

Nutrition Therapy for Active Crohn's Disease

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Abstract

Exclusive enteral nutrition (EEN) is a first line treatment for active paediatric Crohn's disease (CD) but is used less frequently in adult patients. A meta-analysis of six controlled trials in adults found EEN to be less efficacious than corticosteroids at inducing CD remission. In contrast, paediatric studies have found EEN to be an effective treatment. Paediatric EEN regimens often use a polymeric nutrition formula provided orally; this regimen has not been studied in a cohort of adults with active CD.

This research aimed to investigate patient acceptability of EEN, the efficacy of EEN and a sequential EEN and partial enteral nutrition (PEN) regimen in young adults, and the impact of these treatments on disease activity, inflammatory and nutrition markers, quality of life and the gut microbiota. This research also aimed to investigate health professional awareness and perception of EEN in the treatment of CD.

Thirty five patients with CD attending hospital outpatient appointments were surveyed. Patients blind tasted an elemental and a polymeric formula then ranked them on measures of palatability. Patients were also asked whether they would consider using EEN, if it could induce disease remission, to treat active CD. Patients rated polymeric formula more palatable and the majority of patients would consider using EEN to treat moderate to severe CD symptoms.

Two prospective non-randomised clinical trials of eight weeks of EEN or a sequential EEN and PEN regimen were undertaken. Thirty-eight patients aged 16 to 40 years old with active CD were recruited. Twenty-five patients started EEN of which 17 (68 %) responded or achieved disease remission. Thirteen patients started PEN and eight (62 %) responded or achieved disease remission. There were significant improvements in serum CRP and insulin

like growth factor-1. EEN or PEN treatment was completed by 23/38 (61 %) and resulted in significant reductions in anxiety and depression and improvements in quality of life.

Faecal samples were collected prior, during and after EEN or PEN treatment. The faecal microbiota of six patients who used EEN was characterised. EEN treatment shifted the phylogenetic structure of the faecal microbiota and changes in the abundance of specific species were observed.

An electronic survey was completed by 58 dietitians and 42 gastroenterologists working in New Zealand. Most dietitians and gastroenterologists had limited experience using EEN but believe EEN is an appropriate treatment option for some adult CD patients. Health professionals would like more guidance on how to use EEN and more evidence to support its use with adults.

Based on the findings in this thesis, EEN effectively induces disease remission in young adults with active CD and its use should be considered within the profession and presented as an option to patients. There is also a need for further research investigating therapies, such as PEN, which may be more appealing and efficacious for a greater number of adult patients. Patients who are interested in an alternative to corticosteroids should be offered nutrition therapy with the support of an IBD multidisciplinary team.

Preface and Acknowledgements

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List of Abbreviations

App	Application
AuSPEN	Australasian Society of Parenteral and Enteral Nutrition
BIA	Bioelectrical impedance analysis
BLAST	Basic local alignment search tool (microbial database)
BMI	Body mass index
BMR	Basal metabolic rate
CD	Crohn's disease
CDAI	Crohn's disease activity index
CHL	Canterbury Health Laboratories
CHO	Carbohydrate
CI	Confidence interval
CRP	C-reactive protein
CS	Corticosteroids
DGGE	Denaturing temperature gradient gel electrophoresis
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra acetic acid
EEN	Exclusive enteral nutrition
EER	Estimated energy requirement
EF	Elemental formula
ELISA	Enzyme-linked immunosorbent assay
EN	Enteral nutrition

ESR	Erythrocyte sedimentation rate
FC	Faecal calprotectin
GI	Gastrointestinal
HADS	Hospital anxiety and depression scale
HADS-A	Hospital anxiety and depression scale – anxiety subscale
HADS-D	Hospital anxiety and depression scale – depression subscale
HBI	Harvey Bradshaw Index
HC	Healthy control
HRQOL	Health related quality of life
HBI	Harvey Bradshaw Index
IBD	Inflammatory bowel disease
IBDQ	Inflammatory bowel disease questionnaire
IBS	Irritable bowel syndrome
IGF-1	Insulin-like growth factor 1
IL	Interleukin
ITT	Intention to treat
IU	International unit
kcal	Kilocalorie
LOCF	Last observation carried forward
M	Molar
MRI	Magnetic resonance imaging
N/A	Not applicable

NCBI	National Center for Biotechnology Information
NDT	Nasoduodenal tube
NE	Niacin equivalents
NGT	Nasogastric tube
nm	nanometre
NZ	New Zealand
NZSG	New Zealand Society of Gastroenterology
OPG	Osteoprotegerin
OR	Odds ratio
OTU	Operational taxonomic unit
PBS	Phosphate buffered saline
PCDAI	Paediatric Crohn's disease activity index
PCR	Polymerase chain reaction
PEN	Partial enteral nutrition
PP	Per protocol
QIIME	Quantitative Insights Into Microbial Ecology
RIA	Radioimmunoassay
RANK	Receptor activator of NF- κ B
RANKL	Receptor activator of NF- κ B ligand
RE	Retinol equivalents
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid

rpm	Revolutions per minute
SD	Standard deviation
SDS	Standard deviation score
SEN	Supplementary enteral nutrition
PF	Polymeric formula
PHARMAC	Pharmaceutical Management Agency
SCFA	Short chain fatty acids
SIBDQ	Short inflammatory bowel disease questionnaire
TE	Tocopherol equivalents
TGGE	Temperature gradient gel electrophoresis (and TGGE)
TGN	Thioguanine nucleotide
TNF	Tumour necrosis factor
TPMT	Thiopurine S-methyltransferase
TRAIL	TNF-related apoptosis inducing ligand
T-RLFP	Terminal restriction fragment length polymorphism
UC	Ulcerative colitis
UK	United Kingdom
UVB	Ultraviolet B
USA	United States of America
VHAI	Van Hees activity index
% TE	Percentage of total energy

Chapter 1

Introduction

1.1 Crohn's disease

Crohn's disease (CD) and ulcerative colitis (UC) are inflammatory bowel diseases (IBD) characterised by recurring and remitting inflammation, which leads to chronic symptoms such as diarrhoea, abdominal pain and rectal bleeding.⁽¹⁾ CD, unlike UC, can affect the lining of the gastrointestinal tract anywhere from the mouth to the anus but most commonly disease is isolated to the terminal ileum, the colon, or both the ileum and the colon. CD presents as transmural inflammation, which means that it may affect the whole depth of the intestinal lining, not just the mucosa, as seen in UC. In addition, CD may be present in multiple locations in the bowel with healthy sections of bowel separating them, which is referred to as skip lesions. There is currently no known cure for CD or UC and therefore current treatments are focused on minimising inflammation and extending periods of disease remission.

CD can be diagnosed at any age, however the peak age of diagnosis is between 15 and 30 years of age, leading to many years of disease and associated morbidity. A decade ago Canterbury, New Zealand (NZ) had one of the highest incidence rates of CD in the world at 16.5/100,000/year.⁽²⁾ The incidence and prevalence of IBD is increasing world-wide⁽³⁾ and NZ is not immune from this trend. In 2014 the annual incidence of CD in Canterbury, NZ had increased 1.5 fold in a decade to 26.0 per 100,000.⁽⁴⁾ The drivers of this increasing incidence of disease are not yet fully understood but are hypothesised to be a complex interaction between genetic factors, the gut microbiome, the western diet and industrialism, to name a few.^(3, 5)

People with CD commonly also suffer from poor nutritional status during periods of active and inactive disease.⁽⁶⁾ Optimal management of CD requires a multidisciplinary approach involving a gastroenterologist and/or surgeon, specialist gastroenterology nurses, dietitians, psychologists and other specialties.⁽⁷⁾ In NZ, patients are often diagnosed and managed by a specialist gastroenterologist in a tertiary centre. However, dietary outpatient care may be provided by dietitians in tertiary centres or those working in provincial centres. The role of UK paediatric and adult dietitians in the management of IBD patients has been well documented,^(8, 9) but the role(s) of NZ dietitians in the management of CD patients is not defined in the literature.

1.2 Treatment of Crohn's disease

The treatment of CD often involves a multi-pronged approach including therapies to treat active disease and therapies to maintain disease remission. The most common therapies are described in the following paragraphs.

1.2.1 Immunomodulatory Medications

1.2.1.1 Corticosteroids

The standard first line treatment in adults with active CD is corticosteroid (CS) therapy, which is effective at inducing remission in approximately 60 – 68 % of patients.⁽¹⁰⁾ CS suppress the immune response in the intestines thereby reducing acute inflammation. However, CS therapy has many well-documented acute side-effects and numerous long term adverse effects due to repeated or continual use.⁽¹¹⁾ Also, CS resistance can occur in 8-22% of patients and CS dependency occurs in 15-36% of patients.⁽¹²⁾ Alternative therapies that can effectively induce disease remission whilst avoiding the short and long term side effects of CS are desirable for patients and treatment providers.

1.2.1.2 Thiopurines

Maintenance of disease remission is often managed with immunosuppressant thiopurine medications (Azathioprine, 6-Mercaptopurine). These medications are prescribed in varying doses depending on the individual's thiopurine S-methyltransferase (TPMT) enzyme activity and serum thioguanine nucleotide (TGN) metabolites. Side effects mostly occur soon after initiating the treatment and may include nausea, vomiting and anaemia. As with all immunosuppressant medications, patients are more susceptible to infections.

1.2.1.3 Biologics

Biologic therapy can be used to induce and maintain disease remission. In NZ, subcutaneous adalimumab (trade name Humira) and intravenous infliximab (trade name Remicade) are Pharmaceutical Management Agency (PHARMAC) funded for patients who have refractory disease despite trying standard therapies. These medications inhibit the action of a key cytokine in the inflammatory process: tumour necrosis factor (TNF)- α . Their use is associated with improved quality of life⁽¹³⁾ and greater rates of mucosal healing, which is considered the optimal outcome for CD therapy and thought to improve the disease course.⁽¹⁴⁾ However, the patient needs to take the medication regularly and 23-46 % of patients will lose response to the medication after one year.⁽¹⁵⁾ Research into new biological treatments and combination therapy with immunosuppressant medications is on-going.

1.2.2 Nutrition Therapy

The most common nutritional therapy used to treat active CD is exclusive enteral nutrition (EEN). EEN is the ingestion of an elemental or polymeric nutritional formula for a period of four to eight weeks along with the exclusion of solid and liquid foods and drinks apart from water. EEN treatment protocols may also allow ice blocks, boiled sweets and black tea and coffee, and flavourings may be added to the nutrition formula.⁽¹⁶⁾

The replacement of usual foods and fluids with an liquid formula was initially used to rest the gut and thought to improve inflammatory markers and clinical symptoms by reducing the exposure of the intestines to foreign proteins.⁽¹⁷⁾ This may in part be the case but it is now also known that there is/are active ingredients in enteral formula which exert their effect via a number of mechanisms. *In-vitro* studies show the elemental and polymeric formula have positive effects on intestinal tissue cytokine response^(18, 19) and reduce the permeability of the gut lining.⁽²⁰⁾ *In vivo* studies of EEN in paediatric⁽²¹⁾ and adult⁽²²⁾ cohorts have confirmed *in-vitro* findings. Patients treated with EEN achieve reductions of pro-inflammatory cytokines which were associated with histologic and endoscopic healing of the gut.^(21, 22) Mucosal healing consequent to EEN have been confirmed in a subsequent study where EEN was found to result in higher rates of mucosal healing than corticosteroid treatment.⁽²³⁾ EEN treatment also results in significant changes to the intestinal microbiome,^(24, 25) although the impact of these changes on intestinal inflammation is not yet fully understood.

In children, EEN has been shown to be an effective and feasible alternative to CS⁽²⁶⁾. In addition to avoiding the adverse effects of CS exposure, EEN provides additional benefits over and above those provided by CS. Consequently, EEN is now recommended as a first line therapy for children newly diagnosed with CD and is often used to treat a disease flare in children with existing disease.⁽²⁷⁾ EEN therapy is associated with higher rates of mucosal healing,⁽²⁸⁾ changes in the intestinal microbiome,⁽²⁴⁾ greater weight gain,⁽²⁹⁾ improved vitamin D status,⁽³⁰⁾ enhanced bone turnover,⁽³¹⁾ an early rise in the nutrition marker IGF-1,⁽³²⁾ and better quality of life after treatment.⁽³³⁾ The administration of supplementary enteral nutrition (SEN) once disease remission is achieved has also been shown to be beneficial in maintaining remission compared with a free diet in children⁽³⁴⁾ and Japanese adults.⁽³⁵⁾

However, in adult CD populations, EEN is generally not viewed as a first line therapy for newly diagnosed CD or those with a flare of pre-existing CD. Many clinical guidelines^(36, 37) only recommend EEN if a patient declines drug therapy or as an adjunctive therapy to

support nutrition, rather than as a primary therapy. These guidelines are primarily based on the 2007 Cochrane systematic review of six randomised controlled trials,⁽³⁸⁾ which found a pooled OR of 0.33 (95 % CI: 0.21-0.53) in favour of CS and concluded that CS were superior to EEN in the induction of remission of disease. The use of EEN with adult populations will be discussed in more detail in section 1.4.

1.2.3 Surgical Treatment

Medical treatments are the first line approach for CD but when these fail or cannot provide adequate resolution of symptoms, surgical intervention is required. Surgery is not a curative procedure for CD and recurrence of disease post-surgery is common. Population-based studies show that 30 – 50 % of patients will require surgery within 10 years of diagnosis.⁽³⁹⁾ There is some evidence to suggest that since the advent of biological treatments rates of surgery have decreased, however the evidence is not yet conclusive.⁽³⁹⁾ It is beyond the scope of this thesis to provide an in-depth discussion of surgery for CD but in general, current surgical practice tends to target the most inflamed part of the bowel rather than removing all diseased parts of the bowel.⁽³⁶⁾

1.3 Holistic Measures of Treatment Success

The success of CD treatments can be assessed by objective and subjective means. The following sections introduce some of the measures typically used in practice, and research, to measure the extent of disease and the success of CD treatments on, not only objective measures of disease, but subjective measures too.

1.3.1 Nutritional Status

Malnutrition is associated with poorer outcomes and poorer quality of life.⁽⁴⁰⁾ The prevalence of malnutrition in patients with CD is high. In a large American study of 50,000 hospitalised patients with IBD the adjusted odds ratio for malnutrition was 5.57 [95 % CI: 5.29 – 5.86] compared to non-IBD hospitalised patients.⁽⁴¹⁾ The aetiology of malnutrition in CD is multifactorial and summarised in Table 1.1.⁽⁴²⁾

The United Kingdom and Australian IBD standards of care both recommend the inclusion of a specialist gastroenterology dietitian in the IBD multidisciplinary team.^(7, 43) However, an international survey of IBD centres suggests currently that dietitian involvement in the IBD team is infrequent.⁽⁴⁴⁾ A 2014 audit of UK IBD centres found that 23 % of centres did not have access to a specialist dietitian.⁽⁹⁾ Improving patient access to a specialist dietitian provides patients with an opportunity to address dietary and nutrition concerns and utilise dietary treatments such as EEN.

Table 1.1. Causes of malnutrition in Inflammatory Bowel Disease.⁽⁴²⁾

Associated with malnutrition	Potential causes of associations
Decrease in oral intake	Restrictive diets, therapeutic fasting By the disease itself: diarrhoea, abdominal pain, nausea and vomiting, etc. Alteration in taste: due to drugs, vitamin and mineral deficiencies, pro-inflammatory mediators Anorexigenous effect of pro-inflammatory mediators
Gastrointestinal losses	Diarrhoea Rectorrhagia/haematochezia Loss of mucus and electrolytes Protein-losing enteropathy
Metabolic disorders	Increase in resting energy expenditure Enhanced fat oxidation
Increase in nutritional requirements	Inflammatory states Increased basal oxidative metabolism Infectious complications Post-surgery
Drug interaction	Corticoids and calcium reabsorption Corticoids and protein catabolism Sulfasalazine and folates Methotrexate and folates Cholestyramine and liposoluble vitamins Antimicrobials and vitamin K Anti-secretors and iron
Poor absorption of nutrients	Reduction in absorptive surface: intestinal resection, enteric fistulas, hypertrophy of the villi, blind loops, bacterial overgrowth Poor absorption of bile salts in ileitis or resection

1.3.1.1 Serum Albumin

Serum albumin is commonly used as a marker of nutrition status in clinical practice.

Albumin is a readily available measure but is not a reliable marker of malnutrition.^(45, 46)

Albumin is an acute phase protein, which decreases in response to inflammation, therefore, during active disease albumin may be falsely low.⁽⁴⁰⁾

1.3.1.2 Body Mass Index

Body mass index (BMI) is also commonly used a marker of nutrition status in clinical practice. BMI is calculated by dividing body weight (kg) by height (m) squared ($BMI = \text{kg/m}^2$). BMI measurements are readily available but do not provide a reliable indication of malnutrition.^(45, 46) BMI is a ratio of weight to height and does not take into account the ratio of lean body mass to fat mass therefore, is not a sensitive marker of malnutrition in overweight patients or patients with oedema.⁽⁴⁵⁾ Alternatively, lean body mass measurements may provide more accurate and functional information on nutritional status but lean body mass measurement methods are either very expensive, in the case of whole-body magnetic resonance imaging (MRI), or have their own methodological limitations in the case of bioelectrical impedance analysis (BIA).^(40, 45)

1.3.1.3 Serum Insulin-like Growth Factor -1

Insulin-like growth factor -1 (IGF-1) is a novel serum marker of nutrition status. It is a hormone predominantly produced by the liver. The production of IGF-1 varies with age and sex and may also be affected by nutrition intake, body composition and inflammation.⁽⁴⁷⁾ In paediatric cohorts, EEN treatment has been associated with an early rise in IGF-1, which could be suggestive of an anti-inflammatory effect as well as improvement in nutritional intake.⁽⁴⁸⁾ Serum IGF-1 has not been investigated in adults using EEN therapy.

1.3.1.4 Serum Vitamin D (25-OHD)

Synthesis of Vitamin D in the skin is reliant on exposure to ultraviolet B (UVB) radiation from the sun. Christchurch is located at latitude 43.5321° S and longitude 172.6362° E which means that during the winter months the sun is far away and there is limited opportunity for the population to synthesise vitamin D. The 2008/09 NZ National Nutrition Survey found that a third of the sample population had insufficient serum vitamin D (25-OHD), less than 50 nmol/L, and that at the end of Winter the serum vitamin D concentrations of New Zealanders in the South Island of NZ were lower than New Zealanders living in the north of NZ.⁽⁴⁹⁾ Patients with CD living in Dunedin, a city further south than Christchurch, have a high incidence of insufficient serum vitamin D.⁽⁵⁰⁾ The serum vitamin D status of patients living in Christchurch has not previously been reported. It has been suggested that low serum vitamin D is associated with disease activity,⁽⁵¹⁾ immune response⁽⁵²⁾ and that vitamin D status may affect response to treatments.⁽⁵³⁾ However, further research is needed to confirm these initial findings.

1.3.1.5 Serum Iron Status

Iron deficiency anaemia commonly occurs in patients with CD with negative effects on quality of life.⁽⁵⁴⁾ Patients at risk of iron deficiency anaemia are pre-menopausal women with poor dietary iron intake,⁽⁴⁵⁾ patients with active disease and presence of chronic inflammation.⁽⁵⁴⁾ Serum ferritin is usually used as marker of iron stores in healthy populations but ferritin is an acute phase protein and therefore can be falsely elevated during periods of inflammation.⁽⁵⁵⁾

1.3.2 Disease Activity Indices

There are three main validated clinical and composite measures of disease activity: Crohn's Disease Activity Index (CDAI), Harvey Bradshaw Index (HBI) and Van Hees Activity index (VHAI). The VHAI is calculated using serum albumin and erythrocyte sedimentation rate, body mass index, abdominal mass, gender, fever, loose stools, bowel resection and CD

complications. The VHAI correlates moderately ($r = 0.67$) with the CDAI⁽⁵⁶⁾ and disease remission is usually defined as a score of less than 120.

The HBI is a simple disease activity measure, which is calculated from a one day history of general well-being, abdominal pain, loose stools and presence of abdominal mass and CD complications.⁽⁵⁷⁾ Clinical disease remission is usually defined as an HBI of less than five.⁽⁵⁷⁾ The HBI correlates well with the CDAI ($r = 0.8$)⁽⁵⁷⁾ and, for this reason, is often used as an activity index in place of the more burdensome CDAI.

The CDAI score combines a seven day history of patient reported subjective symptoms of general well-being, abdominal pain, loose stools with the objective measures of disease including the presence of abdominal mass and CD complications, anti-diarrhoeal use, haematocrit and weight.⁽⁵⁸⁾ Clinical remission is usually defined as a CDAI of less than or equal to 150 points⁽⁵⁹⁾ whereas, clinical response, as a secondary endpoint, is defined as a fall in CDAI of 70 – 100 points.⁽⁵⁹⁾ The CDAI is the activity index that is recommended for use in medical clinical trials by the Clinical Trials Task Force of the International Organization of Inflammatory Bowel Disease.⁽⁵⁹⁾

One the limitations of all of these disease activity scores is that they do not correlate well with objective measures of disease activity such as endoscopic and histology findings.^(60, 61) The development of more accurate surrogate markers of intestinal inflammation is desirable.

1.3.3 Biomarkers of Gut Inflammation

Ileocolonoscopy with biopsies is the gold standard to assess the degree of disease activity or intestinal inflammation but it is an invasive and expensive procedure. In clinical practice, biomarkers are used as a non-invasive and less expensive measure of intestinal inflammation

in comparison to invasive procedures. There are many biomarkers of inflammation (Table 1.2) but the following sections summarise the literature on the most prevalent existing biomarkers and a novel faecal biomarker of CD activity.

Table 1.2. Biomarkers of gut inflammation.

Biomarker	Attributes of biomarker
Serum CRP > 5 mg/L	Sensitive but non-specific acute phase protein ^(62, 63)
Serum ESR > 10 or 15 mm/hr	Changes more slowly than CRP, concentration may differ depending on location of disease ⁽⁶²⁾
Faecal calprotectin > 50 µg/g	Increasingly used in practice. Non-specific protein from many sources including neutrophil accumulation at intestinal inflammation ⁽⁶⁴⁾
Faecal S100A12 > 10 mg/kg ⁽⁶⁵⁾	Used mainly in research setting. Neutrophil derived protein and sensitive measure of disease activity ⁽⁶⁶⁾
Faecal lactoferrin > 7 – 10 µg/mL	Neutrophil derived protein, marker of intestinal inflammation ⁽⁶⁷⁾
Faecal OPG > 62.5 pg/mL	Member of TNF receptor family, associated with moderate to severe intestinal inflammation ⁽⁶⁸⁾

Note: CRP, C – reactive protein; ESR, erythrocyte sedimentation rate; OPG, osteoprotegerin; TNF, tumour necrosis factor

1.3.3.1 Serum C - Reactive Protein

In clinical practice, serum C – reactive protein (CRP) is the most commonly used biomarker of gut inflammation in IBD. It is readily available and relatively inexpensive. CRP is an acute phase protein, which increases rapidly with inflammation and decreases quickly once inflammation has resolved.⁽⁶²⁾ CRP is a sensitive marker in the diagnosis of CD and it correlates well with active CD.^(62, 63) However, there are individual variations in CRP response and some patients with active disease may have a low CRP.⁽⁶³⁾ Serum CRP has been investigated as a predictor of CD relapse and some studies have shown that it predicts disease relapse whereas other studies have not.^(62, 63)

One of the main limitations of CRP is that it is not a specific marker of CD inflammation. As an acute phase inflammatory protein, CRP increases in response to inflammation outside, as well as inside, the gastrointestinal tract. The desire for more specific markers of CD inflammation has resulted in the investigation of faecal makers of inflammation.

1.3.3.2 Faecal Calprotectin

Faecal calprotectin (FC) is commonly used as a biomarker of intestinal inflammation.

Calprotectin (S100A8/S100A9) is a member of the calcium binding S-100 protein family and is found in many cells in the body including epithelial cells. It was coined ‘calprotectin’ due to its ability to bind calcium and its antimicrobial protective characteristics.⁽⁶⁹⁾ Calprotectin is involved in many biological processes, but in CD, it is important in the regulation of the inflammatory process.⁽⁷⁰⁾

Calprotectin is a constituent of neutrophil cytoplasm and is present on the membranes of monocytes and acute phase macrophages.⁽⁶⁴⁾ During inflammation, neutrophils accumulate at the site of intestinal inflammation, at which point calprotectin is released into the gut lumen and ultimately excreted in faeces.⁽⁶⁴⁾

In clinical practice, FC may be used as a diagnostic tool to differentiate between IBD and non-inflammatory conditions such as irritable bowel syndrome (IBS). A systematic review of the utility of FC to distinguish between IBS and IBD found that a cut-off of 50 µg/g had a sensitivity of 93 % and specificity of 94 % in adult populations.⁽⁷¹⁾ FC is also used as a non-invasive biomarker of intestinal inflammation in patients with IBD.⁽⁷²⁾ FC has been shown to be positively correlated with endoscopic disease activity.⁽⁷³⁻⁷⁶⁾ However, a FC of less than 50 µg/g of stool does not exclude the presence of intestinal inflammation.^(67, 76)

1.3.3.3 Faecal Osteoprotegrin

Osteoprotegerin (OPG) is another potential biomarker of intestinal inflammation. OPG is a member of the TNF receptor family and is expressed in many cells in the body including colonic epithelial cells.⁽⁷⁷⁾ OPG can be measured in serum, intestinal cells and faecal samples, although faecal OPG is not stable in stool at room temperature and samples need to be frozen soon after collection to avoid OPG decay.⁽⁷⁸⁾

OPG has been widely researched in the context of bone health and has been shown to promote bone maintenance. In bone, OPG is a decoy receptor for receptor activator of NF-κB (RANK) and binds RANK ligand (RANKL) thus, inhibiting the formation of osteoclasts and therefore inhibiting bone breakdown.⁽⁷⁹⁾ However in IBD it appears that OPG has a pro-inflammatory role in intestinal inflammation.

In vitro research has shown that OPG binds TNF-related apoptosis inducing ligand (TRAIL) and thus inhibits dendritic cell and T cell apoptosis.⁽⁸⁰⁾ In inflamed epithelial cell lines OPG expression has been shown to be increased⁽⁷⁷⁾ and the presence of OPG in cell culture media increased gut permeability and IL-8 cytokine levels.⁽⁸¹⁾

In vivo, OPG has been shown to be associated with moderate to severe CD inflammation.⁽⁶⁸⁾

In a cohort of 82 children with CD and 45 healthy children, serum OPG was elevated in moderate to severe CD but not mild disease however, mucosal OPG was not significantly elevated in biopsy samples compared with control biopsies. Faecal OPG was also elevated in moderate to severe CD but also in cases of mild disease compared to controls.

Notwithstanding this no significant correlation was found between faecal, serum and mucosal OPG nor with paediatric CD disease activity index scores.⁽⁶⁸⁾

The study by Nahidi et al⁽⁶⁸⁾ also assessed change in faecal OPG in ten children subsequent to treatment with EEN. Faecal OPG fell significantly with EEN treatment but levels were still significantly elevated compared with healthy children at treatment completion.

OPG is not currently used in clinical practice as a marker of intestinal inflammation and there is no data available concerning OPG concentrations in adults with IBD.

1.3.4 Health Related Quality of Life Measures

CD is a chronic disease in which the disease symptoms and treatments can have significant impacts on many facets of life. Health related quality of life (HRQOL) measures have been developed to evaluate the impact of diseases and their treatments on physical, emotional and psychosocial functioning.⁽⁸²⁾ HRQOL has been shown to be lower in patients with CD compared to the general population,^(83, 84) and the impact of CD on patient quality of life may be underestimated by physicians.⁽⁸³⁾

Multiple clinical and treatment related determinants of HRQOL scores have been identified. A systematic review and meta-analysis by van der Have et al⁽⁸⁵⁾ concluded that HRQOL is multifactorial and that poor HRQOL is associated with active disease, hospitalisations,

corticosteroid treatment and work disability.⁽⁸⁵⁾ Whereas, better HRQOL scores were associated with biological treatments.⁽⁸⁵⁾ Since this review, further studies have shown improved HRQOL subsequent to biological treatments⁽¹³⁾ and also subsequent to nutritional therapy with EEN.^(33, 86)

A recent review of HRQOL assessment tools identified ten different questionnaires that have been validated for use with IBD patients.⁽⁸⁷⁾ It is beyond the scope of this thesis to discuss all of these measures, however two of the most commonly used measures in IBD research, the Hospital Anxiety and Depression Scale (HADS) and the Inflammatory Bowel Disease Questionnaire (IBDQ), have been reviewed.

1.3.4.1 Hospital Anxiety and Depression Scale

In 1982 a patient self-assessment tool (HADS) was proposed to estimate the presence of depression and anxiety in patients attending non-psychiatric hospital clinic appointments.⁽⁸⁸⁾ Since then the HADS has been administered in many languages,^(84, 89-91) with patients of varying age,^(90, 92) medical conditions,^(90, 91) and with healthy populations.^(89, 90, 92)

The HADS is composed of 14 questions that screen for emotional stress, depression and anxiety. The presence of anxiety and depression are scored separately: possible scores range from 0 to 21. A score of eight to ten suggests borderline anxiety or depression and a score of 11 or more suggests the presence of moderate to severe anxiety or depression.⁽⁸⁸⁾ The sensitivity and specificity of the HADS to identify cases of anxiety and depression is approximately 0.80.⁽⁹³⁾

Previous research has shown that patients with IBD have higher rates of anxiety and depression than the general population.^(94, 95) Table 1.3 summarises the recent literature that has assessed anxiety and depression using the HADS in IBD cohorts. These studies report no

difference in the presence of anxiety and depression between patients with CD and UC;^(84, 94, 96) anxiety is suffered in at least 30 % of patients and depression is present in 10 – 20 % of patients. In patients with CD, anxiety has been significantly correlated or associated with greater disease knowledge,^(97, 98) greater disability,⁽⁹⁹⁾ greater levels of perceived stress,⁽⁹⁴⁾ active disease⁽¹⁰⁰⁻¹⁰²⁾ and functional defecation disorders.⁽¹⁰³⁾ Depression, however, has been correlated with perceived stress and increasing age⁽⁹⁴⁾, active disease⁽¹⁰¹⁾, disease severity⁽¹⁰¹⁾ and functional defecation disorders.⁽¹⁰³⁾

Active CD is associated with greater anxiety and depression symptoms⁽¹⁰⁰⁻¹⁰²⁾ and a few studies have suggested that medications used to treat active disease also impact on anxiety and depression symptoms, although the results are variable. Corticosteroid use has been associated with depression in one study⁽¹⁰⁴⁾ but not in another.⁽¹⁰¹⁾ In contrast, anti-TNF and thiopurine medications have not been associated with increased anxiety or depression.⁽¹⁰¹⁾ A literature search found no studies that have used HADS to document changes in anxiety and depression subsequent to EEN treatment in children or adults.

Table 1.3. Prevalence of anxiety and depression in IBD cohorts using the Hospital Anxiety and Depression Score (HADS).

Study location and year published	Number of patients (CD, UC, mean age, % with active CD)	Criteria applied	Anxiety (% of cohort)	HADS-A Mean (SD)	Depression (% of cohort)	HADS-D Mean (SD)
Rome, Italy 2011 ⁽¹⁰⁵⁾ (abstract)	27 IBD (46 years)	> 10 8 - 10	55 % 30 %	N/A	N/A	N/A
Edinburgh, UK 2011 ⁽¹⁰⁴⁾ (abstract)	582 IBD (326 CD, 256 UC)	> 11	43 %	N/A	N/A	N/A
Sweden 2003 ⁽⁹⁸⁾	489 IBD	>10 8 - 10	16 % 14 %	N/A	N/A	N/A
Sydney, Australia 2012 ⁽⁹⁷⁾	258 IBD (47 years)	> 10 8 - 10	19 % 22 %	N/A	N/A	N/A
Singapore 2015 ⁽⁹⁹⁾ (abstract)	164 IBD (77 CD, 87 UC, N/A)	N/A	24 %	N/A	13 %	N/A
France 2012 ⁽¹⁰¹⁾	1663 IBD (1062 CD, 600 UC, 20)	> 10	41 %	N/A	11 %	N/A

Study location and year published	Number of patients (CD, UC, mean age, % with active CD)	Criteria applied	Anxiety (% of cohort)	HADS-A Mean (SD)	Depression (% of cohort)	HADS-D Mean (SD)
East London, UK 2012 ⁽⁹⁴⁾	204 IBD (101 CD, 103 UC, 42 years)	> 10	33 %	8.60 (3.90)	4 %	4.70 (3.30)
Spain 2013 ⁽⁸⁴⁾	793 IBD (323 CD, 470 UC, 45 years, 39)	>11	11 %	4.93	20 %	7.40
Canterbury, New Zealand 2015 ⁽⁹⁶⁾	54 IBD (29 CD, 22 UC, 34 years, 59 %	> 7	23 %	5.19 (3.36)	9 %	3.15 (2.71)
Adelaide, Australia 2008 ⁽¹⁰³⁾	61 IBD (31 CD, 30 UC, 51 years)	8 or more	39 %	6.57 (3.52)	11 %	4.18 (2.89)
Melbourne, Australia 2015 ⁽¹⁰⁰⁾	81 IBD (56 CD, 25 UC, 35 years)	> 10	23 %	7.21 (4.75)	13 %	5.35 (4.90)
		8 - 10	20 %		9 %	
N/A, not applicable/reported						

1.3.4.2 Inflammatory Bowel Disease Questionnaire

The IBDQ is a commonly used IBD-specific HRQOL assessment tool. It is a 32-item questionnaire developed in 1989 for use in clinical trials to describe how IBD affects the quality of patients' lives.⁽¹⁰⁶⁾ It includes four sections (bowel symptoms, systemic symptoms and emotional and social function) and each question is answered on a seven-point scale.⁽¹⁰⁶⁾ The IBDQ score is the sum of the 32 answers in which 32 represents the worst and 224 is the best HRQOL function. Each of the four dimensions can also be summated individually for further analysis. A score of ≥ 170 corresponds to clinical remission and an increase of ≥ 16 is considered a meaningful response to intervention.⁽¹⁰⁷⁾

Active CD symptoms, including diarrhoea, abdominal pain and fatigue, are likely to impair normal activities. Objective measures of disease activity, including endoscopic disease activity, CRP and FC, are not significantly associated with IBDQ scores whereas, many studies have shown that CDAI correlates well with IBDQ scores.⁽¹⁰⁸⁻¹¹⁰⁾ Also, females often have lower mean IBDQ scores compared to males with similar disease activity.^(111, 112) The CDAI contains objective measures of disease activity (haematocrit, weight loss, anti-diarrheal use, extra-intestinal manifestations) but also, subjective measures (general well-being, intensity of abdominal pain) which probably, in part, explains the strong correlation between CDAI and IBDQ. Mean IBDQ scores of patients with a CDAI of less than 150 (defined as in remission) are greater than those of patients with active disease, however, even when the disease is in remission IBDQ scores are still less than optimal.⁽¹¹⁰⁾

There are numerous medication options available to manage active CD and the aim of all treatments is to reduce inflammation and disease symptoms and achieve disease remission. However, some treatments are associated with better HRQOL than others. For example, some studies show that despite symptom improvements on corticosteroids, IBDQ scores do

not reflect those improvements.^(111, 112) In contrast, other studies have found disease severity, rather than corticosteroid use, had a greater impact of IBDQ scores.⁽¹¹³⁾ On the other hand, biological treatments used to induce disease remission and as a maintenance treatment are associated with sustained improvement in IBDQ scores.^(109, 114)

Few studies have assessed HRQOL in adults with CD using EEN. Research with paediatric populations has shown that the use of EEN is associated with improved HRQOL,⁽³³⁾ even for children who require nasogastric tubes to administer the feed. Similarly, Japanese adults with CD in remission who were using a half elemental nutritional formula diet to maintain disease remission had similar IBDQ scores to those on a free diet.⁽³⁵⁾ The impact of EEN treatment on HRQOL has not been assessed in western adults.

1.3.4.3 Short Inflammatory Bowel Disease Questionnaire

The IBDQ requires 15 – 20 minutes to complete making it an inappropriate tool to use in outpatient clinical practice setting. Subsequently, a short IBDQ (SIBDQ) version, with only 10 questions, was developed in 1996 for use in clinical practice.⁽¹¹⁵⁾ The short version includes questions from each of the four domains of IBDQ and each question is answered on a seven-point scale. The responses to the 10 questions are summed and divided by ten; the final SIBDQ score can range from one (poorest HRQOL) to seven (optimum HRQOL). Occasionally, the sum of the SIBDQ is reported in a fashion similar to that of the IBDQ. In the initial validation study involving 150 patients with CD, the SIBDQ explained 92 % of the variance in the full IBDQ and clinical changes in disease activity were reflected in SIBDQ score variations.⁽¹¹⁵⁾ The SIBDQ correlates well with CDAI (Pearson correlation $r = -0.54$; $p < 0.001$) and a significant change in the SIBDQ is observed between patients with active and inactive disease.⁽¹¹⁵⁾

The SIBDQ has not been used in clinical trials as often as the IBDQ. A recent study combined SIBDQ score with FC as a non-invasive predictor of mucosal healing.⁽¹¹⁶⁾ The study showed that a combined FC of greater than 30 µg/g and a SIBDQ of less than 6 were good predictors of active endoscopic disease with a sensitivity of 81 % and a specificity of 75 %.⁽¹¹⁶⁾ The SIBDQ has also been used in combination with the HBI to predict severe illness requiring hospitalisation or surgery.⁽¹¹⁷⁾ There has been no published studies that have investigated HRQOL using the SIBDQ in adult patients using EN to treat active CD.

1.3.5 Dysbiosis of the Gut Microbiota

The gut microbiota is defined as all the microorganisms present in the digestive tract, of which the greatest number reside in the colon. It is estimated that the gut microbiota contains up to ten times more cells than the human body⁽¹¹⁸⁾ including bacteria, archaea (single-celled microorganisms) and eukaryote (protozoa, yeast and fungi). The gut microbiota is defined as the community of microorganisms residing in the gastrointestinal tract. Whereas, the gut microbiome is the genes encoded by all of the microbial genomes. The gut microbiome is much larger than the human genome and encodes 100 times more genes than the human genome.⁽¹¹⁹⁾

The microorganisms present in the human colon usually live in symbiosis with the host and improve the health of the host through the production of vitamins, fermentation of undigested carbohydrates and subsequent production of short-chain fatty acids, and the metabolism of bile acids, sterols and xenobiotics. However, pathogenic microorganisms may also coexist in the gut and may result in changes in the microbiota and disruptions to the gut environment, for example, after infectious gastroenteritis.^(120, 121) In the case of CD, changes to the gut microbiota, coined dysbiosis, are apparent.^(121, 122) Microbiota dysbiosis is also observed in other chronic diseases including obesity,⁽¹²³⁾ type II diabetes⁽¹²⁴⁾ and IBS.^(122, 125)

The exact cause of dysbiosis in CD is not fully understood and it is not yet clear if this is a result of the disease or precedes the development of disease.

It is currently thought that CD is the result of an abnormal immune response to a gastrointestinal stimulus in a genetically susceptible person.⁽¹²⁶⁾ One of the theories is that dysbiosis is associated with impaired immunity and inflammation of the gut mucosa.⁽¹²⁷⁾ For example, dysbiosis may occur for example, as a result of antibiotic exposure or an opportunistic infection in a person with a genetic mutation on the *NOD2* gene.⁽¹²⁷⁾ The change in the balance of the gut microbiota results in decreased mucin production (the protective layer between the gut lumen and epithelial cell) and decreased antimicrobial peptide production. These impaired defence mechanisms allow microorganisms or other luminal contents to cross the epithelium stimulating an immune response. In depth analysis of the gut microbiota and microbiome is required to allow further investigation of its role in the development and treatment of CD.

1.3.5.1 Tools Used To Characterise the Intestinal Microbiome

The composition of the gut microbiota varies along the gastrointestinal tract. The stomach and duodenum are usually more sparsely colonised compared with the colon⁽¹²⁸⁾ and the microorganisms present within the small and large intestine vary as well.⁽¹²⁹⁻¹³²⁾ Further to this, the tools used to collect the microorganisms alters the microbiota's composition; for example faecal samples contain different species of bacteria compared with mucosal samples.⁽¹³³⁾

Gut bacteria are classified into phyla, class, order, family, genus and species based on their genetic and functional similarities.

