% 1.03, 2.52).

4.1.3.9.3. Blood pressure and hypertension

Though there was no change in diastolic blood pressure with treatment, there was a fall in systolic blood pressure in male coeliacs (mean difference in systolic blood pressure -3.5 mmHg; 95%CI -5.7, -3.3) but not in female coeliacs (Table 4.38).

4.1.3.9.4. Cholesterol profile

There was a mean increase in total cholesterol by 0.17 (95%CI 0.02, 0.31) following treatment of women with coeliac disease though there was no significant change observed in male coeliacs (Table 4.41). This change in total cholesterol was associated with change in HDL cholesterol and LDL cholesterol but no other variates. For each 1 mmol/L increase in total cholesterol there was a rise in HDL cholesterol by 0.9 mmol/L on regression analysis (p = 0.0001).

The higher proportion of female coeliacs with raised total cholesterol (n = 35; 50%) in comparison to male coeliacs (n = 15; 34%) did not reach statistical significance.

The mean levels of total cholesterol remained significantly lower in coeliacs compared to that of the general population following treatment with a gluten-free diet (Table 4.42). Male coeliacs had -1.3 (95%CI for difference -1.0, -1.7) mmol/L lower total cholesterol and female coeliacs had -0.6 (95%CI for difference -0.9, -0.3) mmol/L lower total cholesterol in comparison to the general population.

Male coeliacs were 40% less likely to have raised total cholesterol (observed: expected 0.60; 95%CI 0.30. 0.90) than the general population. Female coeliacs were as likely to have raised total cholesterol than the general population (Table 4.43).

Table 4.41: Change in cholesterol profile with treatment of coeliac disease

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean cholesterol mmol/L (SD)				
Men	44	4.3 (1.0)	4.4 (1.1)	0.2 [-0.1, 0.4]
Women	68	4.8 (1.2)	5.0 (1.2)	0.2 [0.1, 0.3]
Mean HDL cholesterol mmol/L (SD)				
Men	44	1.1 (0.4)	1.3 (0.4)	0.2 [0.1, 0.2]
Women	68	1.4 (0.4)	1.6 (0.4)	0.2 [0.1, 0.2]
Mean cholesterol:HDL ratio (SD)				
Men	44	4.0 (1.3)	3.6 (1.0)	-0.4 [-0.7, -0.2]
Women	68	3.6 (0.9)	3.3 (0.9)	-0.3 [-0.4, -0.2]
Mean triglyceride mmol/L (SD)				
Men	41	1.4 (0.8)	1.4 (0.6)	0.1 [-0.2, 0.2]
Women	66	1.3 (0.7)	1.3 (0.7)	0.0 [-0.1, 0.1]
Mean LDL cholesterol mmol/L (SD)				
Men	37	2.3 (0.9)	2.5 (1.0)	0.2 [0.1, 0.4]
Women	52	2.8 (0.9)	2.8 (0.9)	-0.1 [-0.2, 0.1]

Table 4.42: Cholesterol profile in treated coeliacs compared to the general population

Vascular risk profile co-variate	N	Treated coeliac disease	General population	Mean difference [95% CI]
Mean cholesterol mmol/L (SD)				
Men	44	3.96 (1.10)	5.30 (1.2)	-1.34 [-1.69, -0.99]
Women	70	4.83 (1.70)	5.40 (1.2)	-0.57 [0.86, -0.28]
Mean HDL cholesterol mmol/L (SD)				
Men	44	1.09 (0.35)	1.3 (0.6)	-0.21 [-0.39, -0.03]
Women	70	1.49 (0.64)	1.6 (0.6)	-0.11 [-0.25, 0.03]
Mean LDL cholesterol mmol/L(SD)				
Men	37	2.52 (0.97)	3.6 (0.9)	-1.07 [-0.39, -0.03]
Women	52	2.79 (0.91)	3.6 (0.9)	-0.81 [-1.07, -0.55]

Table 4.43: Prevalence of dyslipidaemia in coeliac disease following GFD

Vascular risk profile co-variate	Observed proportion in general population	Observed number of coeliacs	Expected number of coeliacs	Observed : expected [95%CI]
Raised total cholesterol				
Men	57%	15/44	25.08	0.60 [0.30, 0.90]
Women	61%	35/70	42.70	0.82 [0.55, 1.09]
Low HDL cholesterol				
Men	9.4%	4/44	4.14	0.97 [0.02, 1.92]
Women	1.8%	0/70	1.26	0
Raised LDL cholesterol				
Men	77%	11/37	28.49	0.39 [0.16, 0.62]
Women	74%	21/52	38.48	0.55 [0.32, 0.78]

Women continued to have a higher HDL cholesterol following treatment with a GFD (1.55 (SD 0.39) mmol/L versus 1.29 (SD 0.36) mmol/L). There was a mean change in HDL cholesterol following treatment by 0.16 (95%CI for difference 0.12, 0.21) mmol/L. Though the change in HDL cholesterol appeared to be greater with the villous atrophy (mean change 0.18 mmol/L versus 0.05 mmol/L in mild enteropathy; p = 0.27), it was only change in tTG (p = 0.019) and change in LDL cholesterol that were the co-variates independently associated with change in HDL cholesterol on regression analysis. For every 50 unit decrease in tTG that occurred on exposure to a GFD, there was a 0.02 mmol/L rise in HDL cholesterol.

Male coeliacs continued to have significantly higher prevalence of low HDL cholesterol than women with coeliac disease following treatment with a GFD (9% (n = 4) versus no female coeliacs).

Only male coeliacs had lower HDL cholesterol than the general population (mean difference in HDL cholesterol -0.21 mmol/L; 95%CI -0.39, -0.03) whereas there was no difference between female coeliacs and the general population following treatment with a GFD (Table 4.43).

Coeliacs were as likely to have low HDL cholesterol in comparison to the general population following treatment with a GFD.

The mean LDL cholesterol changed following treatment with a GFD in men but not in women. Mean LDL cholesterol change was 0.19 mmol (95%CI 0.01, 0.37) in men with GFD. The proportion of female coeliacs with raised LDL did not change with

GFD treatment and remained higher than that observed in male coeliacs (n = 11; 30%).

Coeliacs continued to have significantly lower LDL cholesterol with GFD treatment than that observed in the general population with the effect more marked in men than in women. For example, male coeliacs had -1.1 (95%CI -1.4, -0.8) mmol/L lower LDL cholesterol than the general population. Male coeliacs continued to have significantly lower LDL cholesterol than the general population (observed: expected 0.39; 9%CI 0.16, 0.62) with LDL cholesterol was 55% lower than in the general population in female coeliacs following treatment.

Triglycerides continued to be significantly higher in incident male coeliacs than in females though the mean value did not change on treatment with a GFD (Table 4.41).

4.1.3.9.5. Haemostasis and thrombosis profile

The mean fibrinogen following treatment for coeliac disease continued to be significantly higher in women than in men but there was no significant change following treatment (Table 4.44).

The mean change in platelet count following treatment with a GFD was -38.5 (95%CI -51.2, -25.8) x 10^9/L with no difference in the mean change between men and women. Change in platelet count was not associated with change in total cholesterol or HDL cholesterol. However for every 50 unit fall in tTG that occurred on treatment with a GFD there was a 4 x 10^9/L fall in platelet count observed on regression analysis (p = 0.03).

There was a significant change in the mean APTT following treatment for coeliac disease in men but not in women (mean change 0.9 (95%CI 0.3, 1.4) seconds. Change in APTT was not associated with any variable.

There was also a significant change in mean PT following treatment for coeliac disease in men but not in women. Change in PT was also not associated with any variable.

4.1.3.9.6. Inflammatory markers

The mean CRP in female coeliacs but not male coeliacs changed on exposure to a GFD (mean difference in CRP in female coeliacs following treatment to that at diagnosis of coeliac disease -1.5 g/L; 95%CI -2.6, -0.4). Change in CRP was associated with baseline fibrinogen (p = 0.019).

The mean CRP following treatment was no different to that observed in the general population.

4.1.3.9.7. Glycaemic profile

There was no significant change in the mean random glucose nor the mean HbA1c following treatment with a GFD (Table 4.44). Furthermore there was no significant change in the HbA1c following treatment of coeliacs who were also diabetics with a GFD.

Table 4.44: Change in serum vascular profile with treatment of coeliac disease

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean ferritin µg/L (SD)				
Men	41	40.4 (53.0)	64.2 (49.8)	23.8 [9.3, 38.3]
Women	69	25.7 (33.7)	42.0 (35.7)	16.4 [9.6, 23.1]
Mean folate µg/L (SD)				
Men	43	16.3 (55.7)	11.1 (6.7)	-5.1 [-22.6, 12.3]
Women	68	14.9 (40.1)	10.6 (5.3)	-4.4 [-14.1, 5.4]
Mean ESR mm/hr (SD)				
Men	36	11.3 (11.3)	7.6 (6.3)	-3.7 [-7.3, -0.1]
Women	66	22.6 (21.6)	14.0 (16.1)	-8.7 [-13. 4, -3.9]
Mean CRP mg/L (SD)				
Men	42	3.4 (2.5)	3.4 (3.8)	-0.1 [-1.5, 1.3]
Women	62	4.6 (5.2)	3.1 (3.9)	-1.5 [-2.6, -0.4]
Mean glucose mmol/L (SD)				
Men	30	5.2 (0.7)		
Women	56			
Mean HbA1C % (SD)				
Men	24	6.3 (1.6)	6.1 (1.3)	-0.3 [-0.7, 0.2]
Women	27	5.6 (0.8)	5.6 (1.1)	-0.1 [-0.3, 0.2]
Mean leucocyte count 10^9/L (SD)				
Men	47	7.0 (2.3)	6.7 (1.7)	-0.3 [-0.8, 0.2]
Women	80	6.5 (2.2)	6.4 (1.7)	-0.1 [-0.5, 0.4]
Mean albumin g/L (SD)				
Men	47	40.0 (3.5)	41.1 (3.2)	1.1 [0.1, 2.2]
Women	76	39.3 (3.4)	39.8 (3.1)	0.5 [-0.3, 1.3]

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean platelet count 10^9/L (SD)				
Men	47	285.0 (84.2)	248.3 (62.8)	-36.7 [-56.6, -16.6]
Women	80	323.8 (87.6)	284.2 (70.4)	-39.8 [-56.3, -22.8]
Mean haemoglobin g/L (SD)				
Men	47	12.9 (2.2)	14.2 (1.5)	1.3 [0.7, 1.9]
Women	80	11.9 (1.9)	13.0 (1.1)	1.2 [0.7, 1.6]
Mean fibrinogen g/L (SD)				
Men	38	2.6 (0.8)	2.8 (0.7)	0.1 [-0.1, 0.3]
Women	48	2.9 (1.1)	3.0 (1.1)	0.1 [-0.1, 0.2]
Mean APTT seconds (SD)				
Men	38	25.8 (2.1)	26.6 (1.7)	0.9 [0.3, 1.4]
Women	49	25.5 (2.9)	25.9 (1.9)	0.4 [-0.1, 1.0]

4.1.3.9. Change in vascular disease risk profile following treatment of classic, symptomatic and silent coeliac disease

4.1.3.9.1. Weight and body mass index

The gain in weight observed with a gluten-free diet (Table 4.45) was greatest on treating classic disease and least on treating silent disease (mean difference in weight change 1.1 kg; 95%CI 0.1, 2.3). The increase in mean BMI was however similar between classic, symptomatic and silent disease (Table 4.45). 60% of coeliacs presenting with gastrointestinal symptoms were either overweight or obese following 12 months treatment with a gluten-free diet. Over half (55%) of those coeliacs presenting with silent disease were obese or overweight following 12 months treatment.

4.1.3.9.2. Waist circumference and waist: hip circumference

The mean increase in waist circumference and waist: hip circumference observed following 12 months of a gluten-free diet was similar whether coeliacs had presented with classic, symptomatic or silent disease (Table 4.45).

4.1.3.9.3. Blood pressure and hypertension

There was a significant fall in systolic (mean difference -1.9 mmHg; 95%CI -3.5, -0.3) and diastolic (mean difference -2.3 mmHg; 95%CI -4.7, -0.1) blood pressure observed following treatment of coeliacs that had presented with gastrointestinal symptoms (Table 4.45). The smaller changes in systolic and diastolic blood pressure observed on treating silent and classic coeliac disease with a gluten-free diet did not reach statistical significance.

Table 4.45: Change in anthropometric vascular risk profile in classic, symptomatic and silent coeliac disease treated with 12 months of a gluten-free diet

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean weight kg (SD)				
Classic disease	17	63.8 (12.5)	66.7 (12.9)	2.9 [1.8, 4.0]
Gastrointestinal symptoms	68	73.3 (16.6)	75.3 (16.8)	2.0 [1.5, 2.5]
Silent disease	33	69.4 (17.9)	71.2 (17.5)	1.8 [1.1, 2.5]
Mean BMI kg/m^2 (SD)				
Classic disease	17	22.7 (4.3)	23.6 (4.3)	0.9 [0.5, 1.2]
Gastrointestinal symptoms	68	26.3 (4.8)	27.1 (5.0)	0.9 [0.5, 1.2]
Silent disease	33	24.7 (2.1)	25.8 (4.8)	1.2 [0.4, 1.9]
Mean waist circumference cm (SD)				
Classic disease	16	80.3 (9.5)	82.8 (10.7)	2.4 [1.4, 3.4]
Gastrointestinal symptoms	68	87.9 (13.2)	90.2 (13.6)	2.3 [1.5, 3.1]
Silent disease	33	85.3 (12.9)	87.4 (12.7)	2.1 [1.2, 3.0]
Mean waist: hip ratio (SD)				
Classic disease	16	0.88 (0.07)	0.89 (0.07)	0.01 [-0.01, 0.02]
Gastrointestinal symptoms	68	0.88 (0.08)	0.90 (0.08)	0.01 [-0.01, 0.03]
Silent disease	33	0.87 (0.07)	0.88 (0.07)	0.01 [-0.01, 0.02]
Mean systolic BP mmHg (SD)				
Classic disease	16	123.2 (18.6)	122.4 (16.9)	-0.8 [-3.6, 2.0]
Gastrointestinal symptoms	68	124.2 (16.2)	122.2 (15.7)	-1.9 [-3.5, -0.3]
Silent disease	33	126.4 (20.7)	124.7 (16.5)	-1.7 [-4.4, 1.0]
Mean diastolic BP mmHg (SD)				
Classic disease	16	72.0 (10.9)	72.6 (7.3)	0.5 [-3.5, 4.6]
Gastrointestinal symptoms	68	72.6 (10.1)	70.4 (8.1)	-2.3 [-4.7, -0.1]
Silent disease	33	71.9 (8.5)	71.0 (6.2)	-0.9 [-3.4, 1.7]

4.1.3.9.4. Cholesterol profile

The mean increase in total cholesterol was observed on treating coeliacs presenting with classic disease (mean difference in total cholesterol 0.42 mmol/L; 95%CI 0.03, 0.80) with no significant change in total cholesterol observed on exposing coeliacs with silent or symptomatic disease to a gluten-free diet (Table 4.46). There was a small but significant rise in LDL cholesterol associated with treating coeliacs with classic disease with a gluten-free diet (mean difference in LDL cholesterol 0.21 mmol/L; 95%CI 0.02, 0.45). The mean increase in HDL cholesterol following exposure to a gluten-free diet was similar amongst coeliacs presenting with classic, symptomatic or silent disease (Table 4.46).

Table 4.46: Change in cholesterol profile on treatment of classic, symptomatic and silent coeliac disease

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean cholesterol mmol/L (SD)				
Classic disease	18	4.54 (1.21)	4.96 (1.04)	0.42 [0.03, 0.80]
Gastrointestinal symptoms	58	4.70 (1.16)	4.85 (1.18)	0.15 [-0.01, 0.30]
Silent disease	36	4.51 (1.12)	4.57 (1.27)	0.06 [-0.17, 0.29]
Mean HDL cholesterol mmol/L (SD)				
Classic disease	18	1.34 (0.46)	1.49 (0.41)	0.15 [0.04, 0.26]
Gastrointestinal symptoms	58	1.29 (0.39)	1.47 (0.42)	0.18 [0.13, 0.23]
Silent disease	36	1.24 (0.38)	1.39 (0.35)	0.14 [0.03, 0.25]
Mean triglyceride mmol/L (SD)				
Classic disease	18	1.32 (0.53)	1.42 (0.58)	0.09 [-0.17, 0.36]
Gastrointestinal symptoms	58	1.42 (0.83)	1.34 (0.74)	-0.08 [-0.22, 0.06]
Silent disease	36	1.15 (0.61)	1.26 (0.52)	0.11 [-0.08, 0.30]
Mean LDL cholesterol mmol/L (SD)				
Classic disease	15	2.49 (0.92)	2.70 (0.62)	0.21 [0.02, 0.45]
Gastrointestinal symptoms	49	2.61 (0.92)	2.66 (0.93)	0.05 [-0.12, 0.22]
Silent disease	25	2.77 (1.10)	2.71 (1.14)	-0.06 [-0.27, 1.54]

4.1.3.9.5. Haemostasis and thrombosis profile

There was no significant change in fibrinogen or clotting studies with treatment of classic, symptomatic or silent coeliac disease (Table 4.47). The change in platelet count alluded to in section 4.1.3.8.5 was observed to only occur in those coeliacs presenting with silent coeliac disease (mean difference in platelet count -49.5 x 10^9/L; 95%CI -78.0, -21.1).

4.1.3.9.6. Inflammatory markers

Significant rises in ferritin were observed on treatment of symptomatic and silent coeliac disease with a gluten-free diet (Table 4.47). Treating coeliac disease that had presented with gastrointestinal symptoms was associated with 2g/L fall in CRP (mean difference in CRP -1.6 g/L; 95%CI -2.9, -0.2).

4.1.3.9.7. Anaemia

The greatest improvement in haemoglobin was observed on exposing those coeliacs with silent disease with a gluten-free diet (mean difference in haemoglobin 2.5 g/L; 95%CI 1.7, 3.4).

Table 4.47: Change in serum vascular risk profile on treating classic, symptomatic and silent coeliac disease

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean fibrinogen g/L (SD)				
Classic disease	15	3.07 (1.31)	3.23 (1.60)	0.15 [-0.29, 0.59]
Gastrointestinal symptoms	45	2.84 (0.93)	2.87 (0.76)	0.04 [-0.13, 0.20]
Silent disease	26	2.66 (0.74)	2.81 (0.72)	0.15 [-0.01, 0.32]
Mean APTT seconds (SD)				
Classic disease	15	26.0 (2.3)	26.6 (1.1)	0.6 [-0.5, 1.7]
Gastrointestinal symptoms	45	25.7 (3.1)	26.2 (2.3)	0.5 [-0.1, 1.0]
Silent disease	27	25.3 (1.6)	26.1 (1.3)	0.8 [0.2, 1.5]
Mean platelet count 10^9/L (SD)				
Classic disease	20	295.7 (81.8)	271.9 (83.1)	-23.8 [-50.7, 3.2]
Gastrointestinal symptoms	70	298.9 (79.8)	262.1 (66.3)	-36.9 [53.2, -20.5]
Silent disease	37	336.6 (101.6)	287.0 (67.1)	-49.5 [-78.0, -21.1]
Mean ferritin µg/L (SD)				
Classic disease	17	46.7 (55.2)	59.4 (41.1)	12.6 [-15.4, 40.7]
Gastrointestinal symptoms	60	32.1 (41.7)	51.7 (40.8)	19.7 [11.7, 27.6]
Silent disease	33	21.5 (33.8)	43.0 (46.7)	21.5 [9.7, 33.3]
Mean folate µg/L (SD)				
Classic disease	17	6.1 (4.2)	11.5 (6.3)	5.5 [2.2, 8.8]
Gastrointestinal symptoms	60	17.5 (50.7)	10.9 (5.5)	-6.6 [-19.9, 6.7]
Silent disease	34	16.4 (50.6)	10.1 (6.3)	-6.3 [-23.9, 11.3]
Mean ESR mm/hr (SD)				
Classic disease	15	16.4 (13.1)	17.1 (26.1)	0.7 [-10.7, 12.0]
Gastrointestinal symptoms	56	17.3 (15.8)	11.2 (9.0)	-6.1 [-9.8, -2.5]
Silent disease	31	22.1 (26.4)	10.2 (12.4)	-11.9 [-18.9, -5.0]

Vascular risk profile co-variate	N	At diagnosis of coeliac disease	After 12 months of GFD	Mean difference [95% CI]
Mean CRP mg/L (SD)				
Classic disease	16	4.2 (4.7)	4.0 (1.8)	-0.2 [-2.4, 2.1]
Gastrointestinal symptoms	55	4.7 (5.2)	3.2 (3.1)	-1.6 [-2.9, -0.2]
Silent disease	33	3.1 (1.8)	2.9 (2.6)	-0.2 [-1.4, 1.0]
Mean leucocyte count 10^9/L (SD)				
Classic disease	20	7.0 (2.5)	7.0 (2.0)	0.0 [-1.2, 1.2]
Gastrointestinal symptoms	70	7.0 (2.5)	6.5 (1.7)	-0.5 [-1.0, -0.1]
Silent disease	37	5.8 (1.4)	6.3 (1.5)	0.5 [0.1, 0.9]
Mean albumin g/L (SD)				
Classic disease	20	39.2 (2.9)	40.6 (2.6)	1.5 [-0.1, 3.0]
Gastrointestinal symptoms	66	39.7 (3.7)	40.3 (3.4)	0.7 [-0.2, 1.5]
Silent disease	37	39.6 (3.4)	40.2 (3.1)	0.5 [-0.8, 1.8]
Mean glucose mmol/L (SD)				
Classic disease	14	5.1 (0.6)	5.2 (0.8)	0.1 [-0.6, 0.7]
Gastrointestinal symptoms	49	5.0 (0.6)	5.1 (0.7)	0.2 [-0.1, 0.4]
Silent disease	23	5.0 (0.7)	4.9 (0.7)	-0.1 [-0.4, 0.2]
Mean HbA1C % (SD)				
Classic disease	9	6.2 (1.3)	5.8 (0.9)	-0.4 [-0.7, 0.1]
Gastrointestinal symptoms	22	5.7 (0.8)	5.5 (0.6)	-0.2 [-0.4, -0.1]
Silent disease	20	6.2 (1.7)	6.2 (1.6)	0.0 [-0.5, 0.5]
Mean haemoglobin g/L (SD)				
Classic disease	20	12.5 (1.9)	13.6 (1.5)	1.1 [0.4, 1.8]
Gastrointestinal symptoms	70	12.8 (1.7)	13.4 (1.4)	0.5 [0.2, 0.9]
Silent disease	37	11.0 (2.2)	13.6 (1.4)	2.5 [1.7, 3.4]

4.1.3.10. Vascular disease risk profile in coeliac disease

The observed vascular risk profile in this study suggests both protective and adverse associations of incident coeliac disease (Table 4.48). Exposure to a gluten-free diet resulted in an attenuation or indeed reversal of the vascular risk profile in some co-variates (Table 4.48).

