# University of Southampton

Faculty of Medicine

Human Health and Development

**The Nutritional State in Adults Crohn's Disease Remission**

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by

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**The Nutritional State and Quality of Disease Remission in Adults with Crohn's Disease**


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# Abstract

Crohn's Disease (CD) is characterised by a dysregulated intestinal immune response to an altered host microbiota, leading to intestinal inflammation, accumulated tissue injury, and constitutional malaise. Treatment aims to suppress the immune system and induce and maintain clinical and endoscopic remission. Effective medications are available to alter the disease course. However, the response is variable; the benefit may be short-lived, and poor quality of remission may remain due to residual disease activity and symptom burden. A third of remission cohorts report severe fatigue. Fatigue is poorly understood and has limited effective treatment options. Untreated Crohn's disease may alter the nutritional state through loss of appetite, food-related symptoms, increased loss through diarrhoea and alerted requirements through inflammation. The existing literature on the nutritional state of CD is from mixed populations with variable disease activity, often from a time before effective medical therapies.

The central hypothesis of the work described in this thesis was that adults with Crohn's disease in clinical remission may have an under-diagnosed and underappreciated poor nutritional state and those with a poor nutritional state will have a greater degree of disease-related fatigue and worse health-related quality of life.

The systematic review on micronutrient status in adults with CD in remission found nine eligible studies reporting low circulating levels of Vitamins B6, B12, C, D, and Magnesium and Selenium. There was insufficient information to determine the likely prevalence of micronutrient deficiency from blood biochemistry, nor to understand the causes and clinical consequences of the micronutrient blood tests. The first experimental chapter describes a time-limited trial of a nutritional intervention, Exclusive Enteral Nutrition, in 24 adults with CD in clinical and biochemical remission (faecal calprotectin <250µg/g). The nutritional and clinical state was assessed at 3 time-points, from the perspectives of intake (food diary analysis), micronutrient status and body composition (using anthropometry and bioelectrical impedance) before (assessment 1) and after (2) the intervention and on return to the free diet (3).

Assessment 1 identified inadequacies of micronutrient intake in habitual diet, abnormal micronutrient biochemistry, sarcopenia, reduced phase angle (bioelectrical impedance measurement, which when reduced has been correlated to poor outcomes and nutritional risk), and excess fatigue among the study subjects. At Assessment 2 (after the nutritional intervention), there were statistically significant improvements in intake, micronutrient biochemistry, Phase Angle, and a trend towards improving fatigue (SF36 vitality scores). The study suggested that nutritional issues are present in CD remission, and nutritional and fatigue may be amenable to nutritional intervention. This was followed by an observational cohort study in 200 patients with CD in remission to explore the relationship between nutritional state and the quality of remission (as marked by FACIT-F). Complete data was available for 194 subjects. Severe fatigue was evident in 26% of patients; FACIT-F scores correlated with SF36 vitality scores. Conventional screening tools identified very few patients at nutritional risk, whereas more detailed assessments revealed nutritional concerns. Food diary analysis showed that dietary inadequacies were common, most obviously in those with dietary impact factors and food-related symptoms, which were worse among those with severe fatigue. Excess adiposity (18%) and a lack of lean mass (14%) were evident, and the Standardised Phase Angle was reduced. Phase Angle was lower, and excess adiposity was higher in those with severe fatigue. Blood micronutrient biochemistry analysis was inconclusive without reference ranges but revealed a weak correlation between liposoluble antioxidant Vitamins and FACIT-F.

This thesis shows that adults with Crohn's disease may have a variable quality of remission, including excessive fatigue and altered nutritional state. CD remission can have a burden of residual symptoms, and these may be related to the patient's nutritional state and may be amenable to intervention. Future appropriately powered studies into nutritional interventions have the potential to demonstrate a novel avenue of treatment for improving CD remission.

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# Research Thesis: Declaration of Authorship

Print name: Martin James McDonnell

Title of thesis: The Nutritional State in Adults Crohn's Disease Remission

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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   McDonnell M, Sartain S, Westoby C, Katarachia V, Wootton SA, Cummings JRF. Micronutrient Status in Adult Crohn's Disease during Clinical Remission: A Systematic Review. Nutrients. 2023 Nov 14;15(22):4777. doi: 10.3390/nu15224777. PMID: 38004171; PMCID: PMC10674454.

Signature: ..... Date:.....

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# Definitions and Abbreviations

6TGN ..... 6-Thioguanine nucleotides

ACG..... American college of gastroenterology

AIEC ..... Adherent invasive Escherichia Coli

BDA ..... British dietetic association

BIA..... Bioelectrical Impedance Analysis

BMD ..... Bone mineral density

BMI..... Body mass index

BMR..... Basal Metabolic Rate

CD ..... Crohn's disease

CDAI..... Crohn's disease activity index

CDED..... Crohn's disease exclusion diet

CRP ..... C- -reactive Protein

CPET..... Cardiopulmonary exercise testing

DEXA ..... Dual X-ray absorptiometry

ECCO ..... European Crohn's and Colitis organisation

EEN ..... Exclusive Enteral Nutrition

ESPEN ..... European society for clinical nutrition and metabolism

FACIT-F..... Functional assessment of chronic illness therapy fatigue

FCP.....Faecal Calprotectin

FFMI ..... Fat-free mass index

FFQ..... Food frequency questionnaire

FIS ..... Fatigue impact score

FMI ..... Fat mass index

GC ..... Glucocorticoid

GLIM..... Global leadership initiative on malnutrition

Hb..... HaemoglobinH

HBI..... Harvey-Bradshaw index

HC..... Health Controls

HoloTC ..... Holotranscolbalamin

HPLC ..... High-performance liquid chromatography

HR-QoL..... Health-related quality of life

IBD ..... Inflammatory Bowel Disease

IL ..... Interleukin

IQR ..... Interquartile range

JAK..... Janus-associated kinase

MAB..... Monoclonal antibody

MUST..... Malnutrition universal screening tool

MIRT ..... Malnutrition inflammation risk tool

MRI..... Magnetic resonance imaging

NDNS ..... National Diet and Nutrition Survey

NG..... Nasogastric

NIHS..... Nestlé Institute of Health Sciences

NRI ..... Nutritional risk index

NRS 2002..... Nutritional risk screening tool 2002

PAL..... Physical activity level

PRO-2..... Patient-reported outcome 2 score

PROM ..... Patient-reported outcome measure

SaskIBD-NR ..... Saskatchewan IBD-nutrition risk tool

SCD..... Specific Carbohydrate Diet

SES-CD..... Simple endoscopic score for Crohn's disease

S1P..... selective sphingosine 1-phosphate

SOP ..... Standard Operating Procedures

TEE ..... Total energy expenditure

TNF..... Tumour necrosis factor

Treg ..... Intestinal Regulatory T-cells

UC ..... Ulcerative Colitis

UHS ..... University Hospital Southampton

# Chapter 1 Introduction to Thesis

Crohn's disease is lifelong and debilitating, and the nutritional state of individuals and the extent to which this determines the course or impact of the disease on the individuals is poorly understood. Treatments aim to induce remission from intestinal inflammation, but the extent to which individuals achieve good remission from the disease and its sequelae varies.

This thesis will explore the nutritional state in adults with Crohn's disease during remission. It starts with a background literature review on Crohn's disease and its treatments, nutritional treatments for CD, and how nutritional state might be assessed and altered in individuals with the disease. A systematic review of the micronutrient status of patients with CD in remission will follow this. Two experimental chapters then seek to address the lack of understanding from the literature around the nutritional state in CD remission. The first is an exploratory study that used a time-limited trial of nutritional therapy in a well-characterised group of adults with CD in clinical remission. This study, INTICO-1 assessed markers of nutritional state before and after a period of dietary control to understand nutritionally sensitive aspects of CD remission. The findings of this initial trial informed the design of the second trial, an observational prospective study in a larger cohort of patients with Crohn's disease in clinical remission to identify factors associated with differences in the quality of remission.

The central hypothesis addressed in this thesis is that patients with Crohn's disease in clinical remission may have a poor nutritional state that is unrecognised and untreated and that poorly nourished patients have a greater degree of disease-related fatigue and worse health-related quality of life.

# Review of the literature

## 1.1 Introduction

This chapter describes the background literature around Crohn's disease symptoms, pathophysiology, treatments, how CD remission is defined, and how the disease impacts the nutritional state.

Crohn's disease (CD) is a form of inflammatory bowel disease (IBD), characterised by intestinal inflammation and a disordered innate and adaptive intestinal immune system(1). The aetiology of Crohn's disease is unknown but believed to involve a combination of environmental and genetic factors, leading to a state characterised by intestinal barrier dysfunction, microbial dysbiosis, and immune dysregulation.

Following disease onset, which is most commonly in the second to third decade, Crohn's disease is lifelong. It is estimated to affect between 200,000 and 300,000 individuals in the UK and 1.6 million in Europe (2, 3). Those with CD may have periods of disease relapse (known as 'flares') and remission, and may experience significant morbidity from progressive tissue damage and systemic malaise. The systemic, non-specific features of this life-long condition may be debilitating.

Clinicians traditionally treat Crohn's disease with medications and surgery to address intestinal inflammation and its consequences. Crohn's disease therapies typically aim to induce and maintain a state where symptoms and signs of intestinal inflammation are reduced below a certain pre-defined threshold, known as "remission" (4). International consensus guidelines advocate targeting treatments against clinical activity scores alongside faecal biomarkers or endoscopic improvement (5, 6). The most recent 2021 consensus has added an improved health-related quality of life (HR-QOL) to these targets (7). An expanding range of effective medical therapies such as monoclonal antibodies or small molecule-mediated blockade of dysregulated immune pathways, are available. The medications appear most effective when used early in the disease course, and their expanding availability and usage seem to reduce the requirement for surgery. Despite being transformative for some, up to a third of individuals fail to respond to the prescribed advanced therapy. In those who do respond, subsequent

formation of anti-drug antibodies and changes in disease biology may lead to future disease relapse and drug-associated toxicity or contraindicating comorbidities may necessitate their cessation (8-10).

CD remission can be defined using clinical activity scores (of classical disease features), faecal inflammatory biomarkers, or endoscopic scoring systems. These scores have cuts-off to define disease remission. There may be a variability in the degree to which the symptoms or evidence of intestinal inflammation fall below that cut-off, which is the “depth” of remission. These cut-off scores and other factors may also determine another way CD remission may vary - the future risk of relapse, and the “durability” of remission. Published cohort studies from before the broad utility of biologics suggest that around a quarter of those with CD may enjoy prolonged remission without the need for medications, with others having relapsing-remitting disease and some others remaining chronically active and requiring multiple surgeries. In the era of disease-modifying therapies, the course of remission and risk of relapse may still vary between similarly treated individuals meaning that medication-induced remission may also vary in depth and durability. In addition to the variability in the quality of CD remission from the perspective of its depth and durability, its quality may differ from the perspective of persistent symptoms. With a treatment paradigm directed principally towards addressing endoscopic inflammation and its typical symptoms of diarrhoea and abdominal pain, symptoms that remain will be an unmet need for patients. One common symptom throughout the disease course is a determinant of an impaired HR-QOL and is cited among the leading concerns among individuals with CD is excessive fatigue(11, 12). The degree to which remission varies in its durability, depth, and persistent fatigue may thus be considered the quality of CD remission.

The degree of fatigue affecting individuals or populations has been quantified with a range of fatigue and disease-specific patient-reported outcome measures (PROMs). These studies use a variety of PROMs and cut-offs, but consistently report greater fatigue severity among those with CD than the general population and a higher percentage of individuals fulfilling the criteria for excessive fatigue, with an estimated prevalence of 21-68%(13-22). A range of exploratory analyses of these cohorts suggests that anxiety, depression, poor sleep, and increased disease activity are risk factors for fatigue in CD. There is a lack of information on fatigue and its relationship to nutritional status among individuals with CD (14, 23, 24).

It is generally recognised that Crohn's disease can affect the individual's nutritional status, particularly at the outset of the disease during symptomatic flares of increased disease activity and in those for whom surgical intervention is required(25). A poor nutritional state, as defined by a low body mass index (BMI) or recent weight loss, can increase the risk of developing disease and reduce the resilience to disease processes and the response to therapies (26). Dietary factors are implicated among the risk factors for CD onset and may influence the risk of subsequent flares (27, 28). In those with active Crohn's disease, features of the disease itself may modify nutritional status through an altered intake, reduced absorption, and changed demands. The literature surrounding nutritional assessment related to CD has been considered from the perspective of altered intake, availability, form, and function.

From the perspective of intake, disordered eating and food avoidance are well described among CD cohorts, with the most cited reasons being a wish to avoid symptoms or flares of disease (29-31). Appetite, which regulates nutritional intake in health, may also be altered in individuals with active CD (32, 33). Cross-sectional comparative studies of CD subjects report avoidance of nutrient-rich food groups and a consequent reduction in dietary micronutrient adequacy (34, 35).

The availability of micronutrients for the essential building blocks and metabolism of the body may be altered by disease processes. Micronutrient status may be particularly impaired in some individuals with CD, with the most commonly reported deficiencies being iron deficiency and Vitamin D, estimated from meta-analysis to affect 27% and 57.7%, respectively (36, 37). A range of cross-sectional studies of single or multiple micronutrients among individuals with varying degrees of clinical CD activity consistently find individuals with deficiencies in the populations studied. The pattern of inadequacies, how they interrelate and their consequences for disease outcome or quality of remission are not well described.

Overt alteration in CD-associated nutritional form was present in historical cohorts, in which weight loss was among the principal presenting features (38, 39). Improved diagnostic pathways and a population-wide increase in average BMI have coincided with a change in the typical nutritional features at CD diagnosis with a fall in the percentage who fulfil BMI criteria for undernutrition (40).

Alongside height, weight, and BMI, various measures can be used to assess for a relative reduction in an individual's muscle mass or lean mass, an alteration in form correlated to adverse outcomes in various disease states(41-43). In studies of CD subjects, muscle mass and fat-free mass have tended to be lower than matched controls, with a higher percentage of individuals outside modality-specific population norms (44, 45). In CD populations, a reduced lean mass has been correlated to a reduced response to biological medication and a higher risk of disease complications (46, 47).

There is evidence of a CD-associated functional impairment from cross-sectional surveys of IBD populations, which report a higher percentage of physically inactive individuals than the general population and limitations to activity arising due to CD features such as excessive fatigue (48, 49). More objective evidence of reduced physical capability for some individuals with CD has been demonstrated in small studies of muscle strength, muscle fatigue, and cardiopulmonary fitness(50-52).

The literature review suggests that Crohn's disease is characterised by disordered intestinal inflammation and associated tissue injury (1). Treatment aims to reduce intestinal inflammation and its typical symptoms to a state which is defined as disease remission. The remission attained may vary in quality, and many individuals have excessive symptoms of fatigue throughout the disease course. The specific aetiology of excessive fatigue in CD is not known. An impaired nutritional state, with alterations in dietary adequacy, micronutrient availability, and body composition, has been reported among individuals with CD, most typically in newly diagnosed cohorts or among individuals with disease flares, when nutritional therapies may be beneficial in reducing intestinal inflammation. The nutritional state during CD remission has not been well described, nor has the extent to which this may relate to the quality of disease remission.

## 1.2 Crohn's Disease and its Treatment

### 1.2.1 Introduction

This literature review will define Crohn's disease and describe its characteristic symptoms. It will then give an overview of the current understanding of pathophysiology and how different types of therapy are used to control the disease and its sequelae. The review will consider how remission is defined and may vary, specifically regarding risks of disease relapse and will consider the persistence of excessive fatigue. The review finishes by describing the relationship between CD and nutritional status from the perspective of intake, body composition, micronutrient availability, functional capacity, and nutritional therapies.

### 1.2.2 Crohn's Disease- background; definitions, symptoms, and treatment

### 1.2.3 Clinical Features of Crohn's Disease

The presenting clinical features of Crohn's disease are many and varied but generally arise due to inflammation and associated tissue injury in the gastrointestinal tract, principally the small and large intestines. The pattern of symptoms will vary depending on which part of the gastrointestinal tract is affected, and the degree and duration of its involvement. The features of CD that impact nutrition can vary significantly between and within individuals.

There are several sub-types of CD. The classical presentation of the commonest CD sub-type; inflammatory ileal Crohn's is a sub-acute or chronic presentation of diarrhoea, abdominal pain, and weight loss. Most individuals will have inflammatory disease behaviour and associated symptoms at diagnosis. However, approximately a third of patients in historic cohorts had a disease which perforates through or narrows the lumen of the GI tract at the time of diagnosis (known as penetrating or stricturing phenotype, respectively) (10). Such complications of CD can lead to presentation with peritonism or bowel obstruction, complications which may have specific nutritional sequelae such as sepsis-associated anabolism and temporary intestinal failure, respectively (11). These CD complications may lead to nutritional sequelae such as losing lean mass. Although inflammation in CD is principally centred in the GI tract, inflammation is systemic with constitutional malaise common and characteristic inflammatory lesions of the skin, liver, or bones known as extra-intestinal manifestations (EIMs) present in around 25% (53).

Excessive fatigue is a non-specific but common feature at diagnosis as supported by a 2019 IBD inception cohort (233 with CD) which listed this symptom (80.6%) and abdominal pain (affecting 80.4%) as the most typical symptoms in the weeks preceding diagnosis (15). Nutritional issues have also been described at the time of CD diagnosis, with weight loss in adults and growth failure in children recognised disease features (16, 17). Specific deficiencies in Iron, B12, and Vitamin D are among those most frequently recognised supportive laboratory indices in diagnostic workup (18).

The extent to which nutrition affects in Crohn's disease beyond initial presentation is not well described.

### 1.2.4 Pathophysiology of Crohn's disease

Crohn's is a disease of chronic inflammation of the intestines, the principal interface of the host immune system and the microbiome. The characterisation of the complex pathophysiology of IBD such as CD is a multi-dimensional and evolving picture drawn from analysis of the type and behaviour of cells present, genetics, animal models of intestinal inflammation, the composition and metabolic activity of the intestinal microbiome, and pharmacological blockade of aspect of the host immune response (1, 54-57). Such complexity and heterogeneity in the disease process mean that nutritional status can be altered in various ways.

Crohn's is typically centred on the ileum or colon, but the inflammation can affect any part of the GI tract, is classically discontinuous in the parts affected, and can involve the full thickness of the intestinal wall (19). The disease is classically thought of as relapsing and remitting, yetmany increasingly understand it to be a disease of progressive accumulated tissue injury, through persistent inflammation or a failure to resolve and recover from inflammation (24).

Inflammation describes the processes, characterised by blood vessel dilatation, enhanced leak from capillaries, and infiltration with phagocytosing neutrophils that comprise the reaction of the immune system to the threat to the integrity and homeostasis of a tissue or organism from microbial attack or invasion, damage from heat, radiation, or noxious injury (58). In the acute stage, this is the system by which microbial threat and damage are constrained, and debris is removed. In chronic inflammation such as that seen in CD, these processes are unresolved and persist and can lead to a deleterious effect on the structure of the tissue (59).

The proposed pathophysiology of CD has been extensively and expertly summarised elsewhere. Figure 1 is reproduced from Roda et al. summarising the aberrant pathways in the disease onset (60). The three critical aspects of CD pathogenesis, aberrant immune response, intestinal dysbiosis, and altered barrier function are described in further detail in the next section.

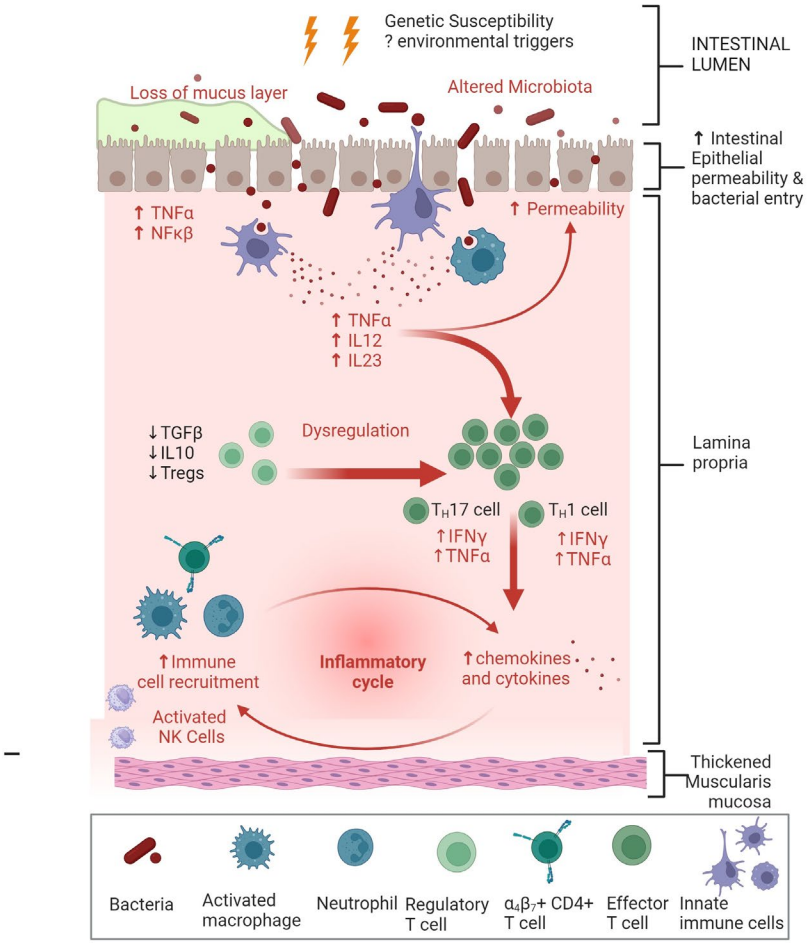


Figure 1: Pathophysiology of Crohn's Disease,

redrawn using Biorender© to summarise the proposed disease mechanisms in CD onset as summarised by Roda et al. (60) A loss of effective barrier function to an altered bacteria of the intestinal lumen (due to genetic susceptibility and a range of environmental insults) lead to activated antigen-presenting cell and macrophages and production of proinflammatory cytokines. Macrophages adopt an inflammatory phenotype and produce the pro-inflammatory cytokines Il-12 and Il23, which activate natural killer (NK cells) and orchestrate chronic inflammation. These proinflammatory cytokines, in turn lead to activation of naïve T-helper TH0 cells and production of TH1 and TH17 cells, with further increase in cytokines such as interferon-gamma (IFγ) and tumour necrosis factor-alpha (TNF-α), the perpetuation of activated adaptive intestinal immunity and the loss of intestinal immune homeostasis

#### 1.2.4.1 Abnormal Immune response

There is evidence of altered innate immune response in CD with an increased infiltration and activation of both macrophages and dendritic cells, monocular phagocytes which have dendrites that pass through tight junctions in the intestinal epithelium to contact luminal bacteria (61). In subjects with CD, these cells show a metabolic switch to a pro-inflammatory phenotype with increased expression of toll-like receptors (TLR), the evolutionarily preserved recognition molecules for bacterial components, and have demonstrated an exaggerated production of pro-inflammatory cytokines. Toll-like receptors are a family of 10 pattern recognition receptors, which reside on the surface of cells of the innate immune system, recognise archetypal signatures of microbes and cellular damage and serve as an antigen-presenting bridge between the innate and adaptive immune response (62). Macrophages in CD with an M1 (inflammatory polarised type) predominate, with fewer M2 macrophages, the subtype which can release cytokines that signal for a resolution of the inflammatory process; resolvins and prostaglandin E-2 (PGE2) to promote tissue restitution (63).

In addition to an altered innate intestinal immune response, chronic intestinal inflammation is characterised by CD4+ (T-helper) cell infiltration (64). In CD, the activated macrophages and dendritic cells produce cytokines such as Il-12, Il-18, and IL-23. They are thought to induce T-helper cell 1 (TH-1), T-helper cell 17 (TH-17), and innate lymphoid cell responses to perceived intracellular and extracellular pathogens, leading to increased production of gamma-interferon, TNF-alpha, IL-2 and IL-17 (56). This T-helper cell response maintains the pro-inflammatory stimulus to the resident macrophages, epithelial and endothelial cells a process of persistent unresolved and inappropriate immune activation (65). The complex, pleiotropic and integrative nature of the different components of the innate and adaptive intestinal immune system and its changes in IBD is shown by the variable response to pharmacological blockade of implicated cytokines (66).

Inadequate counter-regulatory signals are implicated in the loss of homeostatic balance in the intestinal immune system; mutations in the gene for IL-10, which promotes a tolerogenic environment through downregulation of TH-1 cytokines and the expression of stimulatory macrophage signals to bacterial components lead to an early onset paediatric CD and in knockout mice leads to spontaneous enterocolitis (67-69). The activity and presence of a

subset of T-helper cells induce tolerance of the adaptive immune system to luminal contents; the intestinal regulatory T-cells (T-reg) appear to be altered in subjects with CD (65). In CD there seems to be an alteration in the balance between the T-helper cells which recognise, perpetuate, and orchestrate inflammation or the response to a perceived pathogenic threat in the intestinal lumen, and those which recognise and induce tolerance to their specific luminal antigen.

#### 1.2.4.2 Intestinal Microbiome

The intestinal microbiome is the term used to describe the dynamic complex resident ecosystem of micro-organisms that have co-evolved with humans in the lower end of the gastrointestinal tract. The intestinal microbiome protects from harmful pathogens, forms a dynamic intestinal barrier, and regulates immunity, metabolism, and immunity.

The microbiome in CD is believed to exist in a state that is not optimal for the health of the host, a state known as dysbiosis. Changes thought to characterise CD dysbiosis at the bacterial phylum level are a reduction in the relative abundance of the Firmicutes with a relative increase in the Bacteroidetes and Enterobacteriaceae (70, 71). A decrease in diversity (the number of species or operational taxonomic units) also characterises the CD microbiota, as does a temporal instability in the relative composition of bacterial species (72). A reduced α-diversity and temporal instability appear related to Crohn's disease flares (73). Despite the breadth of species and OTUs, specific bacterial species have been proposed as key protective players against intestinal inflammation. One such species is faecalibacterium prausnitzii (F. Prau), a bacterium in the Clostridial cluster IV (also known as Clostridium leptum) genus, which forms the energy substrate and immune-tolerant signals to the intestinal mucosa, through the production of butyrate(74). F. Prau was shown to be present in lower numbers of the mucosal and faecal microbiota of a series of individuals with CD, particularly those with early disease recurrence following surgical resection (75). The relevance to the disease pathogenesis has since been supported by a meta-analysis which shows consistent literature supporting relatively lower abundance of F. Prau in the intestinal microbiota of CD when compared with HC (76).

In contrast to identifying the depletion of beneficial bacterial species in subjects with CD, specific organisms have been proposed as pathobionts for intestinal inflammation. One widely reported family of implicated bacteria are the Adherent invasive Escherichia Coli (AIEC) strains, isolated from the ileal mucosa at higher concentrations in individuals with CD than HC (77). AIEC demonstrates an ability to adhere to and avoid host mucosal defences through enhanced adhesion, activation of pattern recognition receptors on toll-like receptor 5 (TLR5, a subset of toll-like receptors that identify microbial signals and lead to persistent immune activation) together with invasion of and persistence in intestinal macrophages (78). These adherent pathogenic properties of AIEC are intriguingly enhanced in murine models in which putative dietary triggers of CD such as high fat, high sugar, or dietary emulsifiers are added, suggesting a potential microbiota-mediated means through which diet led to intestinal inflammation (79).

Metabolomic analysis of the stool of subjects with CD reveals common changes in the metabolic output of the intestinal microbiome. Consistent changes observed in subjects with CD include lower concentrations of short-chain fatty acids, increased amino acids, aryl carnitines in stool, and evidence of defective bile acid deconjugation (80). These broad metabolic snapshots of faecal contents suggest a net change in the CD microbiome at a functional level (81).

In addition to the extensive research into the bacterial component of the microbiome, there is an emerging appreciation and exploration of CD-specific changes to the fungal and viral components of the intestinal microbiome, referred to as the fungal microbiome and virome, respectively. The virome comprises prokaryotic viruses (principally bacteriophages) and eukaryotic viruses, with the former believed to contribute to the modification of the relative proportions of different bacterial strains through genome integration and modification of bacterial properties or bacteriophage-mediated lysis and the latter through colonisation and modification of the gut mucosa (82). The fungal microbiome has also been observed to differ in CD to HC and UC with specific compositional changes such as increased species diversity and an abundance of Candida species (83).

There is an emerging case that the observed characteristic changes in the microbiota in those with CD are causative for the subsequent dysregulated intestinal inflammation. Evidence supporting dysbiosis in disease onset comes from animal models and observational human

studies. Intestinal bacteria are generally required to develop IBD in susceptible murine models, and the dysregulated mucosal immune changes can be induced by the transfer of human CD faeces into murine hosts(84). In the human setting, the antibiotic metronidazole delays the recurrence of post-resection ileal CD lesions, and factors that can impact the microbiome composition such as reduced dietary fibre, early-life antibiotics, and formula rather than breastfeeding are also correlated to increased risk of subsequent CD (27, 85). A recent prospective study of factors preceding incident CD in 3483 unaffected first-degree relatives has shown characteristic microbiome signatures to precede a diagnosis of Crohn's by up to 5 years (86).

#### 1.2.4.3 Intestinal Barrier

The intestinal barrier is a term used to describe the mucus layer, cells, and their structure that forms the physical layer of protection between the host tissues, the ingested dietary components and the intestinal microbiome. In health, it comprises a bacteria-laden loose layer of intestinal mucous, a thickened adherent mucous layer overlying cells that absorb nutrients while maintaining the mechanical integrity of the epithelium and a homeostatic balance with the commensal components of the intestinal microbiome (87). The cellular makeup of the intestinal epithelium varies along the intestine. It comprises absorptive enterocytes, secretory Paneth and goblet cells (antimicrobial peptides and mucous, respectively), and cells involved in immune surveillance and antigen presentation.

There is evidence of an alteration in the structure and function of the intestinal barrier in individuals with Crohn's disease. An increase in intestinal barrier permeability has been shown to precede a clinical flare in CD (88). An increased permeability is also seen in unaffected first-degree relatives of those with familial CD, linked with common CD-predisposing mutations, and can precede the onset of the disease (89).

From a functional perspective, defects of the anti-microbial components of the intestinal barrier are linked to CD, Paneth cells which form the anti-microbial secretory component of the intestinal barrier, are altered in CD with evidence of reduced α-defensin production in ileal disease and β-defensin production in those with colonic Crohn's disease (90)

Perturbation of the mucus component of the intestinal barrier appears relevant to CD. Knockout of the MUC-2 gene that encodes the dominant secretory mucin of the colon leads to spontaneous colitis in murine models and microarray analysis of colonic biopsies of CD-affected individuals reveals its aberrant ileal expression (91, 92). Murine models also show that dietary emulsifier dissolution of intestinal mucous precedes the occurrence of dysbiosis and intestinal inflammation(93).

Underneath the intestinal mucus lies a structural barrier of epithelial cells, in which alterations have similarly been described in CD. The claudins, junctional proteins that span and regulate the paracellular space between epithelial cells have been altered in their composition and expression profile in the intestines of CD-affected individuals (90).

Collectively, this dissolution of the structure and function of the intestinal barrier leads to bacterial translocation beyond the intestinal lumen, as well as innate and adaptive immune activation, which can be seen systemically through signature serological changes to bacterial cell components (94).

#### 1.2.4.4 Pathophysiology of CD Summary

Crohn's disease pathophysiology can thus be summarised as a dysregulated immune response, altered intestinal barrier function, and an altered intestinal microbiome.

### 1.2.5 Treatments for Crohn's Disease and their potential to alter nutritional state

Current Crohn's disease consensus guidelines recommend that therapy should induce and maintain clinical and endoscopic disease remission, maintain quality of life and avoid disease complications (95). For a given individual with CD, the extent to which these things can be achieved is changing over time, as new medications are approved and evidence about how and when to best use existing medications evolves.

Treatments can involve one or a combination of medical therapies to address and control the dysregulated inflammatory process, surgery to resect or divert the intestinal contents away from inflamed, stenosed, or perforated sections of the bowel, and nutritional therapies as both primary and adjunctive treatment. Monoclonal antibody medications directed towards dysregulated immune pathways have been transformative for Crohn's disease, and as understanding and availability have increased their utility, the requirement for surgery has decreased.

#### 1.2.5.1 Medical Therapies for Crohn's Disease

Medical therapies for CD aim to downregulate the intestinal immune response through immunosuppression delivered systemically through oral, subcutaneous, or intravenous therapies. The mechanism of action and evidence for the most utilised therapies in CD is beyond the scope of this thesis, but the commonly used medications and their current role in an IBD treatment strategy and how they impact nutrition are documented below

The first effective medical treatment for inducing clinical remission in Crohn's disease was Glucocorticosteroids (GC) (96). They are believed to exert their actions upon binding to the cytosolic glucocorticoid receptor(cGR), which leads to the upregulation of anti-inflammatory cytokines and the downregulation of pro-inflammatory cytokines, with more rapid effects through the cGR-dissociated proteins (97). The utility of GC beyond this indication is limited by their acute and chronic multisystem adverse side effects, particularly increased susceptibility to infection and poor wound healing (97, 98). They have limited efficacy at inducing endoscopic remission, with higher mortality and rates of surgery seen in prolonged courses (99). Meta-analysis of GC demonstrates no benefit over placebo in maintaining remission (100).

The subsequent effective medical therapies to enter IBD therapeutics were steroid-sparing immunomodulating medications, Thiopurines and Methotrexate, which are used principally to maintain clinical remission following its induction with GC. Thiopurine actions are modulated by their metabolites, with the therapeutic effect via thioguanine nucleotides 6-TGNs. 6-TGNs are believed to modulate chronic intestinal inflammation through actions that include increased apoptosis of activated T-cells, decreased formation of T-cells and antigen-presenting cell complexes, and downregulation of pro-inflammatory cytokines in macrophages and epithelial cells (101, 102). Methotrexate actions are thought to be mediated through anti-metabolite mediated inhibition of dihydrofolate reductase with a resultant anti-proliferative effect on leukocytes, reduced formation of antibodies and inflammatory mediators, together with the accumulation of adenosine at the site of inflammation (103). As a primary treatment, the immunomodulators have only partial efficacy as a durable and tolerable long-term therapy (104). The long-term utility of immunomodulator medications is further limited by short and long-term side effects, which include an increased risk of cutaneous and haematological malignancies (105).

The management of IBD was transformed by the development of monoclonal antibody (mAB) therapies in the late 1990s. These medications are grown using cell lines to produce humanised antibodies to neutralise parts of the dysregulated immune response and are given intravenously or subcutaneously.

The first such medication was Infliximab, a humanised chimeric mouse-human antigen to soluble and membrane-bound forms of the pro-inflammatory cytokine Tumour necrosis alpha (TNF-α), which showed a dramatic response and remission to the first infusion of the medication and was subsequently shown to be able to induce and maintain Crohn's Disease Activity Index (CDAI)- defined remission (see Clinical Disease Scores for explanation of CDAI) with regular dosing when given in moderate and severely active CD (106, 107). A fully humanised sub-cutaneous anti-TNFα mAB Adalimumab demonstrated similar efficacy in inducing and maintaining remission (108). Subsequent studies reveal the added benefit of a dual Thiopurine immunomodulator therapy and estimate the rate of initial and sustained response to anti-TNFα mAB in CD at 56.8% at 26 weeks{Colombel, 2010 #1531}. While transformative for some, the response to anti TNFα may be short-lived, with the largest

prospective observational study suggesting a primary non-response to treatment in 23%, with 54% of subjects not in remission (defined by HBI, faecal calprotectin and CRP) at 1 year (9).

The pipeline of mAB targeting the intestinal immune response has continued to produce and test drugs with 3 families licensed in the UK in the past decade. The humanised mAB anti-integrin treatment Vedolizumab was licensed in 2014, having demonstrated superiority as an induction and maintenance therapy over a placebo for CDAI-defined remission (110). Vedolizumab's action is mediated through the blockade of α4β7 integrin-mediated trafficking of gut-selective lymphocytes. While its selectivity for the intestinal immune system and lower immunogenicity make Vedolizumab an important medication, the response to the medication is variable, with a meta-analysis of real-world efficacy estimating 30% steroid-free remission at 1 year (111).

Blockade of IL-12 and IL-23 receptors via their shared p40 subunit has become an alternative treatment option for CD. Ustekinumab, an antibody to the shared p40 subunit of the IL-12 and IL-23 receptor, has been licensed for use in UK Crohn's disease since 2017. Working via blockade of the Th1 and Th17 mediated pathways of CD pathogenesis, this medication demonstrated superiority over placebo at inducing and maintaining clinical and endoscopic remission both as a primary biologic therapy and in those who have lost response to or failed to respond to anti-TNF therapy. Real-world observational series Ustekinumab in treatment-resistant CD suggests a clinical response rate of 52% and a long-term remission rate of 42% (112). Most recently, the pipeline of medical therapies continues to offer promising therapies, with antibodies and antagonists to the p19 subunit of IL23, small molecule blockade of Janus-associated kinase-1 (JAK1), and selective sphingosine 1-phosphate (S1P) receptor modulators all demonstrating efficacy over placebo for response and or remission (113-116).

#### 1.2.5.2 Surgery for Crohn's disease

Surgery for CD can impact nutrition both acutely through a catabolic response to the surgery itself and chronically through an alteration in the structure and function of the GI tract. The earliest description of CD as a clinicopathological entity was in a series of subjects who had undergone laparotomy and resection of stricturing or penetrating complications of the disease (117). Before effective medical treatments, surgery was the only way to manage severe

disease and served as a way in which many were diagnosed. It remains indicated for resection of segments obstructed with stricturing disease, medication-refractory disease, drainage, and resection of areas of penetrating disease and their complications, particularly in the perianal region. It can offer an effective alternative to biological therapy for long-term remission in isolated ileal disease(118).

With an improvement in diagnostic modalities and medical therapies, the requirement for surgery at or shortly after CD diagnosis has fallen, and surgery tends to be performed on an elective rather than an emergency basis (119). The longer-term requirement for surgery in CD remains high but falling; a 2013 meta-analysis estimated the 10-year risk to be between 37.7%-57.7%, with a significantly lower risk in more recent studies (120). Resection surgery for Crohn's disease aims to induce remission. It may remove all detectable and symptomatic disease lesions but is not typically curative, with 1-year clinical recurrence in 30% and up to 70% requiring further surgery (121, 122).

Any given cohort of CD subjects in remission may contain some who have undergone one or more surgeries with a varied potential for consequent reduced functional absorptive tissues and may have a variable degree of pre-symptomatic disease recurrence.

#### 1.2.5.3 Summary – Medical and Surgical therapies for Crohn's disease

Therapies for CD have evolved from early surgery with partial downregulation of inflammatory process and incomplete healing from GC to targeted antibody blockade of the dysregulated humoral immune response with biological and most recently small molecule mediations. While the response to medicines can be variable, the resolution of the inflammatory lesions and symptoms can be dramatic and nearly complete, particularly for those given the medications early in the disease journey. Effective medical therapies are now used at the outset of the disease, but the requirement for surgery over the longer term remains high.