Table 1.4 provides three examples of the scientific classification of bacteria from the most prevalent phyla, Bacteroidetes (gram negative) and Firmicutes (gram positive), and one of

the less abundant phyla Proteobacteria. Bacteroidetes and Firmicutes constitute approximately 90 % of the bacteria in the gut followed by Actinobacteria and Proteobacteria.⁽¹³³⁾

Table 1.4. Scientific classification of microorganisms.

Scientific classification	<i>E. coli</i>	<i>F. prausnitzii</i>	<i>B. fragilis</i>
Characteristics	Gram negative, anaerobic, produce vitamin K, some species are pathogenic ⁽¹³⁴⁾	Gram-positive, abundant in healthy people, anaerobic and butyrate producing ⁽¹³⁵⁾	Anaerobic, gram-negative, commensal but may cause infection if translocated from intestines ⁽¹³⁶⁾
Phylum	Proteobacteria	Firmicutes	Bacteroidetes
Class	Gammaproteobacteria	Clostridia	Bacteroidetes
Order	Enterobacteriales	Clostridiales	Bacteroidales
Family	Enterobacteriaceae	Fuminococcaceae	Bacteroidaceae
Genus	<i>Escherichia</i>	<i>Faecalibacterium</i>	<i>Bacteroides</i>
Species	<i>coli</i>	<i>prausnitzii</i>	<i>fragilis</i>

Extensive microbiota data has become available with the advent of genetic sequencing techniques. The early literature investigating the gut microbiota used bacterial culture methods which only identified a limited number of culturable species. Older molecular techniques such as polymerase chain reaction (PCR), terminal restriction fragment length polymorphism (T-RFLP) and denaturing and temperature gradient gel electrophoresis (DGGE and TGGE) allowed profiling of communities, quantification of specific species and estimation of genetic diversity and abundance. Newer genetic sequencing technologies have broadened our depth of knowledge of gut microorganisms, bacterial communities and their functions in the gut.

Initially, sequencing technology focused on the 16S ribosomal RNA, which is a section of relatively stable section ribosomal RNA. Data obtained using 16S rRNA sequencing resulted in the development of specific sequencing probes and has allowed more in depth research into the specific bacterial species. The most recent advance has been next generation sequencing (pyrosequencing or metagenomics), which sequences all of the bacterial genes present in a sample. Such genetic technology is rapidly improving and becoming faster and cheaper. Pyrosequencing can provide data on both the type and the quantity of microorganisms present in a sample. The sequencing data produced by both of these techniques are compared with genetic libraries in order to ascertain which bacteria are present in the sample. DNA sequencing techniques produce a huge amount of data which require specialist bioinformatics skills to interpret and analyse.

1.3.5.2 Dysbiosis in Crohn's disease

It is beyond the scope of this thesis to include a full literature review of dysbiosis in CD but this section summarises the main themes in the literature. There is no one 'normal' CD microbiome profile. Each person has their own unique microbiome,^(132, 133, 137, 138) which is influenced by environmental and genetic factors, antibiotic use and diet. Dietary substrates such as fibre have a significant impact on the composition of the microbiome. A diet very

high in fibre and low in fat and animal protein, such as that consumed by rural African children, is associated with a faecal microbiota rich in Bacteroidetes with a lower relative abundance of Firmicutes.⁽¹³⁹⁾ On the other hand, a typical Western diet high in animal protein, refined carbohydrates and fat and low in dietary fibre is associated with the presence of more Proteobacteria and a greater abundance of Firmicutes than Bacteroidetes.⁽¹³⁹⁾

CD is associated with significant dysbiosis, which likely has a key role in the development and resolution of inflammation. As our functional understanding of the microbiome increases it has become apparent that gut microorganisms can stimulate the immune system and mitigate inflammatory processes.⁽¹⁴⁰⁾ CD dysbiosis is characterised by reduced microbial diversity and reduced abundance of commensal bacteria.^(118, 141, 142) Additionally, recent research has shown that CD is not only associated with bacterial dysbiosis but fungal dysbiosis as well.⁽¹⁴²⁾

More recent literature has used 16S rRNA or metagenomic sequencing techniques to study dysbiosis in children and adults with CD and have found that active CD is often characterised by alterations in abundance of particular groups of bacteria and specific bacterial species. These dysbiosis characteristics are summarised in Table 1.5.

Table 1.5. Characteristics of bacterial dysbiosis in children and adults with active CD.

Classification	Increased Abundance	Decreased Abundance
Phyla	Proteobacteria ⁽¹¹⁸⁾	Bacteroidetes ⁽¹¹⁸⁾
		Firmicutes ⁽¹¹⁸⁾
Order	Enterobacteriales ^(143, 144)	Clostridiales ^(141, 143)
	Fusobacteriales ⁽¹⁴³⁾	Bacteroidales ⁽¹⁴³⁾
Genera	<i>Escherichia</i> ^(121, 142, 143)	<i>Faecalibacterium</i> ^(121, 142, 143, 146)
	<i>Fusobacterium</i> ^(143, 145)	<i>Ruminococcus</i> ^(142, 143)

At a species level, *F. prausnitzii* is probably the most widely researched bacterial species in CD patients. *F. prausnitzii* is a member of the Clostridia class of Firmicutes and in healthy populations is one the most abundant species of the Firmicutes phylum.⁽¹⁴⁷⁾ One of the functions of *F. prausnitzii* is fermentation of dietary fibre and the production of butyrate, a short chain fatty acid (SCFA). SCFAs are a source of fuel for colonocytes and are thought to prevent cell death and maintain the integrity of the intestinal cell lining.⁽¹⁴⁸⁾ Another major producer of butyrate in the gut are the *Roseburia* species (spp).⁽¹⁴⁹⁾ The abundance of *F. prausnitzii* has consistently been found to be reduced in the faecal samples of patients with active CD.^(143, 146) *F. prausnitzii*, and other fibre fermenting and SCFA producing bacteria, are also thought to maintain the health of the intestinal mucus layer and inhibit inflammation via the protective effect of SCFAs.⁽¹⁵⁰⁾

Dietary fibre intake has a significant impact on the abundance of *F. prausnitzii* in faecal samples. In both healthy volunteers and people with active CD the consumption of a fibre deficient liquid diet results in a significant reduction in the abundance of *F. prausnitzii*,^(151, 152) despite reduced gut inflammation in the patients with active CD.⁽¹⁵²⁾ Previous studies have

not yet confirmed whether reduced abundance of *F. prausnitzii* during active colitis is a result of consuming a lower fibre diet during periods of active disease or a function of active disease.

1.3.5.3 Impact of Nutritional Therapy on the Microbiome

Considering that microbiome dysbiosis is prevalent in CD and that gut bacteria appear to have an active role in intestinal inflammation, it would be useful to have a greater understanding of the impact on the microbiome of nutritional therapies, such as EEN, used to manage CD.

The effect of EEN on microbial diversity in children has been studied by various groups and was recently reviewed.⁽¹⁵³⁾ Essentially, treatment with EEN has been shown to further reduce the diversity of the faecal microbiota, along with further reductions in the abundance of fibre fermenting species including *F. prausnitzii* despite children achieving disease remission on the treatment.^(152, 153) After treatment with EEN concluded, and children were re-established on their usual diet, microbiota diversity increased.⁽¹⁵²⁾

As our knowledge of the microbiome and the function of specific bacterial species grows there may come a time when gut bacteria, or microbial metabolites, become therapeutic targets of the clinical management of IBD. Given that a reduction in abundance of SCFA producing bacterial genera are associated with IBD dysbiosis⁽¹⁴³⁾ the use of fibre containing diets as an IBD maintenance treatment has recently been studied. A USA study of 1130 patients with CD and 489 with UC found that a lower dietary fibre intake was associated with a past history of surgery and hospitalisations, being female and having CD.⁽¹⁵⁴⁾ The study also found that in patients with CD (but not UC), those with the highest fibre intake (median intake 23.7 g/day) had an adjusted OR of 0.57 (95 %CI (0.37 - 0.87)) of having a flare within six months compared with those in the lowest quartile of fibre intake (median intake 10.4

g/day). In the study by Brotherton et al.⁽¹⁵⁴⁾, researchers did not explore the reasons for fibre restriction in the study participants but suggest that further studies investigate this practice. They also did not have accurate information on patient disease phenotypes but suggest that prospective studies are needed to further explore the use of dietary fibre in certain disease phenotypes (for example, non-stricturing disease).⁽¹⁵⁴⁾ Furthermore, investigation of the association between dietary fibre intake, abundance of SCFA producing bacteria and CD flares would be helpful to further our understanding of the role nutrition may, or may not, play in gut inflammation.

At present it is not fully understood how dysbiosis contributes in IBD outcomes, however there is much research being conducted investigating the role of the intestinal microbiome in IBD and how manipulating it may alter outcomes. Such research may allow for more targeted and individualised dietary and nutritional interventions.

1.4 Nutrition Therapy in the Treatment of Crohn's Disease

For most adult populations, EEN is not recognised as a first line therapy for newly diagnosed, or pre-existing, CD. European⁽³⁶⁾ and North American⁽³⁷⁾ clinical guidelines recommend EEN if a patient declines drug therapy or as an adjunctive therapy to support nutrition status, rather than as a primary therapy. These recommendations are primarily based on the results of a 2006 Cochrane systematic review of six randomised controlled trials including 192 patients treated with EEN and 160 patients treated with CS.⁽³⁸⁾ The review found a pooled OR of 0.33 (95 % CI: 0.21-0.53) in favour of CS and concluded that CS were superior to EEN in the induction of remission of CD. In contrast to these guidelines, published Japanese experience demonstrates the efficacy of EEN to treat active CD in adult populations.⁽³⁵⁾ It is not clear why the benefits of EEN therapy observed in paediatric populations have not to date been achieved in Western adult populations but, given the potential benefits of the nutrition treatment (as discussed in section 1.2.2) and the limited side effects compared with CS

treatment, it is worthwhile exploring whether EEN could be a useful first line treatment for Western adults.

1.4.1 Exclusive Enteral Nutrition for Adults with active Crohn's Disease

The following sections will outline the current evidence for and against the use of EEN in the treatment of adults with active CD. Also, some of the potential reasons for the discrepancy of results between the adult and paediatric EEN studies are explored.

1.4.1.1 Exclusive Enteral Nutrition Compared to Corticosteroids to Induce Remission

Eleven studies, published between 1984 and 2002, have compared EEN with CS treatment in adults (Table 1.6). Two of these were abstracts^(155, 156) and the rest were full articles. The studies were conducted in Europe, North America and Asia: three in England,^(17, 129, 155) one in Spain,⁽¹⁵⁷⁾ one in Greece,⁽¹⁵⁶⁾ one in Italy,⁽¹⁵⁸⁾ one in the United States of American,⁽¹⁵⁹⁾ one in Japan⁽¹⁶⁰⁾ and three⁽¹⁶¹⁻¹⁶³⁾ were multi-centre European trials. All but two studies enrolled a mix of patients with newly diagnosed CD (naïve to prior treatment) and existing CD. All but one study compared one EN formula with CS therapy.

Table 1.6. Studies of adults that compared EEN with corticosteroid therapy.

Study	Year	Country	Age (SD, range)	No previous treatment (% of EEN group)	Participants (n)		Disease remission (%) (intention to treat)		Significant difference (p value)	Remission criteria	EEN intervention not completed (%)		Disease remission (%) (treatment completed)	
					EEN	CS	EEN	CS			Formula un-palatable	Other reason	EEN	CS
Engelman et al ⁽¹⁵⁵⁾	1993	England	23-54	Not stated	7	4	100%	100%	P = NS	HBI < 6.0	0	0	100%	100%
Gassull et al ^{a (161)}	2002	Europe	31.3 (3.3)	50%	20	19	20%	79%	P = 0.0005	VHAI < 120	5 (25%)	0	27%	79%
Gassull et al ^{b (161)}	2002	Europe	30.8 (4.1)	43.5%	23	19	52%	79%	P = NS	VHAI < 120	4 (17%)	0	63%	79%
Gonzalez-Huix et al ⁽¹⁵⁷⁾	1993	Spain	31.1 (4.1)	47%	15	17	80%	88%	P = NS	VHAI < 120	0	0	80%	88%
Gorard et al ⁽¹²⁹⁾	1993	United Kingdom	31.6 (3.0)	50%	22	20	45%	85%	P < 0.05	HBI – mean < 2	9 (41%)	2	91%	89%
Lindor et al ⁽¹⁵⁹⁾	1992	USA	34.7 (26–64)	33%	9	10	50%	33%	P = NS	CDAI decrease > 100 points	3 (33%)	1	60%	63%
Lochs et al ⁽¹⁶²⁾	1991	Europe	27.5 (1.5)	Not stated	55	52	53%	79%	P < 0.01	CDAI decrease > 100 points or 40%	7 (13%)	0	60%	85%

					Participants (n)		Disease remission (%) (intention to treat)				EEN intervention not completed (%)		Disease remission (%) (treatment completed)	
First author and reference	Year	Country	Age (SD or range)	No previous treatment (% of EEN group)	EEN	CS	EEN	CS	Significant difference (p value)	Remission criteria	Formula un- palatable	Other reason	EEN	CS
Malchow et al ⁽¹⁶³⁾	1990	Europe	30.1 (11.5)	20%	51	44	41%	71%	P < 0.05	CDAI decrease > 100 points 40 %	20 (39%)	0	71%	91%
Mantzaris et al ⁽¹⁵⁶⁾	1996	Greece	Not stated	20%	10	10	40%	70%	P = NS	CDAI < 150 or decrease > 100	0	0	40%	70%
Okada et al ⁽¹⁶⁰⁾	1990	Japan	21.0 (3.3)	100%	10	10	80%	30%	P < 0.01	HBI < 1	0	0	80%	30%
O'Morain et al ⁽¹⁷⁾	1984	England	31.9 (15–60)	100%	11	10	82%	80%	P = NS	HBI – mean < 3	2 (18%)	0	100%	100%
Zoli et al ⁽¹⁵⁸⁾	1997	Italy	33.5 (15.9)	Not stated	12	10	67%	50%	P = NS	HBI < 3	1 (8%)	1	80%	50%

Notes: ^{a, b} = Gassull et al had two EEN arms: ^a – high oleic fatty acid formula, ^b – high linoleic fatty acid formula

Table 1.7. Characteristics of the EEN regimens used in studies that compared EEN and corticosteroid treatment in adults with active CD.

Study	Nutritional product	Type of formula	Duration of EEN (weeks)	Calorie density (kcal/ml)	Nutritional composition (% TE)	Mode of delivery	Calorie intake/day (kcal/kg/day)	Disease remission (%) (treatment completed)	
								EEN	CS
Engelman et al ⁽¹⁵⁵⁾	Peptamen	Semi-EF	2	1.0	Pro 16, CHO 51, Fat 33	Orally	30 – 35	100%	100%
Gassull et al ⁽¹⁶¹⁾	High oleic acid	PF (powder)	4	1.0	Pro 22, CHO 46, Fat 32	Orally and NGT	Not stated	27%	79%
Gassull et al ⁽¹⁶¹⁾	High linoleic acid	PF (powder)	4	1.0	Pro 22, CHO 46, Fat 32	Orally and NGT	Not stated	63%	79%
Gonzalez-Huix et al ⁽¹⁵⁷⁾	Edanec HN	PF	4	1.0	Pro 22, CHO 46, Fat 32	NGT	Not stated	80%	88%
Gorard et al ⁽¹²⁹⁾	Vivonex TEN	EF	4	1.0	Pro 15, CHO 82, Fat 3	Orally, or NGT	2100 kcal/day	91%	89%
Lindor et al ⁽¹⁵⁹⁾	Vital HN	Semi-EF	4	1.0	Pro 17, CHO 74, Fat 9	Orally	40	60%	63%
Lochs et al ⁽¹⁶²⁾	Peptisorb	Semi-EF	4-6	1.0	Pro 16, CHO 69, Fat 15	NGT or NDT	35	60%	85%
Malchow et al ⁽¹⁶³⁾	Survimed	Semi-EF	3–6	1.0	Pro 14, CHO 76, Fat 10	Orally	33	71%	91%

Study	Nutritional product	Type of formula	Duration of EEN (weeks)	Calorie density (kcal/ml)	Nutritional composition (% TE)	Mode of delivery	Calorie intake/day (kcal/kg/day)	Disease remission (%) (treatment completed)	
								EEN	CS
Mantzaris et al ⁽¹⁵⁶⁾	Nutrison HE	PF	4	1.5	Pro 16, CHO 49, Fat 35	NDT	2250 kcal/day	40%	70%
Okada et al ⁽¹⁶⁰⁾	Elental	EF	6	1.0	Pro 19, CHO 81, Fat 1	NDT	40 – 60	80%	30%
O’Morain et al ⁽¹⁷⁾	Vivonex	EF	4	1.0	Pro 15, CHO 82, Fat 3	Orally, or NGT	40 – 60	100%	100%
Zoli et al ⁽¹⁵⁸⁾	Peptamen	Semi-EF	2	1.0	Pro 16, CHO 51, Fat 33	Orally	Not stated	80%	50%

These studies utilised a range of nutritional products, in varying regimens, as summarised in Table 1.7. Eight of the studies used elemental formula (EF) and three studies used polymeric formula (PF). Most formulae were 1 kcal/ml concentration apart from one which used a 1.5 kcal/ml formula. Duration of EEN treatment ranged from 2–6 weeks but most studies used EEN therapy for four weeks. Mode of delivery of the EN formula was either oral, via a nasogastric tube (NGT) if not tolerated orally, continuous feeding via a NGT or nasoduodenal tube (NDT). Nutrition composition of the formulae varied depending on the type and brand of formula used. All formulae had relatively similar amounts of protein (14–22 % of total energy), whereas fat content varied considerably (1–35 % of total energy). Carbohydrate content varied relative to fat content (49–82 % of total energy).

The only study that compared two different enteral formulae and CS was published by Gassull et al.⁽¹⁶¹⁾ They compared two formulae that were the same except for the predominant type of fat: one was high in oleic acid and the other was high in linoleic acid. Study recruitment was ended prematurely because less than 33 % of the high oleic acid formula group had achieved disease remission and the remission rate was significantly different from that of the other treatments.

Corticosteroid protocols also ranged between the evaluated studies. Usual initial CS dosage was between 0.5 mg/kg/day and 1.0 mg/kg/day, with subsequent weaning courses. CS were given orally in two studies^(157, 161) but the route of administration was not specified in the majority of studies. Two studies administered CS and sulfasalazine (anti-inflammatory) concurrently.^(162, 163)

Withdrawals from treatment varied between studies. EEN study group withdrawals were mostly due to unpalatable EN formula (Table 1.6). The number of withdrawals for this reason was as high as 41 % of the EEN group in one study but 0 % in other EEN study groups.

Occasionally patients had to withdraw in situations where urgent surgery was required. Withdrawals from CS groups were much lower. Common reasons cited for withdrawing were side effects, non-compliance with treatment or urgent surgery was required.

All 11 of the studies recorded the disease location of patients. The majority of patients had ileocolonic disease and smaller numbers had ileal or isolated colonic disease. No studies found disease location to be associated with the likelihood of achieving disease remission using EEN or CS therapy.

The age of the participants was recorded differently across the 11 studies. The mean age of patients enrolled in the studies was 27.5–34.7 years old. Inclusion of older adults aged 50 - 70 was not uncommon. Only one study included mostly younger adults (mean 21.0 ± 3.3 years).⁽¹⁶⁰⁾

Disease remission was achieved with EEN therapy on an intention to treat basis in 20–100 % of patients and 30–100 % of patients on CS therapy (Table 1.6). Seven of the 11 studies found no significant difference between EEN and CS treatments to induce disease remission.^(17, 155-159, 161) Of those patients who completed the course of EEN therapy disease remission was achieved in 23 – 100 % of patients and in 30 – 100 % of patients that completed CS treatment.^(17, 129, 155-163) Patients who did not complete the course of EEN therapy were usually withdrawn from the study and started on CS therapy.

1.4.1.2 Factors That May Influence Exclusive Enteral Nutrition Outcomes

There are multiple factors which may influence treatment outcomes in adults treated with EEN and CS. These factors are discussed in detail in the following paragraphs.

The disease remission criteria used by researchers can have a profound impact on the study results. Comparison of disease remission rates between studies is challenging when disease remission is not universally defined. Five of the 11 studies that compared EEN with CS used the HBI to measure disease remission.^(17, 129, 155, 158, 160) Two of the studies that used the HBI did not describe their remission criteria;^(17, 129) however, the mean HBI of participants after the EEN intervention was less than 4, which corresponds with standard interpretations of clinical remission. Another study used a HBI cut off of less than six points with 100 % of participants in both the EEN and CS therapy groups achieving remission in this study.⁽¹⁵⁵⁾ The fourth study to use the HBI used a cut-off of 0–1 points to define disease remission.⁽¹⁶⁰⁾ Of the CS group only 30 % of patients achieved remission using this criterion compared with 80 % of the EEN group. It is unknown if a more liberal cut-off would have increased the number of patients achieving disease remission in the CS group. In all of these five studies at least 80 % of the EEN group participants that completed the course of EEN achieved disease remission using the HBI.

Four of the 11 studies used the CDAI to measure disease remission.^(156, 159, 162, 163) The remission rates of the EEN therapy group in all four studies were low (40 – 53 %), with the two larger studies concluding that, on an intention to treat basis, CS therapy induces disease remission in significantly more patients than EEN therapy.^(162, 163) In two of the studies at least one third of the patients withdrew from the EEN group due to unpalatable formula.^(159, 163) Withdrawals from the CS groups were much lower (20 % or less). Of those that did complete the course of EEN therapy only 40 – 71 % of patients achieved disease remission, whereas remission was achieved in 62 – 98 % of those that completed the course of CS therapy. The disease remission rates of the two studies that used the VHAI to define disease remission were quite different. Gassull et al⁽¹⁶¹⁾ hypothesised that the formula high in linoleic acid, an n-6 polyunsaturated fat, would be less effective than a high monounsaturated fatty acid formula because n-6 fatty acids are pro-inflammatory precursors. Of the 20 patients enrolled in the high oleic acid EEN group only 20 % achieved disease remission after 4 weeks

of therapy, compared with 52 % of the high linoleic acid group and 79 % of those using CS therapy. This research suggests that the type of fat in EEN formulae may affect the efficacy of EEN therapy however, another study that compared similar formulae found no difference between high and low long chain triglyceride formulae.⁽¹⁶⁴⁾ The other study that used the VHA1 to define disease remission found that EEN therapy was as effective as CS therapy: 80 % of those on EEN therapy achieved disease remission compared with 88 % of those using CS therapy.⁽¹⁵⁷⁾

The criteria used to define disease remission should not impact greatly on the results of the study; however, in this case, the studies can be grouped into categories based on the remission criteria applied;

- studies that used the HBI found that EEN therapy was at least as effective as CS therapy in inducing disease remission
- larger studies that used the CDAI found that CS therapy was superior to EEN therapy
- studies with small participant numbers found no significant difference
- studies that used the VHA1 found that there was no significant difference between polyunsaturated PF and CS therapy, apart from the high monounsaturated fat formula study

Based on the observation that only studies using the CDAI found that CS was superior to EEN, future research into the use of EEN with adults should consider using the CDAI to define the effectiveness of EEN to induce disease remission. It should also be noted that there may be differences in study protocols between studies with higher and lower patient numbers that could influence patient outcomes, such as patient support to initiate and maintain EEN therapy.

There is some evidence to suggest that EEN therapy is more effective in newly diagnosed CD patients compared with patients who have existing CD.⁽²⁹⁾ Day et al⁽²⁹⁾ showed that, of 15 newly diagnosed CD patients, 12 (80 %) entered remission after eight weeks of EEN, whereas only seven of the 12 (58 %) children with long-standing disease entered remission ($p > 0.05$). In other paediatric studies with newly diagnosed CD patients disease remission was achieved in 79 – 93 % of those that completed EEN treatment and 70 – 79 % on an intention-to-treat basis.^(28, 165)

Differences in treatment response rates according to time since diagnosis are not limited to EEN therapy. Response and remission rates achieved with biologic therapy are better in children than adults⁽¹⁶⁶⁾ which may, in part, be due to the duration of disease prior to initiation of the treatment. Similarly, adults with a shorter duration of CD are more likely to respond and achieve remission with biologic therapy.⁽¹⁶⁶⁾ The use of immune-modulators early in the disease course in adults and children has also been shown to reduce the probability of long term CS and need for intestinal surgeries.⁽¹⁶⁷⁾

Two studies of adult patients have compared EEN with CS therapy in treatment-naïve patients.^(17, 160) In both studies, 80 % of those treated with EEN achieved disease remission after 4 – 6 weeks of an elemental diet (comparable to remission rates in those treated with CS). Other studies of adult patients comparing EEN with CS have not differentiated between patients with newly diagnosed CD and existing CD in their analyses. One study, with only two newly diagnosed patients, mentioned that both patients responded to EEN treatment⁽¹⁵⁶⁾ but the numbers enrolled in the study were too small to show if there was a statistically significant difference in response to treatment between the two groups. A study of 22 patients treated with EEN found that EEN therapy was as effective in newly diagnosed patients as those with existing disease,⁽¹²⁹⁾ although 40 % of patients did not complete the course of EEN. The authors do not indicate how many of those that completed EEN treatment had existing or newly diagnosed disease. The two larger multi-centre European

trials did not differentiate between those that had and had not received previous CD treatment.^(162, 163)

Longer duration of CD is associated with more complications including tissue scarring, fistulae, abscess formation, strictures, perianal disease and bowel resections.⁽¹⁶⁸⁾ EEN therapy has been shown to induce disease remission by reducing mucosal inflammation.^(23, 169, 170) Complications of CD are often non-inflammatory in nature; therefore, EEN may be less effective in treating these patients. Interestingly, a case series of three children with perianal disease at diagnosis found that EEN (used in combination with surgery and antibiotics) was effective at inducing disease remission and assisted in the healing of perianal disease.⁽¹⁷¹⁾ EEN was used as a maintenance therapy in all three children without the return of perianal disease. A clinical trial has not been conducted to further investigate the potential role of EEN in the management of perianal CD.

Overall, studies in adult patients of EEN compared with CS therapy have not excluded patients with complicated disease. Usual exclusions included imminent surgery, intestinal perforation, ileus, abscesses, massive bleeding, short bowel syndrome with ileostomy and, in some cases, previous surgery. The presence of other complications of existing CD such as scarring, perianal disease or previous bowel surgery is not detailed in the adult literature. It is impossible to ascertain whether those who did not respond to EEN therapy had more or less complications than those who did respond. Furthermore, the studies had only small numbers of patients within each disease sub-group and were unable to conduct in-depth statistical analysis of these sub-groups.

Non-adherence with EEN treatment was a limiting factor in the success of EEN therapy in many studies. A number of reasons for non-adherence of adult CD patients with EEN therapy have been postulated including poor taste of the formula, lack of support and poor

motivation to complete the treatment. The characteristics of the EEN regimens that were used in the studies comparing EEN with CS treatment in adults are summarised in Table 1.7.

Unpalatable EN formula was the most common reason for non-adherence in the studies performed to date. Many early studies that compared EEN with CS treatment used elemental formulas. Although, it is now known that EF and PF are equally effective at inducing remission of disease in children⁽¹⁷²⁾ and adults.⁽³⁸⁾ The difference between PF and EF is that the protein fraction in PF is in its whole form rather than as individual amino acids or peptides in semi-elemental formulae. In addition, EF tend to have a lower total fat content.^(172, 173) EF have a distinctive smell and flavour mostly due to the presence of amino acids, which have a bitter flavour. Bitterness is negatively correlated with palatability, whereas sweetness and sourness are positively correlated with palatability.⁽¹⁷⁴⁾ Fat content may also affect the palatability of the formula.⁽¹⁷⁵⁾ The EF used in the studies were low fat (1 - 3 % of total energy) compared with semi-elemental (9 - 33 % of total energy) and PF (32 - 35 % of total energy). PF are therefore thought to be more palatable. However, there is limited research comparing the palatability of the two formula types. A retrospective study of children who received EF from 1992 – 2001 and children who received PF from 2000 – 2004 found that adherence to treatment did not differ between the two groups but that those receiving PF were less likely to need a NGT inserted to deliver the feed.⁽¹⁷⁶⁾

The mode of delivery of the formula may also play a role in patient compliance. Many studies with high adherence rates administered EF via NG or NDT rather than orally. More recent paediatric studies have encouraged oral intake of PF and use of NG tubes only if needed.^(28, 29, 165) For free living (non-hospitalised) patients, taking the formula orally may be more socially acceptable.

Studies that used EF given exclusively via NG or NDT had low rates of non-adherence (0 – 13 %).^(160, 162) Whereas studies that reported high rates of non-adherence (33 – 41 %) used EF or semi-EF given orally and if a patient did not tolerate EEN orally a NGT was placed.^(129, 159, 163) However, three of the six studies using EF or semi-EF orally reported higher adherence rates.^(17, 155, 158) Two of these studies^(155, 158) only used EEN for 2 weeks and patients were given a peptide based semi-EF (Peptamen) orally rather than an amino acid-based EF. Of the 19 patients using EEN in these two trials, only 1 patient was non-adherent with the treatment. The third study, by O'Morain et al,⁽¹⁷⁾ was one of the first to compare EEN to CS treatment. Patients were asked to take the EF orally for four weeks and if they could not tolerate it a NGT was placed. Of the 11 patients in the EEN group, two (18 %) could not tolerate the formula orally or via a NGT.

Of the three adult studies that used PF, two administered it via NG or NDT with 100 % adherence.^(156, 157) The third study used a PF powder (a high oleic and high linoleic acid formulation) given orally or via NGT if not tolerated orally.⁽¹⁶¹⁾ Non-adherence with the treatment was 17 -25 %. No published adult studies have used a ready-to-drink PF given orally. There are, however, various studies with children that have shown that PF are palatable orally. Borrelli et al⁽²⁸⁾ studied 19 children with CD who drank an isocaloric PF (Modulen) as their sole source of nutrition for 10 weeks. Thirteen children took the formula orally; four required overnight feeding via a NGT, in addition to taking it orally during the day, to meet their nutritional requirements and two children could not manage to take the required volume of formula orally or via a NGT. Of the 17 children that successfully completed the 10 week intervention 15 (88 %) achieved disease remission. Day et al⁽²⁹⁾ studied 27 children with CD who were prescribed EEN with isocaloric PF (Modulen or Osmolite) for up to 8 weeks. Nineteen children managed the required volume of formula orally, five needed to take some of the formula via a NGT and three could not tolerate the required volume orally or via a NGT. Of the 24 children who completed at least 8 weeks of EEN, 19 entered remission (79 %).

Both of these paediatric studies used an isocaloric PF. It appears that the major reason for non-adherence in these cohorts was difficulty tolerating the volume required for nutritional requirements rather than unpalatable formula. It is not clear whether the volume required to meet an adult's nutritional requirements (e.g. 8 - 12 cartons (200 ml) of ready-to-drink isocaloric PF per day) may lead to poor adherence. The use of a concentrated PF, (e.g. 1.5 kcal/ml formula), may help alleviate this issue.

Disease location is thought to affect the efficacy of EEN therapy. In particular, colonic disease may be more refractory to treatment than disease with ileal involvement. However, due to the small participant numbers in most adult EEN studies there has been insufficient statistical power for subgroup analyses. A pooled meta-analysis of mainly adult studies from the 1980s and 1990s found that there was insufficient data to perform subgroup analyses by disease location.⁽³⁸⁾

Some paediatric studies have specifically investigated the impact of disease location on response to EEN therapy. Afzal et al⁽³³⁾ studied 65 children aged 8–17 years old with newly diagnosed CD of which 12 had ileal disease, 39 had ileocolonic disease and 14 had isolated colonic disease. This study found that disease remission was harder to induce with EEN therapy in patients with colonic disease – remission achieved in 50 % compared with 82 % in those with ileocolonic disease and 92 % in those with ileal CD ($p = 0.02$). This study also used colonoscopy to assess mucosal healing after EEN therapy and found that there was no improvement in colonic mucosal inflammation in those with colonic or ileocolonic disease.

Conversely, Buchanan et al⁽¹¹⁾ investigated the effect of disease location on remission rates after EEN therapy and found that colonic CD responded just as well as ileocolonic disease. This study included 114 children (median age 11.6 years), all with recently diagnosed CD.

Nineteen patients had colonic disease, four had ileal disease, 29 had ileocolonic disease, 49 had upper gastrointestinal tract disease and 9 had disease that could not be classified using the Vienna classification. Of those with colonic disease 79 % went into remission after eight weeks of EEN therapy compared with 86 % with ileocolonic disease, 88 % with upper gastrointestinal disease and only 25 % with ileal disease. It should be noted that there were only 4 patients with ileal disease compared with at least 20 in the other three groups. Further evidence is needed to confirm whether CD location affects the efficacy of EEN.

Current guidelines suggest that EEN therapy is more appropriate treatment in paediatric rather than adult patients.^(26, 38) There are no studies with adults that have assessed whether age affects response to EEN therapy. Although, the mean age of adults included in the 11 EEN compared with CS studies was approximately 30 years, the age range varied substantially and was not always published. Of those studies that did publish the age range of patients it was common to include patients aged 20 up to 50 or 60.^(17, 155, 159) It is unknown if age affects response to EEN therapy, or if duration of disease, rather than age, may have a great impact on outcomes. Age may well influence compliance with treatment and further research is required to investigate whether EEN compliance is influenced by factors associated with age, for example social impacts of excluding usual foods and fluids.

1.4.2 Partial Enteral Nutrition for the Treatment of Active Crohn's Disease

There is much evidence to support the use of EEN in the treatment of CD and also positive Japanese data to support the use of supplementary EN, after disease remission is achieved with EEN, to assist in the maintenance of disease remission.^(35, 177) In Japan, CD treatment guidelines recommend patients consume 900-1200 kcal/day from an elemental enteral nutrition formula⁽¹⁷⁸⁾ as a maintenance therapy. This volume corresponds to 50 % of total energy from enteral formula. Two Japanese studies have shown that supplementary EN

reduces disease relapse compared with consuming a free diet or a free diet plus less than 900 kcal/day of EN.^(35, 177)

The use of EN in combination with a solid food diet (partial EN) has been scarcely investigated as a means to induce CD remission. To date, partial EN (PEN) as a treatment to induce disease remission has been investigated in only three prospective studies (Table 1.8). Two of these studies^(179, 180) included children and adolescents and allowed the consumption of unrestricted normal foods in addition to an EF. The third study⁽¹⁸¹⁾ included children and young adults and used PF with a specific exclusion diet which included 18 – 20 g of fibre per day and was gluten, dairy, animal fat, emulsifier and preservative free. The median energy intake from enteral formula was approximately 50 % of total energy in all three studies, although in the Lee et al⁽¹⁷⁹⁾ study there was a large range of 25 – 90% of total energy.

In these three studies,⁽¹⁷⁹⁻¹⁸¹⁾ intention-to-treat disease remission subsequent to PEN varied from 15 – 70 % of patients and, EEN was found to be more effective at inducing disease remission in both of the studies that compared the two treatments.^(179, 180) In the 2006 study by Johnson et al⁽¹⁸⁰⁾ only 15% of patients achieved disease remission, additionally disease remission was only achieved by 42 % of patients on EEN and one third of the patients in this group withdrew from the study early. Given that the remission rates on EEN observed in this study are much lower than the greater than 75 % of paediatric patients quoted in most of the paediatric EEN literature⁽²⁷⁾ it may be expected that treatment success with PEN was poor.

The use of a specific exclusion diet in conjunction with a polymeric formula produced the most positive PEN results and used the most stringent disease remission criteria (Paediatric Crohn's disease activity index (PCDAI) < 7.5 or HBI <4). Disease remission was achieved in 27 of the 40 patients (67 %) that used PEN and 10 patients (25 %) failed to complete the treatment.⁽¹⁸¹⁾ Authors attributed the higher response rate, compared to the previous PEN

study by Johnson et al, to the dietary exclusions. Their justification for this was that seven of the enrolled patients who did not tolerate the polymeric formula followed exclusively the dietary regimen and six of these patients achieved disease remission. Also, another seven patients had previously been using PEN with a free diet but when they changed to using the exclusion diet with PEN their disease responded to the treatment and went into clinical remission.⁽¹⁸¹⁾

The most recent PEN study by Lee et al⁽¹⁷⁹⁾ further supports Signall-Boneh et al's hypothesis. In this study patients were prescribed 80 % of their estimated energy requirements (EER) from EN but were allowed to consume an unrestricted free diet as well. Patients on average consumed 78 % of their EER from formula but then consumed another 73 % of their EER from regular food and drinks. Overall, regular food contributed 50 % TE intake, similar to the other two studies. The authors concluded that EEN is more effective than PEN because EEN primarily excludes aggravating dietary factors rather than just providing adequate amounts of nutrients.

This research provides some evidence that it may be possible for children and young adults to include some food in an EN treatment regimen. The optimal ratio of food to EN is not yet known and more evidence is required to be able to recommend a specific exclusion diet. It is not yet known whether a PEN regimen is applicable to a wider portion of the adult CD population. The EEN research suggests that adherence to EEN is poor with adult populations therefore maybe a more liberal regimen such as PEN would be more appealing to the adult CD population.

Table 1.8. Treatment of active Crohn's disease with partial enteral nutrition.

Study	Year	Country	Age (yrs)	Participants		Formula	Food allowed	Energy from		Disease		EEN vs	Disease remission	
			Mean (range)	(n)		(treatment duration)	during PEN	formula (% TE) Mean (range)		remission (%) (ITT)*		PEN	n (%) (PP)*	
				EEN	PEN			EEN	PEN	EEN	PEN	p value	EEN	PEN
Johnson et al ⁽¹⁸⁰⁾	2006	UK	12 (3.8-16.0)	24	26	Elemental 028 Extra (6 weeks)	Usual table food	98 (89- 100)	47 (39- 58)	42	15	0.035	10/16 (63)	4/16 (25)
Lee et al ⁽¹⁷⁹⁾	2015	USA	12 (7.2-17.8)	22	16	PEN: Peptamen Jr, Peptamen 1.5, EEN: Osmolite, Modulen (8 weeks)	Usual table food	91 (79- 99.5)	53 (25- 90)	59	44	0.03	13/18 (72)	7/15 (47)
Signall- Boneh et al ⁽¹⁸¹⁾	2014	Israel	16 (9-31)	-	47	Modulen or Pediasure (6 weeks)	Specific exclusion diet	-	Aimed for 50 %	-	70	-	-	27/40 (68)

* Clinical remission criteria: Lee & Johnson $\leq$ PCDAI 10, Signall-Boneh PCDAI < 7.5 or HBI < 4

1.5 Summary

Initial reports^(17, 182) demonstrated that EEN was effective in inducing remission in adults with active CD and proposed this intervention as an alternative to CS therapy. However, subsequent larger studies failed to reproduce these results.^(162, 163) Since then many studies have been conducted in paediatric populations and numerous benefits over and above achieving disease remission have become apparent. It therefore seems timely to readdress the disparity between the results achieved in paediatric EEN studies compared to the adult EEN studies. One of the main limiting factors in the success of EEN in the adult literature is the high treatment withdrawal rate. To overcome this the use of polymeric formula provided orally, which has not previously been studied in adult patients, may improve treatment compliance and allow adult patients to reap the many other benefits of EEN that have been shown in children over and above achieving disease remission and improving nutritional status. Additionally, investigating patient interest in such EN treatment is fundamental, as is understanding the experience and knowledge of gastroenterologists and dietitians that would be involved in recommending and managing EN therapy in patients with active CD.

1.6 Hypotheses

The specific hypotheses generated for this thesis all stem from the overarching hypothesis that nutritional therapy modulates gut inflammation in young adults with CD leading to clinical and biochemical remission. The hypotheses that are explored in this research thesis are:

- That polymeric nutritional formula is more palatable than elemental nutritional formula.
- That adults with CD are interested in using nutritional therapies in place of corticosteroid treatments.
- That enteral nutrition therapy induces clinical and biochemical (faecal and serum markers of inflammation) disease remission in adults with active CD.
- That enteral nutrition therapy improves markers of nutritional status in adults with active CD.
- That enteral nutrition therapy is associated with improved HRQOL scores in adults with active CD.
- That enteral nutrition therapy alters faecal microbiota structure in adults with active CD and healthy adults.
- That treatment with a PEN regimen induces disease remission as effectively as EEN in adults with active CD.
- That gastroenterology health professionals have limited experience with, and awareness of, EEN in adult patients with active CD.