Table 4.48: Vascular risk profile in coeliac disease

Vascular disease risk exposure	Incident coeliac disease	Coeliac disease following 1 year of GFD
Current smoking	Incident coeliacs as likely to general population to smoke: - O:E 0.82; 95%CI 0.52, 1.12	
Obesity	56% males and 36% females were overweight or obese at diagnosis of coeliac disease Incident coeliacs are as likely to be overweight as general population: - O:E 0.81; 95%CI 0.58, 1.05	Mean 2.7 kg increase in weight with GFD Coeliacs remain as likely to be overweight as general population
Abdominal truncal obesity	Lower mean waist circumference than general population: - mean difference -5.3 [-0.9, -9.7] cm in male coeliacs - mean difference -4.8 [-1.4, -8.2] cm in female coeliacs 50% less likely to have raised waist circumference than general population: - O:E 0.52; 95%CI 0.33, 0.71 No difference in mean waist: hip circumference than general population 50% more likely to have raised waist: hip circumference than general population: - O:E 1.59 [95%CI 1.02, 2.16] in male coeliacs - O:E 1.52 [95%CI 1.07, 1.97] in female coeliacs	Mean 2.3 cm increase in waist circumference with GFD Only male coeliacs have lower mean waist circumference than general population Higher mean waist: hip circumference than general population - mean difference 0.04 [95%CI 0.01, 0.07] cm male coeliacs - mean difference 0.06 [95%CI 0.4, 0.08] cm in female coeliacs 80% more likely to have raised waist: hip circumference with GFD than general population: - O:E 1.79; 95%CI 1.03, 2.52

Vascular disease risk exposure	Incident coeliac disease	Coeliac disease following 1 year of GFD
Raised blood pressure	Male coeliacs twice as likely to have measured hypertension than general population: - O:E 1.87; 95%CI 1.22, 2.52	Coeliacs as likely to have measured hypertension as general population following GFD No change in diastolic blood pressure with GFD Fall in systolic blood pressure in male coeliacs with GFD (mean difference -3.5 [95%CI -5.7, -1.3] mmHg)
Diabetes mellitus	Coeliacs as likely as general population to have been diagnosed with diabetes	
Raised total cholesterol	Coeliacs have lower mean total cholesterol than general population: - mean difference -1.1 [95%CI -0.8, -1.4] mmol/L in males - mean difference -0.9 [95%CI -0.5, -1.1] mmol/L in females Coeliacs less likely to have raised total cholesterol than general population: - O:E 0.34 [95%CI 0.14, 0.54] in males - O:E 0.63 [95%CI 0.42, 0.84] in females	Coeliacs continue to have lower mean total cholesterol and less likely to have raised total cholesterol than general population
Raised LDL cholesterol	Coeliacs have lower mean LDL cholesterol than general population: - mean difference -1.3 [95%CI -1.0, -1.6] mmol/L in males - mean difference -0.7 [95%CI -0.4, -1.0] mmol/L in females Coeliacs less likely to have raised LDL cholesterol than general population (O:E 0.29, 95%CI 0.11, 0.47 in males; O:E 0.56, 95%CI 0.34, 0.78 in females)	Coeliacs continue to have lower mean LDL cholesterol and less likely to have raised LDL cholesterol than general population

Vascular disease risk exposure	Incident coeliac disease	Coeliac disease following 1 year of GFD
Low HDL cholesterol	Coeliacs have lower mean HDL cholesterol than general population: - mean difference -0.2 [95%CI -0.1, -0.4] mmol/L in males; -0.3 [95%CI -0.2, -0.4] mmol/L in females Male coeliacs are three times as likely to have low HDL cholesterol than general population: - O:E 3.04; 95%CI 1.55, 4.53	Mean HDL cholesterol increases by 0.2 [95% 0.1, 0.2] mmol/L with GFD Coeliacs are as likely to have low HDL cholesterol as general population
Raised triglycerides	Coeliacs have similar mean triglyceride level to the general population and as likely to have raised triglyceride levels	No change in mean triglyceride level with GFD
Raised fibrinogen	Male coeliacs have lower mean fibrinogen than general population: - mean difference -0.3 [95%CI -0.1, -0.5] g/L	No change in mean fibrinogen with GFD
Raised CRP	Coeliacs have similar mean CRP to the general population and as likely to have raised CRP levels	No change in mean CRP with GFD
Anaemia	50% coeliacs were anaemic at diagnosis of coeliac disease	9% coeliacs were anaemia following GFD
Polycythaemia	0.7% incident coeliacs had haemoglobin values >/ 17 g/L	19% coeliacs had haemoglobin values >/ 17 g/L following GFD
Raised glucose	Mean random glucose and HbA1C were no different to that of general population	No change in random glucose or HbA1C following GFD
Non-Caucasian ethnicity	Higher proportion of Caucasians in coeliac cohort than in general population (93% v 89%)	
Poorer socio-economic class	Rate of diagnosis of coeliac disease twice as high in more affluent socio-economic classes	

4.1.4. Discussion

4.1.4.1. Principal findings

The observed vascular risk profile in this study suggests both protective and adverse associations of incident coeliac disease with subsequent exposure to a gluten-free diet resulting in an attenuation or indeed reversal of the vascular risk profile in some co-variates. The lower mean levels of total cholesterol, LDL cholesterol, fibrinogen; the higher likelihood of being from more affluent social class; and the small but significant rise in HDL cholesterol and reduction in blood pressure amongst coeliacs presenting with gastrointestinal symptoms observed following treatment with a gluten-free diet are features of a favourable vascular risk profile. However, the higher likelihood of having abdominal truncal obesity as reflected by higher raised hip: waist circumference ratios amongst incident coeliacs that only worsens following treatment with a gluten-free diet together with the higher proportion of measured systolic hypertension amongst male coeliacs suggests that there are also potentially adverse vascular risk profile features associated with coeliac disease. Having similar triglyceride, CRP, glucose and glycated haemoglobin levels to the general population as well as coeliacs being as likely to be current smokers, overweight or obese to non-coeliacs suggests these exposures are neutral with respect to vascular disease. Identifying and treating adults with silent coeliac disease does not appear to cause a change in their cholesterol profile but was associated with a fall in platelet count and the greatest rise in haemoglobin in comparison to those coeliacs presenting with classic disease or with gastrointestinal symptoms.

4.1.4.2. Limitations and merits

This is the first prospective, longitudinal study where there has been systematic and routine collection of vascular risk profile in a large, unselected and population-based cohort of adults newly diagnosed with coeliac disease and following treatment with a gluten-free diet. While we did not identify every patient diagnosed in all three hospitals during the period we made extensive efforts to do so with 73% of identified incident coeliacs included in our study. We believe it unlikely that the omission of the few patients that will have been missed will have led to a substantial bias in the observed vascular risk profile and changes with treatment we have reported.

22% of recruited adults newly diagnosed with coeliac disease did not complete the full twelve months of the study with losses to follow-up principally due to non-attendance at follow-up appointments made as part of routine NHS care. One could argue that these coeliacs that were lost to follow-up differ in their attitudes, behaviours and health status compared to those coeliacs who attended routine follow-up hospital appointments, introducing selection bias into the study. However, 15 of the 33 coeliacs lost to follow-up attended for coeliac review as part of routine clinical care at three and six months post-initiation of a gluten-free diet and 6 of the 33 coeliacs actively sought referral for follow-up at their local hospitals due to change of address away from the study centres.

Data from the Health Survey for England 2006 [347] was used as a general population comparator for serum markers of vascular disease and possession of vascular disease diagnoses. With 70% of study participants providing a valid blood sample for testing and the study having over two-thirds response rate (68% households and 88% adults of general population random sample participated) it is

regarded as being representative of the whole population at both national and regional level and provides an useful comparator for the study with measurements specifically taken to assess vascular risk profile in the English general population. However with use of medical records in the coeliac study design adding an extra way of ascertaining diagnosed vascular disease there are differences in the two study designs that may contribute to the differences observed.

With laboratory measurements commonly subjected to extensive and repeated validity checks such as comparing observed measurement with an accepted standard, it is likely the serum co-variates in the study reflect the true state of the phenomenon being measured. Formal and standardised approaches were taken to maximise the validity of other measurements such as the use of a visual analogue scale to assess compliance to the treatment and structured interviews and questionnaires to assess previous diagnoses of vascular disease. However non-differential measurement errors are a source of bias in the study. For example, the measurement of anthropometric co-variates such as height and waist circumference. I tried to minimise measurement error in collection of these co-variates through use of standardised protocols; use of exactly the same blood pressure machine, scales, stadiometer and inelastic tape (sections 4.1.2.6.1. and 4.1.2.6.2.). All anthropometric measurements were performed by the same study investigator though different laboratories with different technicians performed the serum measurements. Simultaneous measurements were taken such as for blood pressure by the same study investigator who was appropriately trained to take blood pressure measurements. Digital scales and a digital blood pressure machine were used to minimise observer error. White coat hypertension may also have affected the measured values obtained [426]. The change in vascular risk profile observed in

the study was associated with exposure with a gluten-free diet / treatment of coeliac disease. However the reduction in quantitative vascular risk profile co-variates may also be attributable to regression towards the mean [427]. One could have incorporated into the study design measures to reduce the effect of regression to the mean such as performing duplicate baseline measurements taken on a different occasion from the first one drawn. However due to ethical constraints such as exposing study participants to a "potentially unpleasant procedure of phlebotomy" as raised by the North Staffordshire Research Ethical Committee, as well as to minimise inconvenience to study participants to maximise continued participation in the study this was not done. At the analysis stage rather than taking differences from the baseline value, analysis of covariance could have been performed [428].

There were missing serum values amongst some of the incident coeliacs. For example, only 86 of the 151 (57%) incident coeliacs had LDL cholesterol measured. The principal reason for this is due to local policy of the hospital concerned where total cholesterol and HDL cholesterol were routinely measured by the laboratory concerned. However there is no reason why the lipoprotein metabolism and lipid profile of incident coeliacs at a neighbouring hospital should behave any differently and any increase in the number of incident coeliacs tested for LDL cholesterol should only act to strengthen the reduction in LDL cholesterol observed. Our study only observed what vascular disease had previously been diagnosed prior to the diagnosis of coeliac disease and measured any change in the anthropometric and serum markers of vascular disease though we did not measure more specific indices of atherosclerosis such as intima – media thickness of extracranial carotid arteries. Such measurement of carotid artery intima – media thickness has not only been shown to be

directly related to the risk of coronary artery disease and stroke disease but it is a relatively easy, practical procedure that allows observation of subclinical atherosclerosis as well as providing a surrogate vascular endpoint requiring shorter study periods in comparison to that that may be needed for overt vascular disease events such as acute myocardial infarction to develop [429-431].

4.1.4.3. Comparison with other studies

Our observation that people with newly diagnosed coeliac disease have lower total cholesterol compared with the general population is consistent with our observations in study 3.4. based on historical cohort of coeliacs and is further discussed in the discussion 3.4.4.3. Our findings again indicate that blood lipids may be a disease marker in coeliac disease with low levels of HDL cholesterol and total cholesterol strongly suggestive of untreated coeliac disease.

Our finding of 40% lower LDL cholesterol in males and 20% lower LDL cholesterol in females newly diagnosed with coeliac disease compared with the general population is new though it was suggested in the only comparable study where West et al found LDL cholesterol levels were 7% lower in endomysial-positive people in comparison to endomysial-negative general population controls [170]. It is unclear why the reductions in LDL (>1.5 mmol/l) and total cholesterol (>1.5 mmol/L) observed in our study are somewhat greater than that observed in West et al [170]. This may be a reflection of 'active' coeliac disease amongst our study participants though the underlying mechanism driving it is not clear. The lack of increase in total cholesterol or LDL cholesterol with treatment of coeliac disease together with BMI and weight of the coeliacs being comparable between the two studies suggests that

any mechanism based on intestinal malabsorption is less likely. Atherosclerosis is an inflammatory disease [273] and like other inflammatory and autoimmune conditions such as rheumatoid arthritis can be ameliorated and also regress [432-434]. The low levels of ferritin due to presumed enteropathy-driven processes such as malabsorption together with the absence of raised serum concentrations of CRP which reflect the underlying presence of activated, immune-competent cells typical of systemic inflammation and atherosclerotic lesions [273] observed in our study may signify that we should be measuring other inflammatory factors and cytokines such as TNF-α, IL-10 to support a role for systemic inflammation in the dyslipidaemia of coeliac disease [435-437]. It is also unclear why such low levels of fibrinogen were observed in our study with processes driving elevated levels such as inflammation, smoking, weight gain counteracted by some other mechanisms [438, 439]. However levels of fibrinogen were strongly related to levels of CRP and triglycerides in our study suggesting that systemic inflammation and or inflammatory processes have some part to play in coeliac disease. Disturbed antioxidant mechanisms in untreated coeliac disease [440] may also have a role to play, promoting oxidative modification of LDL cholesterol with subsequent ladening of macrophages with lipids becoming the characteristic foam cells of the atherosclerotic lesion [441, 442].

Adults with contemporary coeliac disease presenting with normal BMI or who are overweight at diagnosis are increasingly the rule rather than the exception as we have observed in our historical cohort studies in section 3 as well as by other authors [443]. Failure to recognise this may contribute to failed and delayed diagnosis of coeliac disease [219] though of further concern is the potential vascular consequences of the abdominal truncal obesity observed in our study with the waist: hip profile worsened

with treatment with a gluten-free diet. Incident coeliacs in our study were as likely to be overweight or obese as the general population though worryingly were 50% more likely to have raised waist: hip circumference. Such abdominal truncal obesity has been associated in the general population with a number of atherogenic conditions such as elevated systolic and diastolic blood pressure, insulin resistance, glucose intolerance, type 2 diabetes, hypertriglyceridaemia, low HDL cholesterol, high serum apolipoprotein B, high plasma fibrinogen all through which the risk of vascular disease is increased [444-446]. Additional mechanisms through which abdominal truncal obesity could also increase the risk of vascular disease include endothelial dysfunction [447] and adverse haemodynamic changes such as the increase in heart rate, stroke volume [448]. In addition to the adverse metabolic consequences of visceral fat, it also has a distinct role as an endocrine organ [449]. Hormones, cytokines, and polypeptides are secreted by adipose tissue, and thus adipocytes are involved in the regulation of energy homeostasis and neuroendocrine, autonomic and immune functions. These secretory products of adipocytes include leptin, TNF-α, plasminogen activator inhibitor-1 and angiotensinogen. Such products may in turn have an important role in the regulation of vascular risk factors. For example, hyperleptinaemia may induce insulin resistance. Increase synthesis of cytokines such as TNF-α may contribute to dyslipidaemia, insulin resistance and haemostatic disorders. A high plasminogen activator inhibitor-1 level is directly related to a prothrombotic state and angiotensinogen to hypertension. There was a mean increase in weight by 2.7 kg and waist circumference by 2 cm with 12 months treatment with a gluten-free diet which only worsened the abdominal truncal obesity profile; such increases were not limited to those coeliacs presenting with villous atrophy or malabsorption. Whether such anthropometric changes are reflective of high sugar and

fat content of prescribed gluten-free foods or disordered fat storage with gluten exclusion [450, 451] it is unclear though such changes are associated with three-fold increased risk of vascular disease events in the general population [251].

Clinically diagnosed coeliac disease is associated with modest though still increased risks of malignancy and mortality [262, 265, 282, 283, 332-334] though we do not know the risk of complications in silent coeliac disease or whether this can be modified by a gluten-free diet. This study provides some evidence of benefit in identifying those people with silent coeliac disease. The improvement in haemoglobin associated with exposure to a gluten-free diet was greatest in those coeliacs presenting with silent disease than those with classic disease or those presenting with gastrointestinal symptoms. There is no adverse change in total or LDL cholesterol associated with exposure of silent disease with 12 months of a gluten-free diet in comparison to the significant and adverse rises in total and LDL cholesterol observed in classic coeliac disease. The small but significant beneficial rise in HDL cholesterol associated with exposure to a gluten-free diet is also seen with silent coeliac disease. However 61% of silent coeliacs possessed raised waist: hip ratio at diagnosis of coeliac disease which worsened with treatment with a gluten-free diet. With the mean 2 kilogram mean weight gain associated with exposing silent coeliacs to 12 months of a gluten-free diet, 55% silent coeliacs were observed to be overweight or obese at 1 year post-initiation of treatment.

4.1.4.4. Summary

The observed vascular risk profile in this study suggests both protective and adverse associations of incident coeliac disease with subsequent exposure to a gluten-free diet resulting in an attenuation or indeed reversal of the vascular risk profile in some co-variates. The lower mean levels of total cholesterol, LDL cholesterol, fibrinogen; the higher likelihood of being from more affluent social class; and the small but significant rise in HDL cholesterol and reduction in diastolic blood pressure amongst male coeliacs observed following treatment with a gluten-free diet suggests coeliacs have favourable vascular risk profile features in comparison to the general population. However, the higher likelihood of having abdominal truncal obesity as reflected by higher raised hip: waist circumference ratios amongst incident coeliacs that only worsens following treatment with a gluten-free diet together with the higher proportion of measured systolic hypertension amongst male coeliacs suggests that there are also potentially adverse vascular risk profile features associated with coeliac disease. Further work is needed to further explore the mechanisms driving the lower cholesterol profiles and the abdominal truncal weight changes.

4.2. Quality of life at diagnosis of coeliac disease and changes following treatment with a gluten-free diet

4.2.1. Introduction

By definition of coeliac disease, there is a chronic inflammatory state of the small intestinal mucosa that heals when dietary gluten is removed [221]. The beneficial effect of a gluten-free diet in people with classic coeliac disease, such as reduction in the malabsorptive state, has been observed since at least the times of Dicke [452]. We now appreciate that these patients with clinically overt coeliac disease only make up the tip of the coeliac iceberg, accounting for the minority of cases of coeliac disease [367, 453]. The majority of coeliacs have few obvious symptoms despite the presence of the enteropathy, have atypical symptoms or have physiological derangements such as iron deficiency anaemia or osteoporosis [453]. It is not clear whether such silent coeliacs have a reduced quality of life or if quality of life changes following treatment with withdrawal of gluten from the diet. Johnson et al (n = 14) observed silent coeliacs had no different life quality at diagnosis nor following 1 year of treatment in comparison to healthy controls though Nachman et al (n = 8) observed silent coeliacs had significantly worse off quality of life in comparison to controls at diagnosis.

The aim of this longitudinal study is to describe the quality of life at diagnosis of coeliac disease in a large contemporary cohort and to observe any change in life quality following treatment with a gluten-free diet.

4.2.2. Methods

4.2.2.1. Study design

Longitudinal observational study.

4.2.2.2. Study population

Data was collected on quality of life in adults with incident coeliac disease and following their treatment with a gluten-free diet that had attended Nottingham University Hospital, Royal Hallamshire Hospital and Derbyshire Royal Infirmary as described in section 4.1.2.2.

4.2.2.2.1. Classification of coeliac disease within study population

The quality of life between different 'glaciers' of the coeliac iceberg as first defined in study 4.1 in section 4.1.2.2.1. are given below again for further clarity:

Classic symptoms
Those coeliacs presenting with weight loss and diarrhoea.

Gastrointestinal symptoms
Those coeliacs presenting with gastrointestinal symptoms including diarrhoea but in absence of weight loss (would be included as having 'classic symptoms'), weight loss in the absence of diarrhoea (would be included as having 'classic symptoms'), constipation, IBS syndrome, nausea, bloating, steatorrhoea, acid reflux, heartburn, vomiting, abdominal pain.

Silent coeliac disease
Those coeliacs presenting with no gastrointestinal symptoms; or physiological derangements such as anaemia, osteoporosis, deranged liver chemistries in the absence of gastrointestinal symptoms.

4.2.2.3. Inclusion criteria

Adults with incident coeliac disease.

4.2.2.4. Exclusion criteria

Significant co-morbidity that would prevent the study participants being well enough to take part.

4.2.2.5. Outcome measures

- Quality of life in incident coeliac disease presenting with classic symptoms, gastrointestinal symptoms and with silent coeliac disease
- Quality of life at diagnosis of coeliac disease and in comparison to the general population (all coeliacs, coeliacs with classic symptoms, coeliacs with gastrointestinal symptoms, coeliacs with silent disease)
- Change in the quality of life between diagnosis of coeliac disease and following 12 months treatment with a gluten-free diet (all coeliacs, coeliacs with classic symptoms, coeliacs with gastrointestinal symptoms, coeliacs with silent disease)
- Quality of life in coeliacs following 12 months treatment with a gluten-free diet compared to that of the general population
- Presence of gastrointestinal symptoms at diagnosis of coeliac disease and following 12 months treatment with a gluten-free diet.

4.2.2.6. Outcome ascertainment

4.2.2.6.1. Quality of life

Quality of life was assessed with the Short-Form (SF-36) questionnaire. SF-36 is a 36 item questionnaire which measures health functioning on eight scales and is among the most widely used, reproducible validated measure of quality of life in studies of patients [454, 455] and the general population [456] with good internal consistency. The questionnaire is self-administered. A copy of the questionnaire may be found in Appendix 7.4.

Figure 4.25 shows the structure of the SF-36. It consists of 36 questions, 35 of which are compressed into eight multi-item scales. Physical functioning (PF) is a ten-question scale that captures abilities to deal with the physical requirement of life, such as attending to personal needs, walking, and flexibility. Role-physical (RP) is a four-item scale that evaluates the extent to which physical capabilities limit activity. Bodily pain (BP) is a two-item scale that evaluates the perceived amount of pain experienced during the previous four weeks and the extent to which that pain interfered with normal work activities. General health (GH) is a five-item scale that evaluates general health in terms of personal perception. Vitality (VT) is a four-item scale that evaluates feelings of pep, energy, and fatigue. Social functioning (SF) is a two-item scale that evaluates the extent and amount of time, if any, that physical health or emotional problems interfered with family, friends, and other social interactions during the previous four weeks. Role-emotional (RE) is a three-item scale that evaluates the extent, if any, to which emotional factors interfere with work or other activities. Mental health (MH) is a five-item scale that evaluates feelings principally of anxiety and depression. Hence, in the SF36 scoring system, the scales are assessed quantitatively, each on the basis of answers to two to ten multiple choice questions,

and a score between 0 and 100 is then calculated using the summated ratings method on the basis of well-defined guidelines, with a higher score indicating a better state of health [454].

