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**UNDERNUTRITION IN INACTIVE
AND MILDLY ACTIVE CROHN'S
DISEASE:
AN UNRECOGNISED PROBLEM?**

*A thesis submitted to the University of Dublin, Trinity College for
the degree of*

DOCTOR OF PHILOSOPHY (Ph.D.)

by

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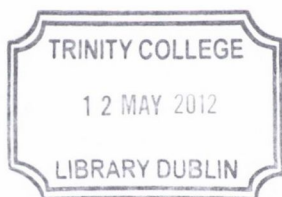
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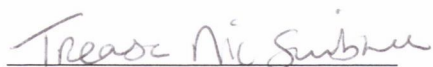
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A handwritten signature in dark ink, reading "Treasa Nic Suibhne", written over a horizontal line.

Treasa Nic Suibhne

SUMMARY

Crohn's disease (CD), the incidence of which is increasing worldwide, is a chronic transmural inflammatory disease that can affect any part of the gastrointestinal tract. It follows a relapsing remitting course and results in symptoms such as weight loss, diarrhoea and abdominal pain. Undernutrition is a common feature of active disease, with weight loss and malnutrition apparent during flare-ups and periods of high disease activity affecting up to 85% of patients. On the other hand, during disease remission or at times of low disease activity, undernutrition is not so apparent and can be difficult to diagnose as most patients in remission look and feel well and lack the typical signs normally associated with malnutrition. However, as subclinical inflammation and symptoms predisposing patients to undernutrition can persist, and treatments such as corticosteroids can add to the risk of undernutrition (as they cause muscle wasting and bone loss but can also lead to weight gain and redistribution of body fat); undernutrition can occur and remain un-noticed due to weight gain.

Therefore, the overall aim of this study was to investigate the nature and prevalence of undernutrition in outpatient CD patients with inactive or mildly active disease and compare them to healthy age-, sex- and socio-economically matched controls and reference standards. The research was broken down into three studies; focusing on undernutrition and body composition, vitamin D status and lifestyle factors. 100 patients and 100 healthy controls were interviewed using comprehensive case record forms and questionnaires, underwent a detailed nutritional assessment involving anthropometry and provided a fasting blood sample for measurement of inflammatory (CRP, TNF α) and nutritional (serum albumin, total protein, vitamin D) serum markers.

A high prevalence of reduced skeletal muscle function, as assessed by handgrip strength was observed in this group of young adult CD patients (mean age 35.7 ± 10.9), most of whom were in clinical remission, had normal BMI, normal muscle and fat stores, normal serum albumin levels and were at low risk of undernutrition when assessed by the MUST nutritional screening tool. Patients of short stature with large bowel disease and a higher corticosteroid usage (82% reduction for those not receiving corticosteroids) appeared at higher risk of reduced muscle function, which was associated with increased fatigue and decreased energy levels in this group. Vitamin D deficiency was also a common observation in these CD patients, similar to that of matched controls. CD patients with long standing disease, those who smoked and who had lower dietary intake of vitamin D were most at risk of deficiency. Calcium and vitamin D intakes were similar in CD patients and controls but intakes in CD patients were well below those recommended for the prevention of osteoporosis in CD. Significant differences in physical activity (significantly lower in CD patients), anxiety and depression (significantly more prevalent in CD patients), smoking (more prevalent in CD patients) and alcohol intake (significantly lower in CD patients) were observed between CD patients and controls.

In conclusion, it is evident from this research that undernutrition is an issue in CD outpatients with inactive or mildly active disease, particularly in terms of muscle function and vitamin D status. Worryingly, undernutrition is likely to remain undetected if current routine measures continue to be employed, therefore, comprehensive screening and diagnostic approaches need to be introduced, particularly at times of low disease activity, if undernutrition and its deleterious affects are to be combatted.

ACKNOWLEDGEMENTS

My supervisor, Dr. Maria O'Sullivan, for her guidance and direction, and for providing the funding that enabled me to carry out this research and to present my work in Ireland and overseas.

Prof. Colm O'Morain a thug an deis domh an taighde seo a dheanamh agus a chuidigh go mor liom on tús agus a thug an deis domh taisteal thar lear chun mo obair a chur i lathair.

The wonderful staff of the Gastro department – Amanda, Orla, Mark, Yvonne, Angie, Ramona, Brian and Malik. A special thank you to Mary, Pauline and Niamh who welcomed me every Wednesday (and many more days besides) and let me harass their patients.

The Crohn's disease patients, the true stars of the show, as without them, this study would not have been possible. Their unending enthusiasm and willingness to share their experiences with me was an invaluable source of encouragement throughout the entire project.

The controls for giving so freely of their time, and their breakfast! Special thanks to OKI, Trulife and Jacobs Biscuits for allowing me to invade their premises.

Those who helped with the blood taking (especially Susan O'Connell and Sam O'Connor). The girls in the Phlebotomy Department, AMNCH who trained me in the art of blood taking and who bravely donated their arms for those first few practice runs.

Marty and Gerry in the Biochemistry Department, St James' Hospital for the vitamin D analysis. Dr Elaine Hand for all her assistance with the statistical analysis. Karen Mc Dermott, for all her hard work during her undergraduate project, and for her starring role in CSI: Tallaght!

The many friends I've made during my time in the research labs. Those who have moved on to greener pastures; Almath, Sean, John, Gerry, Alice, Suren, Gwen, Paul and Noor. And to all still holding the fort - Elaine, Susan, Ellen, AnnaMarie, Kate, Abdur, Stephen, Shirley and Amgad – no matter how bad things were going your 'words of wisdom' got me through the day with a smile. I will really miss you all. Special thanks to Ellen Shiel, my partner in crime, we made it!

Dr. Joan, Sandra and Maedhbh for giving us all honorary member status of the Department of Paediatrics. To Denise, Jimmy and Stephen who keep the show on the road.

Anne-Marie Baird (soon-to-be Dr.) whose shoulder was always available when things were not going to plan (which was most days!), to Fiona Roulston who, without complaint, listened to how things were going (or not) every single day!, and to Dr. Kathleen Mooney, who proved there is light at the end of the tunnel.

Mamaí agus Daidí, do bhur tacaíocht agus grá i dtolamh. Beidh mé i mo 'net contributor' gan mhoill - promise! Bríd agus Cathal, do na iasachtaí, siúloidí ar an trá agus Snow Patrol!

Glenn, mo ghrian 'is mo ghealach, for holding my hand every step of the way. Couldn't have done it without you.

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LIST OF ABBREVIATIONS

AMNCH	Adelaide and Meath Hospital incorporating The National Children's Hospital
AFA	Arm Fat Area
AMA	Arm Muscle Area
BAPEN	British Association for Parenteral and Enteral Nutrition
BMD	Bone Mineral Density
BMI	Body Mass Index
BSG	British Society of Gastroenterology
°C	degrees Celsius
CDAI	Crohns Disease Activity Index
CI	Confidence Interval
cm	Centimetre
CRF	Case Record Form
CRP	C-reactive Protein
DEXA	Dual X-ray Absorptiometry
ELISA	Enzyme Linked ImmunoSorbent Assay
EPAQ2	EPIC Physical Activity Questionnaire
EPIC	European Prospective Investigation into Cancer
ESPEN	European Society for Parenteral and Enteral Nutrition
FFM	Fat Free Mass
FFQ	Food Frequency Questionnaire
gms	Grams
HADS	Hospital Anxiety and Depression Scale
HGS	Handgrip Strength
IBD	Inflammatory Bowel Disease
IBDQ	Inflammatory Bowel Disease Questionnaire

IL-10	Interleukin 10
IOIBD	International Organisation of Inflammatory Bowel Disease
IU	International Units
kcal	Kilocalories
kg	Kilogram
kPa	KiloPascal
MET	Metabolic Equivalent
mg	milligrams
ml	millilitre
mm	millimetre
MUAC	Mid Upper Arm Circumference
MUST	Malnutrition Universal Screening Tool
N	North
ng/ml	Nanogram per millilitre
nm	Nanometre
NS	Non Significant
OCP	Oral Contraceptive Pill
pg/ml	Picogram per millilitre
RDA	Recommended Daily Allowance
rpm	Revolutions per Minute
TNF α	Tumour Necrosis Factor Alpha
TSF	Triceps Skinfold Thickness
UC	Ulcerative Colitis
μ g	Microgram
μ l	Microlitre
VDR	Vitamin D Receptor

PUBLICATIONS FROM THIS THESIS

Book Chapters

Can we treat Inflammatory Bowel Disease with Diet? T Nic Suibhne, CA O'Morain, M O'Sullivan, In IBD: translation from basic research to clinical practice (Falk symposium 140). Colombel, Gasche, Scholmerich, Vucelic. Kluwer Academic Publishers, UK. 2004 Chapter 14: 138-146

Papers

O'Sullivan M, Nic Suibhne T, Cox G, Healy M, O'Morain C. High Prevalence of Vitamin D Insufficiency in Healthy Irish Adults. Irish Journal of Medical Science. 2008;177(2):131-4

Prevalence and Risk Factors for Vitamin D Deficiency in Patients with Crohns Disease. Nic Suibhne T, Cox G, Healy M, O'Morain C, O'Sullivan, M –submitted

Vitamin D Insufficiency is Associated with Lower IL-10 levels in Patients with Crohn's Disease. Kelly P, Nic Suibhne T, O'Morain C, O'Sullivan M – submitted

Abstracts

Reduced muscle function and muscle stores are common in quiescent Crohn's Disease. T Nic Suibhne, C O'Morain, M O'Sullivan. Gastroenterology 2006; 130(4) Suppl

High prevalence of vitamin D deficiency in patients with inactive Crohn's disease. T Nic Suibhne, C O'Morain, M O'Sullivan. Gastroenterology 2006; 130(4) Suppl

High prevalence of vitamin D insufficiency in inactive Crohn's Disease

T Nic Suibhne, C O'Morain, M O'Sullivan. Endoscopy 2006; 38 (Suppl)

Undernutrition and reduced muscle function is common in young patients with Crohn's disease. T Nic Suibhne, C O'Morain, M O'Sullivan. Endoscopy 2006; 38 (Suppl)

Protein Undernutrition in Quiescent Crohn's Disease: An unrecognised problem? T Nic Suibhne, C O'Morain, M O'Sullivan. Clinical Nutrition 2005; 24(4) Suppl

Protein Undernutrition in Crohn's Disease: An unrecognised problem? T Nic Suibhne, C O'Morain, M O'Sullivan. Gastroenterology 2005; 128(4) Suppl

Protein malnutrition in inflammatory bowel disease: An unrecognised problem? T Nic Suibhne, C O'Morain, M O'Sullivan. Endoscopy 2004; 36 (Suppl)

Calcium intake in Crohn's disease (CD): Are we meeting the nutritional guidelines? T Nic Suibhne, C O'Morain, M O'Sullivan. Endoscopy 2004; 36 (Suppl)

CHAPTER 1:

GENERAL INTRODUCTION

1.1. Crohn's Disease

Crohn's disease (CD), together with ulcerative colitis (UC), are two similar but distinct clinical entities, encompassed under the heading of inflammatory bowel disease (IBD). While it is estimated that 1 in every 100 people will suffer from IBD during their lifetime (1), the cause of this debilitating chronic illness remains unknown. Reports of isolated cases of IBD were initially identified during the 19th and 20th centuries (2), but it was not until 1932 that CD was first definitively described as a

'disease of the terminal ileum, affecting mainly young adults, characterised by a subacute or chronic necrotizing and cicatrizing inflammation'

by Dr. Burrill B. Crohn, Dr. Leon Ginzburg and Dr. Gordon Oppenheimer in their landmark paper "Regional ileitis: A pathologic and clinical entity" (3).

1.1.1. Pathology

CD is a chronic transmural disease that can affect any part of the gastrointestinal tract, with the terminal ileum and proximal colon most commonly affected (4). Macroscopic gross features of CD include epithelioid granulomas, discontinuous crypt distortion, focal cryptitis, 'fat wrapping' and discontinuous inflammation (5). The length of the segments involved is variable and these segments are separated by uninvolved 'skip lesions'. Strictures (thickening of the bowel wall leading to luminal narrowing) and fistulae (abnormal connections between the gut lumen and other organs) can also present over the course of disease (6).

1.1.2. Presentation and Clinical Features

CD usually presents in late adolescence or early adulthood (with a second peak in later life) and is marginally more common in females (7). Chronic diarrhoea, although not always present (8), is the most common presenting symptom (9) together with abdominal pain and weight loss, which present in 60-70% of patients (10). Patients, at diagnosis and throughout the course of disease, are often confronted with a range of systemic problems termed 'extraintestinal manifestations', which can occur in up to 46% of patients (11). Complications of the joints (arthralgia and arthritis), skin (erythema nodosum and pyoderma gangrenosum) and eyes (uveitis and episcleritis) are most common, and tend to follow the clinical course of CD while manifestations of the hepatobiliary system including ankylosing spondylitis and primary sclerosing cholangitis do not correlate with disease activity (12). CD patients are also at increased risk of developing pancreatitis (13), thromboembolic (14) and neurological complications (15).

The risk of colorectal cancer in CD has been debated with some studies showing an overall normal risk (16, 17) and others showing an increased risk (18-20) of approximately 2.5-fold compared to the general population (21, 22). Patients with disease onset at a young age and with extensive colonic disease (21, 22) appear at highest risk and should, therefore, be candidates for screening strategies (23). An increased risk for small bowel cancer seems highly probable with two recent meta-analyses observing significantly increased risk ratios in all included studies (21, 22) while increased risk has also been reported for extraintestinal cancers and lymphoma in patients with CD (22).

Reports suggest that CD is associated with an increased mortality rate (24, 25), particularly after 10 years diagnosis (26), although others disagree suggesting rates to be similar to that of the general population (27). A recent large epidemiological follow-up study in Cardiff, (the longest to date with a median follow-up of 24 years) observed a significantly increased overall mortality rate of 29%; patients diagnosed before the age of 20 showed worst prognosis and a reduced life expectancy compared with the general population (28).

1.1.3. Diagnosis

No single 'gold standard' test exists for the diagnosis of CD, therefore, diagnosis is based on a combination of clinical presentation, endoscopic appearance and radiological, histological and surgical findings (29). This combined approach is not without fault and misdiagnosis does occur. A change of diagnosis from CD to UC or vice versa can arise in 10-15% patients within the first year of diagnosis (30). Disease of the colon can lead to a diagnosis of 'indeterminate colitis' in 10% of cases (31), where a definite diagnosis of either CD or UC is not possible.

Colonoscopy, together with multiple biopsy samples from the terminal ileum and five colonic segments, including the rectum, are the first line procedures to establish the diagnosis of CD (30). Microscopic evidence from surgical and mucosal biopsy specimens is then used to verify diagnosis of CD, with three features (as in Table 1.1.) present in the absence of granulomas, or one feature in the presence of an epithelioid granuloma confirming a diagnosis of CD (30). A full medical history and general clinical examination with laboratory investigations including full blood count and C-reactive protein are also required (30).

Table 1.1. Characteristic macroscopic and microscopic features for the diagnosis of CD

<i>Macroscopic features</i>
Ileal disease
Rectum typically spared
Confluent deep linear ulcers, aphthoid ulcers
Deep fissures
Fistulas
Fat wrapping
Skip lesions
Cobblestoning
Thickening of the intestinal wall
Strictures
<i>Microscopic features</i>
Transmural inflammation
Aggregated inflammatory pattern
Transmural lymphoid hyperplasia
Submucosal thickening
Fissures
Sarcoid granuloma
Enteric nervous system abnormalities
Relatively unchanged epithelia

Adapted from (30)

Once CD diagnosis is made, further investigations are recommended to assess the location and extent of the disease, as ileum or proximal small bowel inaccessible by the endoscope can be involved. At present, radiological procedures such as small bowel enema and small bowel follow through are the preferred methods to exclude small bowel disease (32, 33).

Newer diagnostic tools are showing promise. Technical developments have led to the ability of computed tomography (CT) enterography and magnetic resonance imaging (MRI) to detect complications such as abscess, strictures and fistulae (34). Wireless video capsule endoscopy (WCE) provides direct imaging of the entire small bowel mucosa, and initial studies report, that compared to small bowel barium radiography (35, 36) and colonoscopy (37, 38), WCE is more sensitive in establishing diagnosis and disease extent. A recent meta-analysis determined an increased yield of 40% for WCE over barium radiography and 15% over colonoscopy with ileoscopy with patients with established CD, although no statistically significant differences were observed in new patients with suspected CD (39). However, in a study of patients with suspected CD, who remained undiagnosed following standard imaging techniques, WCE findings were diagnostic in 40% of patients (40) and more recently high sensitivity and specificity values of 93% and 84%, respectively, have been reported for WCE detection of small bowel inflammatory changes (41). While further large prospective studies are needed to substantiate the diagnostic importance in CD, the use of WCE is nonetheless appealing as it is less invasive and more comfortable for patients.

Serological testing for IBD has also emerged over recent years, with the detection of the anti-saccharomyces cerevisiae antibody (ASCA) and the

anti-neutrophil cytoplasmic antibody with perinuclear staining (pANCA) the most commonly used in differentiating IBD from non-IBD (42-44). Antibodies to the outer membrane porin C (OmpC) of the bacteria *Escherichia coli* have also been studied, but with less sensitivity (44). CD is highly associated with ASCA, identifying approximately 40-50% of patients (44, 45) (while pANCA is correlated to UC). Results must be used in combination with clinical presentation and other diagnostic tools, as false positive test results can occur (46), which has limited its use in routine clinical practice to date.

1.1.4. Disease Activity

CD shows a relapsing remitting disease course, and as yet no marker can reliably predict recurrence of CD. Assessment of disease activity often relies on a combination of laboratory markers and disease activity indices.

The most commonly used serum inflammatory marker is C-reactive protein (CRP), normally produced in small quantities (<1mg/L) by hepatocytes. CRP is an acute phase protein with a short half-life of 19 hours, which correlates well with disease activity (47, 48), endoscopical and histological inflammation (49) and shows a strong heterogeneity for CD (50). Following an acute phase stimulus such as inflammation, CRP levels rise with levels of 10-40mg/L indicating mild inflammation and levels of 50-200mg/L indicating severe active inflammation or bacterial infection (51). Observations suggest that baseline CRP levels may be modulated by the CRP gene (52, 53), however, results are conflicting (54). Low CRP levels can also persist during active disease (55), highlighting limitations with its use. It has been suggested that more sensitive cut-off values allowing for 100% sensitivity should be introduced (50). High sensitivity CRP assays are now available, and

have been most widely studied in relation to heart disease (56), but as yet little information is available regarding its use in CD (57).

A number of activity indices have been developed for the assessment of CD (58), but the Crohn's Disease Activity Index (CDAI) developed in 1976 (59) remains the most popular and is often used as the 'gold standard' (58). It is routinely used to distinguish between inactive and active disease in adult patients, and for characterising disease activity (Table 1.2.), but as it is a subjective measure based on symptom reports, does not necessarily correspond to inflammation in the gut. Remission is defined by a CDAI score of less than 150, with scores above this cut-off corresponding to various stages of active disease (Table 1.2.). In children, a paediatric version of the Crohn's Disease Activity Index (60) is used.

A retrospective study exploring the relationship between CRP, CDAI, and endoscopic activity in CD patients concluded that neither CRP or CDAI are reliable measures of disease activity as no significant differences in endoscopic activity were observed between patients with high or normal values of either measure (61). However, while these measures may have their limitations, they are the most commonly used at present and are recommended by the European Crohns and Colitis Organisation (ECCO) expert consensus group (30).

Other general markers are used but are not as helpful in assessing disease activity. Erythrocyte Sedimentation Rate (ESR), which reflects changes of plasma protein concentration and packed cell volume of erythrocytes, is less accurate in measuring intestinal inflammation as it rises less rapidly than CRP, and can take several days to decrease after inflammation is resolved.

Table 1.2. Disease Activity assessed by CDAI

Mild	Equivalent to a CDAI of 150-220: <i>e.g.</i> Ambulatory, eating and drinking, <10% weight loss. No features of obstruction, fever, dehydration, abdominal mass or tenderness. CRP usually increased.
Moderate	Equivalent to a CDAI of 220-450: <i>e.g.</i> Intermittent vomiting, or weight loss >10%. Treatment for mild disease ineffective, or tender mass. No overt obstruction. CRP raised above normal.
Severe	Equivalent to a CDAI of >450: <i>e.g.</i> Cachexia (BMI<18), or evidence of obstruction or abscess. Persistent symptoms despite intensive treatment. CRP increased.

Adapted from (30)

It can also be affected by age and conditions such as anaemia (62) and is more closely correlated with colonic rather than ileal disease (63). White cell count and platelets both increase during acute phase response but are not specific to CD. Also, white cell count can be affected by treatments such as azathioprine (64), while the wide range of normal values for platelet count makes it less useful. Albumin, a negative acute phase protein, decreases during inflammation but can also decrease due to malnutrition and malabsorption (65).

Due to the influence of various conditions on serum markers, interest in the use of faecal markers has risen in the hope of providing greater specificity. Faecal calprotectin, which is directly proportional to neutrophil migration to the gut, shows most promise to date, as it is very stable and resistant to degradation (48), correlates well with endoscopic and histological activity (66) and has been reported to predict relapse (67, 68). Unfortunately, while it is a sensitive marker of inflammation in the gastrointestinal tract, it is not specific for CD with increased levels also detected in neoplasia, infections and polyps (69). It can also be affected by the use of non-steroidal anti-inflammatory drugs (70) and the aging process (71).

1.1.5. Disease Classification

“The Holy Grail of Crohn’s disease – aside from finding the elusive cause and cure – is understanding its ‘natural history’; that is, how it develops, presents, evolves, and responds to different therapies”

Sachar, 2006 (72)

With this goal in mind, attempts have been made to determine patterns and behaviour of disease in the hope of predicting its subsequent course. In Vienna (73), and most recently in Montreal (74), formal and

standardised phenotypic classifications of CD have been constructed. Patients are allocated to categories based on their age at diagnosis, disease location and disease behaviour (Table 1.3.). The location of CD appears to be a relatively stable phenotype with only 10–15% of patients displaying a change in their disease location after 10 years of diagnosis (6). Contrastingly, disease behaviour is more susceptible to changes and varies dramatically as disease duration progresses with 46% of patients exhibiting a change in behaviour; the most notable change occurring from non-stricturing non-penetrating disease (present in approximately 80% of patients at diagnosis) to either structuring or penetrating disease (6, 75). The integration of serological and genetic markers into the classification system was discussed in Montreal but considered too premature due to a lack of evidence regarding any specific marker and limited sensitivity of available markers (74).

Accurate classifications of CD can, potentially, be used in clinical practice for assessing disease prognosis and in choosing appropriate medical therapy (76). A European population based clinical follow-up of 380 CD patients reported that phenotype at diagnosis (based on the Vienna classification) could reliably predict surgical and non-surgical recurrence rates with those with upper gastrointestinal disease and diagnosed at a young age most at risk (77).

1.1.6. Epidemiology

The incidence of IBD has increased steadily over the second half of the 20th century (78), and it is suggested that 1 in every 100 people in the UK and many parts of Europe will suffer one or more prolonged attacks of IBD in their lifetime (1).

Table 1.3. Vienna (73) and Montreal (76) classification for CD

	Vienna	Montreal
Age at diagnosis	A1 below 40 years A2 above 40 years	A1 below 16 years A2 between 17 and 40 years A3 above 40 years
Location	L1 ileal L2 colonic L3 ileocolonic L4 upper	L1 ileal L2 colonic L3 ileocolonic L4 isolated upper disease
Behaviour	B1 non-stricturing, non-penetrating B2 stricturing B3 penetrating	B1 non-stricturing, non-penetrating B2 stricturing B3 penetrating 'p' perianal disease modifier

Most centres from Europe and the United States observed increasing incidence rates of CD throughout the 1950s and 1960s (79), which ceased at the beginning of the 1980s and subsequently followed a flat course (1, 80).

CD is more common in developed countries (although no or little data is available for developing countries) with incidence rates of $5.8/10^5$ in North America (81), $8.3/10^5$ in the United Kingdom (82), $8.6/10^5$ in Scandinavia (83), $16.5/10^5$ in New Zealand (84) and the highest reported incidence rate at $20.2/10^5$ observed in Canada (85). The incidence of childhood or juvenile onset CD is also increasing (86), with incidence during 1998 and 1999 reported at 5.2 per 100,000 per year in children in Ireland and the UK aged younger than 16 years (87).

Traditionally, CD has been slightly more prevalent in females compared to males (7, 88) but this appears to be changing. Most incident paediatric reports in recent years show an emerging male predominance (89-92), with 62% of newly diagnosed paediatric cases surveyed in the UK and Ireland of male gender (87).

Geographical variation also exists for CD through a north-south gradient with higher incidence in northern Europe ($7.0/10^5$) compared to southern Europe ($3.9/10^5$) (93), and in the northern states of America compared to southern states (94), although this gradient effect has recently been questioned (95). A north-south gradient has also been reported for the prevalence of genetic factors with lower rates of the known CD associated mutations observed in the northern European populations of Norway, Scotland and Ireland in comparison to more southern countries such as Germany (96, 97).

1.1.7. Treatment

As the aetiology of CD remains unknown, no causal treatment yet exists. The therapeutic goal in CD is, therefore, to induce and maintain clinical remission, with the patient requiring lifelong medication regimes.

1.1.7.1. Induction of Remission

Corticosteroids consistently appear superior for the induction of remission in mild to moderately active ileal or ileocolonic CD (98-100). Controlled ileal release budesonide, while slightly less efficacious (101), is preferable to conventional corticosteroids such as prednisolone as it is associated with less adrenal suppression and other glucocorticoid side effects (101). However, some patients react adversely to corticosteroids (steroid intolerant patients), experience little or no improvement in disease activity (steroid-resistant patients), or suffer relapse in disease activity after dose reduction (steroid-dependent patients).

For these patients or patients with more active disease, newer biologic therapies such as infliximab, a chimeric mouse/human monoclonal antibody against tumour necrosis factor alpha, offer an alternative option in inducing remission (102). In the first key trial of infliximab, administered as a single infusion of 5 mg/kg for active Crohn's disease, 33% of patients entered remission compared to 4% of controls and 81% of patients treated with infliximab had a clinical response compared with 17% of controls (102). The optimum induction regime for infliximab for active Crohn's disease appears to be three infusions at 0, 2, and 6 weeks as this practice is associated with reduced immunogenicity and a slight increase in effectiveness over a single dose (103).

1.1.7.2. Maintenance of Remission

The mainstay of maintenance therapy focuses on the use of immunomodulators, namely azathioprine and its metabolite 6-mercaptopurine (64), and the folate antagonist methotrexate (104), which have been shown to maintain remission in 42% of patients (at 15 months) (105) and 65% of patients (at 40 weeks) (104), respectively. Symptomatic relapse is inclined to occur after azathioprine or 6-mercaptopurine are stopped, so therapy with these agents should be continued indefinitely in most patients (106). However, these drugs are not universally effective, are slow to act and are associated with significant adverse effects, therefore, regular toxicity monitoring is required. Although 5-aminosalicylates are associated with fewer side effects, a Cochrane review found no evidence to suggest that they offer any benefit compared to placebo in maintaining remission (107) and should not be prescribed for remission maintenance in CD (103).

As observed in the ACCENT I trial, infliximab is also successful as a maintenance treatment, with up to 45% of patients maintaining remission at 30 weeks (108). Scheduled therapy with infliximab has proved more successful than episodic treatment (109), with infusions at regular 8-weekly intervals recommended. Increasing dosage from 5 mg/kg to 10 mg/kg or shortening the dose interval from every 8 weeks to every 6 weeks or even every 4 weeks also appears to increase the number of patients successfully maintained.

About 20% of patients fail to respond to infliximab (110), with those with increased CRP (111), pure colonic disease (112) and undergoing concomitant immunosuppressive treatment (112, 113) more likely to respond favourably; smokers are less likely to respond (113). In some patients, treatment with infliximab results in the formation of

antibodies against infliximab (114). While infliximab remains the only licensed biologic therapy in Europe and the United States for treatment in CD (115), for patients failing to respond or losing response to infliximab, other therapies are in late-stage development. These include Certolizumab pegol (CDP870), a polyethylene-glycolated Fab' fragment of anti-TNF (116); Onercept, a recombinant form of the natural human soluble p55 TNF receptor (117); Adalimumab, a recombinant, fully human, immunoglobulin G1 monoclonal antibody TNF inhibitor (118), and Natalizumab, a monoclonal antibody against alpha 4 integrins (119).

Enteral nutrition has proved successful in inducing and maintaining remission in certain CD patients, particularly paediatric patients (see Section 1.4.3.) while the potential of other nutritional therapies, such as fish oils and n-3 fatty acids (120), are currently under investigation.

1.1.7.3. Compliance to Treatment

As with other chronic conditions, non-adherence to maintenance therapy in CD is widespread, with rates ranging from 10-50% reported (121-124). Azathioprine, often prescribed in conjunction with infliximab or after surgery appears to be associated with better compliance rates than mesalazine (125-127), consistent with the observation that complicated disease course increases adherence rates (127).

1.1.7.4. Surgery

Unfortunately, medical therapy is not always successful and approximately 70% of CD patients require surgery for unresponsive disease within ten years of initial diagnosis (128). While surgery can be highly beneficial in addressing complications and restoring quality of life, recurrence rates are also high, varying between 16% (129) and 60%

(130) at 5 years, and rising to 44% at 10 years (131) and 94% at 15 years (130), with patients undergoing an average of 2.5 surgical procedures (132). Disease activity requiring resection appears to diminish with time as demonstrated in a recent long-term follow-up study which observed no necessity for repeat surgery after 25 years after initial resection (133). Disease recurrence is highly associated with smoking (134, 135) and more common in penetrating disease (136, 137) with lesions most often reappearing at the original site of anastomosis (138-140).

1.2. Aetiology of CD

The pathogenesis of CD remains unknown but mounting evidence suggests that the disease

'results from a genetic predisposition to excessive or abnormal interaction with an environmental stimulus, most likely part of the normal luminal bacterial flora which in turn leads to excessive immune activation and chronic inflammation'

Silverberg *et al*, 2005 (74) on behalf of the
Working Party, Montreal World Congress of
Gastroenterology 2005

1.2.1. Genetic Factors

The contributory role of genetic factors in CD was first highlighted by indications from epidemiological studies on familial and twin studies. Familial aggregation studies have shown the relative risk to a sibling of an affected individual to be between 15 and 35 (141) and greater concordance rates have been observed in monozygotic twins than dizygotic twins (142). The higher prevalence of CD in the Jewish population (141), which is maintained over time and geographical location, also points to a genetic predisposition.

Several susceptibility loci have been linked to CD (143-145) but *CARD15/NOD2*, the first contributory gene to be identified for CD (146-148) remains the most replicated region of linkage, through confirmation in different populations (149), including Ireland (150). *CARD 15/NOD2*, which is situated on chromosome 16q12, binds to bacterial lipopolysaccharides and, through regulation by its leucine-rich repeat (LRR) region, activates nuclear factor κ B (NF- κ B) (148), a transcriptional factor in immunoinflammatory responses (151). In CD,

three main mutations occur in the LRR region, altering the response to bacterial components and inducing inappropriate NF- κ B activation (148).

CARD15 mutations, which show association with ileal disease, younger age at onset and fibrostenosing disease (152), are present in approximately 30% to 50% of CD patients (144). Lower prevalence is observed in African Americans (153), while no variants have been identified in Japanese patients (154). CARD15 variants have also been observed in unaffected first degree relatives of CD patients (155) indicating that other factors are involved in disease expression.

1.2.2. Bacterial and Microbial Factors

Infectious pathogens such as *Mycobacterium avium* paratuberculosis (MAP), which are observed in intestinal biopsies and sera of IBD patients (156, 157), have been implicated in the pathogenesis of IBD. But recently, attention has shifted away from pathogenic microbes with evidence (158) suggesting that commensal gut flora are the most likely target of the immune response in CD.

This hypothesis is supported by observations of increased numbers and concentration of bacteria in the mucosal epithelium of IBD patients compared with controls (159) and IBD lesions occurring most often in segments with increased concentrations such as the colon and ileo-caecal valve. While surgical diversion of the faecal stream is associated with a symptomatic improvement in CD, restoration of the faecal stream induces disease recurrence (160). The emergence of a beneficial effect of probiotics on IBD adds to the role of gut flora in IBD (161). Observations from studies on IBD animal models, showing that intestinal inflammation fails to develop in a germ-free environment but

that colitis quickly appears once normal gut flora is introduced (162) has led to the widely accepted concept '*no bacteria, no colitis*'.

1.2.3. Immune Function

'The ultimate goal of an effective inflammatory response is to eliminate the offending agent and then disappear once the cause of inflammation has been eradicated'

Danese & Fiocchi, 2006 (163)

but this appears to fail in IBD due to abnormalities in adaptive, and particularly, innate immunity processes.

IBD is typically associated with a Th1 response, as mucosal T cells are resistant to apoptosis in IBD (164). An abnormal inflammatory response against commensal bacteria is initiated resulting in perpetual pathogenic immune reactivity. Dendritic cells, essential components in the response following detection of commensal bacteria (165), are activated in IBD resulting in increased levels of TLR2, TLR4 and CD40, with increased production of IL-12 and IL-6 (166). TLRs are cell surface molecules that, not only, initiate innate immune responses following detection of microbial infection, but also maintain intestinal homeostasis after identification of commensal bacteria (167). In IBD, an altered expression of TLRs exists with up-regulation of TLR4 and down-regulation of TLR3 (168), which again suggests that an abnormal sensing of bacteria may contribute to disease pathogenesis. Defective innate immunity due to a dysregulated neutrophil response has been observed in the skin of patients with quiescent CD giving support to a primary constitutive abnormality (169).

1.2.4. Environmental Factors

The steep increase in CD incidence over the 20th century has led to an abundance of hypotheses (170-172) relating to environmental factors and CD. Smoking is the best characterised, with the majority of studies observing a clear increased risk among smokers (173), including those subjected to passive smoking (174), and a more severe disease course with higher relapse rates (175, 176). A decreased risk in former smokers has been observed (177), but not confirmed (85, 178), suggesting that increased risk remains even after smoking cessation.

Several other factors have been implicated as having a causative role in CD including oral contraceptive use (85, 178, 179), antibiotic use (180), HRT use (178), BCG vaccination (181), breast feeding (181), low physical activity (182, 183), seasonal variation (184, 185), childhood infection and the 'hygiene hypothesis' (85, 181, 186) and appendicectomy (181, 187) but literature remains inconsistent and any causal relationship is yet to be confirmed.

1.2.5. Dietary Factors

Diet has been an obvious choice when proposing risk factors for IBD, as the gastrointestinal tract is affected, and the increasing incidence of IBD in countries, such as Japan, has been suggested to correlate with the Western lifestyle (188), as positive associations with high fat (189), high protein (188), low fibre (190), and low fruit and vegetable (191) diets have been reported. A wide variety of foods have been implicated as potentially contributing to IBD; these include refined sugar (192), fast food (193), cola drinks (193), cornflakes (194), margarine (195), dairy products (196), coffee (197) and alcohol (197). It has also been proposed that CD is related to allergic inflammation, with stronger response to

food antigens reported in CD patients compared with controls (198), while dietary microparticles found in food additives have also been implicated (199, 200). Unfortunately, due to methodological difficulties, direct causation of IBD by specific diet or food remains to be proven.