Chapter 2

Methods

2.1 Clinical Trial

2.1.1 Recruitment of Patients

A non-randomised pilot intervention clinical trial of patients aged 16 to 40 years old with active CD involving the ileum was undertaken over a 48 month (4 year) period. Patients were eligible for the study if they had active Crohn's disease and were willing to either consume EN (exclusive or partial) for eight weeks or undertake corticosteroid treatment. However, patients were excluded if they had isolated colonic disease, active psychological illness or had taken corticosteroids in the last fortnight. Concomitant use of other CD medications did not limit eligibility. The patient inclusion and exclusion criteria were designed based on literature presented in Chapter 1, section 1.4.1.2. An upper age limit of 40 years old was chosen for two reasons; firstly the Montreal classification (Table 2.1) divides adult patients into two age categories, 16 to 40 years and greater than 40 years therefore by following this construct this research may more easily be compared with similar studies. Secondly, older patients may have had CD for a longer period of time and as discussed in Chapter 1, section 1.4.1.2 patients with long standing disease may respond differently compared with patients with more recent disease onset. Local gastroenterologists referred eligible patients, following patient agreement, to the candidate who was a registered NZ dietitian. Upon receiving the referral the candidate contacted the patient, explained the study, sent written information to the patient as required and arranged an initial appointment if the patient consented to take part. The target for recruitment for the pilot study was 20 patients using corticosteroids, 20 patients using EEN and 20 patients using PEN.

Initially only patients with newly diagnosed CD were eligible for the study. A diagnosis of CD was confirmed by endoscopy and/or histology reports and the location and behaviour of disease was classified according to the Montreal classification (Table 2.1).⁽¹⁸³⁾ However, study recruitment was slower than expected therefore the inclusion criteria were revised to include patients with existing CD having a flare. A patient was defined as having a flare if they had an elevated faecal calprotectin or elevated inflammatory markers (C-reactive protein, erythrocyte sedimentation rate) or a CDAI of greater than 150. This change in protocol required an amendment to the initial ethical approval application (section 2.1.2).

Table 2.1. Montreal classification of Crohn's disease.⁽¹⁸³⁾

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

* L4 can be added to L1-l3 when concomitant upper gastrointestinal disease is present.

p is added to B1-B3 when concomitant perianal disease is present

2.1.2 Ethical Approval

Ethical approval to conduct this clinical trial was given by the Northern B Health and Disability Ethics Committee on 2 April 2013 (ethics reference 13/NTB/11). The initial application was to study patients with newly diagnosed CD. An amendment, which allowed patients with existing disease having a flare as well as those with newly diagnosed disease to be recruited, to the study was approved on 20 March 2014 (ethics reference 13/NTB/11/AM01).

In August 2014 a further amendment to the study protocol was submitted for ethical approval to the Health and Disability Ethics Committee. This amendment was to recruit healthy volunteers with no history of gastrointestinal issues to use EEN for two weeks. This amendment received ethical approval on 23 September 2014 (ethics reference 13/NTB/11/AM03).

A final amendment to the original application was submitted for ethical approval in February 2015. Once at least 20 patients had been recruited to the EEN treatment an amendment to the study protocol was submitted to the Health and Disability Ethics Committee. The original application included two interventions – standard treatment with corticosteroids or treatment with EEN. This subsequent change to the study protocol was to replace the EEN treatment option with PEN treatment. This amendment was approved on 26 February 2015 (ethics reference 13/NTB/11/AM04).

2.1.3 Corticosteroid Treatment

The standard first line treatment for adults with active Crohn's disease is corticosteroids. Patients who chose to use corticosteroids, rather than nutrition therapy, started on a standard reducing dose, as per their gastroenterologist, of 40 mg orally for seven days and then 5 mg less every seven days until the dose had finished. This medication course took eight weeks to

complete. This treatment group had the same assessments as the EN treatment groups and are described in the subsequent sections.

2.1.4 Intervention Treatment: Exclusive Enteral Nutrition

In parallel to corticosteroid treatment, the research targeted the recruitment of 20 patients to the EEN intervention group. Treatment with EEN required the patient to go without usual foods and fluids for eight weeks and drink a commercial polymeric 1.5 kcal/ml oral nutritional formula (Ensure Plus). Ensure Plus is a ready-to-drink formula available in four flavours: chocolate, vanilla, fruits of the forest and banana. The EEN nutritional formula provided patients with all of their calculated nutritional needs. The macronutrient composition of each flavour was the same with the exception of saturated fat which varied from 0.44 to 0.48 g/100 mL. Table 2.2 describes the average nutrition composition of the formula.

Each patient's nutritional requirement was calculated based on their basal metabolic rate (see section 2.1.7.1 for further details) multiplied by a physical activity factor (Table 2.3).⁽¹⁸⁴⁾ Based on this calculation a daily calorie target was set, for example seven x 200 ml tetrapaks (2100 kcal/day). In addition to the prescribed EEN, patients were also encouraged to drink at least 1500 ml per day of additional fluids either as water and/or black unsweetened tea, coffee or herbal tea. Caffeinated tea and coffee were allowed to reduce the potential impact of caffeine withdrawal on initial tolerance of the EEN regimen. Patients were not encouraged to start drinking caffeinated beverages but rather to maintain their usual intake.

The nutritional formula was provided on prescription with a special authority number and was delivered to the patient's home or a specified delivery address. The candidate applied for a special authority number for each patient and wrote the prescription. This particular product is

only partially funded by PHARMAC therefore it has a surcharge. This surcharge was paid by the research study, therefore each patient received the nutritional formula free of charge during the eight week intervention period.

Table 2.2. Average nutrient composition of Ensure Plus (Abbott Nutrition) per 100 ml.

Nutrient	Unit	Quantity	Nutrient	Unit	Quantity
Energy	kJ (kcal)	632 (150)	Carbohydrate, Total	g	20.20
Protein	g	6.25	Sugars	g	6.80
Fat, total	g	4.92	Dietary Fibre, total	g	0
Saturated	g	0.44	Water	g	77.43
Vitamins			Minerals		
Vitamin A (palmitate)	µg RE	88	Sodium	mg	92
Vitamin A (β- carotene)	µg RE	29	Potassium	mg	160
Vitamin D3	µg	2.0	Chloride	mg	110
Vitamin E	mg α TE	2.1	Calcium	mg	120
Vitamin K1	µg	12	Phosphorus	mg	100
Vitamin C	mg	12	Magnesium	mg	30
Folic acid	mcg	40	Iron	mg	2.1
Vitamin B1	mg	0.20	Zinc	mg	1.6
Vitamin B2	mg	0.27	Manganese	mg	0.50
Vitamin B6	mg	0.27	Copper	µg	220
Vitamin B12	µg	0.55	Iodine	µg	22
Niacin	mg NE	2.6	Selenium	µg	8.3
Pantothenic Acid	mg	1.1	Chromium	µg	13.5
Biotin	µg	6.0	Molybdenum	µg	16
Choline	mg	55			

Table 2.3. Activity factors used to estimate daily calorie requirements.⁽¹⁸⁴⁾

Activity level	Males	Females
Sedentary – very physically inactive in work and leisure	1.3	1.3
Lightly active – some walking or intense activity 1-2 x/week e.g. students	1.6	1.5
Moderately active – intense exercise 20-45 mins 3x/week or active job	1.7	1.6
Very active – intense exercise > 60 mins daily or heavy physical job	2.1	1.9

The EEN treatment was initiated over a three day period whereby each day one meal was replaced by one to two tetrapaks of nutritional formula. By day four patients were expected to have only the nutritional formula and no other foods except specified liquids. Tolerance of the treatment was assessed by telephone on day five and then again on day seven to ten depending on how well the patient tolerated the treatment on day five. Tolerance of the treatment was assessed based on the patient's ability to drink an adequate number of nutritional formula tetrapaks without worsening nausea, diarrhoea and/or abdominal pain compared with baseline.

Anecdotally, an experienced paediatric dietitian and paediatric gastroenterologist at Christchurch Hospital had found that EEN can exacerbate existing reflux and nausea symptoms therefore, patients with known upper gastrointestinal CD or with a history of reflux were started on a proton pump inhibitor at baseline to help minimise intolerance of the EEN. Patients with nausea at baseline were started on anti-nausea medication as per their gastroenterologist's direction. If a patient developed constipation, because of the fibre deficient EEN regimen, a stool softener called lactulose was initiated. Patients typically took 20-30 ml of lactulose once daily or as required.

Once established on the treatment, patients were assessed fortnightly at a formal study follow up appointment. Patients were able to contact the candidate by text message or phone call between appointments as necessary. Patients who did not tolerate EEN or did not respond to the treatment within the first four weeks were restarted on their usual diet and referred back to their gastroenterologist for an alternative treatment and therefore did not remain in the study.

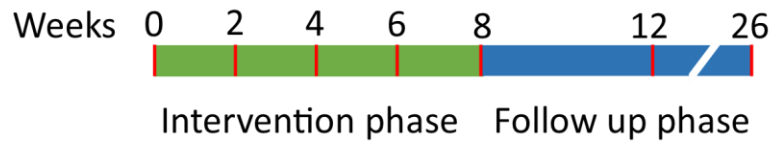


Figure 2-1. Clinical trial data collection time-points.

Patients attended seven study assessment appointments; fortnightly during the eight week intervention phase and at four weeks and four months after completion of EN (Figure 2-1). At each appointment the following were completed: body composition measures, blood and faecal inflammatory markers, blood nutrition markers, symptom questionnaires, quality of life questionnaires. Patients also provided urine and stool samples for subsequent measurement of urinary metabolomics (results not presented in this thesis) and faecal microbiome analysis undertaken in collaboration with the Department of Microbiology and Immunology.

At the end of the eight-week treatment patients reduced their consumption of the nutritional formula and increased their consumption of solid foods and usual fluids. This was usually completed over a three day period. Patients were given the option of continuing to use 200-400 ml per day of nutritional formula as well as their usual diet to assist in maintaining disease remission and to maintain the nutritional quality of their diet. The research study did not fund nutritional formula used subsequent to the study intervention period.

2.1.5 Intervention Treatment: Partial Enteral Nutrition

The target was to recruit 20 patients to the PEN intervention group. Treatment with PEN was also an eight week intervention consisting of two weeks of EEN followed by six weeks of PEN with one small meal per day. Patients underwent the same assessments as detailed in the EEN section 2.1.4. The use of EEN for two weeks was chosen based on a combination of observations. Firstly, the pilot faecal microbiota analysis of six patients who had used EEN (presented in Chapter 7). This analysis showed that there was a change in the faecal microbiota within two weeks of using EEN (Figure 7-1 and Figure 7-2). Secondly, clinical preliminary data from the EEN cohort of the study showed that serum inflammatory markers and symptoms of active disease started to improve with two weeks of EEN treatment. Lastly, the PEN regimen was designed to be a more attractive treatment option than EEN whilst still optimising treatment outcomes. It was felt that two weeks of EEN would be achievable for most patients and just long enough to see improvements in markers of inflammation before regular food was re-introduced.

After two weeks of EEN patients were provided guidelines on how to introduce one small meal per day of solid food in conjunction with EN. The one small meal per day was to provide no more than 33 % of total daily calories with the remaining calories and nutrients provided by the nutritional formula. The ratio of nutritional formula to solid food was calculated based on each patient's intake of nutritional formula during the first two weeks and any change in weight. Each patient chose whether they ate the meal at lunch or dinner and they could alternate the timing of the solid food meal depending on social engagements. Lunch and dinner were chosen over breakfast because these meals are more likely to contain larger amounts of protein and vegetable fibre than breakfast which can help reduce reliance on lactulose as a result of following the fibre deficient EEN diet. Patients were encouraged to eat a balanced meal including foods that

contributed protein (meat, fish, chicken, eggs, and legumes), carbohydrate (rice, bread, pasta, starchy vegetables) and fibre (vegetables).

A list of foods was provided to assist with the transition from EEN to EN plus one meal of solid food. This food list broke foods into three categories based on their fat and fibre content (Appendix A). Patients started at phase 1, which included low fibre bland foods with no pips, seeds and skins. Patients were encouraged to move from phase 1 to 2 and then onto phase 3 based on their own tolerance of the foods. At the end of the eight-week treatment, it was expected that patients would be able to eat a greater range of foods including foods from all three of the food phases. Nutrient intake during the six weeks of PEN was assessed using an electronic food diary. Details of the food diary and nutrient analysis are described in section 2.1.12 of the methods section.

At the end of the eight week treatment patients reduced their consumption of the nutritional formula and increased their consumption of solid foods and usual fluids. Patients were given the option of continuing to use 200-400 ml per day of nutritional formula as well as their usual diet to assist in maintaining disease remission and to maintain the nutritional quality of their diet.

2.1.6 Intervention in Healthy Controls: Exclusive Enteral Nutrition

Healthy individuals who were willing to consume a nutritional formula (Abbott Nutrition, Ensure Plus) as their sole source of nutrition for two weeks were recruited as control subjects primarily for faecal microbiome analysis and secondly to compare changes in body composition subsequent to the intervention and tolerance of the intervention. Subjects had no history of gastrointestinal problems and did not have first degree relatives with CD. The healthy controls (HC) were initially recruited through current and previous EEN study patients (e.g. partners,

flatmates, friends) and then via advertisements at the university. The HC completed two weeks of EEN in the same fashion as patients with CD: three days of phasing in EN and phasing out solid food and then 10 days of EEN. Their nutritional requirements were calculated based on basal metabolite rate data from bioimpedance analysis (details in section 2.1.7.1) and usual physical activity levels (Table 2.3). Tolerance of the EEN was monitored by text message and phone calls at day four or five and then day seven or eight. The HC provided stool samples at baseline, week 2 and week 6 (four weeks after completing EEN). At these time points they also had weight and bioimpedance measurements (detailed in section 2.1.7.1). They did not provide blood samples or answer any quality of life questionnaires.

2.1.7 Body Composition Analysis

At the initial appointment, height was measured using a static tape measure attached to a wall. Participants stood without shoes with their heels against the wall and looked straight ahead. The tape measure was lowered to touch the crown of the head and a height reading was taken. At each appointment, participants had their body weight recorded using electronic scales (Tanita SC-330, Tania Corporation, Japan) without shoes and in light clothing. Participants with CD had a waist and hip circumference taken at baseline and week 8. Waist circumference was measured at the midpoint between the bottom rib and the iliac crest. Hip circumference was taken at the widest part around the middle of the buttocks. A hip to waist ratio was then calculated.

Body composition was measured using bioimpedance analysis (BIA) at each study appointment and skinfold thickness measurements at baseline and week 8 at the end of the treatment. Study appointments were made for a similar time of day each time to minimise the effect of time of day

or hydration on body weight and body water. The methods used for the BIA and skin fold measurements are detailed below.

2.1.7.1 Bioimpedance Analysis

The Biodynamics BIA 450 (Biodynamics Corporation Seattle, Washington, USA) machine used a 50-kilohertz electrical current to measure resistance and reactance in the body and calculates an impedance phase angle. Based on these measurements the machine estimates body composition from established equations.

The BIA 450 machine had a physical activity option but hours of activity were not entered for study patients with the exception of one participant who had a very physical job (builder - entered as 25 hours per week of exercise). The basal metabolic rate was multiplied by an activity factor as described in section 2.1.4 to calculate each person's estimated energy expenditure and thus daily estimated nutrition requirements.

The BIA data were used to compare body composition within and between patients over time. Lean body mass (kg) and fat mass (kg) were expressed as fat-free mass index and fat mass index ($\text{mass (kg)} / (\text{height})^2$) to allow for comparisons between participants and HC of different heights.

2.1.8 Clinical Outcomes – Blood Markers

2.1.8.1 Albumin

Albumin is a marker of nutrition and an acute phase protein, which means that the concentration of plasma albumin falls in response to inflammation. Albumin was monitored at all seven time points during the study and measured by Canterbury Health Laboratories (CHL)

using a bromocresol purple albumin assay kit read of an Abbott C series analyser. The CHL normal range for serum albumin was 32 -48 g/L.

2.1.8.2 Insulin-like growth factor-1

Insulin-like growth factor -1 (IGF-1) is a hormone predominantly produced by the liver. The production of IGF-1 varies with age and sex and affected by nutrition intake, body composition and disease state. IGF-1 was monitored at all seven time points during the study and measured by CHL endocrine laboratory. Initially the laboratory measured IGF-1 using a manual radioimmunoassay method (Endolab RIA). Part way through data collection for the EEN study the laboratory changed the method by which they measured IGF-1 to a commercial kit (iSYS assay by manufacturer Immune Diagnostic Systems (IDS)). The laboratory conducted their own analysis of the two methods (n = 129 samples) and found that the new method correlated well ($r = 0.987$) with the previous manual method however statistical analyses produced a significantly different Deming regression slope (0.949 (0.922 to 0.976) with an intercept of 20.74 (17.75 to 23.72). Consequently, the new method produces slightly higher values (5 % higher) at lower levels of IGF-1. In addition to these differences the standard deviation scores are not comparable because the two test methods have different non-normally distributed age and sex-related reference intervals.

In order to standardise the test results the results from the manual method (Endolab RIA) were transformed to be comparable with the new iSys method results using the following formula: $y = mx + c$. 'Y' being the new result, 'm' the Deming regression slope (0.949), 'x' the manual method result and 'c' the Deming regression intercept (20.74). Calculation of a new standard deviation score for all results allowed comparison of the iSys and transformed manual results. The scores were calculated using a conversion table provided by CHL.

2.1.8.3 Vitamin D

Baseline Vitamin D concentration were assessed by blood 25-hydroxy vitamin D. CHL measured 25-hydroxy vitamin D using high performance liquid chromatography tandem mass spectrometry. In accordance with the NZ Ministry of Health and Cancer Society of NZ consensus statement⁽¹⁸⁵⁾ a level of less than 50 nmol/L was defined as suboptimal 25-hydroxy vitamin D and less than 25 nmol/L as deficient. If a patient had a suboptimal Vitamin D level they were provided with a prescription for cholecalciferol as per their gastroenterologist.

2.1.8.4 C-Reactive Protein

CRP is an acute-phase protein present in serum, which is usually elevated in response to inflammation. CRP was measured at all seven time points during the study by CHL using immunoturbidimetry analysed on the Abbott C series analyser. CHL reported a normal CPR was a value less than 5 mg/L.

2.1.8.5 Erythrocyte Sedimentation Rate

ESR measures the rate at which red blood cells settle in one hour (mm/hr). ESR is a non-specific measure of inflammation. During periods of inflammation there is more fibrinogen present in the blood, which results in red blood cells sticking together and settling faster. ESR was measured at all seven time points in the study by CHL on an automated analyser. CHL reported normal ESR was less than 10 mm/hr in adults aged 30 years and younger and less than 15 mm/hr in adults aged 31-40 years.

2.1.8.6 Haematocrit

Haematocrit is the percentage volume of red blood cells in the blood. It is a marker of anaemia and is included in the CDAI calculation. Haematocrit was measured at baseline, week 4 and week 8 of the study by CHL using an automated analyser. CHL reported a normal haematocrit for females was 0.35 – 0.46 and males was 0.40 – 0.52.

2.1.8.7 Ferritin

Ferritin is a marker of anaemia but in the presence of inflammation ferritin levels are increased. Ferritin was measured at baseline by CHL using chemiluminescent microparticle immunoassay. CHL reported a normal ferritin in the absence of inflammation was greater than 20 µg/L.

2.1.9 Clinical Outcomes – Faecal Markers

Faecal samples were collected to enable analysis of faecal inflammatory markers and changes in the faecal microbiome consequent to EN treatment. Patients provided a stool sample at each study appointment, which they collected at home in a sterile collection pottle no more than 24 hours prior to each appointment and stored in a home refrigerator until their appointment. If patients did not bring a sample to the appointment, they dropped it off, as soon as practical, at the CHL reception. The laboratory stored all samples in a refrigerator until collected for processing. All samples were processed within 24 hours of the patient collecting their sample and processed in a microbiological sterile hood into six aliquots of 0.5 – 1.0 ml of faeces and stored in 2ml Eppendorf tubes at -80°C.

Occasionally a patient could not attend a follow up appointment, usually for geographical reasons. In this situation the patient collected a faecal sample as usual and stored it immediately in the home freezer (-12 to -18°C) in a sterile collection pottle. The patient then brought the

sample, still frozen, to their next appointment. The sample was aliquoted while still frozen to minimize any effects of thawing and then refreezing the sample.

2.1.9.1 Faecal Calprotectin

Faecal calprotectin is a marker of intestinal inflammation. Faecal calprotectin (S100A8/S100A9) of stored stool was measured using a sandwich ELISA technique. A BÜHLMANN fCAL ELISA (BÜHLMANN Laboratories AG, Switzerland) commercial kit was used as per the manufacturer's instructions.

Stool stored at -80°C was thawed and aliquots of 50-100 mg were mixed with an extraction buffer according to the following formula: mg of stool multiplied by 49 = y µL of extraction buffer. The sample was homogenised for 30 minutes on a shaker at the maximum speed. The homogenate was transferred to an Eppendorf tube and placed in a centrifuge for five minutes at 3,000 g. The resultant supernatant was removed and stored at -20°C until further analysis.

The BÜHLMANN fCAL ELISA kit has two working ranges – 10 - 600 µg/g or 30 – 1800 µg/g of calprotectin. Further dilution of samples extended the range to 60 – 3600 µg/g. It was expected that most patients would have elevated calprotectin therefore the extended range of 60 – 3600 µg/g was used. Especially as the normal range for a healthy person without intestinal inflammation is < 50 µg/g. Stool extracts stored at -20°C were defrosted and diluted to 1:7500 with incubation buffer, vortexed and left to equilibrate for at least five minutes. The microtiter plate provided was pre-coated with anti-Calprotectin monoclonal capture antibody. The plate wells were washed twice with 300 µL of wash buffer (100ml of wash buffer concentrate was diluted with 900 mL of deionized water) before 100 µL of blank (incubation buffer), calibrators (standards containing 4, 12, 40, 120, 240 ng/mL of calprotectin), low and high controls (lot

specific native human calprotectin) and diluted sample were added to the plate wells. The plate was covered and incubated at room temperature on a plate rotator set to 450 revolutions per minute (rpm) for 30 minutes. The plate wells were then washed three times with 300 μ L of wash buffer before 100 μ L of enzyme label (anti-calprotectin antibody conjugated to horseradish peroxidase) was added. The plate was covered and incubated at room temperature for 30 minutes on a plate rotator set to 450 rpm. The plate was washed five times with 300 μ L of wash buffer. Next 100 μ L substrate solution (tetramethylbenzidine and hydrogen peroxide) was added to each well and the plate was covered and incubated at room temperature, out of direct sunlight, on a plate rotator set to 450 rpm for 15 minutes after which time 100 μ L of stop solution (0.25 M sulfuric acid) was added. The optical density of each well was determined using a microplate reader (SpectraMAX 190, Molecular Devices, US) set to 450 nm. The lower range of assay was 30 μ g/g calprotectin and the upper range was 1800 μ g/g calprotectin. If samples contained greater than 1800 μ g/g calprotectin the stool extract was further diluted (1 in 450, rather than 1 in 150) with incubation buffer prior to repeating the assay. Further dilution of the faecal extract extended the range of the assay up to 5,400 μ g/g.

2.1.9.2 Faecal Osteoprotegrin

Faecal OPG is also a marker of intestinal inflammation. Stool stored at -80°C was thawed and aliquots of approximately 250 mg were mixed with an extraction buffer in equal parts. The extraction buffer contained phosphate buffered saline (PBS) with 0.5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride, 2.5 μ g/ml leupeptin hemisulfate and 11 μ g/ml aprotinin. Samples were vortexed until stool was well suspended in buffer then homogenised for 30 minutes on a suspension mixer and centrifuged for 10 minutes at 13,500 g. The resultant supernatant was removed and stored at -20°C until further analysis.

Natural and recombinant OPG was measured using a sandwich ELISA technique (DuoSet ELISA, R&D Systems, Minneapolis). The ELISA was run according to the manufacturers' protocol, which is summarized in the following paragraph.

The ELISA was performed at room temperature using a 96-well microplate. After each step the plate was aspirated and washed three times using a wash solution. Initially the plate was coated with a capture antibody (360 µg/mL mouse anti-human OPG per 1.0 mL of PBS) and incubated overnight. Next, a reagent diluent (1 % bovine serum albumin in PBS) was added which was incubated for one hour. The sample supernatant or standard (recombinant human OPG) were then added in duplicate and incubated for two hours. A detection antibody (36 µg/mL biotinylated goat anti-human OPG in 1.0mL of reagent diluent) was added and the plate was incubated for two hours before streptavidin horseradish-peroxidase (1.0 ml Step per 11.9ml of reagent diluent) was added to each well. The plate was incubated for 20 minutes after which a substrate solution (equal parts hydrogen peroxide and tetramethylbenzidine) was added. The plate was then incubated for a further 20 minutes and then a reaction stop solution (2.5M H₂SO₄) was added. The optical density of each well was determined using a microplate reader (SpectraMAX 190, Molecular Devices, US) set to 450 nm. The lower detection limit of the assay was 62.5 pg/mL. Results lower than this detection limit are presented as 62.5 pg/mL.

2.1.9.3 Faecal Microbiota

Faecal microbiota analysis of stored faecal samples required additional funding and specialist expertise. A Laurensen Trust Award funded a pilot study to characterise the faecal microbiota of serial faecal samples. Professor Gerald Tannock and his team at the Department of Microbiology and Immunology, University of Otago, Dunedin completed the microbiota analysis, including the DNA extraction and bioinformatics. The methods used to extract the bacterial DNA and characterise the microbiome were the same as the Dunedin team used in a study characterising

the microbiome of human infants fed goats, cow or human milk. Due to the exact nature of the extraction and characterisation process the following sections are quoted from a publication prepared by the Dunedin team.⁽¹⁸⁶⁾

Faecal samples stored at -80°C were sent on dry ice to Dunedin by overnight courier. Upon receipt of the stool samples microbial DNA was extracted using the following method. “A one-tenth (weight/volume) faecal homogenate was prepared in sterile phosphate-buffered saline (pH 7.0). A 500 µl aliquot of homogenate was made up to 1.0 ml with sterile phosphate-buffered saline and centrifuged at 150 *g* for 5 min at 5°C. The supernatant was transferred to a microcentrifuge tube and centrifuged at 5,000 *g* for 5 min at 5°C. The pellet was suspended in 200 µl of lysis buffer (20 mg lysozyme, 80 µl 10 mM Tris-HCl-10 mM EDTA) and incubated at room temperature for 30 min. Fifty microliters of 20 % (weight/volume) sodium dodecyl sulphate (SDS) solution was added together with 300 µl of 50 mM sodium acetate-10 mM EDTA (pH 5.1) solution. The preparation was transferred to a beadbeater tube, and 300 µl of phenol saturated with 50 mM sodium acetate-10 mM EDTA buffer (pH 5.1) was added to the tubes. The sample was shaken at 5,000 rpm for 2 min in a beadbeater. After centrifugation at 14,000 *g* for 10 min at 4°C, the supernatant was transferred to a microcentrifuge tube, and 600 µl of phenol saturated with sodium acetate- EDTA buffer (pH 5.1) was added. Samples were mixed by vortexing for 1 min and centrifuged under the conditions described above. Then, 600 µl of phenol-chloroform-isoamyl alcohol (25:24:1) was added to the supernatant, and the mixture was vortexed for 1 min and centrifuged. This step was repeated once. Then, 600 µl of chloroform-isoamyl alcohol (24:1) was added to the supernatants, which were vortexed for 1 min and centrifuged for 5 min at 14,000 *g*. This step was repeated once. Nucleic acids were precipitated in 1 ml of isopropanol overnight at 20°C. The precipitated nucleic acids were obtained by centrifugation at 14,000 *g* for 20 min at 4°C. They were washed with 1 ml of 80 % ethanol and centrifuged at 14,000 *g* for 10 min, the supernatant was discarded, and the pellet was dried in air

at 37°C. Further purification of DNA was achieved using the Qiagen-AllPrep DNA/RNA minikit.”⁽¹⁸⁶⁾

Purified DNA was “sent to Macrogen (Korea) for unidirectional sequencing from the reverse primer using the Roche-454 genome sequencer with titanium chemistry. Sequences were processed using a combination of methods from both the QIIME version 1.2.1 and RDP pyrosequencing pipeline packages. Sequences were excluded from analysis if they were 250 or 550 bases in length, had an average quality score of 25, contained one or more ambiguous bases, had one mismatch with the sequencing primer, or had a homopolymer run of > 6. Following splitting into barcoded samples and initial quality filtering, the sequences were passed through the QIIME pipeline using default parameters, including chimera checking.”⁽¹⁸⁶⁾

“Species-level taxonomy was obtained by filtering operational taxonomic unit (OTU) tables, containing taxonomic data generated using the RDP classifier, at a genus level; extracting representative sequences; and using BLAST to identify species-level matches within the NCBI database. Biplots, showing principle coordinate clustering of samples alongside weighted taxonomic group data, were generated as part of the beta-diversity analysis in QIIME using family-level summarized OTU tables.”⁽¹⁸⁶⁾

Based on the results of the faecal microbiota pilot study, and due poor recruitment to the corticosteroid group, the candidate designed and executed a study with health volunteers as detailed in section 2.1.6. The primary aim of this study was compare changes in the faecal

microbiota of adults with and without CD consequent to two weeks of EEN. A collaborative funding grant has been submitted to enable the sequencing and analysis of the remaining faecal samples.

2.1.10 Clinical Outcomes: Disease Activity Indices

2.1.10.1 *Crohn's Disease Activity Index*

CDAI is a validated tool to assess disease activity.⁽⁵⁸⁾ It was calculated at baseline, week 4 and week 8. It was not calculated at every follow up appointment because it requires the patient to record their symptoms for the previous seven days. How the CDAI is calculated and what is included in the calculation are detailed in Table 2.4.

2.1.10.2 *Harvey Bradshaw Index*

The HBI is a validated simple index to described CD activity⁽¹⁸⁷⁾ and correlates well with the more detailed CDAI.⁽⁵⁷⁾ It was calculated at all seven study appointments. The HBI is a one day history of symptoms and extra-intestinal manifestations. The items included in the index and how to calculate it are detailed in Table 2.5.

Table 2.4. Calculation of the Crohn's disease activity index.⁽⁵⁸⁾

Assessment	Formula
How many liquid stools have you had on each of the last 7 days?	Sum x 2
Rate your abdominal pain over the last 7 days: None = 0, Mild = 1, Moderate = 2, Severe = 3	Sum x 5
Rate your general well-being over the last 7 days: Well = 0, Slightly below par = 1, Poor = 2, Very poor = 3, Terrible = 4	Sum x 7
Extra-intestinal manifestations: Yes = 1, No = 0. Arthritis/arthralgia, iritis/uveitis, skin/mouth ulcers, perianal disease, other fistula, fever	Sum x 20
Use of anti-diarrhoeal drugs: Yes = 1, No = 0	Value x 30
Abdominal mass: None = 0, Maybe = 2, Definitely = 5	Value x 10
Haematocrit: difference between current value and reference value (Males 46, females 42)	Difference x 6
Weight: current weight and usual weight	100 x (1 - current weight/usual weight)
Crohn's disease activity index score:	Sum of components

Table 2.5. Calculation of the Harvey Bradshaw Index.⁽¹⁸⁷⁾

Assessment	Formula
General well-being (yesterday): very well = 0, slightly below average = 1, poor = 2, very poor = 3, terrible = 4	0 - 4
Abdominal pain (yesterday): none = 0, mild = 1, moderate = 2, severe = 3	0 - 3
Number of liquid stools (yesterday)	As reported by patient
Do you have any lumps in your abdomen? None = 0, maybe = 1, yes = 2, yes, and tender = 3	0 - 3
Do you have any of these other symptoms? Arthralgia, uveitis, erythema nodosum, aphthous ulcers, pyoderma gangrenosum anal fissure, new fistula, abscess	1 point for each extra-intestinal manifestation
Harvey Bradshaw index score	Sum of components

2.1.11 Clinical Outcomes: Health Related Quality of Life

Patients with CD have lower HRQOL than the general population. The determinants of HRQOL are varied but include disease activity, hospitalisations and medications.⁽⁸⁵⁾ In this study quality of life was assessed before, during and after treatment using two validated self-assessment QOL tools: HADS⁽⁸⁸⁾ and SIBDQ.⁽¹¹⁵⁾

2.1.11.1 Hospital Anxiety and Depression Score

The HADS questionnaire contains 14 questions: seven questions relate to anxiety and seven questions relate to depression (Appendix B).⁽⁸⁸⁾ The questionnaire was administered at all seven appointments. Each question was scored from 0 to 3 and the sum for depression questions and anxiety questions calculated. A normal score for depression and anxiety was less than seven; borderline abnormal was eight to ten and greater than 11 abnormal. Intra and interpatient HADS scores were compared over time.

2.1.11.2 Short Inflammatory Bowel Disease Questionnaire

The SIBDQ is a ten question questionnaire (Appendix C) which is a shortened version of the 36 question IBDQ. The short questionnaire correlates well with the long version⁽¹¹⁵⁾ and was chosen to reduce patient burden. Patients were asked to complete the SIBDQ at all seven study appointments. Patients' responses were scored from one to seven and then the mean response of all ten questions was calculated. Intra and interpatient SIBDQ scores were compared over time. A clinically significant HRQOL response is a SIBDQ of more than 4.7 points.⁽¹¹⁵⁾

2.1.12 Clinical Outcomes: Nutrient Intake

Patients recruited to use PEN were encouraged to obtain one third of their nutrition from solid foods and two thirds from the nutritional formula during the last six weeks of the intervention

phase. At each fortnightly appointment patients were asked to give written examples of the meals that they had eaten during the previous fortnight. Patients completed an electronic food diary for at least 4 days during the six weeks of PEN. Patients recorded a minimum of two non-consecutive days, including one week day and one weekend day, during the first fortnight and two non-consecutive days during final fortnight of the treatment. An electronic food diary was chosen over a traditional paper based food diary as the patient group was relatively young (16 – 40 years old) and likely to be technology competent.

2.1.12.1 *Electronic Food Diary*

Dietary intake during PEN was recorded using a free smart phone/tablet application (App) “Evernote”. If patients did not have their own device Apple iPods were available on loan. The “Evernote” App has been used as an electronic food diary in paediatric research studies with children aged 16-19 years and 8 to 10 year olds and has been validated with these populations against a traditional paper based food diary (unpublished data), however it has not yet been validated for use with adult patients. “Evernote” allowed the researcher to set up an electronic food diary template that was shared with each study participant. Once the study participant had installed the App on their device the patient opened the App and took a photograph of their meal, and another photograph of any leftovers, and entered details of the meal into the App. These details included how the food was prepared, cooked, brand names or photographs of meal ingredients and also the volume of nutritional formula and other fluids consumed that day. The electronic food diaries were reviewed by the candidate and if further information was required to more accurately calculate nutrient intake patients were questioned at their next study appointment. The data recorded in the App was used to calculate average estimated nutrient intake during the PEN treatment.

2.1.12.2 *Nutrient Analysis*

Nutrient analysis during EEN treatment was calculated by multiplying the average number of cartons of nutritional formula consumed each day by the nutrient composition of each carton (Table 2.2) plus any additional fluids drunk (water, black tea or coffee). Nutrient intake during PEN treatment was estimated from electronic food diary data plus nutritional formula consumption.

The electronic food diary data were entered into dietary analysis programme Kai-culator. This programme is a University of Otago, Department of Human Nutrition (Dunedin), in-house nutrient analysis programme. The food diary data was entered as individual foods/fluids or as meal ingredients. The candidate and another NZ dietitian, who has extensive experience with this particular nutrient analysis programme, entered the four-day food diaries of two patients in duplicate. Duplicate entry allowed for comparison of portion size estimates between both research dietitians and comparison of food item selection from the nutrient analysis programme. There were small disparities between the entries which were mostly related to the choice of food items from the nutrient analysis programme, for example lettuce inner leaves rather than lettuce mixed leaves or choosing an existing programme recipe rather than entering a new recipe. These differences were discussed and decision was made on how best to interpret food diary data and chose the most appropriate nutrient analysis programme items. These decisions were then applied to the subsequent diary data entered.

2.2 Stakeholders in Enteral Nutrition Treatment for Active Crohn's Disease

Stakeholders in the delivery of enteral nutrition include, but are not limited to, the patient who is using the treatment, the dietitian who manages the EN treatment and the gastroenterologist who recommends EN treatment and manages the overall treatment plan for the patient. The following sections outline the methods used to survey these three stakeholders.

2.2.1 Survey of Patients with Crohn's Disease

A common assumption is that PF is more palatable than EF however there is limited research to support this assumption. This study aimed firstly to compare the palatability of polymeric and elemental nutrition formulae and secondly to gauge the acceptability of these formulae as a treatment option for active CD.

2.2.1.1 Study Outline

Patients diagnosed with CD attending gastroenterology outpatient clinics at Christchurch Hospital, Christchurch, NZ were invited to take part in the study. They were an unselected group of patients. Patients consented to take part in a taste test to compare the palatability of two different nutritional formulae. Patients that consented to take part in the study were asked to taste 10ml of an EF (Alitraq, Abbott Nutrition, NZ) and 10ml of a PF (Ensure Plus, Abbott Nutrition, NZ). Alitraq is a powdered nutritional formula specifically designed for acute gastrointestinal dysfunction/malabsorption. The protein source is a combination of peptides and free amino acids. It was prepared according to the manufacturer's instructions to a concentration of 1.0 kcal/ml. Ensure Plus (1.5 kcal/ml) was provided in a ready-to-drink form. Both formulae were vanilla flavoured and were served chilled. Participants were blind to the

names and characteristics of the formulae. Participants tasted each formula in alternating order and given 10ml of water to rinse their mouth between samples. These products were chosen because they are both vanilla flavoured milk based formulae, whereas other EFs available in NZ are fruit flavoured or unflavoured. The University of Otago Ethics Committee approved the study.

2.2.1.2 Questionnaire Design

The questions (Appendix D) used in this study were based on those used by Makai et al. ⁽¹⁷⁴⁾ This report used a 15 item scale to assess palatability. The current study used a selection of five of these items. Participants were asked to rate each drink on a 5 point Likert scale as follows: (1) difficult to drink/easy to drink, (2) unpleasant flavour/pleasant flavour, (3) poor mouth feel/good mouth feel, (4) weak aftertaste/strong aftertaste, and (5) not acrid/acrid. We also asked two additional questions in a slightly different format. Participants were asked to indicate whether they preferred one drink over the other and asked to indicate how likely it is that they could drink 200ml of each drink 6 – 8 times per day on a scale of 0-10. These seven questions were selected as they appeared to be most relevant to palatability.

Participants answered a series of questions based on the assumption that using either of the drinks for EEN may put their disease into remission. These questions included whether they would rather use EEN for eight weeks or take a course of corticosteroids for eight weeks. They also indicated if they would consider using EEN for eight weeks if it could put their disease into remission if they had severe, moderate or mild symptoms due to active CD.

2.2.2 Survey of Dietitians

Dietitians are an integral part of the multidisciplinary care of patients with CD. Dietitians are the health professional most likely to manage patients whilst using EEN and therefore New Zealand dietitians were surveyed to understand to their use and perception of EEN as a treatment for active CD.

2.2.2.1 Survey Design

The survey questions (Appendix E) were adapted from a survey used with North American physicians to understand their attitudes and use of enteral nutrition to treat paediatric CD.⁽¹⁸⁸⁾

The survey platform was QuestionPro Online Survey Software Application (www.questionpro.com). It contained single choice, multiple choice and open-ended questions. Question branching was included so that dietitians only answered questions relevant to their experience with CD patients and enteral nutrition. An Australian dietitian who frequently uses EEN and a colleague who has experience with online survey design pretested the survey. The final version of the survey incorporated their feedback. A limitation of the survey design is that pre-testing was not completed with clinical dietitians with a small CD patient load or limited experience using EEN.

The focus of this survey was to understand the current practice of NZ clinical dietitians compared to clinical dietitians internationally and current IBD standards of care. Therefore, the survey did not require ethical approval but the data collected from survey respondents were managed ethically and anonymity of survey respondents was maintained.

2.2.2.2 Participants

NZ dietitians that were members of the national professional association, Dietitians NZ, were invited to complete the survey anonymously. This member group is composed of dietitians working in clinical, public health, research, food service and pharmaceutical industry settings. The web-based survey link was disseminated via Dietitians NZ's weekly electronic newsletter to 488 active members. An electronic link took dietitians to the online survey. Three weeks after the initial newsletter advertisement dietitians were reminded to complete the survey.