Nutritional assessment of an individual with CD must, therefore, consider the disease phenotype, previous surgery, current and prior medications, and the degree of response to the

other medical therapies. These factors, past and present, may affect the degree to which the form and function of the intestinal tract are impaired.

### 1.2.6 Nutritional interventions as a treatment for Crohn's disease

Recognising when and how nutritional interventions are used in CD, the effect of those interventions, and their putative mechanisms can help reveal nutritional processes that are likely to be modifiable for intervention. This section will propose the principles and mechanisms underpinning nutritional interventions used to treat the disease.

Nutritional interventions in adults with CD, such as oral nutritional or micronutrient supplementation, may be prescribed to mitigate the effects of the disease on the individuals and replace inadequacies of intake as a result of altered requirements. Interventions may also directly target the disease process, intestinal inflammation and altered barrier functions.

Nutritional interventions, when used as a primary treatment, work on the principle of providing additional energy or nutrients to the individual, removing or reducing harmful elements from their intake, or a combination of both. For example, enteral nutrition (EEN), when all food is replaced with a formula taken either orally or via a nasogastric (NG) tube, provides both energy and nutrients whilst also avoiding components of the diet that may obstruct or worsen the metabolic state of the gut. Nutritional therapies may also be used in CD as an adjunct to medical therapies to maintain or supplement dietary intake in those at risk of malnutrition or before or after surgery.

#### 1.2.6.1 Exclusive Enteral Nutrition for Crohn's Disease

The first reports of EEN use for CD were in the 1970s, with 'elemental feeding', so named due to single amino acids rather than whole proteins, used in a perioperative setting. Elemental EEN aims to meet nutritional requirements before surgery while avoiding any pain, damage, or obstruction of the antigens or matter of habitual diet and produce a low faecal bulk (123). Subsequent studies of elemental EEN demonstrated a reduction of clinical and biochemical features in active CD, and equivalence to corticosteroids in a comparative controlled trial of CD induction therapies (124, 125). A 1994 series reported the induction of biochemical, clinical, and endoscopic remission in ileal CD with whole protein 'polymeric' EEN (126). EEN may also

be provided from formulae containing oligopeptides, known as “semi-elemental”, but the palatable whole polymeric protein formulae remain the most widely used with elemental EEN an option for those with intolerance or allergies to polymeric or constituents.

EEN as a primary CD therapy provided via an NG tube or oral feed is estimated to induce clinical and endoscopic remission in 75-80% of paediatric CD cases {Ashton, 2019 #1241}. There is a smaller body of literature on EEN in adult CD; a 2018 Cochrane analysis summarised 27 studies, concluding a similar efficacy to corticosteroids for inducing clinical remission, with no difference in efficacy seen between polymeric, elemental, and semi-elemental feeds (127). EEN is recommended for 4-6 weeks as first-line therapy to induce remission in paediatric CD in European consensus guidelines in the adult setting is recommended in UK guidelines as an option in subjects wishing to avoid corticosteroids (128, 129). EEN has demonstrated beneficial effects such as the restoration of paediatric growth trajectories, induction of clinical and endoscopic remission, and the restoration of barrier function (130).

The putative mechanisms through which enteral formulae induce clinical remission have been explored through observation and in-depth characterisation of treated subjects and their microbiome alongside animal and cell culture models (131). EEN restores intestinal immune homeostasis and barrier function (132, 133). This seems to work in part through the provision of elements within the formula to modulate the dysregulated cellular inflammation which characterises IBD and results in restoring intestinal barrier function (134, 135). The observed changes in intestinal bacteria following EEN suggest that the changes may, in part, be due to a reduction of the pathogenic elements of the microbiome (136). The microbial-host cross-talk, which provides nutrients and regulates intestinal immunity, appears from metagenome analysis to be influenced by EEN (137). Periods of EEN have been observed to correct one or more pre-existing micronutrient deficiencies in the circulating pool, which has been proposed as one of the means through which systemic benefits such as restoration of growth trajectories and lean tissue deficits occur (138).

The benefits of EEN as a treatment in active CD may be in providing additional therapeutic elements in the nutritional formula. EEN, however, also involves the complete removal of the habitual diet and any thus constituent elements which are potentially harmful to those with CD. The hypothesis that this removal of pathogenic components of diet is a significant contributor

to its benefits is supported by the ineffectiveness of part usual diet, part enteral nutrition so-called “partial Enteral Nutrition” (pEN) as an induction agent for active CD(139). Attempts to remove the harmful elements of habitual diet, either with or without pEN are described below.

#### 1.2.6.2 Diets for Crohn’s Disease

A diet promoted as a means to control Crohn’s disease was the Specific Carbohydrate Diet (SCD), after a publication of the experiences of Elaine Gottschall (140). This diet, developed initially to manage coeliac disease, excludes complex starchy carbohydrates, lactose-containing dairy processed foods, grains, processed meat and refined sugars and encourages the intake of easily digestible carbohydrates, and probiotic-rich foods (141). This diet was subsequently shown in small observational studies in the paediatric setting to reduce clinical and endoscopic activity (142, 143). The extent to which the proscriptive nature of the diet is required has been questioned by SCD comparison to the Mediterranean diet with a recent prospective trial which found equivalent efficacy in reducing clinical disease activity and faecal inflammatory markers among some subjects with active CD (144).

Several diets have been recently proposed as potential alternatives to EEN to induce or maintain CD remission. Observational data have linked the Mediterranean diet with a reduced onset of Crohn’s, a reduced risk of flare and an improved health-related quality of life (145, 146). The Crohn’s Disease Elimination Diet (CDED) has elements like the SCD and attempts to prescribe the exclusion of food items implicated in animal models of intestinal inflammation. The CDED avoids or reduces the consumption of foods containing elements linked in murine or cell line studies to inducing inflammation, dysbiosis or degradation of the intestinal barrier, such as animal or dairy fats, wheat, red or processed meat, emulsifiers, artificial sweeteners, carrageenans, and sulphites (147, 148). The CDED diet was shown in a comparative unblinded randomised controlled trial (RCT) to have similar effects to EEN on the microbiome, faecal inflammatory markers with similar remission and response rates at 6 weeks, with better tolerance and superiority to partial enteral nutrition and free diet arms in maintaining remission to week 12 (149). The CDED diet has since shown equivalent efficacy to partial enteral nutrition to induce clinical remission as monotherapy for active CD (150).

The dietary approach instead of EEN has been attempted with low fibre, high protein, and micronutrient tablet supplemented “CD-Treat” diet, which elicited similar changes in the faecal microbiome and metabolome in healthy controls, improved a murine model of colitis, and showed some beneficial changes in an open-label study of active paediatric CD (151).

These diets are similar to previous published, such as the specific carbohydrate diet, which permits fresh fruit and legumes, yet excludes starchy vegetables.

#### 1.2.6.3 Summary- nutritional therapies as a disease modifier

Interventions to control nutritional intake by excluding habitual foods and replacing this with a liquid formula appear to be an effective therapy for inducing clinical and endoscopic CD remission. EEN has been observed to cause changes in the composition and metabolic activity of the intestinal microbiome, restore barrier function and institute mucosal healing. Systemic improvements beyond the bowel, such as improved lean mass and growth in paediatric EEN settings, suggest whole-body nutritional restitution supported by a change in serum micronutrient levels post-EEN. There is an emerging case for elements of habitual diet to be harmful in CD, and exclusion diets appear to replicate some of EEN’s microbiome and clinical changes. The disease process and some of its sequelae in active disease appear to be related to and modified by nutrition.

### 1.2.7 Summary of Crohn's Disease

Crohn's disease is a disease of intestinal inflammation and accumulated tissue injury. There is evidence of a loss of homeostasis between the intestinal immune system and microbiome with chronic dysregulated intestinal inflammation, reduced barrier function, and changes in bacterial composition and metabolites. Onset is typically in late adolescence or early adulthood and the disease is lifelong thereafter. It is traditionally considered a disease of the industrialised world, and its onset is believed to be due to a combination of one or more environmental triggers and genetic susceptibility loci. The extent and location of disease lesions and their behaviour can have distinct phases and subtypes that account for variability within and between individuals regarding the disease's consequences.

CD management was historically with courses of corticosteroids to induce clinical remission and surgical management of complications. With the emergence of effective medical therapies, particularly advanced therapies such as biologics, the treatment recommended at the early stages of the disease is to induce and maintain clinical and endoscopic remission with medications. Biological therapies have been transformative for many patients, but primary non-response and loss of response through immunogenicity or intolerance through drug toxicity mean that treatment failure is common. The requirement for surgery has fallen but may still be necessary for those who do not respond to medical therapies, and surgery may be the only effective management for stricturing or penetrating disease.

Modifying nutritional intake through EEN effectively induces remission in active CD. The most significant evidence base for EEN in CD is in the paediatric setting, where it is used more frequently as a primary therapy. Key features of the disease, such as altered barrier function and dysbiosis change in response to EEN, suggest that nutritional intake either through the provision of harmful elements or through inadequacy, is relevant to the disease process. Systemic changes in paediatric CD suggest whole-body nutritional deficits in CD and that these are modifiable to dietary intervention by providing additional nutrients and substrate or excluding harmful ones.

## 1.3 Remission in adult Crohn's disease

Remission refers to the diminution or reduction in the severity and symptoms (152). In assessing and managing CD, remission can be defined in clinical, radiological, endoscopic, or biochemical terms. Consensus guidelines typically recommend directing medical therapies toward achieving and maintaining one or more of these remission forms. Endoscopic and histological remission have become increasingly incorporated into guidelines and trial outcomes (7, 153).

The following section describes how the commonly used scoring systems define clinical, endoscopic, and biochemical disease remission in current practice. This section then describes how remission may or may not persist over time and the consequences of that persistence for tissue injury, a term known as "disease trajectory".

### 1.3.1 Clinical Disease Scores

The term "remission" in IBD consensus guidelines is typically defined by the **Crohn's Disease Activity Index (CDAI),** attaining this CDAI-defined remission was the primary endpoint in the licensing trials of recently approved biological medications.

The CDAI was reported in the 1970s as a means to quantify the severity of Crohn's disease symptoms and signs that were felt to be common and relevant to subjects with CD and used to standardise the assessment and recognition of the clinical disease features, and set out a definition for quiescent disease and severe disease (154). The CDAI takes a 7-day diary of stool frequency, the severity of abdominal pain, and general well-being alongside the single time-point assessment of the presence of extra-intestinal manifestations, pyrexia, weight loss, anti-diarrhoeal consumption, palpable abdominal mass, and haematocrit. Each component is weighted and contributes to an activity index score. CDAI remains widely used with a score of ≤150 defining remission and a fall of ≥100 post-therapy defining response for clinical drug trials and international consensus guidelines (4). Conversely, clinical relapse for CD drug trials is defined as a total CDAI score >150 and an increase of ≥70 during the period of observation (155). The individual with a CDAI score of <150 may still have some degree of diarrhoea,

abdominal pain, or fatigue but below the maximum permitted threshold of symptoms to define remission.

The **Harvey-Bradshaw Index (HBI)** is a simplified clinical disease that does not require a 7-day stool and symptoms diary. The HBI was developed for ease of day-to-day utility, with its definitions of remission, active or severe disease, clinical relapse, and response(156). The HBI includes a score of 1 for each liquid bowel motion, assigns a grade of 0-4 for abdominal pain, 0-4 for general well-being, and abdominal exam with additions for common disease complications or external intestinal manifestations of inflammation for the preceding day. A score of <5 defines remission, 5-7 mild disease activity, 8-16 moderate, and >16 severe disease activity. As with the CDAI, an individual with HBI-defined remission may have reduced reported well-being and some degree of abdominal pain or diarrhoea. The level of persistent symptoms within a CD remission population may be above that of a healthy matched population with a persistent burden of symptoms.

The HBI is widely used in clinical practice to assess response to disease therapy and monitor for disease flare. It is widely used as a pragmatic assessment of clinical remission in observational or 'real-world' studies (157). CDAI and HBI may be used to define a clinically significant increase in disease symptoms, which may be defined as a 'clinical relapse' or 'flare' of Crohn's disease, or if elevated, they may define active and severely active clinical disease in individuals (6).

A further simplified assessment of CD clinical activity used in clinical trials is offered by the patient-reported outcome 2 (**PRO-2**). PRO-2 comprises a score for the degree of abdominal pain and the number of stools. Targeting treatment against these clinical measures alongside an objective grading of endoscopic disease activity and serum or stool inflammatory markers is advocated in international consensus statements on treating to targets and this combination is being used for current clinical drug trials (95). PRO-2 had clear criteria for symptoms associated with intestinal inflammation but did not assign a score for general well-being so it will fail to capture the variability of symptoms for the systemic features of CD.

### 1.3.2 Endoscopic Disease scores

CD remission, active disease, and response to treatment can also be defined by scoring symptoms to standardise the assessment of the severity of endoscopic changes. An endoscopic response to treatment is increasingly used as a target for CD therapy. It is a primary outcome alongside CDAI response for large ongoing phase III trials for CD medications (158). The simplified endoscopic score (SES-CD), the most widely used endoscopic score, grades the extent, ulceration, depth, and surface area of endoscopically involved bowel segments and generates a composite score, with definitions for clinically relevant response and remission (159).

Primary endoscopic outcomes in these more recent CD trials aim at treatment towards 'mucosal healing', a term that describes the endoscopic resolution of inflammation. There is emerging evidence that attaining mucosal healing early in the disease journey predicts longer-term clinical remission and avoidance of disease complications (160, 161). While a more objective measure of the underlying tissue injury that characterises recognised key features of the disease process, therapy directed solely at the visible endoscopic changes may risk failing to recognise or treat constitutional and debilitating extra-intestinal features of CD that occur during periods of active disease and remain after the resolution of these issues.

While clinical trials typically use one or more of these remission definitions at 12 weeks and 1 year to demonstrate the efficacy of medical therapies, Crohn's disease is a lifelong illness. There is a need to understand with or without these treatments whether the variability in the attainment of these states of remission, the variable persistence of these state time and the consequences of this variability for tissue injury and disease complications. The following section will describe these features and the term "disease trajectory" and how this has been observed and classified in cohorts of adults with CD in different treatment eras.

### 1.3.3 How frequently is disease remission attained and maintained in CD cohorts- "Disease Trajectory"

Prospective CD cohort studies from Norway (IBSEN cohort), Sweden and Denmark attempted to classify individuals by whether and for how long individuals attain clinical remission in 5-10

years after diagnosis. These studies, in part from an era before the widespread availability and use of biological medications, are consistent in identifying a subset (14-32%) of individuals whose disease runs an indolent course that does not have flares or disease complications, and another group of individuals with chronic relapsing or persistently active disease (162-164).

More recently, again from the IBSEN cohort in an era with the increased availability of biological medications, the disease trajectory of 432 subjects over 10 years was classified into six phenotypes grouped according to their clinical activity scores at three monthly intervals: 'Active to remission' (25.5%), 'Remission to active' (3.2%), 'Chronic continuous' (5.8%), 'Moderate-severe chronic intermittent' (27.8%), 'Mild chronic intermittent' (9.5%), and 'Quiescent' (28.2%) (165) . Notably, 90% of those in the quiescent group were never treated with an immunomodulator or biological therapy.

In the UK, disease activity and progression over time have been explored in a large inception CD cohort in Lothian, Scotland, which first identified faecal Calprotectin (FCP) in follow-up as predictive of disease complications (166). Using FCP as a disease activity marker, this cohort's analysis identified three clusters among incident CD cases between 2005 and 2017: cluster 1- a rapid resolution of inflammation, cluster 2- persistent inflammation and cluster 3 - persistent resolved inflammation after 1 year. The findings are consistent with IBSEN in supporting biological therapies as disease trajectory modifiers, as they show a higher biological use in group 1 compared with groups 2 and 3 (167). Other published work from the same Lothian cohort shows that the later study subjects had higher biological use and a lower requirement for surgery in the years following diagnosis (168).

These cohorts were variable in their definitions of remission and flares but consistent in showing inflammatory disease features to be most marked at CD onset, with a variable course of remission and relapse thereafter. Therefore, an episode of remission may start a prolonged phase of quiescent disease or a window between further episodes of detectable disease burden and progressive tissue injury.

#### 1.3.3.1 Attainment of disease remission in CD cohorts and biological medication and variability in durability (risk of relapse) and depth of remission

Which course an individual with CD will follow appears to depend on medication strategy and timely use. A recent large prospective study demonstrated that "top-down" treatment with dual anti-TNFα and immunomodulator therapy early in the disease course was more effective than a step-up approach stratified by either biomarker (T-cell transcriptome) or endoscopic inflammation at maintaining steroid-free remission, reducing surgery and hospital admissions at 1 year (169). The broader use of biologics earlier in the disease course, when the rate of response seems to be greatest, has led to a simultaneous increase in the time to first CD surgery (170, 171).

The durability of CD biological medication-induced remission is not guaranteed long-term and is estimated at 50% in systematic reviews (172). Around a third of initial responders to anti-TNFα are estimated to lose response at 12 months and two-thirds at 3 years (9, 173). Some individuals treated with biologicals who then stop are at risk of subsequent relapse, while others will have a medication-free long-term clinical remission.

### 1.3.4 The variability in the quality of CD remission- depth and durability

The resolution of the endoscopic and clinical features in response to therapy can vary, and that variability may determine how long the state of remission may persist. From the perspective of endoscopic appearances, individuals defined as being "in remission" may vary in the extent to which the inflammation is resolved following treatment. Full resolution of endoscopic ulceration or inflammation has been defined in clinical trials as "mucosal healing", and this, when attained early in clinical trials, has been shown in meta-analysis to be correlated with longer-term clinical remission, endoscopic remission, and reduced risk of CD-related surgery (161). This desirable feature of a prolonged clinical or endoscopic remission is thus considered "durable".

Alongside the concept of an individual with CD having a remission of greater durability or persistence is "deep remission". This term was introduced to define individuals who, at a given time, have had a diminution of the disease process, which means that they fulfil both clinical

and endoscopic definitions of remission. Attaining this "deep" remission (clinical and endoscopic resolution) was shown in prospective clinical trials to be associated with a reduced risk of future CD hospitalisation and surgery disease-related disability (174, 175).

Whether or not deep remission determines the durability of that remission or whether they are both markers of individuals whose disease process has reduced is unclear. The following section offers a proposed course of disease trajectory for CD that considers the current treatment paradigm in the context of existing published cohorts.

#### 1.3.4.1 Proposed disease course phenotypes by attainment and maintenance of remission.

The studies described above support the benefits of medication in attaining a remission that resolves the endoscopic changes and clinical features. However, there is a need to think of the trajectory of Crohn's disease, which can be considered using cohort studies from before the era when these medications were widely available.

Individuals with CD (not treated with biological medications, if indicated) can conceptually be grouped by disease activity patterns over time into three broad disease phenotypes, drawn as three groups, labelled groups 1, 2 and 3 in the top half of Figure 2. The graphs plot clinical inflammatory disease activity markers, such as CDAI, over time, with the red line denoting cumulative tissue injury. In a cohort without biological therapies, graph 1 represents an individual with an initial flare of active disease, then stable remission, graph 2 an individual with chronic relapsing disease, and graph 3 represents an individual with chronic continuously active disease, with those in groups 2 and 3 showing a greater degree of accumulated intestinal tissue injury (red line).

Those in group 1 will be at low risk of clinical relapse (when the disease score goes above the remission threshold); in that sense, their remission will be a **"durable"** remission. The further below the threshold (shown in the dashed line) that defines remission an individual is, the lower the ongoing degree of inflammation or associated symptoms. This would mean a lower burden of persistent inflammation and typical symptoms; an individual with a CDAI score of 20 will have a lower symptom burden than one with a score of 149, but both may be defined as "in

remission". Regarding disease activity, remission is **"deeper"** in someone with a CDAI of 20 than 149.

Graph 2 shows the disease course of an individual's "chronic relapsing" CD. When the individual following this disease course has a CDAI or disease marker falling below the threshold for remission, they will, compared to those following graph 1, have a remission that is less **"durable"** to future flares. There may also be a period before the subsequent relapse when those in group 2 will have a greater persisting degree of inflammation and its associated symptoms, and their remission will, in this sense, be less **"deep".**

Groups 4, 5, and 6 in the lower half of the diagram show three additional proposed broad disease course categories which may be present in CD cohorts with widespread use and timely availability of biological medications or small molecules. Group 4 attains remission more quickly than group 1 due to timely and effective biologics use and, therefore, has a lower degree of intestinal injury. Those following this disease course will have a durable remission (low risk of flare) and **deep** (low persistence of inflammation and associated typical CD symptoms). Group 5 shows a proposed disease course of an individual treated with and who responds to a biological medication that subsequently loses its effect on the disease with a consequent flare of inflammation or clinical features. This may be due to immunogenicity, drug toxicity, or changing disease biology. Following the resolution of the first flare, when the marker of disease activity falls to that of "remission", the individual in the group will have a remission which is at a lower risk of flare (more durable) than those of group 5 at the same time point. Moreover, this remission may have lower inflammation or clinical disease activity (a deeper remission).

An individual's CD remission can, therefore, vary concerning the degree to which the inflammation and classical features are below the threshold that defines active disease ("depth") and the risk for future relapse ("durability"). There may be other symptoms that are persistent throughout the disease course. In this thesis, it is proposed that the degree to which these are present will, in addition to depth and stability, determine the **"quality"** of an individual's CD remission. Common and debilitating CD symptoms that persist into CD remission cohorts are described in the next section

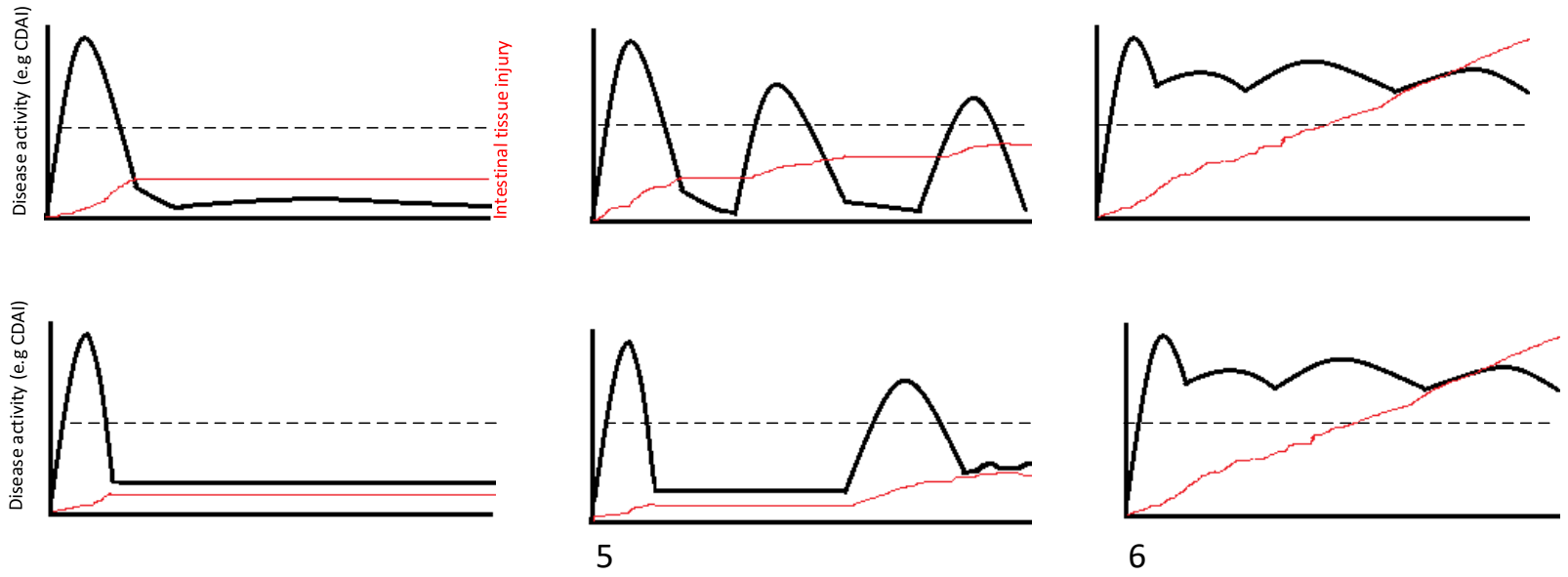


Figure 2 Proposed disease courses of clinical/inflammatory activity over years of CD from diagnosis.

Thick black line represents disease activity, dashed line represents cut-off for remission (below), and the red line represents cumulative tissue injury. Group 1: Stable remission (pre-biologics), Group 2: Chronic relapsing (poor quality remission), Group 3 chronic active (no remission) with progressive tissue injury. Group 4 (stable remission on biologics), Group 5 initial remission with biologics with delayed relapse following the loss of response to medication, and Group 6 (biologics refractory disease).

### 1.3.5 Which features may persist among individuals with CD remission and how are they considered in consensus guidelines?

For individuals with CD fulfilling these definitions of clinical or endoscopic remission, residual features that characterise the disease are not consistently recognised and considered by consensus IBD guidelines. Quiescent disease, in terms of clinical activity or endoscopic activity scores, does not equate to the absence of symptoms, with 60% of "quiescent" cohorts reporting chronic pain (176). Excess fatigue is a leading presenting feature in adult CD, which can remain present in up to 50% of patients in remission and determine health-related quality of life (HRQOL). Still, the specific management of this, alongside reducing clinical and endoscopic remission, is variable (177).

The European Crohn's Consensus Organization (ECCO) guidelines on medical therapies for CD do not address therapeutic targets beyond medications to induce and maintain CDAI or endoscopy-defined remission and do not advocate for assessment or treatment of fatigue (5). An individual successfully treated according to these guidelines may, by the nature of the thresholds used, have some persistent degree of symptoms, such as abdominal pain or reduced reported sense of general well-being. A guideline on assessing and treating anaemia exists, but lacks recognition of the CD features that may remain in those in remission (178).

After advocating for medication directed toward objective evidence of inflammation, the British Society of Gastroenterology (BSG) IBD consensus guidelines list fatigue and pain, skeletal health, and anaemia as associated disease features, advocating for their monitoring and treatment. No specific recommendations other than addressing anaemia and optimising medications for remission are offered for fatigue, and no specific ways of assessing fatigue are recommended (129).

The American College of Gastroenterology (ACG) CD guidelines have a similar focus on attaining and maintaining endoscopic clinical remission with medication or surgery, without a mention of assessment for or treatment of features that may be present in those in remission (179).

Excessive fatigue is a leading presenting feature of CD (177). It may, in part, contribute to a CDAI or HBI score with a symptom associated with but not specific to intestinal inflammation disorders. Therapies directed solely towards endoscopy or PRO-2 may fail to recognise this common feature of CD and may fail to address those needs in a population judged to be in remission.

#### 1.3.5.1 Fatigue is a persistent feature of CD

Fatigue is synonymous with exhaustion, tiredness, or reduced capacity for work. When referring to a muscle, “fatigue” describes the state arrived at by prolonged and intense contractions (180). When used to describe the feeling experienced by an individual, fatigue has been defined as an “overwhelming sense of tiredness, lack of energy and a feeling of exhaustion associated with impaired physical and/or cognitive functioning”. It is a recognised feature of both CD and UC (181). Excessive fatigue is consistently listed among the top concerns of individuals with IBD and is associated with impaired health-related quality of life (11, 12)

Patient Report Outcome Measures (PROM) questionnaires that are specific to capturing fatigue symptoms or that contain a fatigue or energy component have been designed to quantify fatigue and, if the score is outside a reference range, to identify whether an individual has excessive fatigue. Using these measures in populations with CD and comparing the results against matched controls or reference ranges allows researchers to compare whether the average degree of fatigue is more significant in that population and to establish the prevalence of individuals with excessive fatigue. Such comparisons in a cross-sectional study of newly diagnosed CD subjects reported excess fatigue in 48% compared with 7% of a matched control population (13). The increased degree of fatigue among those with CD when compared with a matched HC population has been replicated using the Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F) score and the Fatigue Impact Score (FIS), both fatigue-specific PROMs, which assign a score to the severity of characteristic fatigue symptoms and reduce physical capacity (14, 22). The FIS comparison study also found a greater degree of objectively dynamometer-measured quadriceps muscle fatigue among CD subjects, particularly those with more significant fatigue symptoms (50).

A longitudinal analysis of the Manitoba IBD cohort's clinical disease activity and fatigue symptoms found fatigue to worsen over the 2 years of study follow-up among recently diagnosed subjects (15). This persistence of fatigue beyond initial diagnosis and in those deemed stable on treatment was again replicated with the IBD partners study of 2429 subjects with IBD (1605 with CD), which found 65.8% to have a FACIT-F at a level that met the author’s definition for fatigue, with CD subjects on average more fatigued than those with UC (16).

Cross-sectional PROM studies of CD or IBD cohorts are consistent in finding individuals with excessive fatigue, with an estimated prevalence of 21-68%(13-21). Fatigue is not just

problematic for those with active CD. A synthesis of studies in which clinical remission was an inclusion criterion reported a prevalence of excessive fatigue scores in 36% (182).

Excessive fatigue thus appears to be a problem that may diminish with the resolution of a flare but persists in clinical remission for many, but not all, subjects with CD. Limited evidence shows the inter-relationship of objective muscle fatigue and generalised symptoms of excessive fatigue. Establishing which patients are most likely to experience excessive fatigue and the putative causes has been attempted with regression analyses of patient and clinical factors. The data are consistent in finding fatigue scores to be higher among those with higher clinical activity scores (HBI and CDAI), poorer reported sleep quality and symptoms of depression or anxiety(14, 21, 23, 183).

The consistent correlation of clinical activity scores with more significant fatigue may partially be due to including a patient-rated score for general well-being in both CDAI and HBI. A patient rating their “general well-being” in both a fatigue PROM and clinical activity score may have similar answers, thus confounding the correlation between disease activity and fatigue. This confounder of any putative contribution of clinical activity as a factor in whether or not an individual experiences fatigue is not acknowledged in narrative synthesis studies of the determinants of fatigue(23). The relationship between the clinical activity score and excessive fatigue may also not reflect a relationship with underlying intestinal inflammation. In a cross-sectional study of UK IBD outpatients, this, but not faecal calprotectin, was higher among those assessed to have excess fatigue (20). Clinical activity scores appear to contribute to only part of the variability in fatigue scores (184).

The relationship between biochemical markers of nutritional status and fatigue in individuals with CD is poorly understood. From the perspective of first principles, micronutrients provide the nutritional substrate and cofactors for adequate cellular energy supply. They are essential for the effective formation of oxygen-transporting molecules, are the basis of oxidative phosphorylation through mitochondrial cofactors and prevent tissue damage through anti-oxidative mechanisms for optimal health and well-being. In other disease areas and populations, a poor micronutrient status has been linked incontrovertibly and repletion studies and supplementary studies in vulnerable populations with a reduced physical capacity and mental well-being (185).

At the interface between nutrient provision to the individual and diet is the intestinal microbiota, which communicates with the central nervous system through bacterial-derived

metabolites/neurotransmitters and their immune system regulation. Indeed, GI symptoms and dysbiosis have been linked to future diagnoses of neurodegenerative conditions, such as Parkinson's disease (186). Dysbiosis has been linked more directly to chronic fatigue syndrome, and higher serum levels of proxy markers of dysbiosis have been associated with worsened chronic fatigue symptoms (187) (188).

Anaemia, which determines cellular oxygen delivery and may arise from inadequate availability of Iron, B12, B6, Glycine or Copper, where deficiency is associated with more significant fatigue in some, but not all, studies, whereas markers of iron status were not associated (24). Serum Vitamin D concentration has been explored as a primary outcome in a large Norwegian sectional study and found unrelated to fatigue score (189). The most comprehensive reported exploration of biochemistry alongside fatigue severity was in a large Spanish multicentre study of fatigue in IBD outpatients, comparing concentrations of haemoglobin, ferritin, vitamin B12, folate, and vitamin D between those with and without excessive fatigue symptoms, finding no difference (21). Data on the relationship of other micronutrients with fatigue symptoms in CD are limited to a cross-sectional study that found being on a B-supplement to predict lower fatigue, but did not include biochemical analyses (14). The extent to which nutritional status determines fatigue and targets for micronutrient intervention will be better understood by a more comprehensive biochemical analysis of individuals with CD who have been appropriately stratified for disease activity alongside a broader panel of analytes.

#### 1.3.5.2 Treatments for Fatigue in IBD

Evidence for potential treatments to address fatigue in CD is limited. Fatigue PROMs performed longitudinally in an individual can quantify the degree to which fatigue symptoms change following an intervention.

This approach has been used as a secondary outcome measure using the fatigue symptom scores in the phase III trials of biological medications. The data from studies looking at three different modes of drug action, anti-TNF treatment, anti-p40 (Ustekinumab) and anti-JAK1 (Upadacitnib), show that fatigue scores respond to treatment to a greater degree than with placebo (190-192). This suggests that if left unresolved, intestinal inflammation may lead to excessive fatigue and that addressing it through effective medical therapies can, via various pathways, lead directly or indirectly to improved fatigue.

Concerning nutrient availability as a mediator of CD fatigue, high-dose Thiamine has been trialled in IBD subjects with chronic fatigue. This demonstrated a clinically significant reduction in fatigue symptoms in a pilot study replicated with a crossover placebo trial (193). The crossover design showed a clinically significant decrease in fatigue symptoms in 55% of the first group treated and 75% of the second group compared with 25% and 35% seen after 4 weeks of placebo. A subsequent maintenance trial failed to demonstrate the benefits of a lower dose of Thiamine over the placebo for fatigue symptoms at 12 weeks (194).

Other reported interventional studies for fatigue symptoms in IBD include a pilot randomised control trial comparing Omega-3 supplementation, exercise intervention, and placebo, which found a worsening of FACIT-F scores in the omega-3 treated subject and no effect of the exercise intervention, and small psychotherapy intervention studies which showed small transient benefit (23, 195).

### 1.3.6 Summary - CD Remission and "quality of remission"

Remission in Crohn's disease is generally defined by reducing the classic symptoms and signs associated with untreated intestinal inflammation. Clinical trials and international consensus guidelines set out the target of CD therapy as clinical remission, defined principally by stool frequency and abdominal pain or CDAI (based mainly on these features), but are increasingly using objective evidence of reduced inflammation via endoscopy or inflammatory markers as endpoint or targets for therapy.

While defined cut-offs are used to define CD remission, they may be variable, specifically in their depth (resolution of inflammation and associated symptoms) and durability (risk of future flare). Furthermore, some symptoms remain present during phases of remission. Defining remission in a disease state with ongoing symptoms will mean those symptoms are not looked for, diagnosed, or addressed and may be an unmet need. An example of this in CD is excessive fatigue, a leading presenting feature of the disease that affects an estimated one-third of subjects during subsequent remission. Excessive fatigue is a leading concern for CD patients but is not adequately considered in consensus guidelines on CD therapy.

Fatigue is partly related to active disease with poor sleep, and psychological symptoms are related. The relationship between nutritional status and fatigue or quality of disease remission is poorly described. There are limited interventional studies for addressing fatigue; addressing inflammation through advanced therapies, high therapeutic doses of Thiamine and psychological interventions may benefit, suggesting CD fatigue is complex and multifactorial in aetiology.

This thesis proposes that CD remission may vary in one or more of its **depth, durability, or residual fatigue**. The depth of remission has a considerable but incomplete overlap with durability and to a lesser extent, fatigue. Individually and collectively, these contribute to the quality of CD remission, and interventions should be targeted at attaining remission and at a remission of the highest possible quality. The proposed concept of **quality of remission** and its determinants are represented in Figure 3.

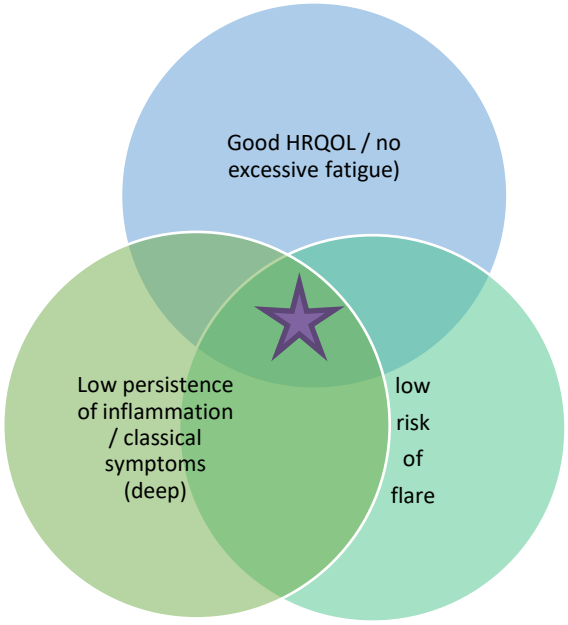


Figure 3: Diagram to represent proposed components of high-quality CD remission

- star representing high quality CD remission. The star represents good quality remission- one that is deep, durable and minimal fatigue

## 1.4 Nutritional and Nutritional Status in Crohn's Disease

*Nutrition* is the science that describes and interprets the provision of energy and nutrients to support human life and health. Nutritional status refers to the extent to which the demands for energy and nutrients are met. Nutrition can be considered from the level of the cell or tissues or, much more broadly with respect to an entire population.

This section will introduce nutrition and nutritional status in CD remission and set out broad categories to consider existing published research and a conceptual framework for their study.

Diarrhoea, abdominal pain, and systemic symptoms from inflammation may be related to, contribute to, or arise from a poor nutritional status. Malnutrition, characterised as low weight, was a common feature in CD at diagnosis in historical cohorts (196) (197). Sarcopenia and specific nutrient deficiencies are not usually systematically diagnosed in clinical practice, leading to a failure to recognise and characterise nutritional problems and, hence, inadequate treatment. The inability to identify the potential complexity of the nutritional diagnosis can be partly attributed to a lack of awareness and the inability to make and interpret the measurement of nutritional state. This, together with inadequate data recording using inappropriate systems (i.e., persistent use of paper notes), makes it virtually impossible to access the relevant clinical and nutritional information to enable longitudinal follow-up of quality. Against this limited diagnostic opportunity, restricted dietetic services of high-quality limit effective care.

The impact of nutritional status on any aspect of the disease is generally poorly understood. There is a failure to acknowledge and differentiate the energy and nutritional needs for maintenance: to make good specific losses associated with different aspects of the disease process, the complicated changes accompanying systemic inflammation; and the pattern of nutrients required to correct tissue deficits. An objective assessment of how much nutritional adaptation has impaired resilience is not readily available. This poor understanding can be attributed to a predominance of underpowered cross-sectional studies, often confounded by inadequate characterisation of important patient variables such as disease activity, medications, disease phenotype, and co-existing medical conditions. Further, the pattern of needs for energy and nutrients will change with the different stages of the disease: through periods of active disease, during recovery and healing of tissue after treatment of a flare, or to sustain wellbeing during remission.