While it is possible that the dietary factors studied so far are not true risk factors for CD, it is possible that genuine differences do exist but remain undetected. To date, most data has been assembled through observational studies, which could have led to bias, as they relied on retrospectively recalled information dating from several years before the onset of illness. On the other hand, some studies have investigated the post-illness diet, which is of limited use when studying risk factors for disease development as dietary habits and food choice are likely to have changed since the onset of the illness. Large prospectively collected dietary information is needed to definitively confirm, or otherwise, proposed associations between dietary factors and CD by establishing how dietary patterns prior to onset of CD symptoms relate to disease risk.

1.3. Nutrition and CD

1.3.1. Aetiology of Undernutrition in CD

Undernutrition is a frequent complication in CD and can result from one or, more often, a combination of factors.

1.3.1.1. Decreased Oral Intake

Decreased oral intake, frequently observed in active CD (201), can result from anorexia associated with chronic inflammation (202) and reduced appetite due to altered appetite parameters (203). Patients often report food intolerances and avoid eating altogether or follow self-imposed dietary restrictions (204, 205) in the hope of controlling symptoms such as abdominal pain and diarrhoea and reducing disease relapse. Patients in remission do not appear to have decreased caloric intake when compared to healthy controls (206-208).

1.3.1.2. Increased Nutritional Requirements

Increased resting energy expenditure has been reported in both active (209, 210) and inactive CD (211, 212), although not all are in agreement (213, 214). Increased lipid oxidation (212), diet induced thermogenesis (210) and rates of protein breakdown (215) have also been observed.

Studies in adults and children have concluded that any hypermetabolism in CD is clinically insignificant (211, 216) and that routinely used equations (217) are sufficiently accurate for predicting energy requirements in CD patients (218). However, in contrast, a paediatric study of inactive CD found the Schofield equation to be unreliable as energy requirements were underestimated by over 500 kilocalories per day in 40% of the children (219), although discrepancies were mostly due to increased physical activity rather than

hypermetabolism. As no specific guidelines exist regarding estimation of energy requirements, clinical judgement needs to be exercised on a case-by-case basis. During active disease in hospitalised patients, an additional stress factor of 0-10% of the basal metabolic rate based on the Elia nomogram (220) can be added. During inactive disease, no additional energy is needed as long as normal body weight is being maintained.

1.3.1.3. Decreased Absorption

Absorptive capacity can be reduced following intestinal resections, or from chronic inflammation and bacterial overgrowth (particularly in the presence of fistulae and/or strictures) (221). However, malabsorption is also suggested to be the major contributor to underweight in CD patients with inactive disease (222). When comparing underweight CD patients with normal weight CD patients (all in clinical remission) with similar energy intakes and energy expenditure, increased malabsorption was observed in both groups, but underweight subjects had a statistically significant increased malabsorption (21%) compared to the well-nourished subjects (11%) (222). Intestinal absorption should, therefore, be measured in patients with inactive disease who fail to gain weight and be considered when estimating energy requirements.

1.3.1.4. Excessive GI Losses

Nutrients are lost through the inflamed and ulcerated gut. Fluid, electrolytes and trace elements can be lost through vomiting, diarrhoea and blood loss (223), while nitrogen loss occurs through exudation from intestinal mucosa and faecal loss (224), and as a result of protein-losing enteropathy (225).

1.3.1.5. Inflammation

More recently, attention has focused on the possible role of inflammation in the aetiology of undernutrition in IBD, due to the metabolic effects of increased production of inflammatory cytokines and immune mediators. Excessive production of cytokines, which is observed in the peripheral blood (226, 227) and intestinal mucosa (228, 229) of CD patients, is one of the main causes of cachexia (230). Cachexia-like changes (disproportionate muscle loss) in body composition have been observed in CD patients (231). The link between inflammatory cytokines and decreased nutritional status was highlighted in a study of adult CD patients which observed that tumour necrosis factor- α (TNF α) and interleukin-1 β (IL-1 β) were significantly and negatively correlated with anthropometric measurements of muscle and fat (227).

The integrity of the growth hormone/insulin-like growth factor-1 (GH/IGF-I) axis is essential for normal linear growth (232) and regulates nitrogen balance and tissue growth in adulthood (233). Evidence of deregulation of the IGF system has been reported in adults and children with IBD (234-237) and linked to active inflammation (233). A study of young CD patients found that markers of the IGF system were negatively related to inflammatory markers (CRP, IL-1 β and IL-6) (238), implying that active inflammation modifies the IGF system, which in turn can lead to disturbances in growth. Similar results were observed in adult CD patients where negative correlations existed between the IGF system and CRP, but also albumin, suggesting a link between inflammation and undernutrition via the IGF system (239). Furthermore, 30-40% of linear growth impairment in experimental colitis was reported to occur as a direct result of the inflammatory process (as assessed by IL-6) acting principally on the IGF system (240).

1.3.2. Nutritional Problems in CD

Undernutrition and weight-loss are well-known features associated with CD, appearing in about 65%-75% of patients (241). Undernutrition is most overt during active disease, with undernutrition observed in 40% of hospitalised IBD patients (242), but is also common at time of diagnosis (243) and during remission (206). However, data suggests that up to a third of patients with inactive CD are overweight (244, 245), therefore, excessive weight gain can also occur and may mask changes in lean body mass, bone mass or nutritional deficiencies.

1.3.2.1. Body Composition Alterations

Studies on the body composition of CD patients have yielded varying results with some reporting a lower lean body mass (214, 231, 246) and others observing no differences (212, 247, 248) in both active (214) and inactive (246, 249) disease, when compared to healthy control subjects. Body composition changes are also observed in paediatric CD with significant deficits in lean mass but preserved fat mass (consistent with cachexia) observed in a study of 104 children and young adults (231). Preliminary data also observed a high prevalence of sarcopenia (61%) in young adult CD patients in remission (250), a muscle-wasting condition normally associated with the aging process. Sarcopenia was highly correlated to osteopenia in these patients suggesting that these two phenomena share synergistic deleterious mechanisms. This proposition was highlighted in a recent study of CD patients, which reported that muscular mass and activity, rather than overall body weight, were the most important determinants of bone mass in CD (251). It is also reported that muscle function is affected in CD patients (206), even in the absence of changes in muscle mass (248).

Alterations in fat mass are also an area of debate as decreased (208, 212, 249) and increased (252) fat mass have been reported in some studies while others have observed no differences (231, 248) when compared with controls. CD is associated with an increase in central fat accumulation and more intra-abdominal fat (252) and a higher ratio of intra-abdominal to total body fat compared with controls (253). Changes in substrate utilisation causing an increase in lipid oxidation (210, 214, 249) and a decrease in glucose and protein oxidation (214) have been observed in CD, possibly contributing to changes in fat mass.

Body composition in adult male subjects appears to be more affected than females (206, 243, 246), an observation also noted in children and adolescences, as male subjects show more body composition deficits than females.

1.3.2.2. Micronutrient Deficiencies

Deficiencies of both the water-soluble (206, 208, 243, 254) and fat-soluble (227, 255-257) vitamins, and trace element minerals (206, 208, 258, 259) have been reported in CD patients, occurring in the remission phase (206, 208) of the disease, as well as in active disease (227). The prevalence of common vitamin and mineral deficiencies are highlighted in Tables 1.4. and 1.5.

Depletion of the fat-soluble vitamins D and K, which frequently occur due to bile acid malabsorption and steatorrhoea (260, 261), can increase the risk of bone disease (262). Deficiency of vitamin E and vitamin C, both powerful antioxidants, can lead to oxidative stress due to elevated production of reactive oxygen species that occurs in chronic inflammation (263).

Table 1.4. Common vitamin deficiencies observed in CD patients

	Prevalence (%)	Functions	Consequences	References
Vitamin A	15-29	- Visual acuity - Immune function - Antioxidant	- Xerophthalmia - Night blindness - Impaired immune response	(206, 208, 255, 268)
Vitamin D	18-70	- Calcium absorption - Phosphate excretion - Immune function	- Osteomalacia - Muscle weakness - Lower immune status	(268-270)
Vitamin E	8-44	- Antioxidant - Free radical scavenger	- Peripheral neuropathy - Skeletal myopathy - Haemolytic anaemia	(206, 208, 255)
Vitamin K	+	- Blood coagulation - Bone calcification	- Hypothrombinaemia - Increased bleeding ? Low BMD	(256, 269, 271)
Vitamin B ₆	35-46	Amino acid metabolism	- Anaemia - Hyperhomocysteinaemia	(208, 268)
Folate	0-24	DNA synthesis	- Megaloblastic anaemia - Hyperhomocysteinaemia - Neural tube defects	(208, 268)
Vitamin B ₁₂	15-52	DNA metabolism	- Pernicious anaemia - Neurological damage	(208, 268, 264)
Vitamin C	50, 84	- Antioxidant - Iron absorption - Collagen synthesis	- Scurvy - Impaired wound healing ? impaired immune function	(206, 208)

Table 1.5. Common trace mineral deficiencies observed in CD patients

	Prevalence (%)	Functions	Consequences of deficiency	References
Iron	15-35	▪ O₂ transport ▪ Electron transport	▪ Anaemia ▪ Poor concentration ▪ Fatigue	(208, 268)
Calcium	6	▪ Bone metabolism ▪ Dentition ▪ Muscle contraction ▪ Neurotransmitter release	▪ Osteoporosis ▪ Poor blood clotting	(208)
Zinc	5-64	▪ Protein synthesis ▪ Control of differentiation	▪ Growth retardation ▪ Alopecia ▪ Impaired immune function ▪ Poor wound healing	(206, 208, 268)
Selenium	26-77	▪ Antioxidant ▪ Thyroid function ▪ Immune function	▪ Cardiomyopathy ▪ Skeletal myopathy ▪ Hypothyroidism	(206, 272)
Magnesium	2-52	▪ Skeletal development ▪ Calcium homeostasis	▪ Muscle weakness ▪ Hypocalcaemia ▪ Neuromuscular dysfunction	(206, 208)

Vitamin B12 deficiency, particularly common in patients who have undergone terminal ileum resection (264) and folate deficiency have both been related to high levels of homocysteine in CD (265, 266), a recognised risk factor for thrombotic events (267).

Recurrent anaemia is a common problem in CD, affecting up to 30% of patients at any one time (273), and is predominantly due to iron deficiency and anaemia of chronic disease but also deficiencies of folate and vitamin B12 (274). Fatigue associated with anaemia is a major determinant of quality of life in CD patients (275, 276). Zinc deficiency is also prevalent in CD (206, 277), due to diarrhoea and fistula drainage (278), and has been associated with poor sperm function and male infertility in CD patients (279).

Screening for nutritional deficiencies should be integrated into the routine assessment of CD patients, and supplementation offered where required, as most deficits can be reversed or improved with supplementation (280, 281).

1.3.2.3. Bone Disease

In CD patients, bone loss is a frequently encountered complication that can ultimately lead to osteopenia and osteoporosis (282, 283). Assessment of bone mineral density (BMD) should ideally be by the 'gold standard' method, dual-energy X-ray absorptiometry (284). BMD measurements are generally reported as T-scores, which is the number of standard deviations from the mean of a young adult population, with osteopenia defined as a bone density between 1 and 2.5 standard deviations below the mean, and osteoporosis defined as a bone mineral density that is 2.5 standard deviations below the mean. Approximately 32%-50% of CD patients are osteopenic (285-288), with 11%-23%

osteoporotic (285-287, 289). The clinical significance of these conditions is an increased susceptibility to fracture. Most, but not all (290) agree that the overall risk of fractures is increased in CD with relative risk scores ranging from 1.1-1.7 (291, 292), while the prevalence of fractures varies considerably, from 7% to 41% (291, 293-295).

As with the general population, age, gender and body weight are believed to be major determinants of BMD in CD, as the combined effect of these factors accounts for up to 50% of the variability in BMD (286, 289, 296-298). Other factors believed to potentially play a role include corticosteroid use (285, 286, 298, 299) intestinal malabsorption (300), intestinal resection (301), disease duration (301), smoking (302), systemic inflammation (303, 304), hormonal status (305), dietary intake (301, 306, 307) and genetic factors (308). Unfortunately, as these factors tend to be present concurrently in many patients, it is difficult to study them independently and determine their actual contribution to reduced BMD in CD. Previous vertebral fracture also appears to be a strong predictor for future fractures (292), with the risk increased by up to 10-fold (309). This risk remains even when BMD is normal (310) and can occur in the absence of a low BMD (311, 312). In a case control study of 224 patients, 19% of patients with normal BMD had fractures (311), while in another study of 181 steroid-free patients, 55% of patients with fractures had normal BMD and no correlation was observed between fracture rate and BMD (312).

Normal bone in healthy adults is in a state of equilibrium, the rate of osteoblastic bone formation equaling the rate of bone resorption by osteoclasts. In CD, there appears to be an increase in bone resorption as supported by consistent findings of raised levels of bone resorption markers such as deoxypyridinoline and cross-linked N-telopeptides of

type 1 collagen (313-316). While some authors suggest that there is no compensatory increase in bone formation (313, 317, 318), increased levels of bone formation markers such as osteocalcin and bone specific alkaline phosphatase have been demonstrated (271, 319). These conflicting results suggest that multiple processes may contribute to bone loss in CD with low bone formation and increased bone resorption occurring at different phases of the disease. There also exists a potential link between inflammation and bone homeostasis as cytokines (e.g. interleukin 6, interleukin 1, tumour necrosis factor) released from the inflamed intestine can directly influence osteoblast and osteoclast function (320), through the activation of the RANKL/OPG system (304), which shifts the balance of bone formation and resorption towards bone resorption with consecutive bone loss.

In response to this increased risk of bone disease, the British Society of Gastroenterology (321) and the American Gastroenterological Association (322) have published detailed guidelines for the prevention and treatment of osteoporosis in IBD. The recommendations are aimed at maintaining optimal bone mass by ensuring that patients receive general advice about weight-bearing exercise, smoking, alcohol excess and adequate dietary calcium and that optimal nutritional bone sparing therapies are prescribed when needed. Bisphosphonates, which inhibit bone resorption, are currently the most effective drugs available, and are considered for patients with osteoporosis (T-score < 2.5), previous fractures and patients on long-term corticosteroid medication (282, 321). Although, these drugs can be associated with side-effects such as nausea, diarrhea and constipation, they are generally well-tolerated and are considered safe in CD (323, 324). The absorption of bisphosphonates in CD is considered adequate as a study of 19 patients displayed BMD increases of 2% after 6 months of treatment together with significant

decreases in bone resorption markers (325). A subsequent 12-month placebo controlled trial of 31 CD patients also found that risedronate improved BMD by 2% at the spine and hip compared to calcium alone (324). Risedronate is also reported to reduce the occurrence of both vertebral and non-vertebral fractures (323).

1.3.2.4. Vitamin D Status

Vitamin D deficiency, which is prevalent in CD (270, 326, 327), is also an important risk factor for bone disease in CD (257, 328). Vitamin D exists as two main forms, ergocalciferol (vitamin D2) which is obtained by irradiation of plants (329) and cholecalciferol (vitamin D3) which is formed in the skin following exposure to sunlight or ultraviolet light (330) and is present in a limited number of foods such as fish, cod liver oil, egg yolk and offal (331). In the liver, vitamin D3 is hydroxylated into 25-hydroxyvitamin D3 (25(OH)D) and then in the kidney into 1,25-dihydroxyvitamin D3 (1,25(OH)₂D) (329), the active metabolite which stimulates calcium absorption from the gut (332).

Vitamin D deficiency causes higher secretion of parathyroid hormone due to low serum 1,25(OH)₂D and low serum calcium, resulting in increased bone turnover and bone resorption. Subsequently, bone loss, mainly from cortical bone, can occur, contributing to the pathogenesis of osteoporosis (333). Risk factors for vitamin D deficiency include pigmented skin (334), low sunshine exposure (335), malabsorption (257), obesity (336) and increasing age (337). Unfortunately, for many countries including Ireland the sun is not strong enough to promote cutaneous synthesis of vitamin D during winter months (338), therefore, individuals must rely on food sources but as these are relatively few, intakes are generally low (339). A study of 66

premenopausal women with IBD (340) found inadequate vitamin D intakes in 58% of subjects while mean intake was only 76IU per day.

Assessment for vitamin D deficiency involves the measurement of circulating concentration of 25(OH)D (341) but to date controversy exists regarding appropriate cut-off values to distinguish deficiency from sufficiency. Evidence now suggests that much higher levels of 25(OH)D are required than was previously thought (342) and experts highlight the need for clear definitions and recommendations for adequate intakes of vitamin D to maintain blood levels within a safe range (343).

1.3.2.5. Growth and Development in Children

Children with CD display the similar problems as adult patients with changes in body composition (231, 344, 345) and micronutrient deficiencies (255, 326, 346) also present. In addition to these changes, however, undernutrition is particularly important in children and adolescents as growth, development and sexual maturity can be impaired. Growth failure in paediatric CD has been linked to delays in diagnosis (347), age at diagnosis (348), jejunal inflammation (8), disease severity (349) and genetic influences (350).

Growth is often compromised at diagnosis, with those with longer delay from onset of symptoms to diagnosis displaying more problems with growth (347). Data from a prospective study of newly-diagnosed paediatric patients with CD in Great Britain and Ireland found that weight loss was present in 58% of children at diagnosis and 27% were below the 3rd centile for weight (8). A population based study in Denmark also observed significantly lower height and BMI z-scores in CD children at diagnosis compared to children with UC and healthy

controls (351). In younger children, failure to thrive is often a presenting feature occurring in 44% of children under five years (352) and in five out of seven children in a case series of CD patients diagnosed before 2 years of age (353).

Impaired linear growth occurs in approximately 35% of paediatric CD patients and can often precede the diagnosis (354). Most studies report a favourable catch-up growth and most children reach their target adult height (355, 356) but in some cases results in a reduced adult height (357). A retrospective study of 123 adult CD patients, diagnosed before 16 years of age, observed a mean deficit of 2.4cm below target adult height, with 19% of patients displaying a deficit of over 8.0cm (357). A study of 135 CD patients observed that patients with onset of disease before puberty were significantly shorter when compared to patients with disease onset in puberty or adulthood (356).

Bone development is a major cause of concern in paediatric patients with CD as bone mass is accumulated during childhood and peaks in early adulthood (358), therefore, impaired bone mass in childhood can affect adult skeletal health (359). Decreased bone mineral density is observed in paediatric patients and is associated with increased steroid exposure and poorer nutritional status (344). However, as with adult patients (360), altered bone mass is present in steroid naïve children at diagnosis (361). The majority of children were not meeting recommended dietary intakes for energy, calcium and vitamin D highlighting the importance of nutritional factors. Delayed puberty, common in CD patients (362) has also been associated with significantly impaired bone mineral accrual in young patients with CD (344, 363).

Childhood CD is also associated with delays in the onset and progression of puberty (362). A cohort of 70 adults with juvenile onset IBD stated that menarche was not reached until age 16 years or older in 73% of females with disease onset prior to puberty, with 27% not reaching menarche until their early twenties (355). Delayed puberty was also reported in a cross-sectional study of 104 children and young adults with CD as patients were on average 1.4 and 1.5 years older than matched controls within Tanner stages 2 and 4 (231).

Impaired growth appears to be more severe in young males than in female children (345, 364). Males had significantly lower values for weight, height and skeletal age than females despite similarities in disease activity, duration and lifetime steroid exposure (345). Gender, therefore, may confer risk for impaired growth in CD in childhood and adolescence.

1.4. Effect of Treatments on Nutritional Status

1.4.1. Corticosteroids

While corticosteroids are highly effective in inducing clinical remission in patients with active CD (98-100), they are not recommended for long term use due to their association with various adverse effects involving the majority of body systems, including the musculoskeletal and metabolic systems (365).

Steroid-induced myopathy, which occurs in 7%-60% of patients receiving corticosteroid treatment for various reasons (366), is an insidious condition of proximal muscle weakness and muscle wasting; myalgia or muscle tenderness is usually not present. Although, the duration or dosage of corticosteroids required to exert an effect on muscle is variable, doses above 40 mg/day of prednisolone and of 30 days of duration (367) have been associated with increased risk, while cumulative doses are significantly related to the development of myopathy and muscle atrophy (368). The pathogenesis of steroid myopathy is not fully understood. Corticosteroids induce protein wasting by inhibiting protein synthesis, primarily in type II muscle fibres (369) and enhancing proteolysis of skeletal muscle (370). High concentrations of muscle glycogen are reported in chronic corticosteroid treatment as muscle glycogen synthesase activity is enhanced and glycogen utilisation is decreased (371). Corticosteroids also interfere with oxidative metabolism leading to higher protein oxidation rates (372, 373) and lower fat oxidation rates (373, 374), which has been shown in CD patients receiving prednisolone (373).

The limited evidence available on the association of corticosteroid intake in CD to muscle wasting and reduced muscle function offer

conflicting results; corticosteroids were found to be associated with lower lean body mass in some (207, 231, 244, 246), but not all reports (227, 375), while lifetime prednisolone dose was not correlated to muscle strength (206, 248). These conflicting reports may be due to differing methodologies used, particularly in the measurement of corticosteroid usage and cumulative use, and the fact that disease activity status of patients varied between studies which could have led to the negative effects of cumulative steroids being counteracted by reduction in inflammation in some patients. Also, the question exists of whether muscle atrophy induced by corticosteroids is partially reversible (376, 377), with some suggesting that irregular intakes of steroids, common in CD, allow muscles to 'recover' between treatments, facilitating body composition and muscle metabolism to normalise (248).

Detection of myopathy or muscle atrophy is difficult, and is hidden even more so by changes in body composition associated with corticosteroids. Corticosteroids stimulate food intake (375), of protein and carbohydrate in particular (375), through direct action on the central regulation of appetite (372). This results in positive energy balance, promoting fat deposition (378), weight gain and obesity (372). In CD, body weight and BMI has been shown to be significantly higher in patients receiving prednisolone treatment compared to those who were corticosteroid free (373). This excess weight can, unfortunately, mask signs of muscle wasting, allowing it to ensue unnoticed, particularly as overt signs or symptoms of myopathy are usually absent.

As discussed previously, there is an increased risk of bone disease in CD (85, 284); this risk is amplified by the use of corticosteroids (283,

379). Long-term corticosteroid therapy is associated with an osteoporotic fracture rate ranging from 20%-60% (380). Corticosteroids induce bone loss through a variety of mechanisms including enhancement of bone resorption through inhibition of calcium absorption in the intestine and promotion of calcium excretion in the kidneys, resulting in secondary hyperparathyroidism (381, 382). Corticosteroids also directly inhibit bone formation as demonstrated by reductions in osteocalcin, a sensitive marker of bone formation (314, 383). Bone loss is most evident during the first few weeks to months of corticosteroid therapy (384). While the rate of bone loss slows down thereafter, it continues at a rate that is 2–3 times higher than the normal rate of age-related bone loss (385). Data suggests rates of bone loss of approximately 2–4% per year in untreated patients (386), considerably more than expected in similar healthy populations.

The use of corticosteroids in CD has been shown to be associated with lower BMD measurements (285-287, 298, 301, 312, 387) and an increased risk of fracture (291, 388, 389). In CD patients with established bone disease (osteoporosis or osteopenia), higher usage of corticosteroids have been observed compared to those without bone disease (287, 390). Among all variables tested in a multivariate study, only corticosteroid use was statistically significant in predicting diminished BMD (387). Significant inverse correlations have been demonstrated between BMD and corticosteroid use in the previous year (287, 391) and total lifetime dose (312), while patients not treated with corticosteroids showed similar BMD to that found in healthy subjects (298). In terms of development of fractures, prior corticosteroid use appears to influence risk (388). A population based study reported that corticosteroid use within 2 years before fracture was evident in 54% of CD subjects who fractured compared to 22% of CD subjects who did not fracture (299).

However, not all are in agreement that corticosteroid use is a predictor of fracture risk (312).

Corticosteroid-induced bone loss is related to both the dose and duration of therapy. Doses below 2.5mg per day do not appear to increase bone loss or risk of fracture (389, 392), while doses above 7.5mg per day significantly increase risk of fracture (321). While some previous guidelines have recommended that corticosteroid induced bone loss begins at a threshold of 7.5mg (393), lower daily doses of 2.5-7.5mg of prednisone do result in an increased relative fracture risk of 1.77 compared to controls (392). In a large case control study of 124 655 patients, including CD patients, increased risk of hip fracture was observed with doses of oral steroids above 2.5mg per day (389).

Debate surrounds the issue of duration of corticosteroid use in CD. While current corticosteroid use is known to cause diminished BMD (298, 387), cumulative doses have been inversely associated with BMD in several studies (286, 298, 317), but not in others (285, 313). This discrepancy appears related to the fact that bone loss associated with corticosteroid treatment may be partially reversible. After a two-month standard tapering dose of prednisolone, significant bone loss was observed in fifteen CD patients compared to baseline levels; after eight months, BMD was not significantly lower (307). In addition, cessation of corticosteroid treatment appears to be followed by rapid recovery in terms of BMD and fracture risk (380). However, as cumulative doses have been associated with lower BMD (286, 298, 317), it is suggested that irreversible bone loss occurs with repeated exposure to corticosteroids and that frequent intermittent courses may be more damaging to bone than continuous corticosteroid therapy (298, 317, 394).

The BSG guidelines for the prevention of osteoporosis in CD (321) include specific recommendations in terms of corticosteroid use. It is advised that all patients requiring corticosteroid are prescribed the lowest dose for the shortest time possible and that oral budesonide should be favoured instead of prednisolone, where possible, due to lesser association with bone loss (395). All patients prescribed corticosteroids should be simultaneously prescribed vitamin D daily for the duration of the treatment. BMD should be measured and repeated at regularly, depending on the outcome, and bisphosphonate offered (in addition to vitamin D) if T-score less than -1.5.

In children, corticosteroid therapy can lead to growth retardation and significant suppression of linear growth (370), primarily through its effects on both the release and actions of growth hormone (396). Treatment with growth hormone has been suggested to counteract some of the negative effects of corticosteroids (397). Corticosteroid-dependent children with CD displayed an improvement in body composition (increased fat-free mass and decreased fat mass) and an increase in linear growth velocity from 3.5cm to 7.7cm after 6 months concomitant treatment with growth hormone (398).

1.4.2. Infliximab

Infliximab is not only valuable in the achievement and maintenance of remission of CD (102, 108) but recent studies have shown it to be beneficial in promoting growth (399), to favourably effect bone metabolism (400) and to improve health related quality of life (401). Compared to traditional corticosteroid therapy, infliximab has a profoundly positive impact on growth and nutrition, particularly in children.

Clinical response to infliximab was associated with improved linear growth in 32 children with chronically active CD (402). During follow-up of the 28 patients who responded to infliximab, all showed significant improvements in weight and BMI, while therapy restored normal linear growth velocity and increased height centiles for patients treated prior to or in early puberty (402). A prospective study of 18 children with severe CD observed dramatic improvements in weight and linear growth after 6 months, particularly in patients who had received retreatment every 8 weeks (399). In addition, all patients were able to discontinue corticosteroid treatment within 4 weeks of beginning infliximab therapy (399), further reducing risks of compromised nutritional status.

Treatment with infliximab has also been shown to have significant benefits in terms of bone metabolism (403-405) as it improves calcium homeostasis (403), increases bone mineral density (400) and reduces fracture risk (400). Changes in bone loss are associated with both increases in bone formation (403, 404) and decreases in bone resorption (405) and can occur after a single infusion of infliximab (404, 405). After one infusion, CD patients displayed normalisation of bone markers after only 8 weeks, with an increase of 14-51% in bone formation markers and a significant decrease of 11% in bone resorption markers (405). This finding was also observed in a prospective study of 24 CD patients where significant increases in bone formation markers persisted for up to 4 months after one infusion (404). A study of CD patients treated with infliximab every 6-8 weeks for 1 year reported significant increases in bone mineral density at the spine and hip, regarded relevant for the reduction of fracture risk (400). The improvement occurred even in patients receiving concomitant corticosteroid therapy (400) suggesting that infliximab can neutralise

the negative effects of corticosteroids on bone. Administration of scheduled infusions every 8 weeks could, therefore, lead to long-term improvements in bone metabolism.

1.4.3 Enteral Nutrition

Although initial research reported that enteral nutrition (EN) was as effective as corticosteroids in inducing remission (406), more recent meta-analyses show that while EN is effective in inducing remission in about 60% of cases, it is significantly less effective than steroids in inducing remission in adult patients (407) and, is therefore, not often used as primary therapy in adults, apart from in Japan (408). However, EN appears effective in the treatment of children (409) and also confers additional benefits in terms of growth and nutritional status (410).

EN has been shown to have a positive influence on growth and nutritional parameters in both adults and children (411-415). A prospective study of 8 children with CD and growth failure observed that chronic intermittent EN (1 out of every 4 months for 1 year) reversed growth failure, as weight and height significantly increased compared to a control group (416). Compared to 8 weeks corticosteroid treatment, linear growth recovery was significantly greater in 37 children treated with EN (417). Nutritional markers also show improvements after EN therapy (418) with serum albumin levels reportedly increasing from a mean of 32mg/dL to 44mg/dL after 8 weeks treatment (419) and serum iron also increasing significantly after 8 weeks treatment (417).

The provision of optimal nutritional intake through EN offers a distinct advantage for children with CD. Eight growth-retarded CD adolescents with a mean weight and height gain of 0.38kg and 1.4cm, respectively,

and were only ingesting 55%-80% of their daily required energy intake observed significant increases in weight (11.75kg) and height (6.98cm) after the addition of 1000-1500ml of EN through nocturnal nasogastric feeding (420). Another additional benefit of enteral nutrition is related to the avoidance of corticosteroid therapy (416, 421). Preliminary results from a retrospective study of 130 newly-diagnosed CD children treated with EN reported that 69% of the children avoided corticosteroid therapy during the first year after diagnosis (421). A long-term follow-up study of 44 newly-diagnosed children reported that 47% of children have avoided corticosteroids for 1-7 years, and that their introduction was postponed by 68 weeks in those who did have to resort to corticosteroids (419).

It is suggested that changes in growth-related proteins following EN precede repletion of nutrition status and are, therefore, related to decreased inflammation rather than improvement in nutritional status. Twelve children treated with exclusive EN over 6 weeks showed a rapid change in inflammatory markers following commencement of EN with ESR and IL-6 levels virtually normal by day 3 while PCDAI, CRP and IGF-I normalised by day 7. Nutritional measurements of weight, muscle and fat stores, however, did not alter significantly until at least day 14 of treatment (236). More recently, this observation has been replicated in adult patients as changes in inflammatory markers and intestinal barrier function in 21 adults with active CD occurred before any significant changes in nutritional parameters of body composition and serum markers were noticed (after 3 to 4 weeks) (415). These findings, therefore, prove that changes in inflammation do not occur as a consequence of improved nutritional status; nutritional improvement is an additional benefit.

1.5. Aims and Objectives

1.5.1. General Aims

The overall general aim is to investigate the nature and prevalence of undernutrition in outpatient CD patients with inactive or mildly active disease and compare them to healthy age-, sex- and socio-economically matched controls and reference standards. This aim will be achieved through three separate studies with the following specific aims:

Chapter 3:

- To define the nature and prevalence of undernutrition in non-hospitalised CD patients attending outpatient clinic using standard measures, anthropometry and handgrip strength
- To compare this patient population to healthy age-, sex- and socio-economically matched controls and reference standards
- To identify clinical factors associated with undernutrition that best identifies patients with or at risk of undernutrition

Chapter 4:

- To determine the prevalence of vitamin D deficiency in non-hospitalised CD patients (males and premenopausal females) attending outpatient clinic and to:
 - compare this patient population to healthy age-, sex- and socio-economically matched controls
 - identify clinical factors associated with low serum vitamin D that best identifies patients with or at risk of deficiency
- To evaluate calcium and vitamin D intakes in non-hospitalised CD patients attending outpatient clinic and to compare this patient population to:
 - healthy age-, sex- and socio-economically matched controls
 - recommended dietary allowances for the Irish population

- intakes recommended by specific guidelines for the prevention of osteoporosis in CD patients

Chapter 5:

- To assess the following non-dietary lifestyle factors

- Physical activity patterns
- Alcohol consumption
- Smoking
- Depression and anxiety

in non-hospitalised CD patients attending outpatient clinic compared to age- and sex-matched healthy controls

1.5.2. Specific Objectives

(i) The prospective recruitment of the following groups:

- 100 CD patients with inactive or mildly active disease from the IBD outpatient clinic at AMNCH
- 100 age, sex and socio-economically matched healthy controls

(ii) Design of a database to include relevant patient background information including:

- Medical history
- Nutritional and dietary information
- Demographic information
- Previous/current laboratory medicine reports
- Quality of life
- Anxiety and depression

(iii) The use of anthropometry to assess nutritional status and body composition

(iv) The measurement of nutritional (serum albumin, total protein and vitamin D) and inflammatory (CRP, TNF α) markers in serum

(v) To investigate the effect of confounding variables and clinical factors on nutritional status and risk of undernutrition.

CHAPTER 2:

GENERAL MATERIALS AND METHODS

2.1. Recruitment

The recruitment phase of the study consisted of the recruitment and interviewing of 100 CD patients and 100 age-, sex- and socio-economically matched healthy control subjects (See Figure 2.1. for recruitment design process).

2.1.1. Patient Recruitment

Patients were recruited from the IBD outpatient clinic at the Adelaide and Meath Hospital, Dublin including the National Children's Hospital (AMNCH) from February 2004 to August 2005. At every IBD outpatient clinic, all patients meeting the inclusion criteria were approached and informed about the study, and provided with a study information leaflet (Appendix 1). These patients were then contacted by telephone one week later and if they wished to participate in the study, an appointment was made for them to attend the hospital for the study visit.