2.2.3 Survey of Gastroenterologists

Gastroenterologists are specialists in the management of gastrointestinal disorders including Crohn's disease. The gastroenterologist, in conjunction with the patient, develops a treatment plan for the long term and acute management of CD and may refer their patients onto other health professionals, including dietitians and IBD nurse specialists to implement and oversee treatment plans. Therefore, understanding gastroenterologists' perception of EN and its role, if any, in the treatment of their patients with active CD is important.

2.2.3.1 Survey Design

The survey questions (Appendix F) were adapted from a survey of North American physicians to understand their attitudes and use of enteral nutrition to treat paediatric CD.⁽¹⁸⁸⁾ The survey platform used was QuestionPro Online Survey Software Application (www.questionpro.com). The survey contained single choice, multiple choice and open-ended questions. Question branching was included so that gastroenterologists only answered questions relevant to their experience with CD patients and enteral nutrition. Two gastroenterologists (one adult and one paediatric specialist) pretested the survey and committee members of the NZ Society of Gastroenterology reviewed it prior to distribution to NZ Society of Gastroenterology members.

The focus of this survey was to understand the current practice of NZ adult compared with paediatric gastroenterologists and compared to gastroenterologists and IBD standards of care internationally. Therefore, the survey did not require ethical approval. However, the data collected from survey respondents was managed ethically and anonymity of survey respondents was maintained.

2.2.3.2 Participants

The survey was distributed through the NZ Society of Gastroenterology as most NZ gastroenterologists are members of the society. Society members include consultant gastroenterologists and surgeons, gastroenterology registrars as well as scientists and other health professionals. The NZ Society of Gastroenterology disseminated the web-based survey link via email list to 130 full members (consultants and trainees) in July 2015. An electronic link took members to the online survey and they received a reminder to complete the survey three weeks after the initial newsletter advertisement.

2.3 Statistical Analysis

The results from clinical trials and questionnaires are presented as percentage of responses, medians and ranges. Means and standard deviations have not been used because many of the variables were not normally distributed and therefore non-parametric statistical analysis based on medians and range have been used. The clinical trial was designed as a pilot study, the results of which could be used to inform a randomised controlled trial, therefore a power calculation was not required. Last observation carried forward (LOCF) intention to treat and per protocol analysis were conducted with the EEN and PEN study results. Statistical analyses were conducted using Mann-Whitney U test (test statistic U), Wilcoxon paired signed rank test (test statistic W), Fisher's exact test, Chi-squared test and Spearman correlation. Statistical significance was present with $p < 0.05$. Open-ended questions were summarised using thematic analysis. Statistical tests and graphs were prepared in Prism 6 version 6.05 (GraphPad Software Inc).

Chapter 3

Patient Perspective of Nutritional Therapy

3.1 Introduction

EEN is not widely recommended for adults with active CD,⁽³⁷⁾ mostly as a result of a Cochrane meta-analysis⁽³⁸⁾ of seven studies that found CS to be superior to EEN for the induction of disease remission. Six of the seven studies in the meta-analysis included adult patients and half of these studies had 25 to 39 % of patients withdraw due to unpalatable EN formula.^(159, 161, 163) One reason for this may have been that these studies used an EF provided orally whereas recent research in children has mainly used PF administered orally with few withdrawals due to poor palatability.^(28, 29) Also, changes in EF and PF formulations in recent years may have led to enhanced taste characteristics and increased palatability.⁽¹⁸⁹⁾

Elemental formula (comprising amino acids and/or peptides) is often assumed to be less palatable than PF primarily based on its distinct smell and flavour. PF (containing whole proteins) may also have a distinct smell and flavour but it is often less marked. The palatability or taste preference of nutritional formulae has been examined^(174, 176, 190, 191) and taste preferences between patients and healthy controls are often similar.⁽¹⁹²⁻¹⁹⁴⁾ Milk-based supplements are rated more highly than fruit based products in most patient groups and countries.^(190, 194, 195) Few studies, however, have compared patient preferences or palatability of EF compared with PF^(174, 176) and there is a lack of research that compares palatability of PF and EF in adults with CD.

3.2 Aims and hypotheses

The aim of this study was, firstly, to compare the palatability of PF and EF in a sample of NZ adults with CD and, secondly, to gauge the acceptability of these formulas as a treatment option for active CD instead of corticosteroids. It was hypothesised that:

- Polymeric nutritional formula is more palatable than elemental nutritional formula
- Adults with CD are interested in using nutritional therapies in place of corticosteroid treatments

3.3 Methods

A blind taste test of one EF and one PF was conducted with patients with CD attending a Christchurch Hospital gastroenterology outpatient clinic. Patients were asked to score each formula on characteristics associated with palatability and asked which they preferred. In addition, patients were asked if they would consider using a nutritional formula to treat active CD. The complete study methods are detailed in Chapter 2 section 2.2.2.

3.4 Results

3.4.1.1 Participant characteristics

Thirty-five patients agreed, and no patients declined, to take part in the study. The median age of patients was 39 years old (range 19 - 77 years): 63 % were males and 71 % (n = 25) had previously used prednisone. One patient had previously used EEN under the paediatric gastroenterology team to treat CD symptoms. Several patients had previously used nutritional formulae to supplement their usual diet.

3.4.1.2 Palatability ratings

PF was rated as being easier to drink, had a more pleasant flavour, good mouth feel and was less acrid compared to the EF (Wilcoxon matched pairs signed rank test, $p < 0.001$ for each variable) (Figure 3-1). However, there was no statistically significant difference in aftertaste between the two formulae ($W = -146.0$, $p = 0.09$).

Overall, the participants preferred the PF (91 %) to the EF (9 %) ($W = 535.0$, $p < 0.001$) and felt that they would be more likely to be able to drink 200ml of the PF six to eight times per day (median 8 (range, 1 to 10) than the EF (median 2 (range, 0 to 9) (Figure 3-2).

3.4.1.3 Acceptability of enteral nutrition

Of the 25 patients who had previously used prednisone to treat CD flare ups, 15 (60 %) indicated that they would rather use EEN for 8 weeks than take another course of prednisone, eight (32 %) indicated that they would rather use prednisone than EEN for 8 weeks and two (8 %) were unsure which they would prefer.

All patients were asked if they would consider using EEN, if it could put their disease into remission, to treat active CD symptoms. A greater proportion of patients would consider using EEN to treat active disease (Chi-square 29.95, $p < 0.0001$), especially if they had moderate or severe symptoms (Figure 3-3).

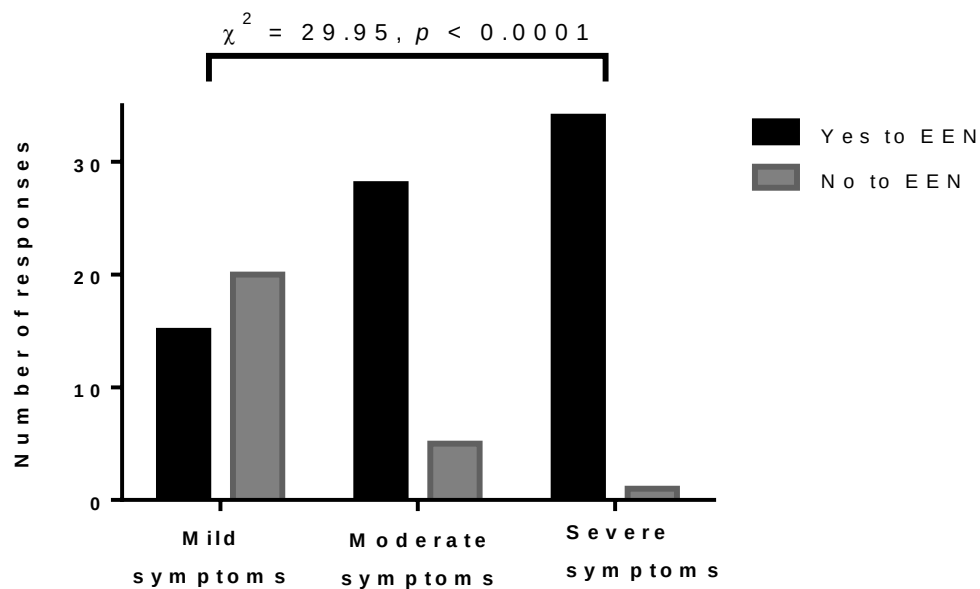


Figure 3-3. Number of patients that would consider using EEN, if it was efficacious, for 8 weeks to treat mild, moderate and severe Crohn's disease symptoms (n = 35).

3.5 Discussion

The palatability of enteral formulae has been studied previously^(174, 176, 190, 191, 193, 194) but comparisons between PF and EF palatability has not been examined widely.^(174, 176) This study used a convenience sample of patients who were attending gastroenterology outpatient appointments at Christchurch Hospital, NZ. The study design and questionnaire were both based on that of Makai et al.⁽¹⁷⁴⁾ in which they used a selection of 1.0 kcal/ml EF, peptide-based formula and PF all of which were unflavoured or coffee and fruit flavoured. Vanilla flavoured milk-based formulae were used in the current study because the non-milk based PF available in NZ is not nutritionally complete therefore unsuitable for EEN. A more calorie dense (1.5 kcal/ml) formula was chosen as it would be challenging for adult patients with CD using EEN to meet their calorie needs orally using only a 1.0 kcal/ml formula.

Makai et al⁽¹⁷⁴⁾ found that some brands of EF were more palatable than others, that peptide-based formulae scored highly for after-taste and acidity and that overall flavoured formulae were more palatable than unflavoured formulae. Only one of the three EF brands scored similarly for palatability compared with the PF. In the current study participants also found the EF less palatable than the PF and rated it more acrid, difficult to drink, disliked the flavour and considered it highly unlikely that they could drink enough of it to meet their nutritional requirements. Most participants scored the vanilla flavoured PF highly for drinkability and flavour. Makai et al⁽¹⁷⁴⁾ proposed that the poor palatability of EF is related to the bitterness and saltiness of the formula. They found that fruit flavoured EF was more acceptable and proposed that it was probably due to organic acids in the fruit flavourings suppressing the bitterness of the free amino acids.⁽¹⁷⁴⁾ The EF used in this study was a mixture of peptides and free amino acids. Peptide-based formulae have been rated poorly for palatability compared with free amino acid formulae.^(174, 193) In this study if there was a milk-based free amino acid flavoured EF available it may have compared more favourably with the PF tested.

One of the aims of this study was to assess patient interest in EEN as a potential CD treatment. Patients whom had previously used CS were asked if they would rather use EEN for eight weeks or take another course of CS. Of the 25 patients who had previously used steroids, 60 % indicated that they would consider using EEN. CS are just one medication that may be used to treat active CD and, like most CD medications, have many side effects including mood changes, glucose intolerance, puffy face, acne, reduced linear growth and steroid dependence.⁽¹¹⁾ Although EEN is burdensome on patients one of its many advantages is the relative lack of side effects and avoidance of drugs with many side effects. High treatment side effects scores are associated with lower patient quality of life.⁽¹⁹⁶⁾ This may explain why patient quality of life has been found to improve post EEN^(31, 33) even in those using nasogastric tube feeding.^(33, 86, 196) This study suggests that patients are interested in avoiding the short and/or long term side effects associated with CS treatment.

Patients were also asked if they would consider using EEN to treat severe, moderate or mild disease. More than 80 % indicated that they would use EEN to treat moderate and severe disease. This result was not surprising given that post prandial abdominal pain and diarrhoea are often common symptoms of active disease. Interestingly, 43 % of patients indicated that they would consider EEN to treat even mild symptoms. It appears that adults are interested in using EEN, whether to avoid side effects of other CD medications or for some other reason. This was not able to be explored further in the current study.

This study provides some insight into the impact that CD symptoms and medication side effects may have on patient quality of life. Forfeiting all solid and liquid food and fluids for eight weeks and consuming only a liquid nutritional formula and water is relatively burdensome on patients,

however the majority of patients in this study would consider following this regimen to treat moderate or severe CD symptoms. Although data on patient disease severity was not collected, 70 % of these patients had previously used steroids and two-thirds of those appeared to appreciate that the potential benefits of EEN outweigh the negatives in order to avoid the side effects of steroids they may have previously experienced. EEN requires commitment from the patient: this study suggests that patients who have had negative experiences with other CD medications or suffered from moderate to severe symptoms may consider an alternative treatment such as EEN.

3.6 Conclusion

This study supports the hypothesis that PF is more palatable than EF and that adults with CD are interested in using nutritional therapy in place of CS treatments. Further research involving adults with CD is warranted to ascertain whether EEN is an effective treatment for active CD inflammation.

Chapter 4

Clinical Trial

4.1 Introduction

EEN has been recommended as a first line therapy for children with active CD instead of corticosteroid therapy for more than 10 years.⁽¹⁹⁷⁾ However, in adults EEN is currently only recommended as an adjuvant therapy in adults who are malnourished or unable to use corticosteroids or for those patients for whom corticosteroids are contraindicated.^(36, 37) Previous randomised controlled trials of EEN compared with CS treatment in adult populations predominantly used elemental and semi-elemental formulae.^(17, 129, 155, 158-160, 162, 163) and three of the twelve studies had at least a third of the patients withdraw from EEN due to the reported unpalatability of the formula.^(129, 159, 163) EEN with PF is well tolerated by children with active CD and has been shown to effectively induce disease remission in approximately 70 – 80 % of children.^(28, 29, 198) Polymeric ready-to-drink formula was used with adults for four weeks in two randomised controlled trials comparing corticosteroids and EEN.^(156, 157) Both studies delivered the formula via a tube and reported 100 % compliance with the EEN regimen and disease remission was achieved in 12 of the 15 (80 %) patients in the Spanish study⁽¹⁵⁷⁾ but only 4 out of 10 (40 %) patients in the Greek study.⁽¹⁵⁶⁾ PF has also been used with adults in randomised controlled trials comparing the efficacy of EF with PF. These studies found that EEN with PF induced disease remission in approximately 60 – 70 % of patients.^(172, 173, 199)

Some paediatric EEN protocols allow small amounts of nutrient deficient foods and fluids, including small amounts of clear fluids, boiled sweets and formula flavourings.⁽¹⁶⁾ These additions to the regimen do not appear to limit the effectiveness of EEN to induce disease remission in the paediatric population. Whereas, the addition of larger amounts of solid food, in addition to the EN formula, does appear to be a less effective means of inducing disease

remission compared with EEN.^(179, 180) A regimen which sequentially combines EEN and PEN and limits the amount of additional food may be more efficacious than a prescribed volume of EN plus unlimited normal diet. Such a regimen has not previously been studied with paediatric or adult patients with active CD.

4.2 Aims and Hypotheses

The aim of this research was to investigate the feasibility and effectiveness of EEN as a treatment for young adults with active CD. The aim of conducting the PEN trial was to investigate if a sequential EEN and PEN treatment is as effective as EEN at inducing disease remission. It was hypothesised:

- That in a population of young adults with active CD, EEN therapy induces clinical and biochemical disease remission.
- That treatment with a novel PEN regimen induces disease remission and markers of disease activity as effectively as EEN in young adults with active CD.

4.3 Methods

The complete clinical trial methods are detailed in Chapter 2, Section 2.1 and will only be described in brief in the following paragraphs. The clinical study was composed of two phases. The first phase of the clinical trial was to recruit 20 patients with active CD who chose to use EEN and an additional 20 patients who used corticosteroid therapy to act as a control group. Patients who met the study inclusion criteria and were interested in participating in a research study using EEN or corticosteroids to treat active CD were referred into the study by Christchurch Hospital gastroenterologists. The EEN group used EN exclusively for eight weeks

with the exclusion of all usual foods and liquids apart from water and black, unsweetened tea and coffee. The patients using CS therapy consumed their usual diet.

The design of the second phase of the clinical trial was based on the outcomes of the first study and the protocols used in recent PEN literature. This phase was the same as the first phase except patients used a sequential regimen of EEN followed by PEN to treat active CD rather than EEN. The PEN group used EN exclusively for two weeks and then reintroduced a small lunch or evening meal plus EN for the remaining six weeks. Patients were asked to eat a small meal, approximately half of their usual portion size, which would theoretically equate to no more than one third of their daily energy intake. There were no restrictions on foods which could be included in the meal although patients were encouraged to eat a balanced meal similar to what they would eat prior to starting the treatment. Patients were asked to continue to only drink water and tea or coffee throughout the PEN phase but could add milk to hot drinks if preferred.

4.4 Results

4.4.1 Patient Recruitment

4.4.1.1 Exclusive Enteral Nutrition Group

Patient recruitment into phase one of the clinical trial started in May 2013 and finished 22 months later in February 2015. During this period 27 patients were referred by Christchurch gastroenterologist's public and private clinics. Two patients decided not to initiate EEN treatment (both did not think that they would be able adhere to the treatment protocol). A mean of 1.14 patients per month were recruited to use EEN to treat active CD.

4.4.1.2 Partial Enteral Nutrition Group

Patient recruitment into phase two of the clinical trial started in March 2015 and ended in December 2015. During the ten months of recruitment, 13 patients with active newly diagnosed and existing CD were recruited to use PEN (a mean of 1.3 patients per month). Patient recruitment was stopped prior to recruiting 20 patients to allow adequate time for the six month study follow up to be completed prior to this thesis submission. All patients referred by their gastroenterologists for PEN consented to initiate the treatment.

4.4.1.3 Corticosteroid Group

Patient recruitment into the CS arm of the study was open for the duration of both the EN studies (32 months) and during this time two patients were recruited into the study. One of these patients, female less than 18 years old, initially consented to use EEN but withdrew from the study within the first week and then started on CS. The other patient, a male aged > 35 years old, chose to use CS rather than EEN from the start. Possible reasons for the limited number of referrals to the CS study group are discussed in section 4.5 of this chapter.

Both of the patients who used CS had newly diagnosed CD, were on no CD medications, one had ileal disease and the other ileocolonic disease. At baseline they had a CDAI of 150 – 220, a normal CRP of 5 mg/L, an ESR < 15 mm/Hr, a normal faecal OPG of 62.5 pg/mL and FC was not performed. Due to the very small numbers in this study group the results of the CS patient group have not been compared with the outcomes of patients using enteral nutrition. In summary, after eight weeks of CS treatment both patients had a CDAI < 150, CRP had increased slightly to 6 and 11 mg/L, ESR was < 15 and 16 mm/Hr and faecal OPG had not changed. At week 26 both patients were on a biologic CD medication.

4.4.2 Baseline Characteristics

The two EN intervention groups were similar at baseline and there were no statistically significant differences between the clinical or biochemical data of the two groups (Table 4.1).

Table 4.1. Baseline characteristics of patients referred for enteral nutrition therapy.

Baseline characteristics	EEN (n = 25)	PEN (n = 13)	<i>p</i>
	Median (range) or n (%)	Median (range) or n (%)	
Age	23.3 (15.8, 38.4)	19.2 (16.5, 38.2)	0.443
Sex - female	18 (72)	12 (92)	0.222
Ethnicity – NZ European	24 (96)	12 (92)	1.00
Born in NZ	23 (92)	12 (92)	1.00
Occupation			0.885
High school student	6 (24)	2 (15)	
Tertiary institution student	5 (20)	2 (15)	
Paid employment	11 (44)	7 (54)	
Other	3 (12)	2 (15)	
Highest qualification			0.148
Post-secondary qualification	11 (44)	2 (15)	
Secondary or no qualification	14 (60)	11 (85)	
Current smoker	3 (12)	0 (0)	0.538
Family history of CD	5 (20)	3 (23)	1.00
Duration of disease, years	0.07 (0.0, 14.4)	0.06 (0.1, 4.3)	0.675
Newly diagnosed	21 (84)	9 (69)	0.407
Disease location			0.554
L1 ileal	12 (48)	9 (69)	
L2 colonic	0 (0)	0 (0)	
L3 ileocolonic	13 (52)	4 (31)	
L4 modifier added to L1-L3	3 (12)	3 (23)	

Baseline characteristics	EEN (n = 25)	PEN (n = 13)	
	Median (range) or n (%)	Median (range) or n (%)	
Disease behaviour			0.958
B1 non-stricturing/penetrating	20 (80)	10 (77)	
B2 stricturing	3 (12)	2 (15)	
B3 penetrating	2 (8)	1 (8)	
p perianal disease modifier	2 (8)	1 (8)	
Disease activity (CDAI)	154 (39, 498)	239 (65, 321)	0.255
< 150	10 (40)	4 (31)	0.266
150 – 220	8 (32)	2 (15)	
> 220	7 (28)	7 (54)	
Disease activity (HBI)	5.0 (0, 14)	5.0 (2, 11)	0.994
< 5	12	5	
≥ 5	13	8	
Concurrent medication			0.418
none	13 (52)	3 (13)	
mesalazine	9 (36)	6 (46)	
immunosuppressant	3 (12)	3 (23)	
biological	1 (4)	1 (8)	
CRP (mg/L)	9.0 (3, 158)	25.0 (3, 71)	0.199
< 5 (mg/L)	8 (32)	3 (23)	
Platelets (x 10 ⁹ /L)	337 (196, 733)	411 (256, 695)	0.181
ESR (mm/hr)	14 (2, 104)	23.0 (7, 68)	0.152
Faecal calprotectin (µg/g)	1025 (60, 3600)	1175 (60, 3600)	0.579
Faecal OPG (pg/mL)	106.1 (62.5, 1632.2)	62.5 (62.5, 838.4)	

4.4.3 Treatment Withdrawals

4.4.3.1 Exclusive Enteral Nutrition Group

Figure 4-1 illustrates the flow of patients through the two EN intervention treatments. EEN treatment was initiated by 25 patients, 11 patients withdrew from EEN treatment with the remaining 14 completing the eight-week treatment. Four patients withdrew from EEN in the first two weeks; two young females tried EEN for less than a week and decided that CS would be a better treatment option for them and changed to CS treatment, one patient who was pregnant had worse nausea on EEN and introduced some food back into her diet, and one patient required urgent surgery as a result of a perforated bowel. A further seven patients withdrew from the study between week two and eight of the intervention period for various reasons; three patients did not tolerate the treatment, one patient was drinking soya based Ensure Plus to comply with her vegan beliefs and had diarrhoea which resolved when she stopped taking the drinks, one patient required a NGT to be placed to help her achieve adequate nutrition intake but the tube split and she opted not to have another tube placed and another patient was tolerating EEN well but developed nausea after three weeks of treatment which resolved when she stopped taking the nutritional formula. One patient did not manage to do EN exclusively and ate throughout the intervention period and one patient used EEN for five weeks but restarted eating usual food in response to a stressful situation at work. The seventh patient was changed to CS treatment at week four because there had been no improvements in his clinical or inflammatory markers. Of the 11 patients who withdrew, six were under 18 years old whilst 8 were female and 3 were male.

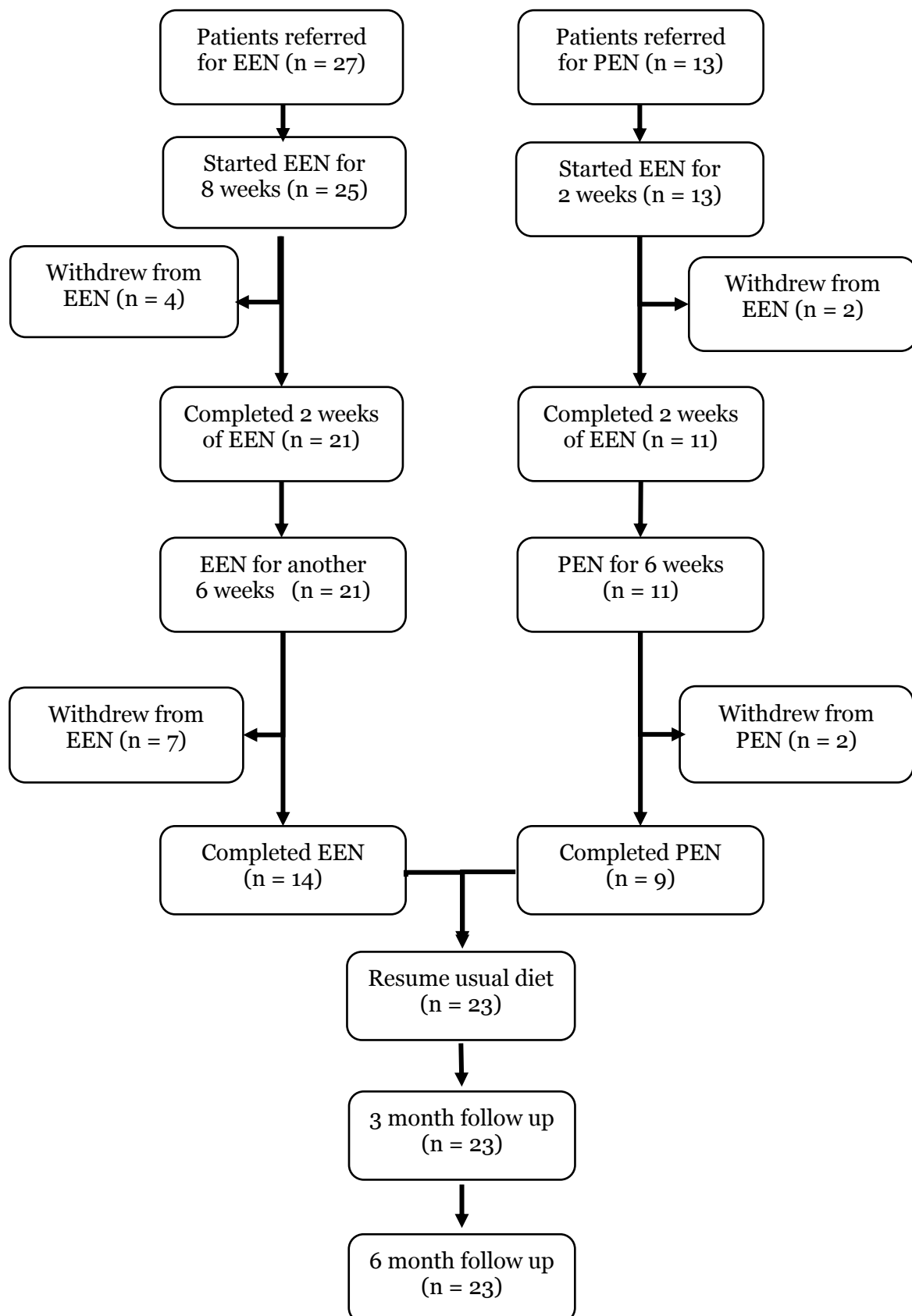
4.4.3.2 Partial Enteral Nutrition Group

PEN treatment was initiated by 13 patients; four patients withdrew and 9 patients completed the eight-week treatment. Two of the four patients who withdrew from the study withdrew in the first two weeks because they were unable to initiate EEN due to nausea in one case and diarrhoea and abdominal pain in the other case. A further two patients withdrew on PEN, one patient had a focal sealed ileal perforation when she was referred for PEN and flared after the

two weeks of EEN when food was reintroduced. She went back onto EEN until she had a bowel resection three weeks later. The second patient had not responded to the treatment at week four and was started on CS therapy instead. Of the four patients who withdrew, two were under 18 years old and three were female.

Total treatment withdrawals due to intolerance of the formula (nausea and/or diarrhoea or inability to consume an adequate amount) of EN were 28 % of EEN patients and 15 % of PEN patients. The EEN and PEN treatments were completed by 56 % and 69 % of patients respectively. There was no statistically significant difference in the proportion of patients who completed EEN compared to the PEN treatment (fisher's exact test, $p = 0.502$).

Figure 4-1. Flow diagram of patients recruited to use exclusive or partial enteral nutrition.



4.4.3.3 *Patients Who Completed EN Treatment Compared With Those Who Withdrew From EN Treatment*

The patients who withdrew (n = 15) from the trial were different compared with the patients who completed (n = 23) EN treatment. Patients who withdrew from EN treatment had a higher median baseline CDAI compared with those who completed EN treatment (Figure 4-2).

However, there was no significant difference in the median CRP or FC of the two groups ($p > 0.05$). The median age of patients who withdrew was younger (17.2 years old) than patients who completed the treatment (23.1 years old) but the age difference did not reach statistical significance ($U = 113.5$, $p = 0.079$) (Figure 4-3). However, the proportion of patients who were of secondary school age (< 18 years old), was significantly greater (fisher's exact test, $p = 0.012$) in the group who withdrew from treatment (Figure 4-3). There was no difference in the proportion of patients who had a CD flare within six months of initiating EN treatment (8/23) compared with the patients (7/15 patients) who withdrew from EN treatment and started an alternative treatment.

4.4.4 Response to Enteral Nutrition Therapies

Patients recruited to use EEN and PEN to treat active CD had similar changes in serum and faecal markers of inflammation and disease activity scores using LOCF intention to treat and per protocol analysis (Table 4.2).

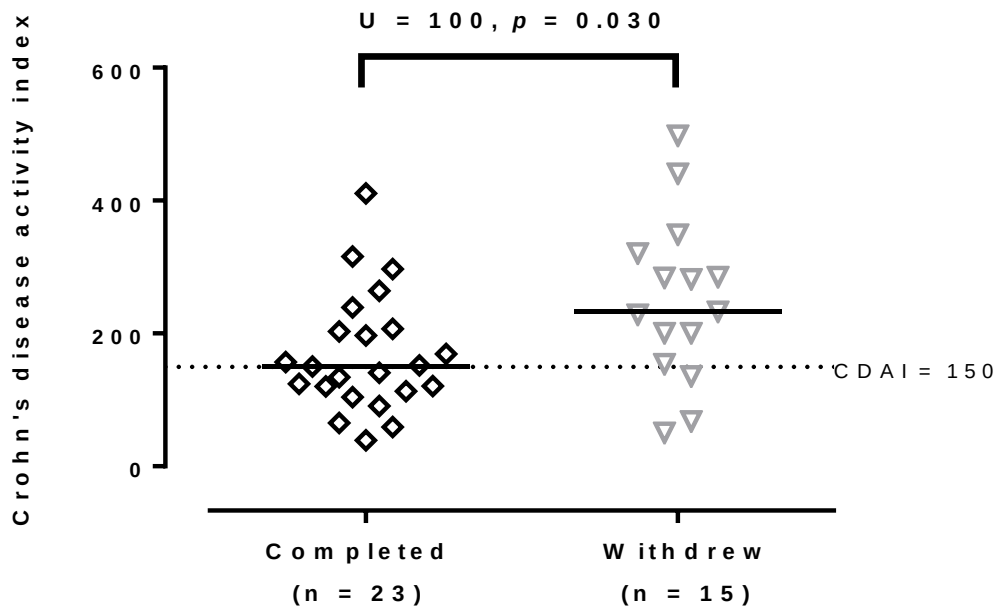


Figure 4-2. Baseline Crohn's disease activity index of patients who completed and withdrew from enteral nutrition therapy.

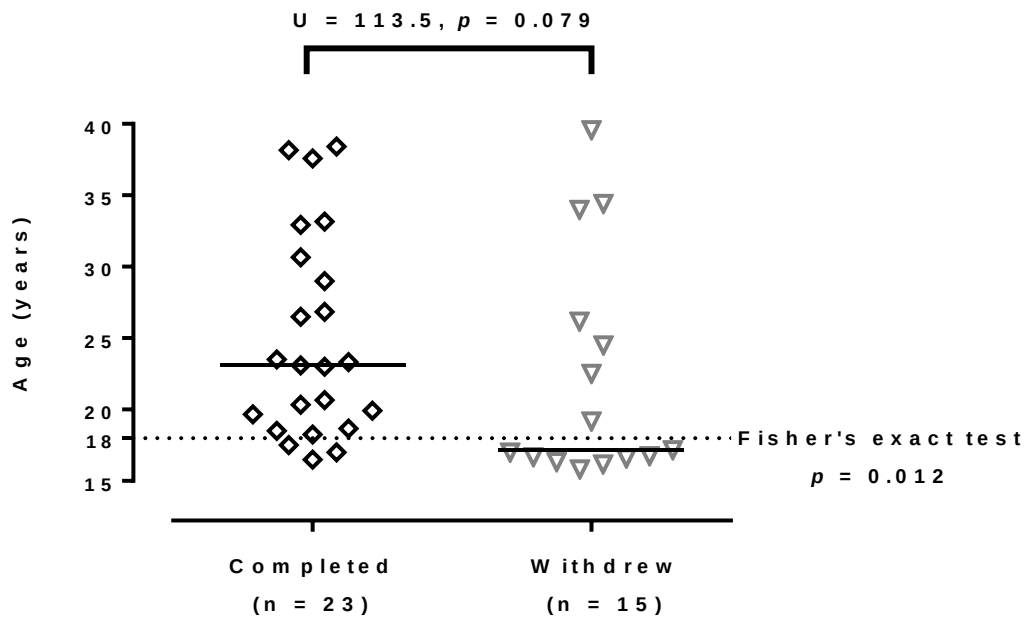


Figure 4-3. Age of patients who completed and withdrew from enteral nutrition therapy.

Table 4.2. Change in disease activity parameters from baseline to treatment completion of patients using enteral nutrition therapy.

Clinical indicators of treatment response	Intention to treat			Per protocol		
	Median change (range)			Median change (range)		
	EEN (n = 25)	PEN (n = 13)	p value	EEN (n = 14)	PEN (n = 9)	p value
CDAI	-17 (-375, 196)	-38 (-261, 34)	0.379	-87 (-375, 18)	-84 (-261, -17)	0.568
HBI	-3 (-9, -8)	-2 (-6, -1)	0.763	-3 (-9, 0)	-2 (-5, 0)	0.632
CRP (mg/L)	-1 (-120, 18)	0 (-46, 12)	0.896	-2 (-33, 18)	-5 (-46, 5)	0.963
ESR (mm/hr)	0 (-37, 9)	0 (-18, 19)	0.696	-1.5 (-37, 9)	1 (-18, 18)	0.311
FC (µg/g)	0 (-3013, 1851)	-128 (-1945, 720)	0.709	-78 (-3235, 456)	-128 (-1945, 1084)	0.541
Faecal OPG (pg/mL)	0 (-1570, 352)	0 (-776, 0)	0.443	-34.9 (-1597, 352)	0 (-776, 0)	0.220

Note: change in each parameter is calculated by subtracting the value at week 8 from the baseline value.

A negative number corresponds with an improvement in that parameter.

4.4.4.1 LOCF Intention-To-Treat Analysis of EN Therapy Outcomes (n = 38)

LOCF analysis of patients who were recruited to use EEN found that CDAI significantly decreased from baseline to week 8 ($W = -110.0, p = 0.025$) as did CDAI of patients recruited to use PEN ($W = -59.0, p = 0.006$) (Figure 4-4). At week 8, of the 25 patients referred for EEN, 17 (68 %) had a CDAI of less than 150 or had a fall in CDAI of more than 70 points. Treatment with PEN resulted in 8 out of 13 (62 %) patients achieving a CDAI of less than 150 or a fall in CDAI of more than 70 points. There was no difference in the response of patients with newly diagnosed CD compared with those with existing CD ($p > 0.05$). Due to the similar response to both EEN and PEN treatment (Table 4.2) the two groups have been combined in the following paragraphs to analyse the effect of EN therapy on disease activity.

LOCF analysis of all patients referred for EN therapy (n = 38) resulted in a statistically significant improvement in CDAI score over the eight week treatment ($W = -324.0, p = 0.0005$). There was also significant improvement in serum CRP within two weeks of EN ($W = -189, p = 0.003$) and this change was maintained at week 8 ($W = -198, p = 0.010$). There were no significant changes in serum ESR, FC or faecal OPG consequent to the EN therapies ($p > 0.05$ for all).

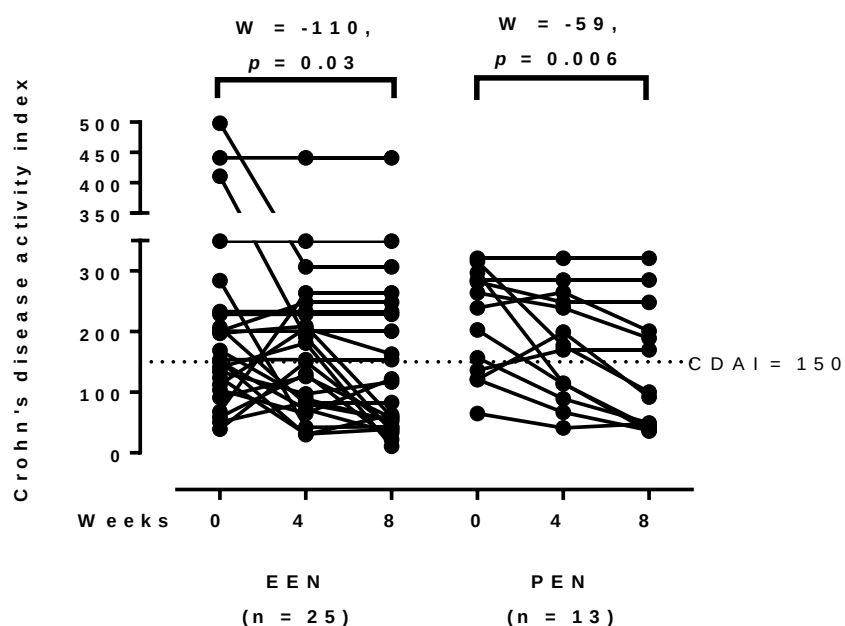


Figure 4-4. Change in Crohn's disease activity index of all patients referred for enteral nutrition therapy.

4.4.4.2 Per Protocol Analysis of EN Therapy Outcomes (n = 23)

Per protocol analysis of the 23 patients who completed either EEN or PEN therapy also resulted in statistically significant improvements in disease activity scores and serum CRP but no significant changes in the other serum or faecal markers of inflammation. The CDAI scores of patients who used EEN or PEN significantly improved (Figure 4-5) during the eight week treatment. CDAI score had started to improve at week 4 but statistically significant improvements were not observed until treatment completion at week 8. At treatment completion 13 of the 14 (93 %) patients using EEN had a CDAI < 150 as did seven of the nine (78 %) patients who used PEN. There were no significant differences in the proportion of patients who achieved disease remission with EEN compared with PEN or CDAI at week 8 ($p > 0.05$).

As a result of the comparable improvements in CDAI consequent to EEN and PEN, changes in markers of inflammation of the two groups have been combined (n = 23) to improve the

statistical power. Serum CRP at baseline was elevated ($\text{CRP} \geq 5 \text{ mg/L}$), or became elevated during treatment, in 17 of the 23 (74 %) patients who completed the EN treatment. The median serum CRP at baseline was 9 mg/L (range, 3 – 71) and fell to a median of 5 mg/L (range, 3 – 22) after two weeks of EEN and then fell further to a median of 4 mg/L (range, 3 – 61) by week 8 of either EEN or PEN treatment (Figure 4-6). There were no significant changes in serum ESR during EN therapy.

FC improved after two weeks of EEN therapy but the initial improvement was not sustained throughout EN treatment (Figure 4-7). The median FC fell from 830 $\mu\text{g/g}$ (range, 60 – 3600 $\mu\text{g/g}$) to 570 $\mu\text{g/g}$ (range, 60 – 3564 $\mu\text{g/g}$) after two weeks of treatment and then increased to 587 $\mu\text{g/g}$ (range, 60 – 3569 $\mu\text{g/g}$) at week 8. FC did not significantly increase in the PEN patients ($n = 10$) in response to introducing a small meal of solid food after week 2. The change in FC during EN treatment was not correlated with change in disease activity.

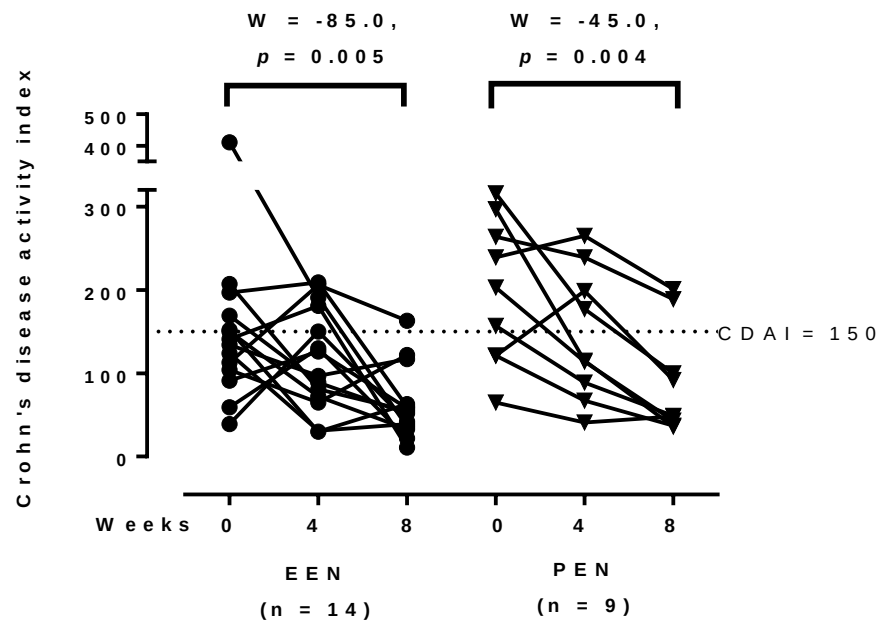


Figure 4-5. Change in Crohn's disease activity index during enteral nutrition therapy.