### 1.4.1 A framework for approaching nutrition in Crohn's Disease

Nutrition and nutritional status are inherently complex and multidimensional concepts but will be considered at the level of the individual with Crohn's disease in the following ways:

1. Intake (how dietary intake is affected).
2. Micronutrient biochemistry
3. Form (how the composition and structure of the body is affected)
4. Function (how nutrition and nutritional status are related to what an individual can do).
5. How are individuals with an impaired nutritional state identified in current practice?

#### 2.4.3.2 Evidence for alteration of nutritional state – Intake in subjects with CD

The quality and quantity of the diet will determine the body's supply of energy and nutrients. Cross-sectional studies of outpatient IBD populations across Europe and North America have attempted to capture the prevalence of restrictive eating using a range of pre-designed questionnaires. These consistently demonstrate that food avoidance is a commonly reported feature of IBD, with between 83% - 90% affected(30, 31, 198). Subjects' reasons for restrictions have been variably captured using these different questionnaires, but most commonly include the intended avoidance of symptoms and flares of disease. (30, 31, 199). A 12-month study of prevalent symptoms in a Canadian IBD cohort reported a loss of appetite in 19% of 426 with CD(200). The potential mechanisms for altered appetite in CD have been explored mechanistically in a smaller number of subjects, with exploratory studies finding the relative serum concentrations of anorectic and orexigenic hormones to be altered in subjects with active CD compared to matched controls(33).

More direct evidence of alteration of dietary intake in CD comes from studies of dietary recall, which compare intake to those of matched healthy controls. A large cohort of Dutch IBD patients (493 subjects, of which 268 had CD, 51% in remission) whose intake was assessed via a food frequency questionnaire (FFQ) demonstrated significantly reduced consumption of potentially nutritious food groups compared to their matched controls (201). Common changes were also seen among 256 subjects in the Manitoba IBD cohort (52% with CD) when compared to age and sex-matched subjects, with this study again finding subjects with IBD reporting lower consumption of potentially nutrient-rich food groups(34). The same research group previously

demonstrated the relevance of such dietary changes in an outpatient IBD cohort, with a correlation between the calculated intake and serum levels of serum B6, Folate, and B12 (35). The presence of food restriction and consequent dietary micronutrient inadequacy in adult CD was similarly reported in a Portuguese cohort of CD subjects when the exclusion of milk, fruit, and vegetables was seen among the CD population, which had a reduced dietary Calcium, Vitamin C, D, E, and K as a consequence(202).

#### 1.4.1.1 Evidence for alteration of nutritional state – Availability; Micronutrient status in CD

Vitamins and trace elements, referred to as micronutrients, are chemical compounds needed by the body's cells as cofactors or substrates for its essential reactions of life and growth. A micronutrient generally cannot be manufactured by the individual and must therefore be consumed in the individual's diet, absorbed and made available to the cells and tissues of the body. An individual's micronutrient status is the extent to which their cells and tissues have the optimal availability of micronutrients for that individual's health and well-being. Food is a complex matrix of micronutrients; micronutrients are utilised and manufactured by the host microbiota, and each has multiple complex and integrated functions for the body's cells. An individual's status of a given micronutrient can be considered at the level of intake, ranging from dietary adequacy to the level of bioavailability to the target tissue of interest. Deficiency of a given nutrient is a state of overt adverse functional consequences for an individual that arises due to the reduced availability of that nutrient in the presence of an otherwise adequate nutritional state.

Based on depletion/repletion studies, clinical syndromes that characterise a state of such deficiency have been described and reported for a variety of micronutrients. Insufficiency of a micronutrient is a term that may be used in a less overt clinical situation in which the individual still had a sub-optimal state due to a nutrient's availability being limited. One way the status of a given nutrient in an individual may be assessed is through the sampling of the transport pool to identify whether the concentration of that nutrient puts the individual at risk of deficiency. For some micronutrients, consensus guidelines have set out a serum or erythrocyte concentration below which an individual is at risk of deficiency. In many reported CD studies, blood tests that demonstrate an individual's transport pool concentration of a given micronutrient to be below such a cut-off are used as the definition of deficiency, and thus the percentage deficiency in the population is defined by this.

To explore whether micronutrient status is impaired in those with CD, studies can compare the plasma or erythrocyte levels of one or more micronutrients in subjects and compare to a matched healthy population. The extent to which micronutrient deficiency or insufficiency is present in those with CD can also be determined by comparing and reporting the percentage of individuals with concentrations below levels compatible with deficiency.

#### 1.4.1.2 Evidence for alteration of nutritional state – Form and Body Composition in Crohn's Disease

Weight loss was initially described in the earliest adult CD cohort studies. The US National Cooperative Crohn's Disease Study reported weight loss in 85% of the cohort at the time of diagnosis, and European multicentre studies from the EC-IBD group in the 1990s reported weight loss as a presenting symptom among 60% of adults with CD (38, 39). In more recent IBD cohorts, improved access to effective diagnostic modalities has generally reduced the time between the onset of pathology and its symptoms and a specialist diagnosis. Early diagnosis reduces the degree of tissue injury at diagnosis and the profile of presenting symptoms. Nevertheless, in a recent US cohort study of presenting symptom profiles in adult IBD, weight loss remains among the presenting clinical features, affecting 63% with ileocolonic disease (203). As the weight of the population in Western Europe has increased, so too have those diagnosed with IBD, reducing the relative percentage of those fulfilling the criteria for malnutrition by BMI. The commonest BMI-defined abnormality in a 2013 study of adult Irish CD subjects was overweight or obese (>25kg/$m^2$), affecting 40% of subjects, compared to 52% of a matched population (40).

A persistent alteration in form as a feature of a CD-associated poor nutritional state is most evident among those diagnosed in childhood. UK cohort studies report that paediatric CD cohorts' age-adjusted height SDS (height 'Z 'scores) is low at the time of diagnosis and can remain low at 1 year after treatment (204, 205). Alteration in form as a feature of CD was demonstrated in a 2006 study that compared the measured final height of 123 UK children diagnosed with CD before age 16 with the target height estimated from their parental height, which showed a mean deficit of 2.4cm (206).

More subtle structural consequences of CD and its associated nutritional state may be seen as changes in body composition (207). A reduction in the absolute or relative size of an individual's lean mass or muscle mass in the context of disease or malnutrition is a well-described feature associated with a poor prognosis (208). Age-related reductions in muscle mass and function, sarcopenia, have been described in the elderly population as a predictor of morbidity. Such disease-associated reduction in lean or muscle mass may also be a feature of adverse nutritional state and inflammatory disease in younger patients, and there is an increasing incorporation of assessments of muscle mass in international nutritional guidelines to identify those most at risk of adverse outcomes (209). Sarcopenia, whether as a cause of adverse

outcomes or a common feature of individuals at risk of one, has been correlated to an increased length of hospital stay and adverse outcomes in a range of critical care populations (43, 210).

In CD, computerised tomography (CT), dual-X-ray absorptiometry (DEXA) and bioelectrical impedance analysis (BIA) to estimate the absolute or relative size of lean mass, skeletal muscle mass, or fat-free mass and compare this to values derived from matched controls (211-214). Systematic reviews of such body composition studies find a lower lean or fat-free mass in the majority of CD cohorts in comparison with matched health controls or population norms (44) (45). In CD populations, a reduced fat-free or lean mass has been correlated to a reduced response to anti-TNFα medications, a greater need for surgery, and complications from surgery (46) (47, 215, 216). Sarcopenia has also been shown in a CD population to predict another alteration in form, namely a reduced bone mineral density (BMD) (217).

Osteoporosis, which is defined by the World Health Organisation as a BMD <2.5 SD below the mean for young healthy sex-matched controls, appears to be more common among those with CD than in the general population (218, 219). In CD populations, it has been linked through correlation studies with both disease factors, such as previous surgeries and glucocorticoid treatment, as well as with markers of micronutrient availability, such as hyperhomocysteinemia and reduced serum Vitamin K levels (220, 221) (222). Such relationships suggest that a reduced lean mass and a reduced bone mineral density in CD may be the physical manifestation of a disease-associated impaired nutritional state.

#### *1.4.1.3* **Evidence for alteration of nutritional state- Function**

In subjects with CD, a poor nutritional state can be considered on a functional level. This may be considered and assessed in several ways: through reported or recorded physical activity levels or through direct measurements of muscle strength or cardiorespiratory capacity. Adequacy of an individual's physical capacity may also be indirectly considered through employment status.

The relationship between CD and physical activity appears to be a complex one. While a narrative review of the relationship between physical activity and CD concluded no consistent relationship across a few studies, the US nurse's studies found a protective role for increased exercise levels for incident diagnosis 2 and 4 years after assessment in two large cohorts (223, 224). Once diagnosed, CD or IBD also appears to impact physical activity; a large European multicentre study found that 1/3 of subjects had reduced their physical activity levels 18 months after their diagnosis (225). Cross-sectional studies that include individuals of longer disease duration are also consistent in finding that a large proportion of the population reduces their physical activity levels as a result of their IBD symptoms, with fatigue the most cited barrier to activities (48, 49).

Excessive fatigue symptoms in CD and IBD have also been explored against objective functional measures of muscle strength, cardiorespiratory fitness, and muscle fatigue. Van Langenburg found a moderate correlation between fatigue symptoms and objective quadriceps muscle fatigue among a CD cohort, with a significantly greater degree of muscle fatigue than matched HC (50). Cardiopulmonary exercise testing (CPET) of CD subjects also found objectively lower cardiorespiratory fitness among those whose PROMS classified them as having excessive fatigue than those who did not (52). A CPET study of paediatric IBD subjects reported lower than reference range fitness levels among subjects, and a comparison of the pre-operative phase of adults undergoing colorectal surgery showed a lower age-adjusted anaerobic threshold than subjects with other surgical indications, suggesting a disease-specific reduction in physical capacity in these CD populations (51, 226).

CD-associated impairment of physical capacity may have financial and career implications. A large cohort study of Norwegian IBD subjects demonstrated that there was a higher percentage of work-related disability claimants among adults with CD than in the general population (227).

#### 1.4.1.4 Evidence for addressing the nutritional state: nutritional therapies in adult Crohn's Disease

The evidence available to inform the nutritional care of patients with CD is relatively weak. The guidelines that have been proposed most usually apply to active disease and have as their objective the achievement of clinical remission. Exclusive Enteral Nutrition (EEN) is a specific therapy in which adequate energy and a complete pattern of all nutrients are assured at a level of maintenance intake, and it has been shown to induce clinical remission. However, there is a lack of evidence on the extent to which nutritional support can better achieve mucosal healing or sustain well-being during prolonged periods of remission. The objective evidence to guide judgment on how, what, and when to feed is based on variable clinical experience that is uncertain in its application from one context to another. There is insufficient information to develop formulations considering the specific nutritional requirements associated with the different aspects of the disease process(es). The importance of variability in the nutritional state amongst individuals or within the same individual at different stages may be of considerable significance for other aspects of care, for example, to account for the recognised variability in response to biological interventions.

#### 1.4.1.5 Nutritional Care and Nutritional Screening in Crohn's Disease

In recognition of the nutritional issues that adults with Crohn's may have, an approach to identify, assess further, diagnose and address nutritional issues for subjects with CD has been proposed by UK, European and US dietetic or nutritional societies. These guidelines include advice about adults with ulcerative colitis, Crohn's and IBD in general. They generally advocate with strong consensus but with a low grade of evidence that all patients with IBD should be screened for malnutrition and those deemed to be at nutritional risk undergo a more comprehensive dietetic assessment of intake, form and micronutrient blood tests. They do not specify which screening tools, assessments or blood tests.

Guidelines are not clear on how screening should be applied or whether there is an evidence base to support this. The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends with good practice points (their lowest grade of evidence) and 100% consensus screening for malnutrition at the time of diagnosis and then at regular intervals but does not specify which tool, how often screening should take place or whether any particular patient groups are at particular risk. From a UK perspective, the 2023 BDA expert consensus guidelines have strong consensus but low grade of evidence that adults with IBD use the Malnutrition Screening Tool (MUST). MUST is one of several generic nutritional screening tools explored in IBD populations. IBD but not Crohn's specific screening tools have also been designed and applied to IBD populations. These tools, the criteria they involve and their utility in published literature are briefly described below.

**MUST** is a generic nutritional screening tool that is standard of care in UK hospital inpatients. It was developed to identify individuals at moderate or high risk for malnutrition and recommends that those with a score of >1 (high risk) receive an immediate intervention. It incorporates a score for a reduced body mass index (BMI), weight loss over 5% in the previous 3-6 months and if a patient is acutely unwell and hasn't been eating for 5 days. In IBD populations, it has been found to correlate with disease activity scores (Harvey Bradshaw Index). It has been applied to patients in a range of settings, with the percentage of individuals assessed to be at risk dependent on the setting, with the prevalence of individuals assessed by MUST to be at risk ranging from 15-33% in outpatient settings to 80% in a group of patients awaiting an intestinal resection (216, 228-230).

**MUST** relies on BMI, and in populations in which there is a high incidence of overweight or obesity, or in which disease is diagnosed and managed promptly, it is unlikely to score many

individuals at risk. In a recent outpatient cohort of 173 IBD patients, when combined with an assessment of lean mass, it failed to identify individuals with a third of those with sarcopenia (229).

To capture both nutritional state and inflammation and better predict the risk of CD deterioration, an IBD-specific scoring system, the **malnutrition inflammation risk tool (MIRT**), was developed. MIRT correlated the cut-offs and scores for low BMI and percentage weight loss from MUST with a score for CRP in 55 subjects with a deterioration in Crohn's disease (composite of steroids, treatment escalation, admission and surgery) at 6 months in (231).

Further use of the MIRT score as a predictive tool has not since been reported, and a study which applied screening and assessment tools to newly diagnosed IBD patients found similar sensitivity to MUST against ESPEN criteria for malnutrition and failed to predict malnutrition and 12 months (230).

Other nutrition scores besides MIRT have previously incorporated markers of nutritional state and inflammation markers as clinical risk predictors. Incorporating a score for a low albumin, as a negative acute phase reactant to reflect an individual's inflammatory state to nutritional scoring systems, predicts length of stay in adults with IBD (232).

A score for both the inflammatory state as assessed by albumin and the nutritional state as assessed by recent weight loss contributes to another non-IBD specific nutritional screening tool, cited in international nutritional guidelines, the Nutritional Risk Index (NRI) (233). The NRI has been reported in a Crohn's population and has been correlated to response to Infliximab and thus, like MIRT been proposed as a tool to assess the risk of adverse outcomes (234).

Another widely cited generic BMI-based tool among those advocated by international guidelines alongside MUST is the Nutritional Risk Screening 2002 (NRS-2002) (235). The **NRS-2002** predicts response to nutritional interventions and scores someone as being "at nutritional risk" if they have one or more of a BMI <20.5kg/$m^2$, weight loss in the previous 3 months, reduced dietary intake in the previous 7 days or are a current intensive care inpatient. It was also applied to the study of IBD patients undergoing surgical resections, finding all subjects in the cohort to be at risk of malnutrition (>1) (216). More recently, in a study describing the development and validation of an IBD-specific screening tool, the IBD Nutritional Screening tool **NS-IBD,** which was also used for IBD surgical candidates, this time in elective cases (described below), **NRS-2002** reported 33% of subjects at high risk (236). The prevalence of NRS 2002-positive patients

within cohorts seems to reflect the disease activity in the cohort, with a much lower prevalence (14%) reported among mixed outpatients. As a general marker of high nutritional risk and poor outcome, it may have utility as a predictor of overall risk and has been correlated to medium to long-term mortality in cohorts of adults with mixed gastroenterological disorders.

The **NS-IBD** is among a group of recently developed IBD-specific nutritional screening tools, which look to go beyond weight loss and a reduced body mass to screen for and or diagnose nutritional issues (237) (228, 238). It was developed in 62 IBD cases undergoing surgery and incorporated scores for chronic diarrhoea, the number of gastrointestinal symptoms and previous surgery alongside weight loss and BMI, finding a better AUROC for a concurrent Global leadership initiative on malnutrition (GLIM) diagnosis of malnutrition and a significant correlation to length of stay but not surgical outcome. There has not been a replication to prove or disprove the utility of the NS-IBD as a predictor of risk (236).

Among these, IBD-specific nutritional screening tools looking to go beyond BMI, developed specifically for IBD is the **Saskatchewan IBD–Nutrition Risk (SaskIBD-NR Tool)** (228). It was designed by dietitians, recognising the risk of underdiagnosing nutritional issues in populations in which overweight and obesity are likely to be common. The SaskIBD-NR Tool assigns a score for 1) weight loss, 2) gastrointestinal symptoms in the previous two weeks, 3) poor intake due to reduced appetites and 4) food group restriction. In the cohort form in which the score was first described, the total score from these four domains was more sensitive and specific for identifying a diagnosis of malnutrition from a subsequent, blinded comprehensive dietitian nutritional assessment than the BMI-based score generated from MUST. It has since been explored in a prospective cohort of two Canadian IBD centres with some predictive value for subsequent hospitalisation (239). In the UK setting, a more careful consideration of nutritional state that considers disease burden (whether the individual is having a flare of IBD), food restriction and concerns around nutrition alongside a low BMI and unintended weight loss, the IBD-Nutritional Screening Tool (IBD-NST) has recently been developed for self-screening of nutritional state via electronic patient portals, which may be a valuable tool to identify nutritional risk among adults with CD.

#### 1.4.1.6 Nutritional Screening and Assessment Tools in Crohn's - Conclusions

Nutritional screening tools have a utility to identify those individuals with the highest nutritional risk. Still, the evidence on which specific tools are most suitable in populations with CD in remission is inadequate to inform practice.

Generic nutritional risk scores tend to use BMI and weight loss, which, when considered in isolation, would identify only those individuals with the highest risk. They are, therefore, most relevant for delayed diagnosis, inpatient and pre-op settings (where active disease and disease complications are present) to identify those with overt malnutrition who require immediate intervention. Beyond these settings, such as outpatient CD populations, when treatment has aimed to induce remission, overweight and obesity are now common in North American and European IBD populations. Therefore, nutritional risk scores relying on a reduced BMI are likely to find few individuals at nutritional risk, so despite their recommendation in consensus guidelines, screening for patients to assess and treat with tools such as MUST and NRS-2002 is unlikely to consider many subjects at risk.

Assessing only those "at risk" via BMI-based tools may limit more comprehensive nutritional assessments and treatments to a small proportion of the CD remission population.

Nutritional scores incorporating a marker of inflammatory status may have added the ability to predict adverse CD outcomes, suggesting that CD activity and undernutrition contribute to poor outcomes. More recent attempts to identify nutritional risk using patient-reported nutritional risks such as issues of intake (dietary impact factors, poor appetite or food restriction) and signs of disease activity (such as symptoms of a flare) have been developed. These may identify individuals with a normal or elevated BMI as being at risk, leading to a larger proportion of CD populations being more comprehensively assessed and treated for nutritional issues. As more effective treatments and diagnostics change the burden of disease at the time of diagnosis and in outpatient populations, such tools should, if validated, be used to aim for a more comprehensive assessment of nutritional risk to improve the nutritional state of the population beyond just addressing and treating overt malnutrition.

## 1.5 Conclusions

Crohn's is a disease of relapsing-remitting intestinal inflammation and accumulative tissue injury. Once diagnosed, CD is lifelong, and treatments, which can be medical, surgical, or nutritional, aim to induce and maintain a state whereby the intestinal inflammation and associated symptoms are in remission.

Clinical disease activity scores, faecal biomarkers, or endoscopic features can define CD remission. Such episodes of CD remission, whether spontaneous or induced by treatment, can vary in the risk for flares (durability), resolution of inflammation and associated symptoms (depth), and persistence of constitutional symptoms such as excessive fatigue. Excessive fatigue is a recognised feature of CD at diagnosis but can remain present through the disease course and may affect up to a third of adult CD cohorts in remission.

The understanding of Crohn's disease and its nutritional state comes from studies of newly diagnosed individuals, subjects with active disease and may be from an era before the widespread availability of advanced therapies. Crohn's disease is associated with an impaired nutritional state as indicated by poor intake, biochemical evidence of reduced micronutrient availability, altered body form and symptoms of fatigue or impaired physical functioning. Altered micronutrient biochemistry, weight loss, and responsiveness to nutritional interventions among those with active CD have been described, but how nutritional state is altered during subsequent remission and the implications of such alterations on the quality of that remission are not known. Screening tools that tend to focus on only those individuals with weight loss and low body mass Index will only identify those with overt malnutrition and may fail to consider nutrition from these perspectives in CD remission.

CD remission may be short-lived or involve persistent debilitating symptoms, particularly excessive fatigue, and an associated impaired health-related quality of life. The causes and treatment of a poor-quality CD remission and the extent to which it is determined by nutritional state are poorly understood and need further investigation.

# Chapter 2 Systematic review: Micronutrient status in adult Crohn's disease during clinical remission:

## 2.1 Background

Undernutrition is a recognised feature of adult Crohn's disease (CD), both at the time of diagnosis and during subsequent flares when overt nutritional inadequacy is manifested through weight loss (1). Following induction of disease remission, dietary restrictions and food avoidance to control CD and its symptoms may continue to alter nutrient and energy intake (30). Restrictive eating, both clinician and patient-imposed, a loss of absorptive capacity through scarring and resections, increased losses, and an altered metabolic demand from chronic inflammation will put the individual with CD at additional risk of nutritional insufficiency throughout the disease course (240-243).

Micronutrients are substances that provide structural components or the cofactors or substrates for the essential reactions of life. They generally cannot be manufactured by the individual. They must be consumed in the diet or manufactured by host microbiota, absorbed, and made available to the cells and tissues of the body. CD, its treatments, and sequelae may impact these processes and impair the ability of an individual to match their requirements for one or more micronutrients. An impaired micronutrient status may adversely affect an individual's wound healing, functional capacity and body structure, putting them at increased risk of cardiovascular disease (42, 244-246).

While international consensus guidelines exist for clinicians on diagnosing and managing iron deficiency anaemia in IBD, there are no guidelines around monitoring or treating other micronutrients in individuals with CD (178). ESPEN consensus guidelines for nutritional care of inflammatory bowel disease (IBD) advocate regular checking for micronutrient deficiencies, but do not specify which micronutrients to monitor or how frequently (247).

Current management of Crohn's disease seeks to induce and maintain remission (regardless of how this is defined), avoid disease-related complications, and maintain quality of life with medication, surgery, or nutritional therapy (5, 7). Alongside this care, individuals with CD require monitoring and, if needed, support of nutritional status. Blood tests of micronutrient status

need to be targeted towards the correct patients and clinically relevant markers of micronutrient state. The potential for impaired micronutrient status in CD appears to be recognised, but the literature has not been appraised. There is a need to bring together, organise, and appraise the relevant literature on micronutrient status in CD remission to aid clinical decision-making and improve care.

This review synthesises the available evidence on the relationship between impaired micronutrient state and micronutrient insufficiencies in subjects with Crohn's disease during clinical remission. The primary objective is to identify and integrate the current understanding of micronutrient blood tests for insufficiency in adults with CD during clinical remission, in terms of the prevalence (versus laboratory ranges), comparison to healthy controls (HC), contributing factors and clinical consequences of poor micronutrient status.

## 2.2 Methods

### 2.2.1 Protocol and Registration

This review was performed according to PRISMA guidelines with support from the university library services. The review was not registered on Prospero.

### 2.2.2 Eligibility Criteria

The review was conducted to answer the following questions: What is the evidence for micronutrient insufficiency in adults with Crohn's disease during clinical remission, and how does micronutrient status compare with healthy controls? Using a PICO strategy, we assigned "P" as adult outpatient CD populations in clinical remission, "I" as blood tests to determine micronutrient status (expressed as a percentage below a given cut-off or a concentration, "C" as matched healthy controls or pre-defined cut-offs for deficiency and "O" as Prevalence of micronutrient deficiency (below pre-defined laboratory ranges) or the serum/ plasma micronutrient status in comparison with matched controls. Due to a published meta-analysis on iron status in CD, this was not included in the synthesis (248).

Secondary outcomes, if available, were 1) whether any subgroup analysis was performed to identify associations with disease characteristics (e.g., disease activity score, biomarker) or subgroups (e.g., those with bowel resections), 2) whether any outcome measures of

micronutrient state were recorded or 3) whether any additional special test of micronutrient status or stores was performed.

### 2.2.3 Exclusion Criteria

The following exclusion criteria were used: studies reporting mixed IBD cohorts (without separate reporting of CD subjects), studies that did not report the number or percentage of subjects with a deficiency or had no healthy control comparison, or those studies with no recorded description of clinical disease activity score of the CD cohort.

The version of the systematic review included in this thesis excludes any studies where the measures of micronutrient state included subjects who were not in disease remission. This differs from a version of the review (published https://www.mdpi.com/2072-6643/15/22/4777), which also included (A) studies in which subjects in clinical remission were not reported separately on the condition that <50% of the cohort had clinically active disease (e.g. CDAI ≥150) or (B) the mean / median disease activity score is above the threshold that defines active disease (e.g. CDAI ≥150) (249).

### 2.2.4 Search Strategy

The following databases were searched in June 2021: COCHRANE, OVID MEDLINE, OVID EMBASE

EBSCO CIHAHL and WEB OF SCIENCE. Additional studies were identified using the references of relevant articles. These detailed search strategies are saved in Appendix A1: Search Technique.

### 2.2.5 Study Selection

Results were saved as a dedicated Endnote library, and duplicates were then removed. Titles and abstracts were scanned to select full texts. The extracted full texts were then independently checked by MM and SW against exclusion criteria. The reviewer(s) then recorded whether the study met inclusion criteria in a spreadsheet of extracted studies and the results compared. Discrepancies were resolved by re-review and discussion.

### 2.2.6 Data Extraction

The following data were extracted into a data table: study first author, year of publication, study size, whether the study compared to reference ranges or HC and micronutrient(s) of the study, and the micronutrient(s) reported in that study (see Table 1). A table compiling the studies fulfilling the review inclusion criteria for each micronutrient was made (see page Appendix A3: Data extracted from eligible studies). In each nutrient's table, additional information was also recorded for studies comparing to reference ranges: the percentage of CD subjects with low blood concentration and the cut-off used to define this and HC studies, the micronutrient concentration in the CD and HC groups, and statistical comparisons between groups. While SI units were not reported in all eligible studies, to avoid altering data, the units of measurement were not converted from those originally reported. Alongside the extracted information, it was then recorded whether the study supports the micronutrient's status being impaired among subjects with CD in remission and whether the study supports the micronutrient being impaired in CD in remission compared to HC. Data extracted for secondary outcomes were: (A) any correlation of micronutrient level with other disease markers, (B) subgroups of patients or disease outcome in CD subjects, and (C) whether any special tests were performed.

### 2.2.7 Quality and risk of bias:

Quality was assessed against a checklist based on the principles for observational studies, and the potential confounders of observational studies and those specific to assessing micronutrient status in CD were also considered (250). This checklist aimed to systematically capture whether modifiers of micronutrient status were documented and whether the population would be representative of a current cohort. The review-specific confounders added to this checklist included the adequate reporting of subjects' inflammatory status (as it can alter micronutrient blood tests), previous resections, and micronutrient supplement usage, whether blood tests were prospective, the clarity of the data, how remission was defined, and the percentage of subjects in remission. The components of the study quality checklist are listed in Appendix 2: Study quality checklist.

The results of the quality checklist for each study were used to classify the studies as low, medium, or high quality in the context of the question addressed in this review. Studies were judged to be low quality if the data were not reported (e.g., only on a graph) or if the confounding variables (resections, inflammatory state, supplement usage or recruitment) were poorly

described. Medium-quality studies had clear information but only partial reporting of the study subjects' confounding variables. High-quality studies had clear data and a thorough description of study subjects and confounding variables. Only those of medium or high quality were used to answer the review objectives. The study quality results for each category and overall study classification are displayed in Table 2.

Only those of medium or high quality were used to answer the review objectives. The year of the study and whether the cohort contained individuals who were not in clinical disease remission, as well as recognised sources of heterogeneity relevant to the study outcomes, were included in the extracted data tables and referred to in the summary statements for each micronutrient.

Table 1: Summary of studies fulfilling the review inclusion criteria,

the type of study and the micronutrients reported in that study ("X" denotes that the publication listed contains information on the Vitamin or Mineral of the column, or the type of study described in the column)

| | | | | | Liposoluble Vitamins | | | | Hydrosoluble Vitamins | | | | | | | Minerals | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Author** | **Year** | **n CD** | **% low in CD** | **CD vs HC** | **A** | **D** | **E** | **K** | **B1** | **B2** | **B3** | **B6** | **B9** | **B12** | **C** | **Ca** | **Cu** | **Mn** | **Mg** | **PO4** | **Se** | **Zn** |
| Schoelmerich (251) | 1985 | 54 | X | X | X | | | | | | | | | | | | | | | | | X |
| Geerling (252) | 1999 | 62 | | X | X | | X | | | | | | | | X | | X | | | | X | X |
| Schoon (222) | 2001 | 32 | | X | | | | X | | | | | | | | | | | | | | |
| McCarthy (253) | 2005 | 44 | X | X | | X | | | | | | | | | | | | | | | | |
| Valentini (214) | 2008 | 91 | X | X | | | | | | | | | X | X | | | | | X | | X | X |
| Basson (254) | 2015 | 44 | X | | | X | | | | | | | | | | | | | | | | |
| Ward (255) | 2015 | 381 | X | | | | | | | | | | | X | | | | | | | | |
| De Castro (256) | 2019 | 31 | X | | | | | | | | | | X | X | | | | | X | | | X |
| MacMaster (257) | 2021 | 59 | X | | X | X | X | X | X | X | | X | X | X | X | | X | X | X | | X | X |

## 2.3 Data Synthesis

The extracted data from medium or high-quality studies were summarised for each micronutrient. For each nutrient, statements were made regarding 1) the range of estimated prevalence of CD individuals below the laboratory reference range, 2) the comparison of CD cohorts to HC, 3) the cohort characteristics, and 4) any exploratory correlations of micronutrient versus outcome or sub-groups. Due to the heterogeneous nature of the studies and the small number of studies for most micronutrients, data syntheses such as calculating the mean prevalence of individuals below the laboratory reference range or the mean difference between CD and HC across different studies were not performed. As the data were not synthesised for a meta-analysis, no sensitivity analyses or methods to determine the certainty of the results were performed.

## 2.4 Results

The initial search returned 7512 studies, of which 2327 were duplicates. The remaining 5185 titles and abstracts were screened, and 5059 were excluded. 126 remaining articles were retrieved for full-text review, of which 85 were excluded (see Figure 4 for reasons). 13 studies met the inclusion criteria for the published review, including information on 16 micronutrients, see Table 1.

9/13 studies reported the percentage of a CD population below a laboratory reference range, and 8/13 compared the micronutrient concentration(s) against a group of matched healthy controls (HC).

The eligible studies reported between 1 and 14 micronutrients but tended to report on 1 or 2 nutrients, and 6 were single-nutrient studies. Only four studies offered a broader micronutrient panel (more than 4 micronutrients), and these tended to be in small populations, reporting on between 54 and 94 subjects (214, 252, 257, 258). One of these broader micronutrient panel studies was from the era of biological therapies (257). Studies tended to include limited numbers of subjects, with just 2 of the 13 eligible studies (both single-nutrient studies) reporting on more than 100 subjects (259, 260).

Following the assessment of quality, 4 of the 13 selected studies were judged to be of low quality. They were not included in the discussions and estimates around the evidence for each micronutrient being impaired among subjects with CD in remission(258, 260-262). Five of the ten medium or high-quality studies included in the data synthesis were published after 2010. Those published before this time were considered as ‘pre-biologic’.

A list of the eligible studies, the number of CD subjects in each, the type of study, and the micronutrients included in these studies is described in Table 1. The quality of these studies, against the quality checklist (see Appendix A2: Study quality checklist), is summarised in Table 2: Study quality. The extracted data for each micronutrient is recorded in data tables in Appendix A3, and these tables are summarised by the nutrients below. Due to the large number of confounding variables and heterogeneity between studies, a quantitative synthesis of data and summary statistics was not performed.

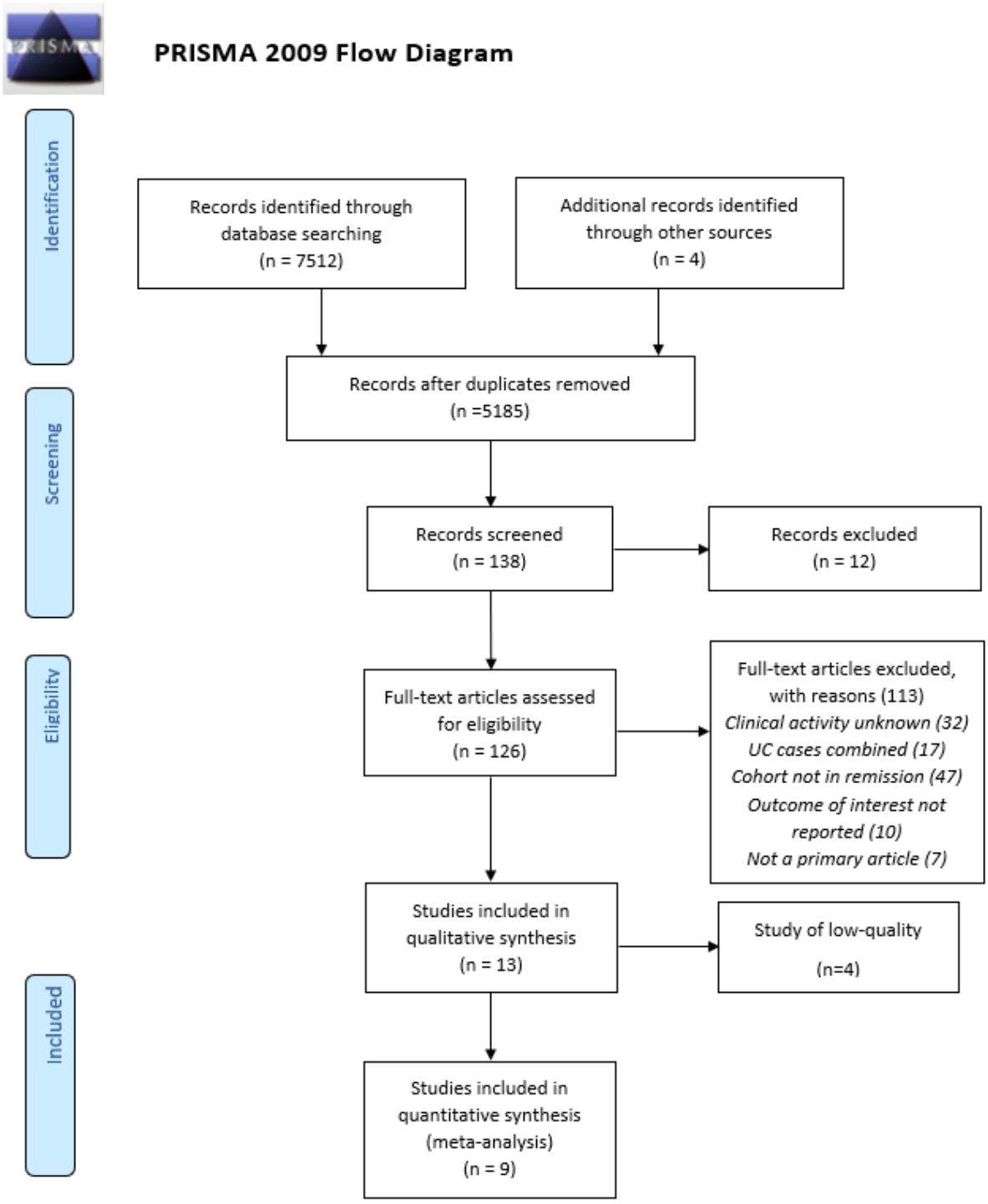


Figure 4: Consort Diagram for Systematic Review- exclusions and reasons

### 2.4.1 Studies fulfilling the review criteria

Table 2: Study quality/risk of bias.

Assessment against criteria set out in Appendix A2 Studies of Medium/High quality were included in estimates of prevalence and HC comparison statements. (Abbreviations: "?" denotes that this was unclear in the publication, "n/a" denotes not applicable to this study as no separate reporting of remission, Crp—C-reactive protein, resect—prior resections, supp—supplement use, Prosp—was the study prospective? sep rem—separate reporting of remission, % rem desc—Percentage of subjects in remission described.

| | | | Reporting of confounders | | | Confounders representative of current IBD cohorts | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study** | **Year** | **Primary** | **CRP** | **resect** | **supp** | **CRP** | **resect** | **supp** | **Recruit-ment** | **Prosp** | **Clear Data** | **Overall Quality** |
| Schoelmerich (251) | 1985 | Yes | No | Yes | Yes | ? | High | Yes | Yes | Yes | Yes | Medium |
| Geerling (252) | 1999 | No | Yes | Yes | No | Yes | High | ? | Yes | Yes | No | Medium |
| Schoon (222) | 2001 | No | No | Yes | Yes | ? | High | Yes | Unclear | Yes | Yes | Medium |
| Duggan (261) | 2004 | No | No | Yes | Yes | ? | Yes | Yes | Unclear | Yes | No | Low |
| McCarthy (253) | 2005 | Yes | Yes | Yes | Yes | Yes | Yes | Excluded | Yes | Yes | Yes | High |
| Filippi (258) | 2006 | Yes | Yes | Yes | No | Yes | No | ? | Yes | Yes | No | Low |
| Valentini (214) | 2008 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | High |
| Jorgensen (260) | 2013 | No | No | No | Yes | ? | ? | Yes | Yes | Yes | No | Low |

| Study | Year | Primary | CRP | resect | supp | CRP | resect | supp | Recruit-ment | Prosp | Clear Data | Overall Quality |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Basson (254) | 2015 | Yes | Yes | Yes | Yes | Yes | Yes | Excluded | Yes | No | Yes | High |
| Ward (255) | 2015 | Yes | No | Yes | No | ? | Yes | ? | Yes | Yes | Yes | Medium |
| De Castro (256) | 2019 | Yes | Yes | Yes | Yes | Yes | Yes | Excluded | Yes | Yes | Yes | High |
| Zhao (262) | 2019 | No | No | Noe | ? | ? | ? | ? | ? | Yes | No | Low |
| MacMaster (257) | 2021 | Yes | No | Yes | Yes | ? | Yes | Yes | Yes | Yes | Yes | Medium |

### 2.4.2 Micronutrient status in CD compared with HC and reference ranges by nutrient.

In this section, the data from the 9 eligible studies is extracted into tables for each of the reported micronutrients. This is followed by summary statements on the prevalence and HC of these studies and a narrative synthesis of the evidence from the studies, which included subjects of variable disease activity.

#### 2.4.2.1 Vitamin A (3 studies)

Two studies compared the Vitamin A concentrations among CD with HC; neither study found a difference (251, 252). Two studies explored the relationship of Vitamin A concentration with CDAI; one found a strong negative correlation, and the other found no difference between those with or without a CDAI >150) but did find that when the subjects who were in remission and subsequently relapsed were re-assessed, the serum Vitamin A was lower (251, 252). One study explored the relationship of Vitamin A with subsequent relapse, finding no relationship (257). Dietary Vitamin A intake was assessed in one study, and there was no difference in intake between those with CD and HC (258).