The scales of SF-36 are summarized into two dimensions. The first five scales make up the "physical health" dimension, and the last five form the "mental health" dimension. The scales vitality and general health are parts of both dimensions. Hence, each dimension includes three specific and two overlapping scales [454].

The SF-36 also includes a question about self-evaluation of change in health during the past year (reported health) that does not belong to any score or dimension or the total SF36 score [454].

The scores of the two dimensions and the total SF36 score are based on mathematical averaging of the scale components. Scores are continuous and are therefore suitable for collating into groups and deriving mean scores for comparison between groups at a specific time point or within groups over time. The QualityMetric software [454] also contains internal validity checks to ensure high quality, reproducible results.

Figure 4.25: SF-36 scoring system; scales and dimensions

Scale 1: Physical functioning
3. Vigorous activities
4. Moderate activities
5. Lift, carry groceries
6. Climb several flights
7. Climb one flight
8. Bend, kneel
9. Walk one mile
10. Walk several blocks
11. Walk one block
12. Bathe, dress

Scale 2: Role-Physical
13. Cut down time
14. Accomplished less
15. Limited in kind
16. Had difficulty

Scale 3: Bodily Pain
21. Pain – magnitude
22. Pain – interfere

Scale 4: General Health
1. General health rating
36. Excellent
34. As healthy as anyone
33. Sick easier
35. Health worse

Scale 5: Vitality
23. Pep / life
27. Energy
29. Worn out
31. Tired

Scale 6: Social functioning
32. Social – extent
20. Social – time

Scale 7: Role-Emotional
17. Cut down time
18. Accomplished less
19. Not careful

Scale 8: Mental Health
24. Nervous
25. Down in dumps
26. Peaceful
28. Blue / sad
30. Happy

Dimension A: Physical Health

Dimension B: Mental Health

2. Change in reported health

4.2.2.6.2. Presence of gastrointestinal symptoms

Generic instruments for assessing health-related quality of life do not assess specific aspects of a gastroenterological disease such as the presence and or severity of gastrointestinal symptoms nor the implications of its treatment (such as perceived social and financial restrictions of a gluten-free diet). At the time of designing this study, there was no disease-specific health-related quality of life questionnaire for people with coeliac disease developed and validated. We systematically collected data on the presence or absence of gastrointestinal symptoms including diarrhoea, abdominal pain, bloating, constipation, indigestion using questions from the validated ROME II questionnaire [457, 458]. The questions used of the ROME II questionnaire may be found in Appendix 7.5. The data was collected by means of completion of a questionnaire. In this questionnaire, we also screened for perceived restrictions of both the disease and its management using standard questions (Appendix 7.5).

4.2.2.7. Assessment of compliance with gluten-free diet

Compliance with the gluten-free diet was assessed clinically and immunologically as described in section 4.1.2.7.

4.2.2.8. Controls

Using the norm-based scoring of the SF-36 questionnaire, the quality of life profile of people newly diagnosed with coeliac disease was compared with that of the 1998 American general population. The norm-based scores in the American general population have a mean of 50 and a standard deviation of 10. All quality of life scores of the coeliac cohort using norm-based methods that are below 50 can be interpreted as below the American general population norm; scores above 50 are above the American general population norm. Meaningful comparisons across the scale and

component scores can thereby be made directly and one can observe which domains of health were observed to be most affected by having coeliac disease.

4.2.2.9. Potential confounders

Age, sex, socio-economic class.

Occupational social class and Index of Multiple Deprivation 2007 (IMD07) score were used as measures of socio-economic class with data collected using a standard questionnaire as described in sections 4.1.2.6.4. and 4.1.2.9.

4.2.2.10. Statistical analysis

The mean quality of life SF-36 measures in adults newly diagnosed with coeliac disease were compared with the norm-based scores of the general population using unpaired t-tests to examine for any difference in the mean values. Paired t-tests were used to examine changes in quality of life SF-36 measures from baseline in incident coeliacs to following 12 months treatment with a gluten-free diet.

We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed suing STATA SE 9.2 [TexCorp].

4.2.2.11. Sample size and power

Nachman observed a 27 point increase in physical functioning and 35 point increase in SF-36 quality of life scores following treatment of 59 incident coeliacs presenting with classic symptoms with a gluten-free diet [226]. In contrast there were no significant changes in SF-36 quality of life measures in 8 incident coeliacs presenting with silent disease following treatment [226]. Our sample size aim was to recruit 150

study participants in order to have sufficient power to detect any change in quality of life SF-36 measures in a contemporary cohort including both classic and silent coeliac disease following treatment with a gluten-free diet.

4.2.2.12. Ethical approval

North Staffordshire Local Research Ethics Committee gave ethical approval (reference 06/Q2604/91) to this study in October 2006. Nottingham University Hospital gave research and development approval (reference 06GM012) to this study in January 2007. Derby Hospitals NHS Foundation Trust gave research and development approval (reference DHRD/2007/005) in April 2007. Royal Hallamshire Hospital gave research and development approval (reference STH14597) in March 2007.

4.2.3. Results

4.2.3.1. Study population

151 adults newly diagnosed with coeliac disease were recruited to participate in the study with the study population described further in section 4.1.3.1.

4.2.3.2. Demography of cohort

The demography of the cohort was described as in section 4.1.3.2.

4.2.3.3. Presenting features of incident coeliac disease

The presenting features of the 151 incident coeliacs are described in section 4.1.3.3.

4.2.3.4. Quality of life in adults newly diagnosed with coeliac disease

The quality of life as reflected by SF36 score and broken down into individual scales and dimensions in all incident coeliacs and according to type of coeliac disease is shown in the following table.

Table 5.49: Quality of life in incident classic, symptomatic and silent coeliac disease

Quality of life	All incident coeliacs (n = 151)	Classic disease (n = 22)	Symptomatic disease (n = 85)	Silent disease (n = 44)
Mean SF36 score* (SD)	59.0 (10.6)	48.7 (19.3)	58.4 (19.2)	65.1 (18.6)
Mean SF36 (SD) scales*:				
Physical functioning	69.5 (26.5)	58.1 (29.4)	70.9 (26.6)	72.3 (24.0)
Role: physical	58.5 (50.8)	41.7 (37.4)	61.8 (59.8)	60.2 (33.8)
Bodily pain	57.3 (30.3)	49.8 (27.4)	51.4 (28.6)	72.3 (30.2)
General health	48.7 (27.7)	44.1 (30.8)	47.6 (27.0)	53.2 (27.7)
Vitality	34.2 (23.8)	26.9 (24.7)	33.8 (22.2)	38.3 (26.0)
Social functioning	68.1 (27.4)	47.0 (23.4)	69.2 (26.5)	76.2 (26.1)
Role: emotional	72.5 (32.5)	55.6 (35.6)	73.8 (30.9)	78.1 (32.1)
Mental health	62.6 (20.8)	62.7 (23.2)	64.1 (20.9)	69.9 (18.9)
Mean SF36 (SD) dimensions*:				
Physical health	53.0 (21.5)	44.7 (23.3)	51.8 (20.8)	59.1 (20.5)
Mental health	57.8 (19.7)	47.5 (18.6)	57.6 (19.4)	63.2 (19.3)

*A score between 0 and 100 is given for each of the SF36 scales, for both SF36 dimensions as well as the overall SF36 score. A higher score indicates a better state of health.

The mean SF36 score was highest in those coeliacs presenting with silent disease and lowest in those presenting with classic disease. Silent incident coeliacs had significantly better quality of life than those presenting with classic disease (mean difference in SF36 scores -16.4; 95%CI -26.2, -6.6); p = 0.001. Silent incident coeliacs had also significantly better quality of life than those coeliacs presenting with gastrointestinal symptoms (mean difference in SF36 scores -6.7, 95%CI -13.7, -0.3); p = 0.05.

Silent coeliac disease appeared to have better quality of life than those coeliacs with classic disease because of:

- better physical functioning (mean difference in scores -14.2; 95%CI -27.7, -0.7),
- less bodily pain (mean difference in scores -22.5; 95%CI -37.8, -7.2),
- better social functioning (mean difference in scores -29.2; 95%CI -42.4, -16.0),
- better emotional status (mean difference in scores -22.5; 95%CI -39.9, -5.1)

resulting in significantly better physical health (mean difference in scores -14.4; 95%CI -25.6, -3.2) and mental health (mean difference in scores -15.7; 95%CI -25.6, -5.8).

Those coeliacs presenting with gastrointestinal symptoms appeared to have better quality of life than those presenting with classic disease due to:

- better physical functioning (mean difference in scores -12.8; 95%CI -25.7, -0.1)
- better social functioning (mean difference in scores -22.2; 95%CI -34.5, -9.9)
- better emotional status (mean difference in scores -18.2; 95%CI -33.3, -3.1)

resulting in significantly better mental health (mean difference in scores -10.1; 95%CI -19.2, -1.0) but not physical health.

Vitality was the lowest scoring scale of the SF36 profile (mean score in all incident coeliacs 34.2 (SD 23.8)) with no difference in vitality scores between coeliacs with silent, symptomatic and classic disease.

The self-reported rating of change of health from when coeliac disease was diagnosed to a year prior to diagnosis varied according to mode of presentation of coeliac

disease. The majority (61.4%; 95%CI 47.0, 75.8) of coeliacs presenting with silent disease reported no change in health from diagnosis to a year prior to diagnosis. In comparison, those coeliacs presenting with classic disease (76.0%; 95%CI 57.8, 94.3) and with symptoms (63.5%; 95%CI 53.2, 73.7) reported that their health was much worse or somewhat worse at diagnosis of coeliac disease compared to a year prior to diagnosis. Only 9% of silent coeliacs avoided eating outside the home such as in restaurants or at friends which was significantly lower than the 38% of classic coeliacs (mean difference in proportion 0.29; 95%CI 0.07, 0.51) and 26% of coeliacs presenting with gastrointestinal symptoms (mean difference in proportion 0.17; 95%CI 0.04, 0.29).

4.2.3.5. Quality of life in adults newly diagnosed with coeliac disease compared to the general population

The quality of life in adults newly diagnosed with coeliac disease was compared to that of the general population (Table 4.50).

Table 4.50: Quality of life in incident coeliac disease compared to the general population

Quality of life	General population	All incident coeliacs (n = 151)	Classic disease (n = 22)	Symptomatic disease (n = 85)	Silent disease (n = 44)
Mean norm-based SF36 (SD) scales*:					
Physical functioning	50 (10)	44.6 (11.5)	40.0 (12.2)	45.3 (11.9)	45.3 (10.0)
Role: physical	50 (10)	43.7 (10.1)	40.1 (10.8)	44.0 (10.0)	44.9 (9.6)
Bodily pain	50 (10)	44.5 (13.7)	40.9 (12.2)	42.4 (13.6)	50.6 (12.9)
General health	50 (10)	39.9 (13.8)	39.4 (18.1)	39.2 (13.1)	41.7 (12.8)
Vitality	50 (10)	38.9 (11.2)	35.3 (11.9)	39.0 (10.5)	40.6 (11.8)
Social functioning	50 (10)	43.5 (12.4)	34.8 (10.7)	44.1 (12.4)	46.5 (11.3)
Role: emotional	50 (10)	46.5 (10.3)	41.2 (11.2)	47.0 (9.8)	48.2 (10.2)
Mental health	50 (10)	44.8 (12.0)	44.0 (14.6)	43.9 (11.9)	46.9 (10.8)
Mean norm-based SF36 (SD) dimensions*:					
Physical health	50 (10)	42.4 (10.8)	39.1 (11.5)	41.9 (10.9)	45.1 (9.9)
Mental health	50 (10)	43.6 (11.4)	37.4 (12.4)	43.7 (11.3)	46.3 (10.4)

* A score between 0 and 50 is given for each of the norm-based SF36 scales and for both SF36 dimensions. A score above 50 indicates a better state of health than that reported by the general population. A score below 50 can be interpreted as a state of health that is worse off in comparison to that reported by the general population.

The overall quality of life of incident coeliacs was significantly lower than that of the general population regardless of whether they had presented with classic, symptomatic or silent disease (Table 4.50).

Those coeliacs presenting with silent coeliac disease had lower physical (mean difference in scores -4.9; 95%CI -8.5, -1.3) and mental health dimensions (mean difference in scores -3.7; 95%CI -7.3, -0.1) than that observed in the general population due to:

- worse physical functioning (mean difference in scores -4.7; 95%CI -8.3, -1.1)
- worse performance in physical roles (mean difference in scores -5.1; 95%CI 8.6, -1.6)
- worse general health (mean difference in scores -8.3; 95%CI -12.2, -4.4)
- lower vitality (mean difference in scores -9.4; 95%CI -13.2, -5.6)

though had no difference in social functioning, bodily pain or performance in emotional roles.

Each of the scales measuring different aspects of quality of life was significantly lower in coeliacs presenting with classic symptoms of coeliac disease compared to the general population:

- worse physical functioning (mean difference in scores -10.0; 95%CI -14.8, -5.1)
- worse performance in physical roles (mean difference in scores -9.9; 95%CI -14.6, -2.4)
- worse bodily pain (mean difference in scores -9.1; 95%CI -4.2, -2.5)
- worse general health (mean difference in scores -10.6; 95%CI -16.1, -5.1)
- lower vitality (mean difference in scores -14.7; 95%CI -19.5, -9.9)
- worse social functioning (mean difference in scores -15.2; 95%CI -19.9, -10.5)
- worse performance in emotional roles (mean difference in scores -8.8; 95%CI -13.6, -4.0)
- worse mental health (mean difference in scores -6.0; 95%CI -11.1, -0.9)

Each and every scale measuring different components of quality of life in coeliacs presenting with gastrointestinal symptoms was also significantly lower than that observed in the general population (data not shown).

4.2.3.6. Continued participation in study and adherence to gluten-free diet

Any losses to follow-up from the study and adherence of the study participants to gluten-free diet are further described in section 4.1.3.8.

4.2.3.7. Change in quality of life following 12 months treatment of coeliac disease with a gluten-free diet

Following 12 months treatment with a gluten-free diet, there was a significant reduction in the prevalence of gastrointestinal symptoms including diarrhoea (mean difference in proportion with diarrhoea following treatment to that at diagnosis -0.45; 95% -0.56, -0.34), abdominal pain, and heartburn.

Following exposure to 12 months gluten-free diet, there was a significant improvement in the quality of life measured in the coeliacs (mean difference in SF36 score 18.5; 95%CI 15.4, 21.6); p = 0.00001 (Table 4.51).

Table 4.51: Change in quality of life following 12-months treatment with a gluten-free diet

Quality of life	At diagnosis of coeliac disease (SD)	Following 12 months GFD (SD)	Mean difference [95%CI]
Mean SF36 score* (SD)			
All coeliacs (n = 118)	59.1 (19.5)	77.7 (16.5)	18.5 [15.4, 21.6]
Classic disease (n = 17)	48.5 (18.8)	67.1 (25.1)	18.7 [8.4, 28.9]
Symptomatic disease (n = 68)	57.4 (18.5)	77.4 (14.1)	20.0 [15.9, 24.1]
Silent disease (n = 33)	67.6 (18.9)	83.2 (14.3)	15.5 [9.9, 21.2]
Mean SF36 (SD) scales*			
Physical functioning			
All coeliacs	70.2 (25.9)	82.9 (22.3)	12.7 [9.5, 16.0]
Classic	60.7 (30.5)	72.3 (27.3)	11.7 [-0.6, 23.9]
Symptomatic	70.4 (25.4)	83.4 (21.3)	13.1 [8.7, 17.4]
Silent	74.2 (24.4)	86.8 (20.8)	12.6 [7.3, 17.9]
Role: physical			
All coeliacs	60.0 (53.9)	79.8 (25.6)	19.8 [10.3, 29.4]
Classic	43.3 (35.9)	65.0 (33.8)	21.7 [5.2, 38.1]
Symptomatic	62.7 (64.4)	80.7 (23.6)	18.0 [2.6, 33.4]
Silent	62.1 (33.1)	84.8 (23.3)	22.7 [12.0, 33.4]
Bodily pain			
All coeliacs	57.3 (30.8)	80.8 (22.8)	23.4 [18.5, 28.4]
Classic	51.6 (28.8)	68.3 (30.3)	16.7 [3.9, 29.4]
Symptomatic	50.1 (28.6)	79.4 (22.0)	29.3 [22.8, 35.8]
Silent	74.6 (29.9)	89.3 (17.2)	14.7 [5.4, 24.0]
General health			
All coeliacs	49.5 (27.8)	67.8 (26.2)	18.3 [14.4, 22.2]
Classic	43.1 (28.8)	59.5 (36.0)	16.5 [5.3, 27.7]
Symptomatic	46.5 (27.0)	67.1 (23.7)	20.6 [15.2, 25.9]
Silent	58.5 (27.1)	73.0 (25.5)	14.5 [7.3, 21.7]
Vitality			
All coeliacs	33.4 (25.0)	58.4 (21.0)	25.0 [20.4, 29.6]
Classic	24.3 (23.1)	52.0 (21.1)	27.7 [11.2, 44.1]
Symptomatic	32.5 (23.1)	57.2 (20.7)	24.7 [18.6, 30.7]
Silent	39.2 (28.6)	63.8 (21.1)	24.5 [16.1, 32.9]
Social functioning			
All coeliacs	68.8 (27.2)	85.8 (19.4)	17.0 [12.6, 21.4]
Classic	48.3 (19.5)	70.1 (29.0)	21.7 [10.2, 33.3]
Symptomatic	67.6 (27.3)	87.0 (16.4)	19.5 [13.5, 25.5]
Silent	80.7 (24.2)	90.6 (16.6)	9.8 [2.2, 17.5]
Role: emotional			
All coeliacs	72.5 (31.3)	93.0 (15.1)	20.5 [15.0, 25.9]
Classic	53.4 (35.3)	84.5 (21.3)	31.1 [14.8, 47.5]
Symptomatic	73.2 (29.8)	92.4 (15.4)	19.2 [12.0, 26.4]
Silent	79.9 (29.9)	98.0 (8.0)	18.1 [7.4, 28.8]

Quality of life	At diagnosis of coeliac disease (SD)	Following 12 months GFD (SD)	Mean difference [95%CI]
Mean SF36 (SD) scales*			
Mental health			
All coeliacs	65.0 (21.4)	74.5 (19.4)	9.4 [6.5, 12.4]
Classic	58.4 (25.7)	68.8 (27.0)	10.4 [-0.5, 20.9]
Symptomatic	63.3 (21.1)	73.5 (18.2)	10.1 [6.1, 14.2]
Silent	71.5 (17.1)	79.0 (17.1)	7.5 [2.9, 12.1]
Mean SF36 (SD) dimensions*:			
Physical health			
All coeliacs	53.2 (21.3)	73.8 (18.9)	20.5 [17.1, 23.9]
Classic	45.5 (22.2)	62.9 (27.3)	17.5 [6.1, 28.8]
Symptomatic	50. 9 (20.1)	73.4 (16.3)	22.5 [18.1, 26.9]
Silent	61.6 (21.3)	79.4 (17.6)	17.8 [11.7, 23.9]
Mental health			
All coeliacs	57.9 (20.1)	75.7 (16.3)	17.8 [14.5, 21.0]
Classic	45.9 (18.4)	66.9 (24.1)	21.1 [10.4, 31.7]
Symptomatic	56.6 (19.3)	75.1 (14.6)	18.5 [14.0, 22.9]
Silent	66.1 (19.4)	80.8 (13.7)	14.8 [9.1, 20.4]

*A score between 0 and 100 is given for each of the SF36 scales, for both SF36 dimensions as well as the overall SF36 score. A higher score indicates a better state of health.

The improvement in SF36 score with treatment with a gluten-free diet was similar whether the coeliacs had presented with gastrointestinal symptoms, with classic disease or with silent disease (mean percentage change in SF36 score in classic disease 38.4%, 95%CI 13.8, 63.0 versus 23.1%, 95%CI 8.7, 37.4).

There was no difference in the degree of change in physical functioning or performance in physical roles with a gluten-free diet whether the initial presentation had been with classic, silent or symptomatic coeliac disease. Improvement in social functioning and general health appeared to be greater amongst those coeliacs presenting with gastrointestinal symptoms than with silent disease. For example, the difference in mean % change between symptomatic and silent disease for social functioning was 19.6%; 95%CI 0.7, 38.5 with p = 0.06. However, improvement in

performance in emotional roles was significantly greater on treating symptomatic disease than that observed on treating silent coeliac disease (difference in mean % change symptomatic versus silent disease 35.6%; 95%CI 6.8, 64.4). Rather unsurprisingly, those coeliacs presenting with gastrointestinal symptoms had much higher improvement in pain control with treatment (mean % change in bodily pain 58.4%; 95%CI 46.6, 70.2) than those coeliacs with silent disease (mean % change in bodily pain 19.7%; 95%CI 6.1, 33.3).

There was no difference in proportion of silent, classic and symptomatic coeliacs who found it difficult to establish whether foods were gluten-free ($p > 0.05$). However both classic and symptomatic coeliacs were more likely to avoid eating outside the home in comparison to those coeliacs with silent disease. For example, 75% (95%CI 53.8, 96.2) of those coeliacs presenting with classic disease avoided eating out in comparison to 27% (95%CI 12.1, 42.5) of those coeliacs with silent disease.

4.2.3.8. Quality of life following 12 months treatment of coeliac disease compared to the general population

The quality of life of the coeliacs following 12 months treatment with a gluten-free diet was compared to the quality of life observed in the general population (Table 4.52).

Table 4.52: Quality of life following 12 months treatment of coeliac disease compared to the general population

Quality of life	General population	All treated coeliacs (n = 113)	Treated classic disease (n = 15)	Treated symptomatic disease (n = 66)	Treated silent disease (n = 32)
Mean norm-based SF36 (SD) scales*:					
Physical functioning	50 (10)	50.6 (10.1)	47.8 (15.3)	50.4 (8.8)	52.3 (9.7)
Role: physical	50 (10)	51.3 (9.8)	49.2 (16.8)	50.8 (6.7)	53.2 (10.8)
Bodily pain	50 (10)	55.1 (10.7)	50.6 (14.5)	54.2 (9.2)	59.2 (10.5)
General health	50 (10)	49.2 (12.6)	45.6 (16.8)	48.8 (11.0)	51.8 (13.2)
Vitality	50 (10)	50.8 (9.8)	47.9 (10.0)	50.4 (9.6)	53.1 (9.9)
Social functioning	50 (10)	51.6 (8.9)	45.5 (13.4)	51.8 (6.6)	54.0 (9.5)
Role: emotional	50 (10)	53.9 (7.8)	53.4 (14.5)	53.0 (4.8)	56.0 (8.4)
Mental health	50 (10)	49.9 (11.7)	47.8 (16.3)	48.9 (10.3)	53.0 (11.7)
Mean norm-based SF36 (SD) dimensions*:					
Physical health	50 (10)	50. 9 (8.8)	46.3 (12.2)	51.0 (7.7)	52.8 (8.5)
Mental health	50 (10)	50.9 (7.8)	47.8 (10.3)	50.7 (7.5)	53.1 (6.6)

* A score between 0 and 50 is given for each of the norm-based SF36 scales and for both SF36 dimensions. A score above 50 indicates a better state of health than that reported by the general population. A score below 50 can be interpreted as a state of health that is worse off in comparison to that reported by the general population.