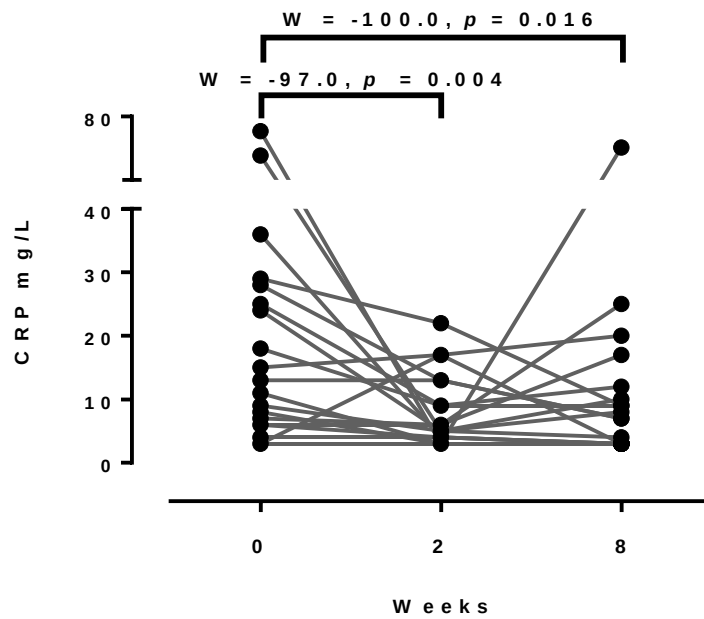


Figure 4-6. Change in serum CRP during enteral nutrition therapy (n = 23).

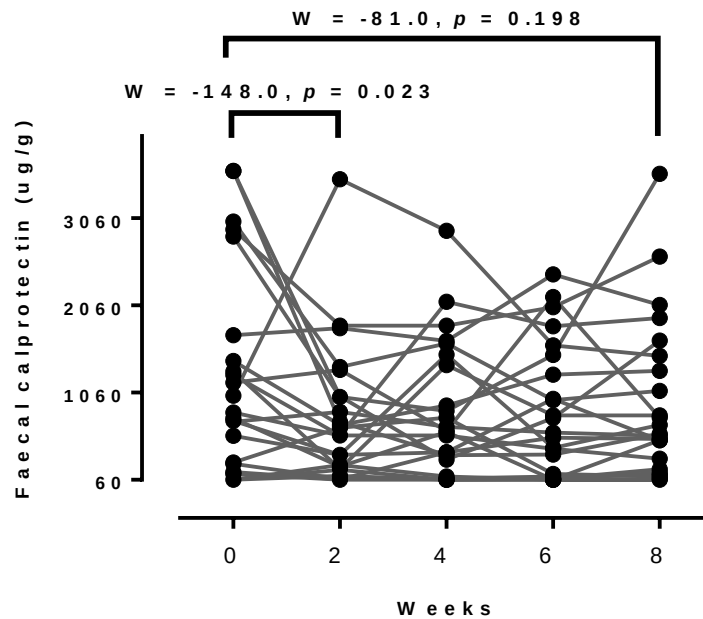


Figure 4-7. Change in faecal calprotectin during enteral nutrition therapy (n = 23).

4.4.5 Follow Up At Six Months

Patients were followed up for four months post EEN or PEN. Four of the 14 patients (30 %) who completed EEN, and four of the nine (40 %) patients who completed PEN, treatment had an escalation of treatment to treat recurrent active disease within four months of finishing EN. One to two cartons of enteral formula was regularly as a supplement to usual diet by five patients (36 %) from the EEN group and three patients (33 %) from the PEN group.

4.5 Discussion

This chapter presents the clinical and inflammatory marker data from the prospective non-randomised clinical trials of EEN and PEN for the treatment of active CD in young adults with ileal or ileocolonic CD. This patient cohort is different from many of the previous adult studies that have trialled EEN for a number of reasons. Firstly, none of the patients in this study had isolated colonic CD. It has been suggested that children with isolated colonic CD do not respond

as well to EEN as patients with any ileal involvement^(198, 200) although another paediatric study has shown that colonic disease does respond to EEN.⁽²⁰¹⁾ A meta-analysis of randomised controlled trials of EEN therapy in mostly adult patients with active CD was not sufficient to adequately determine if disease location affected EEN treatment outcomes.⁽³⁸⁾ The exclusion of patients with colonic CD in the current trials may have contributed to the high proportion of patients achieving a CDAI of less than 150 and/or a clinical response (fall in CDAI > 70) to EN therapy.

The second characteristic which makes this patient cohort unique is that the majority of patients (79 %) were female. Data from Canterbury, New Zealand shows that CD affects slightly more females (59 %) than males and that the incidence and prevalence of CD is higher in females than males between the current study's age inclusion range of 16 – 40 years.⁽²⁰²⁾ Patients were referred into the study by their gastroenterologist and they did not provide data on the number of patients who were offered EN treatment but chose instead to use corticosteroid treatment. These data would have been difficult to accurately collect prospectively, especially as patients were referred from public and private clinics. It is possible that more females chose to try EN therapy, although in Chapter 3 the results of an adult patient survey suggested that both males and females are interested in using EEN rather than corticosteroids to treat moderate to severe CD symptoms. Referrer bias, offering EN treatment to more females, could also have skewed the study sex distribution. Study recruitment occurred over a three year period therefore, the type of patient who was referred may have changed over time as the referring physicians became more familiar with the treatment. A study which focused on adolescents with IBD suggested that EN therapy may be more attractive to female patients due to the potential negative impact of CS treatment on body image.⁽²⁰³⁾ But on the other hand, a large (n = 77) paediatric study of EEN found that females and older adolescents were more likely to withdraw from treatment due to

non-adherence.⁽¹⁹⁸⁾ The exact reasons for the higher number of female participants in the current study are not known but should be explored in subsequent nutrition studies.

This study is also different to previous studies because more patients had newly diagnosed CD and the median age of patients was younger than previous large randomised controlled trials comparing EEN with CS treatment.^(129, 161-163) EEN has been shown to be an effective treatment in children and adults with newly diagnosed disease.^(17, 29, 160) Twenty-nine percent of the study participants were less than 18 years old and three quarter of these patients withdrew from EN treatment for various reasons. A Dutch paediatric study found that patients who withdrew from treatment were older (15.5 years old compared with 13.4 years old) and that non-adherence was more common in females.⁽¹⁹⁸⁾ Adolescence is a challenging period of life and adolescents with IBD face similar social and psychological challenges to adolescents living with other chronic diseases⁽²⁰³⁾ In addition to this, CD diagnosed during childhood is likely to be more extensive than CD diagnosed in adulthood.⁽²⁰⁴⁾ It is likely that the per protocol outcomes of the present study may be better than other adult single centre studies of EN therapy due to the high proportion of patients with newly diagnosed disease but that treatment withdrawals may also be higher as a result of the proportion of older adolescent patients referred for treatment.

Lastly, the patients who were referred to this prospective nutrition intervention study may not be representative of the general population of young adults with CD. Patients were not randomised to the nutrition treatment but opted to use nutritional therapy, in most cases, instead of standard CS therapy. A control group of patients on CS treatment was planned but only two patients were referred to the study over the two and half year study period. The candidate made the following efforts to recruit more patients to the control group: attended weekly gastroenterology department meetings and periodically remind departmental staff about the clinical trial verbally and via email, attended outpatient gastroenterology clinics and reminded

gastroenterologists, trainees and the IBD nurse specialist of the study, screened outpatient notes for eligible patients. On questioning, physicians thought that patients were more likely to be started on CS treatment in primary care by the family doctor rather than waiting for their next outpatient appointment at the tertiary care hospital. Another possible source of CS referrals was patients who were admitted to hospital with a disease flare and required intravenous CS. This group of patients were often started on CS on admission by the house surgeon and were not considered as potential study patients because, in most cases, the house surgeon was not aware of the study and did not inform the study dietitian and/or the patient was too ill to delay treatment until they had been recruited to the research study. A couple of patients were referred for EN therapy from the endoscopy suite but no referrals were received for patients starting on CS treatment. On reflection, recruitment into the control group may have been improved if a trainee doctor was involved in the study, because some physicians commented that they tended to associate the study dietitian with EN treatment rather than CS treatment and therefore did not think of the study when prescribing CS.

The methodology used in this study is unlike most of the other known EN studies with adults. The early adult literature compared EEN with CS treatment and these studies tended to use a NGT to administer the EN formula^(156, 157, 160, 162, 199) or asked patients to drink an EF orally.^(17, 129, 155, 159, 163) More recent studies with adults have also delivered the formula via a NGT^(86, 172, 173) rather than rely on patients to drink adequate volumes orally. Feeding via a NGT allows the required volume of formula to be administered to meet the patients' needs, whereas asking the patient to drink the formula requires patients to adhere to a structured drinking plan. The impact of this method on nutritional markers is discussed in more detail in Chapter 5. This is one of the first studies with young adults that has used a ready to drink 1.5 kcal/mL PF administered orally and the clinical outcomes of this regimen are discussed in the following paragraphs.

The adult EEN literature has been discussed in detail in Chapter 1 section 4: in summary, there was a variable response by adults with active CD to EEN treatment. The two large (51 and 55 patients each) European multi-centre randomised controlled trials of EEN compared with CS found that EEN resulted in a reduction in CDAI of more than 40 % or a decrease in CDAI of greater than 100 points in 41 - 53 % of intention to treat patients and 60 – 71 % of per protocol patients.^(162, 163) The smaller studies of EEN, which included 10 – 15 patients, also reported a variable response to treatment. Intention to treat disease remission, defined as a CDAI < 150, an HBI < 3 or VHAI < 120, was achieved by 40 – 80 % of patients and 40 -100 % of per protocol patients.^(17, 156-158, 160) The per protocol remission rate with EEN was the same or better than that achieved with CS treatment in four of these five small studies but not in the large multi-centre trials. A Cochrane meta-analysis of randomised controlled trials of EEN compared with CS, which included some of the studies already mentioned and one paediatric study, concluded that CS were superior to EEN to induce disease remission in patients with active CD.⁽³⁸⁾ The present study found that EEN therapy induced disease remission (CDAI < 150) in 60 % of patients referred for treatment and 93 % of patients who completed the EEN protocol. The results observed in this study are comparable with those observed in earlier single centre studies of EEN treatment for active adult CD.

This study used a novel PEN regimen of two weeks of EEN followed by six weeks of PEN plus one small meal per day. Two paediatric studies have compared EEN treatment with a PEN regimen. One of these studies, a multi-centre UK trial of 50 children, found that PEN was inferior to EEN as only 15 % of patients who used PEN were in remission (PDCAI < 10) at six weeks compared to 42 % of patients using EEN.⁽¹⁸⁰⁾ This is an unusually low number of children achieving disease remission with EEN as other paediatric studies report remission rates of closer to 80 % with EEN treatment.^(21, 28, 29) One other paediatric study from North America compared

PEN (n = 16), EEN (n = 22) and also anti-TNF α (n = 52) therapy to induce disease remission.⁽¹⁷⁹⁾ A clinical response (PDCAI ≤ 10 or fall in PDCAI of ≥ 15) occurred in 64 % of per protocol patients using PEN (9/15) compared with 88 % of children using EEN (15/18) and 84 % (41/49) of children who used anti-TNF medication. Both paediatric studies concluded that PEN was inferior to EEN to induce disease remission in children with active CD. The novel PEN regimen used in this study with young adults had comparable results to the North American study. The intention to treat response to PEN treatment was 62 % and per protocol response was much higher at 88 % of patients. The possible reasons for this difference in response will be discussed in Chapter 5. The novel PEN regimen used in the current study with 13 adult patients was a feasible and acceptable treatment, which was at least as effective as EEN to induce a clinical response and induce disease remission.

Mucosal healing, defined as no endoscopic disease activity, is associated with longer periods of disease remission.⁽¹⁶⁹⁾ FC has been suggested as a reliable non-invasive measure of endoscopic disease activity^(67, 73, 74, 76) and therefore, improvements in FC as a result of treating intestinal inflammation are used as a marker of mucosal healing. Many paediatric studies have shown that EEN is associated with complete mucosal healing in 58 – 81 % of children.^(23, 28, 170) Paediatric EEN studies have also shown improvements in FC subsequent to EEN, however FC is often still elevated post treatment.^(165, 205, 206) The baseline FC of patients in the current study ranged from 60 – 3600 $\mu\text{g/g}$ and the median was 1104 $\mu\text{g/g}$ (n = 25). There was a significant decrease in FC after two weeks of EEN but this trend did not continue throughout the rest of the treatment and at week 8 the median FC was 587 $\mu\text{g/g}$ (n = 23) still ranged from 60 – 3569 $\mu\text{g/g}$. Few studies have assessed mucosal healing using FC or endoscopy in adults after EEN treatment. A Japanese study used EEN for four weeks in 20 patients and reported endoscopic healing of the ileum and the colon in 39 and 44 % of patients respectively and an endoscopic response in the ileum and colon in 76 – 78 % of patients respectively.⁽²²⁾ This Japanese adult EEN study did not

achieve the same rates of mucosal healing reported in the paediatric EEN studies. However, larger studies of adults with CD that have assessed endoscopic mucosal healing after anti-TNF treatment have reported similar rates of complete mucosal healing as the small Japanese EEN study.⁽²⁰⁷⁾ Currently there is no one medication or therapy that has achieved high rates of mucosal healing in adults with CD. To achieve this goal, future studies should consider using a combination of pharmaceutical and nutritional approaches.

Another potential marker of intestinal inflammation is faecal OPG. A paediatric study investigated changes in serum, mucosal and faecal OPG before and subsequent to a six to eight week treatment with EEN in ten children with newly diagnosed CD.⁽⁶⁸⁾ Faecal OPG was elevated at baseline and was highest in children with more severe disease. The mean OPG decreased from 1994 ± 2289 pg/mL prior to treatment to a mean of 504 ± 551 pg/mL after treatment ($p = 0.002$). The current study with young adults found that at baseline faecal OPG was elevated in 16/38 (42 %) of patients whereas in the paediatric study⁽⁶⁸⁾ none of the children had normal faecal OPG prior to treatment with EEN. In the current studies, the faecal OPG of all of the adults ($n = 23$) after EEN treatment was normal (62.5 pg/mL). This concentration of faecal OPG at baseline and subsequent to EEN was much lower than that observed in the paediatric study.⁽⁶⁸⁾

The difference in the faecal OPG results of the current and the paediatric study⁽⁶⁸⁾ may be a result of methodological issues. OPG is present in very small amounts in stool; equivalent to a millionth of the concentration of FC. Both this study and the paediatric study⁽⁶⁸⁾ used the same stool extraction and ELISA protocol as described in Chapter 2. Due to the low concentration of OPG in the faeces the faecal samples could not be diluted more than two fold during the extraction process. The one to one dilution of faecal sample to extraction buffer meant that more solid stool samples did not mix well despite samples being vortexed and homogenised. If the

extraction buffer does not come in contact with all parts of the stool all of the OPG present may not be extracted into the supernatant. Unfortunately the samples could not be re-tested due to the limited number of aliquots of stored stool and the relatively large volume of faecal matter required to yield an adequate volume of supernatant to use in the ELISA. The commercial OPG kit is primarily designed to measure serum OPG and based on the above methodological issues probably needs to be optimised to ensure that OPG is effectively extracted from stool samples of varying consistencies.

4.6 Conclusion

Nutrition-based therapies are probably not appropriate for all patients but with adequate multidisciplinary support, EEN and PEN therapy are feasible and successful treatments for selected patients who are interested in using a nutritional approach to treat active CD. However, not all patients will tolerate the treatment and withdrawals should be expected. The results of these two pilot clinical trials support the hypotheses that EEN therapy induces clinical remission and partially induces biochemical disease remission and that a PEN regimen is as effective as EEN in young adults with active CD. EN therapies should be considered as a feasible and effective option for young adults with active CD.

Chapter 5

Clinical Trial: Nutrition

5.1 Introduction

The treatment of active CD with EEN reduces inflammation and has additional benefits over and above inducing disease remission. In children, these benefits may include improvements in nutrition status.⁽²⁰⁸⁾ The benefits of EEN on nutrition status are also recognised in malnourished adults with CD in whom EEN is considered an appropriate adjuvant treatment option.^(36, 37, 209) Children with active CD treated with PEN have achieved comparable energy and nutrient intakes and had similar improvements in body weight as children using EEN, but PEN did not reduce inflammation and induce disease remission as effectively as EEN treatment.^(179, 180) PEN with a specific exclusion diet has been used in adults (n = 11) with active disease in one study.⁽¹⁸¹⁾ The study reported no significant changes in weight or serum albumin. The literature to date in children suggests that PEN has a similar effect as EEN on nutrition status but the impact of the two treatments on nutrition status have not been compared in an adult cohort.

Malnutrition is prevalent in patients with active CD and disease in remission.^(41, 210, 211) BMI is a simple measure of nutrition status but it does not differentiate between fat mass and functional muscle mass. Adults with CD do have altered body composition compared with a healthy population⁽²¹²⁾ therefore, BMI may not be a good indicator of malnutrition and/or sarcopenia in people with CD. This is especially pertinent as the average BMI of the NZ population increases.⁽²¹³⁾

Poor nutrition status is reflected not only in altered body composition but also poor micronutrient status. Common micronutrient deficiencies include folic acid, calcium, vitamin D

deficiency, iron deficiency anaemia, zinc and, in patients with a previous small bowel resection, vitamin B12 deficiency.^(45, 209) Serum albumin may also be low in patients with poor nutrition status however, albumin is an acute phase protein which decreases in response to inflammation therefore, may not truly reflect nutrition status during active CD.⁽⁴⁰⁾ A less common marker of nutrition status is IGF-1. In older adults IGF-1 is a sensitive predictor of muscle strength⁽⁴⁷⁾ and in children with CD low IGF-1 is associated with growth retardation.⁽²¹⁴⁾ Adults with CD have lower IGF-1 concentrations than age-matched healthy controls.⁽²¹⁵⁾ EEN treatment in children with active CD is associated with an early rise in IGF-1, which could be suggestive of an anti-inflammatory effect as well as improvement in nutritional intake.⁽⁴⁸⁾ Changes in serum IGF-1 has not been investigated in adults using EEN therapy.

5.2 Aims and Hypotheses

This chapter presents the nutrition-related results from the three prospective clinical trials of EEN and PEN in patients with active CD and EEN in a HC group. These studies aimed to assess various markers of nutrition status and to primarily document the baseline nutrition status of young adults with active CD compared with a comparable healthy population. Secondly, these studies aimed to evaluate the impact of EEN and a novel PEN therapy on dietary intake and nutrition status in both adults with active CD and healthy adults. It was hypothesised:

- That enteral nutrition therapy improves markers of nutritional status.

5.3 Methods

The clinical trial methods have been described in full in Chapter 2.1 and summarised again in Chapter 4.3. This section describes the methods used to measure adherence to the PEN regimen.

All patients and healthy volunteers were prescribed EN based on the BMR calculated as part of the BIA assessment multiplied by a physical activity factor.⁽¹⁸⁴⁾ These calculations provided an estimated energy requirement (EER), for example seven cartons (2100 kcal) of nutritional formula per day. If a person's EER was 7.4 cartons of nutrition formula per day an intake range was recommended, for example 7 – 8 cartons (2100 - 2400 kcal/day), and patients were asked to drink more cartons on days when they were more active. For the purposes of calculating EN intake as a percentage of EER the mid-point of a cartons/day range was assumed to be the EER, for example the EER for a person recommended 7 – 8 cartons/day (2100 - 2400 kcal/day) was 2250 kcal/day.

The patients who were prescribed PEN completed an electronic food record during the last fortnight of the PEN treatment. The purpose of the food record was to estimate the calorie and macronutrient intake from solid foods compared with the EN formula. The full methods describing the electronic food record and the analysis of the food record data are detailed in Chapter 2 section 2.1.12. In brief, oral intake from foods and fluids recorded in the electronic food record were entered into the University of Otago, Dunedin, dietary analysis programme “Kai-culator” (Department of Human Nutrition, University of Otago, NZ version 1.14f). The average intake from the dietary records was calculated as a percentage of total nutrition intake.

The patients using PEN could introduce a small meal once a day for the last six weeks of the treatment. There were no restrictions on the type of food which patients could consume for this meal. Each patient was provided with some guidelines for reintroducing foods. The guidelines suggested introducing easily digestible foods that are low in fat and fibre and increasing the variety of foods and the fat and fibre content of the meal as tolerated.

Each patient recorded their average EN intake fortnightly as cartons of formula per day and were encouraged to disclose if they had eaten or drunk anything in addition to what was recommended.

5.4 Results

5.4.1 Serum Markers of Nutrition Status at Baseline

Patients referred for treatment of active CD with EEN or PEN had similar median serum haemoglobin, ferritin, vitamin D and IGF-1 concentrations at baseline (Table 5.1) however, the median concentration of albumin was lower in the PEN group (Figure 5-2). All serum markers of nutrition, except vitamin D, were correlated with serum CRP (Spearman correlations, $p < 0.01$). Nineteen (50 %) of the patients with CD had lost weight (1.5 to 24 kg) in the three months prior to starting EN therapy. The baseline IGF-1 SDS of this group of patients was significantly lower than those who had not lost weight prior to starting treatment (Figure 5-1). There was no significant difference in the baseline BMI of patients who had, or had not, lost weight prior to starting EN therapy (Mann Whitney test, $p = 0.588$).

Serum vitamin D of patients referred for EN therapy varied significantly between the winter (May and October) and summer (November to April) months ($U = 58.0$, $p = 0.0007$). The median Vitamin D of patients starting EN in the winter months was 43.0 nmol/L (range, 17.0 to 101 nmol/L) compared with 72.0 nmol/L (range, 42 to 106 nmol/L) for patients starting treatment in the summer months. A serum vitamin D of less than 50 nmol/L (defined as insufficient vitamin D⁽¹⁸⁵⁾) was present in 12 of the 19 patients (63 %) in the winter months compared with four of the 17 patients (24 %) in the summer months. Vitamin D deficiency (defined as less than 25 nmol/L⁽¹⁸⁵⁾) was present in four patients (24 %) starting EN therapy in

the winter months and no patients starting EN therapy in the summer months. Two patients were excluded from this analysis; one had recently returned from a two week winter holiday in Fiji and another took high dose (50,000 IU/day) vitamin D supplements. There was no difference in the median CDAI of patients referred for EN therapy during the summer and winter months.

Table 5.1. Baseline serum markers of nutrition status of patients using enteral nutrition therapy.

Serum markers	EEN (n = 25) Median (range) or n (%)	PEN (n = 13) Median (range) or n (%)	All patients (n = 38) Median (range) or n (%)	EEN vs PEN <i>p</i>
CRP (mg/L) ⁺	9.0 (3.0, 158.0)	25.0 (3.0, 71.0)	12.0 (3.0, 158.0)	0.199
Albumin (g/L)	41.0 (34.0, 49.0)	34.0 (26.0, 45.0)*	39.0 (26.0, 49.0)	0.022
IGF-1 (SDS)	-0.3 (-2.7, 0.6)	0.0 (-2.9, 0.9)	-0.2 (-2.9, 0.9)	0.826
Haemoglobin (g/L)	130.0 (88.0, 151.0)	122.7 ± 11.6	126.0 (88.0, 151.0)	0.282
< 115 (g/L) for females	4 (22)	3 (25)	7 (23)	
< 130 (g/L) for males	1 (13)	1 (100)	2 (25)	
Ferritin (µg/L)	50.0 (12.0, 315.0)	24.0 (14.0, 170.0)	48.0 (12.0, 315.0)	0.262
< 20 (µg/L)	3 (12)	4 (31)	7 (18)	
25-hydroxycholecalciferol (nmol/L)	59.0 (17.0, 106.0)	72.0 (24.0, 101.0)	61.5 (17.0, 106.0)	0.569
< 50 (nmol/L)	12 (48)	4 (31)	16 (42)	

SDS – standard deviation score,

* Mann-Whitney U test comparing baseline data of EEN group with PEN group, *p* < 0.05

⁺ CRP is included in this table as a marker of inflammation. The presence of inflammation reduces serum albumin, haemoglobin, and ferritin and may also affect IGF-1 and vitamin D concentrations.

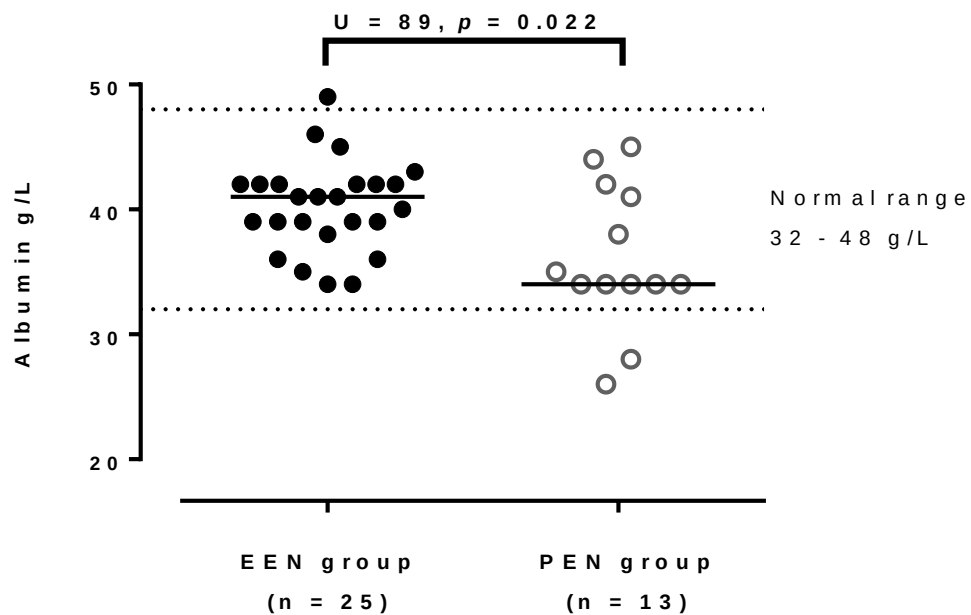


Figure 5-2. Baseline serum albumin concentration in patients referred for enteral nutrition therapy.

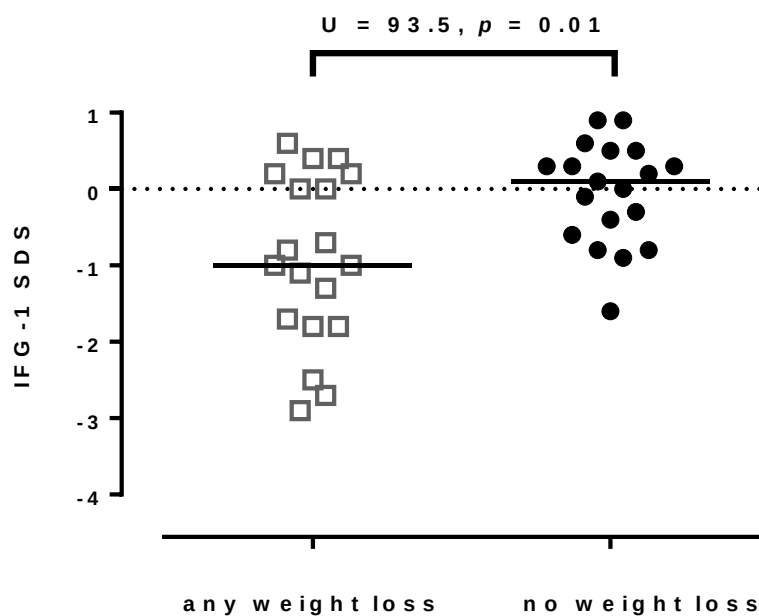


Figure 5-1. Baseline serum IGF-1 of patients who had, and had not, lost weight prior to starting enteral nutrition therapy.

5.4.2 Baseline Dietary Exclusions

Patients with active CD referred for EN therapy often excluded foods or drinks from their diets. Twenty-two (58 %) patients reported excluding at least one food or drink from their diets and 11 (29 %) excluded more than one food or drink. The most common dietary exclusions were dairy (9 patients), alcohol (8 patients), wheat/gluten (6 patients), lactose (6 patients), caffeine (5 patients) and high fat foods (4 patients). Two patients commented that they excluded foods high in fermentable carbohydrates, two were following a low residue diet to manage bowel strictures and three patients did not tolerate either apple, citrus or high fructose corn syrup.

5.4.3 Baseline Body Composition

There were no significant differences in the body composition of patients recruited to use EEN or PEN. Only one male was recruited to the PEN group therefore, the body composition data for the EEN and PEN intervention groups have been combined into one CD patient group presented in Table 5.2. The CD patient group had similar body composition parameters compared with the HC group (Table 5.2).

The median BMI of patients with active CD and HC was similar and there was no statistically significant differences in the distribution of participants across the BMI categories as illustrated in Figure 5-3.

Table 5.2. Baseline body composition characteristics of healthy controls and patients with active CD.

Body composition measures	CD group (n = 38) (Male = 8, Female = 30) Median (range)	HC group (n = 21) (Male = 7, Female = 14) Median (range)	CD vs HC <i>p</i>
Weight (kg)	M: 64.1 (53.8, 110.7) F: 63.2 (41.0, 114.6)	M: 78.2 (71.1, 127.6) F: 66.1 (50.8, 97.5)	0.091 0.420
Height (cm)	M: 177 (167, 190) F: 165.5 (153, 179)	M: 178.5 (175, 191) F: 167.5 (157, 176)	0.518 0.398
BMI (kg/m ²)	M: 22.8 (16.8, 30.7) F: 23.6 (16.5, 37.8)	M: 25.4 (21.9, 38.0) F: 23.7 (18.7, 31.8)	0.252 0.795
Fat mass index (kg/m ²)	M: 3.1 (1.9, 9.9) F: 6.6 (2.2, 16.3)	M: 3.8 (2.1, 12.5) F: 6.3 (3.6, 11.5)	0.487 0.667
Fat-free mass index (kg/m ²)	M: 19.2 (14.7, 22.4) F: 16.9 (12.6, 21.5)	M: 21.2 (19.6, 25.5) F: 17.7 (14.7, 20.5)	0.096 0.365

M = male, F = female. Mann Whitney test used to compare differences between HC and patients with CD

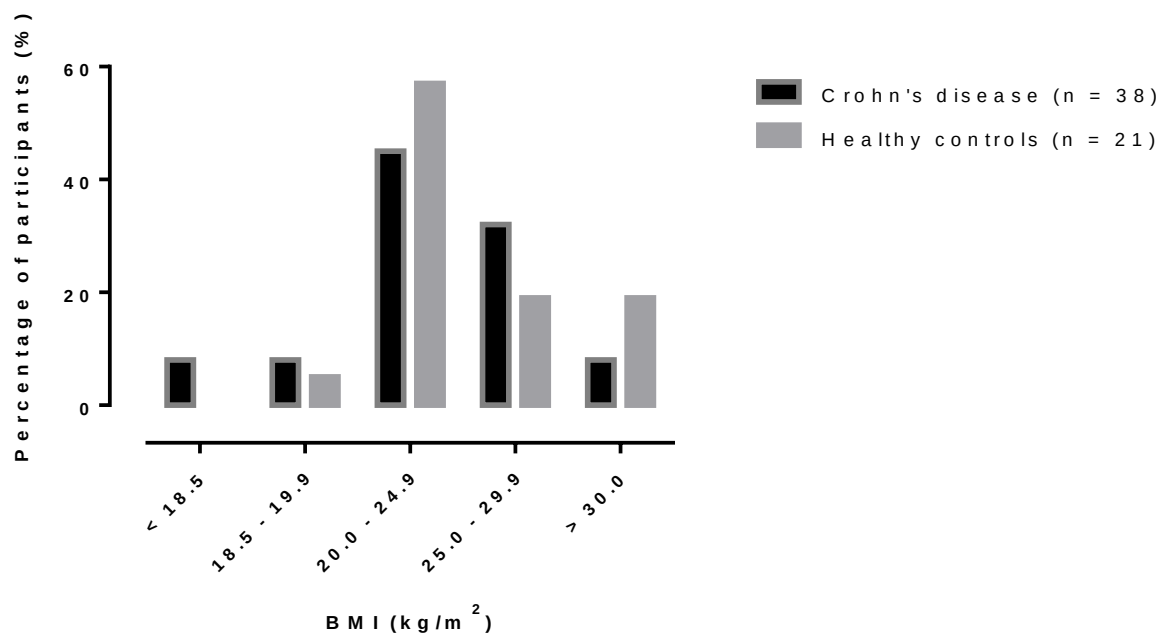


Figure 5-3. Body mass index of healthy controls and patients with active CD

5.4.4 Adherence to the Enteral Nutrition Protocol

EEN was used for two weeks by three study groups, two with active CD (n = 32) and a HC group (n = 17). During this time only two of the 49 participants reported eating any food after the three-day EN phase-in period. No one reported drinking fluids other than tap water and black unsweetened coffee, tea or herbal tea. Black tea and/or coffee was drunk by 17 of the 32 (53 %) of the patients with CD and 11 of the 17 (65 %) of the HC. The remainder of the study participants drank only tap water. After two weeks of EEN, the HC participants re-introduced usual foods and fluids, whereas the patients with CD either continued with EEN for another six weeks or introduced a small meal of usual foods once a day in addition to EN for six weeks (PEN group).

Table 5.3 details the additional food and fluids consumed during EEN and PEN interventions. Three patients in the EEN intervention group were using EEN on Christmas Day and were allowed, by the study coordinator, to have a small meal on this day only. None of these three patients experienced any CD symptoms as a result of eating the Christmas day meal. The additional food that was occasionally consumed was not included as part of the patients' total calorie consumption unless it was habitual, such as the patient in the PEN group who had a row of chocolate every evening. Adherence to the study protocols was greatest during the first two weeks of treatment (Table 5.3). All of the HC group volunteers who used EEN for two weeks were 100 % compliant with the liquid diet. Over the eight EN treatments 3/9 (33 %) of the patients who used PEN and 6/14 (43 %) of the patients who used EEN were 100 % adherent with the treatment protocols. There were no significant differences in the baseline characteristics or outcomes of patients who adhered to the protocols compared with those who did not.

Two patients in the PEN group decided to continue with EEN for a further two weeks and delay reintroducing food. One of these patients had a known focal sealed small bowel perforation and

was awaiting surgery when she started in the study. Upon reintroduction of food at week two the patient flared and it was decided that she would continue on EEN and the date of surgery was brought forward. The patient left the study at week four and had an ileal resection. The other patient decided to use EEN for four weeks because they felt that their body was “slow to respond to treatments”.

Table 5.3. Patient reported deviations from the enteral nutrition protocols.

Intervention follow up	EEN protocol deviations	PEN protocol deviations	Patient adherence to study protocol (%)
Week 2	small snacks throughout the first two weeks (n=1)	nil	EEN – 86 %
EEN (n=21)	½ a biscuit and couple of potato chips (n=1)		PEN – 100 %
PEN (n=11)	a flat white coffee (n = 1)		HC – 100 %
Week 4	small meal on Christmas day (n=2)	Fruit drink (n=1), juice (n=1)	EEN – 66 %
EEN (n=18)	black coffee with ½ tsp sugar (n=1)	Chocolate nightly (n=1), ½ piece of slice (n=1), handful of chips (n=1))	PEN – 55 %
PEN (n=11)	1x can lemonade, little shaved ham and ½ croissant (n=1), cough lollies (n=1)	1x ½ friand and occasionally some chocolate (n=1)	HC – N/A
	2x energy drink, souvlaki, butter chicken curry, 'Subway' (n=1)		
Week 6	1x rice and vegetables (n=1), small meal on Christmas day (n=1), a flat white coffee (n = 1)	Fruit drink (n=1)	EEN – 64 %
EEN (n=14)	1x imperial mint, little cupcake batter (n=1)	1 square of chocolate (n=1), chocolate nightly (n=1)	PEN – 45 %
PEN (n=9)	1x peanut cookie, moro bar, can lemonade, pumpkin/potato mash (n=1)	1x ½ friand and orange juice (n=1)	HC – N/A
		Non-alcoholic punch with some crackers and chips (n=1)	
Week 8 EEN (n=14)	1x diet coke and one small meal (n=1)	Juice (n=2), chocolate once (n=1), 1x biscuit nightly (n=1)	EEN – 64 %
PEN (n=9)	1x glass orange juice, 1x chicken and salad sandwich (n=1) 1x small cup cake (n=1), cough lollies (n=1), potato chips, 1 piece fish, 3x chocolate biscuits (n=1)	1x mandarin & a row of chocolate (n=1)	PEN – 33 %
		Orange drink and two meals per day (n=1)	HC – N/A

Most patients using PEN chose to eat dinner as their one meal per day. This meal was chosen so that it could be shared with family members. Eight of the nine patients completed and uploaded an electronic food record for two to four days. One patient, who lived in a student flat and did not share meals or cooking duties with her flatmates, ate smooth peanut butter sandwiches daily and occasionally had a smooth yoghurt as well. The rest of the PEN group patients tended to eat meals consisting of chicken, red meat and sometimes fish with a carbohydrate food (potato, rice, pasta, bread, pastry) and vegetables either cooked or raw in a lettuce based salad. There was a large range in the calories consumed from the solid food meal of 363 - 1122 kcal which contributed 35.5 % (range, 14.7 to 59.9 %) of total energy consumed from both solid food and enteral formula. Patients consumed a median of 1050 kcal/day (range, 750 to 1800 kcal/day) of EN formula which equates to a median of 62 % (range, 29 to 103 %) of EER. Most patients consumed less kilocalories than their EER and there were no significant differences in the energy intake between patients using EEN and PEN or HC using EEN (Table 5.4).

Table 5.4. Average caloric intake as a percentage of estimated energy expenditure during the first and last two weeks of enteral nutrition therapy.

	EEN	PEN	HC
	Median (range)	Median (range)	Median (range)
% EER at week 2	93 (64, 117)	90 (69, 129)	88 (75, 105)
	(n = 21)	(n = 11)	(n = 17)
% EER at week 8	93 (64, 114)	80 (61, 124)	n/a
	(n = 14)	(n = 8) ⁺	
% kcal from food	n/a	35.5 (14.7, 59.9)	n/a
		(n = 8) ⁺	

n/a: not applicable.

⁺ n = 8 because one patient did not upload their electronic food record.

5.4.5 Change in Serum Nutrition Markers

Serum markers of nutrition, albumin and IGF-1, were measured fortnightly during EN therapy and at week 12 and 26 after therapy. These data were analysed using a LOCF and a per protocol method. The LOCF analysis found that patients who used EEN had a statistically significant improvement in serum IGF-1 concentration during treatment ($W = 125.0, p = 0.01$) and serum albumin ($W = 112.0, p = 0.034$). There was also a non-significant trend towards decreased serum CRP ($W = -87.0, p = 0.060$). Per protocol analysis with the 14 patients who completed the EEN treatment yielded similar results (Table 5.6). There were no significant changes in serum albumin, IGF-1 or CRP in the PEN patient group during treatment using LOCF ($n = 13$) or per protocol analysis ($n = 9$). Serum albumin and IGF-1 were no longer correlated with serum CRP at week 8 (Spearman correlation, $p > 0.05$).

There were no statistically significant differences in median serum CRP or IGF-1 between patients who used EEN compared with PEN treatment. Change in albumin was calculated to correct for differences in baseline albumin between the treatment groups. Patients in the EEN group trended towards a greater improvement in median serum albumin compared with patients in the PEN group ($W = 32.0, p = 0.050$). Figure 5-4 shows the individual variation in serum albumin in response to the EN treatments. The change in serum albumin was not correlated with change in CRP in the EEN group ($p = 0.171$) or the PEN group ($p = 0.344$).