To summarise, the extent to which Vitamin A status is impaired in CD remission is not well described by the literature, but the limited data suggests that the prevalence of deficiency is low. There are also few studies where Vitamin A status in CD is compared to HC; these studies do not show a difference. The limited exploration of the dietary contribution of poor intake reveals that dietary inadequacy is common in CD and HC but may be a determinant factor of deficiency.

#### 2.4.2.2 Vitamin D, specifically 25 OH Vitamin D (4 studies)

Five studies reported the prevalence of low serum concentration (defined as deficiency and /or insufficiency) of 25 OH Vitamin D (25 OHD) in CD cohorts, and six compared with HC (253, 254, 257, 258, 260-262). Four were considered low quality and not included in the synthesis statements(258, 260-262).

The three studies that reported the proportion of CD subjects with a low 25 OH-D (defined as between <25 and <50mmol/L) reported a percentage prevalence of low Vitamin D in these populations ranging from 14% to 34% (253, 254, 257). One HC comparator study eligible for the synthesis found a significant difference in 25 OH-D levels between CD subjects (29% lower) and

HC (253). Exploratory correlation analyses of the studies included consistently identified negative correlations of 25 OHD level with clinical disease scores (254, 260, 262). The 2021 study explored the correlation with CRP, FCP, albumin and risk of flare in the next 12 months, finding no relationship (257). There was some evidence of dietary inadequacy among CD subjects (258). 25 OHD was lower in winter months and related to supplement use, disease duration, and subtype (254, 260).

The limited literature consistently demonstrated that Vitamin D deficiency, as marked by 25 OH-D concentrations below 50 nmol/l, is common in Crohn's disease remission. A comparison of 25 OH-D concentrations between CD and HC cannot be made using the limited literature (a single study).

#### 2.4.2.3 Vitamin E (2 studies)

One eligible study reported the percentage of CD subjects with a Vitamin E below a reference range (257). The study defined remission using CDAI (<150) and corrected for serum cholesterol, defining a low Vitamin E as <3.5µmol/mmol cholesterol reported 2% below the reference range. One eligible study compared Vitamin E in CD remission to HC. From a pre-biological era, serum Vitamin E (not corrected for serum cholesterol) was found to be lower among 50 inactive CD subjects than 70 matched HC (252). Neither of these studies found any relationship to clinical or biochemical disease markers or risk of a 12-month flare.

The prevalence of a reduced serum Vitamin E level among subjects with CD remission cannot be estimated from the limited literature (a single study). There is also insufficient literature comparing Vitamin E status among those with CD in remission to HC.

#### 2.4.2.4 Vitamin K (2 studies)

One study reported the percentage of CD subjects with a Vitamin K level below a laboratory reference range (which was corrected for serum triglycerides); this identified 2% of subjects below the laboratory range (257). One eligible study compared Vitamin K status among subjects with CD and matched HC. This study reported serum Vitamin K and, as a marker of Vitamin K status, the level of Undercarboxylated Osteocalcin (222). Vitamin K levels were lower among CD subjects than HC, and the marker of Vitamin K status, undercarboxylated osteocalcin, was elevated among those with CD. This marker was inversely correlated in regression analyses to a reduced bone mineral density (BMD) in subjects' lumbar spines.

The literature on vitamin K status in CD is limited, and a low serum Vitamin K prevalence among those with CD cannot be established. It is inadequate to establish whether levels are lower than the general population. One study of moderate applicability and medium quality suggests that Vitamin K status is more impaired among those with CD than the general population and may negatively impact skeletal health.

#### 2.4.2.5 Thiamine (B1) (1 study)

One study compared whole blood Thiamine concentration among a CD remission population with a laboratory reference range (257). This study, from 2021, with HBI≤4 as a study inclusion criterion, defined a low Thiamine as <275ng/g Hb and found no subjects below the reference range. There were no eligible HC studies.

There is insufficient evidence of low thiamine among CD subjects to estimate the prevalence of low serum thiamine among subjects with CD during remission or determine whether levels are lower than in the general population.

#### 2.4.2.6 Riboflavin (B2) (1 study)

One study reported on the percentage of subjects with Riboflavin levels below a hospital reference range (<1.0nmol/g Haemoglobin (Hb)), finding 1/59 (2%) of subjects as low (257). No studies have compared Riboflavin status in CD with that of HC.

There is insufficient evidence on the prevalence of reduced Riboflavin levels among CD subjects and, in comparison to the general population.

#### 2.4.2.7 Niacin (B3) (no studies)

No studies that met the inclusion criteria reported on Niacin.

#### 2.4.2.8 Pantothenic acid (B5) (no studies)

No studies that met the inclusion criteria reported on Pantothenic acid.

#### 2.4.2.9 Pyridoxic Acid (B6) (1 study)

One eligible study reported the percentage of individuals in a CD population with a below-range red blood cell pyridoxic acid (257). The study found 10% below the cut-off of <250pmol/g Hb (to correct for inflammatory status). This study did exploratory subgroup analysis and found red

blood cell B6 concentrations to be lower among subjects with a stricturing rather than inflammatory disease phenotype, but to have no relationship to disease activity markers or risk of 12-month flare.

The limited literature consistently supports a low serum B6 in subjects with CD in remission, and that dietary intake and serum levels are related. No studies of sufficient quality meet the review's inclusion criteria to compare B6 concentrations between CD populations and HC.

#### 2.4.2.10 Biotin (B7)

No studies that met the inclusion criteria reported on Biotin.

#### 2.4.2.11 Folic Acid (B9) (3 studies)

Three studies reported the percentage of CD subjects with serum folate below a laboratory reference range (214, 256, 257). The concentration to define low folate was not the same across the studies. Two studies found no subjects with serum folate below the range (214, 256). The most recent study found 9% below the reference range of 3ng/ml, which was between the range used for the other two studies (257). One eligible study compared serum Folate in a CD remission population against HC and found no significant difference (214). Exploratory and correlation studies found Folate to be lower among those with endoscopic or MRI activity in one study and unrelated to CRP, FCP or albumin in another (256, 257).

The literature on the prevalence of low folate in Crohn's disease remission is inconsistent in terms of how a low level is defined and whether there are individuals with a low level in the population. There are no eligible studies to compare folate status in CD remission subjects to HC. The exploratory analyses suggest that low folate blood tests may be partly related to disease activity.

#### 2.4.2.12 Cobalamin (B12) (4 studies)

Four studies reported on the percentage of subjects with either a low B12 (below a reference range) or other biochemical evidence of an impaired B12 status (such as a Holotranscobalamin (holoTC) below the reference range or methylmalonic acid (MMA) above the reference range) (214, 256, 257, 259). All four studies identified CD subjects with evidence of impaired B12 status (20, 37, 49, 55). The highest prevalence (33%) was among a group of CD subjects in clinical remission, which defined an impaired B12 status as either a holoTC below 2 or a holoTC

between 25 and 50 with a paired MMA >280 (259). The study with the lowest reported percentage of B12-deficient subjects (4%) defined this as hydroxocobalamin <197pg/ml and remission through both radiological and endoscopic criteria (256).

One eligible study compared serum B12 concentration with HC, and no difference between CD and HC was reported (214). In the exploratory correlations, Ileal resections <20cm, >20cm and active ileal disease were identified as a risk factor for a B12 below the reference range odds ratio (3.0, 6.7 and 3.9, respectively) (259). Two other studies explored the relationship of B12 with disease activity and found no relationship (256, 257).

The evidence of impaired B12 status in CD remission is heterogeneous but consistently supports the view that some individuals with CD have low B12 concentrations. There are too few HC studies to determine whether B12 status is lower in CD remission than in HC. The correlation analyses consistently support ileal resections and ileal inflammation being a risk factor for B12 deficiency (259).

#### 2.4.2.13 Vitamin C (2 studies)

One eligible study reported the percentage prevalence of subjects with Vitamin C below a reference range, and there was one HC comparator study (252). The prevalence study reported 17% of subjects below the reference range (<15µmol/L), and the comparator study found serum Vitamin C to be 18% lower among CD subjects than HC (257). The correlation analyses showed no relationship to clinical disease activity, CRP, FCP, serum albumin, or risk of flare (252, 257). One study reported a lower Vitamin C intake among CD subjects than matched HC, but did not report the relationship to blood biochemistry (258).

One recent well-designed study supports the view that some individuals will have a low serum Vitamin C in CD remission, but the literature is minimal. The single HC comparator study in 1999 found Vitamin C lower in CD than in HC. Dietary inadequacy may exist among CD populations, but its relationship to blood concentrations has not been explored.

#### 2.4.2.14 Calcium (no studies)

No studies that met the inclusion criteria reported on Calcium.

#### 2.4.2.15 Copper (2 studies)

One study reported the percentage of CD subjects with a serum Copper below laboratory reference ranges, and another compared serum Copper to HC (252, 257). The prevalence study from 2021 reported 5% below the reference range (<10µmol/L men, <11µmol/L women) (257). The HC study was from the pre-biologic era and reported no difference in copper levels between CD subjects and HC (252).

#### 2.4.2.16 Manganese (1 study)

One medium-quality study reported the prevalence of a low serum Manganese in a cohort of individuals, all in clinical remission, and found no subjects below the laboratory reference range; there were no HC studies (257).

#### 2.4.2.17 Magnesium (3 studies)

Three eligible studies reported the percentage of subjects with serum Magnesium below the laboratory reference range (214, 256, 257). Of the three medium- or high-quality studies, one was in the pre-biological era and reported a low magnesium level among 28.7% of subjects (214). The two more recent studies, one from 2019, which required either radiological or endoscopic evidence of “deep remission”, and the other from 2021, which required a CDAI <150, reported low Magnesium in 15% and 7%, respectively (256, 257).

The pre-biologic era prevalence study also compared serum Magnesium concentration in a CD population with HC and found no significant difference (214).

The small body of literature, which includes recent well-characterised cohorts of subjects in CD remission, consistently finds that populations with CD in remission contain individuals with a low serum Magnesium. The literature comparing serum Magnesium concentrations in CD to HC is limited.

#### 2.4.2.18 Phosphorous (no studies)

No eligible studies were reported on the prevalence of low phosphorus status or its comparison to HC.

#### 2.4.2.19 Selenium (3 studies)

Two studies compared Selenium to a reference range (214, 257). One, from a pre-biological era, reported low serum concentration (defined as <0.59µmol/L) in 61% of the cohort (214). A more recent study (2021) found 5% of subjects to be below the serum cut-off (<0.75µmol/L) (257).

The pre-biological era prevalence study also compared concentrations to HC, finding Selenium concentration to be lower among men (vs. HC) but not women (214). The other eligible comparator study, also from a pre-biological CD cohort, found serum Selenium concentrations lower among CD than HC (252).

Two studies explored the relationship of Selenium levels to disease activity. One found no significant correlation (252). The other study compared faecal and serum inflammatory markers and risk for CD flare in the next 12 months, finding a positive correlation to serum albumin and a negative correlation to CRP and no relationship to faecal calprotectin or risk of flare (257).

A small body of literature suggests that Selenium deficiency may be prevalent among CD populations during clinical remission. The literature on whether Selenium levels are lower among those with CD than the rest of the population is small and inconsistent.

#### 2.4.2.20 Zinc (5 studies)

Four eligible studies reported the percentage of CD subjects with Zinc concentrations below laboratory reference ranges (214, 251, 256, 257). Two were pre-biological, and two were post-biological. All studies reported subjects with low zinc levels, with prevalence ranging from 4-45%. The highest prevalence of subjects with a low serum Zinc was in a biologic-era study, which required endoscopic or radiological evidence of remission and had the highest cut-off (<12µmol/L) (256). Three eligible studies compared Zinc concentration between CD subjects and HC (214, 251, 252). Two of these studies found Zinc levels significantly lower among CD subjects than HC. The more recent three studies, which also had the lowest reported prevalence of plasma Zinc below the reference range, found no difference in HC (214). In the exploratory analyses, plasma Zinc concentration was negatively correlated to CDAI and positively correlated to Albumin, yet unrelated to CRP, Calprotectin, or endoscopic/radiological activity in another (251, 256, 257). Of note, plasma serum Zinc level predicted a shortened time to clinical relapse in a 12-month prospective cohort study (257).

The limited literature consistently supports subjects with serum Zinc levels below the laboratory reference range among subjects with CD during remission. The literature comparing Zinc levels in subjects with CD is inconsistent.

### 2.4.3 Summary statements for Micronutrient status in CD

Taken together, the limited number of eligible studies support the view that in adult CD populations during clinical remission, there are likely to be individuals with low circulating levels of the following nutrients: Vitamins B6, B12, C, D, and the minerals Magnesium, Selenium and Zinc.

Of these, the estimated percentage prevalence of individuals at risk of deficiency is most secure for Vitamins D and B12, for which there is a larger body of literature (3 and 4 eligible studies, respectively). The eligible studies of Vitamin A, B1, B9 and E and the mineral Copper reported a percentage prevalence of less than 5%, suggesting that the risk of these deficiencies is likely lower. No eligible studies were identified for the nutrients Vitamins B2, B3, B5, and B7, as well as the minerals Calcium, Manganese, and Phosphorous, to estimate the percentage prevalence of low values.

The eligible HC comparator studies were fewer but found Vitamins C, E, and K to be lower among CD remission populations than controls (1 eligible study per nutrient). Vitamin D was lower in one of two studies, and single HC studies for Vitamins B9, B12 and the minerals Magnesium and Copper reported no difference between CD and HC.

The ranges of reported percentage prevalence of low micronutrient values in comparisons to laboratory ranges and HC for eligible studies for each micronutrient are summarised in the table below (Table 3).

Table 3: Summary of Prevalence of Insufficiency in CD remission and comparison to Healthy Controls for Eligible Studies.

No eligible studies were identified for either prevalence or HC comparison studies for Vitamins B2, B3, B5, B7 and the minerals Calcium and Phosphorous

| | **Prevalence Studies** | | | | **Healthy Control (HC) Studies** | | |
|---|---|---|---|---|---|---|---|
| **Micronutrient** | **Number of studies (total number of subjects)** | **Range of reported lower cut-offs** | **Reported prevalence (%)** | **Evidence of deficiency in CD population** | **Number of Studies vs HC (n)** | **Number of studies in which CD < HC** | **Evidence supports CD < HC** |
| (Vitamin A) | 1 (59) | 1.0 µmol/L | 2 | Yes (<5%) | 3 (106) | 0/2 | No |
| Vitamin D | 3(143) | 25-50nmol/L | 14 - 37 | Yes | 2 (65) | 1/2 | Uncertain |
| Vitamin E | 1(32) | 3.5µmol/mmol | 1 | Yes (<5%) | 1 (62) | 1/1 | Yes |
| Vitamin K | None | - | - | Unknown | 1(32) | 1/1 | Yes |
| Vitamin B1 | 1 (59) | 275ng/g Hb | 0 | No | 0 | 0 | Unknown |
| Vitamin B6 | 1(59) | 250pmol/g Hb | 10 | Yes | 0 | - | Unknown |
| Vitamin B9 | 3(181) | 2.8-3.9ng/mL | 0-9 | Some (1/3) | 1 (91) | 0/1 | No |
| Vitamin B12 | 4 (562) | Low holoTC or high MMA or B12 197-200pg/ml | 4-33 | Yes | 1 (91) | 0/1 | No |
| Vitamin C | 1(59) | 15 µmol/L | 17 | Yes | 1 (62) | 1/1 | Yes |
| Copper | 1(59) | 10µmol (m)<br>11µmol (f) | 5% | Yes | 1(62) | 0/1 | No |
| Manganese | 1(59) | 70nmol/L | 0 | No | 0 | - | Unknown |
| Magnesium | 3(181) | 70-80nmol/L | 7-28 | Yes | 1(91) | 0/1 | No |
| Selenium | 2 (150) | 0.59-75µmol/L | 5-61 | Yes | 2(156) | 2/2 | Yes |
| Zinc | 4 (235) | 10-11µmol/L | 4.3-45 | Yes | 3(207) | 2/3 | Uncertain |

## 2.5 Discussion

This review identified a small body of literature on micronutrient status among subjects with CD remission, compared to normal reference ranges and against matched HC. For most of the 9 Vitamins and 5 minerals and trace elements in the review, there were insufficient studies, both in number and quality, to address the primary research questions of the prevalence of biochemical signs of micronutrient deficiencies and whether the nutrient was lower in CD than HC. Micronutrient studies in CD tend to combine these results with active disease or UC, reducing the number of eligible studies to determine micronutrient status in CD remission.

The published systematic review of this work broadened the inclusion criteria to allow cohorts of variable disease activity (if at least half the cohort was in remission), which increased the eligible studies to 31(249). The tighter inclusion criterion for this review has reduced the number of eligible studies for several nutrients, potentially excluding essential information needed to understand the causes and prevalence of an altered micronutrient state in adult CD remission.

Despite the challenge and limited information, the eligible papers support the view that indicates that some, but not all, patients with CD in clinical remission will have a low circulating concentration of the following micronutrients: Vitamins B6, B12 and C, D, and the minerals Magnesium, Selenium and Zinc. Of these, only the data for Vitamin B12 and D were from more than one study and quality to draw firmer conclusions about whether this is likely to represent CD subjects in remission in general and the probable proportion of individuals likely to be at risk for deficiency. A widely cited narrative synthesis of micronutrient status in adult CD describes micronutrient deficiencies as “common” in adult CD. Still, it is unclear what this means in numerical terms or the threshold for this term (263). A consistent and precise definition of “common” with respect to percentage prevalence and a broader literature are needed to support and inform the clinician of decisions around the scope of blood monitoring that are necessary and justifiable in the IBD clinic.

The second key question of the review was whether the micronutrient status was lower among the CD population in remission than the general population. The data available to answer this was particularly limited. There were either no eligible studies or only one study for more than half of the micronutrients. There were just two studies for the commonly monitored Vitamin D, one of which found no difference between CD and HC. A meta-analysis that concluded that

levels were lower in CD than HC may reflect the inclusion of individuals with active disease, which is a risk factor for a lower 25-OH D (264, 265).

Most studies were from heterogeneous populations regarding treatment and previous surgery, making the findings less generalisable for different patient types. The information was generally taken from cross-sectional studies of mixed CD populations. The quality of the studies was limited by incomplete reporting of co-founders, such as recruitment methods, inflammatory markers, previous surgery, and supplement consumption in the CD cohorts. The estimated prevalence of low circulating levels of other micronutrients in CD remission and HC comparisons were further limited by differences in the choice of a lack of standardisation of cut-off and the number of recent studies of adequate quality.

The review excluded studies in which some of the subjects were not in clinical remission or the results of those in remission were not reported separately. While this will have removed the confounders associated with active CD, such as increased inflammation, altered demands, or changes in dietary intake during flares of disease, many of the individuals in these studies may have been in disease remission and contributed to the estimates of prevalence or results compared to HC. Excluding these studies may have excluded important information from the limited literature on micronutrient status in the CD remission populations. The broader inclusion criteria of mixed CD activity cohorts for the published systematic review meant that the number of eligible studies was greater and included important information on the relationship between micronutrient status and factors such as disease activity, dietary intake, and CD subtype (249).

The review also excluded the data when individuals with UC were combined in the results. This may have excluded well-designed studies that better understood the micronutrient state in adult CD or IBD. One study with combined UC and CD found distinct eating habits and antioxidant vitamin status in active and remission IBD compared with HC (266).

Micronutrients are not consumed, absorbed, and metabolised in isolation. Yet, none of the studies in the review report on more than one micronutrient or metabolite provide data that accounts for this issue. The studies do not report their findings in such a way as to determine whether those individuals with a low concentration of one micronutrient were more likely to be low on others.

The year of the study is likely to have been a factor in the nutritional status of the cohorts studied. Landmark CD studies shortly before 2010 demonstrated the efficacy of biological therapy as a disease modifier (267, 268). In countries with adequate resources, this led to a more widespread adoption of biological treatments for moderate to severe Crohn's. This is likely to impact the cohort's disease activity, prevalence of overt malnutrition, risk of surgery and thus, micronutrient status. In the narrative summary for each nutrient, 2010 was therefore used as the cut-off for the "pre-biologic" era.

Where explored and reported in the included studies, micronutrient blood concentrations tended to be negatively correlated with disease activity scores. This was a consistent finding in the studies of Vitamin D and folate. Systemic acute inflammation is negatively correlated with circulating micronutrient concentration, and there is an increasing recognition that reduced serum micronutrients in IBD may be inflammation-associated epiphenomena (269, 270). The variable reporting of this between studies is likely to account for the variable prevalence of deficiency seen in the studies included.

There were multiple challenges in drawing generalisable conclusions about micronutrient status in CD remission from the available literature. Eligible studies covered over 30 years of CD cohorts, a time in which the availability of biological therapies has transformed the treatment armamentarium and therapy goals. As such, factors that may impact micronutrient status, such as the degree of inflammation, scarring from previous inflammation and length of resected bowel, may differ according to the era of study. The eligible studies which reported on multiple micronutrients (other than Vitamin D-focused studies) tended to be from the pre-biological era, so the applicability of data on clinically relevant micronutrients such as Thiamine to a current treatment cohort is particularly uncertain.

The studies that reported on the percentage of "deficient" subjects in a CD cohort varied in the cut-off to define this, as well as the analysis method and the reporting unit. To inform the identification of subjects at risk of micronutrient deficiency, studies should report SI units, offer clinically relevant cut-offs and be consistent in what proportion of deficient cases constitutes a "common deficiency". For this thesis, a cut-off of less than 5% was used.

The methods and criteria used will have impacted on estimates of prevalence of impaired micronutrient status. Using B12 status as an example, while most studies measured serum total cobalamin, the study which used measured Holotranscobalamin, likely a more reflective marker of B12 status, and MMA when the holoTC was indeterminate as functional measure of

status, suggested 33% of CD subjects had an impaired B12 status, the highest estimate prevalence from the studies included(259, 271). Such variability between studies in the definitions and adjustments to identify those at risk of micronutrient deficiency is another challenge in synthesising and interpreting the literature.

The critical clinical question of whether the deficiencies seen across a range of micronutrients within study populations occur in the same individuals is not answered in the way the studies are reported. It is not, therefore, clear whether being low in a specific, more commonly checked nutrient or having certain disease features could serve to identify a targeted approach whereby only those adults with CD who are most at risk routinely undergo a broader micronutrient profile. The studies also had limited exploration of which factors, such as diet, previous surgery, or inflammation, may determine micronutrient status. There was a minimal exploration of micronutrients as a potential determinant of clinical outcome, with bone mineral density being the most common issue explored, and just one study compared micronutrient status to the risk of future flare (257).

Nutrition is a key area of interest to patients with IBD. Clinicians must have an evidence base to inform their advice and monitoring. There is a need to establish which individuals within CD populations are most likely to have micronutrient deficiencies during clinical remission, establish which disease and dietary factors may determine this and explore the consequences of this. Evidence for the therapeutic benefit of dietary manipulation by removing potentially proinflammatory elements is emerging. However, discussing what nutrients may likely be dietarily inadequate for individuals with CD and the consequences of those inadequacies needs equal consideration for care (7).

With enhanced biomarker monitoring and effective medical treatments, the treatment paradigm of CD is changing towards goal-directed therapy. IOBD Stride-2 criteria now include the normalisation of health-related quality of life as a long-term treatment target. Understanding how micronutrient status contributes to poor quality of life (7).

There is a need for a broad nutritional and phenotypic characterisation study of micronutrient status against population norms or healthy controls alongside relevant outcomes in an adult CD remission population. Understanding the pattern of inadequacy, determinants of nutrient inadequacy, and the relationship of different micronutrients to one another and their outcomes is needed to establish how to best direct nutritional monitoring and support. Confounders such as age, inflammatory state, and previous surgeries need to be included, as well as diet,

including supplement usage, should be included in such analyses. With more detailed reporting, the extent of the unmet nutritional needs of the CD population can be better determined and used to direct better care and monitoring towards those subjects in the CD population most likely to derive meaningful benefits. This was a comprehensive and systematic review of varied literature on micronutrient status in CD remission. Studies may have been missed, and the addition of several eligible papers identified in the references of the initial extraction suggests that some articles may not have been picked up on database searches.

## 2.6 Conclusions

In conclusion, this systematic review finds that populations of adults with CD during clinical remission are likely to have a range of micronutrient deficiencies. Except for Vitamins D and B12, the evidence base to identify which biochemical deficiencies are likely to be present and the prevalence is insecure. The literature comparing micronutrient status in CD remission to HC is also limited, and it cannot predict which nutrients are likely to be lower in CD than in the general population.

What remains unclear is the micronutrients that are likely to be deficient during CD remission, which patients are likely to be affected by, and the clinical consequences. The systematic literature review has exposed a gap in the literature. There is a need for studies comparing well-characterised and appropriately characterised cohorts of adult patients with Crohn's disease in clinical CD remission cohorts to reference ranges and HC against clinically relevant outcomes to inform patient care.

# Chapter 3 Identifying Nutritional Targets in Crohn's Disease: INTICO-1

## 3.1 Introduction to Experimental Work

The narrative review of the literature on the nutritional state in adults with Crohn's disease has identified that it may be impaired in terms of intake (in terms of adequacy), form (body composition and structure), availability (micronutrient status), and function (health-related quality of life). CD remission is generally considered from the perspective of intestinal inflammation seen on endoscopy and the classical symptoms associated with this. There is evidence for nutritional interventions to induce clinical remission, and nutritional therapies are widely used in the setting of paediatric Crohn's disease.

The subsequent systematic review focussed on studies for which the micronutrient state was reported separately for those in a defined CD remission. It found that CD remission adult populations likely contain individuals with a low circulating micronutrient. Still, the limited number of eligible studies of adequate quality meant that statements on how common these were for each micronutrient (other than Vitamins D and B12) could not be made. The eligible studies also failed to explore the relationship between the dietary causes of altered blood biochemistry and their consequences (e.g. clinical outcome). There was a need to better characterise the nutritional state in a group of adults with CD in confirmed remission from each perspective: intake, form, availability, and function.

The experimental work of this thesis examined the nutritional state from these perspectives in two studies: INTICO-1 and INTICO-2. Briefly, INTICO-1 was a hypothesis-generating study which used a time-limited trial of nutritionally complete intake to characterise the nutritional and clinical state in CD remission, and INTICO-2 explored the areas of interest from INTICO-1 across a larger cohort. Below is the description of the first of these studies, INTICO-1.

## 3.2 Introduction - INTICO-1

The INTICO-1 study was an in-depth characterisation of the nutritional status of a group of adults with ileal Crohn's disease during remission, who would not, in normal circumstances, be assessed as being undernourished or under any nutritional therapies.

The study design aimed to achieve two things:

- Describe a baseline nutritional and disease characterisation of CD remission
- Use a time-limited trial of a period of controlled diet to expose nutritional issues and explore the nutritional state and CD remission

The study also explored the feasibility of nutritional assessment in the UHS CD population. It was a comprehensive characterisation of a small group of individuals. The size of the study was pragmatic, it did not have a control arm, and it was not powered to demonstrate that any observations reflected a larger population or that any changes in response to the intervention were statistically significant. The study did not seek to demonstrate the efficacy of the commercial product as a nutritional intervention, it was used as an attempt to control the diet using a formulation which was nutritionally complete and approved for use in the setting of Crohn's disease.

### 3.2.1 Nutritional Assessment

Nutritional status was considered under the four broad categories: intake, availability, form, and function. Each category and their interaction allowed nutritional status to be considered at the cause, mechanism, and consequence level. Requirements were estimated through resting energy expenditure measurements, and intake was analysed by analysing the habitual diet for macronutrient and micronutrient intake adequacy. The availability of individuals' nutrients was assessed using biochemical analyses of circulating micronutrient pools and comparing these to laboratory-provided normal ranges for healthy populations.

Form was assessed by predicting body composition through its bioelectrical properties compared to healthy matched populations using parameters considered optimal for health and well-being. Function was assessed using patient-reported outcome measures of health-related quality of life.

### 3.2.2 Time-limited trials

A time-limited trial to identify the cause of an issue by its response to its treatment is widely used in medicine. In critical care, an increase in the blood pressure of a shocked patient treated with a fluid bolus will suggest hypovolaemia (272). In gastroenterology, bile acid diarrhoea may be identified using a therapeutic trial of a bile salt binder, and bacterial overgrowth is recognised as a contributor to irritable bowel syndrome by antibiotic therapy (273, 274). In nutritional assessment, a response in plasma retinol concentration to a dose of retinol can be used to diagnose inadequate hepatic stores (275). The INTICO-1 study design used this same principle, with the time-limited trial being a nutritionally adequate intake.

The specific hypotheses of the study were:

1. There was evidence of nutritional inadequacy and poor quality of life in clinical remission (baseline observations on habitual dietary intake)
2. A period of complete nutrition will reveal nutritionally sensitive markers of inadequacy (a change in markers after intervention and return to habitual dietary intake)
3. These markers are related to the quality of remission

## 3.3 Methods

### 3.3.1 Setting

The INTICO-1 study was a prospective, single-centre dietary intervention trial conducted at the outpatient population centre of a single UK centre. The study was an investigator-led collaborative research agreement between the University of Southampton, University Hospital Southampton NHS Trust and Nestlé Health Sciences, with Dr. Stephen Wootton and Dr. Fraser Cummings as Principal Investigators. The protocol was approved by the Berkshire B Research Ethics Council (REC) and the UK Health Research Authority (HRA), IRAS number 215871 and was conducted according to the principles of the National Institute for Health Research (NIHR) Good Clinical Practice (GCP). The study was ERGO-approved (Reference number 41365). The study was conducted at University Hospital Southampton (UHS) between August 2018 and June 2019 by a research team consisting of the author, a research nurse, a clinical trials assistant, and a specialist dietitian.

Subjects were recruited from the IBD outpatient clinic at UHS. Before scheduled outpatient visits, they were pre-screened for suitability using an existing research database and electronic healthcare records or from a list of subjects at the centre (from both the virtual clinic list and main patient cohort) who had previously participated in a dietary study. Subjects identified before clinic visits were informed of the study in writing and approached by a research team member before the clinic, given time to read and discuss the patient information sheet and recruited a member of the research team (see Invitation letter appendix B1).

The sample size (25) was determined pragmatically based on what could be delivered within the funding commitment and the ability to recruit IBD patients in established clinical remission who were willing to undergo multiple assessments and had 7 days EEN. There was no control arm of IBD patients or healthy controls over the same observation period.

### 3.3.2 Study Design

INTICO-1 characterised the nutritional and disease state of CD subjects before and after 7 days of standardisation of intake through the replacement of all food with a formula prescribed at a volume to meet the requirements for energy and nutrients: exclusive enteral nutrition (EEN). Subjects underwent three nutritional assessments: before (Assessment 1) and then after (Assessment 2) EEN, and finally after 14 days of 'washout' on an unrestricted diet (Assessment 3). Nutritional status was assessed by a 7-day food diary analysis followed by the three evaluations of micronutrient status, body composition, and health-related quality of life (HR-QoL).

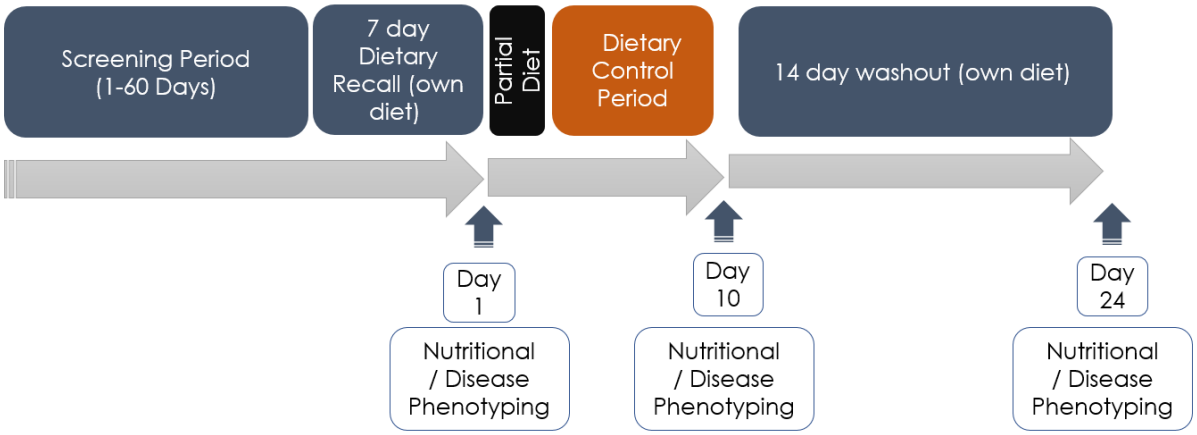


Figure 5: INTICO-1 Study schedule. Timeline of the study schedule.

Blue vertical arrows denote the three study assessments (of nutritional and disease state): Assessment 1 (day 1), Assessment 2 (day 10) and Assessment 4 (day 24). Orange box- period of dietary control

#### 3.3.2.1 Study subjects

The study aimed to capture the nutritional state of subjects whose treatment met the definitions for disease remission, i.e., those considered appropriately controlled on their current treatment. Exclusion criteria sought to remove any other factors that may confound the nutritional assessments.

#### 3.3.2.2 Disease inclusion criteria

- A confirmed diagnosis of ileal or ileocolonic Crohn's disease confirmed by clinical, endoscopic or histological features, in keeping with international guidance.
- Age 18-85
- Clinical remission defined by a Harvey Bradshaw Index (HBI) of <5
- Biochemical remission (faecal calprotectin of <250µg/g)
- Stable on their current medication for 3 months.

#### 3.3.2.3 Disease exclusion criteria

- Prescribed Glucocorticosteroids in the preceding 3 months.
- Not in intestinal continuity (e.g., ileostomy)
- Diagnosis or suspicion of short bowel syndrome.
- Documented symptomatic intestinal strictures in the previous 12 months
- Previous diagnosis of diabetes mellitus.

#### 3.3.2.4 Nutrition-specific exclusion criteria

- The presence of self-reported significant dietary restrictions which may confound results (low residue, gluten-free diet, or veganism)
- Under a current dietetic management plan
- Documentation of or reporting of a previous diagnosis of an eating disorder.
- BMI of <18.5kg/$m^2$

### 3.3.3 Nutritional and Disease Assessments and Intervention

#### 3.3.3.1 Screening visit - Estimation of requirements and energy prescription

Habitual energy requirements were estimated as part of the baseline nutritional assessment to help evaluate the food diary's accuracy and inform the prescription of the dietary intervention. At the screening visit, subjects' probable daily energy requirement for total energy expenditure (TEE) was estimated from their measured Basal Metabolic Rate (BMR) and reported physical activity. BMR was determined by indirect calorimetry (GEM) over 30 minutes after an overnight fast, following the Standard Operating Procedures (SOP) of the Clinical Research Facility. The principle of indirect calorimetry is that oxygen consumption represents the sum of the subjects' oxidative processes and energy consumption (276). Subjects lay underneath a hood ventilated with a known flow rate and gas composition. The oxygen and carbon dioxide concentration difference between the inflow and outflow was measured every minute. The mean oxygen consumption and carbon dioxide production per minute for minutes 5-30 of the study were then converted to a daily estimated resting energy expenditure(277).

The study Specialist Dietitian (CW) calculated an estimated dietary energy requirement to match TEE. This was done with GEM-estimated BMR multiplied by an average physical activity level (PAL) for the day based on each subject's reported typical activity levels using the methods described (278).

Recognising the potential uncertainties in estimating TEE, each subject was provided with a written prescription of their likely minimum and maximum daily energy requirement. They were issued sufficient supplements to match the maximum volume of feed required for each day.

#### *3.3.3.2* Assessment of Intake

##### 3.3.3.2.1 Free diet assessment

Dietary intake was assessed to estimate the micronutrient adequacy of the habitual diet. In the week before assessment 1, subjects were asked to record all food and drink for seven days using Myfood24®, a validated self-completed online dietary recall tool as previously described (279). Myfood24 is a web-based tool that matched the intake to a database of 45,000 foods and generates a daily total energy, macro, and micronutrient amount. Subjects' dietary supplements were recorded, and the micronutrient content of the specific supplements was added to each day's intake. This was extracted and converted to a 7-day average. Subjects

unable to use or access the internet were given a paper food diary with instructions to record the volume and ingredients of each day's intake. This was then checked at visit 2 by a study team member and entered retrospectively into the myfood24 web tool.

#### 3.3.3.2.2 Assessment of intake during dietary control

The intake of the volume of the EEN consumed each day was recorded by the study participants on a patient portal. This volume was combined with the manufacturer's information on the macro and micronutrient concentration per 100ml and added to the study data. Average daily micronutrient data was calculated and expressed (where available) as a percentage of UK reference nutritional intake (RNI) and compared to lower reference nutritional intake (LRNI) (280). This information was required to enable dietary control (see below) and capture detailed energy and nutrient intake information.

#### 3.3.3.2.3 Dietary control

The dietary control stage of the study sought to remove the variability between subjects' free diet and assure adequacy of intake with a nutritionally complete formula for 7 days. The energy requirement for each subject was estimated at screening using an indirect calorimeter (GEM) via the Wier method to estimate BMR with physical activity level multiplier (PAL) calculated according to typical daily activity. The dietary intervention comprised oral nutrition with an oral polymeric feed (Modulen IBD) instead of all food as exclusive enteral nutrition (EEN). EEN was prescribed at various volumes to meet subjects' estimated energy requirements.

A gradual introduction of the diet was used to reduce the symptoms when transitioning from a free diet to an exclusively liquid diet. On day 1, subjects were asked to substitute 1/3 of their usual diet and on day two with 2/3. On days 3 to 9, subjects had 7 days of exclusive nutrition with the supplement instead of food. Water and black caffeinated drinks were permitted. Subjects tasted and were taught how to prepare and take the EEN according to the study dietitian. They were also encouraged to contact the team for study withdrawal if they could not tolerate a safe minimum intake.

#### *3.3.3.3* Assessment of Serum Micronutrients

At each study visit, blood samples were collected to analyse circulating micronutrient levels, nutritionally sensitive metabolites, amino acids and haematology. The presence of systemic inflammation was identified by C-reactive protein, and intestinal inflammation was identified with faecal calprotectin. All samples were taken after an overnight fast. Unless otherwise stated, samples were analysed through the accredited Pathology service at University Hospital Southampton. Samples for B2, B6, and red cell selenium were aliquoted and analysed at the Scottish Trace Element and Micronutrient Diagnostic Research Laboratory. A list of the analysts analysed in UK pathology laboratories is displayed in Table 4 and Table 5.