Following 12 months treatment with a gluten-free diet, those coeliacs with classic disease had no different reported quality of life to that observed in the general population ($p > 0.05$). Treating coeliacs that presented with gastrointestinal symptoms resulted in a significant improvement in their quality of life so that their SF36 scores following 12 months exposure to a gluten-free diet was no different to that observed in the general population. Furthermore, performance within emotional roles (mean difference in norm-based scores 3.0; 95%CI 3.9, 5.6) and pain control was even better in those with treated symptomatic coeliac disease than that observed in the general population. Coeliacs presenting with silent disease had similar or even better reported quality of life than that observed in the general population. Treating silent coeliacs resulted in better social functioning (mean difference in norm-based scores 4.0; 95%CI 0.2, 8.0), better performance in emotional roles (mean difference in norm-based scores 6.0; 95%CI 2.1, 9.9) and less pain than that observed in the general population.

4.2.4. Discussion

4.2.4.1. Principal findings

Silent disease was a common mode of presentation of coeliac disease, affecting one-third of a contemporary and representative cohort of adults newly diagnosed with coeliac disease. Though incident coeliacs with silent disease reported no change in their quality of life prior to diagnosis of coeliac disease, silent coeliacs were as likely to have villous atrophy and physiological derangement to those coeliacs presenting with symptoms or with classic features of coeliac disease. The quality of life reported by incident silent coeliacs was worse than that observed in the general population, principally due to reductions in physical functioning which may be a reflection of underlying malabsorptive and inflammatory processes. Coeliacs presenting with classic disease or with gastrointestinal symptoms have significantly lower reported quality of life in comparison to those presenting with silent disease, with both physical and mental health dimensions affected. The quality of life in coeliacs presenting with classic disease or with gastrointestinal symptoms was worse than that observed in the general population with the difference more marked than that observed in silent coeliac disease. A year's treatment with a gluten-free diet not only caused a significant reduction in the prevalence of gastrointestinal symptoms but caused a beneficial improvement in the quality of life experienced by the coeliacs. Such an improvement with treatment resulted in coeliacs having similar or in some components better quality of life than that observed in the general population. The rate of change of quality of life was similar amongst those coeliacs with silent, classic or symptomatic disease.

4.2.4.2. Limitations and merits

This is the largest, prospective and longitudinal study where there has been systematic and routine collection of quality of life measures in an unselected, contemporary and population-based cohort of adults newly diagnosed with coeliac disease and following treatment with a gluten-free diet. One published study that has assessed a longitudinal change in quality of life was based on only 14 screen detected coeliacs and 17 symptomatic coeliacs [225] whilst the only other published study to evaluate longitudinal change from diagnosis had only 8 coeliacs with silent disease whose quality of life could only be measured at baseline and at a 3-month visit [226]. While we did not identify every patient diagnosed in all three hospitals during the period we made extensive efforts to do so with 73% of identified incident coeliacs included in our study. We believe it unlikely that the omission of the few patients that have been missed will have led to a biased estimate of the quality of life profile and change with treatment we have reported.

22% of recruited adults newly diagnosed with coeliac disease did not complete the full twelve months of the study with losses to follow-up principally due to non-attendance at follow-up appointments made as part of routine NHS care. One could argue that these coeliacs that were lost to follow-up differ in their attitudes, behaviours and health status compared to those coeliacs who attended routine follow-up hospital appointments, introducing selection bias into the study. However, 15 of the 33 coeliacs lost to follow-up attended for coeliac review as part of routine clinical care at three and six months post-initiation of a gluten-free diet and 6 of the 33 coeliacs actively sought referral for follow-up at their local hospitals due to change of address away from the study centres.

At the time of designing this study, a disease-specific health-related quality of life questionnaire for people with coeliac disease had not yet been developed or validated according to recognised guidelines [459, 460]. Though not able to assess specific aspects of a disease such as the potential social and financial restrictions of a gluten-free diet, we elected to use the SF36 questionnaire to assess quality of life in our study as a generic measure of health status, proven useful in estimating the relative burden of a disease, differentiating the health benefits produced by treatment and comparing subgroups of different disease modes [461]. The health concepts measured in SF36 represent the most frequently measured concepts in widely-used health surveys that have been shown to be affect by disease and treatment [462]. It also measures multiple operational definitions of health, including function and dysfunction, distress and well-being, objective reports and subjective ratings and both favourable and unfavourable self-evaluations of general health status [462]. The reliability of SF36's scales and summary measures, estimated using both internal consistency and test-retest methods, is in the order of >0.70 [461]. Validity studies in the literature have compared SF36 with some 225 other measures supporting its performance [461].

Data from the American general population was used as a general population comparator. Norms can be seen to provide anchors to interpret an individual's or a group's score in relation to those of others and thus norm-based scores can be seen as departures from typical values. Several studies have demonstrated the worth of norm-based scoring in assessing the impact of disease in more than 200 different diseases including chronic conditions such as emphysema, diabetes, rheumatoid arthritis, irritable bowel syndrome [463-466]. However, differences have been observed in studies comparing normative data of non-American countries to that of the American

general population sample used in SF36 [467-469] and are a limitation to our study. Such differences are thought to be due to a combination of cultural differences; different age ranges of participants used in the studies (18 – 65 years in American normative data versus 25 – 75 years in Canadian study); and perhaps artefactual effects related to translation such as discrepancies in methods and definitions.

In the absence of a developed and validated questionnaire to assess the disease-specific health-related quality of life for people with coeliac disease, we systematically collected data on the presence or absence of gastrointestinal symptoms including diarrhoea, abdominal pain, bloating, constipation, indigestion using questions from the validated ROME II questionnaire [457, 458] in an attempt to assess specific aspects of coeliac disease. We also systematically collected data on the presence or absence of social and financial restrictions of a gluten-free diet and potential difficulties in following a gluten-free diet using a standard set of questions. Such questions were specifically designed to try to include the dimensions of the construct we wished to measure to maximise the content validity of the questioning. Formal and standardised approaches were also taken to maximise the validity of measurements taken such as the use of a visual analogue scale to assess compliance to the treatment. The change in quality of life observed in the study was associated with exposure with a gluten-free diet / treatment of coeliac disease. However such changes in quality of life observed may also be attributable to regression towards the mean [427] as discussed in section 4.1.4.2.

4.2.4.3. Comparison with other studies

Our observation that adults with newly diagnosed coeliac disease presenting with silent disease (i.e. no gastrointestinal symptoms but have evidence of physiological derangements) have worse quality of life compared with the general population is different to the observations of the two published studies that have evaluated change in SF36 with treatment of silent disease with a gluten-free diet. This may reflect the choice of controls in the study by Johnston et al [225] who had volunteered to participate in a serological screening survey rather than be representative of the general population as a whole. The lack of significant change in SF36 in the 8 silent coeliacs in the Nachman study [226] could be due to the short time treated with a gluten-free diet (only three months) and or reflective of the low sample number. The use of "healthy hospital staff" as controls introducing healthy worker selection bias further limited this study. The odds of reported general health as "good or excellent" was not statistically different between EMA-positive and EMA-negative general population controls though no validated or comprehensive quality of life assessment was performed [170].

The reduction in quality of life in incident coeliacs with silent disease compared to the general population was principally due to reduced physical dimensions such as performance in physical roles, physical functioning and vitality. Though silent coeliacs were as likely to have villous atrophy and elevated tissue transglutaminase titres as those coeliacs presenting with symptomatic disease, silent coeliacs had significantly lower haemoglobin values raising the possibility that the lower haemoglobin could be implicated in impairing physical performance and functioning [470]. Fatigue (overlap of feelings of muscle weakness, tiredness, fatigability [471]) has been reported in adults newly diagnosed with coeliac disease raising the

possibility by some authors that fatigue could be a gluten-related symptom [472] or related to depressive symptoms [473] that ameliorates with gluten exclusion. There was no objective evidence of depression in the silent coeliacs in our study.

Our finding that adults with newly diagnosed coeliac disease presenting with overt symptoms have significantly lower quality of life compared with the general population and coeliacs presenting with silent disease is consistent with the observations by the two other published studies [225, 226]. Though symptomatic coeliacs were as likely to have villous atrophy and elevated tissue transglutaminase titres as those coeliacs presenting with silent disease, one might speculate the reduced quality of life observed in symptomatic disease relative to silent disease could be reflective of both the disease symptoms, dietary restrictions due to fear of exacerbation of disease symptoms, avoidance of social situations due to potential exacerbation of the disease symptoms leading to difficulties in daily social and emotional functioning, dietary restrictions. Our observations of coeliacs with symptomatic disease tending to avoidance of eating outside the home are supportive of such speculation.

4.2.4.4. Summary

Silent disease is a common mode of presentation of coeliac disease with similar likelihood of having villous atrophy and physiological derangements to those coeliacs presenting with gastrointestinal symptoms or with classic features of coeliac disease. Though incident coeliacs with silent disease reported no change in their quality of life prior to diagnosis of coeliac disease, their quality of life is worse than that observed in the general population, principally due to reductions in physical functioning which may be a reflection of underlying malabsorptive and inflammatory processes. Coeliacs presenting with classic disease or with gastrointestinal symptoms have significantly lower reported quality of life in comparison to those presenting with silent disease, with both physical and mental health dimensions affected, and much lower quality of life than that observed in the general population. A year's treatment with a gluten-free diet is associated with a significant reduction in the prevalence of gastrointestinal symptoms amongst coeliacs but also with a beneficial improvement in their quality of life experienced. Such an improvement with treatment results in coeliacs having similar or indeed, in some facets, better quality of life than that observed in the general population. The rate of change of quality of life is similar amongst those coeliacs with silent, classic or symptomatic disease.

Chapter five: Cross-sectional survey of women with coeliac disease

Using the Coeliac UK population-based cohort and the Nottingham, Sheffield and Derby historical coeliac disease cohorts a cross-sectional survey was performed with the aim to:

- describe the breast cancer risk profile in women with coeliac disease and compare to that of the general population

5.1. Understanding the reduced risks of breast cancer in women with coeliac disease

5.1.1. Introduction

Breast cancer is the commonest malignancy in women, comprising 18% of all female cancers and having a prevalence of approximately 2% in the British general population [288]. Established high-risk demographic factors for female breast cancer are:

- advancing age (relative risk > 10 with each 10 year increase during the reproductive years until the menopause)
- Caucasian race (relative risk >5 to Asians)
- higher socioeconomic status (relative risk 2 for social classes I and II to other classes) [289, 291, 292].

Many of the other established aetiological factors of breast cancer are linked to oestrogens with hormonal factors playing a key role as described in section 1.7.6.1. and include:

- early onset of menarche (relative risk 3 for age at menarche before age 11 years) [294, 474]
- later onset of menopause (relative risk 2 for menopause after age 54 years) [293, 294]
- later age at first birth (two-fold increased risk in women who have their first child after the age of 30 years to those who have their first child before the age of 20) [295]
- nulliparity (compared with nulliparous women, women who have had at least one full-term pregnancy have on average 25% reduction in breast cancer risk [296])

- proliferative breast lesions (with atypia at least four-fold increased risk or without atypia has at least two-fold increased risk of breast cancer [297])
- family (a woman's risk of breast cancer is two or more times greater if she has a first degree relative who developed the disease before the age of 50 years with the younger the relative when she developed breast cancer the greater the risk [298])
- current use of oral contraceptives (relative risk 1.24) [289, 302]
- use of hormone replacement therapy for more than 10 years (relative risk 1.34) [289, 302]
- post-menopausal obesity, increasing adult height, high alcohol intake, high intake of saturated fat and folate are also associated with increased breast cancer risk (relative risk 1.3 – 1.5) [303-309]

Regular menstrual cycles (relative risk 0.76; 95%CI 0.62, 0.94 for <1 year to onset of regular menstrual cycles) [300]; breastfeeding (pooled odds ratio 0.84; 95%CI 0.78, 0.91) [299]; severe caloric restriction caused by anorexia nervosa prior to age 40 years (50% lower incidence of breast cancer) [301] have observed to have a protective effect upon breast cancer risk.

Population attributable risk (PAR) estimates suggest that age at first birth at >29 years, nulliparity, menarche before the age of 14 years, family history of breast cancer in 1st degree relative and history of benign breast disease account for the largest fraction of breast cancer cases in published studies (e.g. PAR 29.5% for age at first birth > 29 years and nulliparity in white women in United States ; 95%CI 5.6, 53.3) [292, 310].

Several studies have observed that coeliac disease is associated with more than a 50% reduced risk of breast cancer [265, 282, 283, 287, 318] though the reasons for this reduction are unclear. Later age at menarche and earlier onset of menopause has been observed in women with coeliac disease in studies using small, selected populations [320-323] though a more recent and population-based study observed female coeliacs had similar fertility to that of the female general population though female coeliacs tended to have their babies at an older age [324]. Short stature, low body mass, caloric restriction, fat and folate deficiencies associated with coeliac disease [170, 221, 325] may also be implicated in the apparent reduced risk of breast cancer in women with coeliac disease.

The aim of this chapter is to describe the hormonal, reproductive and anthropometric breast cancer risk profile in female incident and prevalent cases of coeliac disease and compare with that of the general population using age- and sex-matched controls to help understand the reduced risk of breast cancer in coeliac disease.

5.1.2. Methods

5.1.2.1. Study design

Cross-sectional survey.

5.1.2.2. Study population

5.1.2.2.1. Historical cohort

Data was collected on the breast cancer risk profile in incident and prevalent adult women that have attended Nottingham University Hospital, Nottingham; Royal Hallamshire Hospital, Sheffield; or Derby Hospitals NHS Foundation Trust for management of their coeliac disease. The study cohort is further described as in sections 2.2.1, 2.2.2 and 2.2.3., respectively. Consecutive cases of incident coeliac

disease were identified at Nottingham University Hospital using clinical and dietetic records as well as pathology databases. Prevalent cases were identified using clinical coding and dietetic records. I identified 300 women alive with coeliac disease at Nottingham in March 2007. At the Royal Hallamshire Hospital, consecutive cases of incident and prevalent coeliac disease were identified using clinical and dietetic records as well as pathology and immunology databases. I identified 300 women alive with coeliac disease at Sheffield in May 2007. At Derby Hospitals NHS Foundation Trust, consecutive cases of incident coeliac disease were identified using clinical and dietetic records. Prevalent cases of coeliac disease were identified using a computerised coeliac disease database, co-ordinated and maintained by a single gastroenterologist since 1978. There are 781 women with coeliac disease that are alive in the Derby cohort. Extensive efforts were made to identify all incident and prevalent women with coeliac disease at these three centres in order that they could be invited to participate in the study. An adult is aged 18 years or over. A childhood diagnosis of coeliac disease was coeliac disease diagnosed at age 15 years or younger; all other cases were referred to as adulthood-diagnosed coeliac disease.

5.1.2.2.2. Population-based cohort

Coeliac UK is the principal national society for people with coeliac disease offering invaluable dietary guidelines and represents the largest population-based cohort of people with coeliac disease in the United Kingdom. It has over 70,000 registered members [personal email communication with Lawrence Munday, Database Manager, Coeliac UK] from which we selected all women over the age of 35 years who on their membership information had registered a current UK postal address and they had reported that they have coeliac disease. This excluded individuals who were members of Coeliac UK though did not have coeliac disease such as parents, guardians of

affected relatives and also representatives of external organisations such as pharmaceutical companies. We also excluded those individuals who had indicated on joining Coeliac UK that they did not wish to be contacted for research purposes by a third party. We then excluded those women with coeliac disease already included in our study by virtue of being NHS patients within the Nottingham, Sheffield and Derby historical cohorts described above in Chapter 2. This gave a population of 29,954 women to select for our study population from which we did by generating a simple random sample of 9000 individuals using STATA 9.2 software.

5.1.2.3. Inclusion criteria

Females with coeliac disease who are at any stage of their disease.

5.1.2.4. Exclusion criteria

Significant co-morbidity that would prevent the study participants being well enough to take part.

5.1.2.5. Outcome measures

- age at menarche (mean; proportion of women reaching menarche by age 11, 12, 13, 14, 15 and 16 years)
- regularity of menstrual cycles (proportion of women with time from menarche to onset of regular menstrual cycles less than one year; and more than one year)
- age at menopause (mean; median; proportion of women still menstruating at age 45, 46, 47, 48, 49, 50 years)
- age at first full-term pregnancy (mean; proportion of women having first full-term pregnancy at age 19, 21, 25, 29, 33, 42 years)
- nulliparity (proportion)
- breastfeeding (proportion of women never and ever breastfeeding; proportion of women with total breastfeeding duration of 12 or more months)
- family history of breast cancer (proportion of women with one first degree relative with breast cancer, one first degree relative with breast cancer diagnosed under the age of 40 years, two first or second degree relatives with breast cancer diagnosed under the age of 60 years, or three first or second degree relatives with breast cancer)
- previous benign breast disease (proportion of women with atypical epithelial hyperplasia, breast fibroadenosis, solitary breast cyst, diffuse cystic mastopathy, breast fibrosclerosis, mammary duct ectasia)
- socioeconomic group (proportion of women in social class I and II)
- body weight (mean; proportion of women weighing 62.0 – 67.4, 67.5 – 74.9, >/ 75.0 kilograms; mean weight at age 20 years; mean adult weight change)
- height (mean)

- body mass index (proportion of women with normal (18.6 – 24.9 kg/m^2), underweight (\< 18.5 kg/m^2), overweight (25.0 – 29.9 kg/m^2), or obese (>/ 30.0 kg/m^2) body mass index)
- alcohol intake (proportion of women who are non-drinkers; proportion of women consuming >15 units of alcohol each week)
- oral contraceptive use (proportions of women never and ever used and currently using oral contraceptive pill; proportion of women who start taking oral contraceptive pill before the age of 20 years; proportion of women within 1 – 9 years of stopping taking oral contraceptive pill)
- hormone replacement therapy use (proportion of women ever used hormone replacement therapy; proportion of women taking hormone replacement therapy for 5, 10 or 15 years or more)

Below are descriptions of how I have defined the outcome measures used within this study.

Definition 46: Age at first full-term birth

Age in years at birth of their first child.

Definition 47: Total breastfeeding duration

Number of months each child was breastfed for added together.

Definition 48: First degree relative

Mother, sister or daughter.

Definition 49: Second degree relative

Grandmother, granddaughter, aunt or niece.

Definition 50: Atypical epithelial hyperplasia

Refers to fibrocystic change with proliferation of epithelial cells with atypia in the ducts and lobules of the breast (ICD-10 code N60). Answer to question 14b in the questionnaire "Yes – had fibrocystic benign breast disease" and or documentation in the free text space given "atypical epithelial hyperplasia" "sclerosing adenosis" "fibrocystic mastopathy" "ductal hyperplasia" or "lobular hyperplasia".

Definition 51: Breast fibroadenosis

Refers to fibrous changes occurring within the lobules (ICD-10 code N60.2). Answer to question 14b in the questionnaire "Yes – had other form of breast disease" and documentation in the free text space given "fibrosis in breast" "fibroadenosis" "thickening of tissue" "fibrous tissue" "adenosis".

Definition 52: Solitary breast cyst

Refers to the presence of fluid-filled spaces that originate from the terminal ductal lobular unit or from an obstructed duct (ICD-10 code N60.0). Answer to question 14b in the questionnaire "Yes – had other form of breast disease" and documentation in the free text space given "cyst in breast" "cyst" "cyst drained" "benign cyst" "lump in breast fluid drained" "non-malignant cyst".

Definition 53: Diffuse cystic mastopathy
Refers to the presence of multiple fluid-filled spaces originating from the terminal ductal lobular unit or from an obstructed duct diffusely distributed through the breasts (ICD-10 code N60.1). Answer to question 14b in the questionnaire "Yes – had other form of breast disease" and documentation in the free text space given "multiple cysts in breast" "cystic change" "cysts throughout breast" "cystitis in all of breast".

Definition 54: Breast fibrosclerosis
Refers to cystic mastopathy with epithelial proliferation (ICD-10 code N60.3). Answer to question 14b in the questionnaire "Yes – had other form of breast disease" and documentation in the free text space given "fibrosclerosis".

Definition 55: Mammary duct ectasia
Refers to the condition in which there is an obstruction to the lactiferous duct (ICD-10 code N60.4). Answer to question 14b in the questionnaire "Yes – had other form of breast disease" and documentation in the free text space given "duct ectasia" "milk duct blockage" "milk duct problem".

Definition 56: Mean adult weight change
Difference between reported baseline weight on enrolment to the study and recalled weight at 20 years.

5.1.2.4. Outcome ascertainment

Female incident and prevalent cases of coeliac disease were invited to complete a questionnaire that examined the possession of exposures associated with the development of breast cancer. The questionnaire used is found in Appendix 7.6. and is the same questionnaire that was used in the European Prospective Investigation into Cancer (EPIC) multicentre prospective study [475]. There were a number of reasons for using the EPIC questionnaire. It is a standardised and validated questionnaire that can be self-completed [476]. The questionnaire collects detailed information on health and lifestyle, reproductive and sociodemographic characteristics including the participant's reproductive history, oral contraceptive and hormone replacement therapy use, an occupational history as well as life history of tobacco smoking and alcohol drinking. When this breast cancer risk profile in coeliac disease study was being designed, it was thought data from completed questionnaires in the EPIC-Norfolk cohort would be used as a general population control [personal communication with Professor K-T Khaw]. However in the course of this study we have not yet obtained the EPIC-Norfolk data and used alternative data as a control comparator as described in section 5.1.2.4. Like in the EPIC study, the questionnaire was either mailed to the study participants (both hospital-based and population cohorts) or given by hand during attendance at a routine outpatient clinic (hospital-based cohort only). The questionnaire was self-completed. Menarche self-reported in adulthood has been validated elsewhere [477].