Serum markers of nutrition remained stable after EN therapy once food was reintroduced with the exception of serum albumin in the EEN patient group. Serum albumin significantly decreased in both the LOCF ($W = -73.0, p = 0.021$) and the per protocol analyses ($W = -73.0, p = 0.007$).

Table 5.5. Serum albumin, IGF-1 and CRP of patients during and after enteral nutrition therapy using last observation carried forward analysis.

	EEN (n = 25)				PEN (n = 13)			
	Baseline	Week 2	Week 8	Week 26	Baseline	Week 2	Week 8	Week 26
Albumin (g/L)	41.0 (34.0, 49.0)	41.0 (34.0, 46.0)	42.0* (34.0, 48.0)	40.0* (34.0, 46.0)	34.0 (26.0, 45.0)	38.0 (26.0, 43.0)	35.0 (26.0, 44.0)	38.0 (26.0, 42.0)
IGF-1 (SDS)	-0.3 (-2.7, 0.6)	0.0 (-1.8, 2.1)	0.0** (-1.8, 2.1)	0.0 (-1.8, 2.1)	0.0 (-2.9, 0.9)	-0.1 (-1.2, 1.5)	0.2 (-1.1, 1.2)	-0.1 (-1.1, 2.4)
CRP (mg/L)	9.0 (3.0, 158.0)	5.0 (3.0, 102.0)	5.0 (3.0, 102.0)	5.0 (3.0, 102.0)	25.0 (3.0, 74.0)	5.0 (3.0, 74.0)	9.0 (3.0, 74.0)	7.0 (3.0, 74.0)

* Wilcoxon matched-pairs signed rank test of change from baseline to week 8 or week 8 to week 26, $p < 0.05$

** Wilcoxon matched-pairs signed rank test of change from baseline to week 8, $p < 0.01$

Table 5.6. Serum albumin, IGF-1 and CRP of patients during and after enteral nutrition therapy using per protocol analysis.

	EEN (n = 14) median (range)				PEN (n = 9) median (range)			
	Baseline	Week 2	Week 8	Week 26	Baseline	Week 2	Week 8	Week 26
Albumin (g/L)	40.0 (35.0, 49.0)	41.5 (37.0, 46.0)	43.0 * (39.0, 48.0)	40.0 ** (35.0, 46.0)	38.0 (34.0, 45.0)	38.0 (33.0, 43.0)	38.0 (34.0, 44.0)	39.0 (33.0, 42.0)
IGF-1 (SDS)	0.0 (-2.7, 0.6)	0.1 (-1.4, 1.6)	0.4 * (-1.3, 2.1)	0.1 (-1.5, 1.6)	0.1 (-2.9, 0.9)	0.2 (-1.2, 1.5)	0.4 (-1.1, 1.2)	0.0 (-0.7, 2.4)
CRP (mg/L)	8.0 (3.0, 36.0)	5.0 (3.0, 17.0)	3.5 (3.0, 25.0)	4.5 (3.0, 36.0)	13.0 (3.0, 71.0)	3.0 (3.0, 22.0)	7.0 (3.0, 61.0)	3.0 (3.0, 13.0)

* Wilcoxon matched-pairs signed rank test of change from baseline to week 8, $p < 0.05$

** Wilcoxon matched-pairs signed rank test of change from week 8 to week 26, $p < 0.01$

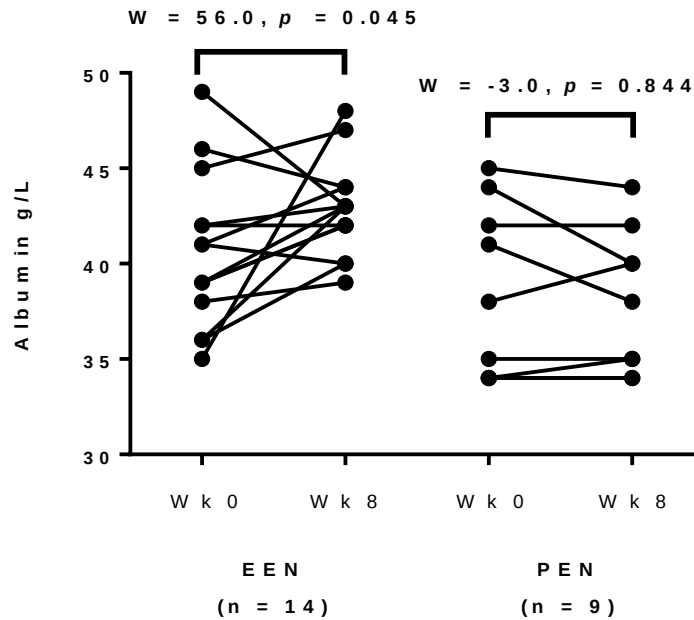


Figure 5-4. Change in serum albumin during enteral nutrition therapy.

5.4.6 Change in Body Composition

During the first two weeks of EEN therapy both patients with active CD and HC lost weight (Table 5.7). There was no significant difference in change in BMI observed between the three groups. The EEN intervention resulted in further weight loss over the next six weeks (Figure 5-5) but there was no significant difference in the change in BMI between those using EEN compared with patients using PEN.

At the completion of the two week intervention in the HC group, and the eight week intervention in patients with active CD, each person resumed eating and drinking their usual foods and fluids. All study participants (patients with CD and HC) were reviewed after four weeks of consuming a usual diet and the median BMI had increased in all groups and was not significantly different from BMI at baseline (Table 5.7).

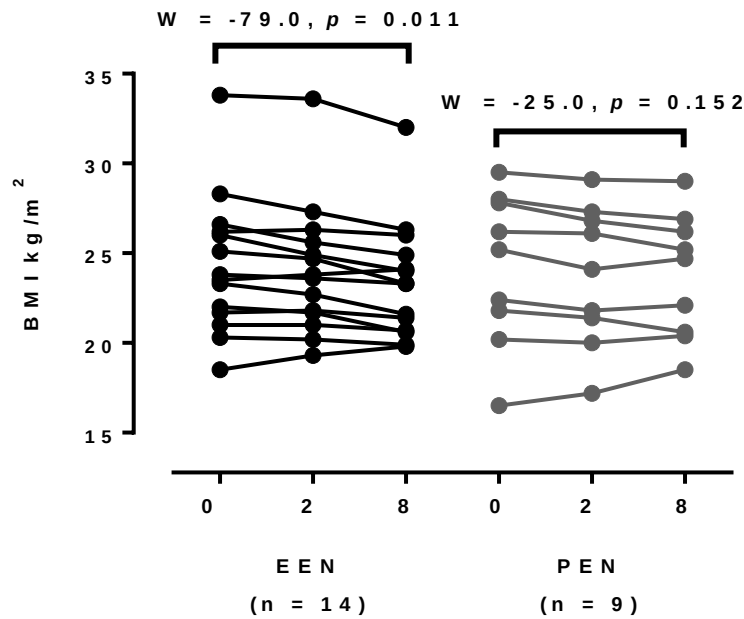


Figure 5-5. Change in BMI during enteral nutrition therapy.

Table 5.7. Change in body mass index from baseline during enteral nutrition therapy and upon reintroduction of usual dietary intake.

	EEN (n=14)	PEN (n=9)	HC (n=17)
	Median (range)	Median (range)	Median (range)
Baseline	23.7 (18.5, 33.8)	25.2 (16.5, 29.5)	23.8 (18.7, 35.0)
Week 2	-0.20 (-1.1, 0.8)	-0.40 (-1.1, 0.7)	-0.30 (-1.1, 0.4)**
Week 8	-0.95 (-2.0, 1.3)*	-0.50 (-1.6, 2.0)	-
After 4 weeks of usual diet	-0.35 (-1.9, 1.9)	-0.50 (-2.3, 2.2)	-0.10 (-2.0, 0.6)

* $p < 0.5$, ** $p < 0.01$ BMI at baseline compared with week 2 or week 8 of enteral nutrition intervention

5.4.7 Dietary Exclusions after Nutrition Intervention

As stated in section 5.4.2 many patients referred for EN therapy excluded certain foods at baseline. Six months later, after completion of EN therapy, six patients (26 %) did not exclude specific foods and 17 patients (74 %) excluded at least one food from their diet. Seven of the 17 who excluded foods at follow up did not exclude foods at baseline. Another seven patients had different food exclusions at baseline compared with follow up at six months. Common food exclusions at follow up were gluten (5 patients), dairy/lactose (4 patients), fatty foods (4 patients), alcohol (3 patients), spicy foods (3 patients) and foods high in fermentable carbohydrates (3 patients).

5.5 Discussion

This chapter presents the nutrition-related data from the prospective non-randomised clinical trial of EEN and PEN for the treatment of active CD in young adults and a trial of EEN in healthy young adults. The baseline and change in nutrition markers consequent to EN therapies will be discussed, followed by discussion of the tolerance of EN therapy between patients with CD and HC and, finally, adherence to the PEN regimen compared with other PEN studies.

Patients with active CD referred for EN therapy during winter months had a high incidence of vitamin D insufficiency. The general population living in the central and lower South Island of NZ also have a high prevalence (18.1 % (95 % CI (12.0 to 26.4))) of vitamin D deficiency (< 25 nmol/L) in August to October and the prevalence of vitamin D insufficiency (< 50 nmol/L) is 46.4 % (95 % CI (37.0 to 55.8)).⁽²¹³⁾ The prevalence of insufficient vitamin D and vitamin D deficiency during the winter months was higher in this study cohort of young adults with active CD than in the general central and lower South Island population during a similar time period. However, the prevalence of low vitamin D was less extreme than has been observed in a comparable population of patients with existing CD from Dunedin,⁽⁵⁰⁾ a city further south (45°

52° S, 170° 30' E) of Christchurch (43° 53' S, 172° 63' E). The Dunedin study measured serum 25-hydroxy vitamin D in 29 patients in winter (June to September) months and during summer (December to March) months and found that 76 % of patients had a vitamin D of less than 50 nmol/L in the winter compared with only 10 % of patients in summer.⁽⁵⁰⁾ The patients included in the Dunedin study were not taking vitamin D supplements and the study found no correlation between disease activity and seasonal variation in vitamin D concentrations. This study did not aim to specifically assess the impact of vitamin D insufficiency or deficiency and its treatment therefore data on sun exposure, sun protective practices, vitamin D status after EN treatment and prescription practices were not collected. Current IBD treatment guidelines recommend vitamin D supplementation for people with IBD living in locations with limited UVB sun exposure⁽²¹⁶⁾, which includes the central and southern South Island of NZ during the winter months.

There were no differences in the baseline body composition of patients with active CD and HC. Previous research has not conclusively shown that patients with recently diagnosed CD have altered body composition.⁽²¹²⁾ A limitation of many studies is the lack of a comparable healthy population. One study which assessed body composition using BIA and did have a comparable control group found that healthy males had significantly higher fat-free mass compared with males with CD, whereas females did not.⁽⁴⁶⁾ There is now research investigating, not only fat-free mass, but skeletal muscle mass and the prevalence of sarcopenia in patients with IBD. Sarcopenia (defined as appendicular skeletal muscle mass (calculated using dual-energy X-ray absorptiometry (DXA)) and hand grip strength of more than one standard deviation below the mean) has been observed in free living patients with CD who have a normal BMI.⁽²¹⁷⁾ BIA provides basic information on patient body composition but in the absence of normal ranges for FMI and FFMI or functional measures of lean body mass BIA may not provide any more insight into the nutrition status of the patient than BMI or weight change over time.

Both the per-protocol EEN and PEN groups had increases in serum IGF-1 and reductions in CRP after two weeks of using EEN. Serum IGF-1 is a marker of nutrition status and has also been suggested as a marker of disease activity^(48, 218) due to its reduced expression in the presence of pro-inflammatory cytokines.⁽²¹⁹⁾ An early rise in IGF-1 has previously been documented in paediatric IBD studies subsequent to EEN treatment.^(48, 220) Both paediatric studies concluded that early improvements in IGF-1 concentration are due to reduced inflammation and improvement in nutrition intake. There is no research in adults with CD that has assessed change in IGF-1 subsequent to EEN therapy. However, the serum IGF-1 concentration of adults with CD and UC has been compared with a HC group in a Greek study.⁽²¹⁵⁾ Greek adults with IBD had lower IGF-1 concentrations and higher IL-6 concentration than the HC group. This study did not report the IGF-1 SDS therefore, it is difficult to compare the mean concentration with data from the current study because IGF-1 normal ranges vary by age and sex. Median serum IGF-1 concentrations in this study cohort increased during EN therapy, despite suboptimal caloric intake of 85 – 91 % of EER and reductions in BMI, and corresponded with a reduction in CRP. These results support the paediatric IBD observations that IGF-1 is more than just a marker of nutrition status and that improvements in serum IGF-1 concentration are also due to reduced gut inflammation.

During the EN treatments adults with CD, as well as healthy adults, consumed less energy than their EER and consequently weight loss was observed. This trend is the reverse of what is usually observed in paediatric studies where weight gain is the goal and many centres use nasogastric tubes to meet caloric requirements.⁽¹⁶⁾ Adults using PEN had a non-significant decrease in BMI during the first two weeks of treatment but once some solid food was introduced the mean BMI stabilised. In order for weight to stabilise a natural assumption would be that patients were meeting their energy requirements however, analysis of the food records show that

mean energy intake was only 85 % of EER compared with 91 % during the EEN phase of the PEN regimen. It is likely that the food records underestimate the average energy intake of patients during the six weeks of PEN treatment. Patients were asked to complete an electronic food diary for four days during the six week intervention. Most patients completed only three days of food record therefore, the nutrient intake from solid food is based on a limited number of days for eight patients. In addition a novel food record was used which has not been validated for use with young adults. The electronic app-based food record has been validated (unpublished data) for use with NZ children and their parents by the Department of Human Nutrition, University of Otago, Dunedin. It was assumed that it would also be appropriate for young adults given the high 'smartphone' ownership by young adult New Zealanders.⁽²²¹⁾ One patient had trouble with the technology and another exceeded their mobile data allowance and used a combination of electronic and paper food records. One of the benefits of the electronic food record is that the investigator can gain further information from the photographs of the meals that may not have otherwise been included in the description of the food by the participant. It may also be a more accurate method of estimating portion size which may not be captured in a paper record. One of the limitations of the photography method is that the person needs to remember to photograph the meal before they start eating, whereas a paper diary could be completed after the meal is consumed if required. There are inherent disadvantages with all methods of food records and under-reporting is always an issue.⁽²²²⁾ Overall the use of PEN regimen appears to limit weight loss during treatment but despite further weight loss during EEN treatment patients regained weight upon reintroduction of food.

The PEN regimen used in this clinical trial is different from those studied previously.⁽¹⁷⁹⁻¹⁸¹⁾ This PEN regimen combined two weeks of EEN with six weeks of PEN in which patients were prescribed a set amount of food to consume in place of a proportion of EN rather than a set amount of formula plus unrestricted usual foods. The mean intake of usual foods during the

PEN phase was 33 % (range, 15 – 60 %) of total energy. There are two paediatric CD PEN studies which have allowed the consumption of usual foods and the patients in both studies consumed more calories from usual foods than the concurrent PEN regimen. In the North American study⁽¹⁷⁹⁾ children using PEN consumed 47 % (range, 10 - 75 %) of total energy from usual foods and the UK study intake of usual foods contributed 53 % (range, 42 – 61 %) of total energy in the PEN and 2 % (range, 0 – 11%) of total energy in the EEN group.⁽¹⁸⁰⁾ The third PEN study included 33 children and 14 adults (aged 19 – 32 years old) and prescribed 50 % of energy from a 1.0 kcal/ml polymeric EN formula and the other 50 % to come from a specific list of foods.⁽¹⁸¹⁾ The study did not report the amount of energy patients ultimately consumed from food. The mean energy intake from solid food in the current study was lower than the other three PEN studies. However, even though patients were asked to consume a small meal (half of their usual portion size) the evening meal still contributed a large proportion of total calories for some patients, particularly those who had a lower total energy intake, such as 600ml of EN formula during the day. In retrospect, patients with lower energy intakes may have benefitted from adding a small volume of EN formula to the evening meal to help reduce their total energy intake of solid food at that one meal. Despite the large range in energy intakes from solid food, the PEN regimen, as discussed in Chapter 4, was as effective as EEN at reducing symptoms of active disease.

The use of exclusion diets, or exclusion of particular foods, in an attempt to manage disease flares may be prevalent in patients with CD.⁽²²³⁾ Single and multiple food exclusion were common in this NZ cohort of young adults with CD, many of whom were newly diagnosed. Food exclusions appeared to change over time suggesting that patients are experimenting with how food may or may not affect their gastrointestinal symptoms or their tolerance of certain foods may be affected by disease activity. This study did not investigate the reasons for excluding

foods from the diet but given the high prevalence of malnutrition in patients with CD it would be interesting to investigate if long term food exclusion behaviour affects nutrition status.

5.6 Conclusion

The current study shows that, in a real world setting, as opposed to the hypothetical setting in Chapter 3, EEN with an oral polymeric ready-to-drink formula is a palatable and mostly well tolerated treatment for adults with active CD. Intolerance of the formula and regimen should be expected and occurs in a proportion of healthy adults and adults with active CD. The treatment of active CD with EEN results in an early and statistically significant continued improvement in serum IGF-1 concentrations and a trend towards increased serum albumin, but a statistically significant reduction in BMI. Conversely, treatment of active CD with PEN resulted in less weight loss and no statistically significant changes in serum IGF-1 or albumin. These data therefore partially support the hypothesis that enteral nutrition therapy improves markers of nutritional status.

Chapter 6

Clinical Trial: Psychological Outcomes

6.1 Introduction

It is well established that many patients with CD have poorer HRQOL^(83, 84) and higher rates of anxiety and depression^(94, 95) than the general population. Previous literature suggests that anxiety is present in up to 40 % of adults with CD^(94, 101, 103, 104) and that up to 10 % suffer from depression.^(99-101, 103) The determinants of HRQOL are varied and may include disease activity, hospitalisations and disease treatments.⁽⁸⁵⁾ The impact of EEN therapy on HRQOL has not been investigated in Western adults with CD.

6.2 Aims and hypotheses

The aim of assessing HRQOL in this study's patient cohort was to document the baseline characteristics of young adults with active CD and to evaluate the impact of EN therapies on HRQOL. It was hypothesised:

- That EEN and PEN therapies are associated with improved HRQOL scores in adults with active CD.

6.3 Methods

HRQOL was assessed before, during and after EN treatment using two validated self-assessment QOL tools: HADS⁽⁸⁸⁾ and SIBDQ.⁽¹¹⁵⁾ The complete methods are detailed in Chapter 2.1.11. In summary, the HADS tool assesses the presence of symptoms of anxiety and depression. A HADS subscale score of less than eight suggests the absence of depression; a borderline abnormal score

is eight to ten and a score greater than 10 is abnormal. A cut-off of greater than eight is recommended to detect the presence of anxiety and/or depression.⁽⁹³⁾

The SIBDQ asks 14 questions related to the impact of disease symptoms on social and personal functioning. The answer to each question is assigned a score of one to seven points, the mean of which is the SIBDQ score. For each question a score of one indicates very poor HRQOL and a score of seven indicates optimal HRQOL.

6.4 Results

6.4.1 Hospital Anxiety and Depression Scale

6.4.1.1 *HADS-anxiety (HADS-A)*

At baseline there was no difference in the median HADS-A score between patients recruited to use EEN or PEN to treat active CD (Table 6.1). Anxiety (HADS-A score of 8 or greater) was present in 47 % of patients with active CD (Table 6.1) and HADS-A scores at baseline were not correlated with baseline disease activity ($p > 0.05$). Baseline HADS-A scores did not differ between patients who were newly diagnosed ($n = 30$) or had existing CD ($n = 8$) nor between patients who withdrew from the study and those who completed EN therapy. EEN therapy for two weeks was associated with improved HADS-A score. Thirty-three patients (87 %) completed two weeks of EEN and the median improvement in HADS-A score during this time was 2.0 points ($W = -283.0, p = 0.0001$). The patients who continued EN therapy and completed either EEN or PEN had further improvements in HADS-A score (Figure 6-1). Nine of these patients had anxiety at baseline and four still had a HADS-A score of eight or more at the end of EN treatment. Median HADS-A scores did not change from week 8 (3.0 (range, 0 – 14)), at the end of the EN treatment, to the follow up appointment at six months (3.0 (range, 0 – 14)) ($W = 0.0, p > 0.999$).

One of the patients who used CS treatment moved from borderline anxiety (score 8-10) to anxiety (score > 10) at week eight of treatment, whereas the other patient had an improvement in anxiety score of five points and moved from a score >10 to a score of < 8 at week eight.

6.4.1.2 HADS-depression (HADS-D)

At baseline, depression, defined as HADS-D score of eight or more, was present in 19 % of the 38 patients recruited to use EEN or PEN therapy and the median HADS-D score was 4.0 (range, 1 – 14) (Table 6.1). Median HADS-D score did not differ between patients with newly diagnosed and existing CD nor between patients who completed EN therapy compared with those who withdrew from the study. There was no statistically significant improvements in median HADS-D score in patients who completed eight weeks of EEN or PEN treatment ($p > 0.05$). Three of the patients who completed EN therapy had a HADS-D score of 8 or more at baseline and HADS-D score was still high in one patient at the treatment completion. HADS-D score was not correlated with CDAI at baseline or treatment completion ($p > 0.05$). The median HADS-D score at follow up at six months (1.0 (range, 0 – 9)) was not significantly different from scores at the conclusion of the EN therapies (2.0 (range, 0 – 9)) ($W = -48.0, p = 0.341$).

For the two patients who used CS a similar result was observed on the depression questionnaire as was seen on the anxiety questionnaire. One patient who had depression at baseline still had depression at week eight and the other patient who had borderline depression at baseline had a five point improvement in the HADS-D score and had a score < 8 after CS treatment.

Table 6.1. Presence of anxiety and depression in patients with active CD at baseline.

	EEN group (n = 25) n (%)	PEN group (n = 13) n (%)	All patients (n = 38) n (%)	Newly diagnosed (n = 30) n (%)	Existing disease (n = 8) n (%)
HADS classifications					
Anxiety (HADS-A > 10)	8 (32)	3 (23)	11 (29)	7 (23)	4 (50)
Borderline anxiety (HADS-A 8 - 10)	4 (16)	3 (23)	7 (18)	3 (10)	1 (13)
HADS-A score (median (range))	7.0 (1 – 14)	5.0 (3 - 15)	7.0 (1 – 15)	6.5 (1 – 15)	9.5 (7 – 15)
Depression (HADS-D > 10)	2 (8)	1 (8)	3 (8)	2 (7)	1 (13)
Borderline depression (HADS-D 8 - 10)	3 (12)	1 (8)	4 (11)	3 (10)	1 (13)
HADS-D score (median (range))	7.0 (1 – 14)	5.0 (1 – 11)	4.0 (1 – 14)	4.0 (1 – 14)	6.0 (1 – 11)

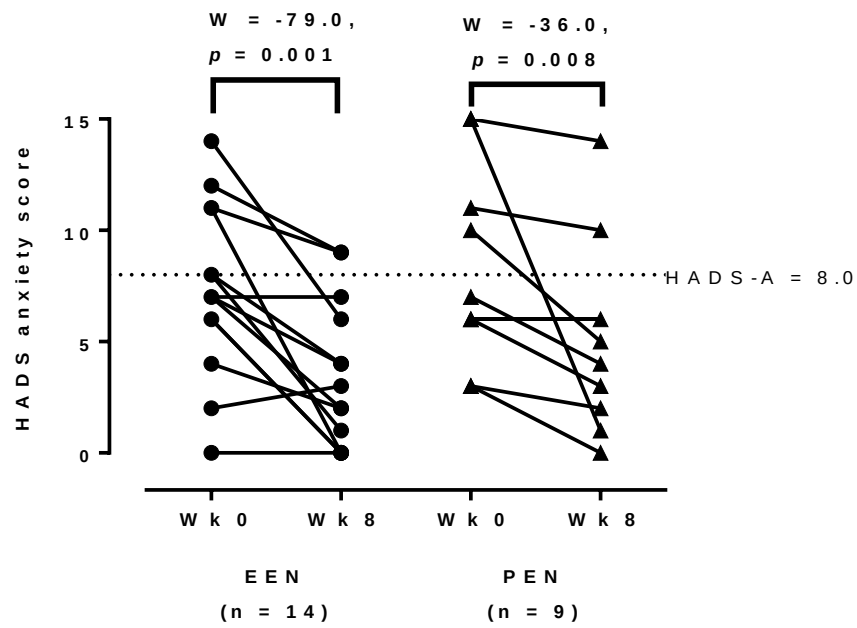


Figure 6-1. Change in HADS-A score during enteral nutrition therapy.

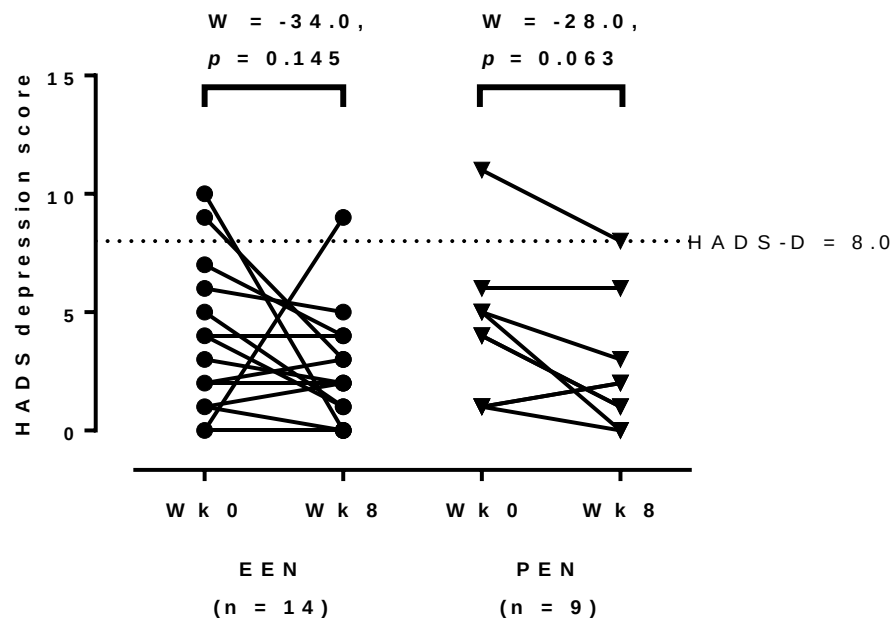


Figure 6-2. Change in HADS depression score during enteral nutrition therapy.

6.4.2 Short Inflammatory Bowel Disease Questionnaire

At baseline the median SIBDQ score of all patients ($n = 38$) with active CD was 4.2 (range, 1.5 to 6.3) and baseline SIBDQ score was significantly correlated with CDAI ($r = -0.418$, $p = 0.009$).

The median SIBDQ of female ($n = 30$) and male ($n = 8$) patients was 4.25 (range, 1.5 to 5.4) and 4.35 (range, 1.5 to 6.3) respectively and was not significantly different ($p > 0.05$).

SIBDQ score increased significantly during treatment with EEN or PEN (Figure 6-3). At treatment completion, 13/14 of the EEN patient group and 7/9 of the PEN patient group had a clinically significant quality of life response (SIBDQ > 4.7) (Figure 6-3) and there was no difference in median SIBDQ at week 8 between the two treatment groups ($p > 0.05$). Upon reintroduction of usual foods and fluid median SIBDQ did not significantly change in either of the treatment ($p > 0.05$).

The two patients who used CS also had improvements in IBDQ score. One patient moved from a score of 4.2 to 4.9, which is clinically significant improvement. The other patient moved from a score of 3.7 to 4.0 at completion of CS treatment.

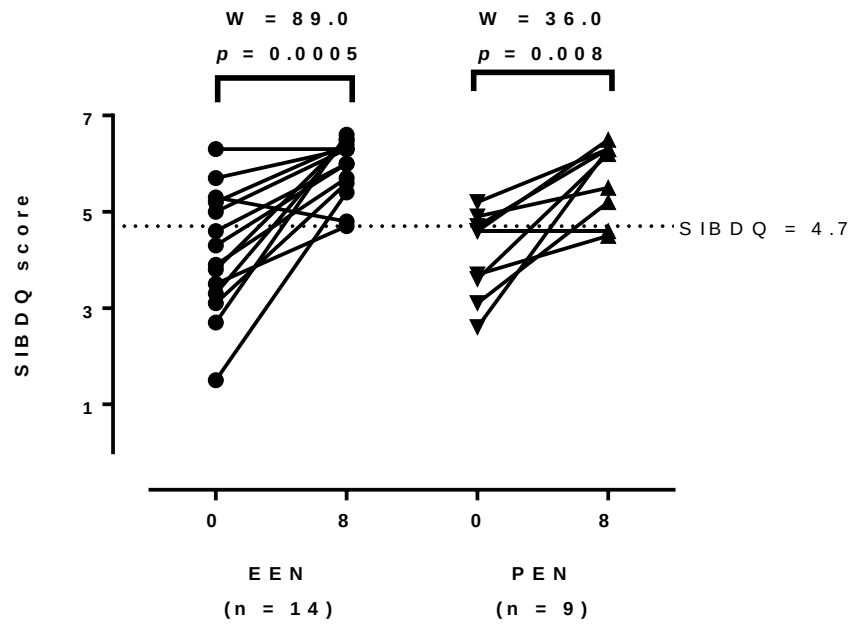


Figure 6-3. Change in SIBDQ score during enteral nutrition therapy.

6.5 Discussion

International research has shown that patients with IBD have higher rates of anxiety and depression^(94, 95) and poorer HRQOL^(83, 84) than the general population. Active disease has been associated with poorer HRQOL⁽⁸⁵⁾ and a greater prevalence of anxiety and depression.⁽¹⁰⁰⁻¹⁰²⁾ Furthermore, some IBD treatments may impact on HRQOL more than others.^(101, 104, 109, 111, 112, 114) The aim of assessing HRQOL in this patient cohort was to document the characteristics of young NZ adults with active CD and to evaluate the impact of EN therapies on HRQOL.

Anxiety, defined as a HADS-A of eight or more, was present in 47 % of patients with active CD prior to starting EN therapies. Anxiety was present in a slightly greater proportion of this patient cohort compared with the international research and present in almost twice as many patients than previously reported in Canterbury, NZ.⁽⁹⁶⁾ Three Australian studies have investigated the presence of anxiety in patients with IBD and have reported that 39 – 43 % of patients scored 8 or more on the HADS-A subscale.^(97, 100, 103) These Australian cohorts contained a mixture of patients with active and in-active existing CD. Anxiety is more likely to be present in patients with active disease,⁽¹⁰⁰⁻¹⁰²⁾ which may explain the slightly higher presence of anxiety at baseline in the current study. This study found no association between CDAI and HADS-A score at baseline or treatment completion but, the median HADS-A score did significantly decrease during treatment as disease activity indices improved non-significantly.

A recent Canterbury, NZ study by McCombie et al,⁽⁹⁶⁾ conducted in the same gastroenterology unit as this research, included 54 patients with newly diagnosed IBD. Anxiety was present in half as many patients in that study compared with the current CD cohort and the mean HADS-A score at baseline was 5.19 ± 3.36 compared with 7.0 (range, 0 to 15) in the current study.⁽⁹⁶⁾ The

discrepancy in the presence of anxiety may be attributed to a range of factors including: the lower average age of the current study's patients (22 years old compared with 34 years old) and/or patients who elect to use EN therapies as an alternative to corticosteroid treatment may be more anxious about the side effects of CD treatments. Potentially the greatest difference between the studies is that in the current study patients were either treatment naïve or had failed other CD treatments when they completed the HADS questionnaire. Patients in the earlier study were newly diagnosed with IBD but 81 % had already started treatment with oral or intravenous steroids when recruited to the study. The current study found that the HADS-A decreased significantly with only two weeks of treatment, which may well account for the lower presence of anxiety observed in the McCombie et al study.

The presence of depression (HADS-D 8 or more) in IBD cohorts varies in the literature from 4 – 22 % of patients.^(84, 94, 100, 101) Previous research has found that the presence of depression is more likely in patients with symptoms of active disease.⁽¹⁰⁰⁻¹⁰²⁾ Prior to starting EEN or PEN treatment, depression was present in 19 % of patients with active CD. The presence of depression and the median HADS-D score reduced consequent to EN therapy and CDAI was not correlated with HADS-D scores at baseline or treatment completion. Previous research has also suggested that symptoms of depression are associated with disease severity.⁽¹⁰¹⁾ Only a few patients in this study cohort had severe CD therefore, no conclusions can be made as to the effect of disease severity on the observed baseline and change in HADS-D. In a larger cohort such a sub-group analysis would be possible.

SIBDQ and IBDQ scores are frequently lower in patients with active IBD,^(108-110, 115) as was apparent in the current study. The median baseline SIBDQ score of 4.25 in this study is comparable to the mean scores (4.34 ± 0.42) of a large study of 150 Canadian patients with active CD (CDAI > 150).⁽¹¹⁵⁾ This Canadian study validated the 10 question SIBDQ with the

more extensive 32-item IBDQ and found that patients with a CDAI of less than 150 had significantly higher mean SIBDQ than patients with active disease.⁽¹¹⁵⁾ A recent study from Canterbury⁽⁹⁶⁾ reported SIBDQ scores of patients with recently diagnosed IBD, many of whom had recently initiated treatment. The mean SIBDQ score at baseline was slightly higher (4.67 ± 1.27) than in the current study, which may be related to recently starting treatment for active CD, but as observed in the Canadian study and the current study, SIBDQ significantly increased as disease activity reduced.

Gender is another variable, independent of disease activity, which has been associated with lower IBDQ scores. Two studies found that female patients with CD had lower mean IBDQ scores than male patients.^(111, 112) This EN therapy study found that the SIBDQ scores of females and males were comparable, although there were only eight males, compared to 30 females, recruited to use EN therapy. Other research studies which have used the SIBDQ have not reported the scores of males and females separately⁽¹¹⁵⁻¹¹⁷⁾ or have reported that there were no significant differences between the scores of males and females.⁽⁹⁶⁾

The use of EEN has been associated with improved HRQOL in a group of European children with CD⁽³³⁾ and Chinese adults with CD.⁽⁸⁶⁾ The Chinese study reported that IBDQ scores of greater than 170 (equivalent to a >4.7 on the SIBDQ scale) were achieved in 10 of the 11 (91 %) patients who responded to EEN treatment.⁽⁸⁶⁾ In the current study, a SIBDQ of greater than 4.7 was achieved in 20 of the 23 (87 %) who completed the treatment. The SIBDQ scores of the three patients with a CDAI of greater than 150 at week eight were all greater than the clinically significant cut-off of an SIBDQ of greater than 4.7 as were the SIBDQ score of 17 of the 20 (85 %) patients who had a CDAI < 150 after eight weeks of EN therapy. No other research groups have yet assessed the impact of EEN treatment on HRQOL of adults with CD.

The HRQOL results of this clinical trial have some limitations. The lack of a control group, due to only recruiting two patients into the study to use corticosteroids, means that no conclusions can be drawn as to the possible impact of the EN therapies on the improvement on SIBDQ score or the presence of anxiety and depression at treatment completion and six months. This limitation may have been mitigated if patients who withdrew from EN treatment were also followed out to six months. In retrospect, HRQOL data from the group of 25 patients who withdrew would have allowed for the comparison of HRQOL parameters between patients who did and did not use a complete course of EN therapy.

6.6 Conclusion

This research suggests that the presence of anxiety and depression in young NZ adults with active CD is equivalent to, or higher, than rates previously reported internationally. This is a unique cohort of patients in that all the patients had active disease and the majority were treatment naïve. The treatment of active CD with EEN or PEN and the subsequent achievement of disease remission resulted in significant reductions in anxiety and depression and clinically significant improvements in the SIBDQ score. These data therefore support the hypothesis that enteral nutrition therapy is associated with improvements in patient HRQOL.

Chapter 7

Faecal Microbiota and Exclusive Enteral Nutrition

7.1 Introduction

Each person has their own unique combination of microbes residing in their gut,⁽¹³³⁾ which are influenced by many variables including, but not limited to, route of birth,⁽²²⁴⁾ breast feeding,⁽²²⁴⁾ antibiotic use^(142, 224) and diet.⁽¹⁴²⁾ Patients with CD have consistently been shown to have a different gut microbiota composition, coined dysbiosis, compared with healthy populations.^(121, 122)

Studying the impact of disease and treatments on the microbiota is challenging due to the natural variability of the gut microbiota. Comparing the results of one study with another is often difficult due to differences in microbiota analysis techniques. Researchers have thus tried to design methods of grouping individuals according to the prevalence and function of phylogenetic groups of bacteria. Data from the Human Microbiome Project has been used to develop one such model called “community types”.⁽²²⁵⁾ Whereas, another research group has developed a similar model referred to enterotypes.⁽²²⁶⁾ The four “community types” do not contain the four most dominant species/families but rather a specific combination of bacteria which differentiate the community types from each other.⁽²²⁵⁾ The characteristics of each community type are summarised in Table 7.1. The grouping of individuals into community types or enterotypes is still being debated and tested with different populations and disease states.^(227, 228)

Gut dysbiosis in patients with Crohn's disease is hypothesised to play a role in the development and recurrence of inflammation.⁽²²⁹⁾ Treatment of active paediatric CD with EEN has been shown to temporarily reduce the diversity of the faecal microbiota despite improvements in disease activity.^(152, 153) One paediatric study has also shown that patients who respond to EEN therapy have different microbial changes compared with patients who do not respond.⁽¹⁴²⁾ There is limited research which explores the effect of nutrition therapy on the faecal microbiota of adults with active CD.

Table 7.1. Characteristics of the four faecal microbiota community types.⁽²²⁵⁾

Community type	Relative abundance				
	Phylum - Bacteroidetes			Phylum - Firmicutes	
	Genus <i>Bacteroides</i>	Genus <i>Prevotella</i>	Genus <i>Alistipes</i>	Genus <i>Faecalibacterium</i>	Family Ruminococcaceae
A	Higher	Absent	Lower	Lower	Absent
B	Lowest	Lower	Lower	Variety of Firmicutes species	Variety of Firmicutes species
C	Lower	Absent	Higher	Higher	Higher
D	Lower than A and D	Higher	Moderate	Moderate	Moderate

7.2 Aims and Hypotheses

The aim of this pilot research was to document changes in faecal microbiota structure consequent to dietary change in adults with active CD. It was hypothesised:

- That exclusive enteral nutrition therapy alters faecal microbiota structure in adults with active CD.

7.3 Methods

A pilot study of serial faecal samples from the first six patients who had completed EEN treatment was funded by a Laurenson Trust Award in collaboration with Professor Gerald Tannock and his team in the Department of Microbiology and Immunology, University of Otago, Dunedin. The methods that related to the collection and storage of stool samples are detailed in Chapter 2 section 2.1.9. Stored stool samples were sent to Dunedin by overnight courier on dry ice for faecal microbiota analysis and interpretation. The methods used to extract the bacterial DNA and characterise the microbiome are described in detail in Chapter 2 section 2.1.9.3.

The sequenced data was analysed using multiple bioinformatics techniques. These included relative abundance of operational taxonomic units, identification of possible bacteria using the operational taxonomic units data, grouping of bacteria into phylogenetic groups, weighted-UniFrac, a beta diversity metric used to assess differences between microbiota communities, and principle coordinate analysis of the phylogenetic data.

7.4 Results

Six patients had serial faecal samples characterised. The first two patients had samples out to six months (seven samples) and the other four had samples up to week 12 (six samples). All of the patients had newly diagnosed CD and were of NZ European ethnicity. The baseline characteristics of the six patients are described in Table 7.2. All six patients completed the eight week EEN treatment and all achieved disease remission (CDAI < 150) at week eight.

Figure 7-1 and Figure 7-2 are three dimensional plots of principle coordinate analysis, which were generated from an algorithm which measures diversity. Figure 7-1 illustrates that the faecal microbiota of each patient is distinct and that phylogenetic structure changes in response to dietary changes and that these changes are unique to each patient. Figure 7-2 suggests that there may be a trend towards patients with ileal CD having a different phylogenetic structure compared with patients with ileocolonic CD.