Table 4: Blood Micronutrient Analyses assessed through accredited laboratories at UHS and Glasgow

| **Analyte** | |
|---|---|
| **Liposoluble Vitamins** | **Hydrosoluble Vitamins** |
| Vitamin A | Vitamin B2 |
| Vitamin D (total) | Vitamin B6 |
| Vitamin E | Folate (B9) |
| **Trace Elements** | Vitamin B12 |
| Calcium corrected | **Hb and Iron Indices** |
| Copper | Haemoglobin |
| Magnesium | Ferritin |
| Phosphate | Transferrin Saturation |
| Selenium | Soluble Transferrin Receptor Index |
| Red Cell Selenium | Serum Inflammatory Markers |
| Zinc | C-reactive protein (CRP) |

Table 5: Amino acid Analysis Completed at UHS Biochemistry

| **Amino acids** | | | |
|---|---|---|---|
| Homocysteine | Glutamine | Valine | Tyrosine |
| Taurine | Proline | Methionine | Phenylalanine |
| Threonine | Glycine | Isoleucine | Ornithine |
| Serine | Alanine | Leucine | Lysine |
| Glutamic Acid | Citrulline | Histidine | Arginine |

#### 3.3.3.3.1 UHS Micronutrient analyses

Vitamin A and E were extracted from serum and measured using a proprietary pre-mixed solution in reaction vials supplied by Chromsystems, then analysed by reverse-phase ultra-high performance liquid chromatography (UHPLC) with detection at 325 nm and 295nm. Vitamin B12 was measured in serum using a competitive binding immunoenzymatic assay (solid phase anti-intrinsic factor alkaline phosphate conjugate). Folate (B9) was assayed in serum using a competitive binding receptor assay. Vitamin D status was assessed using a 25(OH) D assessed using a two-step competitive binding immunoenzymatic assay. B9, B12, and 25-OH D were analysed using the indirect Immunofluorescence seen using the Tri-level Multichem IA Immunoassay, Technopath Product No. IA 310X.

Iron status was assessed using ferritin and the log soluble transferrin receptor index ratio(281). Ferritin was measured in serum using a two-step immunoenzymatic (“sandwich”) assay via the Tri-level Multichem IA Immunoassay, Technopath Product No. IA 310X. Soluble transferrin receptor was measured in plasma using a two-step immunoenzymatic (“sandwich”) assay with the Beckman Coulter Access sTfR, Access sTfR QC2 and QC3 Product No. B11057

Copper, Selenium, and Zinc were measured in serum using inductively coupled plasma mass spectrometry (ICP-MS) using the Perkin-Elmer NexION 300D analyser. The matrix was matched using Sigma bovine serum (Lot no: B9433, Sigma-Aldrich). Three control levels were used for in-house quality control for trace elements.

#### 3.3.3.3.2 Scottish Trace Element and Micronutrient Diagnostic Research Laboratory

Vitamin B2 status was checked by measuring flavin adenine dinucleotide (FAD) in erythrocytes with isocratic high-performance liquid chromatography (HPLC) with a reversed-phase C18 column and fluorescence detection. Vitamin B6 (pyridoxal phosphate) status was also assessed through its concentration in erythrocytes by HPLC, using precolumn semicarbazide derivatisation and fluorescence detection. Vitamin B2 and B6 concentrations in red cells were adjusted to haemoglobin concentration.

Blood results were compared to the clinical laboratory's reported normal ranges.

#### 3.3.3.3.3 Extended Micronutrient Profile Nestle Health Sciences

An extensive micronutrient profile of Hydrosoluble vitamins, liposoluble Vitamins, fatty acids, minerals/trace elements and amino acids was completed through collaborating laboratories at the Nestlé Institute of Health Sciences in Lausanne (see Table 6). These laboratories did not have reference data from matched controls, but as analytes were measured at each of the three nutritional assessment timepoints, comparative statistics were performed,

The following analytes were reported:

Table 6: Extended Micronutrient Profile at Nestlé Institute of Health Sciences

| **Hydrosoluble Vitamins** | **Liposoluble Vitamins** | **Mineral** |
|---|---|---|
| Nicotinamide | Alpha Tocopherol | Iodine |
| Nicotinic acid | Beta Tocopherol | Molybdenum |
| Pantothenic acid | Gamma Tocopherol | Phosphorous |
| Pyridoxic acid | Vitamin K1 | Sulphur |

#### 3.3.3.4 Assessment of Form- Bio-electrical impedance analysis

Body form was assessed using bioelectrical impedance analysis (BIA) using the SECA mBCA 515 (medical Body Composition Analyser) with associated SECA BIA analysis software. In line with the Biomedical Research Centre (BRC) protocols for the standardisation of BIA measurements. The BIA measurements were 1) measured values, i.e. raw impedance data; 2) derived, i.e. estimated body compartments using manufacturer-provided equations; and 3) Standardised, derived measurements corrected for height or converted to SDS or "Z"-scores for age and sex.

Table 7: Bioelectrical impedance analysis measurements

| **Measured** | **Derived** | **Standardised** |
|---|---|---|
| R200 /R5, Phase angle 50KHz | Fat-Free Mass, Fat Mass, Total Body Water (TBW), Extra-cellular water (ECW) | Fat-Free Mass Index (FFMI) Z-score<br><br>Fat Mass Index (FMI) Z-score |

#### *3.3.3.5* Assessment of function - Health-related Quality of Life

Health-related quality of life (HR-QOL) was captured at each assessment using generic (short form 36(SF36) and EQ-5d-5L) and IBD-specific (IBD Control) patient-reported outcome measures (PROMs), which are described below.

##### 3.3.3.5.1 Short Form 36 (SF-36)

The SF36, developed as part of the Medical Outcomes Surgery, assesses health-related quality of life in various medical conditions and interventions (282, 283). It comprises 36 questions with answers scored as grades 0-100 combined to calculate eight scores. The SF36 scores explored in this study were for vitality (SF36VT), physical functioning (SF36PF), Physical limitation (RP) and general health, which are generated from a mean of between 4 and 10 questions. SF36-PF and SF36-VT, generated from 10 and 4-question responses, respectively, have been reported as a measure of fatigue in IBD studies and were the composite scores of interest (182). The SF36 scores were converted to Z-scores using a healthy matched UK

population (284). A sample of the questions, together with the scoring of each question and domain, is included in Appendix B5**.**

#### 3.3.3.5.2 EQ-5D-5L

The EQ-5D-5L is a 5-level recent iteration of a standardised health status measure developed by the EuroQol group. 0-100 Visual Analogue Scale (VAS)(285). The first component of EQ-5D-5L comprises five domains of health today: mobility, self-care, activities, pain/discomfort, and anxiety/depression. The participant assigns a score of 1 to 5 to each domain, with 1 representing the lowest degree of trouble in the domain and five the highest. The second component is a visual analogue score of health today, with 100 representing the best and 0 representing the worst. A sample of the EQ-5D-5L is in Appendix B6.

### 3.3.3.6 Disease Activity Assessments

Disease activity was assessed first at the screening to ensure clinical (Harvey-Bradshaw Index <5) and biochemical (faecal calprotectin <250µg/g) remission. At each subsequent visit, disease activity was checked using serum C-reactive protein and faecal calprotectin as biomarkers of inflammation and Harvey Bradshaw Index and IBD-Control (self-completed) as clinical disease scores.

## 3.3.4 Statistical Analysis

Laboratory values outside the quantification limit were substituted with the upper/lower limit of quantification. Statistical analysis was performed in R version 3.4.4 using Tidyverse, SkimR, GGPlot2, and GGPUBR.

Variables were assessed for normality, and changes across the three assessments were evaluated for significance with ANOVA changes in continuous variables (non-parametric), which were assessed using the Wilcoxon signed-rank test. Variables were assessed for normality, and changes in continuous variables across the three assessments were evaluated for significance with ANOVA or Kruskal-Wallis for parametric and non-parametric continuous variables (non-parametric), respectively. Paired-T and Wilcoxon Rank tests were used to compare changes before and after dietary control. 95% confidence intervals (CI) were calculated using the adjusted Wald method.

## 3.4 Results

This section will start by detailing the subject characteristics and baseline nutritional assessment (Assessment 1) from the perspective of dietary adequacy, micronutrient status, form (BIA), and health-related quality of life.

In the second part of the results section, the nutritional assessments after the dietary intervention (Assessment 2) will be considered, specifically whether the markers of nutritional state and disease state change in response to the dietary intervention, whether these changes persist on return to free diet (Assessment 3) and what this reveals about the nutritionally-sensitive aspects of CD remission.

### 3.4.1 Study Subject Characteristics

Two of the 26 subjects who passed screening withdrew before completing assessment 1. One withdrew due to intercurrent illness (diverticulitis), and the study doctor withdrew the other due to difficulty accessing the venous study blood at assessment 1. Results for the remaining 24 subjects who underwent nutritional assessment are described in the table below.

In brief, 13/24 (56%) of participants were female, with CD duration ranging from 1 to 51 years. 14/24 (58%) of subjects had undergone previous resection surgery, and 14/24 (58%) were on a biological medication. Eight subjects took a multivitamin, and seven were on regular B12 injections.

Table 8: INTICO-1 Study subject characteristics including Montreal classification of Crohn's phenotype.

(A1- age onset ≤16 years, A2 onset 17-40, A3 >40 L1- ileal, L2- colonic L3 Ileocolonic, B1- inflammatory, B2 Stricturing, B3 fistulising, p with perianal involvement)

| **Participant characteristics (n=24)** | |
|---|---|
| Age (mean (range)) | 44.4 (22-74) |
| Gender Male (%) | 11 (45.8) |
| Disease Duration (mean (range)) | 11.6 (1-51) |
| Subtype Age = A2 (%) | 16 (66.7) |
| Subtype Age = A3 (%) | 8 (33.3) |
| **Subtype Location =** L1 (%) | 7 (29.8) |
| **Subtype Location =** L3 (%) | 17 (70.8) |
| **Subtype Behaviour** (%) | |
| B1 | 6 (25.0) |
| B1p | 1 (4.2) |
| B2 | 10 (41.7) |
| B2p | 1 (4.2) |
| B3 | 5 (20.8) |
| B3p | 1 (4.2) |
| **Previous resection surgery** (%) | |
| 0 | 14 (58.3) |
| 1 | 9 (37.5) |
| 2 | 1 (4.2) |
| **Vitamin supplementation** | |
| Multivitamin | 8 (33.3) |

| | |
|---|---|
| Vitamin D | 5 (20.8) |
| B12 injection | 7 (29.2) |
| **IBD Medication** (%) | |
| Biologic single agent | 7 (29.2) |
| Biologic and immunomodulator | 7 (29.2) |
| Immunomodulator monotherapy | 4 (16.7) |
| Nil | 6 (25.0) |
| Harvey Bradshaw Index (median [IQR]) | 1.00 [0.00, 2.00] |
| Calprotectin (µg/L) (median [IQR]) | 35.00 [18.25, 44.75] |
| CRP (mg/L) (median [IQR]) | 2.00 [1.00, 2.25] |
| BMI (Kg/m2) (mean (SD)) | 26.66 (5.18) |

### 3.4.2 Baseline Assessment- nutritional state on free diet

#### 3.4.2.1 Common Dietary Inadequacies in Cohort

Self-reported food intake recorded using food diaries was analysed for all 24 subjects. Four subjects wished to use paper food diaries, so the study team added their dietary intake to the database. Of the 24 subjects, one admitted incomplete intake reporting and recorded energy intake that was implausibly small (42% of their calorimetry estimated BMR) and was removed from the dietary analysis. Another subject submitted an incomplete food diary and withdrew during the dietary intervention, so it was removed from the dietary analysis. The results of the remaining 22 are presented below.

Food diary recall analysis estimated the total energy intake at a Median of 1701 kcals (IQR 1507-1962 kcal), which was 1.01 (IQR 0.8-1.18) times greater than the calorimetry estimated resting energy expenditure( see section 3.3.3.1 for method used), this was below the estimated total energy expenditure for subjects, suggesting that the food diary may have been under-reported in terms of either food eaten or portion size. The analyses estimated the median protein intake at 72g (IQR 64-90g), in which 8/22 (36%) subjects were below their RNI of 0.75g / protein per Kg of body weight.

Table 9: Macronutrient content of intake before Assessment 1

(free diet assessed by Myfood24)

| **Nutrient (LRNI)** | Median (IQR) |
|---|---|
| Energy intake (kcal) | 1701 (1507-1962) |
| Energy Intake / measured BMR (PAL) | 1.01(0.86-1.77) |
| Protein (g) | 72(64-90) |
| Intake below RNI for protein 0.75g/Kg (%) | 8(36) |
| Carbohydrate (g) | 195(168-233) |
| Of which Sugar (g) | 80 (62-99) |

Where available from the food diary and Dietary Reference values, the derived estimates of nutrient intake with and without the inclusion of micronutrient supplements were compared to UK LRNI (280). Food diary analyses revealed a range of dietary inadequacies within this cohort. These were most evident for Selenium, Potassium, and Pyridoxic Acid, where 55%, 18% and 18% of the cohort recorded intakes estimated below the LRNI. Other nutrients of concern were Magnesium (14%), Riboflavin (14%), Iodine (14%), Zinc (9%), and Niacin (9%). There was just one subject whose estimated intake failed to match the LRNI for Calcium and B12; all subjects met their LRNI for Sodium, Iron, Thiamine and Vitamin C. One subject's Calcium intake met the LRNI with the addition of the supplements consumed, but no other dietary inadequacies were corrected. Vitamin A and E were not reported as they do not have reference ranges for LRNI.

#### 3.4.2.2 Supplement usage

Subjects reported various prescribed and non-prescribed supplements, which the study dietitians added to the dietary analysis. These included Vitamin D3 in four subjects, a multivitamin in three subjects, Omega 3 with Vitamins A/D/E in two subjects, and subjects who took Vitamin B complexes or a supplement with Magnesium, B Vitamins, and Vitamin C, respectively. Comparisons to LRNI performed before and after adding supplements to recorded intake revealed that these corrected one dietary inadequacy (Calcium) in one subject.

Table 10: Dietary adequacy vs LRNI from myfood24 analysis

| Nutrient (LRNI) | Number (%) with intake below LRNI from diet alone | Number (%) with intake below LRNI from diet plus supplements |
|---|---|---|
| Thiamine B1 (0.23mg/1000kcal) | 0 | 0 |
| Riboflavin B2 (0.8mg) | 3(14) | 3 (14) |
| Niacin B3 (4.4mg/1000kcal) | 2(9) | 2(9) |
| Pyridoxic acid B6 (11µg/g protein) | 4(18) | 4(18) |
| Folate B9 (100µg) | 2(9) | 2(9) |
| Hydroxocobalamin B12 (1µg) | 1(5) | 1(5) |
| Vitamin C (10mg) | 0 | 0 |
| Calcium (400mg) | 1(5) | 0 |
| Iodine (70µg) | 3(14) | 3 (14) |
| Iron (Men all ages, women over 50: 4.7mg) (women 18-50: 8.0mg) | 0 | 0 |
| Magnesium | 3(14) | 3(14) |
| Potassium | 4(18) | 4(18) |
| Selenium | 12(55) | 12(55) |
| Sodium (575mg) | 0 | 0 |
| Zinc (Men: 4.5mg, women: 4.0mg) | 2(9) | 2(9) |
| Total results <LRNI in group | 37(11) | 36 (11) |

#### 3.4.2.3 Assessment of free diet dietary micronutrient adequacy

The low estimated energy content of the free diet suggested underreporting intake, reducing the security of quantitatively assessing micronutrients. Accepting these limitations, analyses of estimated total micronutrients against requirements were performed to identify patterns of nutrients whose intake was at risk and to visualise a qualitative assessment of each individual's dietary quality.

To explore each individual's adequacy of intake across a range of micronutrients before and after the inclusion of supplements in calculations, the derived estimate of nutrient intake reported by the analytical software and for which there was a UK RNI and expressed as a heat map for each individual as a percentage of RNI. Plotting the data in this way allows the map to be explored in two dimensions – firstly, for a given nutrient across the group (reading across the row) or nutrient intake within a given individual (reading down the column). As can be seen in nutrients that were most likely to be limited in the diet (light pink through dark pink were vitamins K and D, Folate, selenium, Iodine, Iron, Magnesium, and Potassium. Some individuals were likelier to report low intakes of multiple nutrients (subjects 8, 12, 15, 19, 20, and 21).

Supplements led some subjects to meet the RNI, principally for Vitamin D (3 subjects) and brought the intake up to the level of the RNI across multiple micronutrients for subject 9. Some supplements (for subjects 15 and 22) increased intake from above the RNI to more than 500% of the RNI (coloured darker green).

This qualitative visualisation of dietary micronutrients demonstrated that individuals with nutritional inadequacies tended to have this across multiple micronutrients. Subjects 8, 12, 15, 19, 20, and 21 were identified as having various micronutrients for which intake was less than 50% of RNI. Except for vitamin D and one subject's multivitamin, the unprescribed supplements used by the groups tended to make little difference concerning whether subjects met the RNI or LRNI.

#### 3.4.2.4 Comparison to the general population

To contextualise the extent to which the cohort's intake may differ in micronutrient adequacy from that of the English population, the data were compared to the most recent National Diet and Nutrition Survey (NDNS) dietary survey from 2020 (286). Briefly, the NDNS estimated intake of a sample of 800 individuals and provided a median intake of energy, folate, iron, calcium,

Vitamin D and sodium. Briefly, the NDNS estimated the daily intake from 4x 24-hour food recalls from a sample of 900 members of the UK population. The estimated daily calorie intake for adults (aged 19-64) in the NDNS was like that of the 19 subjects in this age group who completed food diaries (1775kcal, 1739kcal, respectively), suggesting a similar estimated total intake and that quantitative comparison of estimated daily intake was more secure.

While statistical methods were not used, the median of the micronutrients in the NDNA data is compared to those of the 19 INTICO-1 subjects aged 19-64. Vitamin D and Folate intake were lower than that in the NDNA. The iron and Calcium intakes were closer, and the estimated Sodium intake in the INTICO subjects was greater than that of the NDNS.

Table 11 Numerical comparison of estimated daily micronutrient intake between NDNS and INTICO-1 subjects

(for nutrients analysed in NDNS)

| | **Median estimated NDNS Intake in adults aged 19-64 (2020)** | **Estimated intake from INTICO subjects aged 19-64 (n=19)** |
|---|---|---|
| Vitamin D (µg/day) | 2.2 | 1.4 |
| Folate (µg/day) | 231 | 179 |
| Iron (mg/day) | 9.8 | 9.2 |
| Calcium (mg/day) | 746 | 688 |
| Sodium (mg/day) | 1931 | 2558 |

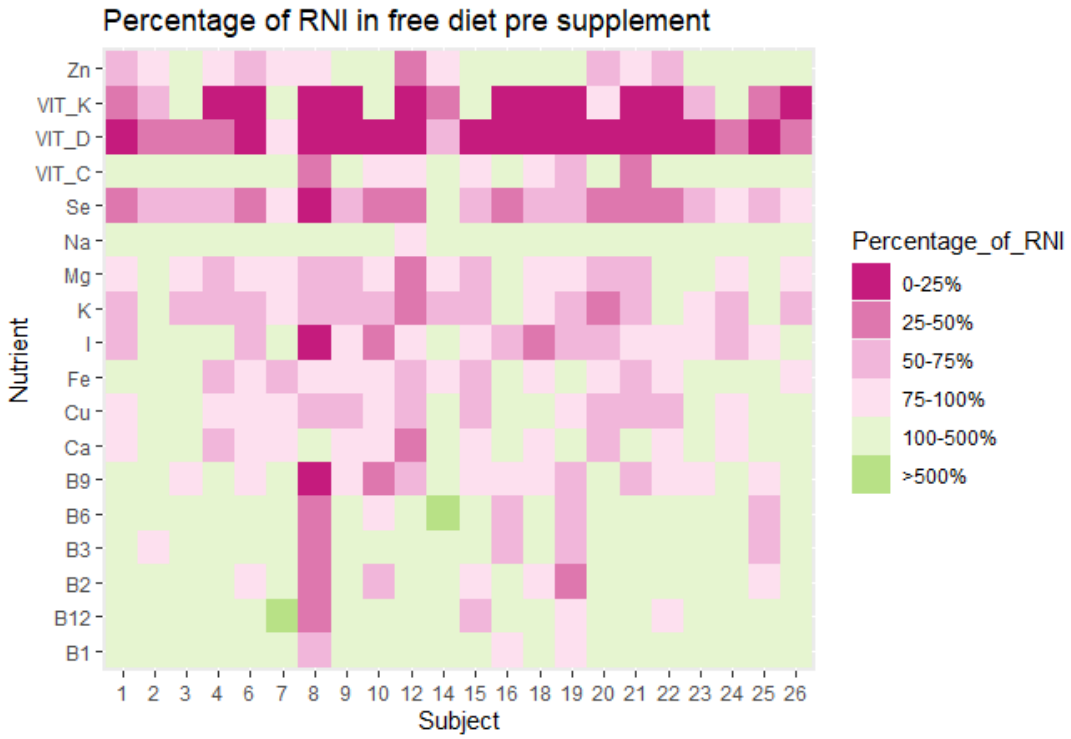


Figure 6: Dietary analysis of free diet micronutrients as a percentage of RNI pre-supplement

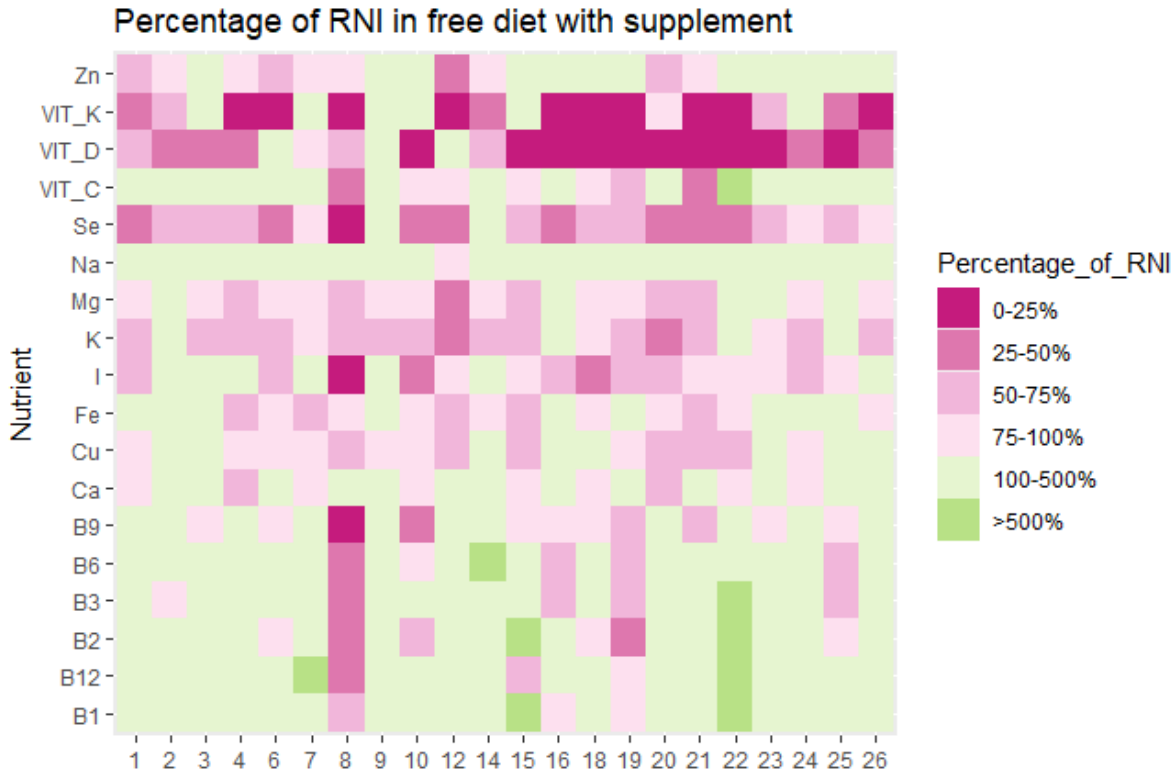


Figure 7 Dietary analysis of free diet as percentage of RNI after addition of supplement

#### 3.4.2.5 Baseline Assessment- Biochemical indices of micronutrient blood status

At baseline (Assessment 1), multiple individuals had one or more blood tests below laboratory normal ranges. Among the 23 individuals, 26 results suggested the risk of deficiency, with the commonest being Vitamin D in 5/23 (21.8%), Copper in 4/23 (17.4%), Vitamin B12 in 4/23 (17.4%) log soluble transferrin receptor index (a marker of iron deficiency) and folate (both in 3/23 (13.0%)).

The extent to which subjects had abnormal blood tests varied. One subject (20) had six results suggesting a risk of deficiency, two individuals had three, and eight individuals had none. Each subject's blood results are displayed in Figure 9 with each nutrient arranged in rows and each subject in columns. Pink marks a blood test at a range of possible deficiency, and green marks a result in the normal range.

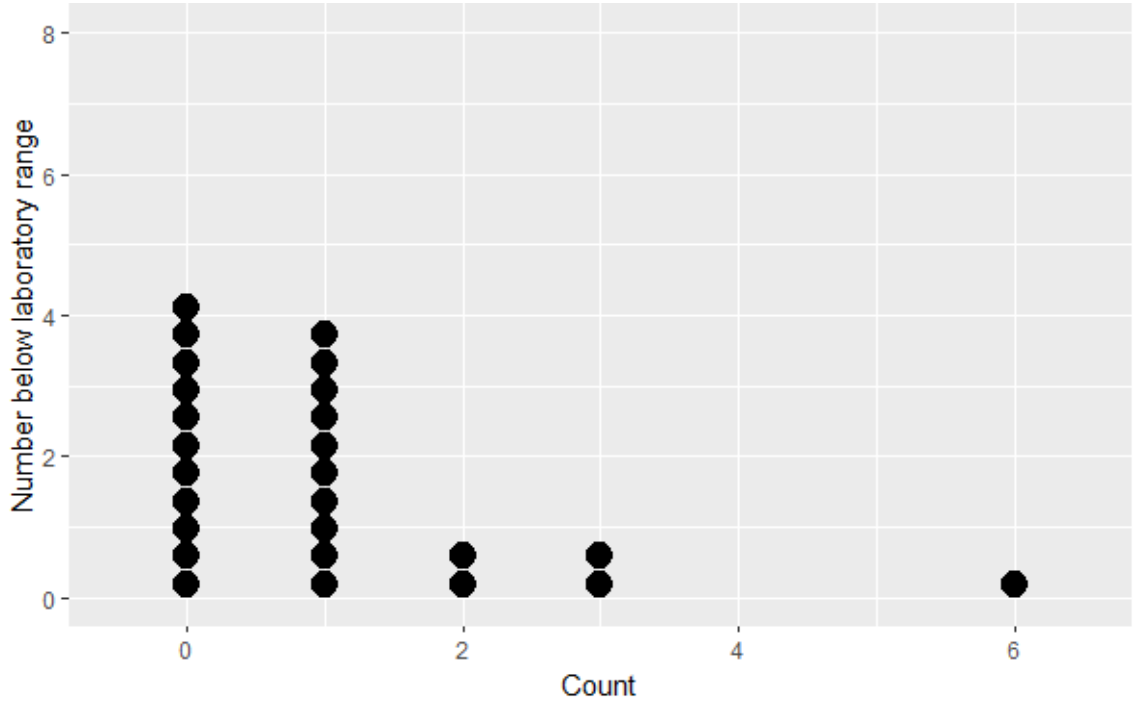


Figure 8: Number of micronutrient analytes below clinical laboratory normal range at baseline (pre-diet)

Table 12: Prevalence of blood tests below normal range at assessment 1 (baseline, on an unrestricted or habitual diet) *soluble transferrin receptor index elevated in iron deficiency.

| **Analyte (normal range)** | **Number (%) below NR** |
|---|---|
| **Liposoluble Vitamins** | |
| Vitamin A (1.07-.3.55µmol/L) | 1 (4) |
| Vitamin D total (50-374nmol/L) | 5 (21) |
| Vitamin E (13.2-46.4µmol/L) | 0 |
| **Hydrosoluble Vitamins** | |
| Vitamin B2 (1.0-3.4nmol/g Hb) | 0 |
| Vitamin B6 (250-680pml/g Hb) | 1 (4) |
| Folate B9 (2.9-20.6ng/ml) | 3(13) |
| Vitamin B12 (160ng/L) | 4 (21) |
| **Trace Elements** | **Number (%) below NR** |
| Calcium corrected (2.20-2.60mmol/L) | 1 (4) |
| Copper (12-26µmol/L) | 4 (17) |
| Magnesium (0.70-1.00nmol/L) | 0 |
| Phosphate (0.80-1.50nmol/L) | 1 (4) |
| Potassium (3.5-5.3nmol/L) | 0 |
| Selenium (0.80-2.0µmol/L) | 2 (8) |
| Sodium (133-143nmol/L) | 0 |
| Zinc (11-24µmol/L) | 1(4) |
| **Hb and Iron Indices** | |
| Haemoglobin (120-150g/L) | 0 |
| Ferritin (11-307µg/L) | 0 |
| Transferrin Saturation (16-45%) | 1 (4) |
| Soluble Transferrin Receptor Index (>12 in iron deficiency) | 3 (13) |

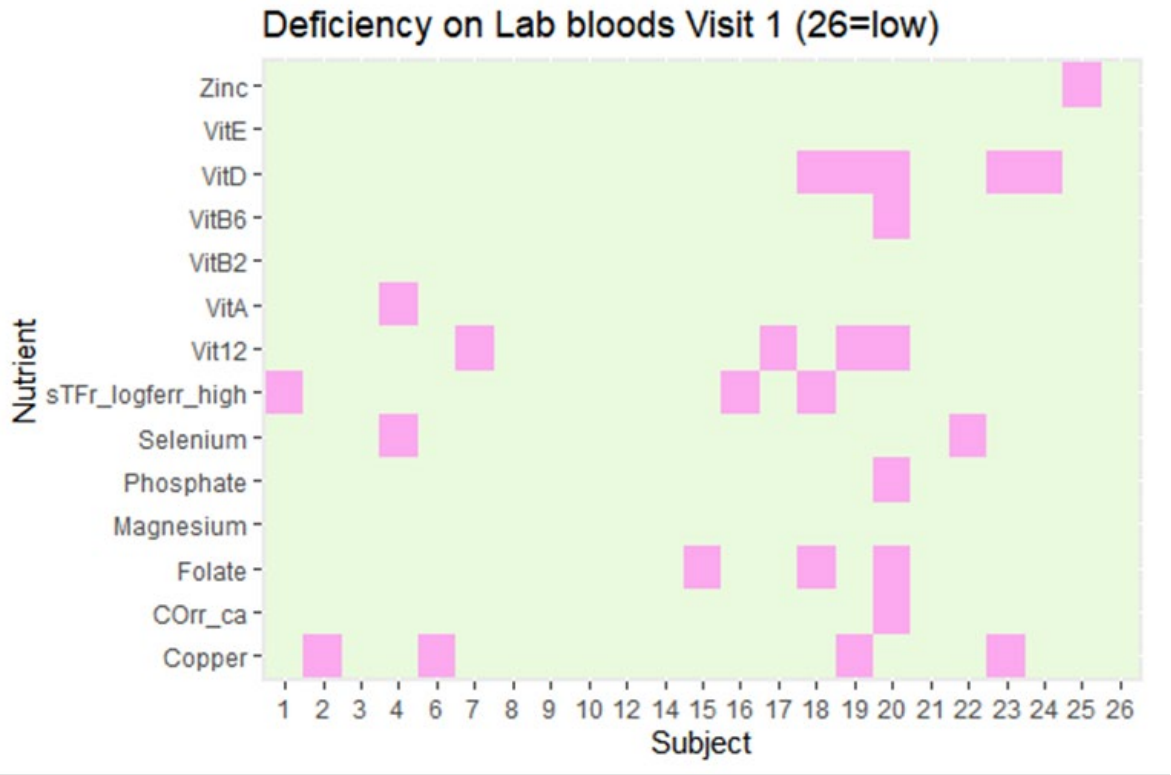


Figure 9: Blood tests below the normal range (coloured pink) by subject (column) and nutrient (row)

#### 3.4.2.6 Baseline nutritional state- Form

At assessment 1, as assessed by body mass index (BMI), 6/24 subjects were overweight (≥25.0 Kg/m$^2$), and 8/24 were obese (≥30.0 Kg/m$^2$). One subject who had lost weight since his screening assessment was underweight by BMI (< 18.5 kg/m$^2$). Women tended to be overweight by BMI (Median BMI 29.2kg/m$^2$).

| Anthropometric Measure | Median (range) Men | Median (range) Women |
|---|---|---|
| Height (m) | 1.76(1.53-1.66) | 1.63(1.61-1.86) |
| Weight (kg) | 69.5(54.5-93.3) | 72.6(54.7-119.7) |
| Body Mass Index (kg/m$^2$) | 23.2(18.4-34.8) | 29.2(20.5-34.3) |
| **Estimated from BIA** | | |
| Fat Mass (kg) | 11.9(12.2-47.7) | 31.5(4.5-45.3) |
| Fat Mass Index (kg/m2) | 4.6(1.7-13.1) | 12.0(4.8-17.5) |
| Fat Mass Index- Z score | -0.74((-1.9-2.7) | 1.1(-1.2-2.8) |
| Fat-Free Mass (kg) | 57.8(45.8-77.1) | 42.8(37.6-48.8) |
| Fat-Free Mass Index (kg/m2) | 19.3(15.1-22.9) | 16.2(13.6-18.1) |
| Fat-Free Mass Index- Z score | -0.33(-3.23-2.07) | -0.33(-1.94-1.37) |

Table 13: Body composition at Assessment 1 (baseline)

#### 3.4.2.6.1 BIA data-derived values to Estimate body composition

Using BIA to estimate body composition identified lean mass deficits within the population. Five subjects (20.1%) had an estimated fat-free mass index Z-scores (fat Mass corrected for height, age and sex) below the 15$^{th}$ centile, with 2/24 (8.3%) below the 5$^{th}$ centile. Two men and two women were above the 95$^{th}$ percentile of age, and their sex-matched fat-mass index (see Figure 10) Two men and two women met their respective malnutrition (GLIM) criteria for a reduced lean mass as assessed by fat-free mass index (<17.0 Kg/m$^2$ and <15.0 Kg/m$^2$, respectively) (287). Women tended to have an increased age and sex-corrected fat mass Index (Median FFMIz 1.1), and men tended to have a relatively reduced fat-free mass index (FFMIz -0.33).

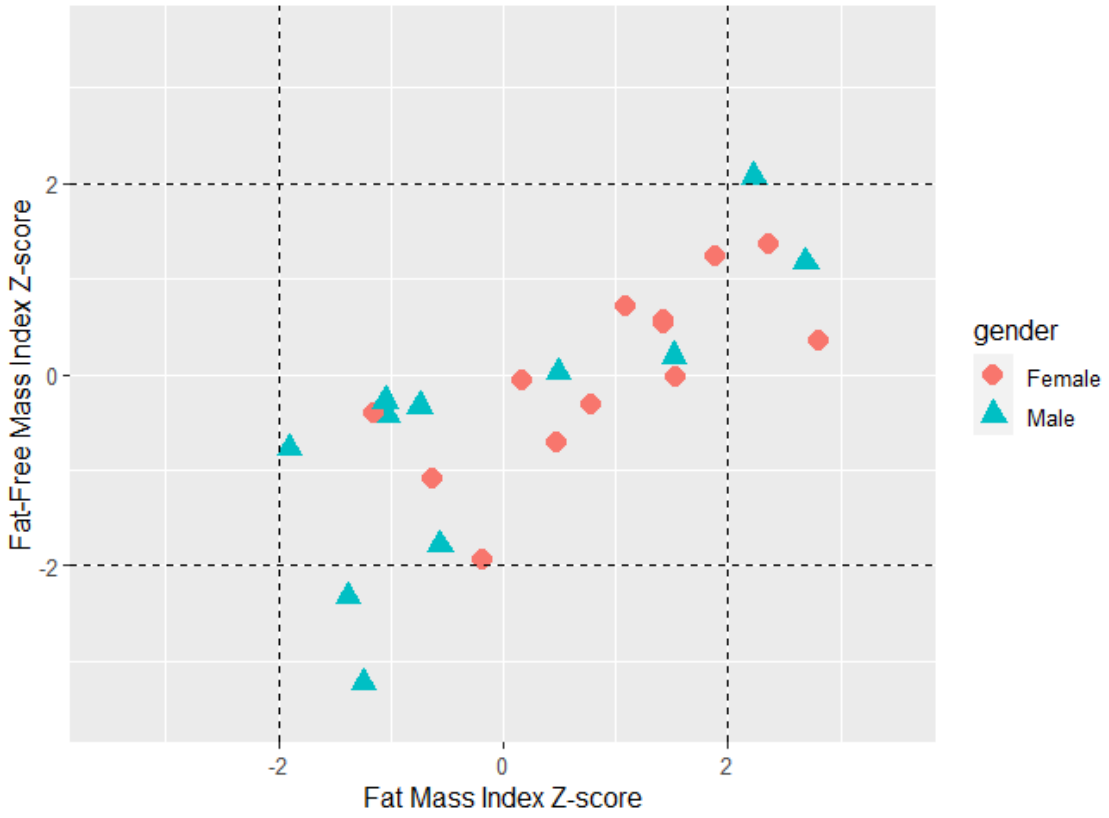


Figure 10: Fat Mass Index Z-score vs Fat-free Mass Index Z-score by gender for study subjects

Dashed lines represent 5th and 95th centiles of Fat Mass Index "Z" score and Fat-Free Mass "Z" score, respectively

#### 3.4.2.6.2 Raw BIA Data

For subjects aged below 70 (n=23), the phase angle at 50KHz was compared to the BIA manufacturer's age and sex-matched population reference data. The Median phase angle Z-score of the cohort was -0.37 (a lower phase angle being correlated elsewhere to a poor nutritional state and adverse outcome), with most of the values below the 50$^{th}$ centile (i.e. below average for the reference population); one subject had a Z-score below the 5$^{th}$ centile, and six were below the 15$^{th}$ centile.