As a patient support group, it is likely that there are members of Coeliac UK that have coeliac disease. However, family of affected coeliacs (such as mothers of affected children, wives of affected husbands) that do not have coeliac disease are also members of Coeliac UK. People with functional bowel disturbances whose symptoms

are helped with dietary exclusion such as wheat though do not have coeliac disease could also be members of Coeliac UK to utilise the dietary support offered by the organisation. The membership database has a field where gender is recorded though in some members this field may be not completed or completed incorrectly. In addition to those questions contained in the original EPIC questionnaire, we included screening questions to ensure the study participant was female and they had coeliac disease.

Social class by current or last known occupation of the female coeliac was coded according to the Registrar General's classification [422] and grouped into categories: professional (social class I), managerial (social class II), non-manual skilled (social class IIIN), manual skilled (social class IIIM), manual semi-skilled (social class IV), manual unskilled (social class V) [422].

5.1.2.5. Controls

Data from a number of different British birth cohort studies and other cohorts were used as a general population comparator.

5.1.2.5.1. The Office of National Statistics Longitudinal Study

The Office for National Statistics (ONS) Longitudinal Study has linked the birth registration and census records since 1971 for a 1% sample of all women in England and Wales [478]. Women born in two 'birth cohorts' approximately ten years apart are used in the study – women born in 1954 – 1958 ('1950s birth cohort') and 1964 – 1968 ('1960s birth cohort') thus attaining ages 23 – 27 years in the Census years 1981 and 1991 respectively, depending on the woman's exact year of birth within the five-year birth cohort. The 1960s birth cohort allow for estimation of the distribution of

age at first childbearing for the ages up to 33 years. The 1950s birth cohort however allow for estimation of the distribution of age at first childbearing through almost the entire range of potential ages of first childbearing and thus was the 1950s birth cohort was used as a general population comparator for proportion of women having first full-term pregnancy using the age bands used in the ONS Longitudinal study (19, 21, 25, 29, 33, and 42 years) [479]. When using the ONS Longitudinal Study 1950s cohort dataset as a control comparison to coeliac cohort, only those coeliacs born between five years before and five years after the age at entry were included in the analysis i.e. between 1949 and 1963.

5.1.2.5.2. Office for National Statistics

Proportion of female coeliacs that are nulliparous are compared to that of the general population using Birth Statistics from the Office for National Statistics [480]. Population estimates of women by ethnic group in 2007 in England from the Office for National Statistics [481] were used to compare the Caucasian proportion of the coeliac cohort to that of the general population. The 2001 Census was used to provide general population estimates of socioeconomic status by occupation of women aged between 16 – 74 years as a comparator to the socioeconomic status by occupation of women with coeliac disease of the same age [482].

5.1.2.5.3. Medical Research Council National Survey of Health and Development

The first maternity survey in Great Britain took place in 1946, based on 13,687 of the 16,695 births that took place between March 3 and 9 of 1946 [483]. Medical officers of health in 453 of 458 local authorities in England, Wales and Scotland at that time agreed to send health visitors to interview the mother at 8 weeks after the birth. The Medical Research Council (MRC) National Survey of Health and Development

(NSHD) is a birth cohort study consisting of a socially stratified sample of 2547 women (and 2815 men) of these births [484, 485]. There have been twenty-two follow-ups of the whole cohort, with the most recent being at age 53 years when 1563 women provided information representing 61% of the original sample with the remainder either living abroad (11% of the original cohort), had withdrawn from the study at earlier follow-ups (12%) or had died (9%). The cohort remains nationally representative in most respects [486]. Dates of all live births have been collected throughout the adult life of the cohort with age at first birth grouped into <20 years, 20 – 29 years and /> 30 years [487]. The proportion of female coeliacs having first full-term pregnancy using these age bands was compared to that of the MRC NSHD data. Proportion of female coeliacs still menstruating was compared to that of the MRC NSHD data using the same age categories as in the control dataset (45, 46, 47, 48, 49, and 50 years) [488, 489]. Mean height of female coeliacs was compared to that of the MRC NSHD 1946 and 1958 birth cohorts [490]. Proportion of female coeliacs of social class I and II was compared to that of the MRC NSHD data [491]. When using the MRC NSHD dataset as a control comparison to coeliac cohort, only those coeliacs born between five years before and five years after the MRC NSHD age at entry were included in the analysis i.e. between 1941 and 1951.

5.1.2.5.4. 1958 British Birth cohort

This cohort included all children born in England, Scotland and Wales in the week of 3 – 9 March 1958 [492] with information obtained on 11,714 (98.2%) births of the target population (17,733 births). Major follow-ups of surviving children in this longitudinal follow-up study (National Child Development Study (NCDS)) were conducted at ages 7, 11, 16, 23 and 33 years [493]. Age at menarche for the female

coeliacs was compared to that of the NCDS data using the same age categories as in the NCDS control dataset (-11, -12, -13, -14, -15 and -16 years) [494]. Data on survey performed at 33 years in 1991 when information was collected on 11,405 (n = 5308 women) members was used for a general population comparison for adult height (mean) [495]. When using the NCDS dataset as a control comparison to coeliac cohort, only those coeliacs born between five years before and five years after the NCDS age at entry were included in the analysis i.e. between 1953 – 1963.

5.1.2.5.5. EPIC cohort

The EPIC cohort consists of about 370,000 women (and 150,000 men) recruited between 1992 – 1998 in ten Western European countries of age range 45 – 70 years and of mean age 57.5 (SD 9.9) years [476]. As described in section 5.1.2.4. all study participants provided extensive standardized questionnaire data as well as anthropometric measurements. Mean age at menarche and mean age at menopause in the female coeliac cohort were compared to that data from a random sample of the EPIC cohort [308]. Age at first full-term pregnancy (<22 years, 22 – 24, 25 – 27, 28 – 30, and 31+ years) from EPIC-France dataset was also used as a general population comparator [496]. Mean recalled weight at 20 years, baseline weight and mean adult weight change in the female coeliac cohort were compared to 24,515 UK-EPIC control data [497]. Mean weight, height were compared to that of 40,273 EPIC control data [309]. When using the EPIC dataset as a control comparison to coeliac cohort, only those coeliacs aged between 45 – 70 years were included in the analysis.

5.1.2.5.6. UK National Case-Control Study Group

This study included incident cases of breast cancer in 3 discrete time periods (1 January 1982 – 31 December 1985; 1 January 1988 – 30 June 1989; 1 July 1990 – 30

June 1991) identified through regional cancer registries were age-matched to controls randomly selected from the list of the case's general practitioner. Data on use of oral contraceptives, regularity of menstrual cycles, family history of breast cancer from this study was used as a general population comparator to the female coeliac data [300].

5.1.2.5.7. Infant Feeding Surveys

The Infant Feeding Survey is the national survey of infant feeding practices [498-502]. Surveys have been conducted every five years since 1975 and based on an initial national representative sample of babies born in the United Kingdom (in the Infant Feeding Survey 2000, 9,500 mothers of babies born in the United Kingdom were studied). The main aim of the survey is to provide national estimates on the incidence, prevalence and duration of breastfeeding and other feeding practices adopted by mothers from the birth of their baby up to around nine months. The survey also collects information on the smoking and drinking behaviour of mothers before, during and after pregnancy. As well as national estimates the survey is also designed to provide individual estimates for the four countries of the United Kingdom. The survey uses a panel design, with three stages of data collection being carried out over a 9-12 month period in order to capture feeding practices at different ages. Wave 1 is carried out when the babies are approximately 6 - 10 weeks old, Wave 2 when they are approximately 4 - 5 months old, and Wave 3 when they are approximately 8 - 9 months old.

The sampling frame in each country consisted of all registrations for births on the selected dates that were received by the appropriate registration office within a

specified sampling period up to a maximum of eight weeks after the birth [501]. The samples in each country were selected from births occurring in a given range of dates between August and October of the year of the survey and were designed to be representative of all births in these periods. The number of days chosen varied between countries, and depended on the estimated number of births in each social class group which would be registered within the sampling period and other details of the sampling scheme in each country. Typical response rate for the surveys was approximately 70%. For example, for the Infant Feeding Survey 1995 72% of the original sample of women responded to the first stage questionnaire; response at the second stage of the survey was higher than at the first stage, ranging from 86% in Scotland to 91% in Northern Ireland (the improvement in response rate was thought largely attributable to the interviewer follow-up of non-respondents); and at Stage 3, the total response was 88%.

Data from 1980, 1985, 1990, 1995 and 2000 Infant Feeding Surveys were used as a general population comparator to the incidence and prevalence of breastfeeding amongst women with coeliac disease. Incidence of breastfeeding was defined in the Survey as the proportion of babies who were breastfed initially. This included all babies who were put to the breast at all, even if this was on one occasion only. Prevalence of breastfeeding was defined as the proportion of all babies who were being breastfed at specific ages. When using the Infant Feeding Survey datasets as a control comparison to coeliac cohort, only those coeliacs giving birth 2 years before or 2 years after or during the year of the Survey were included in the analysis. For example, when using the 1980 Infant Feeding Survey as a general population

comparator, women who gave birth between 1978 – 1982 were included in the analysis.

5.1.2.5.8. Million Women's Study

The Million Women Study is a population-based cohort study, recruiting women aged between 50 – 64 years of age. Participating UK National Health Service Breast Screening Programme screening centres sent a questionnaire at the time they were sent their usual invitation for routine breast screening. The questionnaire is included with each woman's invitation for breast screening and returned at the time she was screened. It includes questions about lifestyle and sociodemographic factors, reproductive history, past use of oral contraceptives, use of HRT, past medical history and family history of breast cancer [503]. The proportion of coeliacs with a mother and or sister with a history of breast cancer was compared to that in the Million Women Study. When using the Million Women Study as a general population comparison cohort, only female coeliacs aged between 50 – 64 years of age were included in the analysis.

5.1.2.6. Potential confounders

Age, socio-economic class.

5.1.2.7. Statistical analysis

Descriptive analyses with calculation of mean and median were performed. Comparisons between the female coeliac cohort and the general population controls were performed using Chi-squared tests for binary outcomes and between continuous data such as mean age at menarche using unpaired t-tests.

We considered a p-value of 0.05 to represent statistical significance in all tests. All analyses were performed using Stata SE 9.2 [TexCorp].

5.1.2.8. Ethical approval

5.1.2.8.1. Hospital-based cohort

North Staffordshire Local Research Ethics Committee gave ethical approval (reference 06/Q2604/91) to this study in October 2006. Nottingham University Hospital gave research and development approval (reference 06GM012) to this study in January 2007. Derby Hospitals NHS Foundation Trust gave research and development approval (reference DHRD/2007/005) in April 2007. Royal Hallamshire Hospital gave research and development approval (reference STH14597) in March 2007.

5.1.2.8.2. Population-based cohort

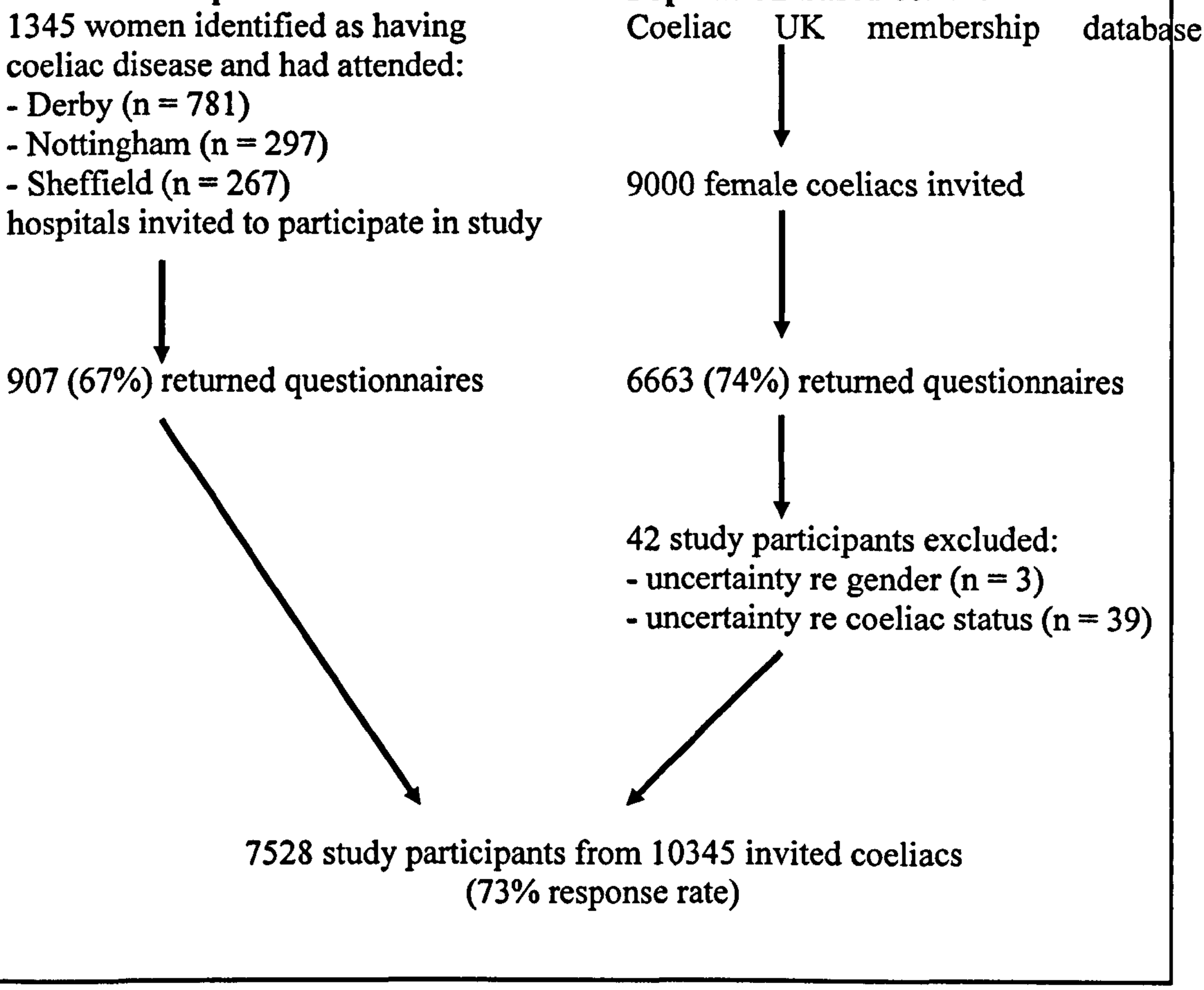
Nottingham Medical School Research Ethics Committee gave ethical approval (reference J/9/2007) to this study in January 2008.

5.1.3. Results

5.1.3.1. Study population

907 of the invited 1345 (response rate 67.4%) women of the historical cohort and 6663 of the invited 9000 (response rate 74.0%) women of the population-based cohort completed the study questionnaire. Following excluding 42 participants derived from the population-based cohort due to uncertainty re their gender (n = 3) and answering that they did not have coeliac disease in the screening questions (n = 39), 7528 women with coeliac disease were therefore included in our study (Figure 5.26).

Figure 5.26: Response rate to study



5.1.3.1. Demography of cohort

The mean age at entry to the study was 58.6 (SD 12.5) years with the median age 59 (interquartile range 49 – 67) years. The mean age at diagnosis of coeliac disease was 42.5 (SD 15.7) years with 91.3% (n = 6872) of the coeliacs diagnosed in their adulthood years. 97.9% (n = 7370) coeliacs reported they were taking a gluten-free diet with the mean duration of consuming a gluten-free diet 28.8 (SD 18.7) years. 96.9% female coeliacs were Caucasian which was a significantly greater proportion than that of the English general population (89.5%); p = 0.00001 [424]. The proportion of Caucasian coeliacs was similar in the hospital-based and population-based cohorts.

5.1.3.2. Social class

The proportion of women from social classes I and II based on current or last occupation was significantly greater in the coeliac cohort (n = 2243) than in the 2001 Census general population [482] comparator (48.0%; 95%CI 46.5, 49.4 versus 34.2%; 95%CI 34.2, 34.3, respectively); p = 0.00001. A similar trend was observed when using the MRC NSHD study [491] as a comparator (Table 5.53). The proportion of female coeliacs from affluent social classes was similar in the hospital-based and population-based cohorts.

5.1.3.3. Age at menarche

The mean and median age at menarche was 13.4 (SD 1.7) years and 13 (interquartile range 12 – 14) years, respectively. On comparison to the EPIC controls, there was no difference in mean age at menarche (13.3 (5^{th} – 95^{th} centile: 11.0 – 16.0) versus 13.3 (5^{th} – 95^{th} centile: 11.0 – 16.0), respectively); p > 0.05 [308]. However a significantly greater proportion of coeliac women had started menstruating early in life in

comparison to the general population [494] controls (16.8%; 95%CI 15.1, 18.4 versus 2.2%; 95% 1.8, 2.6, respectively); p = 0.00001 (Table 5.53).

Table 5.53: Social class, age at menarche and regularity of menstrual cycles in coeliac disease and comparison cohorts

Variable	Coeliac disease cohort N	%	Comparison cohort N	%	p-value
Social class[a]					
- I and II	644	26.4	281	20.4	0.00001
Age at menarche[b] (years)					
- 11	328	16.8	97	2.2	0.00001
- 12	274	14.0	584	13.2	0.3
- 13	452	23.1	1063	24.0	0.5
- 14	396	20.3	1492	33.7	0.00001
- 15	263	13.5	868	19.6	0.9
- 16	133	6.8	221	5.0	0.0036
- Missing data	39	2.0	102	2.3	
Time from menarche to onset of regular menstrual cycles[c]					
< 1 year	1990	67.8	1157	78.1	0.00001
>/ 1 year	782	26.7	325	21.9	0.00001
Missing data	163	5.5			

a MRC National Survey of Health and Development 1946 (n = 1373) was used to provide general population estimates of socioeconomic status by occupation [491]. Only those coeliacs born between 5 years before and 5 years after the MRC study age at entry were included in the analysis i.e. between 1941 and 1951 (n = 2441)

b National Child Development Study (NCDS) 1958 cohort (n = 4427) was used as a general population comparator for age at menarche [494]. Only those coeliacs born between 5 years before and 5 years after the NCDS study age at entry were included in the analysis i.e. between 1953 and 1963 (n = 1927).

c Data on regularity of menstrual cycles from the UK National Case-Control Study Group was used as a general population comparator [300].

5.1.3.3. Regularity of menstrual cycles

69.5% (n = 5235) had regular periods within months from starting menarche. Women with coeliac disease took longer to have regular menstrual cycles from the onset of menarche in comparison to the general population [300]. A significantly lower proportion of women in the coeliac cohort took less than one year from menarche to

onset of regular menstrual cycles in comparison to the control cohort (67.8%; 95%CI 66.1, 69.5 versus 78.1%; 95% 76.0, 80.2, respectively); p = 0.00001 (Table 5.53). Conversely, a significantly greater proportion of women with coeliac disease took more than one year to onset of regular menstrual cycles from the menarche onset in contrast to the control cohort (26.7%; 95%CI 25.1, 28.2 versus 21.9; 95%CI 19.8, 24.0, respectively); p = 0.00001.

5.1.3.4. Age at first full-term birth

6599 (87.7%) of the coeliac cohort were parous with the mean age at first full-term birth 25.7 (SD 4.9) years. A lower proportion of women had their first full-term birth aged 30 years or over in the coeliac cohort in comparison to the control [487] cohort (25.3%; 95%CI 23.7, 26.9 versus 33.3%; 95%CI 32.5, 34.1, respectively); p = 0.00001. Though relative to the comparison cohort [479], coeliac women had a lower proportion having their first full-term birth by 19 years, they appeared to 'catch up' with a higher proportion having their first full-term birth by 30 years (Table 5.54).

5.1.3.5. Nulliparity

12.2% (n = 920) of the coeliac cohort were nulliparous. Coeliac women were less likely to be nulliparous in comparison to the comparison [480] cohort (proportion nulliparous 9.3%; 95%CI 8.1, 10.5 versus 12.4%; 95%CI 10.5, 14.3); p = 0.0042.

Table 5.54: Age at first full-term birth, nulliparity and incidence of breastfeeding in coeliac disease and comparison cohorts

Variable	Coeliac disease cohort N	%	Comparison cohort N	%	p-value
Age at first full-term birth (years)[a]					
- 19	233	8.4	1638	13.7	0.00001
- 21	719	25.8	1280	10.7	0.00001
- 25	674	24.2	2774	23.2	0.3
- 29	459	16.5	2284	19.1	0.0013
- 33	269	9.7	1280	10.7	0.1
- 42	3	0.1	801	6.7	0.00001
Missing data	28	1.0			
Nulliparity[b]					
- Nulliparous	227	9.3	145	12.4	0.0042
Missing data	9	0.1			
Incidence of breastfeeding[c]					
- 1980	287	73.2	2516	67.0	0.0124
- 1985	276	76.0	3036	65.0	0.00001
- 1990	242	71.2	2965	64.0	0.0075
- 1995	180	81.1	3127	68.0	0.00001
- 2000	109	80.2	3863	71.0	0.0199
Missing data	50				
Incidence of breastfeeding by social class[c]					
1995					
- I	14	93.3	275	91.0	0.8
- II	62	76.5	943	82.0	0.2
- IIIN	40	78.4	255	72.0	0.3
- IIIM	5	71.4	724	65.0	0.7
- IV	31	83.8	280	58.0	0.0021
- V	-	-	82	50.	-
2000					
- I	12	80.0	336	91.0	0.2
- II	40	81.6	1153	84.0	0.7
- IIIN	24	75.0	367	79.0	0.6
- IIIM	6	100	909	65.0	-
- IV	11	91.7	359	62.0	0.0355
- V	-	-	129	59.0	-

a ONS Longitudinal Study 1950s cohort (n = 11958) used as comparison dataset [479]. Only those coeliacs born between 5 years before and 5 years after the ONS study age at entry were included in analysis i.e. between 1949 and 1963 (n = 2788).

b Birth Statistics, Office for National Statistics, provided general population data[480]

c Data from the 1980, 1985, 1990, 1990, 1995 and 2000 Infant Feeding Surveys were used as a general population comparator for breastfeeding practices [496-500]. Only those coeliacs giving birth 2 years before or 2 years after or during the year of the survey were included in the analysis.

5.1.3.6. Breastfeeding

3972 (60.2%) of the 6599 parous women with coeliac disease breastfed. The mean total number of months breastfed was 10.9 (SD 10.4) months with 21.0% (n = 1388) of the parous coeliacs breastfeeding for a total of 12 months or more. The incidence of breastfeeding was significantly greater in the coeliac cohort in comparison to the control cohort [496-500] (Table 5.54). For example, the incidence of breastfeeding of babies in 1980 in the coeliac cohort was 73.2% (95%CI 68.8, 77.6) versus 67.0% (95%CI 65.5, 68.5). In comparison to the general population cohort, breastfeeding incidence did not appear to fall with more deprived socioeconomic status in the coeliac cohort (Table 5.54). If anything the converse was observed with higher proportions of coeliacs breastfeeding from more deprived social classes than the proportions of coeliacs breastfeeding in least deprived social classes (91.7% of coeliacs of social class IV (below average class) who gave birth between 1998 – 2002 breastfed in comparison to 62% of the women in 2000 Infant Feeding Survey used as a general population comparator).