At baseline, four of the six patients with active CD had a type C faecal microbiota community and the other two patients had a type B or type D community structure (Figure 7-3). The patient with a type B community followed a vegan diet at baseline and the patient with a type D community consumed a high energy diet. The abundance of the four bacterial genera or family accounted for 40 – 66 % of the microbes present in the stool samples. After eight weeks of all consuming the same fibre deficient oral nutrition formula, all patients had a type C community structure characterised by the absence of *Prevotella* and lower abundance of *Bacteroides* (Figure 7-3). The combined relative abundance of the four bacterial genera or family also reduced in most patients.

The diversity of the faecal microbiota samples tended to cluster by diet. Figure 7-4 and Figure 7-5 are principle coordinate analysis plots which show that generally the microbiota at weeks 0, 12 and 26, when patients are eating a usual diet, cluster together and the microbiota during EEN (weeks 2, 4, 6, 8) cluster separately. These plots indicate that while each patient has their own unique microbiota, treatment with EEN results in a taxonomic shift in all patients. Despite each patient having a unique faecal microbiota there was a small group of bacterial species present in all of the patient faecal samples, which varied in abundance significantly between the EEN treatment and when patients were eating their usual diet (Figure 7-6).

Table 7.2. Baseline characteristics of patients whose faecal microbiota was characterised.

Sex	Age	Disease location	Baseline BMI	IGF-1 SDS	Baseline CDAI	Baseline CRP	ESR	Baseline FC
M	30.7	L3	25.1	0.2	169	18	7	3019
F	26.8	L1	20.3	-0.8	104	3	2	129
M	23.0	L3	26.2	0.3	39	9	8	564
F	19.7	L3	23.3	0.5	150	6	21	1421
F	18.5	L3	23.8	-2.7	207	11	25	3838
M	17.0	L1	18.5	-1.7	59	24	11	765

Note: M, male; F, female

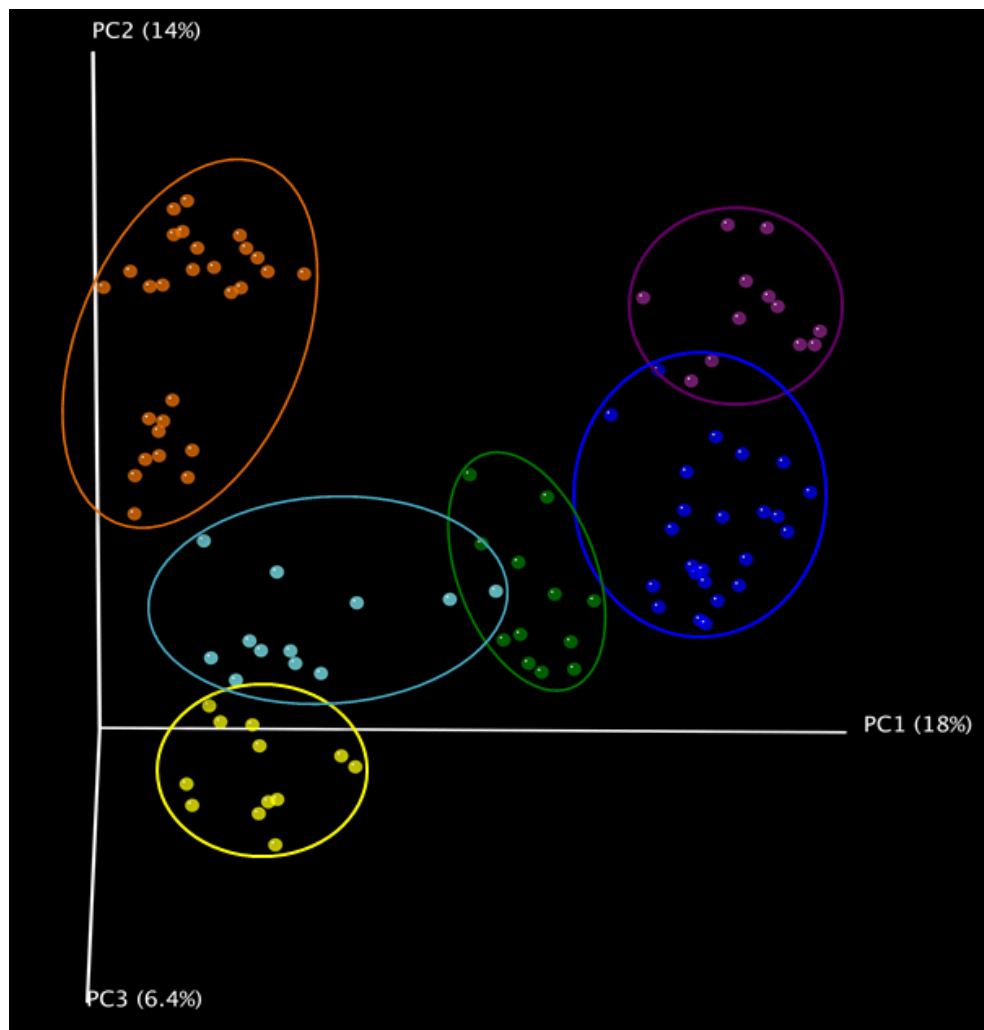


Figure 7-1. Principle coordinate analysis plot of faecal phylogenetic data across all EEN study time points and grouped by patient.

Note: Each coloured dot corresponds to a patient sample and each patient is represented by a different colour.

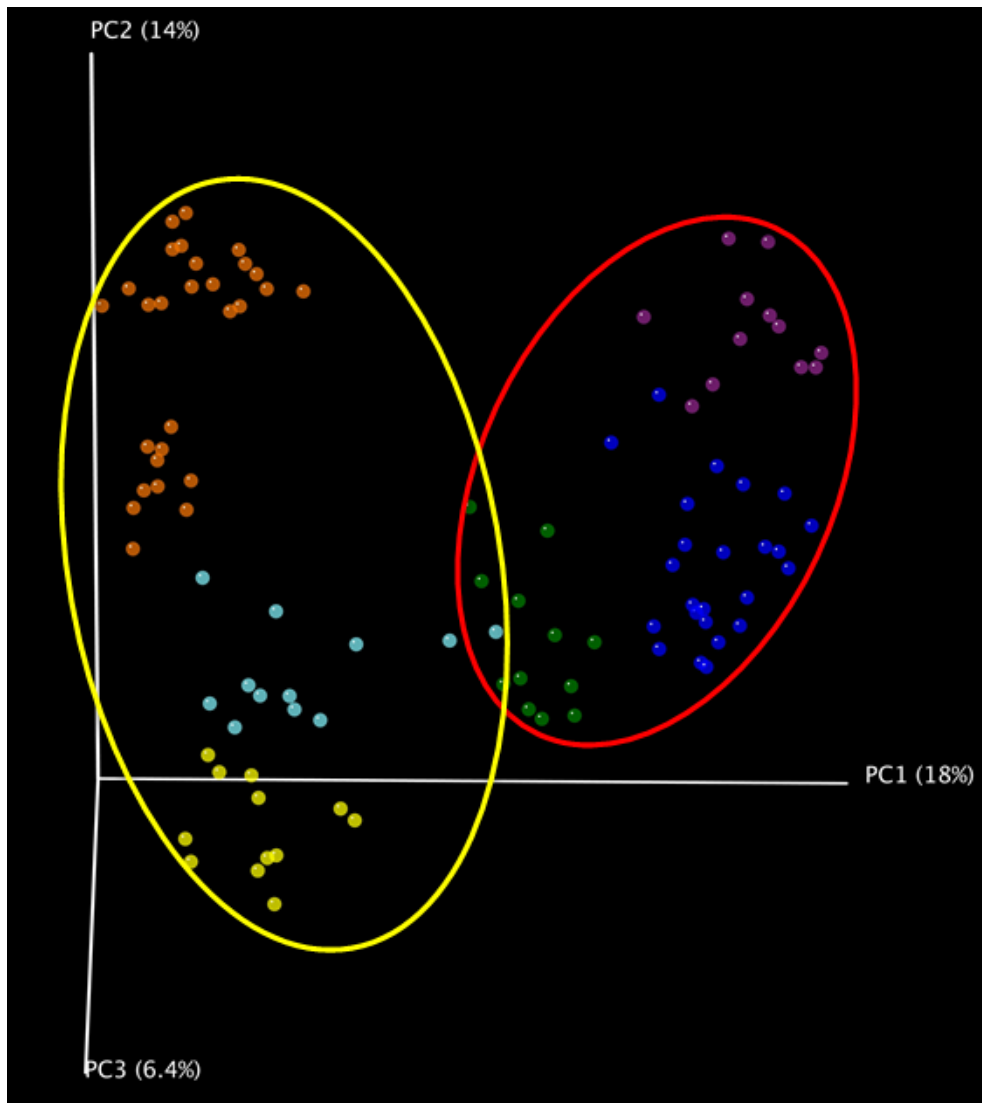


Figure 7-2. Principle coordinate analysis plot of faecal phylogenetic data across all EEN study time points and grouped by disease location.

Note: Each coloured dot corresponds to a patient sample and each patient is represented by a different colour. The dots inside the yellow oval have ileal CD and the dots inside the red oval have ileocolonic CD.

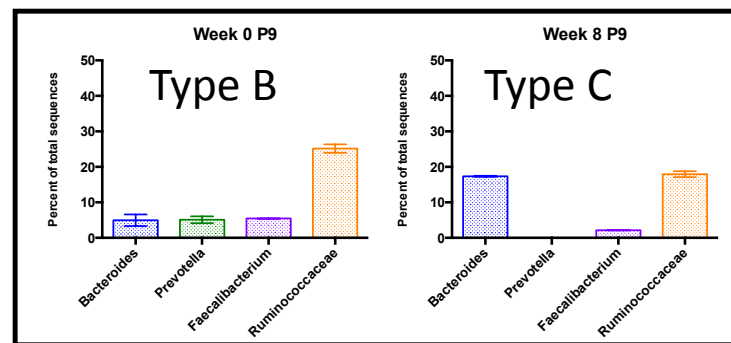
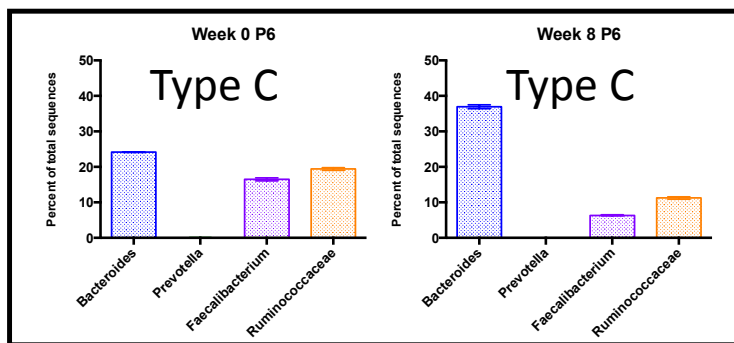
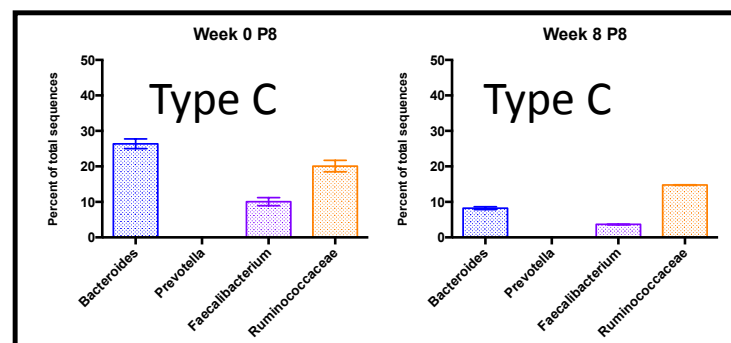
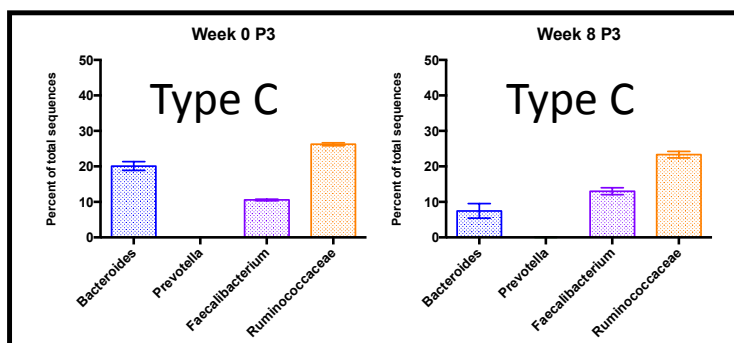
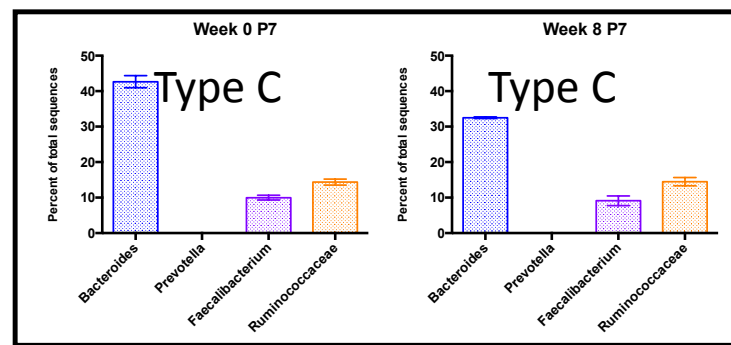
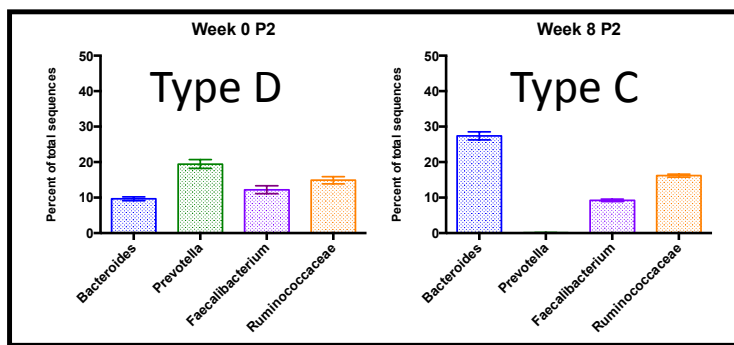


Figure 7-3. Phylogenetic community type of faecal samples at baseline and after EEN treatment.

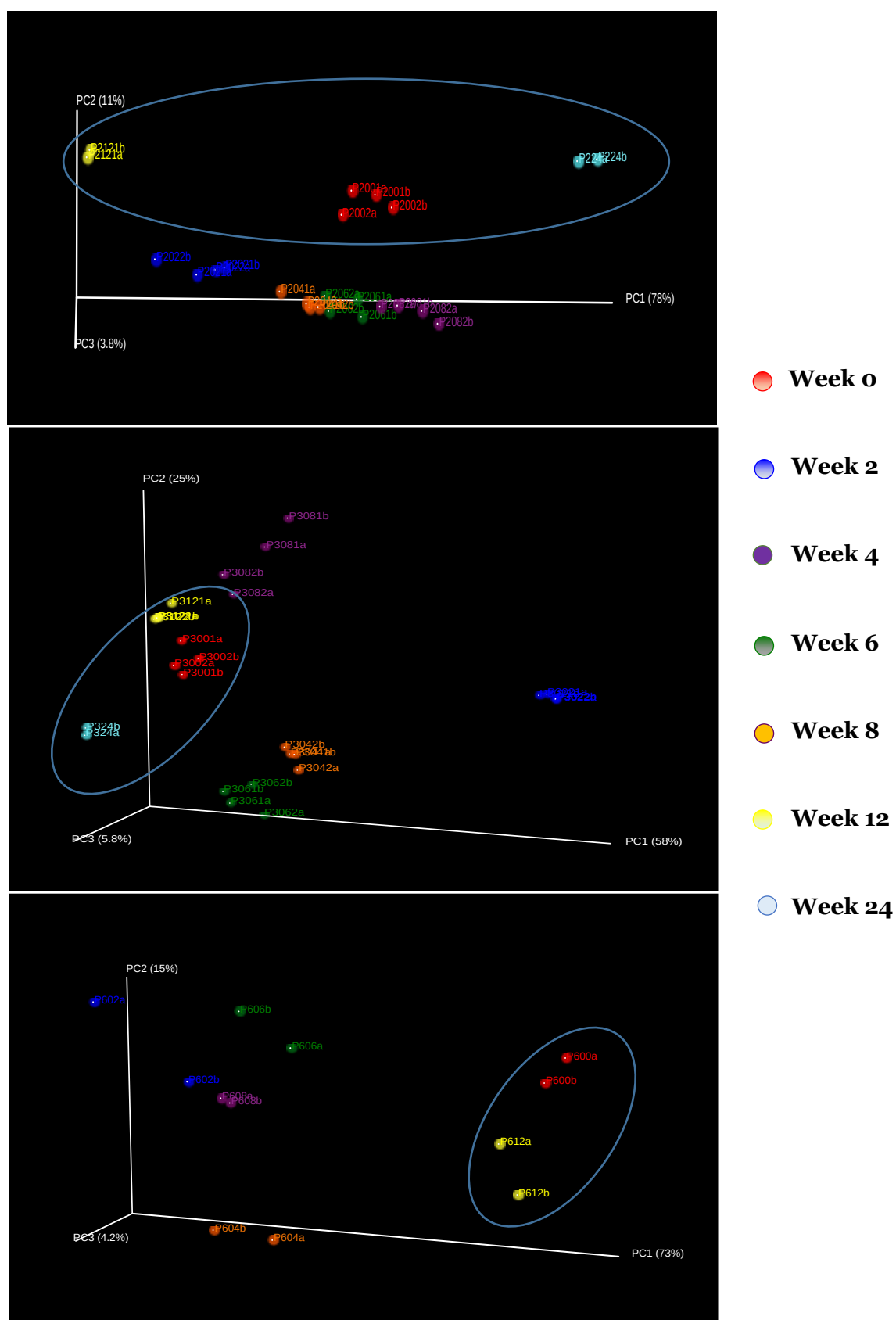


Figure 7-4. Principle coordinate analysis of beta diversity data from the first three patients.

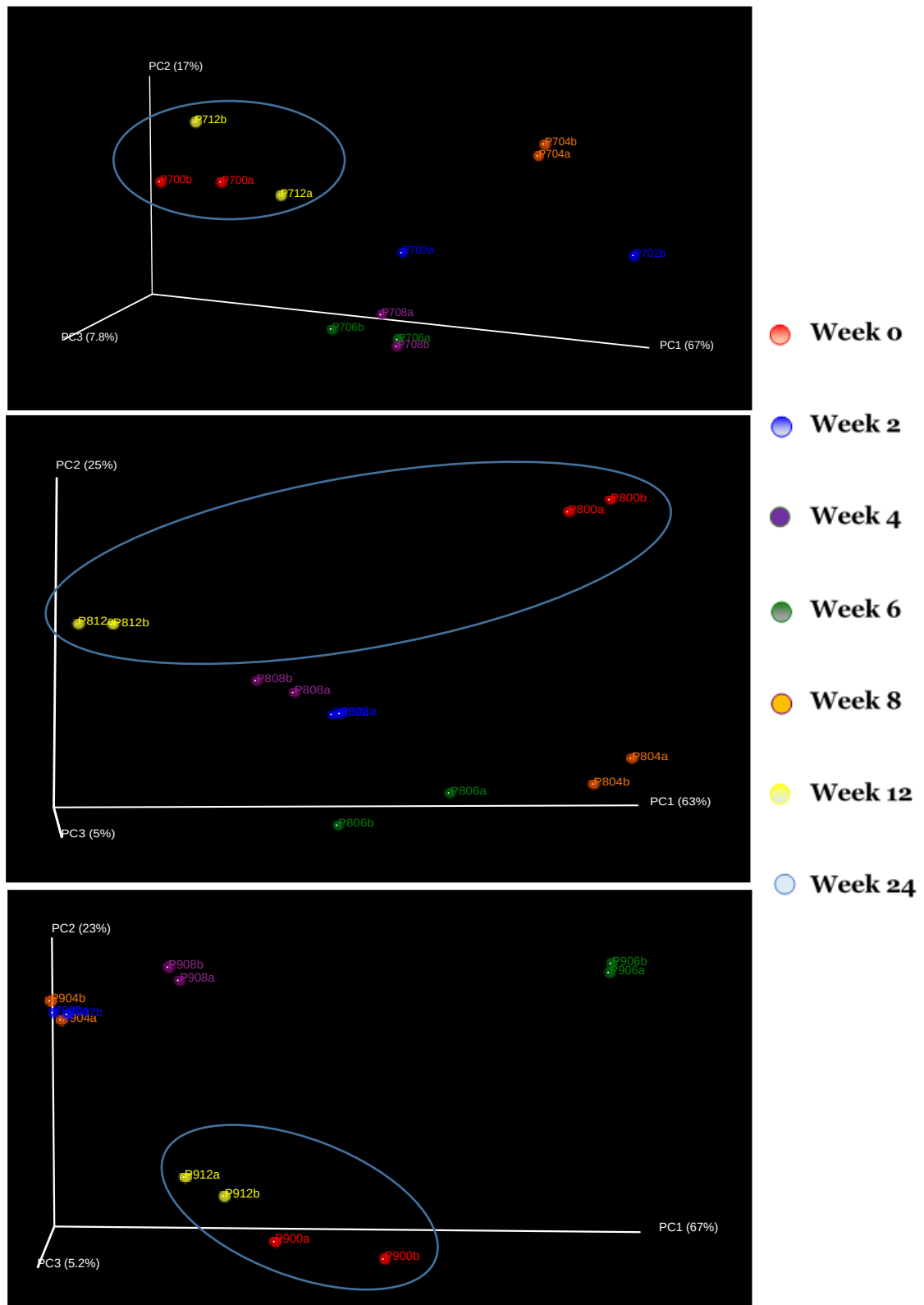


Figure 7-5. Principle coordinate analysis of beta diversity data of the last three patients.

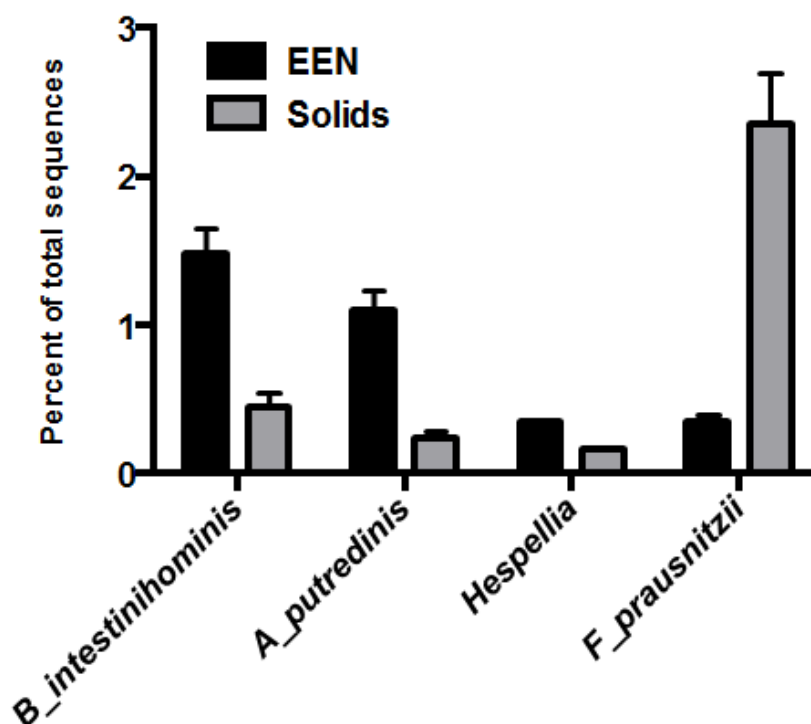


Figure 7-6. Bacterial species which varied significantly between EEN and usual diet phases.

Note: *Hespellia* includes *H. porcina* and *H. stercorisuis*

7.5 Discussion

High throughput sequencing of faecal samples from patients with CD found that the faecal microbiota of each patient were unique. The finding is in keeping with most microbiota research, which reports high faecal microbiota inter-personal variation.^(132, 133, 137, 138) Despite inter-personal variation, many studies have found that samples from patients with CD are different from healthy controls.^(142, 144, 230) Principle coordinate analysis suggested that the phylogenetic structure may vary depending on the site of intestinal inflammation. One other study also noted that the abundance of particular bacteria was different in patients with ileocolonic disease compared with other disease locations.⁽¹⁴⁴⁾ A study of 35 Korean patients with CD reported that *Gammaproteobacteria* was more abundant in faecal samples of patients with ileocolonic disease.⁽¹⁴⁴⁾ Perhaps, the phylogenetic structure of the faecal

microbiota is affected by site of inflammation and this hypothesis should be further tested with the whole enteral nutrition study cohort of 38 patients.

Faecal microbiota composition varied during the EEN phase compared with when patients were consuming their usual diet. This trend has also been observed in other studies which have investigated changes in faecal microbiota in children with CD using EEN.^(142, 152, 231) As was observed in the current study, once children started eating usual foods again their faecal microbiota tended to return to a similar structure as observed at baseline.^(152, 232)

Community type or enterotype characteristics are strongly associated with usual dietary habits⁽²²⁷⁾ and possibly by country of residence.⁽²³³⁾ Baseline dietary intake was not collected in the current study because the focus of the study was on how the faecal microbiota changed in response to a liquid diet and once usual foods and fluids were introduced again. It was assumed that patients returned to their usual dietary habits after the nutrition intervention. The patients' baseline energy requirements and food exclusion habits were collected. Two of the six patients had a different community type at baseline characterised by the presence of *Prevotella* bacteria. *Prevotella* are more prevalent in samples from people who eat a diet high in carbohydrate,⁽²²⁷⁾ which was most probably the case in the study patient with the type D community type. This patient had a very active job and consequently a high energy intake and would therefore have consumed much larger amounts of carbohydrate foods than the other study participants. The patient with a type B community followed a vegan diet, which has also been associated with a greater abundance of *Prevotella* bacteria.⁽²²⁷⁾ Whereas, a *Bacteroides* dominant enterotype, or community type C, is associated with higher intakes of animal protein and saturated fat⁽²²⁷⁾ which happens to reflect the typical NZ diet.⁽²¹³⁾

After eight weeks of EEN, all patients had a faecal microbiota consistent with community type C. All six patients had at least a small reduction in the Ruminocacaecae. One

paediatric CD study of five children found that a reduction in the abundance of Ruminocacaecaea was associated with achieving disease remission.⁽²³¹⁾ The absence of *Prevotella*, the main characteristic of community type C, has not been reported previously, although changes in the *Bacteroides/Prevotella* have been reported.^(25, 142, 152) There has been no other published studies in adults or children with CD which have reported the community type of patients. It is not yet known if the community types observed in this pilot study are characteristic of other populations of patients with CD.

The significance of the changes in the small number of specific bacterial species described in Figure 7-6 is not yet known. At the time this work was undertaken in 2014, little was known about the functions of three of these four bacteria but as high throughput sequencing becomes more accessible more research groups are investigating the faecal microbiota composition and functions. The first bacterium which was significantly different in faecal samples collected during EEN treatment compared with when patients were consuming their usual diet was *Barnesiella intestinihominis*. *B. intestinihominis* has been shown to slow the growth of *Escherichia coli* subsequent to antibiotic treatment.⁽²³⁴⁾ and has been proposed as a potential probiotic to use with antibiotic treatment⁽²³⁴⁾ This bacterium is also more prolific in the presence of the oligosaccharide 2'-fucosyllactose which it utilises as an energy source.⁽²³⁵⁾ *B. intestinihominis* preferentially uses 2'-fucosyllactose, one of the human milk oligosaccharides which has recently been approved to be added to infant formula. Infants consuming infant formula containing 2-fucosyllactose have a faecal microbiota closer to that of breast fed infants.⁽²³⁶⁾ The formula used in the current study was a fibre free formula but there are products available which have added fibre, usually in the form of inulin, soy/oat fibre, soy polysaccharides and/or fructo-oligosaccharides. The use of fibre containing nutrition formula has not been investigated in patients with active CD but given the potential protective effects of commensal bacteria which utilise dietary oligosaccharides perhaps fibre containing formulas should be considered for supplementary nutrition when disease is in remission.

There are limited data available on the function and abundance of *Hespellia* and *Alistipes putredinis* in healthy people or patients with CD. *Hespellia porcina* and *H. stercorisuis* are anaerobic bacteria which use simple sugars such as sucrose and glucose as energy and can hydrolyse starch.⁽²³⁷⁾ The nutrition formula used in this study contained partially hydrolysed and hydrolysed starch and sucrose as its carbohydrate sources. The abundance of *Hespellia* in faecal samples doubled during the liquid diet phase compared to when patients were consuming their normal diets. The significant changes in these bacteria may have been related to substrate availability. Further comparisons with the larger cohort and with a healthy population are required to explore this hypothesis.

A. putredinis has been reported to be abundant and prevalent in faecal samples of healthy Dutch children.⁽²³⁰⁾ The Dutch study of 60 children with newly diagnosed CD observed that that the abundance of *A. putredinis* in all patients was very low or non-existent compared with healthy children.⁽²³⁰⁾ Patients were offered EEN treatment but the paper did not comment if the abundance of this bacterium in particular increased, as seen in the current study. The researchers observed that the faecal microbiota of patients with CD approached that of healthy controls as patients achieved disease remission.⁽²³⁰⁾ No other studies were found that referred specifically to the abundance of *A. putredinis* in the faecal microbiota of patients with CD.

In healthy adults *Faecalibacterium prausnitzii* is one of the most abundant faecal bacterial species but has been shown to be reduced in faecal samples from patients with active CD.^(143, 146) *F. prausnitzii* ferments dietary fibre, produces butyrate and has been shown to have anti-inflammatory effects.⁽¹³⁵⁾ This pilot study shows that the abundance of *F. prausnitzii* decreased on the liquid diet, a result which has been observed in many EEN studies.^(152, 231) Faecal *F. prausnitzii* also reduced significantly in healthy volunteers consuming a fibre-free and fibre containing nutrition formula for 14 days.⁽¹⁵¹⁾ The changes in *F. prausnitzii* may be a

consequence of the reduction in dietary fibre intake during the liquid phase of the diet. In this pilot study, the abundance of *F. prausnitzii* increased once patients resumed their usual diet. The analysis of the faecal samples of patients with active CD who use PEN will help to understand if the addition of whole food dietary fibre to regimen alters the abundance of fibre fermenting genus or species such as *F. prausnitzii*.

Presently, the implications of changes in the faecal microbiota are not fully understood, however it appears that reductions in the abundance and diversity of bacteria consequent to EEN treatment are associated with improvements in disease activity. Once more is known about the function of specific bacteria and how bacteria interact with each other and their environment the implications of these changes may be further interpretable.

7.6 Conclusion

Each person has their own unique faecal microbiota and not all patients with active CD have the same faecal microbiota community type. Treatment of active CD with EEN can modify the community type or phylogenetic structure of the faecal microbiota and changes the abundance of specific bacterial species. The impacts of these changes are not yet fully understood.

Further faecal microbiota research with the remaining faecal samples of patients with CD, and healthy controls who used EEN for two weeks, will be completed once additional funding and expertise is secured. The further studies will add to the available pilot study data and will allow for comparisons between healthy adults and adults with CD using an EEN or PEN regimen to treat intestinal inflammation.

Chapter 8

Gastroenterology Health Professionals

8.1 Introduction

Optimal management of CD requires a multidisciplinary approach involving the gastroenterologist and/or surgeon, specialist gastroenterology nurses, dietitians, psychologists and other specialties.^(7, 43) In NZ, patients are often diagnosed and managed by a specialist gastroenterologist in a tertiary centre. However, much of NZ's population lives outside of cities with a tertiary hospital and outpatient care is provided by gastroenterologists and dietitians working in secondary care hospitals, private practice and/or in smaller provincial centres.

Traditionally the dietitians' role in the management of IBD is to optimise nutritional status through the management of poor oral intake, weight loss, micronutrient deficiencies and control of symptoms, including functional symptoms. In addition, the paediatric dietitian's role is likely to include management of EEN.⁽⁶⁾ The use of EEN by North American gastroenterologists is influenced, in part, by the physicians' previous experience with the treatment.⁽¹⁸⁸⁾ The use, and perceptions, of EEN by NZ gastroenterologists and dietitians has not previously been explored.

8.2 Aims and Hypotheses

The first aim of this research was to understand the current role(s) of NZ dietitians in the management of CD patients and to outline their experience with the use of EEN for patients with active CD. The second aim was to understand NZ gastroenterologists' perceptions, knowledge and experience of EEN. It was hypothesised:

- That gastroenterology health professionals have limited experience with, and awareness of, EEN for adult patients with active CD.

8.3 Methods

The methodology used to understand of the roles, perception and experience with EEN in the treatment of CD of NZ health professionals, namely dietitians and gastroenterologists, is detailed in Chapter 2 sections 2.2.2 and 2.2.3. In summary, NZ dietitians and dietitian students and gastroenterologists and gastroenterology trainees were contacted via their respective member organisations. A web-based survey link was disseminated via Dietitians NZ's weekly electronic newsletter during August and September 2013. Gastroenterologists were sent a web-based survey link via email by the NZ Society of Gastroenterology executive officer during July and August 2015.

The dietitian and physician survey questions were adapted from a survey used with North American physicians to understand their attitudes and use of enteral nutrition to treat paediatric CD.⁽¹⁸⁸⁾ The survey platform was QuestionPro Online Survey Software Application (www.questionpro.com). The surveys were pre-tested with relevant professionals and their feedback was incorporated into the final version of the two surveys.

The surveys did not require ethical approval; however, the data collected from dietitians and physicians were managed ethically, and anonymity of survey respondents was maintained.

8.4 Results

8.4.1 Demographics of IBD Health Professionals

The dietitian survey was started by 102 of the 488 active Dietitians NZ members, which is a response rate of 21 %. Of the 102 dietitians who started the survey, six did not complete it and 25 (25 %) did not see patients for gastrointestinal (GI) complaints. The remaining 77 dietitians (16 % of the total membership) saw a patient with a GI related complaint at least a

few times a year and 58 dietitians saw a patient with CD at least a few times a year. The characteristics of dietitians who saw patients with CD are described in Table 8.1.

The physician survey was sent to approximately 110 members of the NZSG. These members were gastroenterologists, 10 – 15 surgeons and 15 – 20 gastroenterology trainees. The survey was started by 48 members and completed by 42, which is a response rate of 38 %. The characteristics of gastroenterologists and trainees are described in Table 8.1.

Table 8.1. Characteristics of the New Zealand dietitians and gastroenterology physicians who were surveyed.

Characteristics of physicians and dietitians	Physicians (n = 42) n (%)	Dietitians (n = 58) n (%)
Experience:		
Registrar/< 2 years	12 (29)	9 (16)
Consultant < 5 years/2 – 5 years	1 (2)	14 (24)
5 – 10 years	6 (14)	14 (24)
10 – 20 years	13 (31)	9 (16)
> 20 years	10 (24)	12 (20)
Patient group:		
Adults	33 (79)	48 (83)
Paediatrics	6 (14)	10 (17)
Adults and paediatrics	3 (7)	n/a*
Location of practice:		
Public hospital	41 (98)	45 (78)
Private hospital	5 (12)	3 (5)
Private practice	22 (52)	8 (14)
Academic practice	7 (17)	0 (0)
Public community setting	0 (0)	8 (14)
Food service	0 (0)	2 (3)
Work in more than one setting	24 (57)	17 (29)
Geographical location:		
Northland	0 (0)	2 (3)
Auckland	16 (40)	15 (26)
Waikato	4 (9)	4 (7)
Bay of Plenty	2 (5)	1 (2)
Gisborne	0 (0)	0 (0)
Hawke's Bay	2 (5)	2 (3)
Taranaki	0 (0)	3 (5)
Manawatu/Whanganui	0 (0)	5 (9)
Wellington	4 (9)	10 (17)
Nelson/Marlborough	1 (2)	4 (7)
Canterbury/West Coast	8 (18)	8 (14)
Otago	3 (7)	2 (3)
Southland	2 (5)	2 (3)

* The survey did not ask dietitians to specify if they saw both paediatric and adult patients.

8.4.2 Dietitian Gastroenterology Case Load

The majority of dietitians saw a patient with CD a few times a year ($n = 31$) and eight dietitians (three paediatric and five adult dietitians) saw at least one patient with CD every fortnight (Figure 8-1). The dietitians who saw patients with CD more frequently worked in public hospitals in larger centres which have a tertiary hospital. A sub-group analysis found that the frequency of presentation of patients with CD did not differ between paediatric and adult dietitians ($\chi^2 (2, n = 58) = 2.72, p = 0.25$). All dietitians commonly received patient referrals for weight loss: 90 % of paediatric dietitians and 71 % of adult dietitians. However, paediatric dietitians received significantly more referrals for EEN (fisher's exact test, $p < 0.0001$) and micronutrient deficiencies (fisher's exact test, $p < 0.004$) compared with adult dietitians (Figure 8-2).

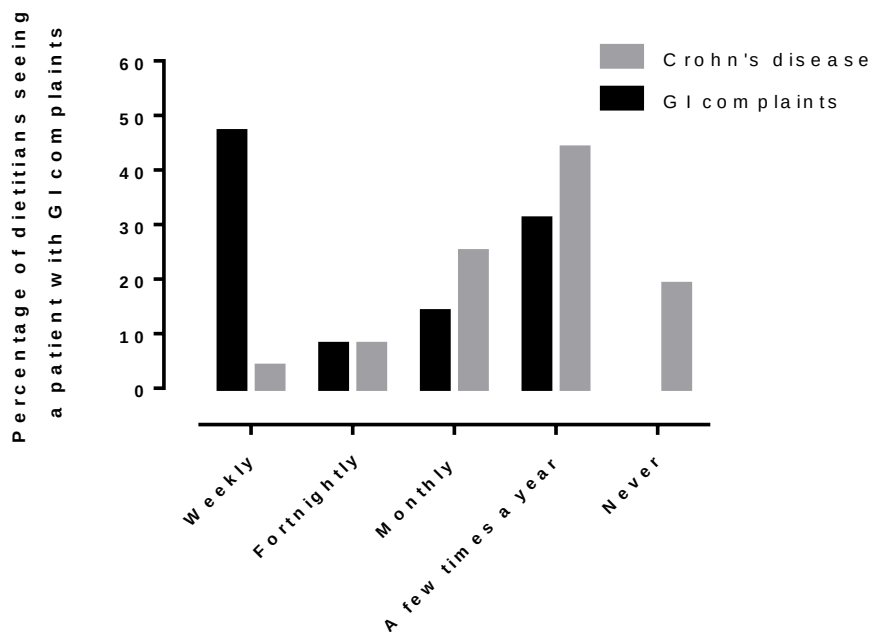


Figure 8-1. Difference in frequency of presentation of patients with CD and general gastrointestinal complaints (n = 77).

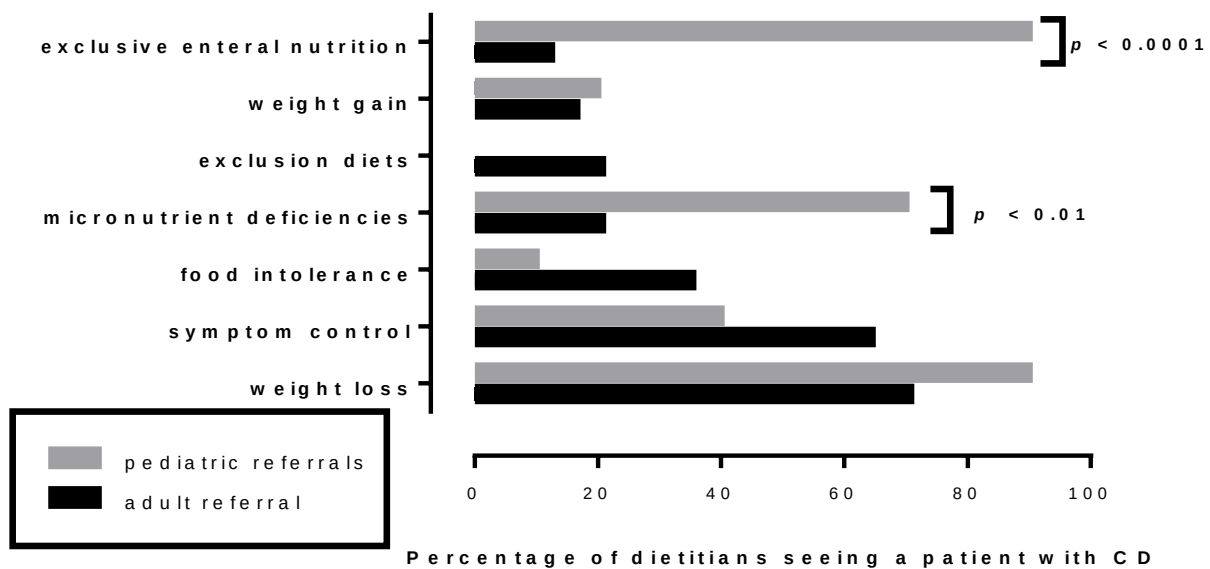


Figure 8-2. Common reasons patients with CD were referred to adult (n = 48) and paediatric dietitians (n = 10)

8.4.3 Health Professional Awareness of Exclusive Enteral Nutrition

Most dietitians, 10 (100 %) paediatric and 38 (79 %) adult dietitians, who saw patients with CD were aware of EEN as a treatment option for active CD. Dietitians were asked whether they believed EEN was an appropriate treatment for their patient group and if there were any disadvantages to the treatment. Five paediatric dietitians and 25 adult dietitians answered these questions. All five of the paediatric dietitians thought that EEN should be offered as a treatment option to selected patients. Of the 25 adult dietitians, 80 % believed that EEN may be beneficial for selected patients, two thought there was currently insufficient evidence to support its use with adults, and two dietitians were unsure if EEN would be of benefit for their patient group.