2.1.1.1. Patient Inclusion Criteria

To be eligible for study inclusion, patients had to:

- Be over 18 years of age and under 65 years of age
- Have definitively diagnosed CD based on clinical, radiological, endoscopic and/or histological findings according to the criteria of Lennard-Jones (29)
- Be diagnosed at least three months prior to study date
- Have sufficient English language ability to carry out the study requirements
- Be ambulatory and have sufficient mental capacity to carry out the study requirements

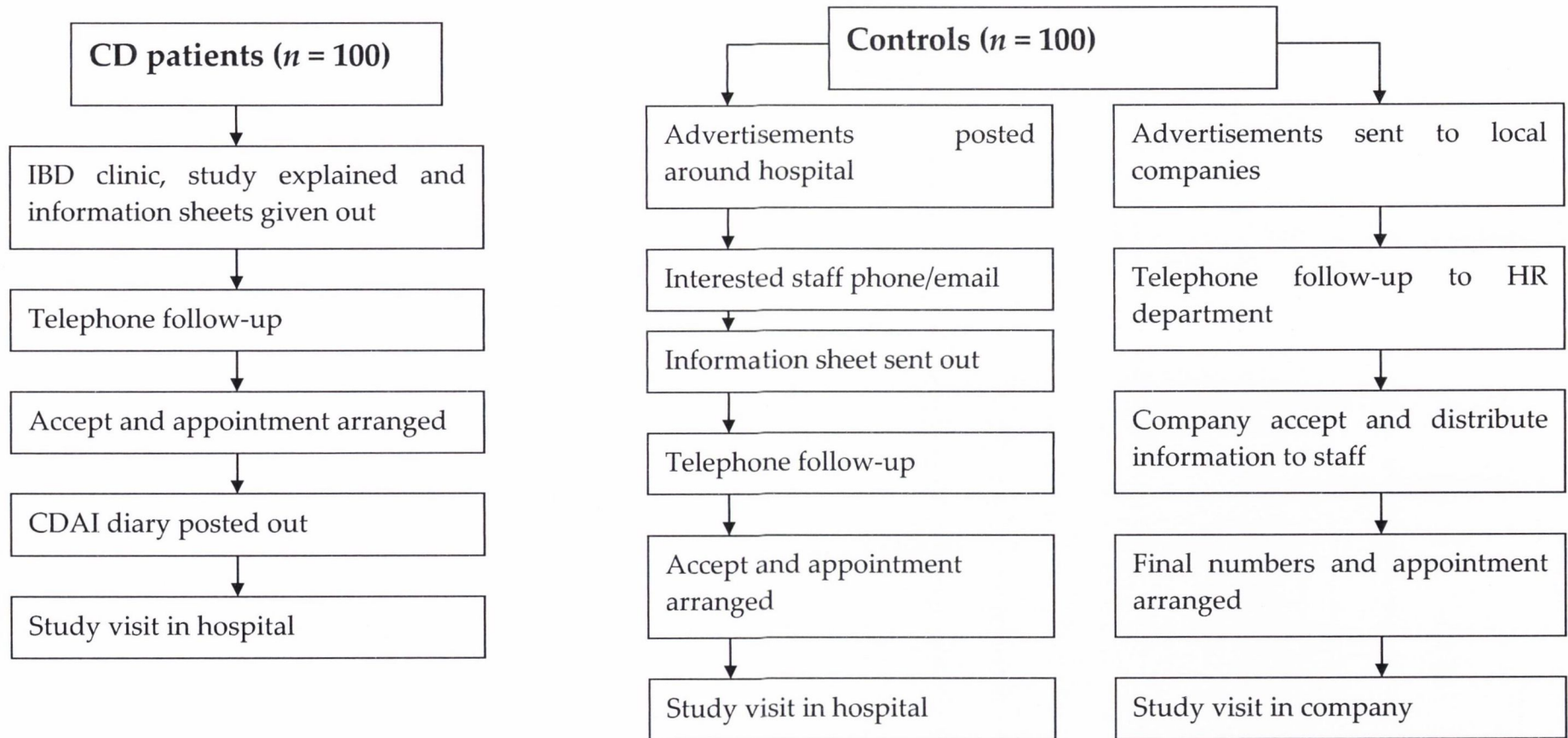


Figure 2.1. Recruitment design process

2.1.1.2. Patient Exclusion Criteria

The following exclusion criteria applied to all patients:

- A diagnosis of indeterminate IBD and/or UC
- Unwilling/unable to give written informed consent
- Physical disability or impairment, which would not allow for anthropometric measurements to be carried out

2.1.2. Control Recruitment

Control subjects were recruited from hospital staff and from local businesses in the Tallaght area between November 2004 and November 2005. Posters were erected throughout the hospital and notices were placed on the internal email notice board. Any eligible staff member who came forward and wished to participate in the study, after reading the study information sheet (Appendix 2), was booked in for a study appointment.

Letters and study information were sent out to approximately 20 businesses in Tallaght. Follow-up phone calls were made to the human resources department of each company to answer questions and to ascertain if the company was willing to participate. If the response was positive, further information and posters were sent by post and email and these were distributed internally within the company to all staff. When final numbers of participants were clarified, a date was organised for the company to be visited to allow for the study to be carried out onsite.

2.1.2.1. Control Inclusion Criteria

To be eligible for study inclusion, control subjects had to:

- Be over 18 years of age and under 65 years of age

- Have no history of IBD or have any first degree relative with IBD
- Have no medical condition affecting bone or muscle or any condition known to affect nutritional status
- Have sufficient English language ability to carry out the study requirements
- Be ambulatory and have sufficient mental capacity to carry out the study requirements

2.1.2.2. Control Exclusion Criteria

The following exclusion criteria applied to all control subjects:

- Unwilling/unable to give written informed consent
- Physical disability or impairment, which would not allow for anthropometric measurements to be carried out

2.1.3. Matching by socio-economic Status

Patients and controls were matched by socio-economic status (as well as by age and gender). Each subject was classified to one of ten specific socio-economic groups according to his/her occupation, based on the system used in the 2002 Census of Ireland (422). Unemployed or retired persons were classified according to their former occupation and employment status. Students or persons working in the home were classified to the socio-economic group of the person on whom they were deemed to be dependent. Table 2.1. shows all subjects classified according to socio-economic group.

As controls were carefully selected to allow for matching of the above demographics and improved validity (423), it is possible that systematic error may have been inadvertently introduced. Also, the equal number of patients and controls (100 to 100) may lead to a reduced statistical power.

Table 2.1. CD patients (n = 100) and control subjects (n = 100) classified according to socio-economic status

	CD (n = 100)	Controls (n = 100)
Socio-economic group	%	%
A: Employers and Managers	7	10
B: Higher Professional	4	8
C: Lower Professional	12	23
D: Non-manual	42	34
E: Manual skilled	18	16
F: Semi-skilled	9	8
G: Unskilled	1	0
H: Own account workers	0	0
I: Farmers	0	0
J: Agricultural workers	1	0
Z: Unknown	4	1
Disability	2	0

No significant difference in median group values, using Mann-Whitney test ($P = 0.300$)

2.1.4. Ethics

The study was approved by the St. James's Hospital and Federated Dublin Voluntary Hospitals Joint Research Ethics Committee, after submission of a detailed study protocol. Each participant gave written informed consent (Appendix 3) prior to study inclusion.

2.1.5. Study Interview and Assessment

All study appointments were conducted in the morning between 8.00am and 12.00pm after an overnight fast. Each patient appointment lasted approximately 45 minutes and consisted of a detailed interview with case record forms, a nutritional assessment and collection of a fasting blood sample. Appointments for control subjects took approximately 20 minutes to complete due to a shorter interview.

2.2. Study Design

The study consisted of three individual studies;

- Study 1: focusing on undernutrition and body composition
- Study 2: focusing on bone and vitamin D
- Study 3: focusing on lifestyle characteristics

The overall study design is outlined in Figure 2.2.

2.3. Case Record Forms and Questionnaires

The study interview was based on case record forms (CRFs) (Appendix 4a and 4b), which were initially pre-piloted on medical and laboratory staff to assess ease of administration. The CRFs asked questions in relation to medical history and medication usage, nutritional supplements and dietary information, demographic details and healthcare consumption.

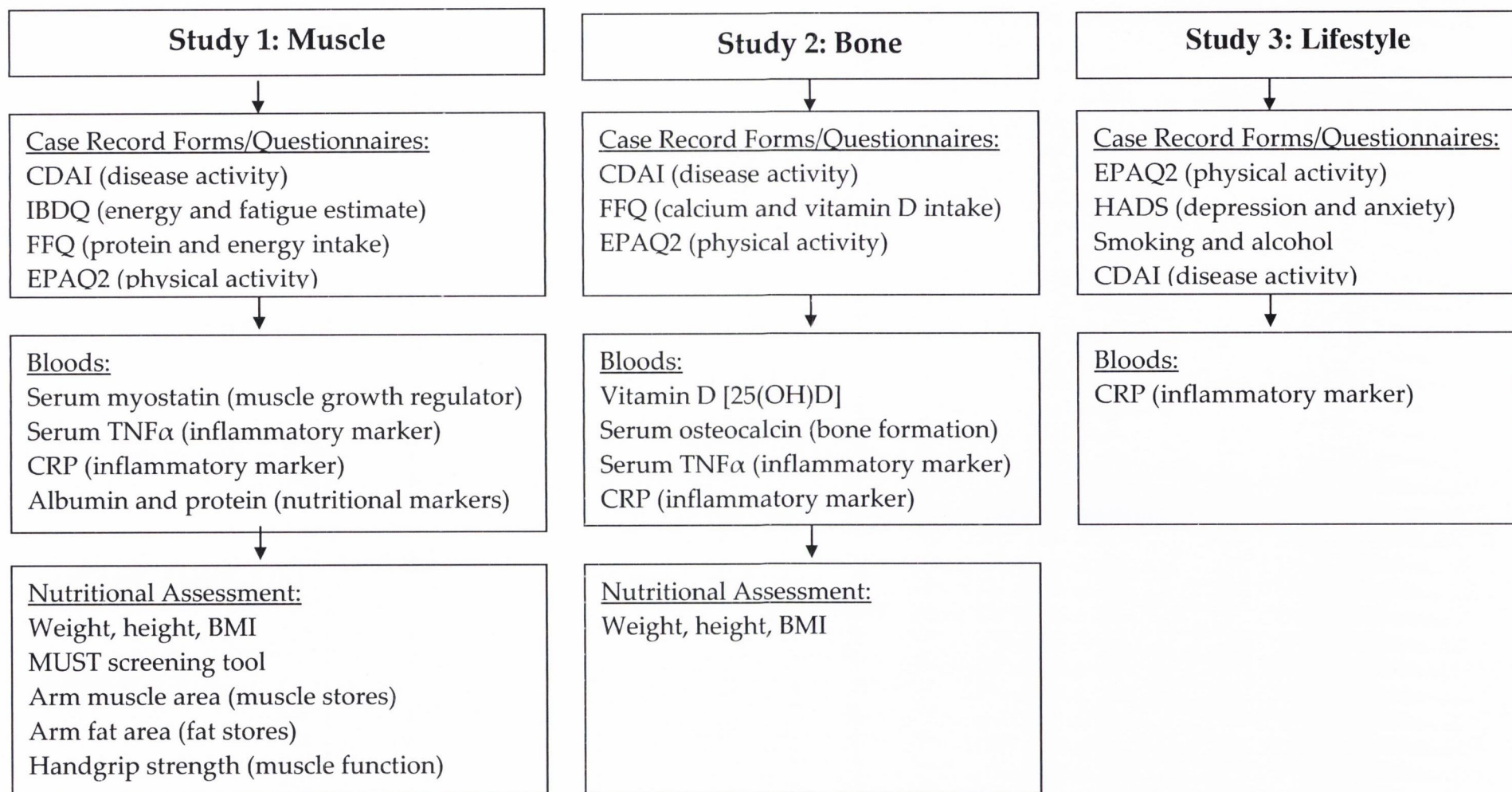


Figure 2.2. Flowchart outlining study design

Information collected from each patient at interview was subsequently validated against the patient's medical records to ensure that information was correct and accurate. A number of validated questionnaires were also used to assess disease activity, energy and fatigue, depression and anxiety, physical activity and dietary intakes of calcium, vitamin D and protein.

2.3.1.Crohn's Disease Activity Index

The Crohn's Disease Activity Index (CDAI) was developed in 1976 (59) to measure clinical disease activity in patients with CD. Although, methodological variations in its use have been highlighted (424), the CDAI remains the 'gold standard' index of disease activity in CD and has recently been certified by the International Organisation of Inflammatory Bowel Disease (IOIBD) (425). It is the most widely used activity index (58) and recent evidence has shown that the assisted retrospective evaluation is of similar accuracy as the standard prospective evaluation, making the CDAI all the more attractive (426). The CDAI is both subjective and objective and consists of an algorithm of eight weighted variables (Appendix 5); four questions (number of liquid stools, severity of abdominal pain, general well-being and use of diphenoxylate or loperamide) based on symptoms and well-being over a seven-day period, are answered prospectively by the patient using a diary while the remaining five variables, based on clinical and biochemical findings are evaluated by the physician. When completed the CDAI gives a numerical score which is used to define disease activity as inactive (CDAI score < 150), active (CDAI >150) or very active (CDAI > 450).

2.3.2. Energy and Fatigue Estimates

Estimates of energy and fatigue levels were assessed using the Inflammatory Bowel Disease Questionnaire (IBDQ) (Appendix 7), which is a specific measure of health related quality of life (HRQOL) in CD. The IBDQ was initially developed in 1989 as a measure of health status and therapeutic efficacy in clinical trials in IBD (427) and is now the most widely used HRQOL instrument in IBD as it is a sensitive marker of changes in the HRQOL of IBD patients over the short-term (428) and long-term (429). The IBDQ shows a close correlation with the CDAI (428-430), and recently, cut-off values indicating remission (≥ 170 points) and clinical response (≥ 32 points) have been proposed for the IBDQ (428). Initially, the IBDQ was developed as an interviewer-administered instrument, but subsequent validation has proved the IBDQ to be a reliable tool when self-administered (431) and for use in various national populations (432).

The IBDQ contains 32 questions relating to four dimensions of quality of life; bowel function (10 questions), systemic symptoms (5 questions), social functioning (5 questions) and emotional status (12 questions). Each answer is graded from 1 to 7 points resulting in a total IBDQ score ranging from 32 to 224, with higher scores indicating better quality of life.

2.3.3. Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) was developed in 1983 by Zigmond and Snaith (433) to identify anxiety and depression disorders in non-psychiatric medical patients. Symptoms related to serious mental disorders are excluded, as are those related to physical symptoms such as weight loss and fatigue. The HADS has subsequently

been translated and validated in various patient populations and in the general population (434, 435), with high internal reliability and concurrent validity (435). A review of 200 papers on HADS in 1997 (434), followed by a second review of 747 papers in 2002 (435), both concluded that the HADS had similar validity and reliability to other more comprehensive tools for the screening of anxiety and depression disorders, and acceptability also appears high amongst patients (436).

HADS is a self-administered and contains fourteen questions, divided into two subscales, which are separately summed to yield two totals - one for anxiety and one for depression (Appendix 8). Each question has four possible answers, which are scored from 0-3, giving an overall total of 0-21 for each subscale. The scores are then translated into a specific degree for each disorder, as follows:

<8: classified as 'normal'

8-10: classified as 'borderline'

>10: classified as 'morbid'

2.3.4. Physical Activity

Physical activity was assessed using the European Prospective Into Cancer (EPIC) Physical Activity Questionnaire (EPAQ2) (questionnaire available at <http://www.srl.cam.ac.uk/epic/questionnaires/epaq2/>), developed as part of the European Prospective Investigation into Cancer-Norfolk Cohort (EPIC-Norfolk), a large population based cohort of UK subjects (437). EPAQ2 is a self-completed questionnaire, which estimates past-year self-reported physical activity from three sub-dimensions; activity at work, home and recreation. The questionnaire was validated against a measure of cardio-respiratory fitness and heart rate monitoring (437), which is highly correlated with the 'gold standard' method of indirect calorimetry (438). The questionnaire

reports estimated energy expenditure from physical activity in units of metabolic equivalent (MET) per week. A MET, the ratio of the energy cost of a particular activity to resting metabolic rate (439) is multiplied by participation (hours/week) to calculate energy expenditure. The questionnaire was not completed during the study interview, but posted out, with a return stamped addressed envelope, to the subjects at home. The subjects, with the help of instructions, then completed and returned the questionnaire. The response rate was excellent with 66 CD patients (66%) and 68 controls (68%) completing and returning the questionnaire. Hours spent television viewing and participating in vigorous activity (activity with a MET score >5) were also calculated from EPAQ2. Answers to the questionnaire were inputted into an associated data entry and processing program (Microsoft Access, 2000) to calculate estimated energy expenditure.

2.3.5. Calcium and Vitamin D Intakes

Dietary intakes of calcium and vitamin D were assessed using a short self-administered food frequency questionnaire (FFQ) (440). While FFQs are associated with various disadvantages including recall bias and under- or over-estimation (441, 442), they remain widely used (443, 444) due to their ease of administration and low cost. The FFQ was derived from the Block-National Cancer Institute Health Habits and History Questionnaire (HHHQ) (445). The 22 foods and beverages listed in the full HHHQ as being rich in either calcium and/or vitamin D were included in the short FFQ; good correlation was observed between the short FFQ and longer version and the short FFQ also correlated strongly with a 7-day food diary (440). The self-administered screening instrument (Appendix 9) is designed to measure a participant's usual food intake during the previous year and for each item on the list, participants were asked to indicate their usual consumption, choosing

from nine frequency categories. The servings were specified in terms of household units (e.g. glass, cup, spoon). Responses were then entered into an associated data entry and processing program (Microsoft Access, 2000), which calculated daily intakes of calcium in milligrams (mgs) and vitamin D in international units (IU). Intakes were compared to published Recommended Dietary Allowances for the Irish population for calcium (800mg/day) and vitamin D (0-10µg/day) (446).

2.3.6. Protein and Energy Intakes

Dietary intakes of protein and energy were assessed using the semi-quantitative FFQ developed for the European Prospective Investigation into Cancer Study (questionnaire available at <http://www.srl.cam.ac.uk/epic/images/ffq.jpg>), which has been validated in a large population based cohort of UK subjects (447-449). The questionnaire, designed to establish average use of foods during the preceding year, consists of 131 items and nine frequency choices from which participants were asked to indicate their usual consumption. The servings were specified in terms of standard portion sizes in household units (e.g. glass, cup, spoon). Responses were then entered into the Q-Builder nutritional analysis software program (Tinuviel Software, UK), which calculated daily intakes of protein in grams (gms) and energy in kilocalories (kcal).

2.4. Nutritional Assessment

All anthropometric measurements were obtained in the morning after an overnight fast by the same trained observer using the same standard equipment.

2.4.1. Height

Height was measured to the nearest 0.1cm using a Leicester portable stadiometer (Seca Ltd, Birmingham, UK). All subjects were measured in light indoor clothing without shoes. Subjects were instructed to “stand up tall and look straight ahead” with feet together and back and heels against the upright bar of the stadiometer. The horizontal bar was brought down to rest on the subject’s head and the measurement noted.

2.4.2. Weight

Body weight was measured to the nearest 0.1kg using an electronic portable personal scales (Seca 888, Seca Ltd, Birmingham, UK). Subjects were weighed in light indoor clothing without shoes.

2.4.3. Body Mass Index

Body Mass Index (BMI) was calculated from weight and height measurements using the equation, $BMI (kg/m^2) = Weight (kg) / Height (m^2)$ and classified according to standard internationally accepted cut-off values (450) as follows:

16 and under: Severe malnutrition

16.1-16.9: Moderate malnutrition

17.0-18.4: Mild malnutrition

18.5-19.9: Marginal malnutrition

20-24.9: Healthy weight

25-29.9: Overweight

30-34.9: Moderately obese

35-39.9: Severely obese

40 and over: Morbidly obese

2.4.4. Arm Anthropometry

Anthropometry is universally accepted as the most applicable, inexpensive and non-invasive method to assess body size and composition (451), with the upper arm most frequently chosen for measurement due to convenience and accessibility. Upper arm muscle area (AMA) reflects muscle protein reserves while upper arm fat area (AFA) gives an indication of body fat stores (452-455). Both are derived from calculations using estimates of mid upper arm circumference (MUAC) and triceps skinfold thickness (TSF).

2.4.4.1. Mid Upper Arm Circumference

MUAC measurements were carried out according to the method of Bishop *et al*, 1981 (454). A single measurement was obtained from the right arm of each subject using a Holtain flexible tape measure calibrated in mm (Holtain Ltd, Pembrokeshire, UK). With the subject's arm held at a 90-degree angle with the palm facing upwards, the midpoint was marked between the acromial process of the scapula (bony protrusion surface of the upper shoulder) and the olecranon process of the elbow (bony point of elbow). The circumference of the arm was measured to the nearest mm at the marked midpoint with the right arm hanging relaxed at the subject's side.

2.4.4.2. Triceps Skinfold Thickness

TSF was measured in triplicate according to the method of Bishop *et al*, 1981 (454) using a Holtain skinfold callipers (Holtain Ltd, Pembrokeshire, UK). A fold of skin plus subcutaneous tissue (without underlying muscle) was formed over the triceps muscle, at the previously marked midpoint, with the thumb and the index finger of the non-dominant hand. The thickness of the fold was measured with

the skinfold callipers held in the dominant hand, whilst continuing to grasp the skinfold with the non-dominant hand. Measurements were recorded to the nearest mm a few seconds after applying the callipers. The procedure was repeated twice more and the average of the three measurements was calculated.

2.4.4.3. Arm Area Calculations

AMA and AFA were calculated from the recorded measurements of MUAC (See Section 2.4.4.1.) and TSF (See Section 2.4.4.2.) according to the following equations (452):

$$AMA (mm^2) = \frac{[MUAC (mm) - TSF (mm)\pi]^2}{4\pi}$$

$$AFA (mm^2) = A - AMA$$

{Where A is upper arm area, derived from the following equation:

$$[\pi/4] \times [MUAC (mm)/\pi]^2\}$$

AMA and AFA estimates were compared to reference standards comprised of age- and sex- specific percentile distributions, based on a cross-sectional sample of 19,097 white subjects derived from the United States Health and Nutritional Examination Survey of 1971 to 1974 (452). No standards are available specifically for the Irish population and current reference data used in the UK and Ireland are based on American subjects (452, 454, 456).

2.4.5. Handgrip Strength

Handgrip strength (HGS), which reflects distal strength and upper limb function, was used as a measure of muscle function as it is quick, easy

to administer and non-invasive. HGS has been found to correlate with strength of other muscle groups and is, therefore, a good indicator of overall muscle strength (457), and is suggested to reliably predict loss of functional status (458). HGS was measured using the Martin Vigorimeter (Martin Medizin Technik, Germany). This instrument has been shown to be 'very precise' when tested with a Universal testing machine under a range of loading forces (459) and reproducible in various settings (460). The Vigorimeter consists of a rubber balloon connected to a manometer via a rubber junction tube. When the balloon is compressed in the hand, the air pressure in the bulb is registered and expressed in kilopascals (kPa). Measurements were made, according to manufacturers instructions, in the non-dominant hand with the forearm placed with the elbow flexed at 90° and the wrist in the neutral position, while the subject was seated in an armchair with elbows supported. The bulb was positioned in the palm of the hand with the air tube extending out between the thumb and index finger, allowing the subject to wrap their fingers around as much of the surface of the bulb as possible. Three measurements were taken with 30 seconds between and subjects were asked to apply as much pressure as possible. The average reading was taken and compared to published reference standards by Merckies *et al* (461), which are stratified by age and gender (See Table 2.2.). A value below the 0.05 quantile value, for the particular age group, was considered to indicate reduced grip strength.

Table 2.2. Grip strength values for healthy adults

	Males		Females	
Age category (years)	0.05 quantile value	Median value	0.05 quantile value	Median value
15-19	72	122	63	90
20-24	89	146	72	106
25-29	101	154	76	112
30-34	110	153	76	112
35-39	115	148	76	109
40-44	115	141	74	104
45-49	111	134	73	98
50-54	105	126	71	92
55-59	94	116	67	84
60-64	83	106	63	76

Taken from Merkies *et al* (461)

2.4.6. Malnutrition Universal Screening Tool (MUST)

Nutritional screening, using specifically developed screening tools (462, 463), aims to rapidly detect individuals at risk of malnutrition or who have poor nutritional status, who can then be referred for more detailed assessment. The Malnutrition Universal Screening Tool (MUST) (464), was developed by the British Association for Parenteral and Enteral Nutrition (BAPEN) for use in adult subjects. It is quick, practical and easy to administer and does not require specific examiner training. The European Society for Parenteral and Enteral Nutrition (ESPEN) have recommended the MUST as the tool of choice in the community setting, as it has excellent inter-rater reliability, concurrent validity and predictive validity (465).

The MUST involves the assessment of three clinical parameters; BMI, unintentional recent weight loss and restricted dietary intake due to presence of acute disease. Responses to each parameter are then rated from 0-2 and overall risk of malnutrition is established by the total of the three responses with 0 = low risk, 1 = medium risk and 2+ = high risk.

Current BMI was calculated from the weight and height obtained during the study visit (See 2.4.3). Unintentional weight loss was calculated on patients who had a previously recorded weight in their medical records for the 3-6 months prior to the study date. Previous weights were not available on control subjects, therefore, unintentional weight loss and a MUST score could not be calculated. As all patients were ambulatory and did have a dietary intake over the 5 days previous to study visit, acute disease was considered to be absent, therefore, each patient received a score of 0 for this question.

2.5. Laboratory Analysis

2.5.1. Serum Processing and Storage

Fasting blood samples were collected into additive-free serum tubes (Vacurette, Cruinn Technologies, Dublin). Samples were left to clot for at least one hour and then centrifuged at 3000rpm for 10 minutes. Serum was aliquoted off into labelled 0.5ml eppendorf tubes and frozen at -80°C until further analysis.

2.5.2. Measurement of Serum TNF α

Serum TNF α was measured using a commercially available immunoassay kit, the Human TNF α UlstraSensitive immunoassay kit (Biosource International, Belgium). Kit optimisation was initially conducted and due to low optical density readings it was decided (with the assistance of the manufacturers technical support staff) to increase the first incubation time to 2.5 hours. All samples were then assayed in duplicate, according to the manufacturers' instructions, and plotted against a standard curve, which was calculated for each assay. Samples giving a reading over 32pg/ml were diluted and re-analysed. In summary, 100 μl of standard, control or 50 μl of sample (diluted with 50 μl of Standard Diluent Buffer), 50 μl of Incubation Buffer and 50 μl of Biotin Conjugate were added to each well of the antibody (specific for TNF α)-coated plate and incubated at 37°C for 2.5 hours. The plate was washed four times with Wash Buffer before addition of 100 μl of Streptavidin–Peroxidase (HRP) Working Solution and incubation for 30 minutes at $20\text{--}25^{\circ}\text{C}$. The plate was washed four times before addition of 100 μl of Stabilised Chromagen, which turned the liquid in the wells blue. After incubation for 30 minutes at $20\text{--}25^{\circ}\text{C}$ in the dark, 100 μl of Stop Solution was added, which turned the liquid yellow. Optical density was read at 450nm on a microplate reader (Bio-tek Instruments

ELx800, Mason Technology Ltd, Dublin), which automatically plotted a standard curve and serum TNF α concentrations, expressed in pg/ml. Results were multiplied by two to correct for the 1:2 dilution of samples at the start of the assay.

2.5.3. Measurement of Serum Myostatin

Serum myostatin, a growth factor involved in the regulation of skeletal muscle growth, was measured using a commercially available immunoassay kit, the Human Myostatin ELISA (Prodomain Specific) enzyme immunoassay (BioVendor GmbH, Heidelberg, Germany). Kit optimisation was initially conducted and it was decided (with the assistance of the manufacturers technical support staff) to use an optical density reading of 405nm (instead of 450nm), as the microplate reader was not able to read the highest standard at 450nm. All samples were then assayed in duplicate, according to the manufacturers' instructions, and plotted against a standard curve, which was calculated for each assay. Validity of the standard curve and sample concentrations were ensured by the use of two quality controls, one low and one high, of known concentration with each assay. In summary, 100 μ l of standard, control or sample were added to each well of antibody (anti-human myostatin prodomain)-coated plate and incubated at 20-25°C for 1 hour while continuously shaking on an orbital shaker (Orbital Incubator SI50, Bibby Stuart Scientific). The plate was washed five times with 0.35mls of Wash Buffer before the addition of 100 μ l of Biotin Labelled Anti-Myostatin Prodomain Antibody Solution and incubation for 1 hour at 20-25°C, whilst continuously shaking. The plate was washed five times before the addition of 100 μ l of Streptavidin-HRP Conjugate and incubation for 30 minutes at 20-25°C, whilst continuously shaking. The plate was again washed five times before the addition of 100 μ l of Substrate Solution and incubation in the dark for 10 minutes at 20-25°C.

100µl of Stop Solution was then added which turned the liquid yellow. Optical density was read at 405nm on a microplate reader (Bio-tek Instruments ELx800, Mason Technology Ltd, Dublin), which automatically plotted a standard curve and serum myostatin concentrations, expressed in ng/ml.

2.5.4. Measurement of Serum Osteocalcin

Serum osteocalcin, a measure of bone formation, was measured using a commercially available kit, the Metra™ Osteocalcin enzyme immunoassay (Quidel, San Diego, USA), which quantifies intact (de novo) osteocalcin. All samples were assayed in duplicate, according to manufacturers' instructions, and plotted against a standard curve, which was calculated for each assay. Validity of the standard curve and sample concentrations were ensured by the use of two quality controls, one low and one high, of known concentration with each assay. In summary, 25µl of standard, control or sample and anti-osteocalcin antibody were added to each well of osteocalcin-coated plate and incubated at 20-25°C for 2 hours. The plate was washed three times with Wash Buffer before the addition of 150µl of enzyme (alkaline phosphatase) conjugate and incubation for 1 hour at 20-25°C. The plate was washed three times before the addition of 150µl Working Substrate Solution and incubation for 35-40°C. 50µl of Stop Solution was then added, which turned the liquid yellow. Optical density was read at 405nm on a microplate reader (Bio-tek Instruments ELx800, Mason Technology Ltd, Dublin). Serum osteocalcin concentrations were calculated from the standard curve and expressed in ng/ml.

2.5.5. Measurement of Serum Vitamin D

Serum vitamin D analysis was carried out in the Biochemistry Department in St. James' Hospital, Dublin, which undergoes regular internal and external quality control checks. The DiaSorin 25-hydroxyvitamin D ¹²⁵I radioimmunoassay kit (DiaSorin Inc, Minnesota, USA) was used to determine the concentration of 25(OH)D. The DiaSorin 25(OH)D assay consists of a two-step procedure. Firstly, 25(OH)D and other hydroxylated metabolites were rapidly extracted from serum with acetonitrile. Following extraction, the treated samples were then assayed using an equilibrium radioimmunoassay procedure, based on an antibody with specificity to 25(OH)D. Samples were incubated with antibody and tracer for 90 minutes at 20-25°C. A second antibody (Donkey Anti-Goat) precipitating complex was added for a 20-minute incubation at 20-25°C, which resulted in phase separation. Following this, addition buffer was added prior to centrifugation to aid in reducing non-specific binding. A calibration curve was plotted using the extent of binding against stated concentrations of the calibration calibrators and concentration of vitamin D calculated and expressed in µg/ml.

2.5.6. Inflammatory and Nutritional Serum Markers

CRP, serum albumin, total protein and haemoglobin were measured by standard procedure in the routine laboratories at AMNCH. Normal ranges, as used in AMNCH, were as follows;

- CRP < 5mg/ml
- Serum albumin 35-50 g/L
- Total protein 60-80 g/L
- Haemoglobin 11.5-15.5 g/dl (females), 13.5-18.5 g/dl (males)

2.6. Statistical Analysis

Descriptive data was expressed as means $\pm$ standard deviation or median (interquartile range). Differences between patients and controls were assessed using the Student's t-test (normally distributed data), Mann-Whitney test (data not normally distributed) and Pearson's chi-square test (proportions). Logistic regression analysis (Chapter 3) and linear regression analysis (Chapter 4) were performed and reported with 95% confidence intervals. All statistical analysis was performed using SPSS Version 12.0, JMP Version 5.1.2 and Stata Version 9.2.

CHAPTER 3:

UNDERNUTRITION AND BODY COMPOSITION

3.1. Introduction

Undernutrition is associated with impaired muscle, respiratory and immune function and can delay recovery from illness and surgery, and is associated with longer stays in hospital and higher health care costs (453). Undernutrition can also have a significant impact on quality of life, particularly in gastrointestinal disease (466).

Undernutrition, a well-recognised complication of active CD, is caused by many factors including poor dietary intake, malabsorption, increased nutrient losses and oxidative stress (467). Undernutrition in active disease has been widely studied, as weight loss and malnutrition are apparent during flare-ups and periods of high disease activity and can affect up to 85% of patients (468).

On the other hand, during disease remission or at times of low disease activity, undernutrition is not so apparent and can be difficult to diagnose as most patients in remission look and feel well and lack the typical signs normally associated with malnutrition. Nonetheless, studies have shown that these patients can, in fact, be malnourished and display changes in body composition (207, 231, 249) and nutritional deficiencies (206, 208).

With progress in the medical management and the advent of newer biologic therapies for CD, more treatment options are available for patients to achieve clinical remission, and therefore, undernutrition can often be overlooked in these patients. Even during periods of remission, patients can still be at risk of undernutrition and have increased nutrient requirements as symptoms predisposing patients to undernutrition can still be present and subclinical inflammation can

persist. Treatments associated with CD can also add to the risk of undernutrition; this is particularly true for corticosteroids, as they not only cause muscle wasting (374) and bone loss (321) but also can often cause weight gain and redistribution of body fat (372), masking any obvious signs of undernutrition. Therefore, we hypothesise that undernutrition is a common feature among non-hospitalised CD patients but that it remains largely unrecognized, particularly in patients in remission with inactive disease.

3.1.1. Specific Aims and Objectives

- To define the nature and prevalence of undernutrition in non-hospitalised CD patients attending outpatient clinic using standard measures, anthropometry and handgrip strength
- To compare this patient population to healthy age-, sex- and socio-economically matched controls and reference standards
- To identify clinical factors associated with undernutrition that best identifies patients with or at risk of undernutrition

3.2. Patients and Methods

3.2.1. *Summary of Methods*

Detailed information regarding recruitment and assessment of patients is outlined in Chapter 2: General Materials and Methods.

In summary:

- 100 CD patients were recruited from the IBD outpatient clinic at AMNCH (See Section 2.1.1.)
- 100 control subjects were recruited from hospital staff and local businesses (See Section 2.1.2.)
- All subjects were interviewed using CRFs (See Section 2.3.), underwent a detailed nutritional assessment (See Section 2.4.) and provided a fasting blood sample (See Section 2.5.1.)
- Information regarding disease activity (See Section 2.3.1.), physical activity (See Section 2.3.4.) and energy and fatigue estimates (See Section 2.3.2.) were obtained at interview using validated questionnaires
- Serum TNF α was analysed using a commercially available ELISA kit (See Section 2.5.2.)
- Serum myostatin was analysed using a commercially available ELISA kit (See Section 2.5.3.)

3.2.2. *Statistics*

Descriptive data was expressed as means $\pm$ standard deviation or median (interquarile range). Differences between patients and controls were assessed using the Student's t-test (normally distributed data), Mann-Whitney test (data not normally distributed) and Pearson's chi-square test (proportions). Logistic regression analysis was performed and odds ratios reported with 95% confidence intervals. All statistical

analysis was performed using SPSS Version 12.0, JMP Version 5.1.2 and Stata Version 9.2.

3.3. Results

3.3.1. Baseline Characteristics of Subjects

There were no significant differences between CD patients and healthy controls in relation to age, gender or socio-economic status (See Section 2.1.3.). CD patients had predominantly small bowel involvement [70% (70/100)] and median disease duration was 6.5 years. Median CDAI was 91.10 (38.26 – 173.86) with 67% (67/100) of patients in remission as defined by a CDAI less than 150. No patient had severe disease (CDAI above 450). Median CRP value was 3.7mg/L (2.9-8.0) with 61% (61/100) of patients below the normal cut-off of 5mg/L.

At the time of the study, 24% (24/100) of patients were receiving oral corticosteroids, with 12% (12/100) taking prednisolone and 12% (12/100) taking budesonide. Forty-one per cent (41/100) had received oral corticosteroids within the previous 12 months; 26% (26/100) receiving prednisolone and 18% (18/100) receiving budesonide [3% (3/100) received both]. In terms of prednisolone use, 19% (19/100) received one course, 3% (3/100) received two courses, 3% (3/100) received three courses and one patient (1%) received four courses during the previous 12 months; total intake over the previous 12 months ranged from 40mgs to 5705mgs, while the average daily dose ranged from 0.1mgs to 15.6mgs. Categorising prednisolone by dosage, 9% (9/100) received low doses (<2.5mgs/day), 12% (12/100) received medium doses (2.5-7.5mgs/day) and 5% (5/100) received high doses (>7.5mgs/day). Forty seven per cent (47/100) had no oral corticosteroid intake at the time of the study or within the previous 12 months. Information regarding corticosteroid use was not available on 12 patients.

Fifteen per cent (15/100) had received infliximab therapy within the previous 12 months and 29% (29/100) had previously undergone surgical resection of the bowel for their disease (Table 3.1.).