5.1.3.7. Age at menopause

5540 (73.6%) of the coeliac cohort reported that they had stopped menstruating with the mean and median age at menopause for the coeliac cohort for those whom had provided information on menopause (n = 5166) was 47.6 (SD 6.4) years and 49 (IQR 44 – 72) years, respectively. Women with coeliac disease had a younger mean age at onset of the menopause in comparison to the general population [308] comparison cohort (47.8 years; 95%CI 47.5, 48.0 versus 49.0 years; 95%CI 48.7, 49.3) p = 0.00001.

5.1.3.8. Oral contraceptive and hormone replacement therapy use

68.7% (n = 5171) coeliacs reported to have ever used the oral contraceptive pill (OCP). 39.7% (n = 2041) of OCP users had started to take the OCP before the age of 20 years. Only 9 coeliacs were current users of the OCP proportion of women with 201 coeliacs within 1 – 9 years of stopping taking the OCP.

2705 (44.8%) coeliac women of the 5540 that had stopped menstruating had ever used hormone replacement therapy (HRT). 1584 (58.6%) of the HRT users in the coeliac cohort had taken HRT for 5 or more years.

5.1.3.9. Benign breast disease

9% (n = 673) of the cohort reported history of breast disease of which fibrocystic change (n = 376) was the most common. 181 of the cohort had been diagnosed with breast cancer. 1% (n = 41) of the cohort reported breast fibroadenosis whereas diffuse cystic mastopathy was reported by 9 coeliacs. 1% of the cohort reported a history of solitary breast cysts. 7 of the cohort had been diagnosed with epithelial hyperplasia whereas 6 reported fibrosclerosis and 8 mammary duct ectasia.

5.1.3.10. Weight, height and body mass index

The mean height in 7304 coeliacs who had provided data was 162.0 (SD 7.2) centimetres (cm). There was no significant difference in observed mean height between women with coeliac disease and that of the general [490] population (161.7 cm; 95%CI 161.4, 162.0 versus 161.8 cm; 95%CI 161.5, 162.1, respectively) born in the 1940s though a small but significant taller height amongst coeliac women born in the 1950s (mean 162.8 cm; 95%CI 162.4, 163.1 versus 162.4; 95%CI 162.3, 162.5, respectively). Using the EPIC-UK cohort [309], coeliac women were shorter than the

general population counterparts (mean 162.1 cm; 95%CI 161.9, 162.2 versus 163.4 cm; 95%CI 163.3, 163.5, respectively) though 2 kilograms heavier (mean weight 66.0 kg; 95%CI 65.6, 66.3 versus 63.9 kg; 95%CI 63.8, 64.0, respectively); p = 0.00001.

The mean BMI of the coeliac cohort was within normal range (24.9 (SD 4.5) kg/m^2). In keeping with the shorter height but heavier weight, the mean BMI of the coeliac women was higher than that of the EPIC-UK comparison cohort (25.1; 95%CI 25.0, 25.3 versus 24.0; 95%CI 23.9, 24.0, respectively); p = 0.00001.

5.1.3.11. Alcohol consumption

77.1% (n = 5804) drank alcohol with mean intake per week 6.0 (SD 5.6) units. 4.4% (n = 330) coeliacs drank 15 or more units of alcohol each week.

5.1.3.12. Family history of breast cancer

The prevalence of breast cancer within the coeliac cohort was 2.6% (n = 195). 23.3% (n = 1750) of the female coeliacs had a family history of breast cancer. 1098 (14.6%) of coeliacs had one or more first degree relative with breast cancer with 79 of these coeliacs having one or more first degree relative with breast cancer diagnosed under the age of 40 years. 28 coeliacs had two or more second degree relatives with breast cancer diagnosed under the age of 60 years. 6 of the coeliac cohort had three first or second degree relatives with breast cancer.

5.1.3.13. Odds of possessing breast cancer risk exposures

The breast cancer risk profile of the coeliac cohort compared to the general population is summarised in Table 5.55.

Table 5.55: Odds of breast cancer risk profile exposure in coeliac versus control comparison cohort

Breast cancer risk exposure	Proportion in coeliac cohort	Proportion in general population cohort	Odds ratio coeliac versus general population cohort [95%CI]	Potential effect of having coeliac disease in comparison to general population upon breast cancer risk profile
Caucasian versus non-Caucasian	5904/6000 98.4%	19032000 /21266200 89.5%	7.22 [5.90, 8.83]	Adverse effect on breast cancer risk with coeliacs more likely to be Caucasian
Menarche <11 years versus >11 years	1112/7416 15.0%	97/4427 2.2%	7.87 [6.38, 9.72]	Adverse effect on breast cancer risk with coeliacs more likely to have earlier menarche
Irregularity of menses within 1y of menarche	1735/2925 59.3%	324/1481 21.9%	5.21 [4.51, 6.01]	Adverse effect on breast cancer risk with coeliacs more likely to have irregular menses
First full-term pregnancy before 30y	2085/2357 88.5%	7976/10057 79.3%	2.00 [1.75, 2.29]	Beneficial effect on breast cancer risk with coeliacs more likely to have babies before 30
Nulliparous	227/2441 9.3%	145/1166 12.4%	0.72 [0.58, 0.90]	Beneficial effect on breast cancer risk that coeliacs less likely to be nulliparous
SES I and II versus III, IV and V	644/2439 26.4%	281/1377 20.4%	1.40 [1.19, 1.64]	Adverse effect on breast cancer risk with coeliacs more likely to be of richer class
Incidence of breastfeeding	287/392 73.2%	2550/3755 67.9%	1.29 [1.02, 1.63]	Beneficial effect on breast cancer risk with coeliacs more likely to breastfeed
Mother and or sister with breast cancer	348/3103 11.2%	10179/113104 9.0%	1.28 [1.14, 1.43]	Adverse effect on risk with coeliacs more likely to have breast cancer family history

5.1.4. Discussion

5.1.4.1. Principal findings

The breast cancer risk profile suggests both protective and adverse associations of coeliac disease. The higher proportion of women being parous, having their first full-term pregnancy before 30 years and breastfeeding in addition to the younger mean age at menopause suggests women with coeliac disease have favourable breast cancer risk profile features in comparison to the general population. However, the higher likelihood of being Caucasian and of affluent social class together with higher proportion having early menarche and irregular menstrual cycles suggests there are also potentially adverse breast cancer risk profile features associated with coeliac disease. Having similar height to the general population and BMI within normal range suggests anthropometric exposures may not explain the apparent reduced risk of breast cancer in women with coeliac disease to the general population.

5.1.4.2. Limitations and merits

This is the first cross-sectional survey where there has been systematic and routine collection of breast cancer risk profile in a contemporary and population-based cohort of women with coeliac disease. While we cannot be certain that we have identified every woman diagnosed or treated with coeliac disease in all three hospitals during the study period to generate the historical cohort component of the study, we made extensive efforts to do so. Coeliac UK is the principal national society for people with coeliac disease and represents the largest assembled and population-based cohort of people with coeliac disease in the United Kingdom with over 70,000 registered members. One could argue that the use of such members who have voluntarily sought help and advice from Coeliac UK may differ in their attitudes, behaviours and health status compared to those coeliacs who are non-members, introducing selection bias into the study. Nevertheless, at the time the study commenced, membership of Coeliac UK was free to join with a freepost paper (also available online) application form which was encouraged to be distributed to all those newly diagnosed with coeliac disease by anyone involved with managing coeliac disease with the incentive of receiving the free and useful food directory. In our study, the reported age at diagnosis and breast cancer risk profile in those coeliacs of the Coeliac UK cohort was similar to that reported by those coeliacs of the historical cohort suggesting it is unlikely the Coeliac UK study participants were unrepresentative of contemporary coeliac disease.

The use of a self-completed questionnaire to ascertain the outcomes of interest was a further source of selection bias in the study with responders likely to differ in their attitudes, behaviours and health status compared to non-responders. However it is difficult to imagine how better ascertainment of outcomes involving such large

numbers of population-based female coeliacs could be achieved. The questionnaire used is a standardised and validated questionnaire was that used in the EPIC prospective study that can be self-completed [475, 476]. Equal time and effort was spent in inviting each potential study participant to take part in the study with two further contacts made to non-responders after a designated lapsed time period using a standardised recruitment protocol. Recall of ages at menarche and pregnancy-related events is subject to recall error and bias because it was not collected at the time of the events. However recall of self-reported menarche and pregnancy-related events in adulthood has been validated with correlation coefficients 0.65 – 0.75 observed in studies comparing recalled measures with prospective measures [477, 504-506]. Validity has been observed to improve by categorising ages such as age at menarche which was used in this study's questionnaire [477].

As a patient support group, it is likely members of Coeliac UK have coeliac disease. However, family of affected coeliacs (such as mothers of affected children, wives of affected husbands) that do not have coeliac disease but are also members of Coeliac UK, principally to make use of the dietary guidelines. People with functional bowel disturbances whose treatment involves wheat exclusion may also be members of Coeliac UK for the dietary advice. With the diagnosis of coeliac disease not specifically validated within the Coeliac UK database, we included several different screening questions to minimise misclassification bias. In addition to the coding of gender in the Coeliac UK electronic database, we included screening questions to ensure that the study participant was female. Like with coeliac status, if there was any uncertainty re gender status the study participant was excluded from the study. Study

participants from the historical cohort were known to have coeliac disease based on findings from histopathology, immunological and clinical records.

British birth cohorts are thought to be national and representative samples of the general population and as such were used as a general population comparator. Data has been systematically collected from early life to adulthood by trained data collectors on infant feeding; occupation, education and training; fertility and physical measures with repeated measures. Knowing the time period of when the coeliacs were born allowed appropriate control groups to be drawn up from the same period in the British birth cohorts. Several different control cohorts were used as the comparator to the coeliac cohort for a particular outcome of interest with findings consistent irrespective of the control cohort used. Some of the British birth cohort studies were somewhat limited by exclusion of births to the unmarried but that was in part dictated by the available technology at the time and lack of access to the Adoption Register at a time when many births to the unmarried were adopted.

5.1.4.3. Comparison with other studies

Our observation that female coeliacs have earlier onset of menopause is in keeping with previous findings [320, 321]. It is unclear why female coeliacs have an earlier onset of age at natural menopause which has been proposed as a marker of ovarian toxicity for environmental and early life factors that may directly or indirectly damage the follicular pool [507]. Whether due to more rapid metabolism of oestrogen, lower oestrogen levels, interference with oestrogen receptor binding, or accelerated follicle ageing by hydrocarbons contained in smoke, cigarette smoking is consistently associated with an earlier age of menopause onset [508-510]. However, two-thirds of the coeliac cohort had never smoked and were significantly less likely to smoke in comparison to age-matched general population controls from 1958 Birth Cohort and EPIC-Norfolk [511]. In our study, female coeliacs were less likely to be nulliparous and had the same parity as general population controls [512] suggesting reproductive characteristics [513] are not implicated in causing earlier age at menopause in coeliacs. Greater weight [509] and taller height [514] predispose to a later age and caloric restriction to an earlier age at menopause [515] though coeliacs having similar height and BMI within normal range suggests that anthropometric exposures may not explain the earlier onset of menopause in coeliacs. Given that the pool of primordial follicles is formed during fetal development, the association of lower birthweight, lower infant and childhood weight, poorer childhood socioeconomic conditions with earlier menopause have suggested early life factors acting *in utero* or in early childhood play a role in determining age at menopause [489, 516]. In our related study based on the same study population, female coeliacs were more likely to have fathers who were from non-manual and more affluent occupational social classes than the general population cohort and thus exposed to more affluent childhood socioeconomic exposures [511].

Our observation that women with coeliac disease are more likely to have a younger age of menarche is in contrast to previous studies [320, 321]. However these studies were highly selected, hospital-based case series of women with coeliac disease with the larger study observing only 130 coeliacs [320]. Age at menarche is an indicator of puberty and onset of sexual maturation, controlled by a complex of known and unknown genetic and environmental factors. Weight, height and velocity of growth are associated with early age at menarche again suggesting favourable early life and childhood environmental exposures such as good nutrition are implicated in menarcheal age [517, 518]. In our related study based on the same study population, female coeliacs were more likely to have fathers who were from non-manual and more affluent occupational social classes than the general population cohort and thus exposed to more affluent childhood socioeconomic exposures [511]. Though we do not know of any delay in leading to the diagnosis of their coeliac disease, our female coeliacs attained similar height to general population comparators with 91% of our study participants being diagnosed with coeliac disease in their adulthood years (mean age at diagnosis of all coeliacs 42.5 years) suggesting that coeliacs tended to have favourable nutritional exposures during early childhood or do not get coeliac disease until they are adults. However, the tendency for coeliacs to have irregular menstrual cycles during their teenage years observed in our study may suggest enteropathy-related nutritional, immunological or some other factors might have come into force.

Despite female coeliacs possessing a number of high-risk aetiological factors such as higher likelihood of being Caucasian (relative risk >5 compared to Asian), of more affluent social class (relative risk 2 for social classes I and II to manual classes), of

having earlier menarche (relative risk 3 for age at menarche before age 11 years) and irregular menstrual cycles (relative risk 1.25 for more than 1 year to onset of regular cycles to less than 1 year), published studies have observed coeliac disease is associated with a more than 50% reduced risk of breast cancer [265, 282, 283, 287, 318]. Whether this is in part due to the favourable breast cancer risk profile offered by coeliac disease such as higher proportion being parous (25% reduced risk compared to nulliparous women), having first-term birth before the age of 30 years (2-fold decreased risk in comparison to age at 40 years), earlier age at menopause (relative risk 2 for menopause after age 54 years) or that traditional aetiological factors have a less of a role to play in coeliac disease in comparison to non-coeliacs it is not clear. Further work is required to further explore non-reproductive and non-hormonal factors such as immunological mediators or gene loci associated with coeliac disease that could be implicated in the reduced risk of breast cancer in coeliac disease.

5.1.4.4. Summary

The breast cancer risk profile suggests both protective and adverse associations of coeliac disease. The higher proportion of women being parous, having their first full-term pregnancy before 30 years and breastfeeding in addition to the younger mean age at menopause suggests women with coeliac disease have favourable breast cancer risk profile features in comparison to the general population. However, the higher likelihood of being Caucasian and of affluent social class together with higher proportion having early menarche and irregular menstrual cycles suggests there are also potentially adverse breast cancer risk profile features associated with coeliac disease. Having similar height to the general population and BMI within normal range suggests anthropometric exposures may not explain the apparent reduced risk of breast cancer in women with coeliac disease to the general population. Whether the overall effect of these factors is to confer a protective effect or there are as yet unknown reasons further work is required to help explain the apparent reduced risk of breast cancer in women with coeliac disease.

Chapter 6: Thesis conclusions

6.1. Principal findings

The principal findings of this thesis are that:

- Coeliacs with mild enteropathy have few biochemical deficiencies at diagnosis of coeliac disease and show no important biochemical improvements following treatment with a gluten-free diet in comparison to those with severe enteropathy coeliac disease.
- There is a strong, independent graded association between the incidence rate of new diagnoses of coeliac disease and socio-economic status with the rate twice as high in adults from affluent areas compared with that in adults living in poorer areas.
- Hypertransaminasaemia is uncommon affecting less than 2% of newly diagnosed adults with coeliac disease and in those coeliacs with an abnormal test the majority normalised following a year of treatment with a gluten-free diet.
- At diagnosis coeliacs have much lower total cholesterol levels than the general population with the observed reduction greater in men (21%) than in women (9%) with no increase in total cholesterol observed on treatment with a gluten-free diet.
- Coeliac disease has both protective (such as lower mean levels of total cholesterol, LDL cholesterol, fibrinogen; the higher likelihood of being from more affluent social class; and the small but significant rise in HDL cholesterol) and adverse effects (such as higher likelihood of having abdominal truncal obesity amongst incident coeliacs that only worsens following treatment with a gluten-free diet) on vascular risk profile.

- The quality of life reported by coeliacs presenting with silent disease, classic disease and with gastrointestinal symptoms is worse than that observed in the general population. However a year's treatment with a gluten-free diet results in coeliacs having similar or in some components better quality of life than that observed in the general population. The rate of change of quality of life was similar amongst those coeliacs with silent, classic or symptomatic disease.

- Coeliac disease has both protective (such as higher proportion of women being parous, having their first full-term pregnancy before 30 years and breastfeeding in addition to the younger mean age at menopause) and adverse effects (higher likelihood of being Caucasian and of affluent social class together with higher proportion having early menarche and irregular menstrual cycles) on breast cancer risk profile

6.2. Interpretation

Clinically diagnosed coeliac disease is associated with morbidity but also benefits to health. Although there are clearly some negative *health effects such* as anaemia and a reduction in quality of life whether presenting with silent coeliac disease or with gastrointestinal symptoms, physiological derangements normalise and *quality* of life improves with a year's treatment with a gluten-free diet. Coeliacs have much lower total cholesterol than the general population with no change on treatment with a gluten-free diet. The lower prevalence of hypertransaminasaemia in incident coeliac disease and the association of hypertransaminasaemia with clinical and histological features of more severe coeliac disease may suggest contemporary coeliac disease has a milder disease spectrum in comparison to earlier periods. There is no obvious physiological benefit in treating mild enteropathy coeliac disease though the risk of morbidity such as malignancy and mortality as well as possible benefits of treatment upon symptoms needs further clarification. Adverse anthropometric profiles such as tendency to be overweight or obese at diagnosis of coeliac disease with a higher likelihood of abdominal truncal obesity highlights the changing face of contemporary disease and may help to explain the increased risk of vascular disease events observed in some but not all studies. Whether the observed association between incidence rate of coeliac disease and affluence is a reflection of the variation in environmental exposures to aetiological factors such as breastfeeding or could be accounted for by differences in uptake and utilisation of health services it is unclear but further work to unravel the contribution of social class to the aetiology and incidence of coeliac disease is required. Either the overall effect of factors such as a reduced higher proportion of women being parous, having their first full-term pregnancy before 30 years and breastfeeding in addition to the younger mean age at menopause confers a

protective effect or there as yet unknown explanations to the apparent reduced risk of breast cancer in women with coeliac disease.

6.3. Recommendations for future work

6.3.1. Mild enteropathy coeliac disease

Further approaches to clarify the impact of mild enteropathy versus severe enteropathy are suggested by this work. In the future, a survival analysis and cancer registry linkage carried out on this cohort would give information about the mortality and malignancy risk in those people with mild enteropathy coeliac disease compared to those with severe enteropathy disease. The cohort size may result in such a study being dependent on a longer follow-up period before a meaningful analysis could be undertaken. Using a similar longitudinal study design to the vascular risk profile study in this thesis, the effect of treatment with a gluten-free diet upon quality of life and symptoms in coeliacs newly diagnosed with mild versus severe enteropathy disease should be performed.

6.3.2. Screening for silent coeliac disease

A number of studies have observed that clinically diagnosed coeliac disease still confers a 1.3 to 2-fold increased risk of all-cause mortality compared with the general population. Recent data suggests people with latent coeliac disease have also an increased risk of death. We have observed people with silent coeliac disease have a reduction in quality of life in comparison to the general population which improves with treatment with a gluten-free diet. However silent coeliacs appear to have the least favourable cholesterol and waist: hip circumference profile at diagnosis with the degree of abdominal truncal obesity worsening with treatment and cholesterol profile

not improving with treatment. It is unclear whether people with silent coeliac disease share similar risks of mortality and vascular events to coeliacs with gastrointestinal symptoms. In the future, a survival analysis of all-cause and vascular-related deaths carried out on this cohort would give information about the mortality risk in those people with silent disease and so help contribute to the discussion as to whether screening for silent coeliac disease should be performed or not.

6.3.3. Incidence of coeliac disease and socioeconomic status

The incidence of coeliac disease by socioeconomic status could be further explored by determining the socioeconomic distribution of people who are being screened for coeliac disease in both a primary care and hospital setting. Such a study could be performed by determining the IMD07 score of each serological test requested by source of referral (general practitioner; hospital-based referral) through searching immunological databases. Further work is underway using the study cohort of chapter 5 to compare the childhood socioeconomic status using father's occupational social class in women with coeliac disease compared to the general population.

6.3.4. Fertility of women with coeliac disease

Data from the study cohort of chapter 5 is being used to examine the fertility experience of women with coeliac disease compared to the general population to further assess the impact of clinically diagnosed coeliac disease.

6.3.5. Vascular risk profile in coeliac disease

We have used anthropometrics such as waist: hip ratio to reflect the presence of abdominal truncal obesity though more accurate quantitation of intraabdominal

visceral fat mass and distribution at diagnosis of coeliac disease and any change with a gluten-free diet could be achieved with the use of dual-energy x-ray absorptiometry (DEXA) or computerised tomography measurements. Carotid artery intima and media thickness at diagnosis of coeliac disease (classic, those with gastrointestinal symptoms, silent disease) and following treatment with a gluten-free diet could be non-invasively measured in a longitudinal cohort study, providing a surrogate marker for a vascular disease outcome thereby reducing the need for a longer follow-up period. The intima and media thickness could also be modelled against predictor variables such as presence of villous atrophy, tissue transglutaminase titres using logistic regression.