Thirty (43 %) dietitians felt they had adequate knowledge and/or skills to manage a patient referred for EEN. Half of the dietitians would find further professional development on the role of EEN in CD useful. They would also like more EEN patient resources, and more scientific evidence to support the use of EEN with adults, together with professional supervision.

Thirty-seven (90 %) gastroenterologists and trainees were aware of EEN as a treatment for active CD. The majority (64 %) considered that EEN was appropriate or sometimes appropriate to induce CD remission in adults and 29 % thought that it was rarely an appropriate treatment for adults. In the case of paediatric CD, a third of physicians answered not applicable, one third considered that EEN was an appropriate treatment and one third were unsure if EEN was an appropriate treatment. Most physicians (55 %) did not work, or had not previously worked, in a unit where EEN was used to treat active CD. EEN was regularly, or very regularly, used in physician's current unit in 19 % of respondents. Physicians considered that EEN treatment had a range of important benefits for the patient (Table 8.2).

Table 8.2. Physicians' perception of the benefits of EEN treatment.

Possible benefits of EEN treatment	Responses
	n (%)
Steroid sparing	31 (94)
Avoid immunosuppression	9 (27)
Improve nutritional status	29 (88)
Improve growth in children	22 (67)

8.4.4 Health Professional Use of Exclusive Enteral Nutrition

Thirty-five dietitians, 10 paediatric and 25 adult dietitians, reported that they had managed a patient with active CD with EEN. These dietitians were geographically spread across NZ and most (26 dietitians) worked in a public hospital. Seventeen dietitians who worked with adult patients had not managed a patient with CD on EEN, and the main reasons for this included that gastroenterologists and surgeons do not refer patients for EEN treatment (13 dietitians), a limited CD patient case load (9 dietitians) and limited experience using EEN (6 dietitians).

Dietitians' clinical experience of using EEN to treat active CD varied from one to fifty patients with a median of three patients (Figure 8-3). Ten dietitians (four paediatric, six adult) had used EEN with five or more patients. One of these ten dietitians commented that their experience was gained while working in the United Kingdom, and one was not currently practicing dietetics. The other eight dietitians practiced in main centres. All four paediatric dietitians who had managed at least five patients on EEN found EEN to be a successful treatment to improve disease symptoms. In contrast, five of the six adult dietitians who had

managed at least five patients on EEN reported that EEN was only sometimes successful in improving disease related symptoms.

Twenty-nine (69 %) physicians reported that they had previously used EEN with their patients of which 21 had used EEN with a patient in the last 12 months. Six paediatric gastroenterologists had used EEN in the last 12 months with 1 to 15 patients and in a median of six patients. Three physicians managed both children and adults with CD and had used EEN with a median of five patients and a range of “less than five” to ten patients. The remaining 12 physicians managed only adult patients and had managed a median of three patients (range, 1 to 8). The physicians who had recently used EEN with their patients were geographically spread across NZ but half (n = 6) of the adult gastroenterologists worked in Canterbury.

The paediatric gastroenterologists reported that 70 – 100 % of their patients achieved disease remission with EEN, the three physicians who manage both adults and children estimated that 50 to 70 % of their patients achieve remission consequent to EEN and the adult gastroenterologists reported that a lower number of patients complete the treatment and that a range of 0 to 75 % of patients had achieved disease remission with EEN.

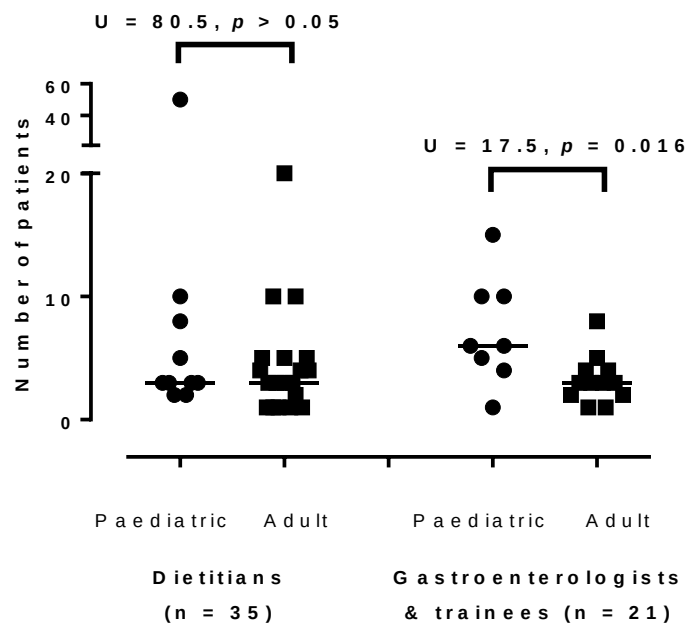


Figure 8-3. NZ dietitian and gastroenterologist experience with EEN for the treatment of active Crohn's disease.

8.4.5 Benefits and Barriers of Exclusive Enteral Nutrition

Dietitians who had used EEN with their patients were asked to comment, in an open-ended question, which patients had responded well to EEN. In their experience EEN was most successful for patients who adhered to the treatment, were motivated, did not have complicated disease, had newly diagnosed disease and/or were young adults. Physicians who had used EEN in the last 12 months reported that they usually considered EEN for children with newly diagnosed CD and patients who are nutritionally compromised. Two thirds also consider EEN for patients with existing disease or moderate to severe disease. Half of physicians (n = 10 to 12) would also consider EEN for adults with newly diagnosed CD and patients with mild disease. Paediatric and adult gastroenterologists had different views on the use of EEN for different disease locations Figure 8-4.

Both dietitians and physicians identified barriers to EEN treatment and these are described in Table 8.3. Other barriers mentioned by dietitians included that the treatment is socially restrictive if tube feeding required and that there are complications associated with tube feeding, children get bored of treatment quickly and that there may be fear associated with returning to usual oral foods and fluids. Other barriers mentioned by physicians included that adults have adequate calorie intake and lose weight and that the treatment does not always work.

Common reasons that physicians had not used EEN were that patients struggle to maintain treatment compliance (65 %), physicians had limited experience using EEN (41 %) and patients had limited social support (33 %). Many physicians somewhat or strongly disagreed that there was insufficient evidence to support the use of EEN with children (60 %) and that there was limited support from dietitians (43 %). Thirty-seven % also disagreed that there was limited evidence to support the use of EEN with adults.

Physicians were asked what would increase the likelihood of them recommending EEN to their patients the key themes were multidisciplinary support and evidence based guidance as illustrated in Figure 8-5.

Table 8.3. Barriers to using exclusive enteral nutrition with patients with active CD.

Downsides of EEN	Physicians (n = 32) n (%)	Dietitians (n = 42) n (%)
Need for treatment adherence	29 (91)	34 (81)
Needs multidisciplinary approach	10 (31)	11 (26)
Cost	6 (19)	8 (19)
More time intensive	12 (38)	6 (14)
Requires social support for patient	13 (41)	12 (29)

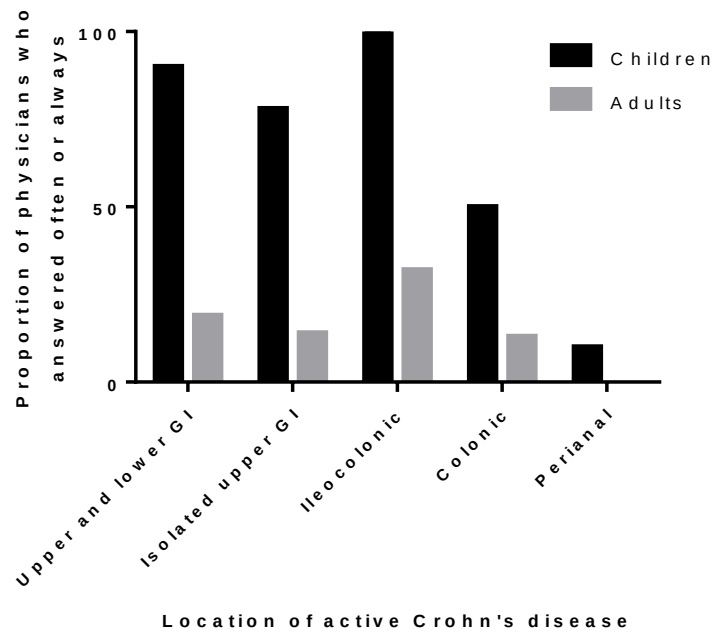


Figure 8-4. Proportion of physicians who would often or always consider using EEN to treat children (n = 10 responses) and adults (n = 16 responses) with active CD in a variety of locations.

Note: A statistical test was not employed due to the low number of responses in each group.

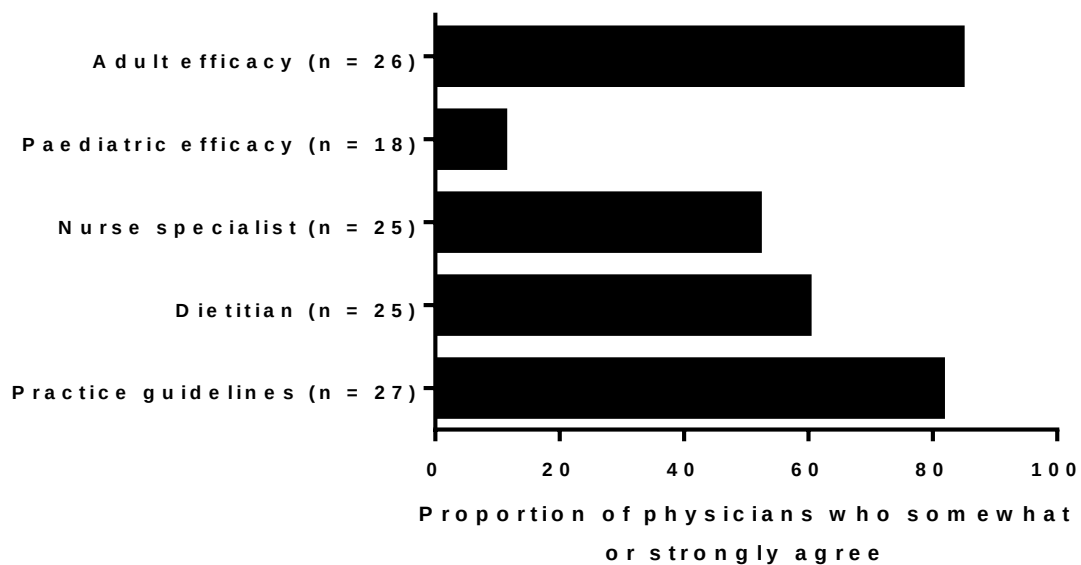


Figure 8-5. Resources required by physicians to increase likelihood of recommending EEN for patients with active CD.

Note: physicians answered using a Likert score of strongly disagree to strongly agree.

8.4.6 Supplementary Enteral Nutrition

The use of SEN by dietitians' to maintain disease remission was variable: 19 % always prescribed it, 40 % sometimes prescribed it, 18 % rarely or never prescribed it, and 23 % said the question was not applicable or did not answer. The prescription of SEN was usually used to treat nutritional concerns rather than as a means of maintaining remission of CD. There was no standard regimen and dietitians tended to use an individualized approach depending on weight, history of weight loss and current oral dietary intake. Similarly, gastroenterologists reported sometimes using SEN and the regimen was often two to three cartons per day depending on the patient.

8.5 Discussion

One of the aims of this research was to understand the role of NZ dietitians in the management of CD patients. This electronic survey suggests that patients with GI complaints frequently present to the NZ dietitians surveyed but patients with CD present much less frequently despite being at risk of poor nutritional status. It is estimated that 1 in 500 to 700 New Zealanders, or approximately 7,000 people, live with CD.⁽²³⁸⁾ Based on the patient caseload of the dietitians which responded to this survey, NZ dietitians only see approximately 7 - 13 % of the CD population. A more detailed survey of NZ dietitians would be required to ascertain if IBD service involvement and routine hospital nutritional screening is related to the observed low dietitian CD patient caseload. Regardless, the low exposure of NZ dietitians to patients with CD is concerning given the prevalence of undernutrition and may lead to poorer outcomes for patients.

Another aim of this research was to quantify NZ dietitians' and gastroenterologists' experience of using EEN for active CD. Thirty-five (60 %) dietitians and 29 (68 %) of physicians had previously used EEN to treat active CD disease. Dietitians would predominately receive referrals for EEN from gastroenterologists so it was expected that similar number of health professionals would have had prior experience with EEN. EEN is

recommended as a first line therapy to treat active paediatric CD,⁽²⁶⁾ and all of the gastroenterologists who manage children and nine of the ten paediatric dietitians reported previous use of EEN. However, half of the paediatric dietitians, had managed less than a total of five patients on EEN whereas paediatric gastroenterologist had used EEN with 1 to 15 patients in the last 12 months. The discrepancy between physician and dietitian responses suggests that either more than one dietitian works with each paediatric gastroenterologist or dietitians who more commonly manage patients on EEN did not complete the survey or have access to the online survey because they are not members of the national dietetic association.

Six of the 48 adult dietitians (12.5 %) commonly received referrals for EEN. The results of a patient survey described in Chapter 3 of this thesis suggests that adults patients are interested in using EEN as an alternative to corticosteroids and the recent British Dietetic Association dietary guidelines for the management of CD suggest that EEN could be considered as alternative to corticosteroids.⁽²³⁹⁾ Further to this recommendation the UK IBD standards⁽⁷⁾ suggest that all patients should have access to all types of nutrition therapy. In NZ, referrals to adult dietitians were lower than was observed in a survey of UK adult acute care dietetic departments where 76/129 (59 %) had received a referral for EEN in the last three months.⁽⁸⁾ The adult dietitians who commonly received EEN referrals were located in a small number of locations in NZ, whereas the paediatric dietitians who commonly received EEN referrals were spread more evenly across NZ. This observation suggests that referrals to dietitians for EEN may vary between hospitals and/or gastroenterologists which is in keeping with the findings of the physician survey. Physicians who have used EEN in the last 12 months and work with adult patients were also located in a small number of locations in NZ.

Internationally the use of EEN by paediatric gastroenterologists varies widely between countries.^(188, 240 , 241) The results of this survey suggest that NZ gastroenterologists often or always considered EEN as a treatment option for paediatric CD. Whereas, physicians who manage adults with CD rarely or sometimes considered using EEN for active CD and to

improve their use of the treatment practice guidelines and more evidence of the efficacy of EEN in the treatment of adults is required. The development, or adoption, of national treatment guidelines and EEN protocols may improve discrepancies in the geographical use of EEN in active CD.

This survey explored not only dietitian and physician use of EEN but also their experience managing patients on EEN and their perception of EEN as a treatment for active disease. Dietitians were optimistic about the effectiveness of EEN to induce disease remission. Forty-five percent perceived that EEN was an effective treatment, and 52 % felt that it was sometimes successful and was best used with specific groups of patients. Physicians would often or always consider EEN as a treatment option for many children and half of physicians working with adults would often consider using EEN for adults with newly diagnosed CD or mild disease. Literature suggests that the level of health professional support for patients using EEN is a major contributing factor to the success of the treatment.⁽²⁴²⁾ Therefore, dietitian and physician perception of the effectiveness of EEN is important, especially as patients need intensive dietetic support to optimise nutritional intake and minimise intolerance of the nutrition formula. It is likely that the involvement of optimistic and proactive health professionals may affect adherence to the treatment and therefore improve treatment outcomes.

The success of EEN, particularly in adults and as was observed in the clinical trial discussed in Chapter 4, is often limited by poor adherence to the treatment. NZ dietitians and physicians identified the need for treatment adherence was the main disadvantage of EEN treatment. Other disadvantages of EEN included the need for adequate social support and a multidisciplinary approach. North American and Australian paediatric physicians also reported compliance as the main disadvantage of EEN treatment.^(188, 240) Further, North American and Australian physicians who rarely used EEN reported that the main barriers to prescribing EEN more were the need for treatment adherence followed by lack of social

support and lack of experience using EEN.^(188, 240) The number of responses in the current surveys were too small to complete a similar analysis but limited experience with EEN also featured as a barrier for NZ dietitians and physicians. The survey of North American paediatric physicians found that physicians were more likely to recommend EEN to their patients if they had worked/trained in a centre that commonly used the EEN.⁽¹⁸⁸⁾ Half of the NZ physicians had not worked in unit where EEN was used to treat active CD and only 19 % currently worked in unit where the treatment was regularly used. Despite physicians believing that EEN has many potential benefits the limited exposure of physicians to EEN treatment in clinical practice may impact the use of the treatment.

Dietitians identified that professional development, especially for those with limited experience utilising EEN, is required and 80 % of physicians selected practice guidelines, alongside more efficacy of use in adults, as the main requirement to increase their likelihood of recommending EEN for patients with active CD. Further education may increase dietitian expertise and optimise treatment outcomes for patients with active CD and practice guidelines and indications for EEN developed in conjunction with the multidisciplinary team may increase the use of this nutrition therapy. Recent paediatric CD guidelines provide physicians with more practical guidance on the use of EEN with children⁽²⁷⁾ and suggest that if there is no response after two weeks and alternative treatment could be started.⁽²⁷⁾ Such guidelines may be helpful for health professionals working with adults interested in using nutrition therapy too.

The main limitation of the dietitian survey is whether or not the respondents are representative of paediatric and adult dietitians in clinical practice in New Zealand. Although this survey was disseminated via Dietitians NZ, the national dietetic professional organization, not all clinical dietitians are members of Dietitians NZ. Due to a lack of non-responder data there may be a bias towards dietitians who are aware of EEN in the treatment of CD and the results may overestimate awareness and utilization of this treatment. Another

limitation is that survey pre-testing was not completed with clinical dietitians who see patients with CD less frequently or have limited experience using EEN. Further surveys or audits that target dietetic departments and private practices that care for patients with IBD are required to further understand the specific role and utilisation of dietitians in tertiary centres that have a high CD patient caseload and also dietitians in private practice dietitians and rural centres who may manage this patient group less frequently.

Likewise, there are limitations with the results of the physician survey. The survey was sent to consultant gastroenterologists and gastroenterology trainees and 29 % of the respondents were trainees therefore their experience with EEN may overlap with that of the consultant the trainee was working alongside. Thirty consultant gastroenterologists completed the survey and represented most (n = 9) of the 13 regions in NZ which suggests that the survey was completed by only some of the gastroenterology physicians. The results may overestimate the usage of EEN with adult patients for two reasons: more physicians with an interest in nutrition therapy may have completed survey and half of the physicians who have used EEN with adult patients were from Canterbury where a clinical trial, presented in this thesis, had been running for the preceding 18 months.

8.6 Conclusion

These surveys provides a broad understanding of the involvement of NZ dietitians in the multidisciplinary care of patients with CD and use of EEN to treat active CD by dietitians and gastroenterologists. This survey has identified that patients with CD present frequently to a few NZ dietitians in limited number of centres. Most dietitians, paediatric dietitians included, have limited experience managing patients using EEN and gastroenterologists working with adults also have limited experience using EEN to treat active CD. Both dietitians and gastroenterologists would like more guidance on the use of EEN therapy and more evidence to support the use of EEN with adults with active CD before recommending EEN to a greater number of patients.

Chapter 9

Conclusion

This thesis has comprehensively explored EEN as a potential treatment option for young adults with active CD. Surveys of key stakeholders involved in EEN treatment, namely patients, dietitians and gastroenterologists, were conducted which identified that patients with CD are interested in nutrition therapy as a treatment option despite a lack of scientific evidence supporting the efficacy of EEN as a treatment for adults with CD.

In an attempt to increase the scientific evidence of EEN treatment efficacy a prospective non-randomised clinical trial of EEN was conducted. This trial of 25 patients found that EEN significantly reduced Crohn's disease activity; 68 % responded to EEN and 52 % of patients achieved disease remission. As has been observed in previous trials with adults, withdrawals from EEN therapy occurred due to intolerance of enteral formula but also for a variety of other reasons. On a per protocol basis, EEN was a very effective therapy with 93 % of patients achieving disease remission along with significant reductions in anxiety and depression and significant improvements in quality of life.

The use of a sequential EN regimen which combined a short course of EEN followed by PEN appears to be a feasible treatment option for adults with active CD. A pilot study of 13 patients suggests that PEN may help to minimise weight loss associated with EEN and may reduce the incidence of treatment withdrawals. Furthermore, 62 % of patients responded to the PEN therapy and 54 % achieved disease remission. On a per protocol basis PEN effectively induced disease remission in 78 % of patients along with significant reductions in anxiety and improved quality of life. However, improvements in FC were not achieved to the same degree as was observed with EEN treatment. Before PEN treatment is used with patients in clinical practice further research is needed to elucidate the optimal combination of solid foods to achieve palliation of symptoms and improvements in faecal inflammatory

markers, PEN treatment also needs to be tested in a larger cohort, and ideally in a randomised controlled trial of PEN and EEN and/or corticosteroids.

NZ gastroenterologists and dietitians believe that EEN is an appropriate treatment option for some patients but would like further evidence to support the use of EEN with adult patients. Both gastroenterologist and dietitians would also like guidance on the use of EEN through practice guidelines and professional support, and more access to members of an IBD multidisciplinary team.

Since the 2007 Cochrane review⁽³⁸⁾ of EEN in the treatment of active CD, many health professionals have not considered nutrition therapies for the management of adult CD. Based on the findings presented in this thesis, the use of EEN to treat adults with active CD should be discussed again within the profession and presented as an option to patients. There is also a need for further research investigating other nutrition therapies, such as PEN, that may be more appealing and efficacious for a greater number of adult patients. EEN effectively induces disease remission, and patients who are interested in an alternative to corticosteroids should be offered nutrition therapy with the support of an IBD multidisciplinary team.

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Appendix A

Food Reintroduction Guidelines

Food groups	First foods (Stage 1)	Next step (Stage 2)	Try last (Stage 3)
Bread	White bread, flat bread, pita, rolls or toast, plain English muffins Fine wholemeal bread	Coarse wholemeal bread Wholemeal pita or flat breads	Very fresh bread Wholegrain bread Bread containing dried fruit, whole grains, nuts or seeds
Cereals	Cornflakes, puffed rice White flour, cornflour Pasta or white rice	Porridge Weet-bix Wholemeal flour Brown rice, couscous	Muesli Bran cereals
Biscuits and cakes	Biscuits, cake, crackers, muffins, pancakes, pikelets, scones or sponge made without coconut, dried fruit, grains, seeds or nuts		Snack bars, biscuits, cake, crackers, muffins and scones containing coconut, dried fruit, grains, seeds or nuts
Fruit	Raw, cooked or tinned, without pips, seeds or skins Fruit juice without pulp Jams or marmalades without seeds or pith	Tinned pineapple	Berries, citrus fruit, fresh pineapple Dried fruit e.g. dates, sultanas and apricots Fruit juice with pulp
Vegetables	Tender vegetables – peeled and cooked e.g. carrot, kumara, parsnip, potato, pumpkin and yam Pureed or creamed vegetable soup Tomato paste or puree	Dahl (not spicy), pureed lentils, hummus Broccoli or cauliflower tops Cabbage, lettuce or silverbeet leaves (no stalks) Courgettes/marrow without skin or seeds, green beans, raw carrots Creamed corn Whole tinned tomatoes with seeds removed	Vegetables with coarse stalks, pips, seeds and skins e.g. corn (including popcorn) cucumber, garlic, onion, peas, raw tomato Baked beans, whole beans and lentils Coleslaw Pickle, relish, chopped chilli, garlic and ginger Fresh herbs

Food groups	First foods (Stage 1)	Next step (Stage 2)	Try last (Stage 3)
Meat, fish, poultry and eggs	Lean chicken, fish, lamb or minced beef Fish tinned in water Eggs Smooth pate or spread	Pork Skinless sausages Roast meat, steak	Tough gristly or fatty meat Chicken skin Sausage skins
Milk products and cheese	Milk, cheese, cottage cheese, cream cheese, sour cream Plain yoghurt, dairy food	Fruit yoghurt (no seeds)	Cheese with seeds or nuts Yoghurt with bits
Nuts and seeds	Smooth peanut butter	Finely ground nuts Tahini	Pumpkin sesame or sunflower seeds Nuts – whole or chopped Crunchy peanut butter
Dessert	Milk puddings, ice cream, jelly, plain yoghurt		Puddings containing coconut, dried fruit, nuts, pips, seeds and skins
Miscellaneous	Oil, margarine, butter Tomato sauce, soya sauce, gravies Mayonnaise Smooth salad dressings Chocolate (remember it's a treat)	Deep fried foods (except those in Stage 3)	Chunky sauces (ie satay), sauces with chilli flakes (ie chilli sauce) Vinaigrette

Appendix B

Hospital Anxiety and Depression Score (HADS)

Please choose one answer for each statement by ticking in the corresponding box. Do not think too long about your answers, and please answer the questions according to how you are currently feeling.

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 as much
 - ☐ Only a little
 - ☐ Hardly 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 so 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 too 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 as 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 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 program

- ☐ Often
- ☐ Sometimes
- ☐ Not often
- ☐ Very seldom

Appendix C

Short Inflammatory Bowel Disease Questionnaire (SIBDQ)

This questionnaire is designed to find out how you have been feeling during the last 2 weeks. You will be asked about symptoms you have been feeling as a result of your inflammatory bowel disease, the way you have been feeling in general, and how your mood has been.

1. How often has the feeling of fatigue or of being worn out been a problem for you during the last 2 weeks? (tick 1 option)
 - ☐ All of the time
 - ☐ Most of the time
 - ☐ A good bit of the time
 - ☐ Some of the time
 - ☐ A little of the time
 - ☐ Hardly any of the time
 - ☐ None of the time

2. How often during the last 2 weeks have you had to delay or cancel a social engagement because of your bowel problem? (tick 1 option)
 - ☐ All of the time
 - ☐ Most of the time
 - ☐ A good bit of the time
 - ☐ Some of the time
 - ☐ A little of the time
 - ☐ Hardly any of the time
 - ☐ None of the time

3. How much difficulty have you had, as a result of your bowel problem, doing leisure or sports activities you would have liked to have done during the last 2 weeks? (tick 1 option)
 - ☐ A great deal of difficulty; activities made impossible
 - ☐ A lot of difficulty
 - ☐ A fair bit of difficulty
 - ☐ Some difficulty
 - ☐ A little difficult
 - ☐ Hardly any difficulty
 - ☐ No difficulty; the bowel problems did not limit sports or leisure activities

4. How often during the past 2 weeks have you been troubled by pain in the abdomen? (tick 1 option)
- ☐ All of the time
 - ☐ Most of the time
 - ☐ A good bit of the time
 - ☐ Some of the time
 - ☐ A little of the time
 - ☐ Hardly any of the time
 - ☐ None of the time
5. How often during the last 2 weeks have you felt depressed or discouraged? (tick 1 option)
- ☐ All of the time
 - ☐ Most of the time
 - ☐ A good bit of the time
 - ☐ Some of the time
 - ☐ A little of the time
 - ☐ Hardly any of the time
 - ☐ None of the time
6. Overall, in the last 2 weeks, how much of a problem have you had with passing large amounts of gas? (tick 1 option)
- ☐ A major problem
 - ☐ A big problem
 - ☐ A significant problem
 - ☐ Some trouble
 - ☐ A little trouble
 - ☐ Hardly any trouble
 - ☐ No trouble
7. Overall, in the last 2 weeks, how much of a problem have you had maintaining or getting to, the weight you would like to be at? (tick 1 option)
- ☐ A major problem
 - ☐ A big problem
 - ☐ A significant problem
 - ☐ Some trouble
 - ☐ A little trouble
 - ☐ Hardly any trouble
 - ☐ No trouble

8. How often during the last 2 weeks have you felt relaxed and free of tension? (tick 1 option)
- ☐ None of the time
 - ☐ A little of the time
 - ☐ Some of the time
 - ☐ A good bit of the time
 - ☐ Most of the time
 - ☐ Almost all of the time
 - ☐ All of the time
9. 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? (tick 1 option)
- ☐ All of the time
 - ☐ Most of the time
 - ☐ A good bit of the time
 - ☐ Some of the time
 - ☐ A little of the time
 - ☐ Hardly any of the time
 - ☐ None of the time
10. How much of the time during the last 2 weeks have you felt angry as a result of your bowel problem? (tick 1 option)
- ☐ All of the time
 - ☐ Most of the time
 - ☐ A good bit of the time
 - ☐ Some of the time
 - ☐ A little of the time
 - ☐ Hardly any of the time
 - ☐ None of the time

Appendix D

Patient Questionnaire

Palatability of drink A

Please rank the drinks for the following qualities.

0	1	2	3	4
Extremely	Slightly	Neither	Slightly	Extremely
Difficult to drink			Easy to drink	
0	1	2	3	4
Unpleasant flavour			Pleasant flavour	
0	1	2	3	4
Poor mouth feel			Good mouth feel	
0	1	2	3	4
Weak aftertaste			Strong aftertaste	
0	1	2	3	4
Not acrid*			Acrid*	
0	1	2	3	4

* Acrid = strong and unpleasant taste or smell

Palatability of drink B

Please rank the drinks for the following qualities.



0	1	2	3	4
Extremely	Slightly	Neither	Slightly	Extremely

Difficult to drink	Easy to drink
--------------------	---------------

0	1	2	3	4
---	---	---	---	---

Unpleasant flavour	Pleasant flavour
--------------------	------------------

0	1	2	3	4
---	---	---	---	---

Poor mouth feel	Good mouth feel
-----------------	-----------------

0	1	2	3	4
---	---	---	---	---

Weak aftertaste	Strong aftertaste
-----------------	-------------------

0	1	2	3	4
---	---	---	---	---

Not acrid*	Acrid*
------------	--------

0	1	2	3	4
---	---	---	---	---

* Acrid = strong and unpleasant taste or smell



Drink A compared with Drink B

Please mark on the line which of the two drinks you prefer more

Prefer drink A

Neutral

Prefer drink B

Thinking about Drink A

On a scale of 0 – 10 how likely is it that you could drink this 6-8 times per day?

0 1 2 3 4 5 6 7 8 9 10

Very unlikely

Highly likely

Thinking about Drink B

On a scale of 0 – 10 how likely is it that you could drink this 6-8 times per day?

0 1 2 3 4 5 6 7 8 9 10

Very unlikely

Highly likely

Nutrition therapy with these drinks can greatly improve disease symptoms and may put disease into remission.



Have you taken prednisone previously for Crohn's disease? ☐ Yes ☐ No

Knowing what you do about prednisone, would you rather take either of these drinks and not eat and drink (other than water) for 8 weeks OR take a course of prednisone for 8 weeks?

☐ Drinks

☐ Prednisone

If these drinks could put your disease into remission would you consider using them and not eating or drinking (apart from water and black unsweetened tea or coffee) for 8 weeks if you had:

severe symptoms? ☐ Yes ☐ No

moderate symptoms? ☐ Yes ☐ No

mild symptoms? ☐ Yes ☐ No

General information about you

Your date of birth _____

Your sex ☐ Male ☐ Female

Thank you for your time.

Catherine Wall

PhD student, University of Otago and NZ Registered Dietitian

Appendix E

New Zealand Dietitian Survey Questions

In which areas do you currently practice?

Public hospital
Private hospital
Private practice
PHO
Community setting
Public health
Research
Food industry
Food service management
Not currently practicing
Other

Where do you practice?

Northland
Auckland
Waikato
Bay of Plenty
Hawke's Bay
Gisbourne
Taranaki
Manawatu-Whanganui
Wellington
Nelson/Marlborough/Tasman
West Coast
Canterbury
Otago
Southland
Outside of New Zealand
Not currently practicing

How many years of dietetic experience do you have?

< 2

2 – 5

5 – 10

10 – 20

More than 20 years

How often do you see patients for gastrointestinal related reasons?

Weekly

Fortnightly

Monthly

A few times a year

Never

How often do you assess patients with Crohn's disease?

Weekly

Fortnightly

Monthly

A few times a year

Never

Who are the majority of your CD patient group?

Paediatric cases

Adult cases

What are the main reasons you see patients with CD? (check as many as necessary)

Weight loss

Micronutrient deficiencies

Exclusion diets

Exclusive enteral nutrition

Food intolerance

Symptom control

Weight gain

Other

Have you heard of exclusive enteral nutrition (EEN) as a treatment for active CD?

Yes

No

Thinking about your patient group, do you think that EEN is an appropriate treatment for active CD?

Yes, for all my CD patients

Yes, for selected CD patients/patient groups

No, there is insufficient evidence to support its use with my patient group

No, it is not appropriate for my patient group

Not applicable

Other

For which patients/groups do you think EEN is an appropriate treatment?

What do you see as the downsides of EEN?

Patient compliance with treatment

Need for multidisciplinary approach

Cost

More time intensive for dietitian

Requires patient to have adequate social support

Other

Have you used EEN with any of your patients?

Yes

No

With approximately how many patients have you used EEN?

What are the main reasons that you have not used EEN? (tick as many as applicable)

Gastroenterologist does not offer it to their patients

Surgeon does not offer it to their patients

There is currently insufficient evidence to support its use with children

There is currently insufficient evidence to support its use with adults

We have limited dietetic resources to support people on EEN

We have limited experience with using EEN

Doctor manages any patients using EEN

Gastroenterology nurse manages any patients using EEN

I don't see many CD patients or Other

What PERCENTAGE of patients that you have treated do you estimate have successfully completed a course of EEN? (just enter the number and not the % sign)

Have you found it to be a successful treatment to induce disease remission?

Yes

Sometimes

No

If sometimes, in which patients have found it to be most successful?

What PERCENTAGE of your patients do you estimate have achieved disease remission with EEN? (just enter the number without the % sign)

Do you use supplementary enteral formula to maintain remission of CD?

Always

Sometimes

Rarely

Never

Not applicable

Please describe your standard maintenance regimen

Do you feel you have adequate knowledge and/or skills to manage patients that may be referred to you for EEN?

Yes

No

Is there anything you require to increase your confidence/ability to manage patients that may be referred to you for EEN?

Patient resources

Professional support

Professional development

More evidence of the effectiveness of EEN as a suitable treatment for children

More evidence of the effectiveness of EEN as a suitable treatment for adults

No, I feel confident in my ability to manage patients referred for EEN

Other

Do you have any general comments on this topic?

Appendix F

New Zealand Physician Survey Questions

Sex

Male

Female

Years of experience

Gastroenterology Registrar

Consultant < 5 years

Consultant 5 – 10 years

Consultant 10 – 20 years

Consultant > 20 years

In which areas do you currently practice? % of time (must add up to 100%)

Public hospital

Private hospital

Private practice

Academic practice

Where do you practice?

Northland

Auckland

Waikato

Bay of Plenty

Hawke's Bay

Gisbourne

Taranaki

Manawatu-Whanganui

Wellington

Nelson/Marlborough/Tasman

West Coast

Canterbury

Otago

Southland

Which patient groups to do manage?

Adults

Children

Adults and children

Have you heard of exclusive enteral nutrition (EEN) as a treatment for active CD?

Yes

No

Exclusive enteral nutrition (EEN) is the provision of all of a patient's nutritional requirements from a nutritional formula (eg. Fortisip). EEN can be managed in an inpatient or outpatient setting. EEN is often prescribed for a period of 6 – 8 weeks and may be used in conjunction with other medication. During the course of EEN the patient is not allowed any solid foods and is usually only allowed to drink additional water and black unsweetened tea or coffee.

Have you previously trained/worked in a unit where EEN is regularly used?

No, EEN was never used

EEN was rarely used

EEN was sometimes used

Yes, EEN was used regularly

Yes, EEN was used very regularly

Do you currently work in a unit where EEN is used regularly?

No, EEN is never used

EEN is rarely used

EEN is sometimes used

Yes, EEN is used regularly

Yes, EEN is used very regularly

Do you consider that EEN has a role as a therapy in the induction of remission for children with active CD?

No, never appropriate

Rarely appropriate

Sometimes appropriate

Yes, it is appropriate

Yes, it is very appropriate

Do you consider that EEN has a role as a therapy in the induction of remission for adults with active CD?

- No, never appropriate
- Rarely appropriate
- Sometimes appropriate
- Yes, it is appropriate
- Yes, it is very appropriate

Have you used EEN with any of your patients?

- Children - Yes/No
- Adults - Yes/No

If answered YES:

What do you see as the most important benefits of EEN?

- Steroid sparing
- Avoid immunosuppression
- Nutritional
- Improve growth in children
- Other:

What do you see as the downsides of EEN?

- Patient compliance with treatment
- Need for multidisciplinary approach (eg Dietitian and Nurses)
- Cost
- More time intensive
- Requires patient to have adequate social support
- Other

What are the main reasons that you have not used EEN? (tick as many as applicable)

- There is currently insufficient evidence to support its use with children
- There is currently insufficient evidence to support its use with adults
- Patients struggle to maintain treatment compliance
- Limited support from dietitian
- Limited support from nursing staff
- Limited experience with using EEN
- Limited social support for patients – maybe likert scale
- Cost
- Mechanisms of action unclear or other

What would you require to increase the likelihood of recommending EEN for your CD patients?

- Practice guidelines
- Access to a dietitian
- Access to a nurse specialist
- More evidence of efficacy in children
- More evidence of efficacy in adults
- Other

For those whose gastroenterologists that have used EEN in last 12 months.

In the last 12 months approximately how many of your patients have been treated with EEN?

For which Crohn's disease patient group/s do you usually consider EEN? Yes/No

- Any patient with CD
- Newly-diagnosed children
- Newly-diagnosed adults
- Existing diagnosis
- Patients who are nutritionally compromised
- Patients with mild disease
- Patients with moderate to severe disease
- Other

Would you consider EEN for children with CD who had disease in the following locations?
never/rarely/sometimes/often/always

- I manage children
- Colonic
- Ileocolonic
- Isolated upper gut disease
- Upper + lower gut disease
- Perianal disease

Would you consider EEN for adults with CD who had disease in the following locations?
never/rarely/sometimes/often/always

- I manage adults
- Colonic
- Ileocolonic
- Isolated upper gut disease
- Upper + lower gut disease
- Perianal disease

What duration of EEN do you usually recommend?

- < 6 weeks
- 6-8 weeks
- 9-10 weeks
- 10-12 weeks
- > 12 weeks

What percentage of patients that you have treated with EEN do you estimate have successfully completed a course of EEN?

Have you found it to be a successfully treatment to induce disease remission?

- Yes
- Sometimes
- No

If sometimes, in which patients have found it to be most successful?

What percentage of your patients do you estimate have achieved disease remission with EEN?

Are the following individuals routinely involved in administration of EEN in your practice?
never/rarely/sometimes/often/always

- Nurse
- Dietitian
- Psychologist
- GP
- Other

How do you judge the success of EEN?

- Clinical (e.g. improvement of symptoms)
- Nutritional (e.g. weight gain)
- Biochemical (e.g. ESR, platelets)
- Radiological
- Disease activity index
- Other: _____

Do your patients supplementary enteral formula after EEN to maintain remission of CD?

Unsure

Always

Sometimes

Rarely

Never

If yes, please describe your standard maintenance regimen?

Do you ROUTINELY use other therapy in conjunction with EEN?

Nil

5-ASA

Steroids

Azathioprine

6MP

Methotrexate

Infliximab

Other

For all gastroenterologists:

Additional comments: Please provide any additional comments or thoughts:

Thank you for your assistance in the completion of this questionnaire.