3.3.2. Nutritional Assessment by Standard Measures

3.3.2.1. Body Mass Index

Weight, height and BMI were similar in patients and controls (Table 3.2.), with the mean BMI of each group falling into the overweight category (25.07 ± 5.47 v 25.43 ± 3.74 kg/m² respectively). Figure 3.1. shows all subjects classified according to BMI. The majority of both CD patients [49% (49/100)] and controls [42% (42/100)] had a BMI in the healthy weight range (20-24.9kg/m²). Most of the remainder of both groups fell into the overweight (25-29.9kg/m²) or obese (BMI >30kg/m²) categories [patients 40% (40/100) v controls 52% (52/100)] with only 11% (11/100) of patients and 6% (6/100) of controls malnourished (BMI <20kg/m²).

3.3.2.2. Malnutrition Universal Screening Tool (MUST)

Using the MUST screening tool (464), 80% (69/86) of patients were classified as low risk, 15% (13/86) at medium risk and 5% (4/86) at high risk of malnutrition. Details on weight loss within the previous 6 months were unavailable on 14 patients; therefore, MUST scores could not be calculated for this group. Looking at weight changes within the previous 6 months, 66% (57/86) of patients had no or non-significant weight loss. Thirteen percent (13%) (11/86) had a significant weight loss (>5% within 3-6months) and 21% (18/86) had gained weight. MUST scores could not be calculated for the control group, as accurate information was not available on previous weights.

3.3.2.3. Serum Albumin and Total Protein

Standard measures of serum albumin and total protein were available on CD patients but not for control subjects. Mean total protein in the CD patient group was $73.76 \pm 5.07 \text{g/L}$ with 92% (90/98) within the normal range of 60-80g/L. Mean serum albumin was $42.61 \pm 4.34 \text{g/L}$ with 93% (91/98) of patients within the normal range of 35-50g/L.

3.3.3. Nutritional Assessment by Anthropometry

3.3.3.1. Muscle Stores

Mean AMA values ($4112.51 \pm 1323.82 \text{mm}^2$ v $4154.68 \pm 1168.18 \text{mm}^2$; NS) showed no difference between CD and controls, respectively (Table 3.2.). Table 3.3. shows subjects categorised according to reference standards derived by Frisancho (452), which also shows no significant differences between groups. The majority of both patients [54% (54/100)] and controls [63% (63/100)] showed AMA values between the 15th and 85th percentile indicating normal muscle stores, although about a third of each group [41% (41/100) of patients and 34% (34/100) of controls] had values below the 15th percentile indicating muscle depletion.

3.3.3.2. Fat Stores

Mean values of AFA were similar between patients and controls ($2666.60 \pm 1123.25 \text{mm}^2$ v $2636.53 \pm 978.19 \text{mm}^2$; NS) (Table 3.2.). On comparison to reference standards (Table 3.3.), no differences were observed between patients and controls. The majority of subjects had AFA values within the normal range of 15th to the 85th percentile [81% (81/100) of patients and 82% (82/100) of controls]. In contrast to muscle stores, more of those outside the normal range had values in the upper

range above the 85th percentile [14% (14/100) of patients and 8% (8/100) of controls], which correlated positively to BMI ($r = 0.70$, $P < 0.001$).

3.3.3.3. Muscle Function

Overall, grip strength values were significantly lower in the CD group compared with controls ($77.64 \pm 22.94 \text{ kPa}$ v $91.44 \pm 24.03 \text{ kPa}$; $P < 0.001$) and significantly more CD patients had values below the normal range (461) when compared with controls [72% (72/100) v 43% (43/100); $P < 0.001\chi$]. Similar results were observed when looking at males and females separately. Grip strength values were lower in CD males than control males across all age groups (Figure 3.2.), and significantly so in the 20-29 year old ($93.21 \pm 21.04 \text{ kPa}$ v $109.48 \pm 18.98 \text{ kPa}$; $P = 0.05$) and the 40-49 year old ($73.77 \pm 15.81 \text{ kPa}$ v $119.96 \pm 17.24 \text{ kPa}$; $P = 0.002$) age groups. Significantly more male patients [70% (28/40)] than male controls [33% (13/40)] had values below the normal range ($P = 0.001\chi$). Female patients also displayed lower grip strength values than female controls across all age groups (Figure 3.2.), and significantly so in the 20-29 year old ($67.43 \pm 14.58 \text{ kPa}$ v $80.80 \pm 18.99 \text{ kPa}$; $P < 0.03$), 30-39 year old ($64.22 \pm 11.40 \text{ kPa}$ v $85.73 \pm 19.69 \text{ kPa}$; $P = 0.002$) and 50-59 year old ($56.70 \pm 10.86 \text{ kPa}$ v $76.89 \pm 18.62 \text{ kPa}$; $P < 0.02$) age groups. Significantly more female patients [73% (44/60)] than female controls [50% (30/60)] had values below the normal range ($P = 0.009\chi$).

3.3.4. Predictors of Muscle Function

Logistic regression analysis was conducted to assess what factors may be associated with reduced muscle function. In controls, height was significantly ($P = 0.04$) associated with reduced function with those of shorter stature at increased risk (Table 3.4.). In patients, any oral steroid usage over the past 12 months was highly associated ($P = 0.01$) as those patients not receiving steroid therapy showed an 82% reduction in the

odds of having reduced muscle strength; Shorter stature ($P = 0.01$) and colonic disease location ($P = 0.01$) were also significantly associated with reduced muscle function in the patient group (Table 3.5.), with large bowel disease accompanied with a tenfold increased likelihood of having reduced function compared to small bowel disease. Age, gender, weight, muscle stores (AMA), fat stores (AFA), protein intake and physical activity participation were not associated with muscle function in either patients (Table 3.5.) or controls (Table 3.4.); there were also no associations observed between inflammatory markers (CRP and $\text{TNF}\alpha$), disease activity (CDAI) or disease duration in patients (Table 3.5).

3.3.5. Associations with Serum Myostatin

Serum myostatin concentrations were measured in a random subgroup of 32 CD patients; median myostatin concentration was 1.76ng/ml (0.26-5.04). Subjects with low grip strength tended to display higher myostatin values compared to those with normal grip strength [2.39ng/ml (0.51-7.11) *v* 0.50ng/ml (0.14-1.74); $P = 0.082$]; this reached significance in male subjects [2.61ng/ml (0.77-7.44) *v* 0.29ng/ml (0.11-1.96); $P = 0.04$] (Figure 3.3.), while no differences were observed in females. In male subjects also, myostatin levels showed a significant negative correlation with grip strength values ($r = -0.52$, $P = 0.04$). A significant positive correlation was observed between myostatin and AFA in the whole group ($r = 0.47$, $P = 0.007$) (Figure 3.3.), and in male patients ($r = 0.61$, $P = 0.01$), with a trend was also noted in females ($r = 0.43$, $P = 0.08$). No association was found between myostatin levels and muscle stores in the group as a whole or when analysed separately by gender.

3.3.6. Functional Capacity

Patients with reduced muscle function showed significantly less overall physical activity levels compared to those with normal muscle function (122.21 ± 55.66 METs *v* 153.51 ± 64.18 METs; $P = 0.05$) (Figure 3.4.). On separate analysis, no differences were observed for recreational or home activity but there was a trend for patients with reduced function to undertake less work activity. Looking at television viewing (hours/week) as a marker of physical activity, no differences were observed between those with normal and reduced function (20.08 ± 12.15 hours/week *v* 19.13 ± 9.69 hours/week; NS).

On assessment of functional questions from the IBDQ relating to fatigue and energy levels, patients with reduced function scored significantly lower than patients with normal function on both fatigue (3.89 ± 1.59 *v* 3.15 ± 1.31 ; $P = 0.02$) and energy (4.46 ± 1.55 *v* 3.87 ± 1.31 ; $P = 0.05$) suggesting that reduced muscle function in these patients was associated with increased fatigue and decreased energy levels (Figure 3.5.).

Table 3.1. Baseline characteristics of CD patients (*n* = 100)
compared to control subjects (*n* = 100)

	CD (<i>n</i> = 100)	Controls (<i>n</i> = 100)	<i>P</i> value
Age, years (mean±SD)	35.7±10.9	37.9±11.0	<i>P</i> = 0.153
Gender (male:female)	40:60	40:60	<i>P</i> = 1.000
Smoking (%)			<i>P</i> = 0.288
Current smoker	25	16	
Ex-smoker	29	32	
Non-smoker	46	52	
Disease location (%)			
Small bowel	46	-	-
Large bowel	28	-	-
Small and large bowel	24	-	-
Upper GI	2	-	-
Disease duration, years [median (range)]	6.5 (3.0– 12.8)	-	-
Family history (%)	20	-	-
CDAI [median (range)]	91.1 (38.2-173.9)	-	-
CRP, mg/L [median (range)]	3.7 (2.9-8.0)	-	-
CDAI <150 (%)	67	-	-
CRP < 5mg/L (%)	61	-	-
Steroids within last year (%)	41	-	-
Immunosuppressants (%)	45	-	-
5ASAs (%)	77	-	-
Infliximab (%)	15	-	-
Previous resection (%)	29	-	-

Table 3.2. Anthropometric measurements in CD patients ($n = 100$) and control subjects ($n = 100$)

	All		Females		Males	
	CD $n = 100$	Controls $n = 100$	CD $n = 60$	Controls $n = 60$	CD $n = 40$	Controls $n = 40$
Weight (kg)	71.18±17.08	72.59±13.91	65.60±13.10 ^a	67.21±11.68 ^b	79.56±19.00	80.66±13.17
Height (m)	1.68±0.09	1.68±0.09	1.61±0.06 ^a	1.63±0.07 ^b	1.77±0.05	1.76±0.05
BMI (kg/m ²)	25.07±5.47	25.43±3.74	25.03±5.08	25.15±3.85	25.13±6.06	25.84±3.58
AMA (mm ²)	4112.51±1323.82	4154.68±1168.18	3412.63±824.73 ^a	3511.10±774.81 ^b	5162.33±1239.24	5120.06±984.26
AFA (mm ²)	2666.60±1123.25	2636.53±978.19	3037.19±1062.39 ^a	3051.70±913.69 ^b	2110.72±983.38	2013.76±709.24

Results presented as means ± SD

^a $P < 0.001$ compared to CD males

^b $P < 0.001$ compared to control males

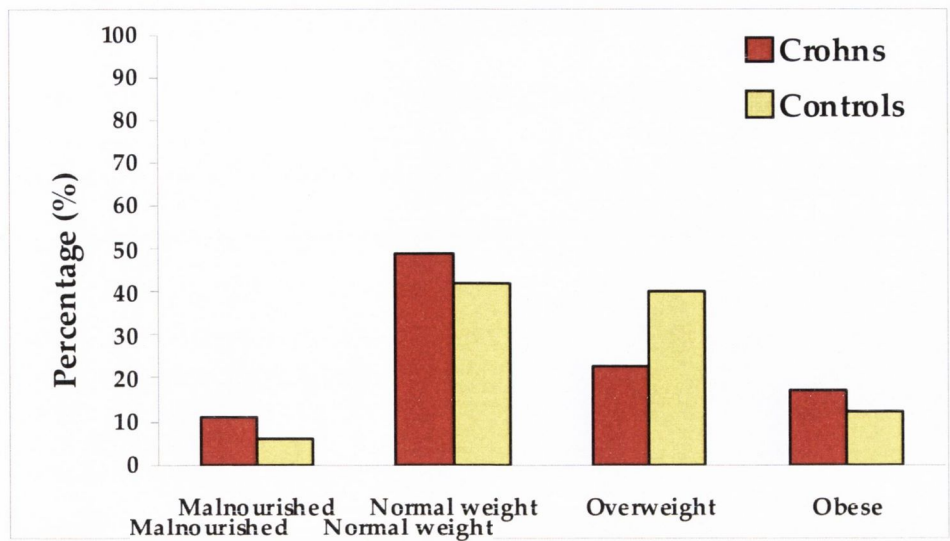


Figure 3.1. BMI classifications of CD patients ($n = 100$) and control subjects ($n = 100$)

Results expressed as percentage of subjects in each group

BMI classifications (450):

Malnourished: BMI $< 20\text{kg/m}^2$

Normal weight: BMI $20\text{--}24.9\text{kg/m}^2$

Overweight: BMI $25\text{--}29.9\text{kg/m}^2$

Obese: BMI $> 30\text{kg/m}^2$

Table 3.3. Muscle and fat percentiles in CD patients (*n* = 100) and control subjects (*n* = 100)

	CD (<i>n</i> = 100)			Controls (<i>n</i> = 100)		
	<15 th	15 th – 85 th	>85 th	<15 th	15 th - 85 th	>85 th
AMA	41%	54%	5%	34%	63%	3%
AFA	5%	81%	14%	10%	82%	8%
	CD females (<i>n</i> = 60)			Control females (<i>n</i> = 60)		
	<15 th	15 th – 85 th	>85 th	<15 th	15 th - 85 th	>85 th
AMA	37%	56%	7%	25%	72%	3%
AFA	5%	82%	13%	10%	85%	5%
	CD males (<i>n</i> = 40)			Control males (<i>n</i> = 40)		
	<15 th	15 th – 85 th	>85 th	<15 th	15 th - 85 th	>85 th
AMA	48%	49%	3%	48%	49%	3%
AFA	5%	80%	15%	10%	77%	13%

Results expressed as the percentage of subjects in each category

Values less than 15th percentile indicate depleted stores

No significant differences between any groups

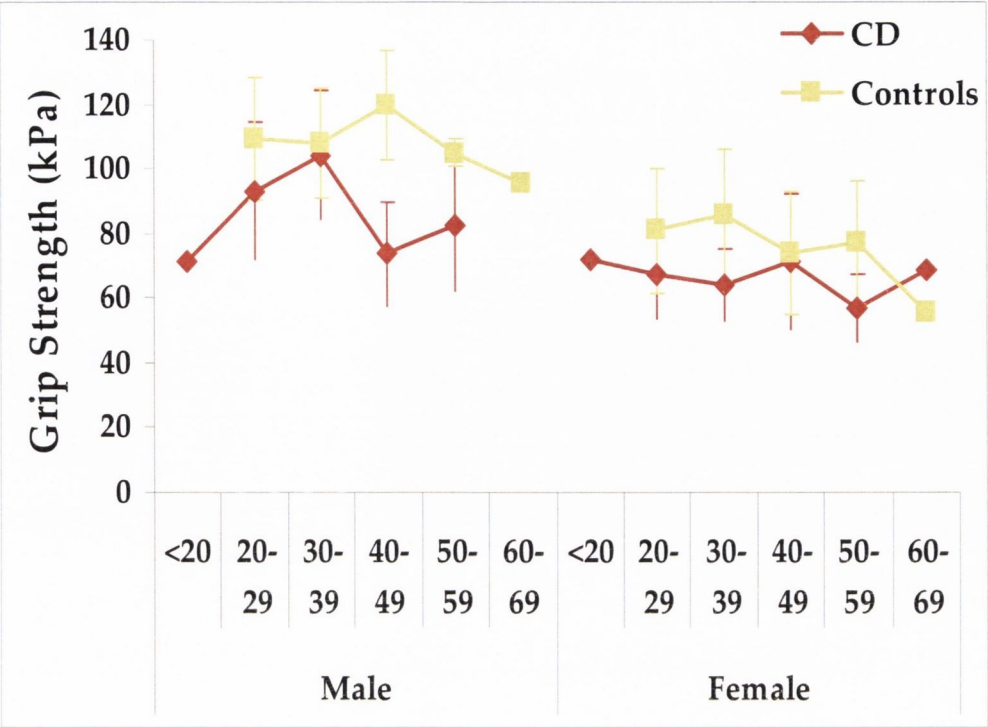


Figure 3.2. Comparison of muscle function, as assessed by hand grip strength (kPa) in CD patients ($n = 100$) and control subjects ($n = 100$)

- a** $P = 0.05$ compared to controls
- b** $P = 0.002$ compared to controls
- c** $P < 0.03$ compared to controls
- d** $P = 0.002$ compared to controls
- e** $P < 0.02$ compared to controls

Table 3.4. Factors associated with reduced muscle function in control subjects (n = 43) using logistic regression analysis

	OR (95% CI)	P value
Age (yrs)	0.98 (0.94, 1.02)	0.260
Gender (female)	0.40 (0.06, 1.96)	0.227
Weight (kg)	1.06 (0.97, 1.15)	0.201
Height (m)	2.57x10 ⁻⁷ (8.54x10 ⁻¹² , 0.007)	0.004 *
AMA (mm²)	1.00 (0.99, 1.00)	0.350
AFA (mm²)	1.00 (0.99, 1.00)	0.943
Protein intake (g/day)	0.95 (0.91, 1.01)	0.109
Physical activity (hrs/wk)	1.80 (0.51, 6.39)	0.362

Results presented as odds ratio (OR) and 95% confidence interval (CI)

P <0.05 considered significant

* Shorter stature associated with reduced muscle function

Table 3.5. Factors associated with reduced muscle function in CD patients (n = 72) using logistic regression analysis

	OR (95% CI)	P value
Age (yrs)	1.00 (0.93, 1.06)	0.876
Gender (female)	0.09 (0.00, 1.80)	0.117
Weight (kg)	1.03 (0.95, 1.12)	0.421
Height (m)	6.83×10 ⁻⁷ (5.47×10 ⁻¹² , 0.08)	0.018 *
AMA (mm ²)	0.99 (0.99, 1.00)	0.310
AFA (mm ²)	1.00 (0.99, 1.00)	0.621
CDAI	1.00 (0.99, 1.01)	0.197
CRP (mg/L)	0.93 (0.85, 1.01)	0.125
Disease duration (yrs)	1.04 (0.92, 1.18)	0.450
No steroid usage	0.18 (0.04, 0.71)	0.014 **
Small bowel disease	2.17 (0.46, 10.15)	0.322
Large bowel disease	10.83 (1.55, 75.60)	0.016 ***
Protein intake (g/day)	1.04 (0.98, 1.10)	0.177
Physical activity (hrs/week)	1.08 (0.25, 4.73)	0.910
TNFα (ng/ml)	1.05 (0.76, 1.46)	0.747

Results presented as odds ratio (OR) and 95% confidence interval (CI)

P <0.05 considered significant

* Shorter stature associated with reduced muscle function

** Higher steroid usage associated with reduced muscle function

*** Large bowel disease associated with reduced muscle function

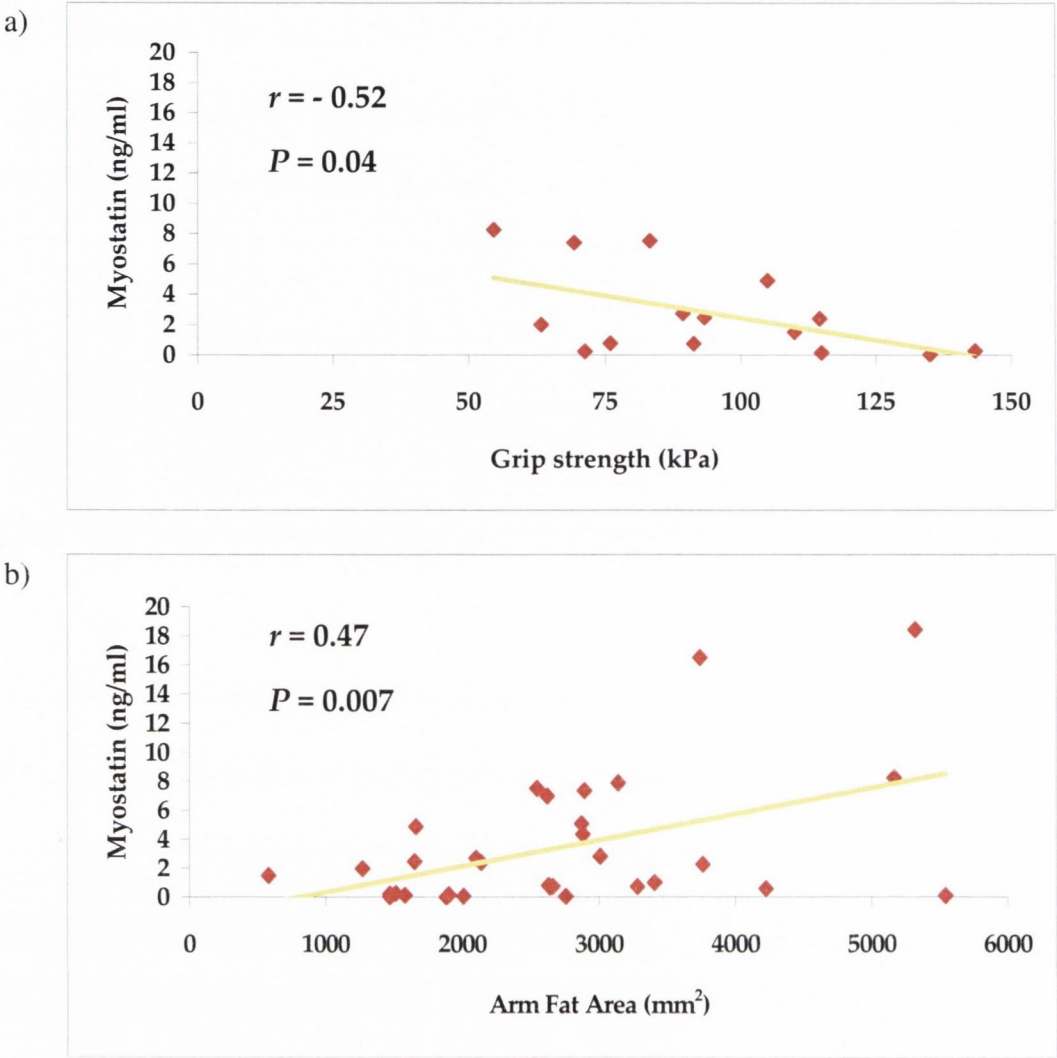


Figure 3.3. Serum myostatin associations in CD patients

- a) grip strength in male CD patients ($n = 15$)
- b) AFA in all CD subjects ($n = 32$)

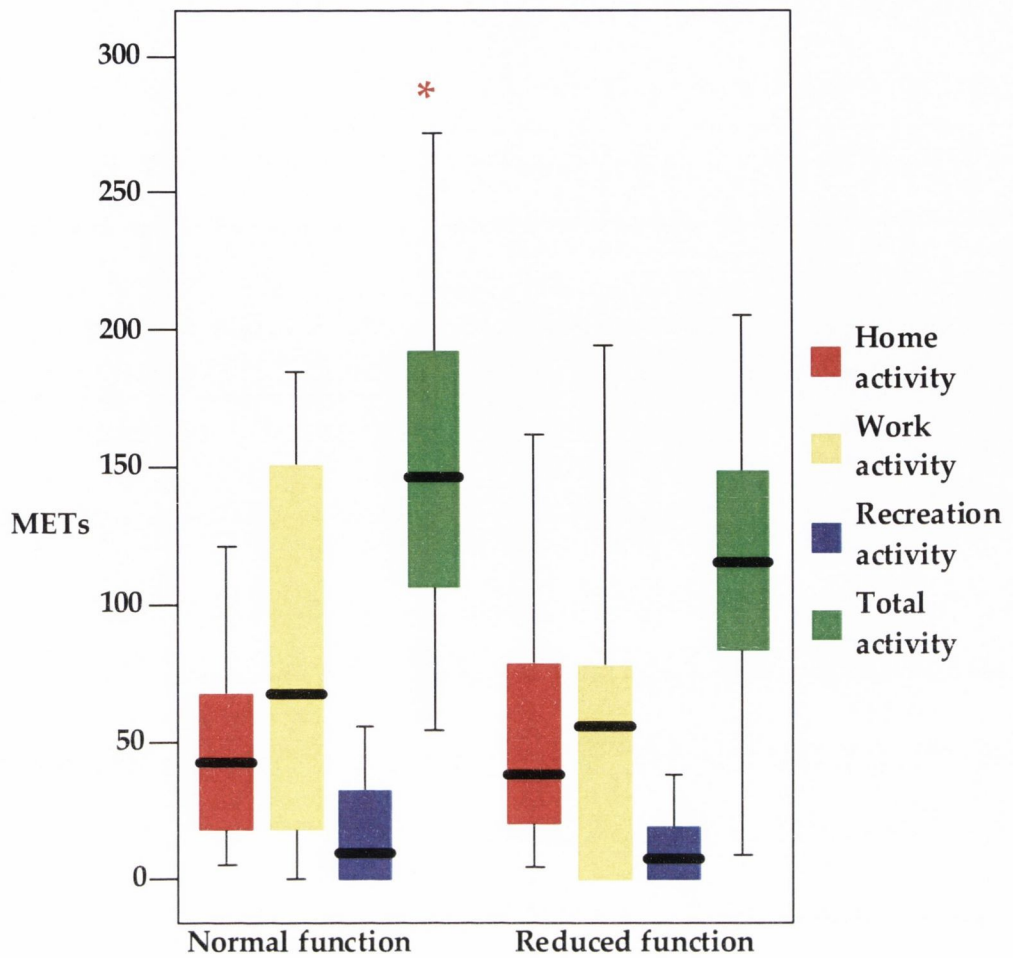


Figure 3.4. Physical activity in CD patients with reduced muscle function ($n = 72$) and normal muscle function ($n = 28$)

METS: Multiples of resting metabolic rate x hours per week, derived from the EPAQ2 (437)

* $P = 0.05$ compared to those with reduced muscle function

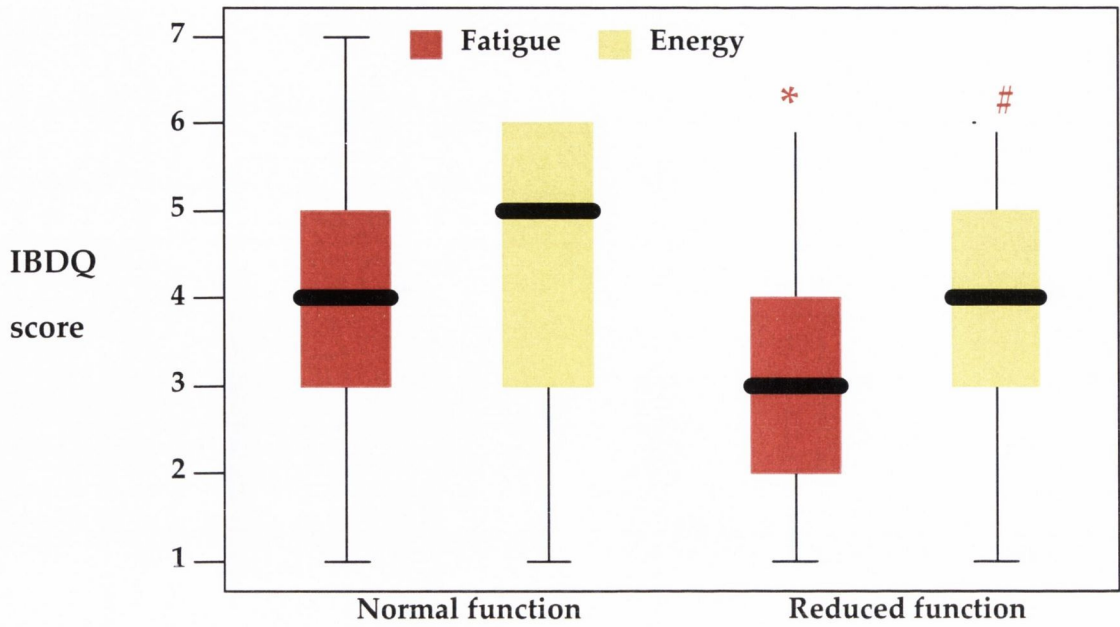


Figure 3.5. Fatigue and energy levels in CD patients with normal (n = 28) and reduced muscle function (n = 72)

Assessed by IBDQ (Questions 2 and 6) with lower scores indicating lesser quality of life i.e. more fatigue and less energy

* $P < 0.05$ compared to normal function

$P < 0.05$ compared to normal function

3.4. Discussion

This study reports a high prevalence of reduced skeletal muscle function, as assessed by handgrip strength, in a group of CD patients, most of who are in clinical remission, have normal BMI, normal muscle and fat stores, normal serum albumin levels and are at low risk of undernutrition when assessed by the MUST nutritional screening tool. Patients of short stature with large bowel disease and a higher steroid usage are at higher risk of reduced muscle function, which is associated with increased fatigue and decreased energy levels.

This study is, to our knowledge, the first to report a high prevalence of reduced handgrip strength in non-hospitalised CD patients, despite normal body composition. Few studies have investigated skeletal muscle function in CD patients but all (206, 248), are in agreement with our results and conclude that reduced muscle function is a feature of CD. Wiroth et al (248) observed reduced lower limb isometric performances in CD patients when compared to controls and female CD patients also had significantly reduced handgrip strength although no differences were observed in male patients. Reduced strength of the hamstring muscles with preserved quadriceps muscle strength was observed in a study of long-standing CD patients currently in remission, but no assessment of handgrip strength or upper limb function was carried out on these patients (206). A recent preliminary study also reported significantly reduced handgrip strength and reduced functional capacity in CD and UC patients in remission (469). In contrast, in newly diagnosed (less than 6 months) CD patients, lower limb muscle function was found to be similar to controls (243) suggesting that muscle function is preserved at time of diagnosis. It is, therefore, plausible that disease progression and associated treatments

may play a role in the development of reduced muscle function in CD. The median disease duration in our current group of patients was 6 years, with 54% diagnosed more than 5 years ago, which is similar to the groups studied by Wiroth *et al* (248) (10 years) and Geerling *et al* (206) (16 years), who also displayed reduced muscle function.

In healthy subjects, muscle function and mass are usually associated (243, 470), with muscle mass being a strong predictor of muscle strength. While newly diagnosed CD patients with normal body composition and normal muscle function show significant correlation between muscle mass and strength (243), muscle function does not appear to be dependent on muscle protein stores in patients with long standing disease, as reduced function can present even when lean mass is preserved. In the current study, reduced muscle function was apparent even though lean mass was not significantly reduced. This is in agreement with previous work, which showed abnormalities in muscle performance despite preserved lean mass (206, 248). The reason for this decrease in muscle strength appears related to metabolic changes in the capacity of energy production in muscle (471) and changes in contractility, relaxation rate and endurance of the muscle (472), causing a decrease of muscle strength, without a corresponding loss of skeletal muscle mass (473).

This observation suggests that skeletal muscle function may, therefore, be used as an early indicator of malnutrition as it appears to be more sensitive to nutritional depletion than body composition, and may detect functional abnormalities in patients with subclinical malnutrition. During a fasting and refeeding study in obese subjects, defined changes in skeletal muscle function were observed when significant changes in serum albumin and anthropometrical

measurements of lean mass and fat mass could not be detected. Furthermore, refeeding resulted in a rapid normalisation of functional parameters (474). Muscle function changes in clinically malnourished patients were also reversible when treated with nutritional support (472) and muscle performance was restored in anorexia nervosa patients well before normal nutritional status was re-established (475). Muscle function indices have also been proven to distinguish between anthropometrically similar groups but who have different nutritional states, such as those who are chronically undernourished and those who are merely underweight but healthy (473). Although not all studies agree with the possibility that muscle strength is an early indicator of malnutrition (458), the proposal is, nonetheless, appealing. Prospective studies are needed to evaluate whether handgrip strength is responsive to changes over time and can, therefore, identify patients at risk; a limitation of the present study was the fact that measurements, including handgrip strength were only carried out at one timepoint.

In study patients, who showed a high frequency of reduced muscle function, standard measures of nutritional screening including BMI, the MUST screening tool and serum albumin deemed the majority of patients to be at no or low risk of undernutrition. Using BMI alone, only 11% of patients would be considered malnourished, with the majority of normal BMI or above, which is not uncommon in inactive disease (207, 208, 244). A high proportion were actually obese (17%) or overweight (23%), which not only masks underlying nutritional problems, but is also associated with increased disease severity (476). According to published guidelines by the European Society for Parenteral and Enteral Nutrition (ESPEN) (465), MUST is the recommended screening tool for use in the community setting and assesses BMI, weight change and recent dietary intake. The MUST

classified 84% of our patients to be at low risk of undernutrition, with 12% at medium risk and only 4% at high risk, as most patients had normal BMI and dietary intake. A recent study suggested that another screening tool, the Subjective Global Assessment, could identify malnutrition-related muscle dysfunction in hospital patients (477). Although all patients classified malnourished according to the SGA did have impaired muscle function, it only classified 26% of the whole group as malnourished when in fact 53% had reduced handgrip values, and therefore, the SGA alone, as is the case with the MUST, cannot identify all patients with reduced muscle function. Serum albumin was within the normal range in over 90% of patients. Although albumin is a useful index of severe protein malnutrition, it does not function well as an early indicator of malnutrition in CD, as serum levels tend to remain normal even when changes in nutritional status are evident (206, 207, 231, 247).

This finding highlights the fact that standard measures of nutritional assessment are not sufficient for use in CD patients, particularly those in remission, as they classify patients as being well-nourished and at no risk of malnutrition even though changes in muscle function (and body composition) can be present. The use of handgrip strength as a routine screening tool is, therefore, an attractive and viable option. Handgrip strength can be measured using a handheld dynamometer, which is easily administered, quick, non-invasive and inexpensive. It is not affected by hydration status as certain anthropometrical measurements can be or by inflammation which can affect markers such as serum albumin. It is a dynamic measure of nutritional status, which can be administered at the bedside and is also ideal for assessing patients in outpatient clinics or in the community who are currently in remission but who may be at risk of becoming malnourished. Indeed, a recent

study (478) supports the use of handgrip strength as a nutrition risk screening tool as patients with lowest handgrip strength values were at increased risk of being nutritionally at risk, while those with higher handgrip strength values had an independent decreased risk of being malnourished (478). It is imperative that these patients are detected and treated accordingly, and in the case of CD patients, so that they can be maintained in the best possible nutritional state during remission and are in a better starting position should their disease relapse.

In the present study, we observed lower grip strength values in females compared to males in both groups and a trend for decreasing grip strength with increasing age, which is in keeping with previous reports (461, 479, 480). Grip strength declines from approximately 30 years of age; mean annual loss amongst healthy people aged 30-70 years is estimated to be 0.5%-1% of the strength at 30 years of age (481). In comparison to the published reference standards, our control group displayed lower grip strength values, with approximately 40% of our controls showing reduced muscle function. This is unlikely to be caused by differences in technique as the protocol described by Merckies *et al*, 2000 (461), which has recently been further recommended (482), was adhered to at all times. The likelihood is that our study group was of shorter stature than the Dutch subjects on which the reference values were based (461), which has a known association with grip strength (470, 483).

In addition, the interpretation of grip strength data and comparison between studies can be difficult due to the use of different tools, units of measurement and age category division. In the present study, grip strength was assessed using the Martin vigorimeter, which measures pressure and therefore dynamic movements in kilopascals (kPa).

Another commonly used device is the Jamar dynamometer which is a static strength measure and records in kilograms (kgs). These instruments have been compared with results indicating a very high correlation between the two measures with Jamar values ranging between 0.45 and 0.52 of the corresponding Vigorimeter values (484). To allow for comparison with other studies of healthy individuals, we derived estimated values for our grip strength in kgs using an arbitrary quotient of 0.5, to enable comparison with studies in which the Jamar dynamometer was used. This provided mean grip strengths in our male controls and female controls of 55.2kg and 39.4kg, respectively, which is more in line with other populations; 48.6kg and 28.5kg for Scottish men and women (479), 46.3kg and 29.3 for English men and women (482), 39.9kg and 25.7kg for Spanish men and women (485) and 62kg and 37kg for American men and women (486). To date, no reference standards are available for the Irish adult population, although normative values have been derived for the elderly Irish population (487), with the authors highlighting that values were significantly different in the Irish group in comparison to contemporary UK data. This paper and previous reports (480, 485) suggest that mean grip strength varies widely across countries and geographical areas (even after adjustment for height), highlighting the need for country specific reference standards to be established for healthy subjects, stratified by age and gender.