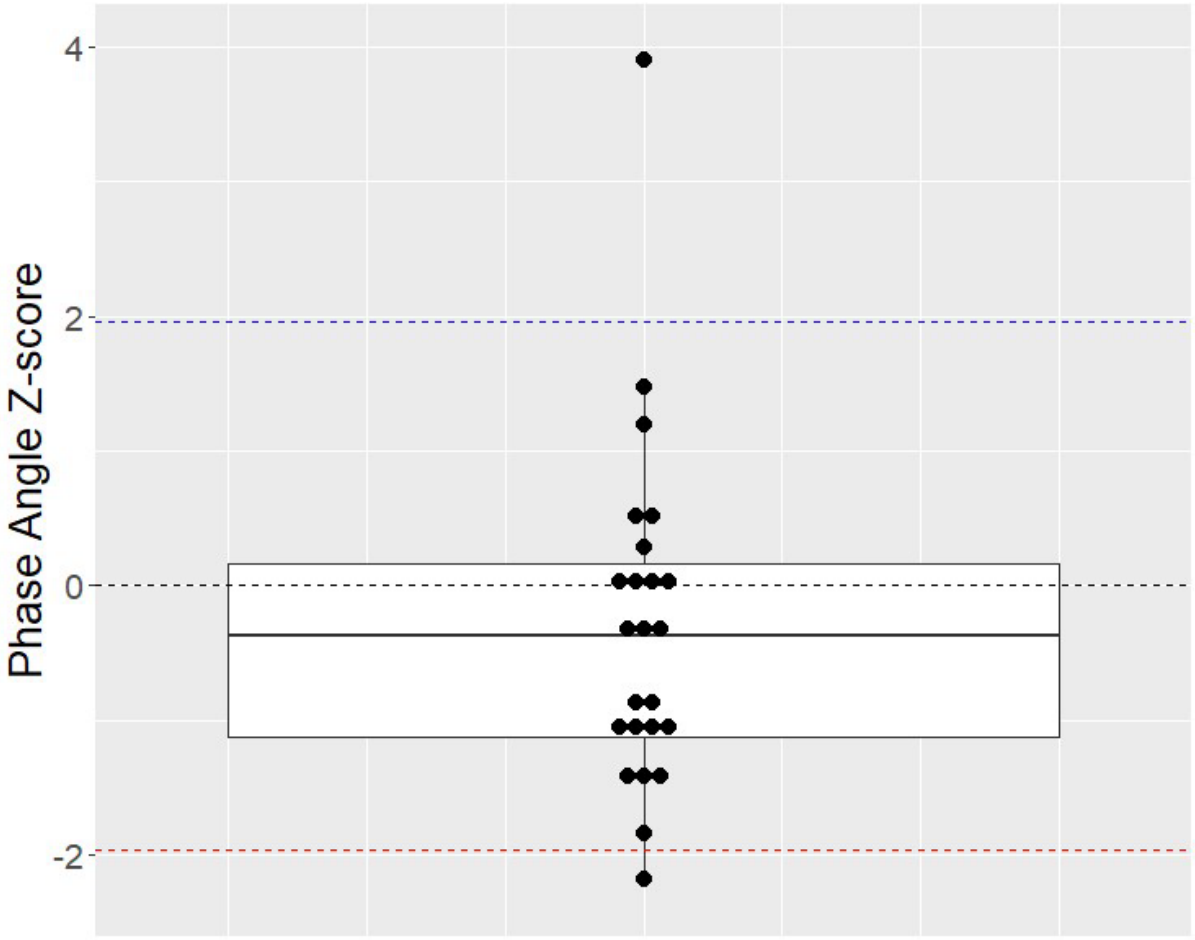


Figure 11: Phase angle Z-score at Assessment 1 (baseline)

#### 3.4.2.7 Baseline Assessment- health-related quality of life (HR-QOL)

##### 3.4.2.7.1 IBD-control PROM

At assessment 1, the patient-reported outcome measures (PROMs) identified evidence of fatigue symptoms and an impaired HR-QOL score. Of the 24 subjects who completed the IBD control PROM at assessment 1, 9 (36%) responded "Yes" (from the options of yes/no/not sure) to the statement that they had been *"often lacking in energy (fatigued)*".

##### 3.4.2.7.2 Short Form 36 (SF-36)

23 subjects completed a baseline SF-36, which generated different domains to assess HR-QOL. Energy/vitality, physical functioning, role limitation from physical symptoms, and general health were compared to values from a healthy UK population. Figure 10 displays the mean Z-score of these domains and the 95% confidence intervals at Assessment 1. Subjects rated their general health below population norms, and there was a trend towards a lower SF-36-VT score.

| SF-36 Domain | Mean Z score (95% CI) |
|---|---|
| Physical functioning (SF-36-PF) | 0.08(-0.20 to 0.36) |
| Vitality (SF-36-VT) | -0.29(-0.74 to 0.15) |
| Role-limited physical (SF-36-RL) | -0.29(-0.97 to 0.38) |
| General health (SF-36-GH) | -0.64(-1.06 to -0.23) |

Table 14: SF36 Domains of interest at baseline, expressed as Z-score

The Z scores for these SF-36 domains at assessment 1, displayed in Figure 12, demonstrating variability in each of them among the study subjects in each of these domains.

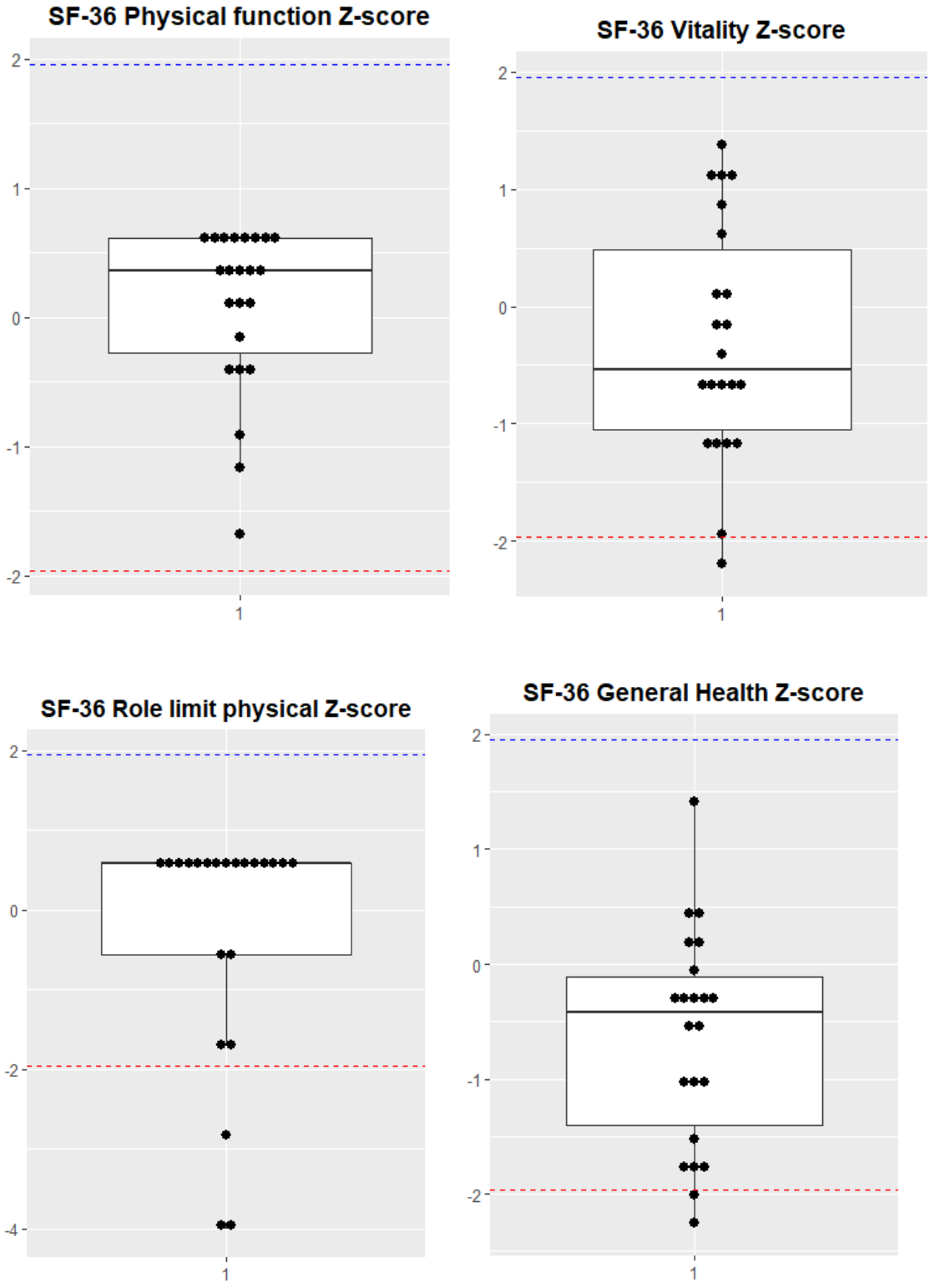


Figure 12: SF36 Domains of Physical functioning, Vitality, role limit, physical and general health, expressed as Z-scores for matched UK population.

Blue dashed line 95th centile, red dashed line 5th centile

### 3.4.3 The effects of a time-limited Trial of Controlled nutrition

One subject withdrew from the study and did not attend further nutritional assessments. The intake, blood tests, and body composition results were removed from the results table and graphs to compare these parameters before and after the intervention.

#### 3.4.3.1 Change of Intake vs Free Diet

Twenty-four subjects commenced the dietary control with exclusive enteral nutrition (EEN). Subjects' daily consumption of EEN was ad libitum, with a suggested range of requirements based on their calorimetry-calculated BMR and typical physical activity levels (PAL multiplier). For the 7 days of the dietary control (EEN), participants recorded their total supplement intake (Modulen IBD®). This means that the daily volume of EEN intake was, in turn, used with the manufacturer's information to calculate the average daily calorie, macronutrient, and micronutrient intake.

One participant contacted the team to report that they could not tolerate the liquid diet at a volume adequate to meet their nutritional needs and was advised to return to regular food.

Three other subjects struggled to consume the recommended amount but felt they had consumed a safe proportion of their target intake and were kept in the study. Two subjects needed more than the upper estimate of their requirements; one contacted the team to ask for more, and the other subject did not contact the team and reported hunger. The macronutrient and micronutrient content of the EEN dietary control, in comparison to the amount estimated from the myfood24 analysis of their free diet for the 23 subjects who completed the diet, is displayed in Dietary adequacy of EEN was expressed as a percentage of RNI per nutrient in each subject and shown in Figure 14. The subjects who could not continue the study did not notify the study team of how much EEN they had consumed. They did not attend the Assessment 2 visit for blood tests, body measurements, or PROMS, which was not included in this figure or any subsequent results.

#### 3.4.3.1.1 Change in intake- macronutrients

The total recorded median daily energy intake increased from 1701 kcal in the food diary analysis to 2004 kcal during dietary control. The free diet estimated PAL was 1.01, which increased to 1.22 during dietary control. The subjects who recorded less than the RNI of protein intake (0.75g per Kg body weight) fell from 36% during the free diet to 18% during dietary control (see Table 15).

Table 15: Macronutrient intake in free diet analysis (My Food24) vs. dietary control

(For continuous variables (i.e. not percentages) Paired Wilcoxon Rank Visit 1 vs Visit 2 P <0.05*, <0.01**, <0.001 ***, <0.0001****)

| **Nutrient (LRNI)** | **Free Diet Analysis** | **Dietary Control** |
|---|---|---|
| | **Median (IQR)** | **Median (IQR)** |
| Energy intake (kcal) | 1664 [1396-1934] | 2007 [1660-2353] ** |
| Energy Intake / measured BMR (PAL) | 1.01 [0.86-1.16] | 1.22 [1.12-1.32] ** |
| Protein (g) | 66 [54-79] | 72 [60-84] |
| Intake below RNI for protein 0.75g/Kg (%) | 8 [36] | 4 [18] |
| Carbohydrate (g) | 193 [161-225] | 220 [182-258] |
| Of which Sugar (g) | 80 [63-98] ** | 60 [49-70] |

#### 3.4.3.1.2 Change in Intake- Micronutrients

The aim of the EEN as a period of nutritional control was to ensure micronutrient adequacy of the diet, which was achieved mainly at the volume consumed by the study subjects. The intake of 15 nutrients was compared in both “free diet” and during “dietary control” against UK LRNIs. These nutrients were chosen if there was a UK LRNI, which was included in the Myfood24 analysis. During the dietary control period, the only nutrients for which the recorded intake fell below the LRNI among the 22 subjects were Sodium in 4/22 (18.2%) and Potassium in 4/22 (18.2%). Across the 15 nutrients analysed, there were eight occasions when the LRNI for a nutrient was not met, compared to 43 from the free diet analysis. The average daily intake and number of individuals whose recorded intake was below the LRNI for each nutrient is compared between free diet, free diet plus supplement and dietary control is compared inTable 16 and

Table 17. The number of individuals with intake below LRNI fell for all nutrients except Sodium, for which there were previously no intakes below LRNI, and Potassium, which was unchanged.

compare intake as a percentage of RNI between the free diet plus supplements and the dietary control, with each nutrient in a row and each subject in a column (see Figure 13 and Figure 14). This demonstrates that, at the volume consumed, the period of dietary control ensured that subjects met RNIs for the analyzed micronutrients, which was not the case with the analysed free diet.

Table 16: Adequacy of Micronutrient intake in free diet analysis (My Food24) vs. dietary control

Number and percentage of the 23 subjects who completed all three visits and had dietary intakes below LRNI by nutrients in free diet, free diet with supplement and during dietary control (EEN). Supplements (which brought one subject's intake in Calcium over the LRNI were included in Assessment 1 estimate of intake)

| **Nutrient (LRNI)** | **Number (%) with intake (including supplements) below LRNI from free diet** | **Number (%) with intake below LRNI during dietary control (EEN)** |
|---|---|---|
| Thiamine B1 (0.23mg/1000kcal) | 0 | 0 |
| Riboflavin B2 (0.8mg) | 3 (13) | 0 |
| Niacin B3 (4.4mg/1000kcal) | 2(8) | 0 |
| Pyridoxic acid B6 (11µg/g protein) | 4(17) | 0 |
| Folate B9 (100µg) | 2(8) | 0 |
| Hydroxocobalamin B12 (1µg) | 1(4) | 0 |
| Vitamin C (10mg) | 0 | 0 |
| Calcium (400mg) | 1(4) | 0 |
| Iodine (70µg) | 3 (13) | 0 |
| Iron (Men all ages, women over 50: 4.7mg) (women 18-50: 8.0mg) | 1(4) | 0 |
| Magnesium | 4(17) | 0 |
| Potassium | 5(21) | 4 (17) |
| Selenium | 13(54) | 0 |
| Sodium (575mg) | 0 | 4(17) |
| Zinc (Men: 4.5mg, women: 4.0mg) | 3 (13) | 0 |
| **Total number of nutrients with intake below LRNI in cohort** | 42 | 8 |

Table 17: Estimated daily micronutrient intake during free diet versus during dietary control

(Paired Wilcoxon Rank Visit 1 vs Visit 2 P <0.05*, <0.01**, <0.001 ***, <0.0001****)

| **Nutrient (LRNI, RNI)** | **Median (IQR) Intake during free diet estimated from food diary** | **Median (IQR)Intake during dietary control with EEN** |
|---|---|---|
| Thiamine B1 (0.23mg/1000kcal, 0.4mg/100kcal) | 1.3 [1.2-1.7] | 2.4 [2.1-3.0] **** |
| Riboflavin B2 (0.8mg, 1.3mg(m), 1.1mg(f) | 1.3 [1.1-1.9] | 2.6[2.3-3.3] **** |
| Niacin B3 (4.4mg/1000kcal,6.6mg/1000kcal) | 16.9 [13.8-20.6] | 24.0[21.5-30.0] *** |
| Pyridoxic acid B6 (11µg/g protein,15µg/g protein) | 1.5 [1.2-1.9] | 3.4[3.0-4.3] **** |
| Folate B9 (100µg,200µg) | 176 [153-300] | 481[429-600] **** |
| Hydroxocobalamin B12 (1µg,1.5µg)) | 3.1 [2.2-4.2] | 6.4[5.7-8.0] **** |
| Vitamin C (10mg,40mg) | 51.4 [36.5—80.4] | 194[173-242] **** |
| Vitamin D (no LRNI,10µg) | 1.4 [1.1-2.8] | 20.0[17.9-25.0] **** |
| Vitamin K (no LRNI,1µg/kg) | 21.1 [8.4-62.5] | 110[98-138] *** |
| Calcium (400mg,700mg) | 693 [596-901] | 1823[1628-2275] **** |
| Copper (no LRNI,1.2mg) | 1.70 [0.75-1.4] | 2.00[1.80-2.50] *** |
| Iodine (70µg,140µg) | 107 [88.8-138] | 200[179-250] **** |
| Iron (M all ages, f over 50): 4.7mg,8.7mg) (f 18-50: 8.0mg,14.8mg) | 8.8 [7.9-11.8] | 22.0 [19.7-27.5] **** |
| Magnesium ((m)190mg,300mg;(f18-50)150mg,270mg, (f>50)190mg,270mg) | 232 [197-256] | 401 [358-500] **** |
| Potassium (2000mg,3500mg) | 2345 [2111-2858] | 2404 [2147-3000] |
| Selenium ((m)40µg,75µg, (f)40µg,60µg) | 33.9 [27.6-46.2[ | 70.1 [62.6-87.5] **** |
| Sodium (575mg,1600mg) | 2546 [2128-2961[ **** | 701 [626-875] |
| Zinc (m): 5.5mg, 9.5mg, (f)4.0mg,7.0mg) | 7.5[6.7-9.1] | 19.2 [17.2-24.0] **** |

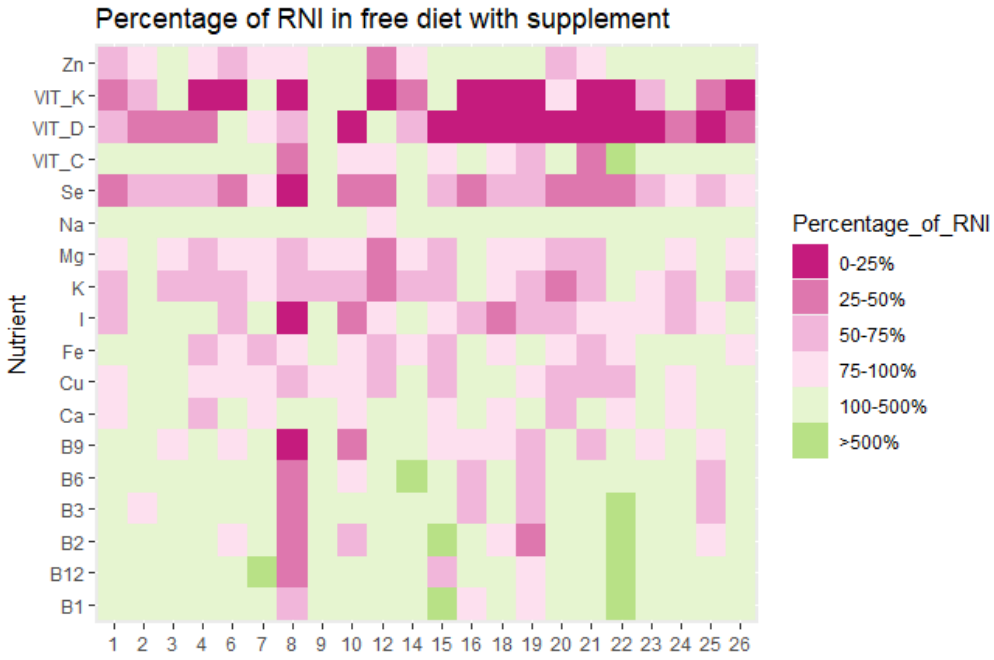


Figure 13: Percentage of RNI by nutrient and subject in free diet and supplement

(Pink denotes less than RNI, darker pink is a lower percentage, green denotes above RNI, darker green is greater than 500% RNI

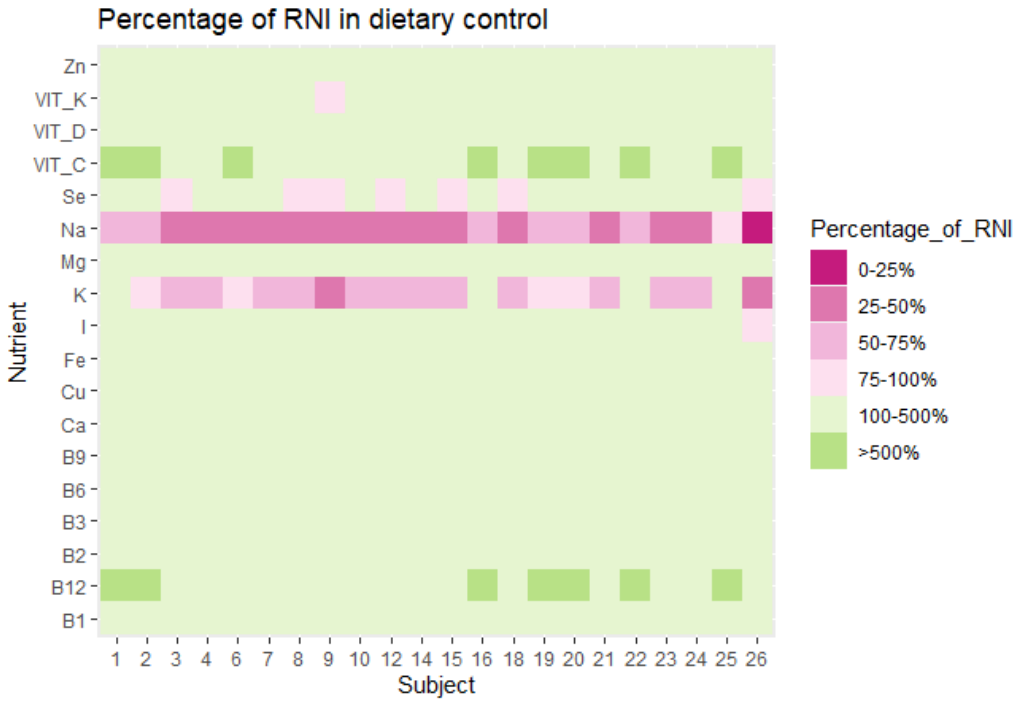


Figure 14: Percentage of RNI by nutrient and subject consumed in dietary control EEN

#### 3.4.3.2 Change in Micronutrient Blood Biochemistry

Twenty-three study subjects underwent repeat nutritional assessments. The period of dietary control (between assessments 1 and 2) led to changes in the blood micronutrient concentration, correcting 38% of the abnormal blood tests present at baseline in the group. Following the period of dietary control, the total number of blood results (across the 15 nutrients studied among the 23 subjects) below the normal range fell from 26 to 16 (a fall of 38%). Four of the 16 abnormal results seen at assessment 2 were newly abnormal (two low Zinc and two low Potassium). Zinc was inversely correlated to the CRP (nil other micronutrients were correlated), which for some was elevated at assessment 2, which may, in part, have accounted for this. The resolution in most of the low blood tests was transient; at assessment 3 (after two weeks of free diet), the blood tests below the normal range re-increased from 16 to 22.

There was a statistically significant increase in the following micronutrients following the dietary intervention (between assessments 1 and 2): Vitamins A, D, E, B2, B6, and B9 (folate): vitamin E, Calcium, Copper, Phosphate, and Selenium. The change appeared to revert upon return to the free diet (assessment 3) for all nutrients except B6 (pyridoxic acid) and B9 (Folate), for which a statistically significant difference remained in the comparisons of assessment 1 to assessment 3. The number of subjects with blood tests below the normal range and the median concentration of each micronutrient at assessment 1 (baseline- post-free diet), assessment 2 (post dietary control), and assessment 3 (return to free diet) are shown below in Table 18

For the micronutrients that were analysed at collaborating laboratories in the Nestle Institute of Health Sciences (NIHS), Alpha Tocopherol, the water-soluble Vitamins Nicotinamide (B3), Pantothenic Acid (B5) and Pyridoxic Acid (B6), 5- Methyl tetrahydrofolate and the mineral Sulphur saw a statistically significant increase following the dietary intervention (Table 19) Of these, only Pyridoxic acid remained higher at assessment 3. Vitamin K, Gamma Tocopherol and Iodine did not change. Riboflavin was below the laboratory level of quantification in fewer subjects after the dietary intervention (1/23) and after washout (10/23) than at assessment 1 (15/23).

For the amino acids, there was a statistically significant rise in the homocysteine between assessments 1 and 2 and a statistically significant fall between visits 1 and 3 and visits 2 and 3 (see Table 19). Ornithine and lysine also showed a statistically significant change between assessments 1 and 2. The other 17/20 amino acids did not show any changes between the visits. After applying a Bonferroni adjustment for the 20 amino acids, the only changes that met

statistical significance were the changes in homocysteine between visits 2 and 3. One study subject with multiple B vitamin deficiencies had a markedly elevated Homocysteine at baseline (53.2µmol/l), which fell progressively to 38.9µmol at visit 3.

Changes in each of the 15 micronutrients analysed in the clinical laboratories over the three assessments are shown in Figure 15, Figure 16, Figure 17, Figure 18 and Figure 19. The changes in the micronutrients analysed in NIHS are shown in Figure 20, Figure 21 and Figure 22, and the amino acids Homocysteine (with and without the outlier subject), Lysine and Ornithine are shown in Figure 23 .

Table 18: Change in micronutrient concentrations over study. (Paired Wilcoxon Rank Visit 1 vs Visit 2 P <0.05*, <0.01**, <0.001 ***, <0.0001****, Paired Wilcoxon Rank Visit 1 vs Visit 3 P <0.05 ‡, <0.01 ‡‡, <0.001 ‡‡‡, <0.0001 ‡‡‡‡, Paired Wilcoxon Rank Visit 2 vs Visit 3 ʃP <0.05ʃ, <0.01ʃʃ, <0.001ʃʃ, <0.0001ʃʃʃʃ)

| **Analyte (normal range)** | **N below range** | **Median (IQR) conc** | **N (%) below range** | **Median (IQR) conc** | **N (%) below range** | **Median (IQR) Conc** |
|---|---|---|---|---|---|---|
| | **Assessment 1** | | **Assessment 2** | | **Assessment 3** | |
| **Liposoluble Vitamins** | | | | | | |
| Vitamin A (1.07-.3.55µmol/L) | 1(4) | 2.3 [1.9, 2.7] | 0 | 2.7 * [2.3, 3.1] | 2(8) | 2.30 ʃʃ [1.9, 2.5] |
| Vitamin D total (50-374nmol/L) | 5(21) | 66.0 [53.5, 86.5] | 2 (8) | 73.0 * [64.5, 86.5] | 5(21) | 63.0ʃʃ [52.0, 78.5] |
| Vitamin E (13.2-46.4µmol/L) | 0 | 25.2 [22.9, 31.6] | 0 | 31.3 **** [26.8, 35.6] | 1(4) | 27.6 ʃ [23.0, 30.80] |
| **Hydrosoluble Vitamins** | | | | | | |
| Vitamin B2 (1.0-3.4nmol/g Hb) | 0 | 1.6 [1.5, 2.0] | 0 | 1.70 ** [1.7, 2.0] | 0 | 1.70 [1.5, 1.9] |
| Vitamin B6 (250-680pml/g Hb) | 1(4) | 318.0 [301.0, 429.0] | 1 (4) | 480.0 **** [441.0, 584.0] | 1(4) | 396.0 ‡ʃʃʃʃ [349.0, 462.0] |
| Folate B9 (2.9-20.6ng/ml) | 3(13) | 7.70 [5.5, 12.1] | 0 | 13.7 **** [11.0, 18.0] | 1(4) | 9.5 ‡ʃʃʃʃ [6.5, 12.4] |
| Vitamin B12 (160ng/L) | 4(17) | 338.0 [207.5, 510.0] | 1(4) | 446.0 [259.0, 613.8] | 1(4) | 344.0 [223.0, 627.5] |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Minerals** | | | | | | |
| Calcium corrected (2.20-2.60mmol/L) | 1(4) | 2.3 [2.2, 2.3] | 0 | 2.3 * [2.3, 2.4] | 1(4) | 2.3 [2.3, 2.4] |
| Copper (12-26µmol/L) | 4(17) | 16.8 [13.1, 18.2] | 2(8) | 16.7 *** [13.7, 22.1] | 4(17) | 16.ʃ [13.2, 19.9] |
| Magnesium (0.70-1.00mmol/L) | 0 | 0.8 [0.8, 0.8] | 1(4) | 0.8 [0.8, 0.9] | 0 | 0.80ʃ [0.78, 0.81] |
| Phosphate (0.80-1.50mmol/L) | 1(4) | 1.0 [0.9, 1.1] | 1(4) | 1.0 * [0.9, 1.3] | 2(8) | 1.0 [0.9, 1.1] |
| Potassium (3.5-5.3mmol/L) | 0 | 4.0 [3.9, 4.2] | 2(8) | 4.1 [4.0, 4.3] | 0 | 4.0 [3.9, 4.3] |
| Selenium (0.80-2.0µmol/L) | 2(8) | 1.1 [1.0, 1.2] | 0 | 1.4 **** [1.3, 1.6] | 1(4) | 1.2ʃʃʃʃ [1.0, 1.3] |
| Sodium (133-143mmol/L) | 0 | 138.0 [137.0, 139.0] | 1(4) | 137.0 [136.5, 139.0] | 0 | 139.0ʃ [137.5, 140.0] |
| Zinc (11-24µmol/L) | 1(4) | 13.5 [12.5, 14.7] | 3(13) | 13.6 [12.9, 14.9] | 1(4) | 12.6 [12.1, 15.6] |
| **Iron status** | | | | | | |
| Soluble Transferrin Receptor Index (>12 in iron deficiency) | 3(13) | 9.2 [6.6, 12.1] | 2(8) | 9.3 [6.5, 10.8] | 2(8) | 9.3 [7.1, 12.8] |
| **Total Abnormal bloods** | 26 | | 16 | | 22 | |

Table 19: Change in NIHS analytes over Nutritional Assessments

| **Analyte (normal range) Ng/ml** | **Median (IQR) conc** | **Median (IQR) Conc** | **Median (IQR) conc** |
|---|---|---|---|
| **Assessment** | **1** | **2** | **3** |
| **Liposoluble Vitamins** | | | |
| Alpha Tocopherol (ng/ml) | 12000 [10,650, 14,700] | 15600 ** [11600, 1810] | 12400 ʃʃ [10140, 13,800] |
| Gamma Tocopherol (ng/ml) | 559.0 [356.9, 814.6] | 427 [302.3, 586.1] | 560.3 [280.9, 695.6] |
| Vitamin K (ng/ml) | 0.51 [0.31, 0.87] | 0.38 [0.32, 0.67] | 0.47 [0.30, 0.65] |
| **Hydrosoluble Vitamins** | | | |
| Riboflavin (ng/ml) | 15/23 <LLOQ (4ng/ml) | 1/23 <LLOQ (4ng/ml) | 10/23<LLOQ (4ng/ml) |
| Nicotinamide (ng/ml) | 37.9 [27.8, 50.8] | 45.9 * [34.3, 55.7] | 34.4 ʃʃ [23.6, 50.46] |
| Pantothenic acid (ng/ml) | 35.2 [29.0, 40.6] | 42.4 *** [37.9, 52.2] | 36.8 ʃʃ [28.1, 41.8] |
| Pyridoxic acid (ng/ml) | 4.27 [3.27, 5.21] | 8.53 **** [6.40, 9.69] | 4.97 ‡ ʃʃʃʃ [4.00, 5.85] |
| 5-Methyl Tetrahydrofolate (ng/ml) | 9.6 [6.8,13.9] | 17.4 **** 12.6,22.7] | 11.7 ʃʃʃʃ [8.7,14.8] |
| **Minerals** | | | |
| Iodine (ng/ml) | 64.1 [58.3, 73.4] | 70.6 [59.6, 78.7] | 63.4 ʃʃʃ [55.9, 68.4] |
| Sulphur (ng/ml) | 1240000 [1170000, 1280000] | 1270000 * [124000, 132000] | 1220000 ʃʃ [1160000, 1275000] |

Table 20: Change in Amino acid blood measurements over Nutritional Assessments

| **Analyte (normal range) Ng/ml** | **Median (IQR) conc** | **Median (IQR) Conc** | **Median (IQR) conc** |
|---|---|---|---|
| **Assessment** | **1** | **2** | **3** |
| Homocysteine (µmol/l) | 7.4 [6.4, 9.0] | 8.1** [6.7, 10.5] | 7.4‡‡ʃʃʃʃ [5.8, 8.3] |
| Taurine (nmol/ml) | 153 [129, 166] | 159 [125, 165] | 143 [127, 169] |
| Threonine (nmol/ml) | 148 [124, 168] | 131 [110, 169] | 143 [115, 165] |
| Serine (nmol/ml) | 131 [119, 151] | 128 [112, 143] | 128 [112, 143] |
| Glutamic Acid (nmol/ml) | 69 [50, 100] | 82 [52, 104] | 67 [52, 92] |
| Glutamine (nmol/ml) | 547 [505, 579] | 547 [523, 608] | 542 [488, 621] |
| Proline (nmol/ml) | 205 [174, 241] | 207 [187, 265] | 215 [199, 252] |
| Glycine (nmol/ml) | 290 [246, 325] | 281 [238, 335] | 283 [250, 331] |
| Alanine (nmol/ml) | 403 [373, 441] | 376 [344, 471] | 421 [392, 468] |
| Citrulline (nmol/ml) | 31 [29, 40] | 30 [27, 39] | 31 [25, 36] |
| Valine (nmol/ml) | 218 [190, 247] | 225 [200, 264] | 214 [195, 261] |
| Methionine (nmol/ml) | 27 [25, 30] | 28 [27, 34.00] | 29 [24, 33] |
| Isoleucine (nmol/ml) | 56 [53, 63] | 61 [51, 69] | 59 [53, 69] |

| | | | |
|---|---|---|---|
| leucine (nmol/ml) | 116 [106, 136] | 117 [108, 137] | 119 [113, 139] |
| Tyrosine (nmol/ml) | 59 [53, 66] | 64 [56, 71] | 61 [56, 69] |
| Phenylalanine (nmol/ml) | 63 [57, 78] | 72 [65, 80] | 63 [60, 76] |
| Ornithine (nmol/ml) | 97 [85, 114] | 88* [71, 104] | 92 [84, 102] |
| Histidine (nmol/ml) | 82 [75, 93] | 80 [78, 93] | 79 [74, 90] |
| Lysine (nmol/ml) | 183 [156, 205] | 206** [167, 231] | 178ʃ [166, 214] |
| Arginine (nmol/ml) | 89 [74, 100] | 87 [71, 97] | 91 [74, 98] |

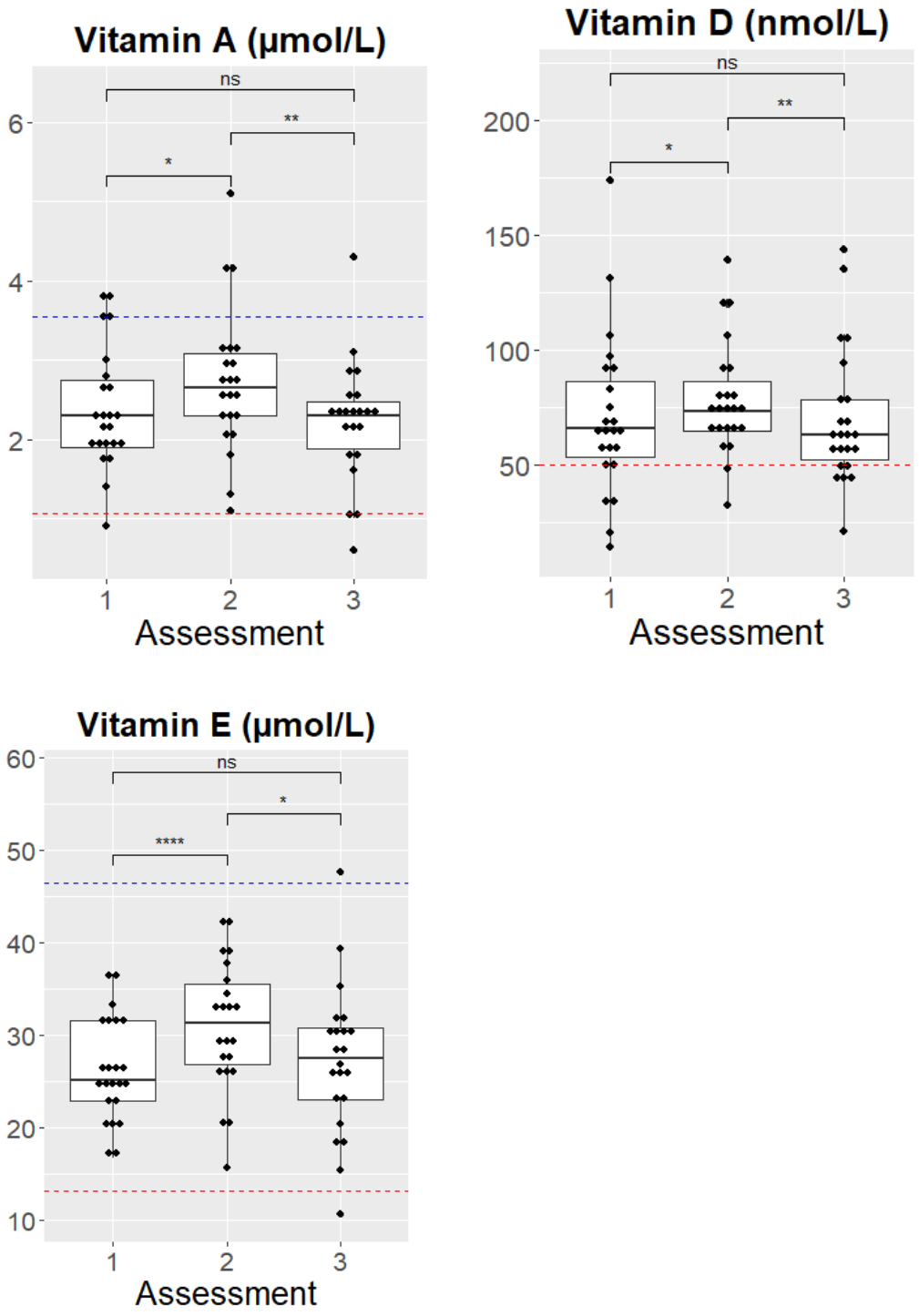


Figure 15: Change in concentration of Fat-Soluble Vitamins (A, D and E) at Assessments 1, 2 and 3

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

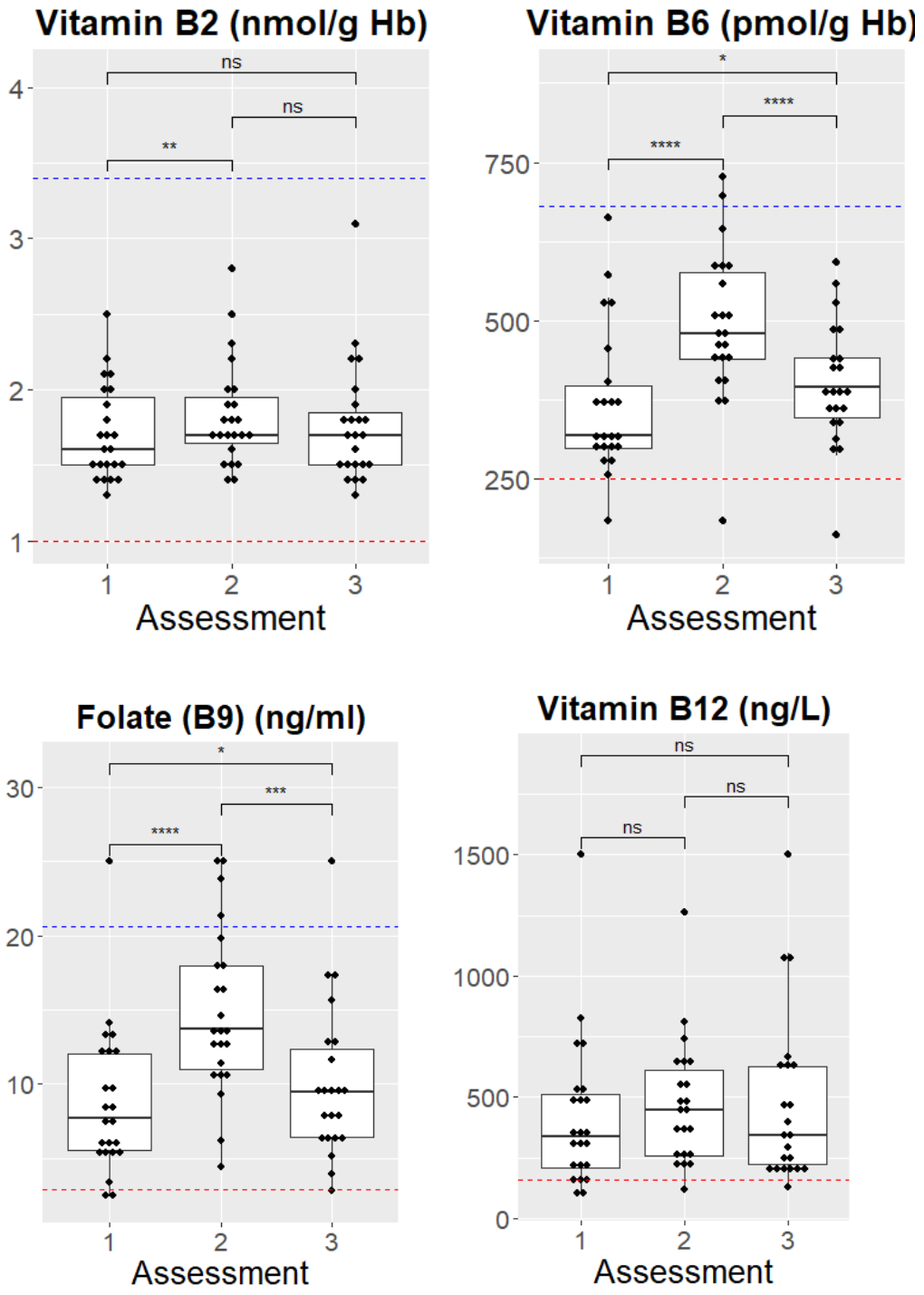


Figure 16: Change in Vitamin B2, B6, B9 (Folate) and B12 at Assessments 1, 2 and 3.