Chapter 7: Appendices

Appendix 7.1. Sheffield geographically defined by super output area

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001611	Sheffield 001	Sheffield 001A
Yorkshire and The Humber	Sheffield	E02001611	Sheffield 001	Sheffield 001C
Yorkshire and The Humber	Sheffield	E02001611	Sheffield 001	Sheffield 001D
Yorkshire and The Humber	Sheffield	E02001611	Sheffield 001	Sheffield 001E
Yorkshire and The Humber	Sheffield	E02001611	Sheffield 001	Sheffield 001B
Yorkshire and The Humber	Sheffield	E02001612	Sheffield 002	Sheffield 002D
Yorkshire and The Humber	Sheffield	E02001612	Sheffield 002	Sheffield 002C
Yorkshire and The Humber	Sheffield	E02001612	Sheffield 002	Sheffield 002A
Yorkshire and The Humber	Sheffield	E02001612	Sheffield 002	Sheffield 002B
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003B
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003A
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003C
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003D
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003E
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003F
Yorkshire and The Humber	Sheffield	E02001613	Sheffield 003	Sheffield 003G
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004A
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004E
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004C
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004D
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004B
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004G
Yorkshire and The Humber	Sheffield	E02001614	Sheffield 004	Sheffield 004F
Yorkshire and The Humber	Sheffield	E02001615	Sheffield 005	Sheffield 005A
Yorkshire and The Humber	Sheffield	E02001615	Sheffield 005	Sheffield 005C
Yorkshire and The Humber	Sheffield	E02001615	Sheffield 005	Sheffield 005D
Yorkshire and The Humber	Sheffield	E02001615	Sheffield 005	Sheffield 005E
Yorkshire and The Humber	Sheffield	E02001615	Sheffield 005	Sheffield 005B
Yorkshire and The Humber	Sheffield	E02001616	Sheffield 006	Sheffield 006A
Yorkshire and The Humber	Sheffield	E02001616	Sheffield 006	Sheffield 006B
Yorkshire and The Humber	Sheffield	E02001616	Sheffield 006	Sheffield 006D
Yorkshire and The Humber	Sheffield	E02001616	Sheffield 006	Sheffield 006E
Yorkshire and The Humber	Sheffield	E02001616	Sheffield 006	Sheffield 006C
Yorkshire and The Humber	Sheffield	E02001617	Sheffield 007	Sheffield 007C
Yorkshire and The Humber	Sheffield	E02001617	Sheffield 007	Sheffield 007B
Yorkshire and The Humber	Sheffield	E02001617	Sheffield 007	Sheffield 007A
Yorkshire and The Humber	Sheffield	E02001617	Sheffield 007	Sheffield 007D
Yorkshire and The Humber	Sheffield	E02001618	Sheffield 008	Sheffield 008C
Yorkshire and The Humber	Sheffield	E02001618	Sheffield 008	Sheffield 008B
Yorkshire and The Humber	Sheffield	E02001618	Sheffield 008	Sheffield 008D
Yorkshire and The Humber	Sheffield	E02001618	Sheffield 008	Sheffield 008E
Yorkshire and The Humber	Sheffield	E02001618	Sheffield 008	Sheffield 008A
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009A
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009G
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009F
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009E
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009D
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009C

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001619	Sheffield 009	Sheffield 009B
Yorkshire and The Humber	Sheffield	E02001620	Sheffield 010	Sheffield 010A
Yorkshire and The Humber	Sheffield	E02001620	Sheffield 010	Sheffield 010B
Yorkshire and The Humber	Sheffield	E02001620	Sheffield 010	Sheffield 010D
Yorkshire and The Humber	Sheffield	E02001620	Sheffield 010	Sheffield 010C
Yorkshire and The Humber	Sheffield	E02001621	Sheffield 011	Sheffield 011D
Yorkshire and The Humber	Sheffield	E02001621	Sheffield 011	Sheffield 011B
Yorkshire and The Humber	Sheffield	E02001621	Sheffield 011	Sheffield 011A
Yorkshire and The Humber	Sheffield	E02001621	Sheffield 011	Sheffield 011C
Yorkshire and The Humber	Sheffield	E02001622	Sheffield 012	Sheffield 012D
Yorkshire and The Humber	Sheffield	E02001622	Sheffield 012	Sheffield 012E
Yorkshire and The Humber	Sheffield	E02001622	Sheffield 012	Sheffield 012F
Yorkshire and The Humber	Sheffield	E02001622	Sheffield 012	Sheffield 012A
Yorkshire and The Humber	Sheffield	E02001622	Sheffield 012	Sheffield 012B
Yorkshire and The Humber	Sheffield	E02001622	Sheffield 012	Sheffield 012C
Yorkshire and The Humber	Sheffield	E02001623	Sheffield 013	Sheffield 013C
Yorkshire and The Humber	Sheffield	E02001623	Sheffield 013	Sheffield 013D
Yorkshire and The Humber	Sheffield	E02001623	Sheffield 013	Sheffield 013E
Yorkshire and The Humber	Sheffield	E02001623	Sheffield 013	Sheffield 013A
Yorkshire and The Humber	Sheffield	E02001623	Sheffield 013	Sheffield 013B
Yorkshire and The Humber	Sheffield	E02001624	Sheffield 014	Sheffield 014B
Yorkshire and The Humber	Sheffield	E02001624	Sheffield 014	Sheffield 014A
Yorkshire and The Humber	Sheffield	E02001624	Sheffield 014	Sheffield 014C
Yorkshire and The Humber	Sheffield	E02001624	Sheffield 014	Sheffield 014D
Yorkshire and The Humber	Sheffield	E02001624	Sheffield 014	Sheffield 014E
Yorkshire and The Humber	Sheffield	E02001624	Sheffield 014	Sheffield 014F
Yorkshire and The Humber	Sheffield	E02001625	Sheffield 015	Sheffield 015E
Yorkshire and The Humber	Sheffield	E02001625	Sheffield 015	Sheffield 015D
Yorkshire and The Humber	Sheffield	E02001625	Sheffield 015	Sheffield 015A
Yorkshire and The Humber	Sheffield	E02001625	Sheffield 015	Sheffield 015B
Yorkshire and The Humber	Sheffield	E02001625	Sheffield 015	Sheffield 015C
Yorkshire and The Humber	Sheffield	E02001626	Sheffield 016	Sheffield 016C
Yorkshire and The Humber	Sheffield	E02001626	Sheffield 016	Sheffield 016D
Yorkshire and The Humber	Sheffield	E02001626	Sheffield 016	Sheffield 016A
Yorkshire and The Humber	Sheffield	E02001626	Sheffield 016	Sheffield 016B
Yorkshire and The Humber	Sheffield	E02001627	Sheffield 017	Sheffield 017D
Yorkshire and The Humber	Sheffield	E02001627	Sheffield 017	Sheffield 017B
Yorkshire and The Humber	Sheffield	E02001627	Sheffield 017	Sheffield 017A
Yorkshire and The Humber	Sheffield	E02001627	Sheffield 017	Sheffield 017C
Yorkshire and The Humber	Sheffield	E02001628	Sheffield 018	Sheffield 018C
Yorkshire and The Humber	Sheffield	E02001628	Sheffield 018	Sheffield 018A
Yorkshire and The Humber	Sheffield	E02001628	Sheffield 018	Sheffield 018B
Yorkshire and The Humber	Sheffield	E02001628	Sheffield 018	Sheffield 018D
Yorkshire and The Humber	Sheffield	E02001629	Sheffield 019	Sheffield 019D
Yorkshire and The Humber	Sheffield	E02001629	Sheffield 019	Sheffield 019A
Yorkshire and The Humber	Sheffield	E02001629	Sheffield 019	Sheffield 019C
Yorkshire and The Humber	Sheffield	E02001629	Sheffield 019	Sheffield 019B
Yorkshire and The Humber	Sheffield	E02001630	Sheffield 020	Sheffield 020A
Yorkshire and The Humber	Sheffield	E02001630	Sheffield 020	Sheffield 020B
Yorkshire and The Humber	Sheffield	E02001630	Sheffield 020	Sheffield 020C
Yorkshire and The Humber	Sheffield	E02001630	Sheffield 020	Sheffield 020D
Yorkshire and The Humber	Sheffield	E02001630	Sheffield 020	Sheffield 020E
Yorkshire and The Humber	Sheffield	E02001631	Sheffield 021	Sheffield 021D
Yorkshire and The Humber	Sheffield	E02001631	Sheffield 021	Sheffield 021C
Yorkshire and The Humber	Sheffield	E02001631	Sheffield 021	Sheffield 021A
Yorkshire and The Humber	Sheffield	E02001631	Sheffield 021	Sheffield 021E

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001631	Sheffield 021	Sheffield 021B
Yorkshire and The Humber	Sheffield	E02001632	Sheffield 022	Sheffield 022E
Yorkshire and The Humber	Sheffield	E02001632	Sheffield 022	Sheffield 022D
Yorkshire and The Humber	Sheffield	E02001632	Sheffield 022	Sheffield 022C
Yorkshire and The Humber	Sheffield	E02001632	Sheffield 022	Sheffield 022B
Yorkshire and The Humber	Sheffield	E02001632	Sheffield 022	Sheffield 022A
Yorkshire and The Humber	Sheffield	E02001632	Sheffield 022	Sheffield 022F
Yorkshire and The Humber	Sheffield	E02001633	Sheffield 023	Sheffield 023C
Yorkshire and The Humber	Sheffield	E02001633	Sheffield 023	Sheffield 023B
Yorkshire and The Humber	Sheffield	E02001633	Sheffield 023	Sheffield 023D
Yorkshire and The Humber	Sheffield	E02001633	Sheffield 023	Sheffield 023E
Yorkshire and The Humber	Sheffield	E02001633	Sheffield 023	Sheffield 023A
Yorkshire and The Humber	Sheffield	E02001634	Sheffield 024	Sheffield 024B
Yorkshire and The Humber	Sheffield	E02001634	Sheffield 024	Sheffield 024A
Yorkshire and The Humber	Sheffield	E02001634	Sheffield 024	Sheffield 024D
Yorkshire and The Humber	Sheffield	E02001634	Sheffield 024	Sheffield 024F
Yorkshire and The Humber	Sheffield	E02001634	Sheffield 024	Sheffield 024E
Yorkshire and The Humber	Sheffield	E02001634	Sheffield 024	Sheffield 024C
Yorkshire and The Humber	Sheffield	E02001635	Sheffield 025	Sheffield 025C
Yorkshire and The Humber	Sheffield	E02001635	Sheffield 025	Sheffield 025A
Yorkshire and The Humber	Sheffield	E02001635	Sheffield 025	Sheffield 025D
Yorkshire and The Humber	Sheffield	E02001635	Sheffield 025	Sheffield 025B
Yorkshire and The Humber	Sheffield	E02001636	Sheffield 026	Sheffield 026E
Yorkshire and The Humber	Sheffield	E02001636	Sheffield 026	Sheffield 026B
Yorkshire and The Humber	Sheffield	E02001636	Sheffield 026	Sheffield 026D
Yorkshire and The Humber	Sheffield	E02001636	Sheffield 026	Sheffield 026C
Yorkshire and The Humber	Sheffield	E02001636	Sheffield 026	Sheffield 026A
Yorkshire and The Humber	Sheffield	E02001637	Sheffield 027	Sheffield 027D
Yorkshire and The Humber	Sheffield	E02001637	Sheffield 027	Sheffield 027C
Yorkshire and The Humber	Sheffield	E02001637	Sheffield 027	Sheffield 027B
Yorkshire and The Humber	Sheffield	E02001637	Sheffield 027	Sheffield 027A
Yorkshire and The Humber	Sheffield	E02001638	Sheffield 028	Sheffield 028B
Yorkshire and The Humber	Sheffield	E02001638	Sheffield 028	Sheffield 028A
Yorkshire and The Humber	Sheffield	E02001638	Sheffield 028	Sheffield 028C
Yorkshire and The Humber	Sheffield	E02001638	Sheffield 028	Sheffield 028D
Yorkshire and The Humber	Sheffield	E02001639	Sheffield 029	Sheffield 029A
Yorkshire and The Humber	Sheffield	E02001639	Sheffield 029	Sheffield 029B
Yorkshire and The Humber	Sheffield	E02001639	Sheffield 029	Sheffield 029C
Yorkshire and The Humber	Sheffield	E02001639	Sheffield 029	Sheffield 029D
Yorkshire and The Humber	Sheffield	E02001640	Sheffield 030	Sheffield 030D
Yorkshire and The Humber	Sheffield	E02001640	Sheffield 030	Sheffield 030A
Yorkshire and The Humber	Sheffield	E02001640	Sheffield 030	Sheffield 030B
Yorkshire and The Humber	Sheffield	E02001640	Sheffield 030	Sheffield 030C
Yorkshire and The Humber	Sheffield	E02001640	Sheffield 030	Sheffield 030E
Yorkshire and The Humber	Sheffield	E02001641	Sheffield 031	Sheffield 031D
Yorkshire and The Humber	Sheffield	E02001641	Sheffield 031	Sheffield 031C
Yorkshire and The Humber	Sheffield	E02001641	Sheffield 031	Sheffield 031B
Yorkshire and The Humber	Sheffield	E02001641	Sheffield 031	Sheffield 031A
Yorkshire and The Humber	Sheffield	E02001642	Sheffield 032	Sheffield 032A
Yorkshire and The Humber	Sheffield	E02001642	Sheffield 032	Sheffield 032F
Yorkshire and The Humber	Sheffield	E02001642	Sheffield 032	Sheffield 032B
Yorkshire and The Humber	Sheffield	E02001642	Sheffield 032	Sheffield 032C
Yorkshire and The Humber	Sheffield	E02001642	Sheffield 032	Sheffield 032D
Yorkshire and The Humber	Sheffield	E02001642	Sheffield 032	Sheffield 032E
Yorkshire and The Humber	Sheffield	E02001643	Sheffield 033	Sheffield 033B
Yorkshire and The Humber	Sheffield	E02001643	Sheffield 033	Sheffield 033D

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001643	Sheffield 033	Sheffield 033C
Yorkshire and The Humber	Sheffield	E02001643	Sheffield 033	Sheffield 033A
Yorkshire and The Humber	Sheffield	E02001644	Sheffield 034	Sheffield 034C
Yorkshire and The Humber	Sheffield	E02001644	Sheffield 034	Sheffield 034A
Yorkshire and The Humber	Sheffield	E02001644	Sheffield 034	Sheffield 034D
Yorkshire and The Humber	Sheffield	E02001644	Sheffield 034	Sheffield 034B
Yorkshire and The Humber	Sheffield	E02001645	Sheffield 035	Sheffield 035B
Yorkshire and The Humber	Sheffield	E02001645	Sheffield 035	Sheffield 035A
Yorkshire and The Humber	Sheffield	E02001645	Sheffield 035	Sheffield 035C
Yorkshire and The Humber	Sheffield	E02001645	Sheffield 035	Sheffield 035D
Yorkshire and The Humber	Sheffield	E02001646	Sheffield 036	Sheffield 036A
Yorkshire and The Humber	Sheffield	E02001646	Sheffield 036	Sheffield 036B
Yorkshire and The Humber	Sheffield	E02001646	Sheffield 036	Sheffield 036C
Yorkshire and The Humber	Sheffield	E02001646	Sheffield 036	Sheffield 036D
Yorkshire and The Humber	Sheffield	E02001646	Sheffield 036	Sheffield 036E
Yorkshire and The Humber	Sheffield	E02001647	Sheffield 037	Sheffield 037C
Yorkshire and The Humber	Sheffield	E02001647	Sheffield 037	Sheffield 037D
Yorkshire and The Humber	Sheffield	E02001647	Sheffield 037	Sheffield 037A
Yorkshire and The Humber	Sheffield	E02001647	Sheffield 037	Sheffield 037B
Yorkshire and The Humber	Sheffield	E02001648	Sheffield 038	Sheffield 038C
Yorkshire and The Humber	Sheffield	E02001648	Sheffield 038	Sheffield 038D
Yorkshire and The Humber	Sheffield	E02001648	Sheffield 038	Sheffield 038B
Yorkshire and The Humber	Sheffield	E02001648	Sheffield 038	Sheffield 038A
Yorkshire and The Humber	Sheffield	E02001649	Sheffield 039	Sheffield 039B
Yorkshire and The Humber	Sheffield	E02001649	Sheffield 039	Sheffield 039C
Yorkshire and The Humber	Sheffield	E02001649	Sheffield 039	Sheffield 039D
Yorkshire and The Humber	Sheffield	E02001649	Sheffield 039	Sheffield 039E
Yorkshire and The Humber	Sheffield	E02001649	Sheffield 039	Sheffield 039F
Yorkshire and The Humber	Sheffield	E02001649	Sheffield 039	Sheffield 039A
Yorkshire and The Humber	Sheffield	E02001650	Sheffield 040	Sheffield 040B
Yorkshire and The Humber	Sheffield	E02001650	Sheffield 040	Sheffield 040D
Yorkshire and The Humber	Sheffield	E02001650	Sheffield 040	Sheffield 040A
Yorkshire and The Humber	Sheffield	E02001650	Sheffield 040	Sheffield 040C
Yorkshire and The Humber	Sheffield	E02001651	Sheffield 041	Sheffield 041D
Yorkshire and The Humber	Sheffield	E02001651	Sheffield 041	Sheffield 041A
Yorkshire and The Humber	Sheffield	E02001651	Sheffield 041	Sheffield 041C
Yorkshire and The Humber	Sheffield	E02001651	Sheffield 041	Sheffield 041B
Yorkshire and The Humber	Sheffield	E02001651	Sheffield 041	Sheffield 041E
Yorkshire and The Humber	Sheffield	E02001652	Sheffield 042	Sheffield 042C
Yorkshire and The Humber	Sheffield	E02001652	Sheffield 042	Sheffield 042D
Yorkshire and The Humber	Sheffield	E02001652	Sheffield 042	Sheffield 042E
Yorkshire and The Humber	Sheffield	E02001652	Sheffield 042	Sheffield 042A
Yorkshire and The Humber	Sheffield	E02001652	Sheffield 042	Sheffield 042B
Yorkshire and The Humber	Sheffield	E02001653	Sheffield 043	Sheffield 043B
Yorkshire and The Humber	Sheffield	E02001653	Sheffield 043	Sheffield 043C
Yorkshire and The Humber	Sheffield	E02001653	Sheffield 043	Sheffield 043D
Yorkshire and The Humber	Sheffield	E02001653	Sheffield 043	Sheffield 043A
Yorkshire and The Humber	Sheffield	E02001654	Sheffield 044	Sheffield 044B
Yorkshire and The Humber	Sheffield	E02001654	Sheffield 044	Sheffield 044A
Yorkshire and The Humber	Sheffield	E02001654	Sheffield 044	Sheffield 044D
Yorkshire and The Humber	Sheffield	E02001654	Sheffield 044	Sheffield 044C
Yorkshire and The Humber	Sheffield	E02001655	Sheffield 045	Sheffield 045D
Yorkshire and The Humber	Sheffield	E02001655	Sheffield 045	Sheffield 045C
Yorkshire and The Humber	Sheffield	E02001655	Sheffield 045	Sheffield 045B
Yorkshire and The Humber	Sheffield	E02001655	Sheffield 045	Sheffield 045E
Yorkshire and The Humber	Sheffield	E02001655	Sheffield 045	Sheffield 045A

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001656	Sheffield 046	Sheffield 046D
Yorkshire and The Humber	Sheffield	E02001656	Sheffield 046	Sheffield 046B
Yorkshire and The Humber	Sheffield	E02001656	Sheffield 046	Sheffield 046E
Yorkshire and The Humber	Sheffield	E02001656	Sheffield 046	Sheffield 046A
Yorkshire and The Humber	Sheffield	E02001656	Sheffield 046	Sheffield 046C
Yorkshire and The Humber	Sheffield	E02001657	Sheffield 047	Sheffield 047C
Yorkshire and The Humber	Sheffield	E02001657	Sheffield 047	Sheffield 047B
Yorkshire and The Humber	Sheffield	E02001657	Sheffield 047	Sheffield 047A
Yorkshire and The Humber	Sheffield	E02001657	Sheffield 047	Sheffield 047F
Yorkshire and The Humber	Sheffield	E02001657	Sheffield 047	Sheffield 047E
Yorkshire and The Humber	Sheffield	E02001657	Sheffield 047	Sheffield 047D
Yorkshire and The Humber	Sheffield	E02001658	Sheffield 048	Sheffield 048C
Yorkshire and The Humber	Sheffield	E02001658	Sheffield 048	Sheffield 048B
Yorkshire and The Humber	Sheffield	E02001658	Sheffield 048	Sheffield 048A
Yorkshire and The Humber	Sheffield	E02001658	Sheffield 048	Sheffield 048D
Yorkshire and The Humber	Sheffield	E02001659	Sheffield 049	Sheffield 049C
Yorkshire and The Humber	Sheffield	E02001659	Sheffield 049	Sheffield 049B
Yorkshire and The Humber	Sheffield	E02001659	Sheffield 049	Sheffield 049A
Yorkshire and The Humber	Sheffield	E02001659	Sheffield 049	Sheffield 049D
Yorkshire and The Humber	Sheffield	E02001660	Sheffield 050	Sheffield 050D
Yorkshire and The Humber	Sheffield	E02001660	Sheffield 050	Sheffield 050F
Yorkshire and The Humber	Sheffield	E02001660	Sheffield 050	Sheffield 050E
Yorkshire and The Humber	Sheffield	E02001660	Sheffield 050	Sheffield 050C
Yorkshire and The Humber	Sheffield	E02001660	Sheffield 050	Sheffield 050B
Yorkshire and The Humber	Sheffield	E02001660	Sheffield 050	Sheffield 050A
Yorkshire and The Humber	Sheffield	E02001661	Sheffield 051	Sheffield 051A
Yorkshire and The Humber	Sheffield	E02001661	Sheffield 051	Sheffield 051E
Yorkshire and The Humber	Sheffield	E02001661	Sheffield 051	Sheffield 051D
Yorkshire and The Humber	Sheffield	E02001661	Sheffield 051	Sheffield 051C
Yorkshire and The Humber	Sheffield	E02001661	Sheffield 051	Sheffield 051B
Yorkshire and The Humber	Sheffield	E02001662	Sheffield 052	Sheffield 052E
Yorkshire and The Humber	Sheffield	E02001662	Sheffield 052	Sheffield 052A
Yorkshire and The Humber	Sheffield	E02001662	Sheffield 052	Sheffield 052B
Yorkshire and The Humber	Sheffield	E02001662	Sheffield 052	Sheffield 052C
Yorkshire and The Humber	Sheffield	E02001662	Sheffield 052	Sheffield 052D
Yorkshire and The Humber	Sheffield	E02001663	Sheffield 053	Sheffield 053D
Yorkshire and The Humber	Sheffield	E02001663	Sheffield 053	Sheffield 053A
Yorkshire and The Humber	Sheffield	E02001663	Sheffield 053	Sheffield 053E
Yorkshire and The Humber	Sheffield	E02001663	Sheffield 053	Sheffield 053B
Yorkshire and The Humber	Sheffield	E02001663	Sheffield 053	Sheffield 053C
Yorkshire and The Humber	Sheffield	E02001664	Sheffield 054	Sheffield 054B
Yorkshire and The Humber	Sheffield	E02001664	Sheffield 054	Sheffield 054D
Yorkshire and The Humber	Sheffield	E02001664	Sheffield 054	Sheffield 054C
Yorkshire and The Humber	Sheffield	E02001664	Sheffield 054	Sheffield 054A
Yorkshire and The Humber	Sheffield	E02001665	Sheffield 055	Sheffield 055A
Yorkshire and The Humber	Sheffield	E02001665	Sheffield 055	Sheffield 055D
Yorkshire and The Humber	Sheffield	E02001665	Sheffield 055	Sheffield 055E
Yorkshire and The Humber	Sheffield	E02001665	Sheffield 055	Sheffield 055C
Yorkshire and The Humber	Sheffield	E02001665	Sheffield 055	Sheffield 055B
Yorkshire and The Humber	Sheffield	E02001665	Sheffield 055	Sheffield 055F
Yorkshire and The Humber	Sheffield	E02001666	Sheffield 056	Sheffield 056C
Yorkshire and The Humber	Sheffield	E02001666	Sheffield 056	Sheffield 056D
Yorkshire and The Humber	Sheffield	E02001666	Sheffield 056	Sheffield 056A
Yorkshire and The Humber	Sheffield	E02001666	Sheffield 056	Sheffield 056B
Yorkshire and The Humber	Sheffield	E02001667	Sheffield 057	Sheffield 057D
Yorkshire and The Humber	Sheffield	E02001667	Sheffield 057	Sheffield 057C