In this study, almost twice as many patients (72%) than controls (43%) displayed reduced grip strength values. Regression analysis showed that height, corticosteroid usage and colonic disease were associated with reduced muscle function in our group of patients. The most interesting of these findings was the 82% reduction in the odds of having reduced function for those patients not on corticosteroids

during the previous year, pointing to a link between corticosteroid use and reduced muscle function in CD. These results are contradictory to previous studies in CD patients (206, 248), which observed no association between steroid use and muscle strength. However, the fact that newly diagnosed patients seem to have a preserved muscle function (243) also highlights a link to corticosteroids as these patients are likely to be steroid naive. The discrepancy in results could be due to the various methods used to assess corticosteroid usage. Both other studies assessed cumulative lifetime steroid usage but this was, unfortunately, not possible in our study due to a lack of reliable accurate data. We had to rely on the available medical notes of each patient to calculate corticosteroid intake but many of our patients had admitted to receiving courses of corticosteroids that were not documented in the medical notes (i.e. from their GP and/or self-prescribing during flare-ups) over numbers of year, therefore, steroid usage in the previous year was examined. The total or average dosage of corticosteroids or the number of courses for the year did not correlate with grip strength, probably due to sample size but splitting the group into users or non-users of oral corticosteroids in the previous 12 months provided evidence of an association, suggesting that even relatively small doses can affect muscle function. This agrees with the hypothesis that doses above 40 mg/day of prednisolone and of only 30 days duration (standard in CD patients) can increase risk (367). While 24% of patients were receiving oral corticosteroids at the time of the study, 41% had taken steroids within the previous year suggesting that steroid-induced myopathy can persist even after the therapy has been stopped, an observation also noted in respiratory patients (376), but it has also been postulated that corticosteroid-induced muscle atrophy is partially reversible (367) and that muscles can 'recover' between treatments. Further research is needed in this important area, particularly as

approximately one third of CD patients become steroid dependent (488). Of course, when considering the effect of corticosteroids, the contribution of inflammation cannot be ruled out as those patients on corticosteroids have, in general, more active disease and greater inflammation, making it difficult to decipher the roles played by corticosteroids and active inflammation. Research has suggested an association of inflammatory markers with muscle strength and mass (489), as high levels of pro-inflammatory cytokines have been associated with both low muscle mass and function (490), while low levels of CRP correlated with high grip strength (491, 492), with CRP levels over 6.1 $\mu\text{g/mL}$ associated with a twofold increased risk of strength loss compared with low CRP levels of less than 1.4 $\mu\text{g/mL}$ (492). In the current study, no associations were observed between CDAI or CRP with muscle function in our patient group, which is in agreement with previous work in CD (248), however, this is an issue which requires further investigation in more studies with larger sample sizes.

Short stature is known to be associated with reduced function (470, 483) as muscle development, and, therefore, muscle strength is dependent on the growth of the long bones of the body. This association was clear in our study, as height was the only variable significantly associated with muscle function in our control group and was also highly significant in our patient group. A surprising result was that disease confined to the colon was ten times more likely to be associated with reduced function than small bowel disease. It is unlikely that colonic disease itself caused a reduction in muscle function but that patients with colonic disease displayed other common factors. Analysis revealed that patients with disease confined to the colon were significantly heavier ($74.80 \pm 14.87\text{kg}$ *v* $67.17 \pm 13.87\text{kg}$, $P = 0.02$) and had significantly

higher AFA ($3003.69 \pm 1203.79 \text{ mm}^2$ v $2488.65 \pm 998.56 \text{ mm}^2$, $P = 0.05$) than those with small bowel disease; these factors most likely caused the negative effect on muscle function. While regression analysis did not determine physical activity to be a predictor of reduced muscle function in our groups, it was observed that patients with reduced grip strength had significantly lower levels of physical activity than patients with normal function. Only one other study has assessed habitual physical activity and its relation to muscle function in CD patients; no association was reported (248). This discrepancy is most likely due to differences in the assessment and classification of physical activity. Also, as self-reported physical activity questionnaires were used, it is possible that subjects may have misreported, a known disadvantage with this method (493). Whether reduced function was a consequence of lower physical activity levels, or whether the reduced muscle function was the cause of lower levels of physical activity remains to be answered. This is an area which needs further research as the positive influences of exercise on grip strength (494) and muscle function (495) are well-recognised; physical activity could potentially be used as an intervention in CD patients in remission.

The inhibition of myostatin, a negative regulator of muscle mass (496), has been linked to muscle strength in mice (497) and humans (498). The present study is in agreement with these observations as serum myostatin levels were significantly negatively associated with muscle function in male CD patients. A significant positive association was observed with AFA in the patient group as a whole, which is also in agreement with previous work, which reported suppression of fat accumulation in myostatin deficient mice (499). As myostatin was assessed in only a subgroup of patients, further research is needed on this topic with larger patient numbers to further clarify the issue.

Reduced muscle function in our patients was associated with a reduced capacity for physical activity and, importantly, increased fatigue and lower energy levels. Fatigue, which affects up to 86% of CD patients with active disease (Minderhoud et al 2007) and 40% of patients in remission (500) adversely affects quality of life, which is decreased in CD when compared to healthy controls (466, 501). Worryingly, the levels of fatigue observed in CD patients in remission are comparable to those reported in cancer patients (500). Various theories have been suggested as a cause for this increased fatigue, but, as yet, no conclusive arguments have been proven as fatigue is rarely explained by biochemical or haematological tests. Anaemia, a well recognised cause of fatigue (274), is a factor in some patients but would not account for all; in the present study only 11% of patients displayed low haemoglobin levels. Cytokine-mediated fatigue has been investigated as a cause as significant associations have been observed between infliximab treatment and fatigue reduction (502), however, no direct correlations could be seen with either CRP or proinflammatory cytokines (502). Hypothalamic-pituitary-adrenal suppression and secondary hypocortisolism, as a result of corticosteroid usage, has also been investigated but yielded negative results as no patients displayed adrenal insufficiency (500). Significant disturbances in sleep patterns have also been reported in CD patients in remission (503), although the reason behind this is unknown. While the origin of increased fatigue remains poorly understood, this issue does need further investigation. It is plausible that reduced muscle function could provide some clarification on this issue.

Hand dynamometry has been shown to be a powerful predictor of future outcomes, with low grip strength associated with an increased risk of premature mortality (457, 504), functional limitations and

disability (458, 505, 506), fractures (507, 508) and complications or increased length of stay after surgery or hospitalisation (509-511). Studies of grip strength in middle age have been shown to strongly predict risk of disability (506) and all-cause mortality (457). A 25-year prospective study of 6059 middle-aged men aged 45-68 reported that hand grip strength, after adjustment for multiple potential confounders, was highly predictive of functional limitations and disability 25 years later (506), highlighting the importance of maintenance of muscle strength in middle age for better health in later life. Good muscle strength in midlife is associated with good muscle strength in old age (512), reducing the risk of becoming disabled, as there is a greater reserve of strength to cope with any chronic conditions that may arise. This is a worrying fact given the high rate of reduced muscle function in our young patient group (mean age 35), at a time when handgrip strength should be at its peak (457, 461).

In conclusion, we observed a high prevalence of reduced muscle function in a group of ambulatory CD patients attending outpatient clinic, most of whom were in clinical remission and were well nourished. High corticosteroid usage was the main factor associated with a reduction in muscle function, which was related to increased fatigue and decreased energy levels.

CHAPTER 4:

VITAMIN D STATUS

4.1. Introduction

CD patients are at high risk of developing low bone mineral density (BMD), osteopenia and osteoporosis (285, 313, 321). Low BMD can affect CD patients of all ages, including children and young adults (344, 361), which persists throughout adulthood, negatively affecting daily living and quality of life. In recognition of the problem, the British Society of Gastroenterology (BSG) published guidelines for the prevention of osteoporosis in CD (321).

Low BMD in CD is often related to corticosteroid usage (285, 312) but studies have shown that BMD can be lowered in newly diagnosed steroid-naïve patients (320, 361), indicating that other factors such as malnutrition (286), smoking (302), inflammation itself (303) and physical inactivity (513) are involved. Dietary factors contributing to lower BMD in CD include vitamin D and/or calcium deficiency (257, 293, 328, 391) due to malabsorption, poor dietary intake or, in the case of vitamin D, low exposure to sunlight.

Vitamin D is essential for calcium absorption and maintaining calcium homeostasis (257) and preventing bone disease (335). Deficiency causes decreased intestinal absorption of calcium leading to secondary hyperparathyroidism, which results in calcium mobilisation from the skeleton, thus, reducing BMD and increasing the risk of osteoporosis (335) and fracture (291).

Numerous studies have shown sub-optimal vitamin D status in CD patients (257, 270, 271, 327, 514), although others have reported otherwise (327, 515). In Ireland, the risk of deficiency is increased as the main source of vitamin D, namely sunlight, is in short supply over the

winter months (338) and few foods naturally contain vitamin D. Only one previous study has focused on Irish CD patients, which reported significantly reduced serum vitamin D and increased deficiency rates in CD compared to controls in both winter and summer (270).

The current study aims to determine vitamin D status in males and premenopausal female CD patients, and to evaluate dietary intakes of calcium and vitamin D to assess whether the guidelines recommended by the BSG are being adhered to.

4.1.1. Specific Aims and Objectives

- To determine the prevalence of vitamin D deficiency in non-hospitalised CD patients (males and premenopausal females) attending outpatient clinic and to:
 - compare this patient population to healthy age-, sex- and socio-economically matched controls
 - identify clinical factors associated with low serum vitamin D that best identifies patients with or at risk of deficiency

- To evaluate calcium and vitamin D intakes in non-hospitalised CD patients attending outpatient clinic and to compare this patient population to:
 - healthy age-, sex- and socio-economically matched controls
 - recommended dietary allowances for the Irish population
 - intakes recommended by specific guidelines for the prevention of osteoporosis in CD patients

4.2. Patients and Methods

4.2.1. *Summary of Methods*

Detailed information regarding recruitment and assessment of patients is outlined in Chapter 2: General Materials and Methods. In addition to the exclusion criteria described in Sections 2.1.1.2. and 2.1.2.2., postmenopausal females were also excluded ($n = 11$ CD patients and $n = 13$ control subjects) from the analysis on serum vitamin D deficiency.

In summary:

- CD patients were recruited from the IBD outpatient clinic at AMNCH (See Section 2.1.1.)
- Control subjects were recruited from hospital staff and local businesses (See Section 2.1.2.)
- All subjects were interviewed using CRFs (See Section 2.3.) and provided a fasting blood sample (See Section 2.5.1.)
- Information regarding disease activity (See Section 2.3.1.) and physical activity (See Section 2.3.4.) were obtained at interview using validated questionnaires
- Dietary intakes of calcium and vitamin D were assessed using a validated short self-administered FFQ (See Section 2.3.5.)
- Serum vitamin D was measured using the DiaSorin 25-hydroxyvitamin D ^{125}I radioimmunoassay kit (See Section 2.5.5.)
- Serum osteocalcin was analysed using a commercially available ELISA kit (See Section 2.5.4.)

4.2.2. *Statistics*

Descriptive data was expressed as means $\pm$ standard deviation or median (interquartile range). Differences between patients and controls were assessed using the Student's t-test (normally distributed data),

Mann-Whitney test (data not normally distributed) and Pearson's chi-square test (proportions). Difference between intakes from diet alone versus diet and supplements were assessed using Wilcoxon Signed Rank test. Variables associated with low serum 25(OH)D were assessed using linear regression analysis, with correlation coefficients and 95% confidence intervals reported. All statistical analysis was performed using SPSS Version 12.0, JMP Version 5.1.2 and Stata Version 9.2.

4.3. Results

4.3.1. Baseline Characteristics of Subjects

Serum vitamin D values were available on 71 CD patients and 64 controls, after exclusion of postmenopausal females. There were no significant differences between CD patients and healthy controls in relation to age, gender or socio-economic status. CD patients had predominantly small bowel involvement [70% (50/71)] and median disease duration was 5 years. Median CDAI was 90.1 (39.0-164.5) with 72% (51/71) of patients in remission as defined by a CDAI less than 150. No patient had severe disease (CDAI above 450). Median CRP value was 4.0mg/L (2.9-10.3) with 59% (40/68) of patients below the normal cut-off of 5mg/L. Over forty per cent [43% (31/71)] had received corticosteroid therapy within the previous 12 months and 27% (19/71) had previously undergone surgical resections for their disease (Table 4.1.).

Complete dietary intake information was available on 97 CD patients and 98 controls and these subjects were included in analysis regarding dietary intakes of calcium and vitamin D. As described previously, these groups were also matched for age (See Table 3.1.), gender (See Table 3.1.) and socio-economic status (See Table 2.1.).

4.3.2. Vitamin D Status

4.3.2.1 Serum 25(OH)D Levels

Mean serum 25(OH)D values showed no difference between patients and controls (47.76 ± 27.27 nmol/L *v* 51.86 ± 24.53 nmol/L; NS). On gender analysis, similar results were observed between male patients and male controls (47.07 ± 23.01 nmol/L *v* 49.25 ± 24.02 nmol/L; NS) and female patients and female controls (48.32 ± 30.61 nmol/L *v* 53.89 ± 25.06 nmol/L;

NS), and also between males and females in both the patient ($47.07 \pm 23.01 \text{ nmol/L}$ v $48.32 \pm 30.61 \text{ nmol/L}$; NS) and control ($49.25 \pm 24.02 \text{ nmol/L}$ v $53.89 \pm 25.06 \text{ nmol/L}$; NS) groups.

A sub-analysis of consumers and non-consumers of any calcium and vitamin D containing supplements showed a trend for patients who consumed supplements to have higher serum 25(OH)D levels than patients who did not ($53.90 \pm 28.42 \text{ nmol/L}$ v $42.72 \pm 25.55 \text{ nmol/L}$; $P = 0.08$). No such trend was observed in the control group ($51.56 \pm 14.79 \text{ nmol/L}$ v $51.91 \pm 25.87 \text{ nmol/L}$; NS).

The effect of seasonality could not be assessed in the current study as most CD patients were recruited in the first six months of the year with control recruitment more evenly spread throughout the year, therefore, comparisons could not be made. An effort to assess any seasonal trend was attempted by classifying subjects into two seasons depending on when blood samples were taken; summer (those recruited from May to October) and winter (those recruited from November to April). Mean serum 25(OH)D was significantly higher in summer compared to winter in the CD group ($60.57 \pm 30.83 \text{ nmol/L}$ v $43.08 \pm 24.53 \text{ nmol/L}$; $P = 0.01$), a trend also observed in the control group ($56.29 \pm 24.99 \text{ nmol/L}$ v $45.80 \pm 22.94 \text{ nmol/L}$; $P = 0.09$) (Figure 4.1.).

4.3.2.2. Prevalence of Vitamin D Deficiency

As no international definition of optimal vitamin D status currently exists, two suggested sets of criteria were used to define vitamin D sufficiency and insufficiency in the present study. Criteria by Lips (341) use a serum 25(OH)D value greater than 50 nmol/L to define vitamin D repletion, between $25\text{--}50 \text{ nmol/L}$ to define mild deficiency, $12.5\text{--}25 \text{ nmol/L}$ to define moderate deficiency and $<12.5 \text{ nmol/L}$ to define

severe deficiency. Criteria by Heaney (516) use a higher cut-off value of 80nmol/L to distinguish between vitamin D deficiency and sufficiency.

Lips criteria

According to these criteria, 63% (45/71) of CD patients were vitamin D deficient while 37% (26/71) were vitamin D replete. Of those patients showing deficiency, 46% (33/71) were mildly deficient and 17% (12/71) were moderately deficient. Controls subjects showed similar ($P = 0.226\chi$), although slightly lower, rates of deficiency than patients with 53% (34/64) of controls vitamin D deficient and 47% (30/64) vitamin D replete. Of those showing deficiency, 39% (25/64) were mildly deficient, 11% (7/64) were moderately deficient and 3% (2/64) were severely deficient (Figure 4.2.). Assessing males and females separately showed similar results with 69% (22/32) of CD males, 54% (15/28) of control males, 59% (29/49) of CD females and 50% (21/42) of female controls showing deficiency. The prevalence of deficiency was similar in consumers and non-consumers of supplements in both the patient [59% (19/32) *v* 67% (26/39); NS χ] and control [56% (5/9) *v* 53% (29/55); NS χ] groups. There was a trend for higher rates of deficiency in winter compared to summer for CD patients [69% (36/52) *v* 47% (9/19); $P = 0.09\chi$], whereas, no such trend was observed in the control group [59% (16/27) *v* 49% (18/37); NS χ].

Heaney criteria

According to Heaney criteria, 89% (63/71) of CD patients were vitamin D deficient while only 11% (8/71) were vitamin D replete. Controls subjects showed similarly ($P = 0.825\chi$) high rates of deficiency with 88% (56/64) of controls classed as vitamin D deficient and only 13% (8/64) vitamin D replete (Figure 4.3.). Assessing males and females separately showed similar results with 91% (29/32) of CD males, 93% (26/28) of

control males, 90% (44/49) of CD females and 83% (35/42) of female controls showing deficiency. The prevalence of deficiency was similar in consumers and non-consumers of supplements in patients [84% (27/32) *v* 92% (36/39); NS χ] and controls [100% (9/9) *v* 85% (47/55); NS χ]. There was no difference in prevalence of deficiency in winter compared to summer for CD patients [92% (48/52) *v* 79% (15/19); NS χ] or controls [93% (25/27) *v* 84% (31/37); NS χ].

4.3.2.3. Predictors of Vitamin D Deficiency

Disease variables (CDAI, CRP, disease duration, disease location, corticosteroid usage and previous surgery) and others known to be associated with vitamin D (age, gender, BMI, calcium intake, vitamin D intake and physical activity) were entered into a linear regression model for both the control group and CD group. For controls, lower serum 25(OH)D was associated with winter season only ($P = 0.07$); no significant associations were observed (Table 4.2.). In the patient group, lower serum 25(OH)D was significantly associated with winter season ($P = 0.004$), longer disease duration ($P = 0.01$) and smoking ($P = 0.05$); a trend was also observed for lower dietary vitamin D intake ($P = 0.07$) (Table 4.3.).

4.3.2.4. Effects of Vitamin D Deficiency on Bone Formation

Serum osteocalcin levels were measured in a subgroup of subjects (56 CD patients with inactive disease and 58 age- and sex-matched controls). Mean serum osteocalcin levels were significantly lower in CD patients compared with healthy controls ($6.58 \pm 3.12 \text{ ng/ml}$ *v* $8.94 \pm 3.26 \text{ ng/ml}$; $P < 0.001$). Similar results were observed on gender analysis; $7.23 \pm 3.16 \text{ ng/ml}$ for CD males *v* $10.37 \pm 3.31 \text{ ng/ml}$ for control males ($P = 0.002$) and $6.17 \pm 3.06 \text{ ng/ml}$ for CD females *v* $8.06 \pm 2.94 \text{ ng/ml}$ for control females ($P = 0.010$) (Figure 4.4.). Vitamin D status was not

associated with serum osteocalcin in either the CD group or control group as no differences were observed in mean values between those with deficiency and those without: $6.45 \pm 2.75 \text{ ng/ml}$ v $6.78 \pm 3.64 \text{ ng/ml}$; NS (CD-Lips criteria), $6.55 \pm 3.02 \text{ ng/ml}$ v $6.81 \pm 3.88 \text{ ng/ml}$; NS (CD-Heaney), $8.54 \pm 2.78 \text{ ng/ml}$ v $9.37 \pm 3.71 \text{ ng/ml}$; NS (control-Lips), $8.95 \pm 2.99 \text{ ng/ml}$ v $8.89 \pm 5.15 \text{ ng/ml}$; NS (control-Heaney). Univariate analysis showed no association between dietary intakes of vitamin D or calcium and serum osteocalcin levels in either patients ($P = 0.988$, $P = 0.297$) or controls ($P = 0.852$, $P = 0.917$).

4.3.3. Calcium and Vitamin D Intakes in CD Patients and Control Subjects

4.3.3.1. Calcium intakes

Median calcium intakes from diet alone [904.98 mg/day ($653.15\text{-}1133.09$) v 966.85 mg/day ($703.54\text{-}1364.02$); NS] and total intake (when supplements added) [1011.79 mg/day ($778.42\text{-}1280.16$) v 1022.76 mg/day ($713.52\text{-}1394.63$); NS] showed similar results in the CD and control groups (Table 4.4.). Gender analysis showed no difference between female patients and female controls, or male patients and male controls. In CD patients, females had significantly lower intakes of calcium from diet alone [856.06 mg/day ($618.05\text{-}1099.97$) v 1014.88 mg/day ($784.06\text{-}1314.88$); $P = 0.03$], but similar total intake (when supplements added), when compared with CD males. No differences were observed between control males and females. In both CD patients and controls, calcium intake significantly increased when intake from supplements was included (Table 4.4.).

4.3.3.2. Vitamin D Intakes

Median vitamin D intakes from diet alone were significantly lower in CD patients compared with controls [1.0µg/day (0.6-1.9) *v* 1.6µg/day (1.0-2.5); $P < 0.001$], although total intakes when dietary supplements were included were similar between groups [2.1µg/day (0.8-6.0) *v* 1.8µg/day (1.1-3.4); NS] (Table 4.5.). Vitamin D intakes from diet alone were significantly lower in female CD patients compared with female controls (0.9µg/day (0.5-1.5) *v* 1.6µg/day (1.0-2.7); $P < 0.001$). No differences were observed between CD males and control males. There were no differences between CD females and CD males regarding intakes of vitamin D from either diet alone or total intake (when supplements added), which was also observed for control females and control males. In both CD patients and controls, vitamin D intakes significantly increased when intake from supplements was included (Table 4.5.).

4.3.3.3. Calcium Guidelines

The Recommended Dietary Allowance (RDA) for calcium for Irish adults over the age of 18 years is 800mg/day (446). There were no differences between CD patients and controls as the majority of both groups [62% (60/97) *v* 64% (63/98); NS χ] reached this target from diet alone, and these figures increased when supplemental intake was included [72% (70/97) *v* 67% (66/98); NS χ]. The mean daily intake of calcium (diet and supplements) in Irish adults as reported in The North/South Ireland Food Consumption Survey (517) was 949mg for males and 742mg for females. Again, the total intake (diet and supplements) of the majority of both patients and controls met these figures [72% (70/97) *v* 64% (63/98); NS χ].

4.3.3.4. Vitamin D Guidelines

There is currently no specific RDA for vitamin D for Irish adults over the age of 18 years. In 1999, it was recommended (446) that the EU guideline of 0-10µg/day of vitamin D (518) should be adopted. Taking 5µg/day as an average target intake, no differences were observed between CD patients and controls as very few [0% (0/97) *v* 3% (3/98); NS χ] reached this target from diet alone. These figures increased when supplemental intake was included, with significantly more CD patients than controls meeting the target [38% (37/97) *v* 17% (17/98); $P = 0.001\chi$]. The mean daily intake of vitamin D (diet and supplements) as reported in The North/South Ireland Food Consumption Survey (339) was 3.7µg for both males and females. Significantly more CD patients met this figure compared with controls [39% (38/97) *v* 21% (21/98); $P = 0.007\chi$].

4.3.3.5. BSG Guidelines for CD Patients

In 2000, the BSG published specific guidelines for CD patients for the prevention of osteoporosis (321). It is recommended that all CD patients should consume 1500mg of calcium daily, from diet or by including supplements if required. Worryingly, even when including intake from supplements, only 22% (21/97) of patients met this target, which was similar to the control group [18% (18/98); NS χ]. The BSG does not have general dietary intake guidelines for vitamin D but recommend an intake of 20µg/day for any patient receiving corticosteroids. While forty two percent of patients (41/97) received corticosteroids within the previous year, none of them met this target intake of 20µg of vitamin D.

Splitting patients into consumers and non-consumers of calcium and vitamin D containing supplements showed that significantly more supplement consumers met the target for calcium compared with non-

consumers [41% (15/37) *v* 10% (6/60); $P < 0.001\chi$], while no patients reached the target for vitamin D (Figure 4.6.).

4.3.3.6. Sources of Calcium and Vitamin D

The use of calcium and vitamin D containing supplements was significantly higher in the CD group compared with controls [38% (37/97) *v* 16% (16/98); $P = 0.004\chi$]. In CD patients, multivitamin preparations (approximately 162mg calcium and 5 μ g vitamin D) were the most common supplement consumed [22% (21/97)], while only 11% (11/97) consumed a specific calcium/vitamin D supplement (approximately 500mg calcium and 5 μ g vitamin D); 5% (5/97) of patients consumed both a multivitamin and calcium/vitamin D specific supplement.

Of those patients and controls consuming supplements, supplements contributed 24% and 20% to total intake of calcium and 64% and 32% to total vitamin D intake. Dietary sources of calcium and vitamin D showed no differences between patients and controls. Milk and dairy products were the main food source of calcium in both patients and controls (70% *v* 71%; NS χ), with bread the second single most important food source in both groups (9% *v* 7%; NS χ). For vitamin D, fish were the main food source in both patients and controls (38% *v* 48%; NS χ), while the single most important food source of vitamin D in CD patients was eggs (27%) and in controls was cereals (26%). There was no difference between males and females regarding dietary sources of either calcium or vitamin D.

Table 4.1. Baseline characteristics of CD patients (*n* = 71) and control subjects (*n* = 64) with serum 25(OH)D values

	CD (<i>n</i> = 71)	Controls (<i>n</i> = 64)	<i>P</i> value
Age, yrs (mean±SD)	34.0±9.4	34.8±8.6	<i>P</i> = 0.588*
Gender (% male)	45 (32/71)	44 (28/64)	<i>P</i> = 0.877**
BMI, kg/m ² (mean±SD)	24.9±5.6	25.4±3.9	<i>P</i> = 0.533*
Smoking (%)			<i>P</i> = 0.067**
Current smoker	30 (21/71)	17 (11/64)	
Ex-smoker	21 (15/71)	38 (24/64)	
Non-smoker	49 (35/71)	45 (29/64)	
Disease location (%)			
Small bowel	48 (34/71)	-	-
Large bowel	27 (19/71)	-	-
Small and large bowel	23 (16/71)	-	-
Upper GI	3 (2/71)	-	-
CD duration, yrs [median (range)]	5 (3 –12)	-	-
CDAI [median (range)]	90.1 (39.0-164.5)	-	-
CRP, mg/L [median (range)]	4.0 (2.9-10.3)	-	-
CDAI <150 (%)	72 (51/71)	-	-
CRP < 5mg/mL (%)	59 (40/68)	-	-
Current medications:			
Immunosuppressants (%)	44 (31/71)	-	-
5ASAs (%)	79 (56/71)	-	-
Infliximab (%)	15 (11/71)	-	-
Steroids within last year (%)	43 (31/71)	-	-
Oral Contraceptive Pill (%)	13 (9/71)	13 (8/64)	<i>P</i> = 0.984**
Previous surgical resections (%)	27 (19/71)	-	-

No significant differences with * Students t-test or ** Pearson’s Chi-square test

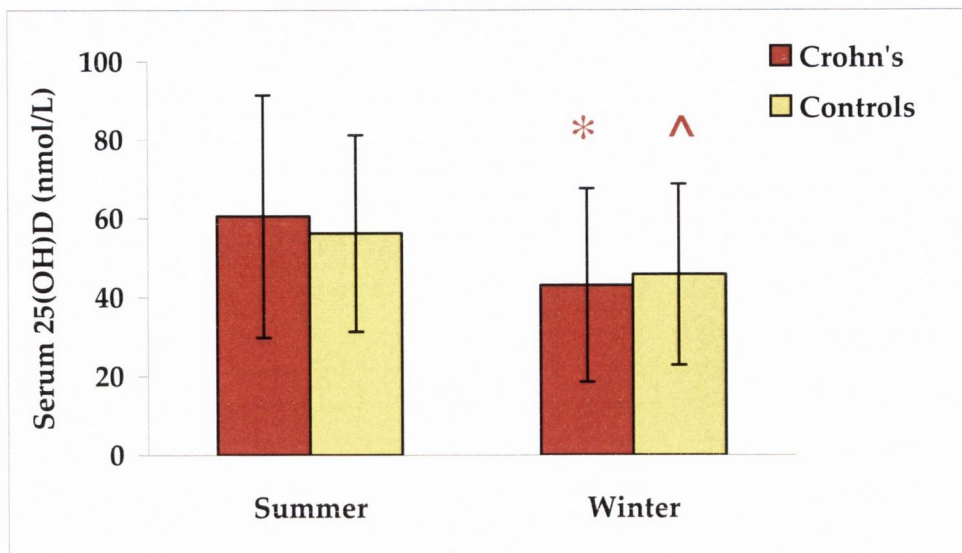


Figure 4.1. Serum 25(OH)D levels in CD patients ($n = 71$) and control subjects ($n = 64$) according to season

Summer: May to October inclusive

Winter: November to April inclusive

* $P = 0.01$ compared to mean summer value for CD, using Students t-test

^ $P = 0.09$ compared to mean summer value for controls, using Students t-test

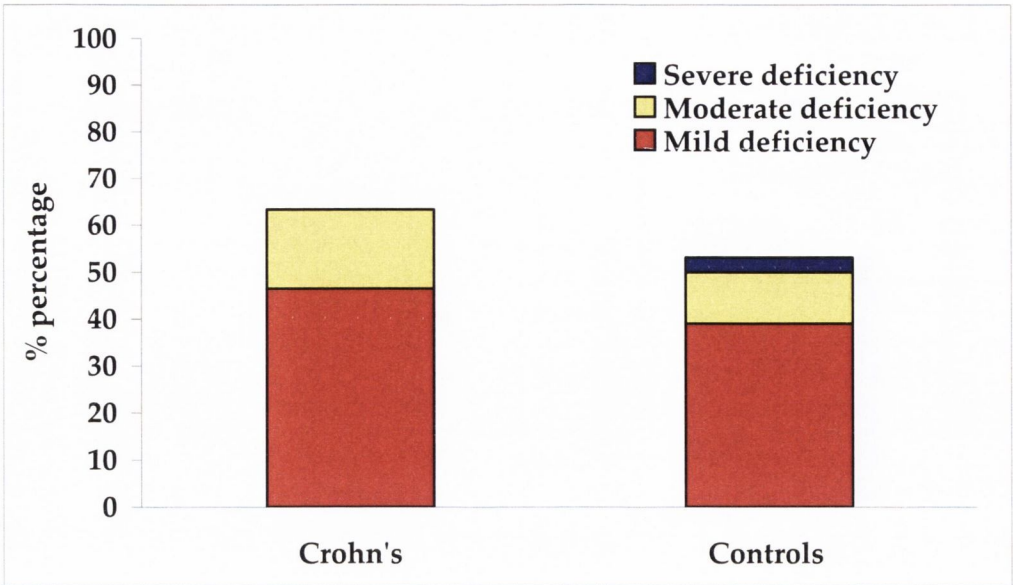


Figure 4.2. Prevalence of vitamin D deficiency in CD patients (*n* = 71) and control subjects (*n* = 64) according to Lips criteria (341)

Results expressed as percentage of subjects in each group that are deficient

Replete: >50nmol/L

Mild deficiency: 25-50nmol/L

Moderate deficiency: 12.5-25nmol/L

Severe deficiency: <12.5nmol/L

No significant differences between groups, using Pearson's chi-square test ($P = 0.226\chi$)

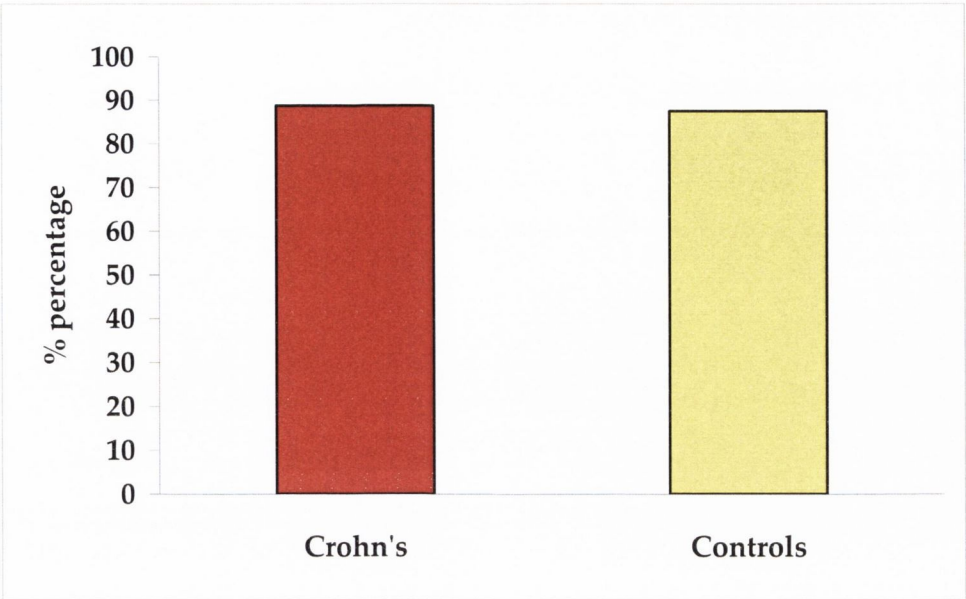


Figure 4.3. Prevalence of vitamin D deficiency in CD patients ($n = 71$) and control subjects ($n = 64$) according to Heaney criteria (516)

Results expressed as percentage of subjects in each group that are deficient

Replete: $>80\text{nmol/L}$

Deficient: $<80\text{nmol/L}$

No significant difference between groups, using Pearson's chi-square test ($P = 0.825\chi$)

Table 4.2. Linear regression model of factors associated with low serum 25(OH)D in control subjects

	<i>R</i> ² (95% CI)	<i>P</i> value
Age (yrs)	0.13 (-0.59; 0.86)	0.71
Gender (female)	7.85 (-4.96; 20.68)	0.22
Vitamin D intake (µg)	-0.36 (-1.68; 1.60)	0.96
Seasonality (winter)	-11.84 (-24.79; 1.09)	0.07 *

Results presented as correlation coefficient (*R*²) and 95% confidence interval (CI), *P* < 0.05 considered significant

Other variables included in analysis but not significant and removed were: BMI, calcium intake, physical activity

* Lower serum 25(OH)D associated with winter season

Table 4.3. Linear regression model of factors associated with low serum 25(OH)D in CD patients

	<i>R</i> ² (95% CI)	<i>P</i> value
Age (years)	-0.32 (-0.95; 0.31)	0.31
Gender (female)	1.06 (-11.10; 13.23)	0.86
Seasonality (winter)	-21.06 (-35.18; -6.95)	0.004 *
Disease duration (yrs)	-1.19 (-2.18; -0.19)	0.01 **
Smoking	13.09 (-0.14; 26.33)	0.05 ***
Vitamin D intake (µg)	1.09 (-0.13; 2.32)	0.07 ****

Results presented as correlation coefficient (*R*²) and 95% confidence interval (CI), *P* < 0.05 considered significant

Other variables included in analysis but not significant and removed were: CDAI, CRP, disease location, previous surgical resections, corticosteroid usage over previous year, BMI, calcium intake, physical activity

* Lower serum 25(OH)D associated with winter season

** Lower serum 25(OH)D associated with longer disease duration

*** Lower serum 25(OH)D associated with smoking

**** Lower serum 25(OH)D associated with lower vitamin D intake

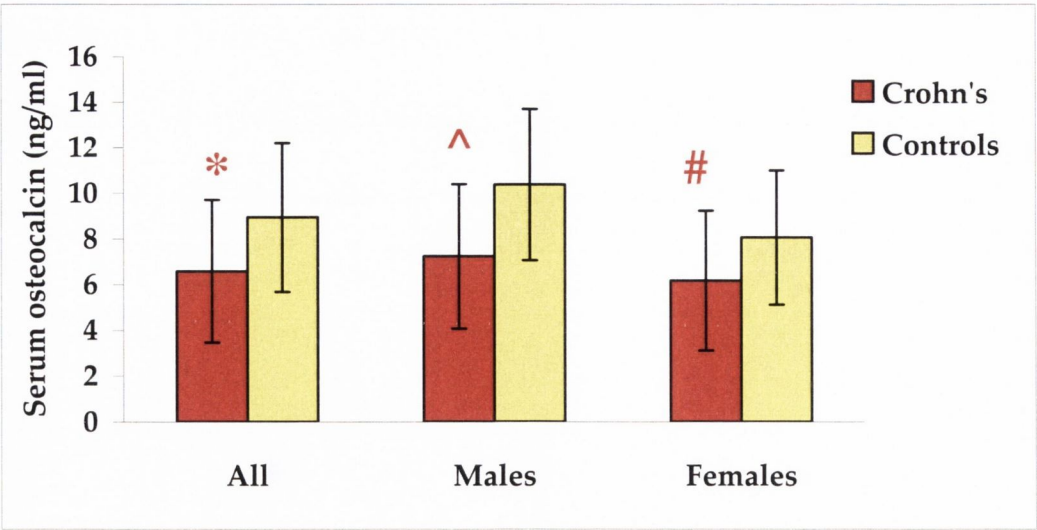


Figure 4.4. Serum osteocalcin concentrations in CD patients (n = 56) and control subjects (n = 58)

- * $P < 0.001$ compared to controls, using Students t-test
- ^ $P = 0.002$ compared to male controls, using Students t-test
- # $P = 0.010$ compared to female controls, using Students t-test

Table 4.4. Calcium intakes (mg), diet alone and total intake (with supplements), in CD patients ($n = 97$) and control subjects ($n = 98$)

	Calcium (mg)	
<i>Total group</i>	CD ($n = 97$) *	Controls ($n = 98$) *
Diet only	904.98 (653.15-1133.09)	966.85 (703.54-1364.02)
Total Intake	1011.79 (778.42-1280.16)	1022.76 (713.52-1394.63)
<i>Females</i>	CD ($n = 59$) *	Controls ($n = 59$) ***
Diet only	856.06 (618.05-1099.97) ^a	930.23 (633.33-1327.96)
Total Intake	971.16 (651.08-1255.48)	1034.83 (641.72-1391.04)
<i>Males</i>	CD ($n = 38$) *	Controls ($n = 39$) **
Diet only	1014.88 (784.06-1314.88)	972.68 (759.81-1380.62)
Total Intake	1058.41 (811.89-1620.70)	972.68 (759.81-1410.86)

Results presented as median (interquartile range)

^a $P = 0.03$; Calcium intake from diet only significantly lower in female CD patients compared with male CD patients, using Mann-Whitney test

* $P < 0.001$, ** $P = 0.034$, *** $P = 0.003$; Calcium intake from diet only was significantly lower than total intake (when supplements added), using Wilcoxon Signed Rank Test

Table 4.5. Vitamin D intakes (µg), diet alone and total intake (with supplements), in CD patients (n = 97) and control subjects (n = 98)

	Vitamin D (µg)	
<i>Total group</i>	CD (n = 97) *	Controls (n = 98) *
Diet only	1.0 (0.6-1.9) a	1.6 (1.0-2.5)
Total Intake	2.1 (0.8-6.0)	1.8 (1.1-3.4)
<i>Females</i>	CD (n = 59) *	Controls (n = 59) ***
Diet only	0.9 (0.5-1.5) b	1.6 (1.0-2.7)
Total Intake	1.5 (0.7-5.6)	2.0 (1.1-3.8)
<i>Males</i>	CD (n = 38) *	Controls (n = 39) **
Diet only	1.4 (0.7-2.0)	1.5 (1.0-2.4)
Total Intake	2.2 (1.3-6.4)	1.7 (1.1-3.0)

Results presented as median (interquartile range)

a $P < 0.001$; vitamin D intake from diet only significantly lower in CD patients compared with controls, using Mann-Whitney test

b $P < 0.001$; vitamin D intake from diet only significantly lower in female CD patients compared with female controls, using Mann-Whitney test

* $P < 0.001$, ** $P = 0.034$, *** $P = 0.002$; vitamin D intake from diet only was significantly lower than total intake (when supplements added), using Wilcoxon Signed Rank Test

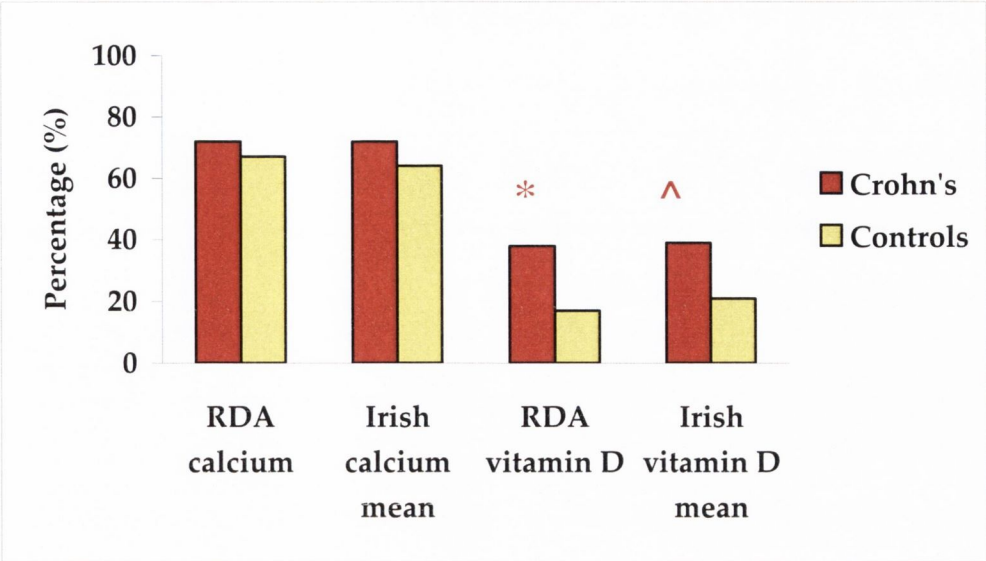


Figure 4.5. CD patients (*n* = 97) and control subjects (*n* = 98) meeting guidelines for calcium and vitamin D from total intakes (supplements added)

Results presented as percentage of each group meeting each guideline

RDA Calcium: 800mg/day (446)

Irish Calcium mean (from North/South Ireland Food Consumption Survey (517): 942mg/day for males, 747mg/day for females

RDA vitamin D: 0-10µg/day, 5µg/day used as average (446)

Irish vitamin D mean (from North/South Ireland Food Composition Survey (339): 3.7µg/day

* *P* = 0.001; significantly more CD patients meeting RDA for vitamin D compared with controls, using Pearson's chi-square test

^ *P* = 0.007; significantly more CD patients meeting Irish vitamin D mean compared with controls, using Pearson's chi-square test

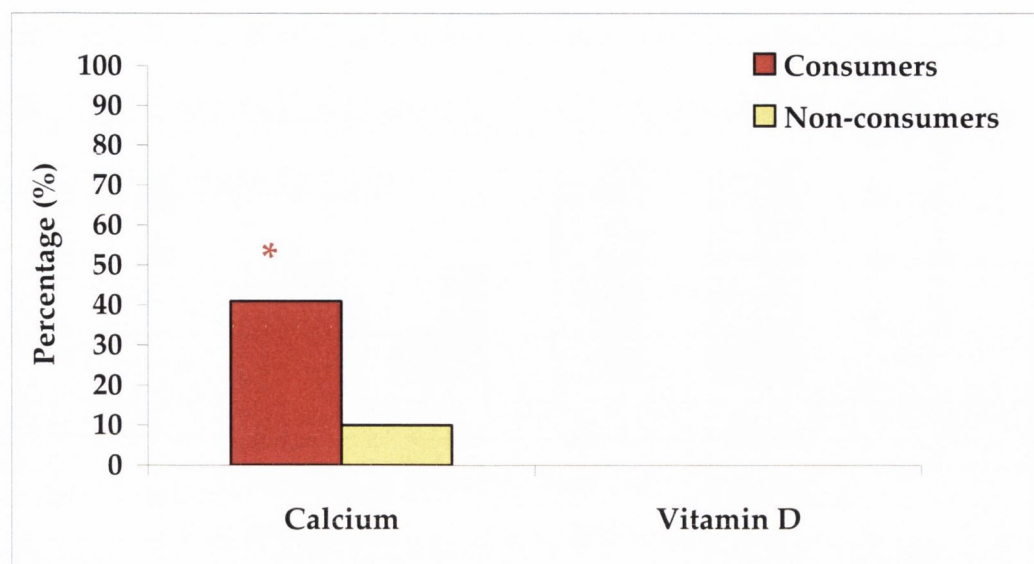


Figure 4.6. CD patients, split into consumers ($n = 37$) and non-consumers ($n = 60$) of supplements, meeting BSG guidelines (321) for prevention of osteoporosis

Results presented as percentage of each group meeting guideline

Calcium BSG Guideline: 1500mg/day for all CD patients

Vitamin D BSG Guideline: 20 μ g/day for any CD patient receiving corticosteroid therapy

(Supplements: any preparation containing calcium and vitamin D)

* $P < 0.001$ compared to non-consumers for calcium, using Pearson's chi-square test

No patients met guideline amount for vitamin D

4.4. Discussion

This study reports a high prevalence of vitamin D deficiency in CD patients attending outpatient clinic, similar to that of matched controls, suggesting that deficiency is a common problem. CD patients with long standing disease, who smoked and had lower dietary intakes of vitamin D were most at risk of deficiency. CD patients had significantly lower serum osteocalcin levels compared with control subjects, which did not appear to be associated with vitamin D status or intake. Calcium and vitamin D intakes were similar in CD patients and controls but intakes in CD patients were well below those recommended for the prevention of osteoporosis in CD.

Vitamin D deficiency is a common phenomenon in CD, in both adults and children (257, 270, 271, 326, 327, 514). The present study observed deficiency rates of 63%-89% for CD patients (similar to controls, 53%-88%), depending on the cut-off point used to define deficiency. To our knowledge, only one other study has focused on Irish CD patients (270). Our results are in disagreement with these findings, which observed significantly lower serum 25(OH)D and significantly higher deficiency rates in CD patients compared with controls in both winter and summer. The serum levels reported by McCarthy et al (270), particularly for control subjects (80 nmol/L in winter and 105nmol/L in summer), appear quite high when compared to our values (52nmol/L) and reported values from Northern Ireland (55.5nmol/L) (519) and Europe (36.1nmol/L, 46nmol/L and 52.5nmol/L) (336, 520, 521). While the circulating concentration of 25(OH)D is the accepted method of vitamin D assessment (522), the lack of standardisation in the analytical methods used to measure 25(OH)D (341) is a factor which could lead to variations in results. Also, the inclusion of postmenopausal females in

the McCarthy (270) study group could have influenced the results. In the current study, only pre-menopausal females and male subjects were included to avoid any effects of menopausal status.

Definition of vitamin D deficiency is currently an area of immense debate (335, 341, 516, 523) with no clear designation of which level of 25(OH)D distinguishes deficiency from sufficiency. A recent review (342) examining the optimal serum 25(OH)D concentration needed for reduction in fracture risk, lower-extremity function, dental health and colorectal cancer concluded that the target should be set at 75nmol/L, with the ideal concentrations set at 90-100nmol/L. Using this recommendation, the criteria by Lips (341) used in the present study significantly underestimate the prevalence of deficiency in both CD and controls, with the criteria set by Heaney (516), which estimated ~90% deficiency in both groups, more appropriate. Apart from the controversy surrounding the definition of vitamin D deficiency, other factors also make the comparison of studies very difficult. Geographical variation exists in serum 25(OH)D levels with higher levels typically found in Asia, Australia and the US compared to Europe (524). In Europe, a north-south gradient also exists, with higher levels in Scandinavia compared to Central Europe (525), which is likely due to higher intakes of vitamin D-containing foods and supplements (526).

Serum osteocalcin, a sensitive and specific marker of bone formation (527), was assessed in a subgroup of controls and CD patients with inactive disease. CD patients displayed significantly lower levels when compared with control subjects, which is in keeping with previous reports (271, 313, 528), but not all (256, 270, 327). Serum osteocalcin levels were not related to vitamin D status or to intakes of vitamin D or calcium, observations previously reported (257, 271, 529). The finding of

lower serum osteocalcin levels provides evidence of reduced bone formation, and therefore, an altered bone turnover in CD, even in inactive disease. It has been suggested that levels of bone turnover can predict future fractures as well as BMD in conditions other than inflammatory disease (530).

Seasonality is a well-known factor in determining vitamin D status (270, 514), as the main source of vitamin D is through cutaneous synthesis on exposure to sunlight (335). The focus of the current study was not that of seasonality, therefore, groups were not matched for season, and accurate comparisons between and within groups was not possible. Despite this, however, a trend for seasonal variation, with lower levels in winter compared to summer, was observed in both patients and controls. Winter season was also negatively associated with serum vitamin D in both the patient and control groups on regression analysis, which has previously been reported (270, 531, 532).

Regression analysis also identified smoking, longer disease duration and low intakes of vitamin D to be negatively associated with vitamin D status in CD patients. Smoking has previously been linked to lower vitamin D levels in healthy adults (531) and CD patients (271, 514). It has been estimated that there is an approximate 10% difference in serum vitamin D values between smokers and non-smokers (533). One reason as to why this may be is inadequate dietary intake (534, 535). A population study of 2319 women observed strikingly lower daily intakes of vitamin D (and calcium) in current smokers compared to women who had never smoked, with over 200mg difference between the groups (535). Also, in CD patients, as smoking is related to a more severe disease course with more flare-ups (192), ongoing inflammation and disease treatments may also contribute to lower vitamin D status.

Long standing disease is also known to increase risk of vitamin D deficiency in CD patients (206, 327), as patients are more likely to have had more flare-ups resulting in periods of malabsorption (306), and possible bowel resections (300). While vitamin D intake was not significantly associated with serum vitamin levels, a trend was observed. It has previously been shown that only vitamin D intakes above approximately 4µg/day can significantly predict serum 25(OH)D (536). The median vitamin D intake in the present study was only 2.1µg/day, which may explain the lack of significance in the relationship between vitamin D intake and serum 25(OH)D.

Calcium intakes from food alone, and total intakes were similar in both CD patients and controls, an observation which is in keeping with previous studies, including those based on Irish subjects (256, 270, 529). Comparing intakes in the present study to those reported in the North/South Ireland Food Consumption Survey (517), which included 1379 Irish adults, showed that approximately 70% of both CD patients and controls met the reported mean intake; a similar percentage met the RDA of 800mg for Irish adults (446). Low calcium intakes are associated with increased metabolic clearance of 25(OH)D leading to deficiency, suggesting that high calcium intakes have a sparing effect on the vitamin (537). But on the other hand, the absorption of calcium is dependent on vitamin D status (332), as without vitamin D only 10-15% of dietary calcium is absorbed by the small intestine, compared to 30% in the sufficient state (335), therefore, individuals cannot benefit fully from their calcium intake (even when it is adequate) unless vitamin D status is also adequate.

As mentioned, sunlight is the major source of vitamin D with the remainder acquired from the diet. However, in northern latitudes of 40-

60°N, including Ireland at 51-55°N, sunlight is not strong enough to stimulate synthesis of the vitamin during the winter months (338), therefore, dietary intake is crucial if vitamin D status is to be maintained. Unfortunately, only a limited number of foods such as fish, egg yolk and meat products, naturally contain vitamin D and few foods in Ireland are fortified with vitamin D. In the present study, no differences were observed in total intake between the patient and control groups (2.1µg/day and 1.8µg/day), which is in agreement with previous reports (256, 515, 520, 529). But, assessing intakes from diet alone (i.e. without inclusion of supplements) showed CD patients to have significantly lower intakes compared with controls, highlighting the importance of supplemental intake in these patients. As no specific RDA exists for vitamin D (446), 5µg/day was chosen as an average target intake. No patients and only 3% of controls met this target from dietary intake alone, and while supplemental intake increased these figures, the majority of both groups were still unable to meet the target. The North/South Ireland Food Consumption Survey (339) reported mean daily intakes (including supplements) of 3.7µg of vitamin D, which is below the average RDA target, highlighting the difficulties in meeting the recommended intake for vitamin D.

The observation that the inclusion of intakes from supplements significantly increased calcium and vitamin D intakes in both patients and controls has previously been observed (270, 340). Supplements contributed approximately 25% to calcium intakes and over 60% to vitamin D intakes in the 38% of patients who were consumers of supplements in the present study. Supplements not only increase dietary intakes but also offer the potential to increase serum vitamin D status (306, 519) and to prevent seasonal bone loss (538). Supplement use by CD patients in the current study was significantly higher when

compared with controls and to usage in the North/South Ireland Food Consumption Survey (539), was similar to other Irish CD patients (270) but lower than Danish patients (257) where use of supplements is high (524). The use of supplements must be substantially increased among CD patients, particularly in winter months but ideally all year round, if the guidelines set by the BSG (321) in relation to prevention of osteoporosis are to be adhered to. The target of 1500mg/day of calcium for all CD patients was met by only 22% of patients while the target of 20µg for vitamin D was not reached by any patient. The use of supplements significantly increased the likelihood of meeting the target for calcium (41% versus 10%) and supplements are certainly required to increase intakes of vitamin D to 20µg/day. This is in agreement with other Irish research, in which only 5% of CD patients met this target intake, and only through the use of supplements (270).

The optimal amount of oral vitamin D necessary to obtain adequate serum vitamin D levels remains to be defined as the usual dietary intake of vitamin D by many populations (524), including healthy Irish adults, as described in the current study, and others (540), is not sufficient to maintain adequate vitamin D status. In the US, a safe tolerable upper intake of 250µg/day (10000IU) has been supported (541), while daily doses of 10µg/day (400IU) have been deemed ineffective (524). doses of 100µg/day (4000IU) increased serum vitamin D to 96nmol/L without any significant increases in either serum or urinary calcium levels (542), although long-term effects of such doses have not yet been properly assessed (543). Others have, therefore, criticised this regime (544), as much lower doses of 20µg/day (800IU) have been shown to correspond to similar increases in serum levels (543), although this increase also appears dependent on an adequate dietary intake (331). From such evidence, it has been estimated that a

daily intake of 20–25µg/day (800-1000IU) should be sufficient to maintain serum levels of approximately 70–100 nmol/L in adult Caucasian populations (342). Whether this is appropriate for CD patients remains to be seen and further studies are needed in this subgroup of individuals.

In the meantime, CD patients receiving corticosteroids must be prescribed a specific calcium and vitamin D containing preparation, as multivitamin preparations do not contain these nutrients in high enough doses. Two tablets containing 10µg (400IU) vitamin D (and 500mg calcium) daily should be prescribed (for example; Calchichew D3 Forte, Ideos). A study assessing the protocols being followed regarding steroid-induced osteoporosis by Irish clinicians in daily practice observed that 25% of gastroenterologists did not prescribe vitamin D/calcium concomitantly with corticosteroids and that serum 25(OH)D was not assessed in any patient prior to commencement of steroid therapy (545). These practices will have to change if the lofty targets set by the BSG are to be met. Serum 25(OH)D should be monitored regularly. It may be worth considering whether the routine use of vitamin D supplements should be a general recommendation for all CD patients rather than only for those patients undergoing steroid treatment. As shown in the present study, serum 25(OH)D was low in the majority of patients, whether receiving corticosteroids or not, highlighting the fact that all patients are at risk of deficiency.

In conclusion, the majority of pre-menopausal females and males with CD are vitamin D deficient, which is associated with longer disease duration, lower vitamin D intake and smoking status. While intakes of calcium and vitamin D are similar to control values, they are well below

those recommended for CD patients and, therefore, the increased use of supplements is required in all patients if these targets are to be reached.

CHAPTER 5:

LIFESTYLE FACTORS

5.1. Introduction

The increase in non-communicable diseases such as diabetes, heart disease and cancer, together with the current obesity epidemic in Ireland (546), has focused attention on the importance of diet and physical activity.

Participation in physical activity has not been widely studied in CD patients, with the little research available focusing on safety aspects and outcomes of exercise in experimental conditions (547-549). Physical activity not only promotes weight loss but maintains muscle mass (550) and bone mass (513). It is possible that physical activity may be reduced in CD patients due to a variety of reasons including symptoms such as pain, fatigue and diarrhoea, while repeated relapses may interfere with patients establishing any regular pattern of exercise; routine participation in this group has not been studied to date.

Psychological stress has been implicated in disease relapse in CD (551, 552), particularly by patients (553), but not all are in agreement with this suggestion (554-555).

Smoking is a known risk factor for the development and exacerbation of CD (556), with smokers experiencing a more severe disease course with higher relapse rates (176). In addition, CD patients who smoke develop more complications (175), undergo more surgical procedures (557) and experience reduced quality of life (558).

Little is known regarding alcohol intake in CD patients, and the little research to date has yielded conflicting information with some

reporting higher intakes compared to controls (206) and others observing lower intakes (243).

The present study aims to assess common lifestyle factors, namely physical activity, alcohol and smoking trends and depression and anxiety in CD patients with inactive or mildly active disease in comparison to healthy control subjects.

5.1.1. Specific Aims and Objectives

- To assess the following non-dietary lifestyle factors
 - Physical activity patterns
 - Alcohol consumption
 - Smoking
 - Depression and anxiety

in non-hospitalised CD patients attending outpatient clinic
compared to age- and sex-matched healthy controls

5.2. Patients and Methods

5.2.1. *Summary of Methods*

Detailed information regarding recruitment and assessment of patients is outlined in Chapter 2: General Materials and Methods.

In summary:

- CD patients were recruited from the IBD outpatient clinic at AMNCH (See Section 2.1.1.)
- Control subjects were recruited from hospital staff and local businesses (See Section 2.1.2.)
- Information on smoking and alcohol intake were collected during interview using CRFs (See Section 2.3.)
- Information regarding disease activity (See Section 2.3.1.), physical activity (See Section 2.3.4.) and depression and anxiety (See Section 2.3.3.) were obtained at interview using validated questionnaires

5.2.2. *Statistics*

Descriptive data was expressed as means $\pm$ standard deviation or median (interquartile range). Differences between patients and controls were assessed using the Student's t-test (normally distributed data), Mann-Whitney test (data not normally distributed) and Pearson's chi-square test (proportions). All statistical analysis was performed using SPSS Version 12.0, JMP Version 5.1.2 and Stata Version 9.2.

5.3. Results

5.3.1. Baseline Characteristics of Subjects

There were no significant differences between CD patients and healthy controls in relation to age, gender or socio-economic status (See Table 2.1.). CD patients had predominantly small bowel involvement [70% (70/100)] and median disease duration was 6.5 years. Median CDAI was 91.10 (38.26 – 173.86) with 67% (67/100) of patients in remission as defined by a CDAI less than 150. No patient had severe disease (CDAI above 450). Median CRP value was 3.7mg/L (2.9-8.0) with 61% (61/100) of patients below the normal cut-off of 5mg/L. Over forty per cent of patients [41% (41/100)] had received corticosteroid therapy within the previous 12 months, 15% (15/100) had received infliximab therapy and 29% (29/100) had previously undergone surgical resection for their disease (Table 3.1.).

5.3.2. Physical Activity Patterns

Complete physical activity information was available for 66 CD patients and 68 age- and sex-matched healthy controls. In overall terms, energy expenditure was significantly lower in CD patients when compared with controls [66.70 METs (19.20-120.00) *v* 94.45 METs (70.85-134.97); $P = 0.002$] (Figure 5.1.). Household activity was similar between CD patients and control subjects [37.95 METs (19.75-78.90) *v* 37.95 METs (24.60-48.60); NS]. Time spent in work (occupational) activity was significantly less in CD patients compared with controls (26.03±17.99 hours *v* 38.02±10.71 hours; $P < 0.001$). Energy expenditure from work activity was significantly lower in CD patients compared with control subjects [60.00 METs (0-89.35) *v* 76.50 METs (59.17-100.15); $P = 0.003$] (Figure 5.2.). Separate analysis was carried out on only those subjects currently in employment, which resulted in no difference in overall

energy expenditure from physical activity [125.95 METs (88.85-166.50) *v* 136.40 METs (108.85-1185.90); $P = 0.08$] (Figure 5.3.).

5.3.2.1. Gender Differences

Male subjects displayed higher overall energy expenditure than female subjects in both the CD group [109.00 METs (67.20-158.30) *v* 51.70 METs (11.70-79.20); $P < 0.001$] and control group [117.45 METs (85.10-196.62) *v* 84.85 METs (56.00-117.42); $P = 0.003$]. Household activity was higher in female subjects compared with male subjects in both the CD [67.40 METs (33.40-98.20) *v* 20.30 METs (8.80-37.70); $P < 0.001$] and control [43.60 METs (32.40-58.95) *v* 23.50 METs (13.82-32.97); $P < 0.001$] groups. Work activity was significantly higher in male subjects compared with female subjects in both the CD [75.20 METs (60.00-124.10) *v* 41.50 METs (0-73.70); $P = 0.001$] and control group [96.70 METs (74.32-132.55) *v* 64.20 METs (54.00-91.95); $P < 0.001$] as were hours spent in work activity (CD: 36.22 ± 14.77 hours *v* 18.98 ± 16.72 hours; $P < 0.001$, Controls: 43.01 ± 8.62 hours *v* 34.92 ± 10.80 hours; $P = 0.002$).

Overall energy expenditure was similar in male CD patients and in male controls [109.00 METs (67.20-158.30) *v* 117.45 METs (85.10-196.62); NS]. Household activity was similar in male CD patients and male controls [20.30 METs (8.80-37.70) *v* 23.50 METs (13.82-32.97); NS]. Although the number of hours spent in work activity was significantly lower in CD males (36.22 ± 14.77 hours *v* 43.01 ± 8.62 hours; $P = 0.04$), energy expenditure from work activity was not different between male CD patients and male controls [75.20 METs (60.00-124.10) *v* 96.70 METs (74.32-132.55); NS].

Overall energy expenditure was significantly lower in female CD patients compared with female controls [51.70 METs (11.70-79.20) *v*

84.85 METs (56.00-117.42); $P = 0.001$]. Female CD patients expended significantly more energy in household activity compared with female controls [67.40 METs (33.40-98.20) *v* 43.60 METs (32.40-58.95); $P = 0.04$]. Female CD patients had significantly lower work activity [41.50 METs (0-73.70) *v* 64.20 METs (54.00-91.95); $P = 0.001$] and spent less time working (18.98 ± 16.72 hours *v* 34.92 ± 10.80 hours; $P < 0.001$) compared with female controls.

5.3.2.2. Age Differences

Overall energy expenditure was lower in CD patients in all age groups when compared to control subjects, and was significantly so in 30-39 year olds [80.20 METs (62.85-149.70) *v* 131.90 METs (91.85-202.35); $P = 0.04$] and 40-49 year olds [39.60 METs (6.00-68.97) *v* 93.60 METs (60.65-116.97); $P = 0.003$] (Figure 5.1.). Home activity was significantly higher in CD patients aged 40-49 years compared to controls of the same age [74.20 METs (34.65-119.32) *v* 40.75 METs (29.07-51.72); $P = 0.03$] but was similar in other age groups. Work activity was significantly lower in CD patients aged 40-49 years compared to controls of the same age [32.10 METs (0-68.35) *v* 66.70 METs (49.82-91.12); $P = 0.02$] but similar in other age groups.

5.3.2.3. Recreational Activity

Leisure activity showed no difference between CD patients and controls [8.00 METs (0-19.67) *v* 13.50 METs (0-31.42); NS] (Figure 5.2.) or when comparing male CD patients and male controls [10.40 METs (0-46.00) *v* 11.85 METs (0-37.60); NS], and female CD patients and female controls [7.40 METs (0-17.10) *v* 13.65 METs (0-30.52); NS] separately. Comparison of males and females showed similar expenditure from recreational activity in both the CD and control groups. Participation in high impact activities was significantly lower in CD patients compared

with control subjects (0.27 ± 0.78 hours *v* 1.15 ± 6.43 hours; $P = 0.03$). Male CD patients tended to spend less time in high impact activities (0.24 ± 0.73 hours *v* 0.60 ± 1.05 hours; $P = 0.06$) and vigorous activity pursuits (0.57 ± 1.22 hours *v* 1.66 ± 2.81 hours; $P = 0.07$) compared to male controls but no differences were observed in female subjects. Compared with controls, CD patients spent less time undertaking high impact and vigorous activity pursuits in all age groups, although these results did not reach statistical significance.

Twenty-one percent (14/66) of CD patients and 16% (11/68) of controls did no form of leisure time physical activity once a week. Of the recreational activities in which subjects participated at least once a week, walking was the most important in both the CD [36% (24/66)] and control [50% (34/68)] group. Table 5.1. and Table 5.2. show the main recreational activities that CD patients and control subjects participated in at least once a week.

5.3.2.4. TV Watching

CD patients spent significantly more time watching TV per week compared with control subjects (21.11 ± 9.65 hours *v* 16.71 ± 8.22 hours, $P = 0.001$). Female CD patients spent significantly longer watching TV per week than female controls (20.86 ± 9.89 hours *v* 15.11 ± 6.80 hours; $P < 0.001$), while no differences were observed in male subjects. To assess whether higher TV watching was due to spending more time in the home, separate analysis of only those in current employment was undertaken. Similar results were observed with female CD patients watching more TV than female controls (21.31 ± 9.47 hours *v* 15.33 ± 6.84 hours; $P < 0.001$), while no differences observed in male subjects. TV watching hours were similar in males and females in the CD group but male controls spent significantly more time watching TV per week than

female controls (19.10 ± 9.59 hours *v* 15.11 ± 6.80 hours; $P = 0.01$). Hours of TV watching were similar in subjects under 40 years but CD patients spent significantly more hours watching TV per week compared with control subjects in the 40-49 age group (22.62 ± 8.31 hours *v* 15.09 ± 6.54 hours; $P = 0.002$) and 50+ age group (28.39 ± 10.32 hours *v* 18.26 ± 8.55 hours; $P = 0.004$).

5.3.3. Alcohol consumption

5.3.3.1. Prevalence of Alcohol Consumption

There were significantly more non-consumers of alcohol in the CD group compared with the control group [26% (26/100) *v* 14% (14/100); $P = 0.034\chi^2$]. Male patients were less likely to be alcohol consumers compared with male controls [75% (30/40) *v* 90% (36/40); $P = 0.07\chi^2$], and female patients were less likely to be alcohol consumers compared with female controls [73% (44/60) *v* 83% (50/60); $P = 0.184\chi^2$] (Figure 5.4.). There was no difference in the percentage of alcohol consumers in either the CD group or controls groups when comparing males with females. In all age groups, CD patients were less likely to be alcohol consumers compared with controls [18-29 year olds: 81% (29/36) *v* 92% (23/25); $P = 0.215\chi^2$, 30-39 year olds: 72% (21/29) *v* 90% (27/30); $P = 0.083\chi^2$, 40-49 year olds: 76% (16/21) *v* 81% (21/26); $P = 0.703\chi^2$, 50+ year olds: 57% (8/14) *v* 79% (15/19); $P = 0.178\chi^2$].

5.3.3.2. Units of Alcohol per Week

Alcohol consumption (in those who were alcohol consumers), measured in units per week, was significantly lower in the CD group compared with the control group [4.00 units/week (2.00-9.25) *v* 7.75 units/week (4.00-13.00); $P = 0.003$]. Alcohol consumption was significantly lower in male CD patients compared with male controls

[6.50 units/week (0.50-12.00) *v* 12.00 units/week (4.25-18.00); $P = 0.04$], and in female CD patients compared with female controls [2.00 units/week (0-4.00) *v* 4.00 units/week (2.00-8.00); $P = 0.001$]. Males consumed significantly more units of alcohol than females in both the CD group [6.50 units/week (0.50-12.00) *v* 2.00 units/week (0-4.00); $P = 0.001$] and the control group [12.00 units/week (4.25-18.00) *v* 4.00 units/week (2.00-8.00); $P < 0.001$]. In all age groups, CD patients tended to consume less alcohol than controls [18-29 years: 5.00 units/week (2.00-10.00) *v* 7.00 units/week (4.00-12.00); $P = 0.17$, 30-39 years: 5.00 units/week (2.75-12.00) *v* 8.00 units/week (4.50-13.00); $P = 0.14$, 40-49 years: 4.00 units/week (2.00-5.87) *v* 5.00 units/week (3.50-12.00); $P = 0.07$, 50+ years: 4.50 units/week (2.00-9.75) *v* 7.50 units/week (3.00-15.00); $P = 0.26$].

5.3.4. Smoking

There were more current smokers in the CD group compared with controls [32% (32/100) *v* 18% (18/100)], and less ex-smokers (stopped smoking at least 1 year ago) [22% (22/100) *v* 30% (30/100)] and non-smokers [46% (46/100) *v* 52% (52/100)] in the CD group compared with controls, although these differences were not significant ($P = 0.06\chi$). Current smoking was significantly higher among female patients compared with female controls [38% (23/60) *v* 13% (8/60); $P = 0.007\chi$] while current smoking was similar in male patients and controls [23% (9/40) *v* 25% (10/40); NS χ] (Figure 5.5.). Current smoking was higher in female CD patients compared with male CD patients [38% (23/60) *v* 23% (9/40); $P = 0.06\chi$], no differences were observed in male and female controls. Smoking rates were similar in CD patients and controls across all age groups.

Current smokers in the CD and control groups were similar in terms of number of cigarettes smoked per day [1-10 per day: 28% (9/32) *v* 33% (6/18), 11-20 per day: 53% (17/32) *v* 61% (11/18), 21+ per day: 9% (6/32) *v* 6% (1/18); NS χ] and in total years of smoking [18.00 years (11.25-28.75) *v* 17.00 years (8.37-23.00); NS]. Cigarettes smoked per day and total years of smoking were similar in males and females on separate gender analysis and across all age groups.

5.3.5. Anxiety and Depression

5.3.5.1. Anxiety

Anxiety scores were significantly higher in CD patients compared with controls (7.53 ± 3.84 *v* 6.18 ± 3.41 ; $P = 0.01$). More CD patients had scores in the borderline or morbidly anxious categories compared with controls [49% (48/98) *v* 35% (34/98); $P = 0.08\chi$] (Figure 5.6.). Female CD patients had significantly higher anxiety scores than male CD patients (8.49 ± 3.95 *v* 6.07 ± 3.19 ; $P = 0.002$) and significantly more female CD patients had scores in the morbid category compared to male CD patients [31% (18/59) *v* 10% (4/39); $P = 0.03\chi$]. No differences were observed between female and male controls. Male CD patients and male controls had similar scores for anxiety. Female CD patients had significantly higher anxiety scores compared with female controls (8.49 ± 3.95 *v* 6.67 ± 3.40 ; $P = 0.009$), and significantly more female CD patients had scores in the morbid or borderline categories [58% (34/59) *v* 37% (22/59); $P = 0.04\chi$]. Anxiety results were similar in younger patients and controls but significantly more CD patients over 50 years had scores in the morbid or borderline category compared with controls of the same age [46% (6/13) *v* 11% (2/19); $P = 0.05\chi$].