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001667	Sheffield 057	Sheffield 057B
Yorkshire and The Humber	Sheffield	E02001667	Sheffield 057	Sheffield 057A
Yorkshire and The Humber	Sheffield	E02001668	Sheffield 058	Sheffield 058B
Yorkshire and The Humber	Sheffield	E02001668	Sheffield 058	Sheffield 058D
Yorkshire and The Humber	Sheffield	E02001668	Sheffield 058	Sheffield 058C
Yorkshire and The Humber	Sheffield	E02001668	Sheffield 058	Sheffield 058A
Yorkshire and The Humber	Sheffield	E02001669	Sheffield 059	Sheffield 059C
Yorkshire and The Humber	Sheffield	E02001669	Sheffield 059	Sheffield 059A
Yorkshire and The Humber	Sheffield	E02001669	Sheffield 059	Sheffield 059D
Yorkshire and The Humber	Sheffield	E02001669	Sheffield 059	Sheffield 059B
Yorkshire and The Humber	Sheffield	E02001669	Sheffield 059	Sheffield 059E
Yorkshire and The Humber	Sheffield	E02001670	Sheffield 060	Sheffield 060A
Yorkshire and The Humber	Sheffield	E02001670	Sheffield 060	Sheffield 060D
Yorkshire and The Humber	Sheffield	E02001670	Sheffield 060	Sheffield 060C
Yorkshire and The Humber	Sheffield	E02001670	Sheffield 060	Sheffield 060B
Yorkshire and The Humber	Sheffield	E02001671	Sheffield 061	Sheffield 061D
Yorkshire and The Humber	Sheffield	E02001671	Sheffield 061	Sheffield 061C
Yorkshire and The Humber	Sheffield	E02001671	Sheffield 061	Sheffield 061A
Yorkshire and The Humber	Sheffield	E02001671	Sheffield 061	Sheffield 061B
Yorkshire and The Humber	Sheffield	E02001672	Sheffield 062	Sheffield 062A
Yorkshire and The Humber	Sheffield	E02001672	Sheffield 062	Sheffield 062B
Yorkshire and The Humber	Sheffield	E02001672	Sheffield 062	Sheffield 062C
Yorkshire and The Humber	Sheffield	E02001672	Sheffield 062	Sheffield 062D
Yorkshire and The Humber	Sheffield	E02001673	Sheffield 063	Sheffield 063D
Yorkshire and The Humber	Sheffield	E02001673	Sheffield 063	Sheffield 063B
Yorkshire and The Humber	Sheffield	E02001673	Sheffield 063	Sheffield 063C
Yorkshire and The Humber	Sheffield	E02001673	Sheffield 063	Sheffield 063A
Yorkshire and The Humber	Sheffield	E02001674	Sheffield 064	Sheffield 064A
Yorkshire and The Humber	Sheffield	E02001674	Sheffield 064	Sheffield 064C
Yorkshire and The Humber	Sheffield	E02001674	Sheffield 064	Sheffield 064E
Yorkshire and The Humber	Sheffield	E02001674	Sheffield 064	Sheffield 064F
Yorkshire and The Humber	Sheffield	E02001674	Sheffield 064	Sheffield 064B
Yorkshire and The Humber	Sheffield	E02001674	Sheffield 064	Sheffield 064D
Yorkshire and The Humber	Sheffield	E02001675	Sheffield 065	Sheffield 065F
Yorkshire and The Humber	Sheffield	E02001675	Sheffield 065	Sheffield 065C
Yorkshire and The Humber	Sheffield	E02001675	Sheffield 065	Sheffield 065E
Yorkshire and The Humber	Sheffield	E02001675	Sheffield 065	Sheffield 065D
Yorkshire and The Humber	Sheffield	E02001675	Sheffield 065	Sheffield 065A
Yorkshire and The Humber	Sheffield	E02001675	Sheffield 065	Sheffield 065B
Yorkshire and The Humber	Sheffield	E02001676	Sheffield 066	Sheffield 066A
Yorkshire and The Humber	Sheffield	E02001676	Sheffield 066	Sheffield 066B
Yorkshire and The Humber	Sheffield	E02001676	Sheffield 066	Sheffield 066C
Yorkshire and The Humber	Sheffield	E02001676	Sheffield 066	Sheffield 066D
Yorkshire and The Humber	Sheffield	E02001677	Sheffield 067	Sheffield 067E
Yorkshire and The Humber	Sheffield	E02001677	Sheffield 067	Sheffield 067D
Yorkshire and The Humber	Sheffield	E02001677	Sheffield 067	Sheffield 067B
Yorkshire and The Humber	Sheffield	E02001677	Sheffield 067	Sheffield 067C
Yorkshire and The Humber	Sheffield	E02001677	Sheffield 067	Sheffield 067A
Yorkshire and The Humber	Sheffield	E02001678	Sheffield 068	Sheffield 068A
Yorkshire and The Humber	Sheffield	E02001678	Sheffield 068	Sheffield 068E
Yorkshire and The Humber	Sheffield	E02001678	Sheffield 068	Sheffield 068F
Yorkshire and The Humber	Sheffield	E02001678	Sheffield 068	Sheffield 068C
Yorkshire and The Humber	Sheffield	E02001678	Sheffield 068	Sheffield 068D
Yorkshire and The Humber	Sheffield	E02001678	Sheffield 068	Sheffield 068B
Yorkshire and The Humber	Sheffield	E02001679	Sheffield 069	Sheffield 069D
Yorkshire and The Humber	Sheffield	E02001679	Sheffield 069	Sheffield 069A

Region	Local Authority	Lower Layer SOA Code	Middle Layer SOA	Lower Layer SOA
Yorkshire and The Humber	Sheffield	E02001679	Sheffield 069	Sheffield 069C
Yorkshire and The Humber	Sheffield	E02001679	Sheffield 069	Sheffield 069B
Yorkshire and The Humber	Sheffield	E02001680	Sheffield 070	Sheffield 070D
Yorkshire and The Humber	Sheffield	E02001680	Sheffield 070	Sheffield 070C
Yorkshire and The Humber	Sheffield	E02001680	Sheffield 070	Sheffield 070B
Yorkshire and The Humber	Sheffield	E02001680	Sheffield 070	Sheffield 070A
Yorkshire and The Humber	Sheffield	E02001681	Sheffield 071	Sheffield 071A
Yorkshire and The Humber	Sheffield	E02001681	Sheffield 071	Sheffield 071E
Yorkshire and The Humber	Sheffield	E02001681	Sheffield 071	Sheffield 071C
Yorkshire and The Humber	Sheffield	E02001681	Sheffield 071	Sheffield 071D
Yorkshire and The Humber	Sheffield	E02001681	Sheffield 071	Sheffield 071B

Appendix 7.2. Vascular diagnoses possessed by incident cases of coeliac disease

This questionnaire is designed to find out about your medical history. Please try to answer every question by writing in the spaces provided or ticking the appropriate boxes.

Question 1

What is your ethnic group?

- ☐ Caucasian
- ☐ Black Caribbean or Black British Caribbean
- ☐ Black African or Black British African
- ☐ Other Black background
- ☐ Asian Indian or Asian British Indian
- ☐ Asian Pakistani or Asian British Pakistani
- ☐ Asian Bangladeshi or Asian British Bangladeshi
- ☐ Chinese or other Ethnic background Chinese
- ☐ Other Asian background
- ☐ Mixed – White and Black Caribbean
- ☐ Mixed – White and Black African
- ☐ Mixed – White and Asian
- ☐ Other Mixed background
- ☐ Other ethnic background

Question 2

Do you ever drink alcohol?

- ☐ No
- ☐ Yes

How much alcohol would you typically drink in a week?

Number of wine glasses per week .....

Number of pints of lager, beer or cider per week .....

Number of glasses of spirits or liquor per week .....

Question 3

Do you smoke?

- ☐ No I have never smoked
- ☐ I don't smoke currently but I have smoked in the past

How much did you typically smoke in a day?

Number of cigarettes / cigars / pipes per day .....

How many years did you smoke?

Number of years smoked .....

- ☐ I smoke cigarettes / pipe / cigars

How much would you typically smoke in a day?

Number of cigarettes / cigars / pipes per day .....

How long have you been smoking?

Number of years smoked .....

Question 4

1. How old were you when you left school? ..... *years*

2. What was the highest school / college level which you reached?

- ☐ Primary school completed
- ☐ Secondary school completed
- ☐ Technical or professional secondary school
- ☐ Grammar school or college
- ☐ University degree
- ☐ Did not attend school

Question 5

Do you own your own home?

- ☐ No
- ☐ Yes

Question 6

1. What is your occupation? .....
2. What was your father's occupation when you were 10 years old? .....

Question 7

Have you ever been told by a doctor that you have, or had, one or more of the following?

1. Heart attack
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*
2. Angina
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*
3. Stroke
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*
4. High blood pressure
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*

     Are you receiving treatment for this?
     - ☐ No
     - ☐ Yes ..... *name of tablets*
5. High cholesterol
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*

     Are you receiving treatment for this?
     - ☐ No
     - ☐ Yes ..... *name of tablets*

6. Diabetes
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*

     Are you receiving treatment for this?
     - ☐ No
     - ☐ Watching diet
     - ☐ Tablets
     - ☐ Insulin
7. Circulatory problems in the legs
   - ☐ No
   - ☐ Yes

     If yes at what age did it first occur? ..... *years*

Appendix 7.3. Compliance with a gluten-free diet

1. How would you describe your compliance to the gluten-free diet? *Please place a vertical mark on the line below to indicate how you would much you stick to the gluten-free diet:*

Do not stick to diet		*Stick to diet all the time*

Appendix 7.4. SF36 questionnaire

INSTRUCTIONS: This set of questions asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. Answer every question by marking the answer as indicated. If you are unsure about how to answer a question please give the best answer you can.

1. In general, would you say your health is: (Please tick **one** box.)

Excellent	☐
Very Good	☐
Good	☐
Fair	☐
Poor	☐

2. Compared to one year ago, how would you rate your health in general now? (Please tick **one** box.)

Much better than one year ago	☐
Somewhat better now than one year ago	☐
About the same as one year ago	☐
Somewhat worse now than one year ago	☐
Much worse now than one year ago	☐

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much? **(Please circle one number on each line.)**

	Activities	Yes, Limited A Lot	Yes, Limited A Little	Not Limited At All
3(a)	**Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	1	2	3
3(b)	**Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1	2	3
3(c)	Lifting or carrying groceries	1	2	3
3(d)	Climbing **several** flights of stairs	1	2	3
3(e)	Climbing **one** flight of stairs	1	2	3
3(f)	Bending, kneeling, or stooping	1	2	3
3(g)	Waling **more than a mile**	1	2	3
3(h)	Walking **several blocks**	1	2	3
3(i)	Walking **one block**	1	2	3
3(j)	Bathing or dressing yourself	1	2	3

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	(Please circle one number on each line.)	Yes	No
4(a)	Cut down on the **amount of time** you spent on work or other activities	1	2
4(b)	Accomplished less than you would like	1	2
4(c)	Were **limited** in the **kind** of work or other activities	1	2
4(d)	Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (e.g. feeling depressed or anxious)?

	(Please circle one number on each line.)	Yes	No
5(a)	Cut down on the **amount of time** you spent on work or other activities	1	2
5(b)	Accomplished less than you would like	1	2
5(c)	Didn't do work or other activities as **carefully** as usual	1	2

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups? (Please tick **one** box.)

- Not at all ☐
- Slightly ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

7. How much physical pain have you had during the past 4 weeks? (Please tick **one** box.)

- None ☐
- Very mild ☐
- Mild ☐
- Moderate ☐
- Severe ☐
- Very Severe ☐

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)? (Please tick **one** box.)

- Not at all ☐
- A little bit ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

9. These questions are about how you feel and how things have been with you during the past 4 weeks. Please give the one answer that is closest to the way you have been feeling for each item.

	(Please circle one number on each line.)	**All of the Time**	**Most of the Time**	**A Good Bit of the Time**	**Some of the Time**	**A Little of the Time**	**None of the Time**
9(a)	Did you feel full of life?	1	2	3	4	5	6
9(b)	Have you been a very nervous person?	1	2	3	4	5	6
9(c)	Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
9(d)	Have you felt calm and peaceful?	1	2	3	4	5	6
9(e)	Did you have a lot of energy?	1	2	3	4	5	6
9(f)	Have you felt downhearted and blue?	1	2	3	4	5	6
9(g)	Did you feel worn out?	1	2	3	4	5	6
9(h)	Have you been a happy person?	1	2	3	4	5	6
9(i)	Did you feel tired?	1	2	3	4	5	6

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives etc.) (Please tick **one** box.)

- All of the time ☐
- Most of the time ☐
- Some of the time ☐
- A little of the time ☐
- None of the time ☐

11. How TRUE or FALSE is each of the following statements for you?

	(Please circle one number on each line.)	**Definitely True**	**Mostly True**	**Don't Know**	**Mostly False**	**Definitely False**
11(a)	I seem to get sick a little easier than other people	1	2	3	4	5
11(b)	I am as healthy as anybody I know	1	2	3	4	5
11(c)	I expect my health to get worse	1	2	3	4	5
11(d)	My health is excellent	1	2	3	4	5

Appendix 7.5. Assessing presence of gastrointestinal symptoms

1. In the last 3 months, did you *often* have discomfort or pain in your tummy? *Please tick one box:*
   No or rarely ☐
   Yes ☐
   Often means that the symptoms were present at least one day in each week

2. In the last 3 months, did you often have heartburn, a burning pain in your chest? *Please tick one box:*
   No or rarely ☐
   Yes ☐
   Often means that the symptoms were present at least one day in each week

3. In the last 3 months, did you have loose, mushy or watery stools during more than three-quarters (3/4) of your bowel movements? *Please tick one box:*
   No or rarely ☐
   Yes ☐

4. Do you avoid eating outside the home such as in restaurants or at friends' meal invitations? *Please tick one box:*
   All of the time ☐
   Most of the time ☐
   Some of the time ☐
   Never ☐

Appendix 7.6. Breast cancer risk profile

This questionnaire is designed to find out about your reproductive history. Please try to answer every question by writing in the spaces provided or ticking the appropriate boxes, except when there is a specific request to skip a section.

Question 1

What is your ethnic group?

- ☐ Caucasian
- ☐ Black Caribbean or Black British Caribbean
- ☐ Black African or Black British African
- ☐ Other Black background
- ☐ Asian Indian or Asian British Indian
- ☐ Asian Pakistani or Asian British Pakistani
- ☐ Asian Bangladeshi or Asian British Bangladeshi
- ☐ Chinese or other Ethnic background Chinese
- ☐ Other Asian background
- ☐ Mixed – White and Black Caribbean
- ☐ Mixed – White and Black African
- ☐ Mixed – White and Asian
- ☐ Other Mixed background
- ☐ Other ethnic background

Question 2

How old were you when you had your first menstrual period?

…….*years of age*

Question 3

How long was it after your first menstrual period did your periods began to occur at regular intervals?

- ☐ Within months
- ☐ After 1 year
- ☐ After 2 years
- ☐ After 3 years
- ☐ After 4 years
- ☐ After 5 years
- ☐ After 5 or more years
- ☐ Only after 1st pregnancy
- ☐ Always irregular in the first 10 years

Question 4

Are you still menstruating?

- ☐ Yes
- ☐ No

  At what age did you stop having your periods? …….*years of age*

Question 5

Have you ever taken an oral contraceptive ('the pill')?

- ☐ No
- ☐ Yes

If you have ever taken the 'pill', how long in total did you or have you been taking it?

- ☐ 1 year or less
- ☐ 1 - 4 years
- ☐ 5 – 9 years
- ☐ 10 – 14 years
- ☐ 15 years or more

How old were you when you started using the pill?

- ☐ 14 years or less
- ☐ 15 - 20 years
- ☐ 21 – 25 years
- ☐ 26 – 30 years
- ☐ 31 – 35 years
- ☐ 36 – 40 years
- ☐ 41 years or more

Question 6

Have you taken or are you taking hormones for the menopause ('HRT')?

- ☐ No
- ☐ Yes

If yes, how long have you taken menopausal hormones ('HRT')?

- ☐ Less than 1 year
- ☐ 2 – 4 years
- ☐ 5 – 9 years
- ☐ 10 – 14 years
- ☐ 15 years or more

How old were you when you first started taking HRT?

- ☐ 40 years or less
- ☐ 41 – 44 years
- ☐ 45 - 50 years
- ☐ 51 - 55 years
- ☐ 56 - 60 years
- ☐ 60 - 65 years
- ☐ 65 - 70 years
- ☐ 71 years or more

Question 7

Have you ever been pregnant?

- ☐ Yes — *Please go to question 8*
- ☐ No — *Please go to question 10*

Question 8

Please give details of all previous pregnancies (including miscarriages) in the order of occurrence (the earliest pregnancy first):

Previous pregnancy	Year of pregnancy	**Outcome of pregnancy**
1st pregnancy		☐ Live birth When baby was born, was he / she born early, later or at expected time? ☐ early ☐ late ☐ expected time ☐ Stillbirth ☐ Miscarriage ☐ Ectopic ☐ Abortion
2nd pregnancy		☐ Live birth When baby was born, was he / she born early, later or at expected time? ☐ early ☐ late ☐ expected time ☐ Stillbirth ☐ Miscarriage ☐ Ectopic ☐ Abortion
3rd pregnancy		☐ Live birth When baby was born, was he / she born early, later or at expected time? ☐ early ☐ late ☐ expected time ☐ Stillbirth ☐ Miscarriage ☐ Ectopic ☐ Abortion
4th pregnancy		☐ Live birth When baby was born, was he / she born early, later or at expected time? ☐ early ☐ late ☐ expected time ☐ Stillbirth ☐ Miscarriage ☐ Ectopic ☐ Abortion

5th pregnancy

- ☐ Live birth
  When baby was born, was he / she born early, later or at expected time?
  - ☐ early
  - ☐ late
  - ☐ expected time
- ☐ Stillbirth
- ☐ Miscarriage
- ☐ Ectopic
- ☐ Abortion
- ☐

6th pregnancy

- ☐ Live birth
  When baby was born, was he / she born early, later or at expected time?
  - ☐ early
  - ☐ late
  - ☐ expected time
- ☐ Stillbirth
- ☐ Miscarriage
- ☐ Ectopic
- ☐ Abortion

7th pregnancy

- ☐ Live birth
- ☐ Stillbirth
- ☐ Miscarriage
- ☐ Ectopic
- ☐ Abortion

Question 9

Did you breast feed?

- ☐ No
- ☐ Yes

  If yes for how long did you breast feed?
  - ☐ 1 week
  - ☐ 2-3 weeks
  - ☐ 4-5 weeks
  - ☐ 6-7 weeks
  - ☐ 2 months
  - ☐ 3 months
  - ☐ 4-5 months
  - ☐ 6-7 months
  - ☐ 8-9 months
  - ☐ 10-11 months
  - ☐ 12 months or more

Did you breast feed for a similar length of time with your other children?

- ☐ Yes
- ☐ No
- ☐ Not applicable – had one child

Question 10

1. Have you had a hysterectomy (womb removed)?
   - ☐ No
   - ☐ Yes

     If yes at what age did it occur? ..... *years of age*

2. Have you ever had an operation to remove one or both of your ovaries?
   - ☐ No
   - ☐ Yes

     If yes at what age did it occur? ..... *years of age*

Question 11

1. Have you ever been told by a doctor that you have had any breast disease?
   - ☐ No
   - ☐ Yes

     What was condition called?
     - ☐ Fibrocystic benign breast disease
     - ☐ Other .....

2. Have you ever been told by a doctor that you have breast cancer?
   - ☐ No
   - ☐ Yes

   If yes, at what age did the breast cancer first occur? ..... *years*

Question 12

Is there anyone in your family that has / has had breast cancer?

- ☐ No
- ☐ Yes

  If yes, which family member had breast cancer?
  - ☐ Mother

    At what age did the breast cancer first occur? ..... *years*
  - ☐ Sister

    At what age did the breast cancer first occur? ..... *years*
  - ☐ Daughter

    At what age did the breast cancer first occur? ..... *years*
  - ☐ Grandmother

    At what age did the breast cancer first occur? ..... *years*
  - ☐ Aunt

    At what age did the breast cancer first occur? ..... *years*
  - ☐ Niece

    At what age did the breast cancer first occur? ..... *years*

Question 13

Do you ever drink alcohol?

- ☐ No
- ☐ Yes

  How much alcohol would you typically drink in a week?

  Number of wine glasses per week .....

  Number of pints of lager, beer or cider per week .....

  Number of glasses of spirits or liquor per week .....

Question 14

1. How old were you when you left school? ..... *years*

2. What was the highest school / college level which you reached?
   - ☐ Primary school completed
   - ☐ Secondary school completed
   - ☐ Technical or professional secondary school
   - ☐ Grammar school or college
   - ☐ University degree
   - ☐ Did not attend school

Question 15

Do you own your own home?

- ☐ No
- ☐ Yes

Question 16

3. What is your occupation? .....

4. What was your father's occupation when you were 10 years old? .....

Question 17

1. What is your height? ..... *feet and inches / metres*

2. What is your weight? ..... *stones and pounds / kilograms*

3. What was your weight when you were 20 years of age?
   ..... *stones and pounds / kilograms*

4. What was your birth weight? ..... *pounds*

Question 18

Do you smoke?

- ☐ No I have never smoked
- ☐ I don't smoke currently but I have smoked in the past
  How much did you typically smoke in a day?
  Number of cigarettes / cigars / pipes per day .....
- ☐ I smoke cigarettes / pipe / cigars
  How much would you typically smoke in a day?
  Number of cigarettes / cigars / pipes per day .....

Chapter 8: References

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