5.3.5.2. Depression

Depression scores were significantly higher in CD patients compared with controls (4.20 ± 3.54 v 3.08 ± 2.64 ; $P = 0.01$). Significantly more CD patients had scores in the borderline or morbid categories compared with controls [18% (18/98) v 4% (4/98); $P = 0.007\chi^2$] (Figure 5.6.). Female and male CD patients had similar scores for depression (4.66 ± 3.81 v 3.51 ± 3.00 ; NS) but significantly more female patients had scores in the morbid category [15% (9/59) v 0%; $P = 0.01\chi^2$]. No differences were observed between female and male controls. Male CD patients had similar scores for depression (3.51 ± 3.00 v 2.71 ± 2.56 ; NS) but significantly more male CD patients had scores in the borderline or morbid categories compared with male controls [15% (6/39) v 3% (1/39); $P = 0.02\chi^2$]. Female CD patients had significantly higher depression scores compared with female controls (4.66 ± 3.81 v 3.32 ± 2.68 ; $P = 0.02$), and significantly more female patients had scores in the morbid or borderline categories [20% (12/59) v 5% (3/59); $P = 0.02\chi^2$]. Depression results were similar in all age groups, except in the 30-39 age group, where significantly more CD patients had scores in the morbid or borderline categories compared with controls [18% (5/28) v 0%; $P = 0.05\chi^2$].

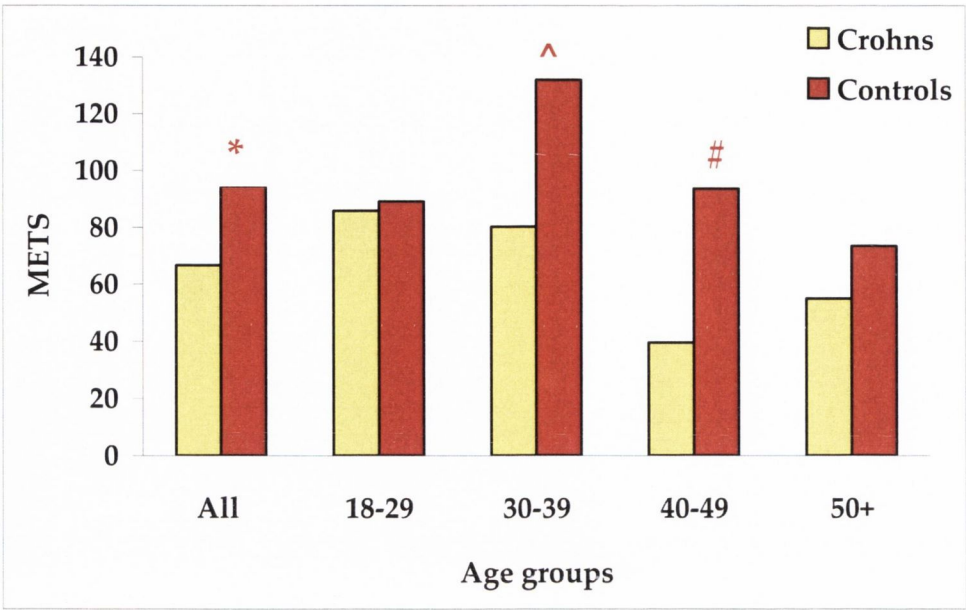


Figure 5.1. Median energy expenditure in CD patients (*n* = 66) and control subjects (*n* = 68)

METS: Multiples of resting metabolic rate x hours per week

- * *P* = 0.002 for CD patients versus controls, assessed by Mann Whitney test
- ^ *P* = 0.04 for CD patients versus controls, assessed by Mann Whitney test
- # *P* = 0.003 for CD patients versus controls, assessed by Mann Whitney test

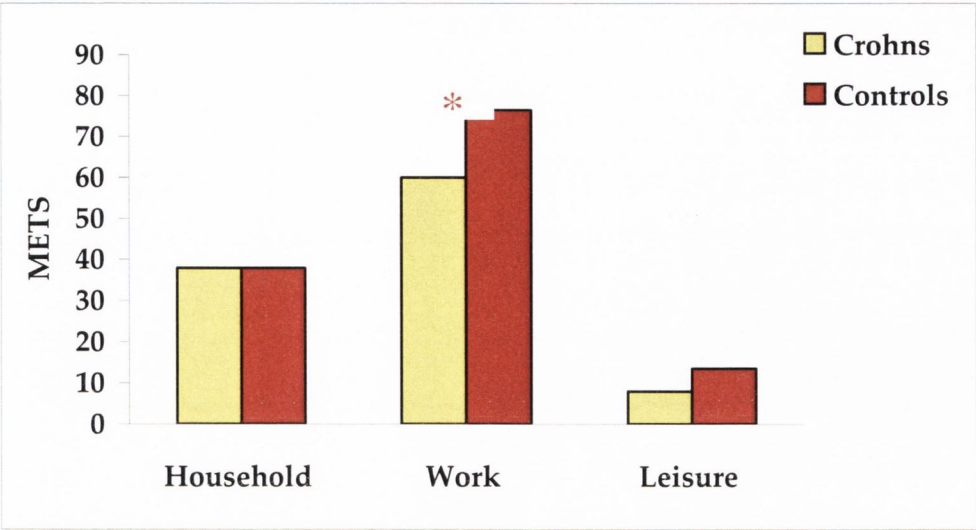


Figure 5.2. Median energy expenditure in household, work and leisure activities in CD patients ($n = 66$) and control subjects ($n = 68$)

METS: Multiples of resting metabolic rate x hours per week

* $P = 0.003$ for CD patients versus controls, assessed by Mann Whitney test

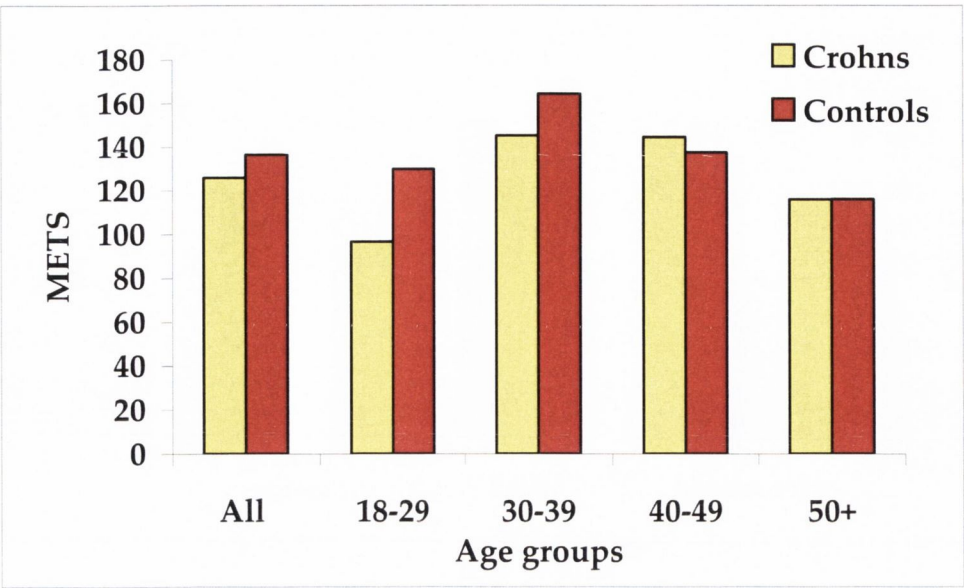


Figure 5.3. Median energy expenditure in CD patients (*n* = 56) and control subjects (*n* = 65) in current employment

METS: Multiples of resting metabolic rate x hours per week

No significant differences between CD patients versus controls, assessed by Mann Whitney test

Table 5.1. Recreational activity in CD patients (*n* = 66)

Activity	% CD patients participating once a week or more
Walking	36
Watering lawn	32
Mowing lawn	21
Weeding or pruning	21
Floor exercises	17
Digging/Shovelling	15
DIY	14
Exercise with weights	12
Musical instrument playing	12
Conditioning exercises	11
Football/Rugby/Hockey	8
Snooker/Billiards/Darts	5
Golf	5
Other aerobics	5
High-impact aerobics	3
Dancing	3
Jogging	3
Horse-riding	2
Netball/Volleyball/Basketball	2
Rowing	2
Tennis/Badminton	2
Bowling	2
Cycling	2
Swimming	2
No exercise	21

Table 5.2. Recreational activity in control subjects (*n* = 68)

Activity	% controls participating at least once a week
Walking	50
Floor exercises	40
Exercises with weights	21
Conditioning exercises	21
Watering the lawn	18
Jogging	18
Other aerobics	15
Weeding/Pruning	13
High impact aerobics	9
Football/Rugby/Hockey	7
Digging/Shovelling	7
Musical instrument playing	7
Backpacking	7
Mowing the lawn	7
Swimming	6
Tennis/Badminton	6
Cycling	3
Competitive running	3
Golf	3
Netball/Volleyball/Basketball	3
Martial arts	3
Snooker/Billiards/Darts	1
Fishing	1
Cricket	1
Rowing	1
Bowling	1
DIY	1
Competitive swimming	1
No exercise	16

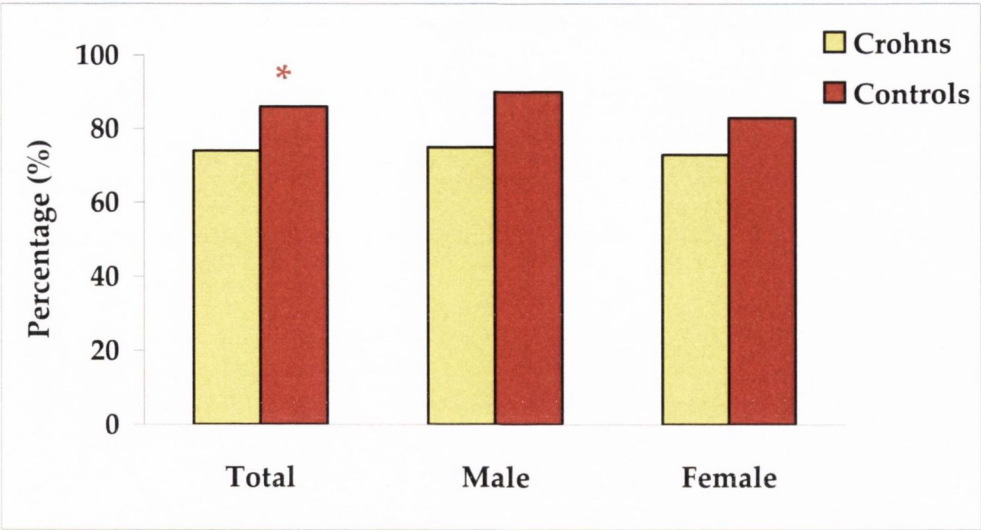


Figure 5.4. Percentage of alcohol consumers amongst CD patients ($n = 100$) and control subjects ($n = 100$)

* $P = 0.03$ for CD patients versus controls, using Pearson's chi-square test

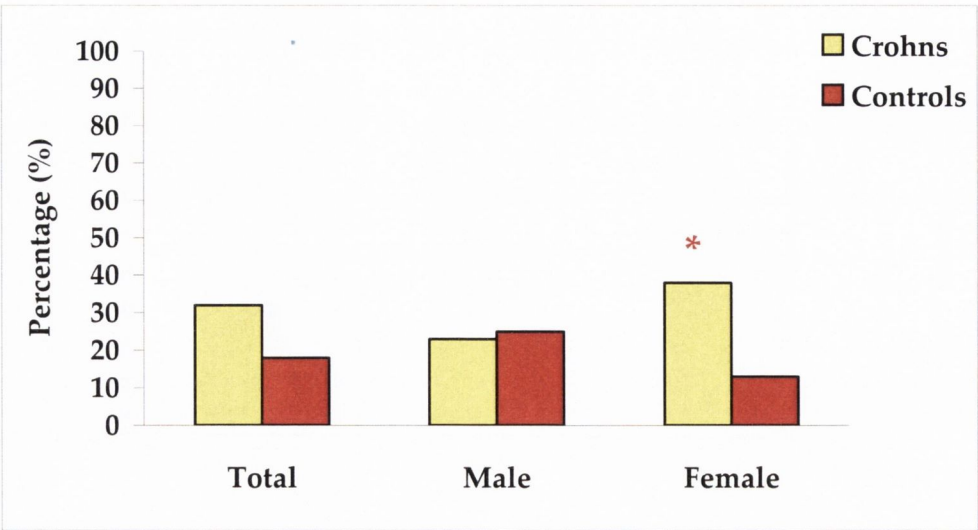


Figure 5.5. Percentage of current smokers amongst CD patients ($n = 100$) and control subjects($n = 100$)

* $P = 0.007$ for female CD patients versus female controls, using Pearson's chi-square test

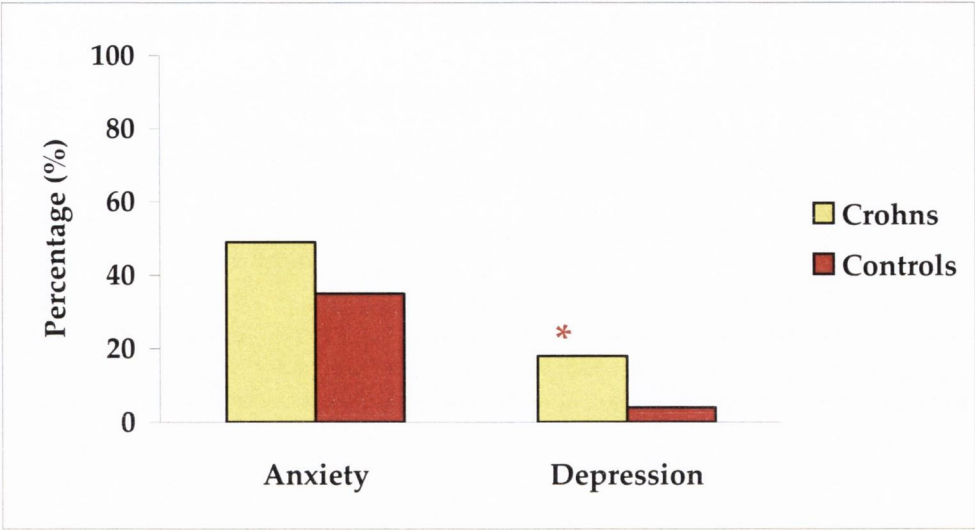


Figure 5.6. Anxiety and depression amongst CD patients ($n = 100$) and control subjects ($n = 100$)

Results presented as percentage of subjects with scores in the borderline (8-10) or morbid (>10) categories for each measurement according to HADS

* $P = 0.007$ for CD patients versus controls, using Pearson’s chi-square test

5.4. Discussion

This study reports significant differences in lifestyle characteristics between non-hospitalised CD patients in clinical remission or with mildly active disease, and age-and sex-matched healthy controls. Overall physical activity was significantly lower in CD patients. Anxiety and depression was significantly more prevalent in CD patients. Smoking tended to be more prevalent in CD patients, and was significantly so in female patients, while alcohol consumption was significantly lower in CD patients compared with control subjects.

This is the first study, to the best of our knowledge, to focus on routine physical activity in the daily lives of non-hospitalised CD patients. While previous research has focused on the effects and safety of exercise (547-548), little is known about usual patterns of physical activity in the daily routines of CD patients. The physical activity questionnaire used in the current study provides a detailed assessment of all areas of daily physical activity (437), giving a clearer picture of an individual's physical activity pattern. CD patients displayed significantly reduced overall physical activity and work activity but similar household and leisure activities when compared with controls. The observation of reduced work activity was due to the fact that a higher proportion of the patient group was not in current employment - students, retired, disability (only 2 patients) or working in the home - compared to controls [15% (10/66) *v* 4% (3/68)], which therefore, caused a decrease in reported work activity. Analysis on only those subjects in employment showed no differences in work activity, and subsequently, no difference in overall activity between patients and controls. Males, as reported previously (559), were generally more active than females, but in different ways, highlighting the fact that men and women cannot be

directly compared in terms of physical activity. In both the patient and control groups, male subjects were significantly more active in work-related activity while females were significantly more active in household-related activity.

Although work-related activity is important in overall energy expenditure, technology has greatly reduced the energy demands of work activity (560), even in manual occupations, therefore, non-work related activity in leisure time is becoming increasingly more important as a health promotion target. Leisure time activity was similar in patients and controls, with a decline in activity with increasing age observed in both groups, which has been reported previously in Irish adults (559). High-impact exercise, which has positive effects on bone, was reduced in CD patients when compared to controls. Exercise-induced bone stress is associated with an increase in bone mineral density (513), and has been recommended for all CD patients to combat osteoporosis (321). However, high-impact exercise can be hard on joints and weak bones and may, therefore, not be suitable in CD patients, especially when extraintestinal manifestations involving the joints are present. Low-impact weight-bearing exercise can also positively affect bone health in CD; a prospective randomised controlled trial in CD patients reported increases in bone mineral density after 12-months of low-impact exercise (549). Walking, which is low-impact and weight-bearing, was the most common form of exercise in the present study, which has been previously observed (559, 561). Along with positive effects on bone, walking has also been shown to improve the physical health, general well-being, quality of life and psychological stress of CD patients with inactive disease who underwent a 12-week programme of 3 walking sessions weekly (548).

TV watching and related pursuits, as reported previously (546), took up a substantial proportion of free time in both CD patients and controls, although this was significantly higher in the CD group. TV watching is associated with lower physical activity and physical fitness (562), and approximately one-fifth of the current subjects (21% CD and 16% controls) undertook no leisure time physical activity at least once every week, which is generally in keep with previous reports (563). Current guidelines for healthy adults recommend 30 minutes of moderate-intensity activity on at least 5 days per week (564). In the current group, only 25% of CD patients and 51% of controls met this guideline. This figure of 51% for control subjects is somewhat higher than a previous report which observed 40% participation rates in a sample of 3080 Irish adults (563); over-estimating cannot be ruled out in the current control group. While the majority of CD patients did not partake in the recommended 30 minutes of moderate-intensity activity each day, many undertook exercise of a longer duration (i.e. football, GAA, musical instrument playing, golf) on 2-3 days per week. The reason behind this remains unclear but it is possible that CD patients succumb to pain, tiredness or other symptoms intermittently that make it difficult for them to exercise every day. Also, as muscle function was significantly lower in our patient group and occupational activity was not significantly different from our control group, this could indicate that managing this level of activity with a reduced muscle function may lead to increased fatigability, and therefore, less participation in non-occupational related activity.

Nonetheless, the general guidelines for physical activity should be recommended for CD patients in clinical remission without any other health issues that may cause contraindication. While exercise is not usually a focus in the therapeutic range for CD, some authors have

suggested it be explored as a treatment strategy. Results from an international survey of 259 IBD patients, including 58 Irish patients, assessing the use of alternative medicine in IBD reported that while exercise was the most commonly used form of alternative medicine (28% of patients), only 3% of Irish patients used exercise as a means of alternative medicine. Interestingly, the authors reported that the Irish patients were the most enthusiastic for alternative medicine (565). A more recent study of 150 IBD patients observed 15% of patients used regular exercise as a form of therapy (566). Walking, which approximately one-third of the current patients undertook at least once a week, and other forms of physical activity should be promoted in CD patients, where possible.

The role of physical activity in CD has not been widely studied to date, despite the suggested benefits of physical activity in other chronic diseases including rheumatoid arthritis, another chronic inflammatory disease (567). Reports also suggest a beneficial protective effect of physical activity in CD. A case-control study reported an inverse relationship between CD and regular physical activity during the previous five years (568), while another observed lower occurrence of CD among outdoor workers with physically active jobs and higher occurrence in those with sedentary jobs (182). Physical activity, as well as enhancing physical health, could confer additional benefits on CD patients due to its associations with immune function (569), inflammation (570), psychological health (571) and stress-management (572). Moderate intensity exercise appears to be well tolerated in CD patients in remission and does not negatively affect gut physiology or disease course (547). Previous studies on exercise capacities of CD patients in remission observed reduced maximal exercise load (573) and reduced muscle performance (206, 248). Indeed, high rates of reduced

muscle function observed in the current group of patients were associated with reduced functional capacity (See Chapter 3). Further research is needed on exercise in CD, particularly regarding how much exercise is appropriate; nonetheless, low-impact weight-bearing exercise such as walking should be encouraged in patients in remission.

The majority of patients hold the belief that psychological stress has a significant influence on their disease activity (553), and while stress and anxiety has been linked to symptom exacerbation and disease relapse (551, 552), not all are in agreement with this suggestion (554, 555). In the current study, significantly higher rates of anxiety and depression, as measured by the HADS, were observed in the patient group compared with controls, which has been previously reported (574). Female patients appeared to be more affected, which agrees with earlier work suggesting that psychological factors in CD play a greater role in females than in males, as females often rate their symptoms as being worse and have more disease-related concerns (575, 576). Psychological stress has also been linked to health-seeking behaviour (577), therefore, it is possible that highly-stressed patients present themselves to outpatient clinics more readily, and thus, could be over-represented in the present study. Psychological symptoms have been shown to contribute to impaired quality of life in CD patients and psychological stress was found to be an independent predictor of the physical function (578). Whether psychological stress and anxiety are associated with disease course in CD remains unknown but it is possible that helping patients to cope with psychological distress could improve quality of life in CD patients.

Smoking, is a well-established risk factor for the development and exacerbation of CD (556), and is associated with reduced quality of life

(575). Almost one-third of the present group were current smokers, which was higher, although not significantly so, than the control group. High alcohol intakes, possibly due to their high sulphurate content, have been shown to predict an increased likelihood of relapse in UC patients (579), but this fact has been disputed (197). The patients in the present study were significantly less likely to be consumers of alcohol compared with controls, and those patients who were alcohol consumers drank significantly less alcohol compared with the control group. This observation is in agreement with Geerling *et al* (243) who studied newly diagnosed patients, but in disagreement with a similar study focusing on patients with long-standing disease (206, 580), although very little work has focused on alcohol in CD. It is possible that patients in the current study consumed less alcohol due to its negative effects on symptoms, which has been reported previously in UC patients (581), due to concurrent medication or patients may not have been socialising in bars, therefore, not drinking as much. Also, as data on alcohol was self-reported, underestimation cannot be ruled out.

In conclusion, physical activity levels are, in general, similar in CD patients compared with controls while psychological stress and anxiety are significantly more common in CD patients. Exercise has been shown to reduce psychological stress (548), and an attempt should be made to incorporate more moderate exercise into patients' daily routine from both the physical and psychological point of view.

CHAPTER 6:

GENERAL DISCUSSION

Summary of Main Findings

This research focused on the prevalence and type of undernutrition present in CD patients with inactive or mildly active disease.

In Chapter 3, a high prevalence of reduced muscle function (72%) was observed in inactive or mildly active CD patients who present with normal BMI, normal fat and muscle stores, normal serum albumin and protein levels and who are well-nourished according to a nutritional screening tool. Higher corticosteroid usage appears to be the only modifiable risk factor associated with reduced muscle function, which is related to fatigue in patients.

In Chapter 4, the majority of inactive CD patients were found to have low vitamin D status (63%), which was associated with smoking and season. While intakes were similar to those of controls, the low use of calcium and vitamin D containing supplements, which failed to meet the guidelines recommended by the BSG, was also noted.

In Chapter 5, higher rates of smoking were observed in CD patients while alcohol intakes were lower. Physical activity patterns in inactive CD patients were similar to those of controls while anxiety and depression were more common in inactive CD patients.

Undetected Undernutrition

Undernutrition, particularly unidentified or undetected undernutrition, is an area that receives a lot of attention in both nutrition and medical circles (453, 465, 582, 583). The lack of a universally accepted definition for malnutrition, together with the lack of a screening tool that can be used as a gold standard makes the issue of detection extremely

complicated. There are over 70 published tools available for the detection or assessment of malnutrition (584) and it is likely that many more similar unpublished tools are also in use.

Clinical recognition of undernutrition appears lower in younger outpatients compared to older patients (585), most likely due to physicians having an awareness of the increased risk in elderly subjects. A study of 1017 outpatients failed to detect undernutrition (based solely on weight) in 88% of young outpatients, a figure similar to the present study, although rates of detection were higher in older subjects (43%) (585). Higher body weights and BMI observed in Ireland in recent years have led to an underestimation of nutritional risk as fewer individuals in developed countries have BMI values which fall below 20kg/m² (546). This is true for the current study as 40% of patients and 52% of controls were overweight or obese, and is in agreement with other studies on CD patients (207, 208, 244) and other chronic conditions traditionally associated with low body weight such as coeliac disease (586). It is evident, therefore, that BMI cannot be solely relied on to detect undernutrition; more detailed assessments are required.

In addition, although body weight and BMI are easily obtained, they do not provide information on body composition and the distribution of fat mass and fat-free mass (FFM). Higher fat mass can mask low FFM, and BMI can fall in the normal range even when FFM is reduced, therefore, underestimating the prevalence of nutritional risk (587-589). In addition, anthropometric reference data in current use is generally derived from measurements obtained 25 years ago in American populations (452, 454), therefore, may not accurately reflect current study populations. Future studies should avail of the use of Dual X-ray Absorptiometry (DEXA) as an assessment of body composition. DEXA

enables the determination of fat mass, FFM and BMD and is suggested to be more accurate and precise than any other non-invasive method (590). This method would have been invaluable in the current study but unfortunately this technology was not available.

Serum albumin is also used routinely despite its low sensitivity, specificity and long half-life, but furthermore often fails to detect undernutrition (206, 207, 247, 587, 589). Approximately one third of chronically ill patients (including CD patients) with normal BMI displayed low FFM and high fat mass when compared to controls and acutely ill patients (587); albumin assessment only identified 6% of these patients.

Reduced Muscle Function

The significance of the physical function of muscle as an indicator of nutritional status is well established (472, 474, 475). Reports also suggest that muscle function can influence the risk of fracture as it causes impaired balance leading to falls (591) and can predict future disability (506) and overall mortality (457).

Physical activity can improve muscle function as exercise interventions have been shown to successfully improve functionality and muscle size (592, 593), while physical activity was found to be the only manipulative predictor of skeletal mass in pre- and postmenopausal women (594). Physical activity is an area that could be targeted in the prevention of muscle loss and preservation of muscle function. Patients in the current study were found to have similar physical activity levels as controls, so it is plausible that increasing activity is amenable, as patients are already relatively physically active. Interventions in young or middle-aged patients could offset the deleterious effects reduced

muscle function in old age. A five-week experimental bed-rest trial observed an average decrease in muscle strength of 20% and bone density of 3% (495). A four-week period of active recovery including weight bearing exercise led to improvements in muscle mass and strength back to normal values, whereas bone loss remained decreased, suggesting a greater impact of inactivity on bone metabolism.

Muscle and Bone Associations

Another important role of muscle is its positive association with bone (595-597), as the preservation of adequate bone strength and density is highly dependent on the maintenance of adequate muscle mass and function. Muscle contractions are proposed to be the most significant influencing factor on bone mineral content in the body (598-600), as muscle contractions provide the largest voluntary loads on bone which is essential for modelling and remodelling which subsequently increase bone strength and mass (598). Correlations between grip strength and bone mineral content and bone mineral density in healthy athletes (601) and stroke patients (602) support the concept that muscle contractions play a significant role in bone strength and mass. Muscle mass starts to decrease by approximately 1% per year following the fourth decade of life (603); changes in bone mass follow a similar course and decrease over the life span (358).

Vitamin D Deficiency and Muscle Strength

Vitamin D deficiency is associated with muscle weakness (604, 605), with a direct correlation noted between vitamin D deficiency and musculoskeletal pain (606, 607). It has been shown in experimental studies that muscle tissue is a direct target for vitamin D metabolites, offering further proof for the association between vitamin D deficiency

and muscle weakness (608). A vitamin D receptor (VDR), binding specifically to 1,25(OH)D₃, has also been found in human skeletal muscle cells, (609, 610) and it appears that muscle strength is influenced by the VDR genotype in the muscle (611). A study of non-obese, elderly women observed a 23% difference in quadriceps strength and a 7% difference in grip strength between the 2 homozygote types of the VDR (611). In young adults with prolonged vitamin D deficiency, associated with muscle weakness, supplementation with vitamin D has been shown to improve symptoms (612, 613). Treatment of vitamin D deficiency produced an increase in muscle strength and a marked decrease in back and lower-limb pain within 6 months (607).

Vitamin D and Immune Function

Mounting evidence (614) suggests that vitamin D is an environmental factor involved in autoimmune diseases such as diabetes (615), multiple sclerosis (616) and IBD (616, 617). Experimental IBD in IL-10 knockout mice was exacerbated by vitamin D deficiency and ameliorated with vitamin D supplementation (618). The link between IBD and vitamin D appears to involve TNF α , as genes associated with TNF α production and signaling were found to be down-regulated by vitamin D (and calcium) treatment in the colonic tissue of IL-10 knockout mice, leading to maximal suppression of IBD (619). Moreover, an *in vitro* study demonstrated that KH 1060, a vitamin D analogue, decreased TNF α levels in peripheral blood mononuclear cells isolated from IBD patients (620). It is thought that vitamin D regulates the differentiation and activity of CD4⁺ T cells, thus, regulating Th1 immune responses, associated with the progression of IBD (618). Polymorphisms in the VDR, which mediates the biologic activity of vitamin D (621), have also been implicated with IBD development. Correlations with increased susceptibility of IBD have been reported (622) and VDR deficiency in

two different mouse models of IBD resulted in severe accelerated inflammation (623).

Other lines of evidence linking vitamin D status and IBD include the geographical (93) and seasonal (624, 625) variations in the incidence and prevalence of IBD. Seasonal variations in the onset and exacerbations of IBD have been observed with higher relapse rates of CD noted in autumn and winter (624). Newly diagnosed patients often present with low vitamin D levels (320, 361), while fish oil, a rich source of vitamin D, has been shown to decrease severity of IBD (626).

While the current study found no association between disease severity and vitamin D status, it was not the focus of the current research and further studies are needed to address this issue. Intervention trials with vitamin D should assess the effects of vitamin D on disease activity but also on muscle function and bone disease.

Study Limitations and Future Work

There are a number of limitations associated with the current study that should be highlighted. Physical activity and nutrient intakes were measured through self-reported questionnaires, known to be open to problems, such as over-reporting of physical activity (493) and underreporting of energy intake (627).

DEXA scanning should be employed as it enables the determination of body composition (bone, muscle and fat). The role of exercise is also an area open to further research as it has positive effects on muscle and bone function, as well as being beneficial to psychological health.

Recommendations From This Work

- Better screening strategies needed for the detection of undernutrition
- ALL patients are at risk of undernutrition
- Vitamin D status should be regularly assessed in all patients
- All patients receiving corticosteroid therapy should receive vitamin D
- All patients should be encouraged to do more physical activity
- All patients should be encouraged to stop smoking

Conclusion

In conclusion, it is evident from the current research that undernutrition is an issue in CD outpatients with inactive or mildly active disease, particularly in terms of muscle function and vitamin D status. Worryingly, undernutrition is likely to remain undetected if current routine measures continue to be employed. Comprehensive screening and diagnostic approaches are crucial in the assessment of undernutrition of CD, particularly when disease is inactive, and these need to be introduced if undernutrition and its deleterious affects are to be combatted.

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8: APPENDICES

PATIENT INFORMATION SHEET AND

CONSENT

Title of study

Nutritional factors: Role in disease outcome in inflammatory bowel disease.

Introduction

Inflammatory Bowel Disease (IBD) is a collective term for two diseases, Crohn's disease and Ulcerative Colitis, which are characterised by inflammation or swelling of various parts of the bowel. A mixture of genetic (hereditary) factors and environmental factors, such as diet and nutrition, are involved in the cause of IBD. These factors are also thought to be involved in disease severity and course, which varies among patients; some people remain in remission for long periods and respond well to medication while others, however, experience more relapses and don't respond so well to medication.

Patients with IBD are often malnourished and this can affect the severity of their disease. We aim to assess the nutritional status of IBD patients attending AMNCH and to determine if poor nutritional status is linked to their disease activity. We will also determine if any links exist between nutritional status and genes known to be involved in the aetiology of IBD.

Procedures

All adult patients with inflammatory bowel disease attending AMNCH will be eligible for participation as long as you:

- are over 18 years of age
- have confirmed determinate IBD (Crohn's disease or ulcerative colitis)
- have been diagnosed for at least 3 months
- have sufficient English language ability to carry out the study requirements
- be willing and able to take part in the study

If you decide to take part in the study you will be asked to come in to the hospital, having fasted from the night before, to be interviewed by one of the research team. This one off visit, which will last approximately 45 minutes, will be made at a time convenient to you. During the interview you will be asked to fill in some questionnaires regarding your medical history and your family history, lifestyle, nutritional and dietary habits. The researcher will take some measurements i.e. height, weight etc, which will be used to determine your fat and muscle stores and body composition. These measurements are non-invasive and will not hurt. At the same visit, a blood sample will be taken, so that we can assess the

level of nutrients in your blood and also to isolate genes known to be involved in IBD.

All information, blood samples and genetic data collected will be labelled by code in a secure database so that only the research team will have access to the information.

Benefits

As the study is aimed at learning more about the factors involved in the outcome of Crohn's disease and ulcerative colitis, the results we discover may be of benefit to IBD patients in the future.

Risks

The study will not put you at any risk, apart from the possibility of slight discomfort during the blood sampling and/or slight bruising afterwards. This will be minimised as much as possible as a trained phlebotomist/physician will carry out the procedure using standard clinical methods.

Exclusion from participation

Your doctor has told you that you cannot be in this study if any of the following are true:

- You are under 18 years old
- Have unconfirmed IBD
- Have insufficient English language ability to carry out the study requirements
- Are not willing or able to take part in the study

Alternative Treatment

This is not a treatment study and will in no way affect your current or future treatments.

Confidentiality

Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the hospital.

Compensation

Your doctors are covered by standard medical malpractice insurance. Nothing in this document restricts or curtails your rights.

Voluntary Participation

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits you had before entering the study.

Stopping the study

You understand that your doctor may stop your participation in the study at any time without your consent.

Permission

This study has approval from the Hospital's Ethical Committee.

Further information

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from:

Prof Colm O'Moráin who can be telephoned at **01 4143851** or any member of the Gastroenterology Research Team.

If your doctor learns of important new information that might affect your desire to remain in the study, he or she will tell you.

CONTROL INFORMATION SHEET AND

CONSENT

We are conducting a nutritional study on patients with a digestive disease and we need healthy people to act as controls for comparison purposes. We need to compare the nutritional status of the patients to healthy people (controls) to prove that any differences we find are due to their disease. We do this by matching each patient with an age- and sex-matched control.

Title of Study

Role of nutritional factors in disease outcome in IBD

What is Inflammatory Bowel Disease (IBD)?

Inflammatory Bowel Disease (IBD) is a collective term for two diseases, Crohn's disease and Ulcerative Colitis, in which the bowel becomes red and inflamed and causes symptoms including abdominal pain, weight loss, diarrhoea, constipation, tiredness and joint pain. Both Crohn's disease and ulcerative colitis are chronic illnesses comprised of flare-ups (relapse) and periods of well-being (remission). The cause of IBD is not yet known but a mixture of genetic (hereditary) factors and environmental factors, such as diet and nutrition, are thought to be involved.

What are we studying?

Patients with IBD are often malnourished and this can affect the severity of their disease. We aim to assess the nutritional status of IBD patients attending AMNCH, which will enable us, to 1) establish the prevalence of malnutrition among the IBD patients 2) determine if poor nutritional status is linked to their disease activity 3) identify patients at risk of malnutrition. We will also determine if any links exist between nutritional status and genes known to be involved in the aetiology of IBD.

What will I have to do?

The study will take place in the morning (after an overnight fast) and will be a one-off assessment, which should last no longer than 20 minutes. There are three parts to the study:

Questionnaires: You will be asked some questions regarding your medical history and family history, lifestyle, nutritional and dietary habits.

Nutritional Assessment: The researcher will take some measurements i.e. height, weight etc, which will be used to determine your fat and muscle

stores and body composition. These measurements are non-invasive and will not hurt.

Blood sample: A fasting blood sample will be taken so that we can assess the level of nutrients in your blood.

Who can act as a control?

Anyone over 18 years of age, of Irish descent in good health can participate. It doesn't matter if you're overweight/underweight, smoker/non-smoker etc. The main exclusion criteria are conditions such as diabetes, coeliac disease and/or a family history of Crohn's disease or ulcerative colitis, as these are the patient groups that we are studying. If you are unsure as to whether you can be included, please contact the research team.

Confidentiality

All information and blood samples collected will be labelled by code in a secure database, that only the research team will have access to. Your identity will remain confidential at all times. Your name will not be published and will not be disclosed to anyone outside the hospital.

Risks

The study will not put you at any risk, apart from the possibility of slight discomfort during the blood sampling and/or slight bruising afterwards. This will be minimised as much as possible as trained phlebotomist/physician will carry out the procedure using standard clinical methods.

Voluntary Participation

You have volunteered to participate in this study. You may quit at any time.

Permission

This study has approval from the Hospital's Ethical Committee.

Further information

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from:

Treasa Nic Suibhne or Dr. Maria O'Sullivan

Tel: 01 6083828 / 087 6758836

Email: ibdstudy@hotmail.com

Ethical Approval has been obtained from St. James's Hospital and Federated Dublin Voluntary Hospitals Joint Research Ethics Committee

CONSENT FORM FOR PARTICIPATION IN GENETIC RESEARCH

Participant Identification Number:	SL_____
Title of Protocol:	Nutrition in IBD
Name of Institution leading the Research:	AMNCH
Research Director:	Prof Colm O'Moráin
Phone Number and Contact Details:	01 4143851

Please initial boxes

1. I have read the attached information sheet on the above project dated _____ and have been given a copy to keep. The information has been fully explained to me and I have had an opportunity to ask questions about the project and understand why the research is being done and any foreseeable risks or consequences involved. I also understand that no guarantee can be given about the possible results.

☐

2. I agree to give a sample(s) of blood / DNA for research in the above project. I understand how the sample will be collected, that giving a sample for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without giving a reason. If I withdraw my consent I understand that my sample will be destroyed unless I otherwise authorise. I understand that I may ask for my samples to be destroyed and that this will be without my medical treatment or legal rights being affected. I agree that the samples I have given and the information gathered by me can be stored and looked after by the Dept of Gastroenterology. I understand that any genetic information obtained will not be made available to me.

☐

3. I give permission for my medical records to be looked at and information taken from them to be analysed in the strictest confidence by the relevant and responsible people from the Gastroenterology Research Team or from organisations supervising the research. I have been told that all medical information / data pertaining to me will be protected by the principles of confidentiality and both national and EU data protection legislation. I have further been told of / shown assurances that this also applies to all medical information / data pertaining to me that are utilised in any non-EU state.

☐

4. I understand that the confidentiality of the sample(s) I donate and information derived therefrom will be protected. I have been told that all medical information / data pertaining to me and derived from the sample(s) will be protected by the principles of confidentiality and both national and EU data protection legislation. I have further been told of / shown assurances that this also applies to all medical information / data pertaining to me and derived from the sample(s) that are utilised in any non-EU state.

☐

FOR OTHER GENETIC RESEARCH:
(Note: New research should be submitted for approval by the Research Ethics Committee before proceeding)

5. I understand that future research using the sample I give may include genetic research aimed at understanding the genetic influences in disease but that such test will not be of predictive / clinical value and that the results of these investigations are unlikely to have any implications for me personally. ☐

6. I understand that I will not benefit financially in any way if this research leads to the development of a new treatment or medical test. ☐

7. I know how to contact the research team if I need to. ☐

_____ Name of participant (BLOCK CAPITALS)	_____ Date	_____ Signature
--	---------------	--------------------

_____ Name of researcher (BLOCK CAPITALS)	_____ Date	_____ Signature
---	---------------	--------------------

_____ Name of witness (BLOCK CAPITALS)	_____ Date	_____ Signature
--	---------------	--------------------

Ethical Approval has been obtained from St. James’s Hospital and Federated
Dublin Voluntary Hospitals Joint Research Ethics Committee

CONTACT DETAILS

Name: _____

Hospital number: _____

Address: _____

Contact phone numbers:

Home: _____

Mobile: _____

GP name: _____

Address: _____

Phone number: _____

Data collected by: _____

Signature: _____

Date: _____

***PLEASE REMOVE THIS SHEET AND TRANSFER TO
CONFIDENTIAL PATIENT FILE***

MEDICAL HISTORY

Diagnosis confirmed by:

- Surgical
- Endoscopy/Histology
- Radiology

Date of first diagnosis: / / (dd/mm/yy)

Age at diagnosis:

Duration of disease: years months

Site of disease:

- Small bowel
- Large bowel
- Small and large bowel
- Upper GI

Most recent colonoscopy:

Date / /
Biopsies taken Yes No
Histology result _____
Histology number _____

Previous IBD surgery:

	Yes	No
If yes, please specify	Year	Reason for surgery
Op 1 _____		_____
Op 2 _____		_____
Op 3 _____		_____
Op 4 _____		_____

Non-IBD surgery:

	No	Yes	Age	Unknown
Appendectomy				
Tonsillectomy				
Other _____				

Has the patient ever suffered any complications in connection with their IBD?

Yes No N/A

If yes, please specify: N/A No Yes Year Ongoing

Intestinal:

Perianal disease

Abscess or ulcer

Fistulae

Strictures

Extraintestinal:

Iritis/uveitis

Erythema nodosum

Psoriasis

Pyoderma gangrenosum

Arthritis/arthralgia

Others:

Sacroiliitis

Sclerosing cholangitis

Ankylosing spondylitis (AS)

Has patient suffered any flare-ups or relapses* in last 12 months?

Yes No N/A

If yes, please specify:

Dates from – to

Treatment

/ / - / /
 / / - / /
 / / - / /

Has the patient suffered any flare-ups or relapses* in last 5 years?

Yes No N/A

If yes, please specify

Dates from - to

Treatment

/ / - / /
 / / - / /
 / / - / /
 / / - / /

*Relapse defined as CDAI > 150, requirement for steroids/Infliximab, increase in medication, surgery, activity shown by investigation

Other medical conditions:

YesNoN/A

If yes, please specify:

Condition

Diagnosed (year, age)

,

Condition

Diagnosed (year, age)

,

Fracture risk:

Has patient ever had any fractures?

YesNoN/A

If yes, please specify:

Fracture site

Date

/ /

/ /

/ /

Family history:

IBD:

YesNoN/A

If yes, please specify:

Relation

Condition

Date of diagnosis

/ /

Other conditions:

RelationDiagnosed

COMMENTS:

Data collected by:

Signature:

Date:

MEDICATION/DRUG USAGE

CURRENT IBD MEDICATION:

Is the patient currently on steroid medication?

	No	Yes	Start date (mm, yy)	Current Dose
IV hydrocortisone			/	_____
Oral prednisolone			/	_____
Oral budesonide:				
		Budesonide	/	_____
		Budenofalk	/	_____
Steroid enemas:				
		Proctofoam	/	_____
		Coliform	/	_____
		Anugesic	/	_____
		Predsol	/	_____
		Scheriproct	/	_____
		Predenema	/	_____
		Predfoam	/	_____
Other:		_____	/	_____

Does the patient currently fit criteria for steroid resistance?

	Yes	No	Unknown
≥2 courses of steroids in last 12 months			
Prior bowel resections			
High initial CDAI			
Perianal disease			
Other			

Is the patient currently receiving Infliximab therapy?

(within last 2 months)	Yes	No
If yes, please specify:		
Date of last treatment	/	/
Indication	_____	
First treatment?	Yes	No
Any reaction?	Yes	No
If yes, please specify:	_____	

Is the patient currently on any of the following medication?

	Yes	No	Start date (mm,yy)	Current Dose
<u>Immunosuppressants</u>				
Azathioprine:				
Ciclosporin:				
Methotrexate:				
Mercaptopurine:				
<u>5-ASAs/Aminosalicylates</u>				
Mesalazine:				
Olsalazine:				
Sulfasalazine:				
<u>Anti-diarrhoeal</u>				
Codeine phosphate:				
Co-phenotrope:				
Loperamide:				
Morphine:				
<u>Other</u>				
Study medication				

PREVIOUS IBD MEDICATION:

Has the patient previously been on steroids?
(in the last 12 months)

YesNo

If yes, please specify:

IV Hydrocortisone

Dates from – to	Regime/dosage	Total
/ / - / /		
/ / - / /		
/ / - / /		
/ / - / /		
Total no of courses	Total steroid equivalence	

Oral prednisolone

Dates from – to	Regime/dosage	Total
/ / - / /		
/ / - / /		
/ / - / /		
/ / - / /		
Total no of courses	Total steroid equivalence	

Oral budesonide

Dates from – to	Regime/dosage	Total
/ / - / /		
/ / - / /		
/ / - / /		
/ / - / /		
Total no of courses	Total steroid equivalence	

Has the patient previously received Infliximab therapy?
(within last 12 months)

YesNo

If yes, please specify:

Date received	Indication	Any reactions?
/ /		Y/N
/ /		Y/N
/ /		Y/N
/ /		Y/N

Has the patient previously been on any of the following?
(within last 12 months)

	N/A	No	Yes	Duration (months)	Dose
<u>Immunosuppressants</u>					
Azathioprine (e.g. Imuran)					_____
Ciclosporin: (e.g. Neoral, Sandimmun, SangCya)					_____
Methotrexate:					_____
Mercaptopurine: (e.g. Puri-Nethol)					_____
<u>5-ASAs/Aminosalicylates</u>					
Mesalazine: (eg Asacol, Ipocol, Pentasa, Salofalk)					_____
Olsalazine: (eg Dipentum)					_____
Sulfasalazine: (eg Salazopyrin)					_____
Balsalazide: (eg Colazide)					_____
<u>Others:</u>					
Ursodesoxycholic acid: (e.g. Destolit, Urdox, Ursofalk)					_____
Ciprofloxacin					_____
Metronidazole					_____
Other IBD _____					_____
Study medication _____					_____

Has patient ever had reaction to or stopped taking medication
because of side-effects?

	Yes	No	N/A
If yes, please specify:			
Drug name	_____		
Type of reaction/side effects	_____		
Duration on drug	_____		

Non-IBD Medication:

Is patient currently taking any other medication, unrelated to IBD?

	Yes	No	N/A
If yes, please specify:			
Drug			
Indication			
When was condition diagnosed?	/	/	
When was drug started?	/	/	
Drug			
Indication			
When was condition diagnosed?	/	/	
When was drug started?	/	/	
Drug			
Indication			
When was condition diagnosed?	/	/	
When was drug started?	/	/	

Contraceptive pill:

Have you ever used a contraceptive pill?

	Yes	No	N/A
If yes, please specify:			
When did you start? (year, age)			
Do you still use the contraceptive pill?		Yes	No
If no, please specify:			
Which year did you stop?			
If used intermittently, how many years of use in total? (years)			

Hormone Replacement Therapy:

Are you currently receiving HRT?

	Yes	No	N/A
If yes, please specify:			
When did you start? (year, age)			

NUTRITION & DIETARY INFORMATION

Question 1. **Do you take any vitamin/mineral supplements?**

	No	Yes	Duration (months)	Name	Dose
Multivitamin				_____	_____
Folic acid				_____	_____
B12				_____	_____
Calcium				_____	_____
Vitamin D				_____	_____
Iron				_____	_____
Other _____				_____	_____

Question 2. **Any confirmed nutritional deficiencies at present?**

	No	Yes	Duration (months)	Blood Level
Anaemic				_____
B12				_____
Calcium				_____
Vitamin D				_____
Other				_____

Question 3. **Have you taken any vitamin/mineral supplements in the last 12 months?**

	No	Yes	Duration (months)	Name	Dose
Multivitamin				_____	_____
Folic acid				_____	_____
B12				_____	_____
Calcium				_____	_____
Vitamin D				_____	_____
Iron				_____	_____
Other _____				_____	_____

Question 4. **Are you currently under the care of a dietician?**
No Yes

If yes, please specify
Name of dietician _____
Duration (months)

Question 5. **Are you currently on a prescribed diet?**
No Yes Duration
(months)

High protein/calorie
Low fibre
Other, specify _____

Question 6. **Are you currently taking any nutritional feeds/supplements?**
No Yes Duration Dose
(months)

Ensure Plus	_____
Enlive	_____
Fortijuce	_____
Provide Extra	_____
Calshake	_____
Calogen	_____
Liquigen	_____
E028	_____
Other _____	_____

Question 7. **Are you currently receiving nutritional support?**
No Yes Duration
(months)

Enteral Nutrition
Total Parenteral Nutrition (TPN)
Elemental diet

Question 8. **Are there any foods that you avoid because of your illness?**

Food	_____
Avoiding for how long	_____
Reason for avoiding	_____
 Food	 _____
Avoiding for how long	_____
Reason for avoiding	_____

Question 9. **Do you eat the following types of food?**

	Yes	No	Since	Reason for not eating?
Milk/Dairy products				_____
Red meat				_____
Bread/Wheat				_____

COMMENTS:

Data collected by: _____
Signature: _____

Date: _____

DEMOGRAPHICS QUESTIONNAIRE

Gender: Male Female

Date of Birth: / / Age: _____
 dd/mm/yy

Ethnicity: Caucasian Black
 Asian Other
 (specify _____)

Jewish background: No
 Yes (>1/4 grandparents are Jewish)
 Partly (1/4 grandparents are Jewish)

Marital status: Single
 Living with partner, unmarried
 Married
 Separated
 Divorced
 Widowed

Children:
Do you have children? Yes No

If yes, please specify:

	Gender	Age	IBD	Other conditions
Child 1	M/F		No/UC/CD	_____
Child 2	M/F		No/UC/CD	_____
Child 3	M/F		No/UC/CD	_____
Child 4	M/F		No/UC/CD	_____
Child 5	M/F		No/UC/CD	_____

Education:
What is your level of education at this moment?
Primary school
Junior/Inter Certificate (15-16 years)
Leaving Certificate (17-18 years)
3rd level education
Postgraduate qualification

Unemployed
Employed
Self-employed
Student
Work in the home
Retired
Other

What is your partner's current/most recent occupation?

Smoking:

If yes,

How many cigarettes do you smoke per day? 1-10
11-20
21 or more

When did you start smoking? (year, age)

Total years of smoking

If no,

Have you ever smoked? Yes No

How many cigarettes did you smoke per day? 1-10
11-20
21 or more

When did you start smoking? (year, age)

When did you stop smoking? (year, age)

Total years of smoking

Do you drink alcohol?	Yes	No
-----------------------	-----	----

If yes, please specify:

How much do you drink per week? _____

How many units of alcohol do you drink per week?

(1 unit = ½ pint beer, 1 single (25ml) measure spirit, 1 (100ml) glass of wine)

Physical activity:

Do you take part in regular physical activities (walking, jogging, cycling, swimming >30 minutes or similar activities)?

Daily

Weekly

Monthly

Other please specify: _____

TV watching:

How many hours per day do you spend watching TV/video/DVD etc?

Weekdays

Weekends

COMMENTS:

Data collected by: _____

Signature: _____

Date: _____

HEALTHCARE CONSUMPTION COSTS

(related to last 12 months)

1. Clinic Visits

Who has mainly taken care of your bowel disease since diagnosis?

A gastroenterologist in a hospital

A gastroenterologist in private rooms*

A surgeon

Your family doctor

Other

*(CO'M/MB/HO'C)

Please specify for the last 12 months:

Total number of consultations with your GP related to complaints of your IBD?

Total number of consultations with your gastroenterologist (in a hospital) related to complaints of your IBD?

Total number of consultations with your gastroenterologist (in private rooms) related to complaints of your IBD?

Total number of consultations with your surgeon related to complaints of your IBD?

Total number of consultations with a dietician?

2. Procedures

Endoscopic: Colonoscopy

/	/
/	/
/	/
Total	_____

Gastroscopy

/	/
/	/
/	/
Total	_____

Radiologic: Barium follow through

/	/
/	/
/	/
Total	_____

Barium enema	/	/
	/	/
	/	/
Total	_____	
Ultrasound	/	/
	/	/
	/	/
Total	_____	
X-ray procedures	/	/
	/	/
	/	/
Total	_____	

3. Hospitalisation

Have you been hospitalised in the last 12 months in relation to your IBD?

Yes No

If yes, please specify:

Dates	Primary indication	Via A&E?
/ / - / /	_____	
/ / - / /	_____	
/ / - / /	_____	
Total no of hospital days		

4. Accident and Emergency Visits

Have you visited an A&E department in the last 12 months in relation to your IBD?

Yes No

If yes, please specify:

Dates
/ /
/ /
/ /
Total no of A&E visits

5. Surgery

Have you had any surgical procedures in the last 12 months in relation to your IBD?

Yes No

If yes, please specify:

Dates	Procedure
/ /	_____
/ /	_____
/ /	_____
Total no of surgical procedures	

6. Total Parenteral Nutrition

Have you received TPN in the last 12 months? Yes

No

If yes, please specify:

Dates

No of days

$$/ / - / /$$

11 - 11

$$/ \quad / \quad - \quad / \quad /$$

Total days on TPN

7. Days lost from work

During the last 12 months,

How many days did you have to report sick from work/school/college due to your IBD?

If you work in the home, how many days were you unable to carry out your work due to your IBD?

Have you been unwillingly (full time or part time) unemployed because of your IBD?

Yes

No

If yes, how many days were you out of work for?

Total days lost from work

8. Disability benefit

Have you ever received a disability benefit because of your bowel disease? Yes No

Yes

No

COMMENTS:

Data collected by: _____

Signature: _____

Date: _____

CONTACT DETAILS

Name: _____

Hospital number: _____

Address: _____

Contact phone numbers:

Home: _____

Mobile: _____

Email: _____

GP name: _____

Address: _____

Phone number: _____

Data collected by: _____

Signature: _____

Date: _____

***PLEASE REMOVE THIS SHEET AND TRANSFER TO
CONFIDENTIAL FILE***

MEDICAL INFORMATION

Any documented medical conditions:

Yes	No	N/A
Condition	Diagnosed	

Previous surgery:

Yes	No	N/A
Type	Year/Age	

Previous fractures:

Yes	No	N/A
Site	Year/Age	

Family history:

Yes	No	N/A
Condition	Relation	

Medications:

Yes	No	N/A	
Medicine	Condition	When started	Dosage

Contraceptive Pill:

Yes	No	N/A	
Name	Started	Stopped	Total years

Hormone Replacement Therapy:

Yes	No	N/A
-----	----	-----

NUTRITIONAL INFORMATION

Nutritional supplements:

Yes No N/A

	No	Yes	Duration	Name	Dose
Multivitamin					
Folic acid					
B12					
Calcium					
Vitamin D					
Iron					
Other					

Are you currently under the care of a dietician?

Yes No N/A

Reason: _____
How long for: _____

Are you currently on a prescribed diet?

Yes No N/A

	Reason	Duration
High protein/calorie		
Low fibre		
Low fat		
Low protein		
Other		

Are you currently taking any nutritional feeds/supplements?

Yes No N/A

Feed	Reason	Duration

Do you eat the following types of food?

	Yes	No	Duration	Reason
Milk/Dairy				
Red Meat				
Bread/wheat				
Other				

DEMOGRAPHIC INFORMATION

Gender: Male/Female

Date of Birth: / / Age: _____

Ethnicity: Caucasian/Black/Asian/Other

Jewish background: No
Yes (>1/4 grandparents are Jewish)
Partly (1/4 grandparents are Jewish)

Marital status: Single/Living with partner, unmarried/Married/
Separated/Divorced/Widowed

Children: Do you have children? Yes/No
If yes, please specify:

	Gender	Age	IBD
Child 1	M/F		No/UC/CD
Child 2	M/F		No/UC/CD
Child 3	M/F		No/UC/CD
Child 4	M/F		No/UC/CD
Child 5	M/F		No/UC/CD

Education:
Primary school
Junior/Inter Certificate (15-16 years)
Leaving Certificate (17-18 years)
3rd level education
Postgraduate qualification

Occupation:
Unemployed
Employed
Self-employed
Student
Work in the home
Retired
Other

Current/most recent occupation? _____
Partner's current/most recent occupation? _____
(Socio-economic group: A/B/C/D/E/F/G/H/I/J/Z)

Smoking:

Yes	No	Ex		
	Per day	Started	Stopped	Total years
Smoker				
Ex-smoker				

Alcohol:

Yes No

How much do you drink per week? _____

How many units of alcohol do you drink per week?
(1 unit = ½ pint beer, 1 single (25ml) measure spirit, 1 (100ml) glass of wine)

Physical activity:

Do you take part in regular physical activities (walking, jogging, cycling, swimming >30 minutes or similar activities)?

Daily
Weekly
Monthly
Other please specify: _____

TV watching:

How many hours per day do you spend watching TV/video/DVD etc?
Weekdays
Weekends

HEALTHCARE CONSUMPTION COSTS

(*related to last 12 months*)

Please specify for the last 12 months:

Total number of outpatient clinic visits

Total number of consultations with your GP

Total number of consultations with a consultant
(hospital/private rooms)

Total number of consultations with a surgeon

Total number of consultations with a dietician

Procedures

Have you had any procedures/tests carried out in last 12 months?

Yes No N/A

Procedure	When	Why	In/Out patient

Hospitalisation

Have you been hospitalised in the last 12 months?

Yes No

Admitted	Discharged	Reason	Via A&E	Total Days
			Total Days	

Accident and Emergency Visits

Have you visited an A&E department in the last 12 months?

No Yes

How many times?

Reason: _____

Surgery

Have you had any surgical procedures in the last 12 months?

Yes No N/A

What procedure? _____

Total Parenteral Nutrition

Have you received TPN in the last 12 months?

Yes No N/A

Started	Stopped	Total Days

Days lost from work

During the last 12 months,

Sick days taken	
Days lost due to forced unemployment	
Total days lost	

Disability benefit

Have you ever received a disability benefit due to illness?

Yes No N/A

Data collected by: _____

Signature: _____

Date: _____

CROHN'S DISEASE ACTIVITY INDEX

PART 1 – TO BE FILLED OUT FROM PATIENT DIARY

Question 1.

How many liquid or very soft stools have you passed each of the last seven days?

Day 1	_____
Day 2	_____
Day 3	_____
Day 4	_____
Day 5	_____
Day 6	_____
Day 7	_____

_____ x 2 =

Question 2.

Have you suffered from abdominal pain during the last seven days?

(0 = none, 1 = mild, 2 = moderate, 3 = severe)

Day 1	_____
Day 2	_____
Day 3	_____
Day 4	_____
Day 5	_____
Day 6	_____
Day 7	_____

_____ x 5 =

Question 3.

Describe your general well being over the last seven days?

(0 = generally well, 1= slightly unwell, 2 = poor, 3 = very poor, 4 = terrible)

Day 1	_____
Day 2	_____
Day 3	_____
Day 4	_____
Day 5	_____
Day 6	_____
Day 7	_____

_____ x 7 =

Question 4.

Have you used Imodium, or any other drugs for diarrhoea in the past seven days?

Yes

No

1 x 30 =

0 x 30 =

COMMENTS:

Data collected by: _____

Signature: _____

Date: _____

PART 2 - TO BE FILLED OUT BY THE DOCTOR

Question 5.
How many of the following 6 categories of complications has the patient had in the last seven days?
(one point for each)

- Arthritis/arthralgia
 - Iritis/uveitis
 - Erythema nodosum/pyoderma gangrenosum/aphthous stomatitis
 - Anal fissure, fistula or abscess
 - Other fistula
 - Fever over 37.8C during last week
- _____ x 20 =

Question 6.
Is there any abdominal mass present?

- None
 - Questionable
 - Definite
- 0 x 10 =
2 x 10 =
5 x 10 =

Question 7.
Haematocrit levels

_____ %

for males: 47 – haematocrit level (_____)

for females: 42 - haematocrit level (_____)

_____ x 6 =

Question 8.
Weight

Body weight . kgs

Height . m

Standard weight . kgs

(1- weight/standard weight) x 100 = _____ x 1 =

Calculated CDAI Total = _____

Clinical remission (< 150)

Yes

No

Relapse (> 150)

Yes

No

COMMENTS:

Data collected by: _____

Signature: _____

Date: _____

QUALITY OF LIFE QUESTIONNAIRE

TO BE FILLED OUT BY THE PATIENT ONLY

INTRODUCTION

- This questionnaire is designed to find out how you have been feeling over the past **2 WEEKS** - your symptoms, mood and general well being.
- Please read each question carefully and **CIRCLE THE NUMBER BESIDE THE ANSWER** that comes closest to how you have been feeling. Circle only **ONE** answer for each question.
- Remember there are no right or wrong answers – we are interested in what **YOU** think.
- Don't spend too long on each question - your immediate reaction is usually the most accurate.
- We would like you to answer all questions – please don't leave any blanks.
- Yours answers will be treated in the strictest of confidence – your questionnaire will be identifiable only by a code.

DATE: _____

TOTAL SCORE: _____

1. How frequent have your bowel movements been during the last two weeks?

- 1 Bowel movements as or more frequent than they have ever been
- 2 Extremely frequent
- 3 Very frequent
- 4 Moderate increase in frequency of bowel movements
- 5 Some increase in frequency of bowel movements
- 6 Slight increase in frequency of bowel movements
- 7 Normal, no increase in frequency of bowel movements

2. How often has the feeling of fatigue or of being tired and worn out been a problem for you during the last 2 weeks?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

3. How often during the last 2 weeks have you felt frustrated, impatient or restless?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

4. How often during the last 2 weeks have you been unable to attend school or do your work because of your bowel problem?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

5. How much of the time during the last 2 weeks have your bowel movements been loose?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

6. How much energy have you had during the last 2 weeks?

- 1 No energy at all
- 2 Very little energy
- 3 A little energy
- 4 Some energy
- 5 A moderate amount of energy
- 6 A lot of energy
- 7 Full of energy

7. How often during the last 2 weeks did you feel worried about the possibility of needing to have surgery because of your bowel problem?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

8. How often during the last 2 weeks have you had to delay or cancel a social engagement because of your bowel problem?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

9. How often during the last 2 weeks have you been troubled by cramps in your abdomen?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

10. How often during the last 2 weeks have you felt generally unwell?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

11. How often during the last 2 weeks have you been troubled because of fear of not finding a bathroom / toilet?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

12. How much difficulty have you had, as a result of your bowel problems, doing leisure or sports activities you would have liked to have done during the last 2 weeks?

- 1 A great deal of difficulty; activities made impossible
- 2 A lot of difficulty
- 3 A fair bit of difficulty
- 4 Some difficulty
- 5 A little difficulty
- 6 Hardly any difficulty
- 7 No difficulty; the bowel problems did not limit sports or leisure activities

13. How often during the last 2 weeks have you been troubled by pain in the abdomen?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

14. How often during the last 2 weeks have you had problems getting a good night's sleep, or been troubled by waking up during the night?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

15. How often during the last 2 weeks have you felt depressed or discouraged?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

16. How often during the last 2 weeks have you had to avoid attending events where there was no bathroom / toilet close at hand?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

17. Overall, in the last 2 weeks, how much of a problem have you had with passing large amounts of gas?

- 1 A major problem
- 2 A big problem
- 3 A significant problem
- 4 Some trouble
- 5 A little trouble
- 6 Hardly any trouble
- 7 No trouble

18. Overall, in the last 2 weeks, how much a problem have you had maintaining or getting to, the weight you would like to be at?

- 1 A major problem
- 2 A big problem
- 3 A significant problem
- 4 Some trouble
- 5 A little trouble
- 6 Hardly any trouble
- 7 No trouble

19. Many patients with bowel problems often have worries and anxieties related to their illness. These include worries about getting cancer, worries about never feeling any better, and worries about having a relapse. In general, how often during the last 2 weeks have you felt worried or anxious?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

20. How much of the time during the last 2 weeks have you been troubled by a feeling of abdominal bloating?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

21. How often during the last 2 weeks have you felt relaxed and free of tension?

- 1 None of the time
- 2 A little of the time
- 3 Some of the time
- 4 A good bit of the time
- 5 Most of the time
- 6 Almost all of the time
- 7 All of the time

22. How much of the time during the last 2 weeks have you had a problem with rectal bleeding with your bowel movements?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

23. How much of the time during the last 2 weeks have you felt embarrassed as a result of your bowel problem?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

24. How much of the time during the last 2 weeks have you been troubled by a feeling of having to go to the bathroom even though your bowels were empty?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

25. How much of the time during the last 2 weeks have you felt tearful or upset?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

26. How much of the time during the last 2 weeks have you been troubled by accidental soiling of you underpants?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

27. How much of the time during the last 2 weeks have you felt angry as a result of your bowel problem?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

28. To what extent has your bowel problem limited sexual activity during the last 2 weeks?

- 1 No sex as a result of the bowel disease
- 2 Major limitation as a result of bowel disease
- 3 Moderate limitation as a result of bowel disease
- 4 Some limitation as a result of bowel disease
- 5 A little limitation as a result o bowel disease
- 6 Hardly any limitation as a result of bowel disease
- 7 No limitation as a result of bowel disease

29. How much of the time during the last 2 weeks have you been troubled by nausea or feeling sick to your stomach?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

30. How much of the time during the last 2 weeks have you felt irritable?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

31. How often during the past 2 weeks have you felt a lack of understanding from others?

- 1 All of the time
- 2 Most of the time
- 3 A good bit of the time
- 4 Some of the time
- 5 A little of the time
- 6 Hardly any of the time
- 7 None of the time

32. How satisfied, happy, or pleased have you been with your personal life during the past 2 weeks?

- 1 Very dissatisfied, unhappy most of the time
- 2 Generally dissatisfied, unhappy
- 3 Somewhat dissatisfied, unhappy
- 4 Generally satisfied, pleased
- 5 Satisfied most of the time, happy
- 6 Very satisfied most of the time, happy
- 7 Extremely satisfied, could not have been more happy or pleased

HOSPITAL ANXIETY & DEPRESSION

SCALE

Instructions: *Please read each item and tick the reply that comes closest to how you have been feeling in the past **WEEK**. Don't take too long over your replies - your immediate reaction is probably more accurate than a long thought-out response.*

1) I feel tense or "wound up":

Most of the time

A lot of the time

From time to time, occasionally

Not at all

2) I still enjoy the things I used to enjoy:

Definitely as much

Not quite so much now

Definitely not so much now

Not at all

3) I get a sort of frightened feeling as if something awful is about to happen:

Very definitely and quite badly

Yes, but not too badly

A little, but it doesn't worry me

Not at all

4) I can laugh and see the funny side of things:

As much as I always could

Not quite as much now

Definitely not as much now

Not at all

5) Worrying thoughts go through my mind:

A great deal of the time

A lot of the time

From time to time but not that often

Only occasionally

6) I feel cheerful:

Not at all

Not often

Sometimes

Most of the time

7) I can sit at ease and feel relaxed:

Definitely

Usually

Not often

Not at all

8) I feel as if I am slowed down:

Nearly all the time
Very often
Sometimes
Not at all

9) I get a sort of frightened feeling like "butterflies" in the stomach:

Not at all
Occasionally
Quite often
Very often

10) I have lost interest in my appearance:

Definitely
I don't take so much care as I should
I may not take quite as much care
I take just as much care as ever

11) I feel restless as if I have to be on the move:

Very much indeed
Quite a lot
Not very much
Not at all

12) I look forward with enjoyment to things:

As much as I ever did
Rather less than I used to
Definitely less than I used to
Hardly at all

13) I get sudden feelings of panic:

Very often indeed
Quite often
Not very often
Not at all

14) I can enjoy a good book or radio or TV programme:

Often
Sometimes
Not often
Very seldom

TOTAL SCORE: Anxiety

Depression

SHORT FOOD QUESTIONNAIRE

This form asks about your *usual* eating habits over the past year

Listed down the left hand side are different foods/drinks.

For each food/drink there are 2 questions to answer:

1) How often do you eat it?

For each food listed, mark the column to show how often, on average, you ate the food during the past year (ranging from never/less than once per month to 2+ times per day)

- Please BE CAREFUL which column you put your answer in.
- Please DO NOT SKIP any foods. If you never eat a food/drink, mark “Never or less than once a month.”

2) How much do you eat?

Mark whether you usually have a small, medium or large serving.

An estimate of a medium portion size is given for each food. If this is about what you normally eat, then mark the medium column.

A small serving is about 1/2 the medium serving size given, or less. If this is your normal portion, mark the small serving column.

A large serving is about one-and-1/2 times the medium serving size shown, or more. If this is your normal portion, mark the large serving column.

Sample: This person ate a medium serving of rice about twice per month and never ate baked beans.

HOW OFTEN										HOW MUCH			
Type of Food	Never or Less than Once Per Month	1 Per Month	2-3 Per Month	1 Per Week	2 Per Week	3-4 Per Week	5-6 Per Week	1 Per Day	2+ Per Day	Medium Serving	Your Serving Size		
											S	M	L
Rice			X							¾ cup		X	
Baked beans	X									½ cup			

Type of Food	HOW OFTEN										HOW MUCH		
	Never or Less than Once Per Month	1 Per Month	2-3 Per Month h	1 Per Week	2 Per Week	3-4 Per Week	5-6 Per Week	1 Per Day	2+ Per Day	Medium Serving	Your Serving Size		
											S	M	L
Broccoli										½ cup			
Green vegetables (other than broccoli)										½ cup			
Pizza, including take-away pizza										2 slices			
Spaghetti, lasagna, other pasta with tomato sauce										1 cup			
Mixed dishes with cheese (omelette, pasta dishes etc)										1 cup			
Cheeses and cheese spreads (including on sandwiches, burgers, wraps; not including cottage cheese)										2 slices/ 2oz/50g			
White bread including sandwiches, bagels, burger rolls, scones etc										2 slices			
Brown bread (wheat, rye, granary) including sandwiches, rolls, scones etc										2 slices			
Biscuits or muffins										2 biscuits /average muffin			
Ice cream										1 scoop/ ½ cup			
Yogurt, frozen yogurt										1 average carton			
Milk on cereal										½ cup			
Oysters										6 medium			
Shrimp										3 oz/ 80g			
Pink salmon										3 oz / 80g			

Tuna, tuna salad, tuna casserole										½ cup			
Liver, including chicken livers										4 oz/110g			
Eggs										1 egg = small 2 eggs = med 3 eggs = large			
High fibre cereals (Shredded wheat, Weetabix, Branflakes etc)										1 average sized bowl			

HOW OFTEN										HOW MUCH			
Type of Beverage	Never or Less than Once Per Month	1-3 Per Mon	1 Per Week	2-4 Per Week	5-6 Per Week	1 Per Day	2-3 Per Day	4-5 Per Day	6+ Per Day	Medium Serving	Your Serving Size		
											S	M	L
Full-fat milk, on its own or used to make milky drinks (<u>not</u> on cereal or in tea/coffee)										8oz. Glass/ 250mls			
Semi-skimmed milk, on its own or used to make milky drinks (<u>not</u> on cereal or in tea/coffee)										8oz. Glass/ 250mls			
Skimmed milk, on its own or used to make milky drinks (<u>not</u> on cereal or in tea/coffee)										8 oz. Glass/ 250mls			
Milk in tea/coffee										1/5 cup			