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

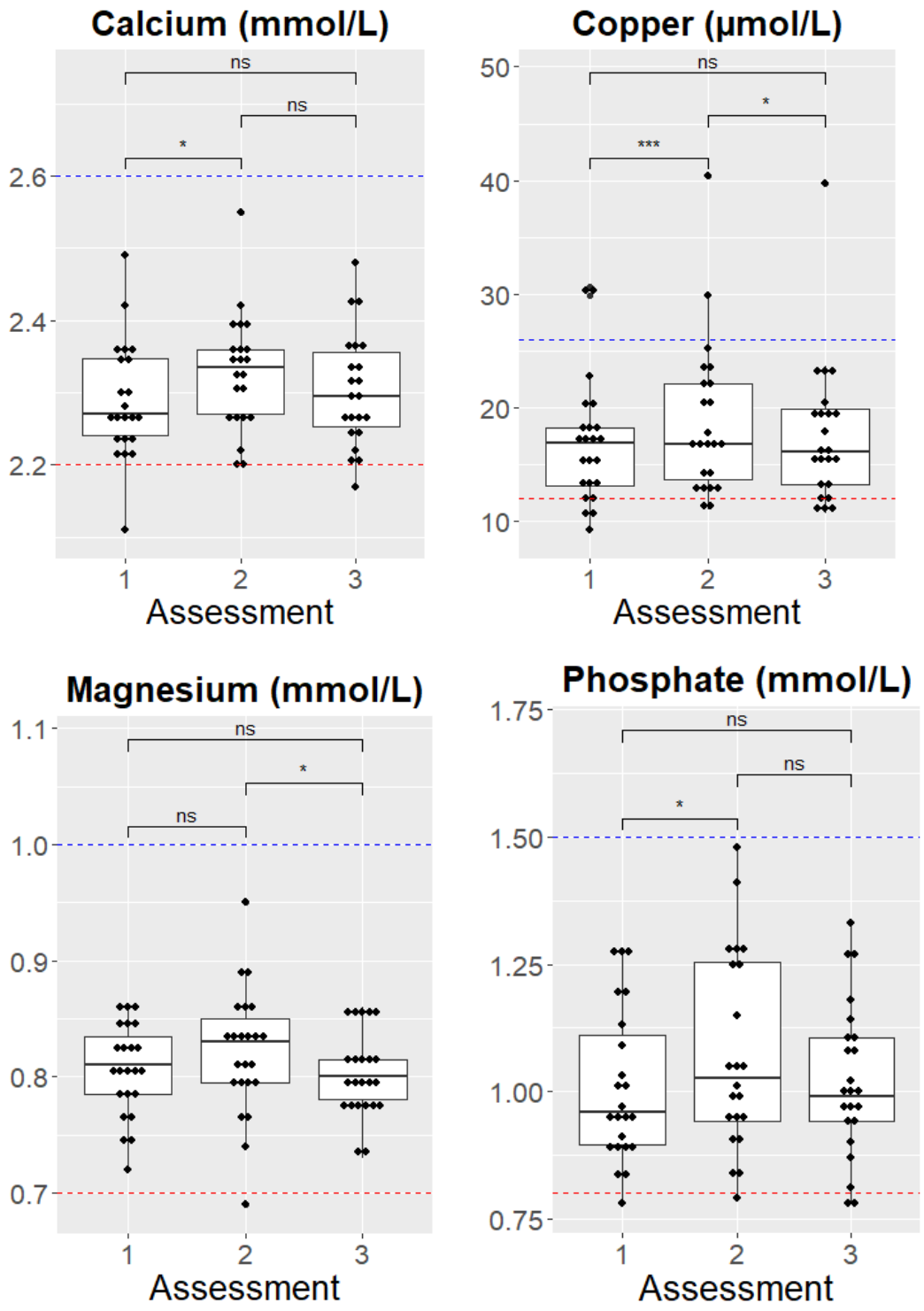


Figure 17: Serum concentrations of minerals (1) at Assessments 1, 2 and 3.

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

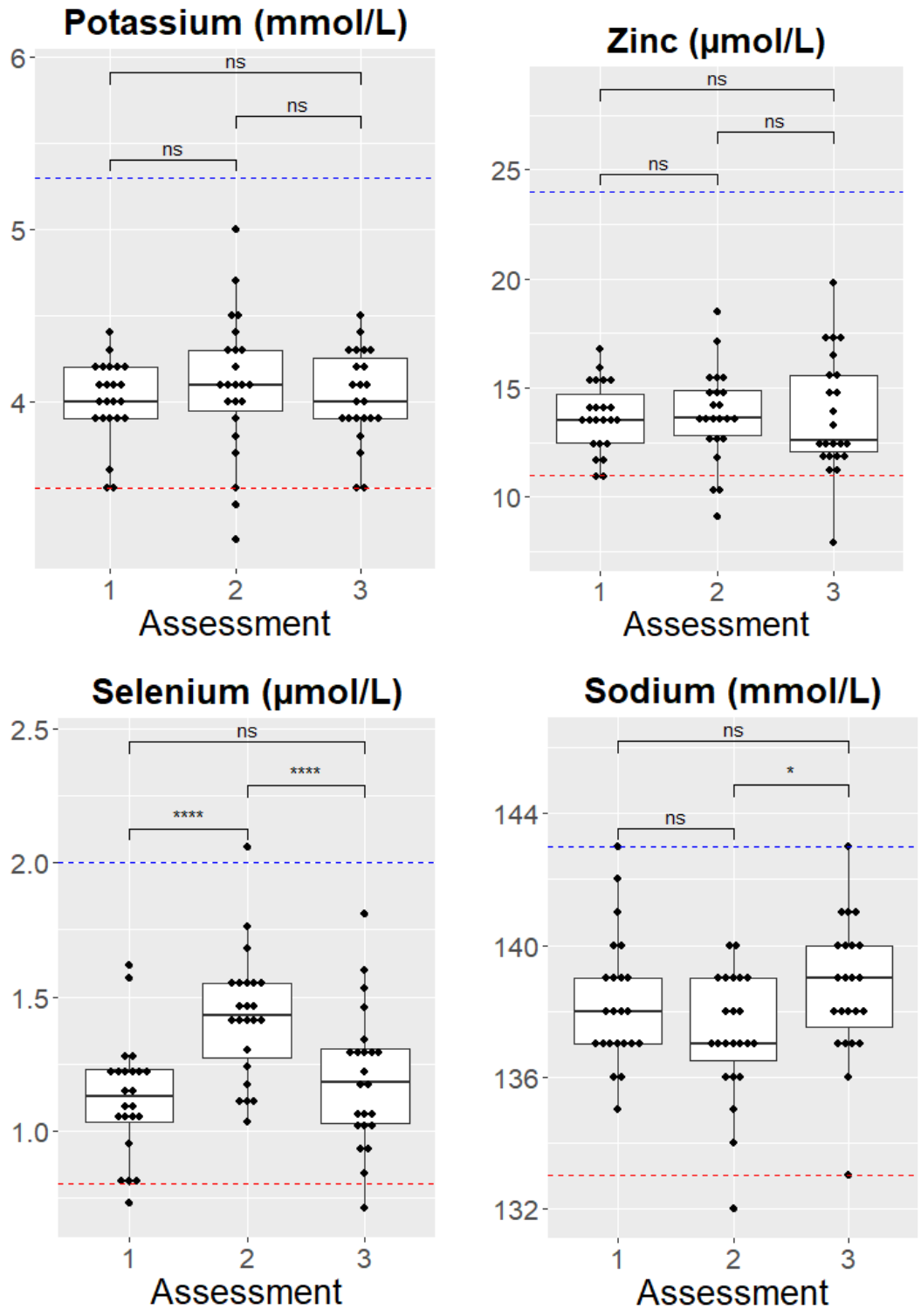


Figure 18: Serum concentrations of minerals (2) at Assessments 1, 2 and 3.

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

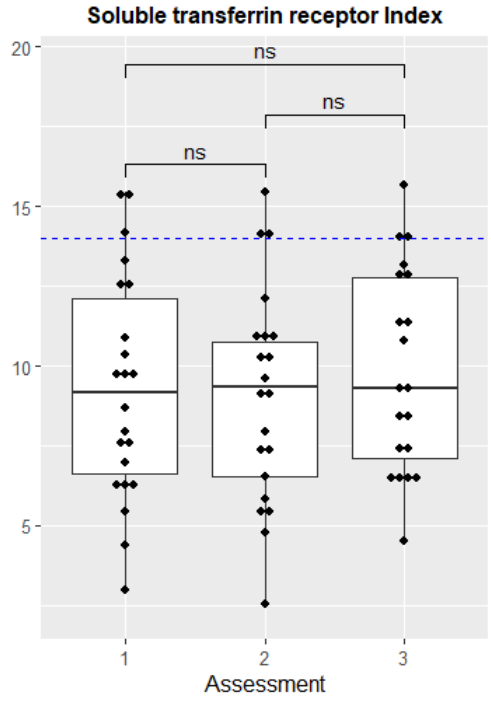


Figure 19: Soluble transferrin receptor index at assessments 1, 2 and 3.

Blue dashed line marks upper limit of normal (14)- increase above 14 suggests iron deficiency Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

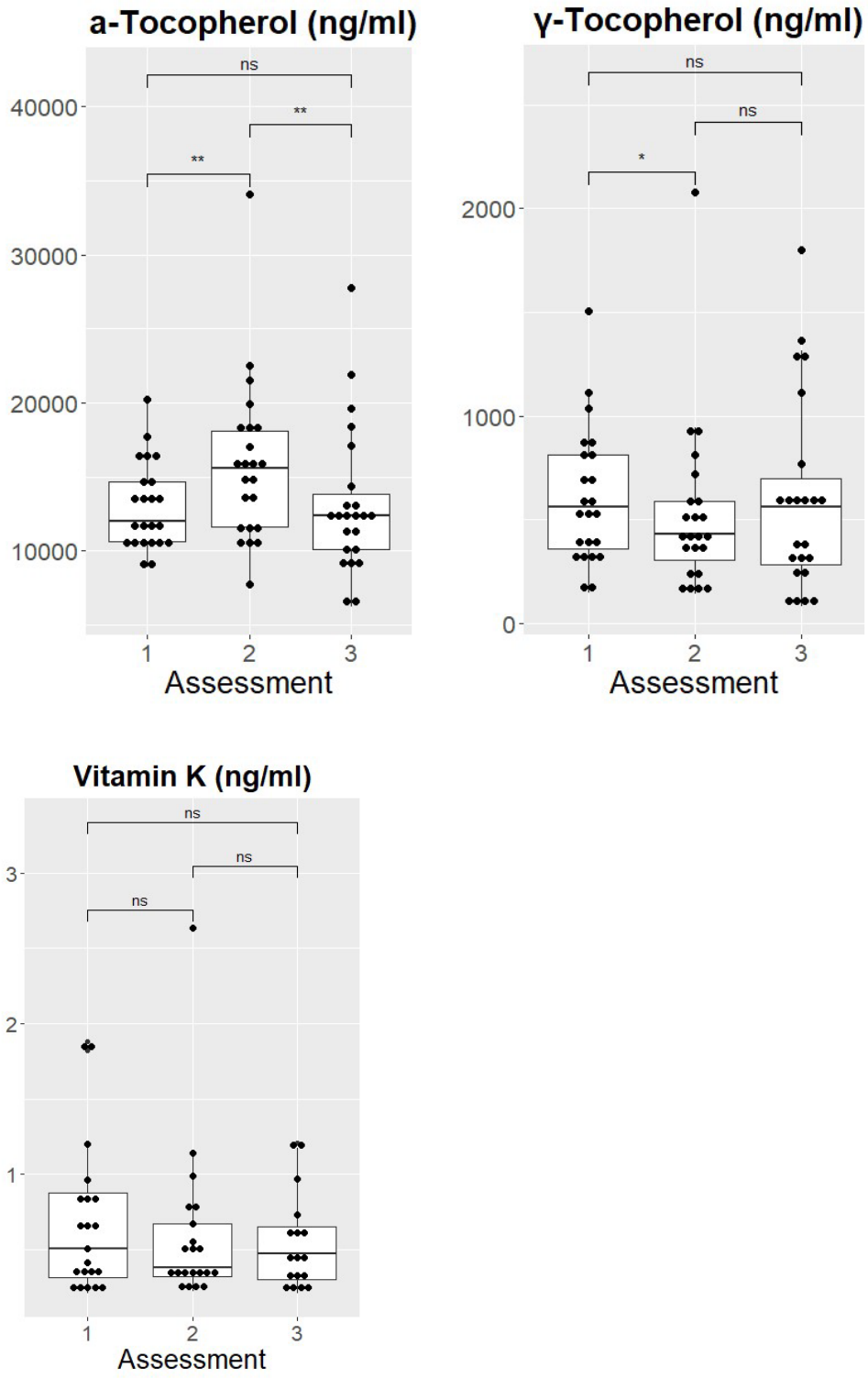


Figure 20 Serum concentrations of nihs liposoluble vitamins Assessments 1, 2 and 3.

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

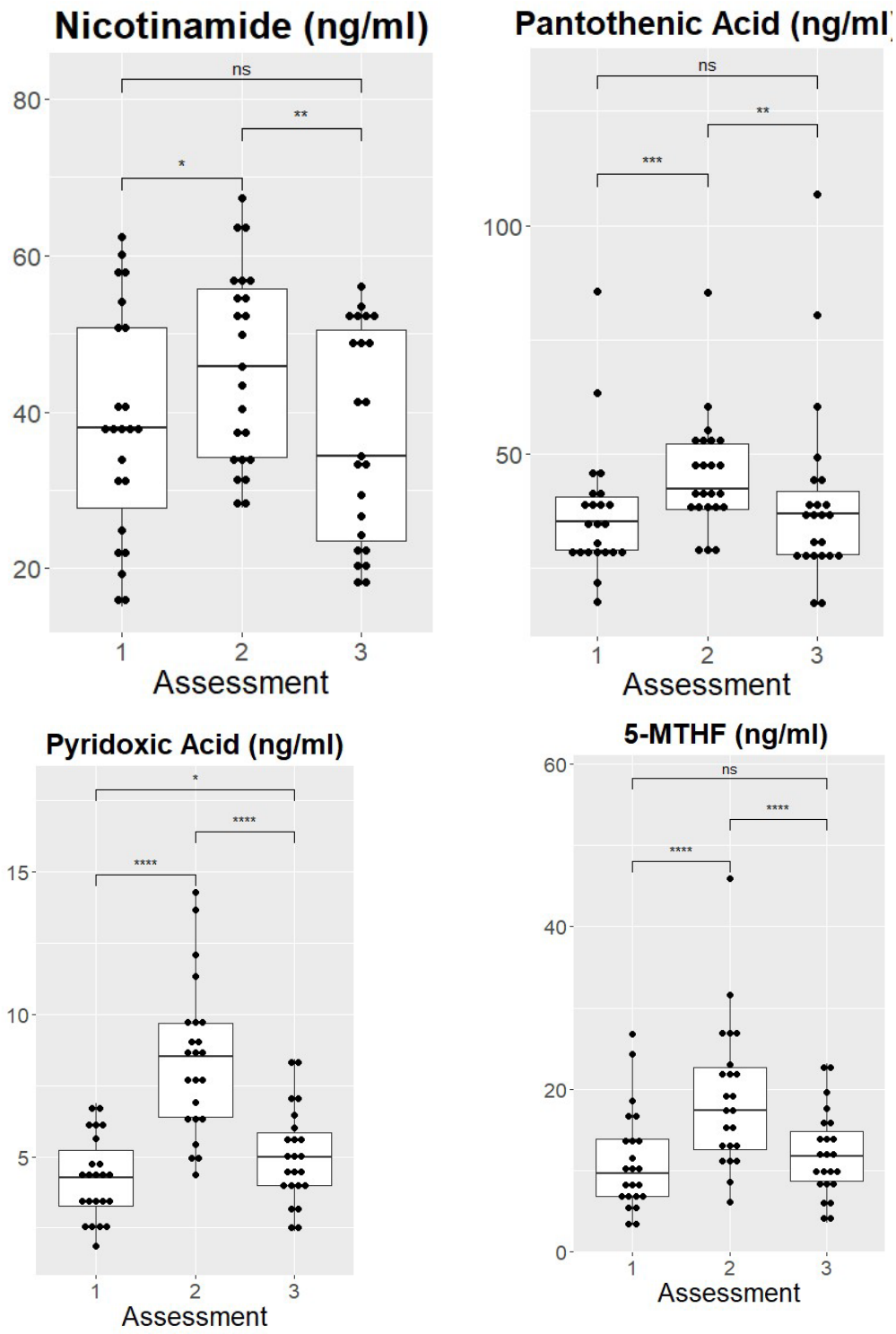


Figure 21 Serum concentrations of nihs hydrosoluble vitamins Assessments 1, 2 and 3.

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

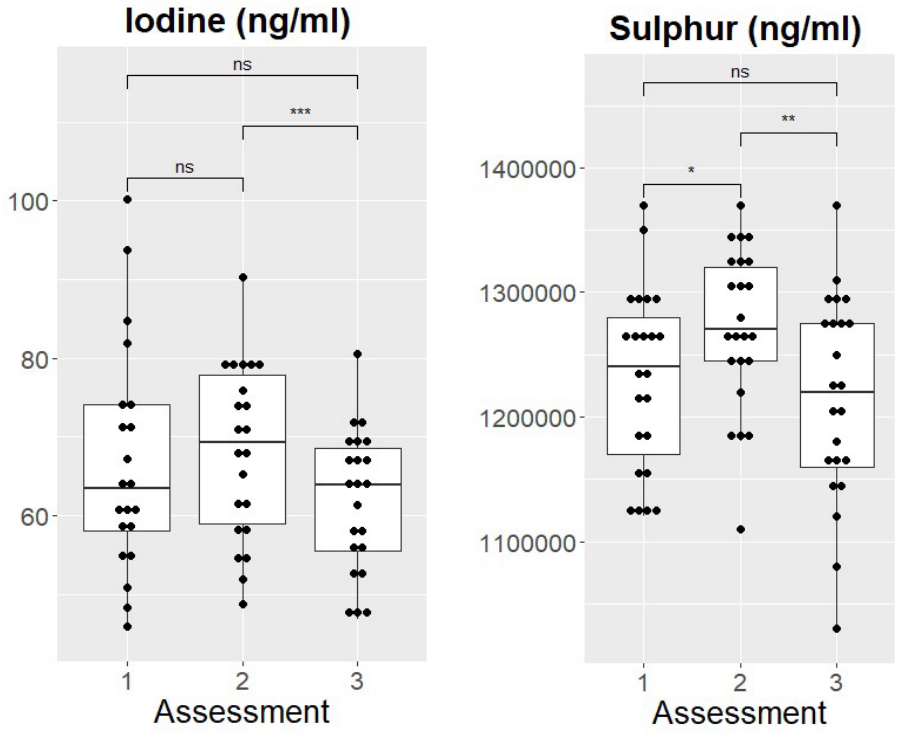


Figure 22: Serum concentrations of nihs minerals Assessments 1, 2 and 3.

Lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line (lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

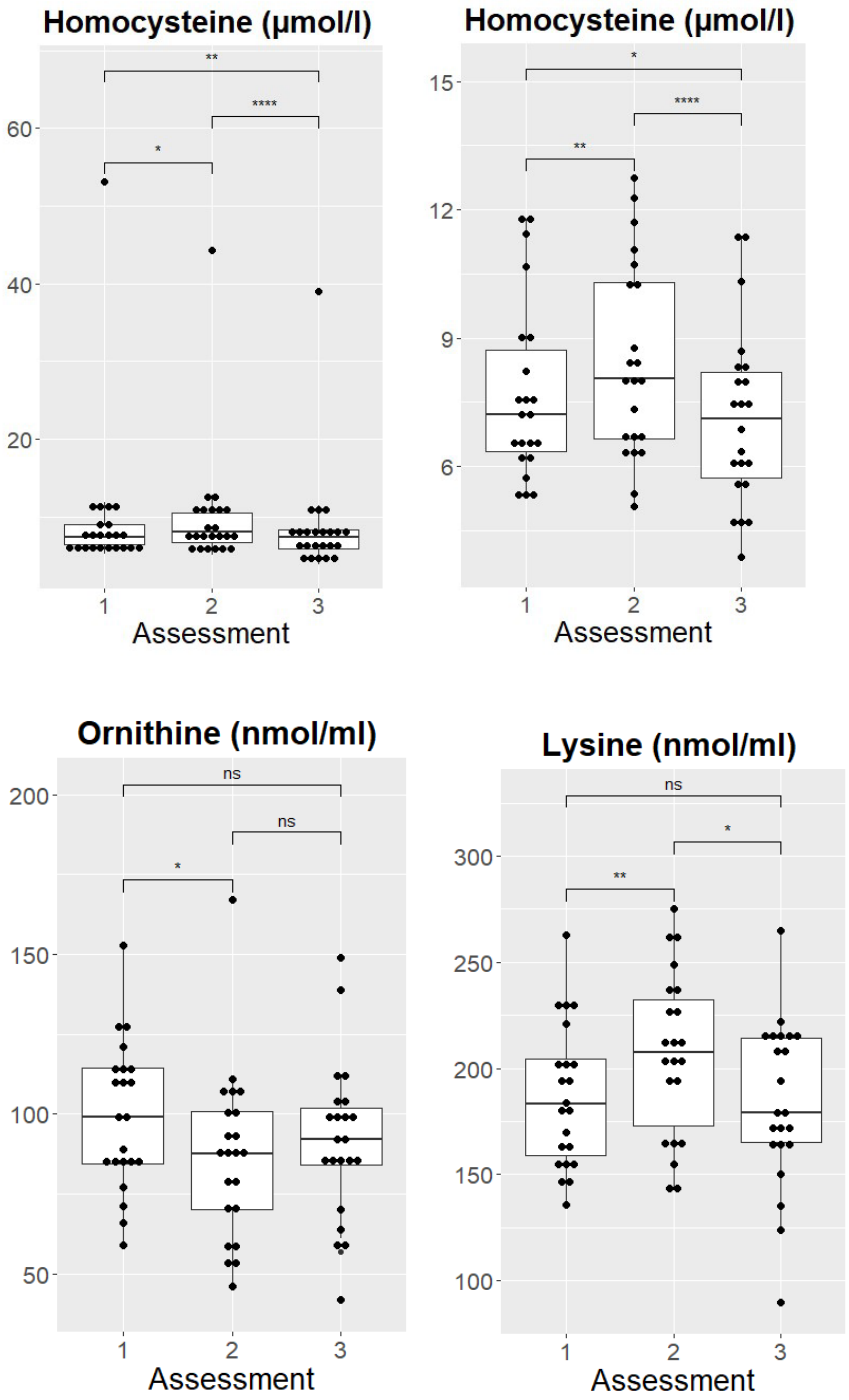


Figure 23 Amino acid changes at visits 1,2 and 3 (top right panel with outlying subject removed)

(lower limit of laboratory normal range marked in dashed red line, upper limit blue dashed line. Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

#### 3.4.3.3 Change in Body Form following dietary control

##### 3.4.3.3.1 Body Weight

Mean body weight fell significantly after the dietary intervention (75.7kg vs 74.6 Kg, $p$<0.001). Subjects' weight increased to baseline levels during washout (mean weight visit 2 vs Visit 3 (74.6 Kg vs 75.5 Kg $p$< 0.01).

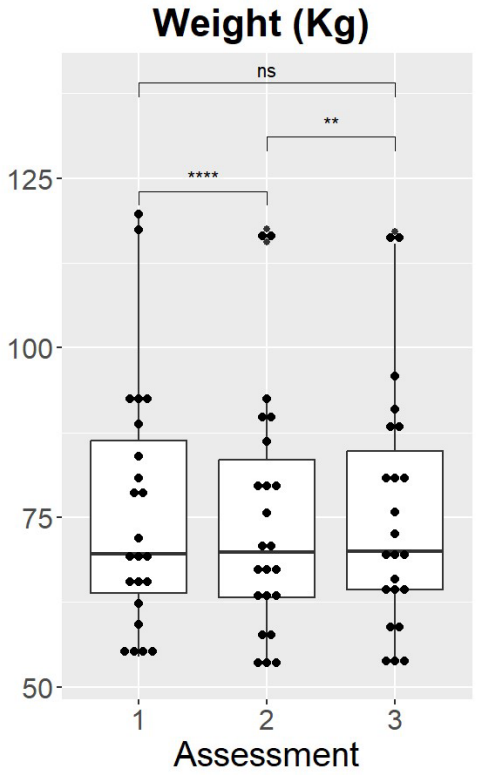


Figure 24: Body Weight at Assessments 1,2 and 3

(Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

#### 3.4.3.3.2 Phase angle

The Phase Angle at 50 KHz appeared to be sensitive to the intervention with an increase after the intervention (a higher phase angle is considered a marker of improved nutritional and clinical state) (Figure 25). The change in phase angle score was primarily due to a change in reactance rather than a change in resistance. There was a statistically significant increase in the Phase Angle of study subjects following the period of controlled nutrition (assessment 1 to assessment 2), which tended to return to baseline levels after subjects had resumed the free diet (assessment 3).

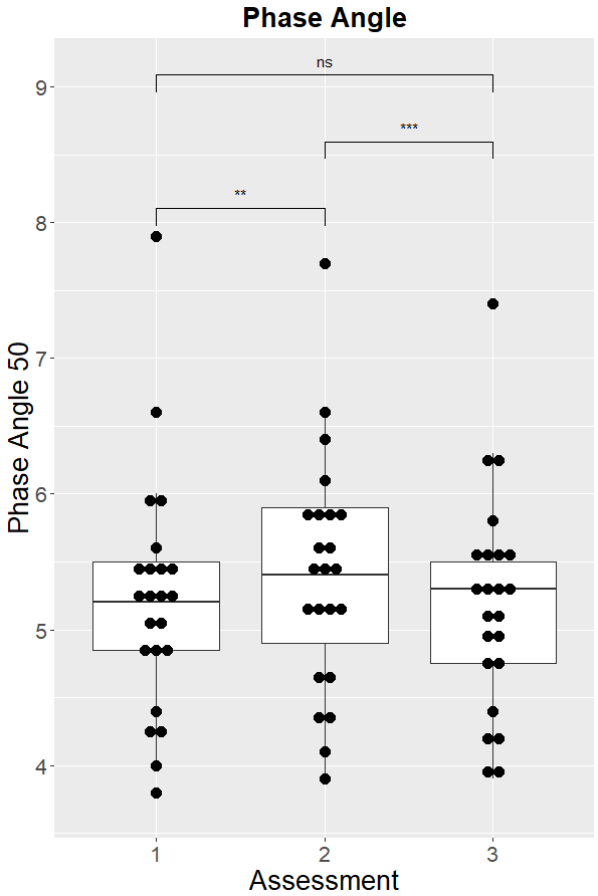


Figure 25: Phase Angle (degrees) at Assessments 1, 2 and 3.

(Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

#### 3.4.3.4 Change in function- health-related quality of life

##### 3.4.3.4.1 SF-36 changes

There was a significant improvement in the SF-36 physical functioning score between assessments 1 and 2 among those who completed the SF-36 at each visit (n=22). There was a variable response to the vitality score (SF-36-VT) after the dietary control, with some clinically significant improvements observed, but the changes among the group did not reach statistical significance Figure 26The SF-36 role-limited physical score did not appear to differ between visits. There was a trend towards a fall in the SF-36 general health score between assessments 1 and 2, but it did not reach statistical significance (see Figure 27).

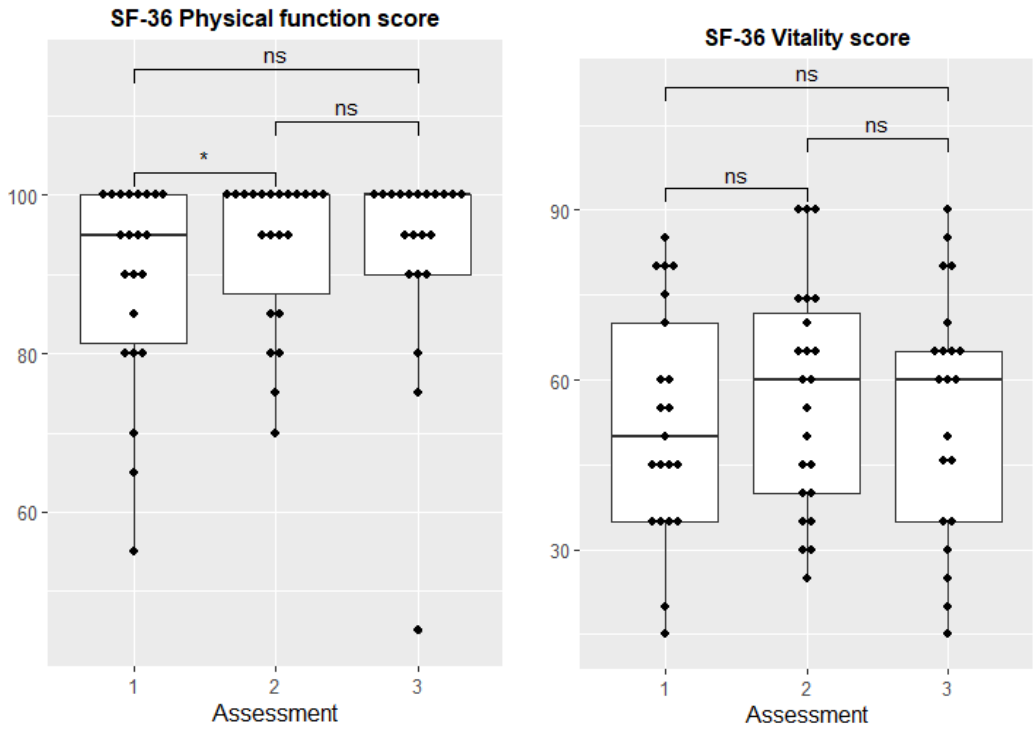


Figure 26 SF-36 Physical functioning score and SF-36 Vitality score at Assessments 1, 2 and 3.

100 represents no impairment of Physical function / vitality Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

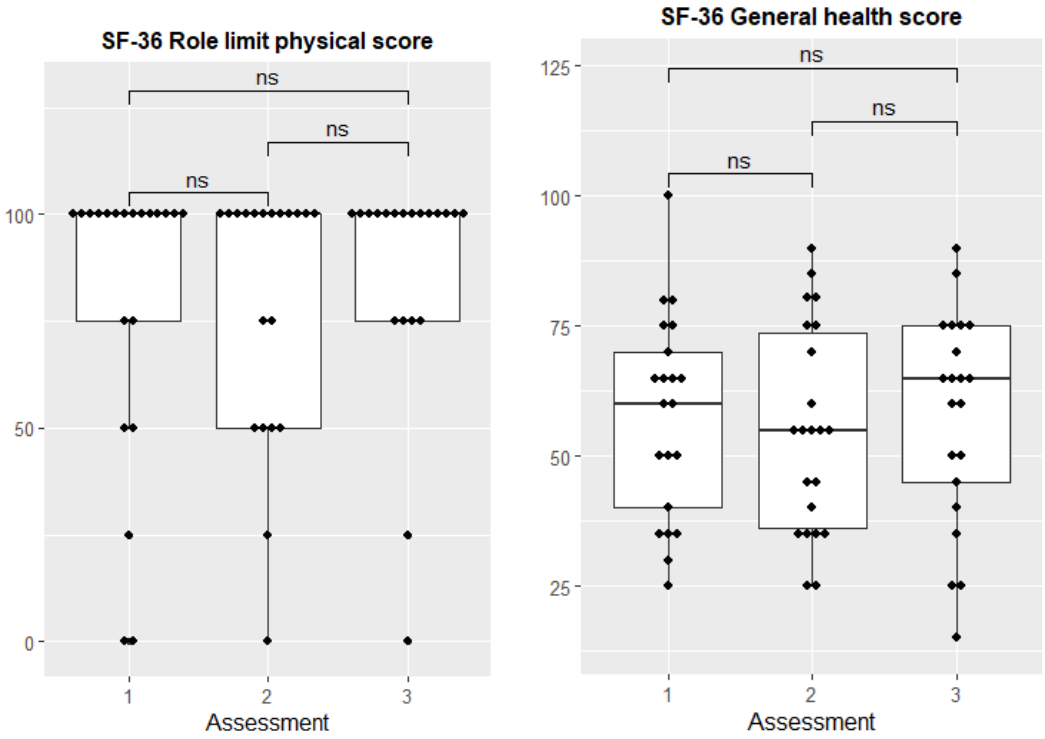


Figure 27: SF-36 Role limited physical functioning score and SF36 General health score at Assessments 1, 2 and 3.

(Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

#### 3.4.3.4.2 EQ-5D-5L

The EQ-5D-5L sub-scores for impaired mobility, self-care and usual activities, pain, anxiety and depression tended to be lower at baselines. There was no statistically significant change from this across the three visits. For the EQ, visual analogue scale for “Your health today” (rated 0-100) the PROM did not show statistically significant changes between the nutritional assessment visits. Three individuals were observed to rate their health today (0-100) at ≤50 at Assessment 2, but not at any other visits (see Figure 28).

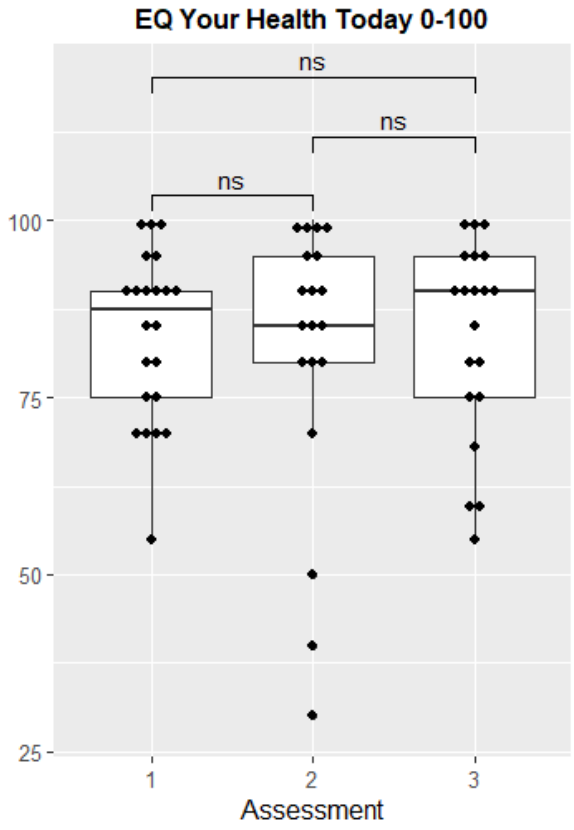


Figure 28 EQ "Your Health Today" visual analogue score at Assessments 1, 2 and 3.

(Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

#### 3.4.3.4.3 IBD Control 8 and IBD-Control visual analogue score (IBD Control VAS score)

The IBD control-8 score did not differ significantly between the nutritional assessments. The number of subjects rating their IBD control at 16 (best control) was 10 at baseline and fell to 3 at assessment 2, then increased to 5, suggesting that the dietary control period reduced their disease control for some. The IBD-VAS score, which in this study was rated by subjects as 0-10 (10 denoting best control), showed a similar pattern, with the 7 subjects rating their disease score at ten out of ten at Assessments 1 and 3 and three doing so at assessment 2.

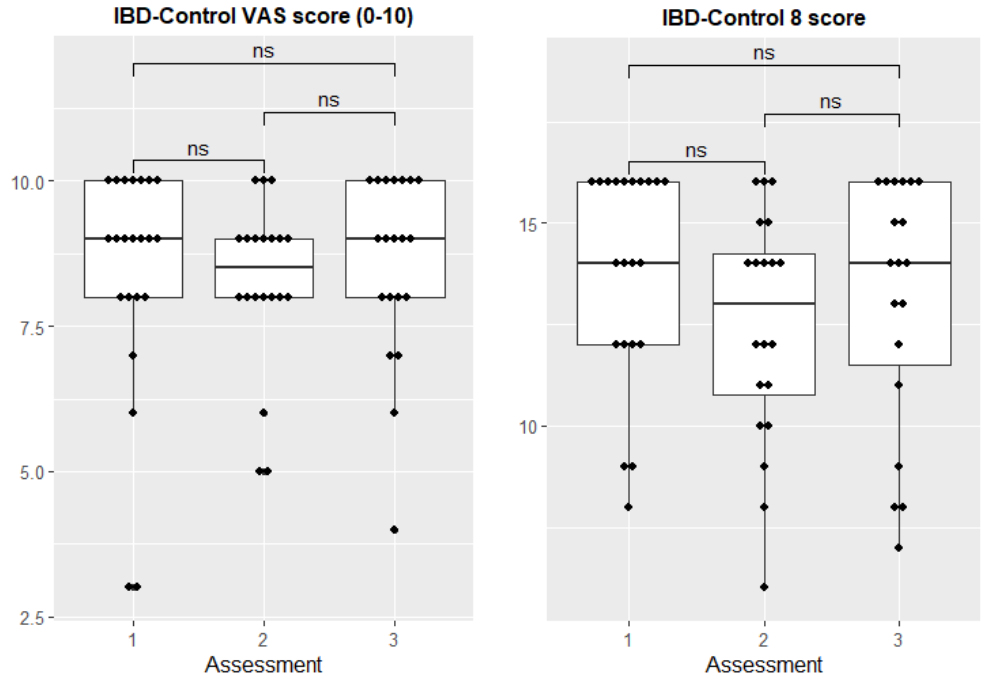


Figure 29 IBD Control VAS score and IBD Control 8 score at Assessments 1, 2 and 3.

(Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS=not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****)

#### 3.4.3.5 Change in markers of disease activity

Disease activity was assessed using the clinical activity score Harvey Bradshaw Index (HBI), alongside serum inflammatory marker C-reactive protein (CRP) and faecal calprotectin (FCP) at both the screening visit (between 7 and 30 days before assessment 1) and the 3 nutritional assessments.

Two subjects had a clinical flare (HBI≥4), and one subject had a biochemical flare (FCP > 250µg/g), between screening and assessment 1. HBI, CRP and FCP tended to remain stable between screening and assessment 1.

The faecal calprotectin showed a statistically significant increase between assessments 1 and 2 and returned to baseline during the washout period (assessment 3).

Four subjects had a transient increase in their faecal calprotectin after the dietary intervention (Assessment 1(Baseline) to Assessment 2 (post-EEN)) over the level consistent with active disease (250µg/g). Among these was the subject in whom FCP had increased to over 250 µg/g between the screening and visit 1. None of these four subjects had an increase in HBI over the level consistent with active disease (≥5), and each of these subjects had a fall in FCP to less than 250 µg/g after returning to a free diet (assessment 3 ('washout', day 24). Two subjects had a marked elevation in their CRP at the final visit, one from a diagnosed urinary tract infection (subject 9, CRP 85mg/L), the other (CRP 50 mg/L) for a clinical (HBI>5) flare in Crohn's due to stopping her Adalimumab (subject 14, FCP 160). Four other subjects had a transient increase over of CRP above the normal range (<5mg/L) after the dietary intervention (day 10, assessment 2), which was reduced by assessment 3 (post washout).

The changes in faecal calprotectin, C-reactive protein and Harvey-Bradshaw Index by subject are displayed in Figure 30 and for the cohort in Figure 31.

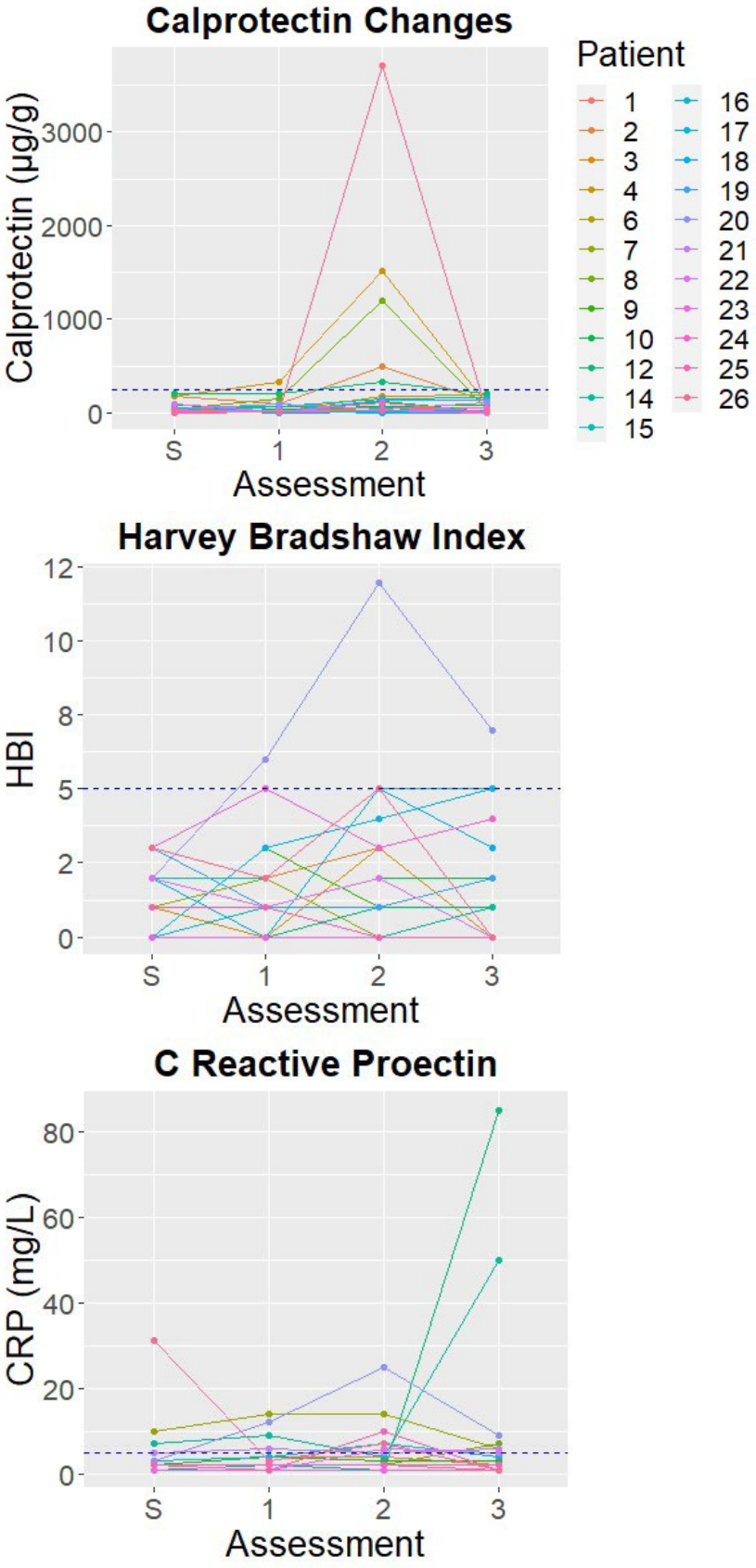


Figure 30: Change in Faecal Calprotectin, Harvey Bradshaw Index and C-reactive Protein by Subjects over the study period

(Screening= S, followed by Assessments 1,2 and 3)

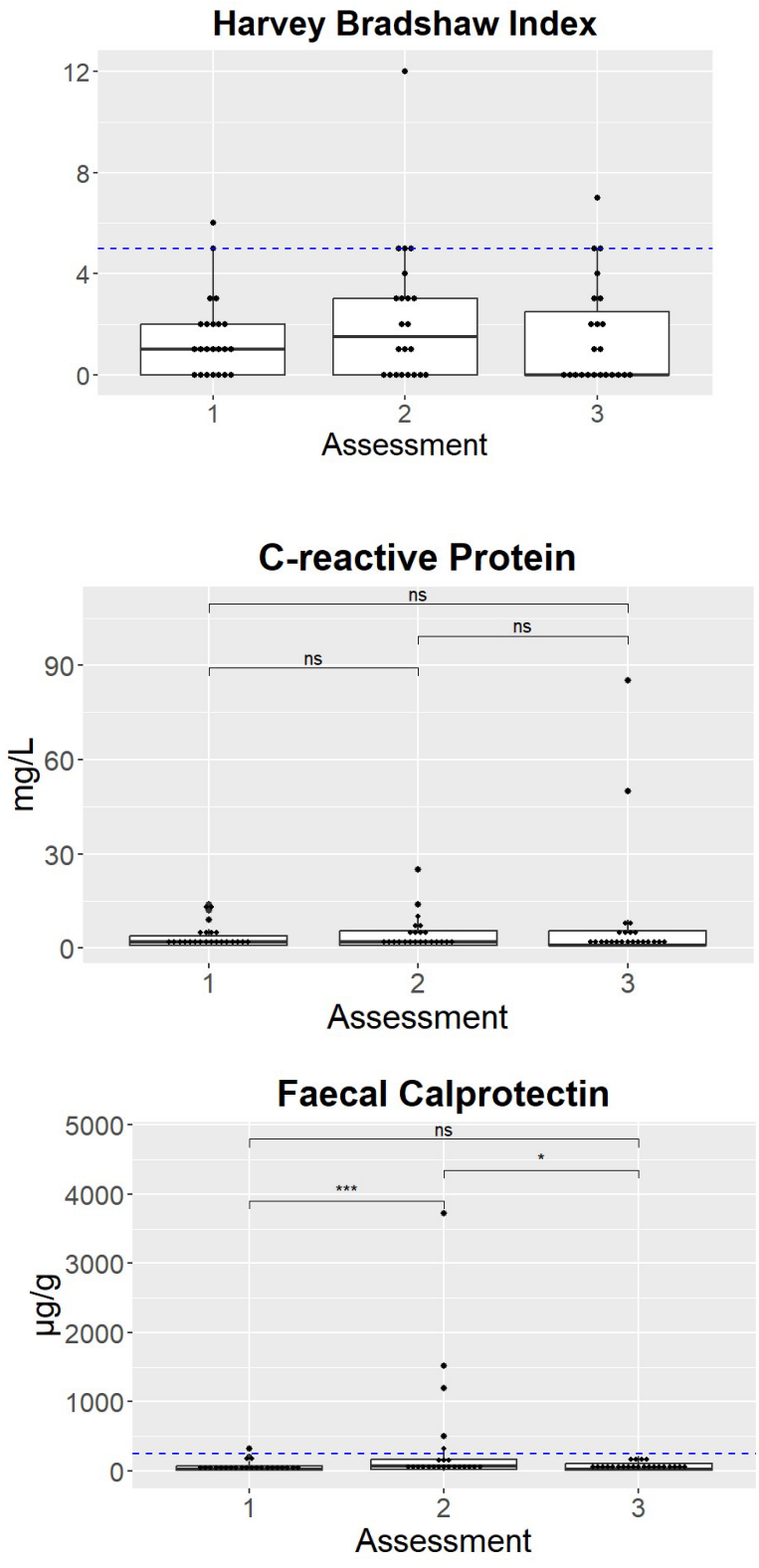


Figure 31: HBI, FCP and CRP at Assessments 1, 2 and 3.

Paired Wilcoxon Rank between visits 1 and 2, 2 and 3 and 1 and 3 NS= not significant, P <0.05*, <0.01**, <0.001 ***, <0.0001****

#### 3.4.3.6 General Observations and reported symptoms

At each assessment visit, subjects were invited to report any new symptoms or intercurrent illnesses and recorded qualitatively in the site file. At assessment 2, subjects were asked explicitly if they had any new symptoms of note or general observations of how they felt on the EEN. They were graded for severity and the degree to which the intervention (changing all food to EEN) was implicated. Common adverse side effects were changes in bowel habits (constipation in 3) and diarrhoea in 2. 5 subjects reported a noticeable improvement in energy levels at visit 2.

baseline.

### 3.4.4 Summary Table of Changes Observed in Response to the Nutritional Intervention

The markers of nutritional and disease state are shown in Table 21. Those variables showing a statistically significant difference between assessments 1 and 2 are listed below in the left-hand column.

Table 21: Study Variables and whether they changed after the intervention

(Assessment 1 to 2) and whether they persisted after the washout return to free diet (Assessment 1 to 3)

| **Variables that changed (with statistical significance) after the provision of nutritional intervention (1 vs 2)** | **Variables that persisted after washout (1 vs 3)** | **Variables with no change** |
|---|---|---|
| **Biochemistry – (all increase unless specified)**<br>Vitamin A, B2, B3, B5, B6, 9, D, E, Minerals:<br>Ca++, Cu, PO4, Se, S<br>Amino acid: Ornithine, Lysine, homocysteine<br>**BIA / anthropometry -**<br>Phase angle<br>Weight (fall))<br>**PROMs-**<br>SF36 Physical functioning<br>**CD activity-**<br>Faecal calprotectin (increase) | Biochemistry –<br>B6, B9, | Biochemistry-<br>Vitamin K, Na, K, Mg, I<br>Amino acids:<br>Taurine, Threonine, Serine, Glutamic Acid, Glutamine, Proline, Glycine, Alanine, Citrulline, Valine, Methionine, Isoleucine, Leucine, Tyrosine, Phenylalanine, Histidine, Arginine<br>PROMS-<br>IBD Control PROM, IBD Control VAS score, EQ5D<br>SF36 Vitality, SF36 role limit physical |

#### 3.4.4.1 Baseline biochemistry as a predictor of response to the dietary intervention

Alongside the profound changes in micronutrient biochemistry seen between assessments 1 and 2, phase angle and the SF-36 physical functioning scores showed statistically significant changes. These two variables reflected some functional benefits of the nutritional intervention, specifically better membrane integrity (phase angle) and a reduced impairment of functional capacity. While the response was statistically significant across the 24 subjects, it was variable. The SF36-vitality (SF-36-VT) also showed a variable response, albeit without statistical significance.

It was proposed that those with biochemical evidence of nutritional inadequacies would show a greater response in functional measures to the provision of nutritionally complete intake. One way of expressing the degree of insufficiency was to use the number of blood analytes below the normal laboratory range at assessment 1 (baseline) as the numerical variable against which the change in SF36-physical functioning or vitality or Phase Angle could be compared. The number of baseline blood tests lower than the laboratory range did not differ between those who had improved their SF36 physical functioning score post-EEN and those who did not. There was no association between the number of baseline blood tests below the laboratory range and the post-EEN change in phase angle. However, those with more than one baseline insufficiency blood all had an increase in the Phase angle post-EEN. Four of the six subjects with one laboratory blood below the normal range increased in phase angle post-EEN, compared to 3/7 with no baseline blood below range. Despite these trends, the correlation was weak ($r$ 0.22) and failed to reach statistical significance.

## 3.5 Discussion of core findings

INTICO-1 was a characterisation of a small cohort of adults with CD in stable clinical remission who underwent nutritional and disease phenotyping at three time points: first on habitual intake, then again after 7 days of consuming an enteral nutrition formulation that is licenced as a balanced complete feed (i.e. sufficient to meet the requirements of most healthy individuals), and after a return to an unrestricted diet of their volition. The three time-point assessments individually and collectively demonstrate evidence of an impaired nutritional state during adult CD remission in this cohort. Furthermore, the time-limited trial of a balanced nutrition formulation was associated with an improved nutritional state as marked by some variables and disease-associated impairment of HR-QOL with nutritional interventions.

Assessment 1 (free diet) identified individuals with evidence of micronutrient inadequacies, reduced lean Mass, altered bioelectrical properties of the body (a low phase angle), and an impaired HR-QOL. By assuring the nutritional adequacy of the diet with a time-limited trial of feeding with a nutritionally complete formula, it has been possible to explore if and how these markers of nutritional status and general well-being may be amenable to nutritional interventions. The period of dietary control for these subjects with CD led to some profound biochemical changes, improvements in BIA markers of nutritional status, and a PROM assessment of physical function. This was a group of subjects who, by being in clinical and biochemical remission and not overtly malnourished by BMI, would not undergo nutritional assessment or treatment. Current UK IBD care pathways would not have been assessed, identified, or treated these potentially nutritionally sensitive components of CD remission in this cohort.

The nutritional characterisation included an assessment of the habitual diet for nutritional adequacy, but this part of the nutritional phenotype data was less secure. The subjects recorded their dietary intake in an online food diary or, in some cases, recorded on paper, and then this was then transferred by the research team. Foods were matched from a database, and portion size was estimated to calculate the diet's energy, macronutrient, and micronutrient content. The total energy intake calculated in this study was similar to the estimation of BMR from calorimetry (i.e. a PAL of approximately 1, when the typical PAL for UK adults is typically 1.49-1.78) (288). This suggests that intake was under-reported through missed food or incorrect portion sizes. Such under-reporting of food intake below what is plausibly required to match total energy expenditure is a common feature and limitation of dietary recall studies (289). In

this study, if the total dietary intake was, on average, underestimated by 40%, this, in turn, will have reduced the estimated micronutrient intake and led some individuals' intakes to be incorrectly identified as falling below the LRNI for some micronutrients. The subjects reported that the dietary analysis website was cumbersome and that they struggled to match certain foodstuffs in the database. Ease of use of dietary capture programs is essential in facilitating accurate intake capture.

Despite the limitations, many dietary inadequacies identified from the dietary record are likely accurate, as evidenced by nutrients such as Niacin (B3) and Pyridoxic acid (B6). The intake of B3 and B6 are expressed relative to the estimated energy and protein content of the diet, so this was controlled for overall under-reporting, yet was still found to be low in 2 and 4 subjects, respectively. Moreover, the LRNI estimates the minimum dietary intake of a nutrient for those with low requirements, so it is likely to be a conservative estimate of the required intake levels. The dietary analysis data, while less secure as a quantitative assessment of micronutrient intake, allowed for a qualitative evaluation of dietary quality and adequacy, showing that the free diet intake of some individuals was low across multiple micronutrients.

The study used a nutritional intervention to standardise intake to explore further the nutritional state and its relationship to CD remission. By prescribing exclusive enteral nutrition at a volume aimed to meet individuals' requirement for energy, the measure of micronutrient adequacy improved so that all subjects met their RNI across all micronutrients apart from Sodium. The 7-day trial of nutritionally adequate dietary intake led to multiple profound changes in markers of nutritional status, specifically improved circulating micronutrient biochemistry and a change in the bio-electrical properties of the body towards levels associated with improved health and nutritional status. The phase angle has been validated as a predictor of operative complications in cancer surgery, predicts subjective global assessment nutritional scores and hospital mortality (290-293). In the subjects in this study, the change was driven by an increase in reactance rather than a change in body composition, which suggests an increased membrane capacitance, which may reflect the nutritional intervention improved membrane function on a whole-body level. It was hypothesised that individuals with more biochemical abnormalities at baseline would have a change in phase angle between assessments 1 and 2. All subjects with more than one baseline biochemical insufficiency showed an increase in phase angle, and there was a weak positive correlation to support this, but the change did not reach statistical significance. This was an exploratory analysis of a small sample and is likely to be underpowered.

There were changes with potential meaningful clinical relevance as some subjects reported a dramatic improvement in longstanding fatigue symptoms at Assessment 2. These observations were supported by the SF-36 physical functioning score, a composite of 10 questions on self-reported physical capacity, which improved between assessments 1 and 2. This was not a study powered to demonstrate whether a particular intervention affected the outcome, and the response appeared variable among the group. Nevertheless, the size of the effect on this self-reported PROM was sufficient among some of the study subjects for the results of the cohort to show a statistically significant improvement in this domain after the intervention. The number of baseline biochemistry results below the normal range did not predict this in our cohort.

Enteral nutrition was used here to ensure a balanced supply of energy and nutrients. The intervention was a commercial product (Modulen®, Nestle Health Sciences) licenced in the UK to treat active Crohn's disease and induce clinical remission. The subjects would be familiar to subjects with IBD and the study dietitian. This was not just an intervention that provided energy and nutrients; polymeric feeds in active CD have been shown to downregulate inflammatory pathways, restore intestinal barrier function, reduce bacterial load in the colon and lead to mucosal healing (136, 294, 295). EEN provides nutrients and potentially beneficial substances while reducing exposure to foodstuffs from the diet, which may compromise barrier function or drive intestinal inflammation. Dietary exclusion of putative pro-inflammatory elements is the cornerstone of many therapeutic diets for Crohn's disease management (e.g. CDED). Nevertheless, an improved micronutrient status is a known beneficial observation of EEN, and the changes seen in biochemistry here were reflective of the increased supply of nutrients within the nutritional intervention (138).

Subjects were permitted to continue caffeinated drinks during EEN based on the experience of the study dietitian with caffeine withdrawal to make the study less burdensome. Subjects would likely have continued on a similar intake of coffee or tea, so this aspect of the study will not have changed any of the observed differences between assessments 1 and 2. Moreover, black tea and coffee will have had a negligible impact on the macronutrient or micronutrient intake, so this is unlikely to have confounded the study observations.

This study also assessed the Crohn's disease 'activity' at screening, baseline, post EEN and washout using a clinical activity score, the Harvey Bradshaw Index (cut-off for remission <5) and a marker of intestinal inflammation, faecal calprotectin (FCP; cut-off for FCP remission <250µg/g) and marker of systemic inflammation, serum C-reactive with HBI and FCP being

requisites for study entry. A notable observation was the profound changes among four subjects in the faecal calprotectin concentration after the dietary intervention. One subject had undergone an increase between screening and Assessment 1 over the threshold used to define remission and increased further post-EEN. This was followed by a fall below the remission threshold after the 2-week washout return to an unrestricted diet. This subject did not have a clinical flare (HBI>4) during this time, which was the case for the other four subjects with a similar marked transient rise in FCP after Assessment 2, which fell below 250 at Assessment 3. Faecal calprotectin is an S100 protein, the dominant cytoplasmic protein of neutrophils, and is a surrogate marker of their presence in the bowel mucosa (296). Its transient rise in these subjects and a statistically significant increase in the group is a challenging observation to explain. The change suggests that the nutritional intervention is modulating the intestinal inflammatory response. In routine care, an increase in FCP >250µg/g would be considered a sign of a flare of intestinal inflammation (as a surrogate for endoscopy), so this study observation is an unexpected response for something considered a treatment to reduce intestinal inflammation. It is unclear whether continuing the EEN would have led to a further increase in FCP and a clinical flare, as the subsequent assessment was after 2 weeks of unrestricted diet. Published data of EEN typically reports a fall at baseline at 6 weeks, so the change seen here may reflect an initial inflammatory response before a subsequent fall. The lack of clinical flare at any of the three assessments and return to baseline FCP at visit 3 in all four subjects who experienced an increase in FCP is reassuring that this change was not a reflection of the 7 days of EEN being harmful. A less optimistic assessment of the intervention was a damaging increase in intestinal inflammation due to the provision of nutrients in the EEN, which was reversed upon return to an unrestricted diet.

Serial weekly measurements of faecal inflammatory markers during EEN and endoscopic assessment would elaborate on the subject further. From a therapeutic perspective, there is emerging evidence of the efficacy of cyclical EEN as an alternative to medication in paediatric Crohn's and limited evidence of partial enteral nutrition as a maintenance therapy post-EEN-induced remission (139, 297).

Remission in CD is defined primarily by the absence of active intestinal inflammation disease and its classically associated symptoms. Under current treatment guidelines, the individuals who completed this study would not be at nutritional risk, nor would their treatment be considered inadequate for optimal health and well-being. This work demonstrated nutritional

issues and suggested that these may change with an intervention to provide a more nutritionally adequate diet, which may improve fatigue symptoms during CD remission.

This study has limitations. It was a small cohort of individuals prepared to change their diet to EEN during clinical remission, an intervention most typically used in subjects with a flare. Selecting people prepared for EEN without the usual motivation of ending a CD flare may have biased recruitment to those with concerns about their nutrition or with a greater burden of symptoms. The dietary capture appears to be incomplete during the free diet, and the higher energy intake we recorded during the dietary control will be due to the comparative ease of recording a total volume of EEN versus matching foods and portion sizes from a database. Using EEN, a recognised agent to induce CD remission, may have treated active Crohn's disease rather than purely being a nutritional intervention. Such distinction between an intervention being purely nutritional rather than medical is a false dichotomy as the disease will impact upon nutritional state and vice versa. The changes in faecal calprotectin suggest that the EEN modulated intestinal inflammation. It is unclear whether this modulation was due to the provision of nutrients, excluding harmful pro-inflammatory habitual dietary elements or an inflammatory addition to the EEN formula.

Finally, the improvement in the SF36 PROMs may have been due to a placebo effect, which can be common in trials of CD therapies(298). Randomisation and blinding in a dietary intake study are impossible, which makes controlling for such an effect an unavoidable limitation of nutritional research.

To conclude, this study found the nutritional state during CD remission and quality of remission to be variable. Our cohort suggests that adult CD populations may contain individuals with a range of nutritional issues which go underdiagnosed and untreated. Our dietary control period suggests that some symptoms during CD remission, such as excessive fatigue, may be related to the nutritional state and amenable to interventions to address this.

# Chapter 4 The INTICO Cohort Study: INTICO-2

## 4.1 Introduction to INTICO-2

The first experimental work, INTICO-1**,** was a feasibility study that used a time-limited trial that examined the nutritional state of 24 adults living with CD during clinical remission before and after a period of dietary control. This study identified individuals with poor-quality diets, altered micronutrient biochemistry, altered bioelectrical impedance properties, and reduced lean mass. Alongside these nutritional issues, INTICO-1 found subjects with symptoms of excessive fatigue and impaired health-related quality of life (HR-QOL). Several individuals reported a marked resolution of symptoms of fatigue. This improvement in the physical function score of the general health-related quality of life domain (SF-36- PF) was an unanticipated observation that, if found to be reproducible, would markedly improve the quality of life of many patients. This apparent improvement in function required further investigation with a fatigue-specific PROM, which would better allow exploration of its causes.

The previous literature on nutritional state and the role of nutritional therapy in CD remission is limited. INTICO-1 assessed the feasibility of nutritional assessment in the population and suggested that adults living with CD may have an impaired nutritional state when in clinical remission. The profound changes seen between assessments suggested that poor nutritional state may be modifiable by nutritional intervention, and the variability of nutritional state may be a determinant of the "quality of disease remission", such as persistent inflammation and fatigue. INTICO-1 had limitations; a small group of subjects (24) were recruited if they were willing to substitute their habitual diet with Exclusive Enteral Nutrition (EEN). As such, it may not reflect all the adult CD remission population as 1) the broad confidence intervals to estimate the true prevalence of deficiency or fatigue, and 2) recruitment on willingness to change diet may be biased towards individuals with baseline nutritional inadequacies.

These limitations, the lack of evidence from previous literature and the indications that the quality of remission (especially fatigue) may be related to an impaired nutritional state supported the need for further study in a larger outpatient population.

### 4.1.1 Study aims- INTICO-2

1. To characterise clinical phenotype, nutritional status and quality of remission across an adult outpatient population living with Crohn's disease when in clinical remission.
2. To determine whether differences in nutritional status during clinical remission were associated with excessive fatigue, as an indicator of the quality of remission.

### 4.1.2 Specific Hypotheses

1. There were individuals with excessive fatigue in the population.
2. There were individuals with undiagnosed impairment of the nutritional state in the population
3. Individuals with an impaired nutritional state would have the greatest fatigue / poorest quality of remission (and vice versa).

## 4.2 Conceptual Framework for INTICO-2

This study explored how the clinical disease state and treatment relate to the nutritional status of Crohn's patients and the remission quality (see Figure 32). There are three considerations. Firstly, how does the clinical disease state relate to the quality of remission (i.e., do those with more severe or active disease have the poorest quality of remission?)? Secondly, how does the clinical disease state relate to the individual's nutritional status (i.e., do those with more severe or active disease have the poorest nutritional state?)? Thirdly, how does the nutritional state relate to the quality of remission (i.e. do those with the poorest nutritional state have the poorest quality of remission?

In addition, a follow-up assessment offered the opportunity to consider how disease state, nutritional state and quality of remission at baseline related to fatigue at 12 months (i.e. the persistence of the quality of remission) and the likelihood of a subsequent flare of disease activity (i.e. time to flare over the 12 months).

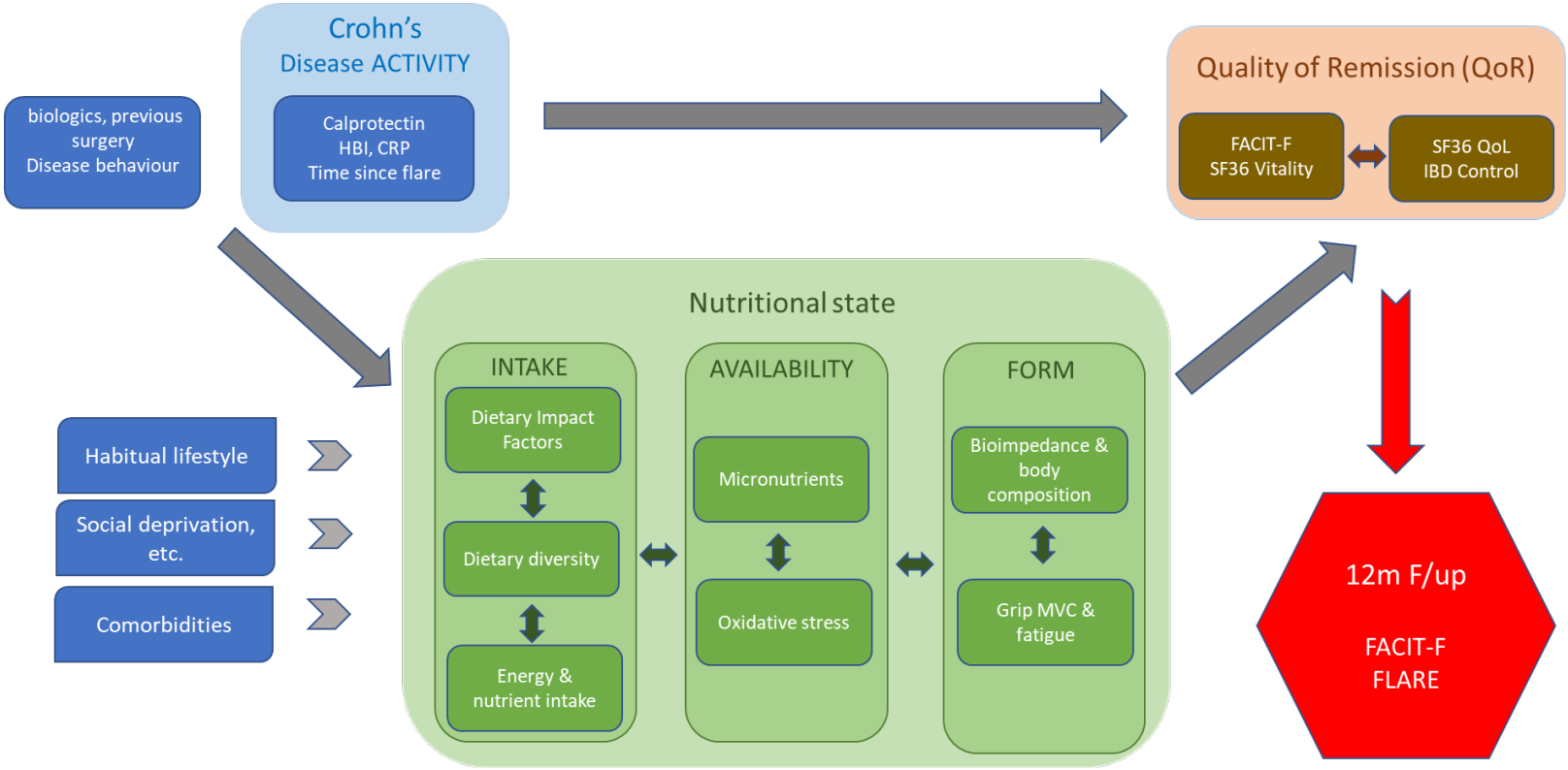


Figure 32: Conceptual approach to the analysis of INTICO-2.

The boxes represent the study data, with the smaller shaded boxes explored first within their larger box and then with study data. The large green box represents the nutritional state, with the smaller green boxes representing how the nutritional state was assessed.

## 4.3 Methods INTICO-2

### 4.3.1 Setting

INTICO-2 was a prospective, observational, cross-sectional cohort study from the outpatient population centre of a single UK centre. The study was part of an investigator-led Collaborative Research Agreement between the University of Southampton, University Hospital Southampton NHS Trust and Nestlé Health Sciences. Following the experience of completing and analysing INTICO-1, I designed this part of the study with input from my supervisors, Dr Stephen Wootton and Dr Fraser Cummings, as principal investigators. The study protocol was approved by the West of Scotland Research Ethics Council (REC) and the UK Health Research Authority (HRA), IRAS number 28390 and was conducted according to the principles of the National Institute for Health Research (NIHR) Good Practice (GCP). The study was ERGO-approved (Reference number 61605).

The study was conducted at the University Hospital Southampton (UHS) between December 2020 and January 2022 by a research team led by the author (MM), which also consisted of another Clinical Research Fellow, Dr Stephanie Sartain (SS), a clinical trials assistant, and specialist dietitians, Catherine Westoby (CW) and Vasiliki Katarachia (VK). The study was designed, received regulatory approval, and was set up during the COVID-19 pandemic. Adjustments were made for remote pre-screening and recruitment. The COVID-19 pandemic delayed the study opening and then twice suspended due to national lockdowns. A materials transfer agreement (MTA) was drawn up for samples analysed through collaborating laboratories at Nestlé Research École Polytechnique Fédérale de Lausanne (EPFL).

#### 4.3.1.1 Recruitment

The study size (n = 200) was based on the number that the research steering committee felt could be recruited from the population at the centre within 12-18 months.

#### 4.3.1.2 Identifying subjects

Pre-screening for potential study subjects was conducted with the support of the Clinical Informatics Research Unit (CIRU) of the University of Southampton, the electronic patient record and a UHS patient portal (MyMedicalRecord (MMR)). Pre-screening was conducted using

the electronic record to identify all subjects with CD in the UHS outpatient population who were likely to be in disease remission and did not meet any exclusion criterion. The search methods and pre-screening checks are described below.

#### 4.3.1.3 Pre-screening search

A keyword search of "Crohn's" was performed in patient letters from the gastroenterology clinics, generating a list of 1866 subjects. The electronic patient records were then checked by MM and CW to see 1) if the EPR confirmed Crohn's disease or 2) if the EPR confirmed the subject met an exclusion criterion.

To further assist in identifying subjects likely to meet an exclusion criterion (see SUBJECT EXCLUSION CRITERIA), this list was subsequently checked against 1) an electronic list of dietetic clinics, 2) electronic lists of subjects who had received IV micronutrients on the day unit infusion lists, 3) electronic lists of having had surgery in the trust and 4) any clinic letters/discharge summaries clinical coding mentioned the keyword "stoma".

Other subjects potentially missed by the keyword search were then identified by cross-checking virtual patient clinic lists and previous study lists. The steps to identify potentially eligible subjects and numbers removed or added at each stage are listed in the Appendix.

Pre-screening identified 1388 subjects who were potentially eligible for the study. These subjects were then invited in five separate waves of invites via post to participate in the study. The letter (see Invitation Letter to INTICO-2) explained the research and invited interested subjects to contact the team in writing via email or phone.

#### 4.3.1.4 Study Design

The study was a cross-sectional study of the CD remission population. It involved screening to confirm clinical and biochemical remission and then within the preceding 3 months, a 7-day food diary followed by a single timepoint assessment of nutritional status and clinical state (see Figure 33).

Nutritional status was assessed from the perspective of 1) intake (electronic food of 7-day intake and dietary impact factor questionnaire (INAT)), 2) micronutrient status (blood biochemistry), and 3) form (height, weight, bioelectrical impedance measures of nutritional status and estimated body composition).

The clinical state was assessed on the same day using 1) disease phenotype (e.g. previous surgery, Montréal Classification, completed by the researcher from the electronic patient record), 2) patient-reported outcome measures of Fatigue (FACIT-F), IBD symptoms (IBD control) and health-related quality of life (HR-Qol) (SF-36) and 3) markers of CD activity (Harvey-Bradshaw Index, C-reactive protein and Faecal calprotectin.

Screening clinical activity scores and patient-reported outcome measures were collected via a specially designed secure patient portal on MyMedicalRecord (MMR).

### 4.3.2 Cohort study

Subjects were invited to enter an observational cohort study to see whether they had a flare of their Crohn's disease in the year after the baseline nutritional assessment.

The cohort study required subjects to record 3-monthly disease scores (Harvey Bradshaw Index and IBD Control PROM) 3, 6, 9 and 12 months after the nutritional study visit. These were completed via MyMedicalRecord or post. Those with a Harvey-Bradshaw ≥5 were flagged to the study team and were then invited to either submit a faecal calprotectin or have an endoscopy / MRI to confirm flare. Individuals experiencing a suspected flare between scheduled assessments were encouraged to contact the nurse helpline or the study team for a clinical assessment with a calprotectin or, if indicated, an endoscopy / MRI to confirm flare. At 12 months, the electronic record was reviewed for any confirmed flare (a clinical flare would be associated with an escalation in treatment, with one or more of a raised faecal calprotectin or endoscopic / CT/ MRI evidence of active disease) over the intervening year. The subjects had a re-assessment of fatigue symptoms with the FACIT-F PROM, a short questionnaire about any changes in their diet since doing the study and were invited to repeat their routine blood tests (Vitamin D and iron status).

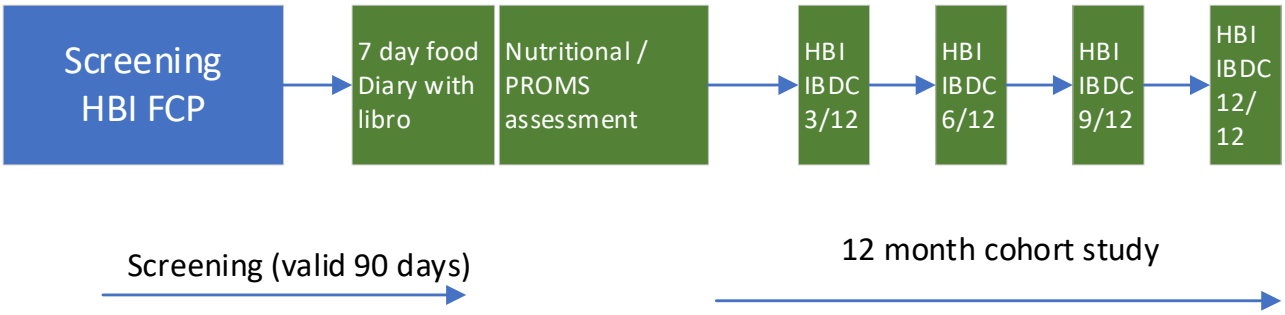


Figure 33: Study Schedule INTICO2-

Blue box denotes screening, green box study assessments. HBI: Harvey Bradshaw Index, FCP: Faecal Calprotectin, PROM: Patient-Reported Outcome Measures, IBDC: IBD Control

### 4.3.3 Study Subjects

As with INTICO-1, the study aimed to assess subjects with confirmed CD in remission who were not currently receiving dietetic input into their care for overt malnutrition, intestinal failure, active disease, or stoma.

#### 4.3.3.1 Inclusion Criteria

- Confirmed diagnosis of Crohn's disease that has been robustly phenotyped using endoscopic, histological, and radiological means in line with international guidelines.
- No history of relapse or change in treatment for at least 3 months.
- Ability to give informed consent.
- Faecal Calprotectin concentration <250µg/g at screening (valid for 3 months)
- Harvey Bradshaw Index <5

#### 4.3.3.2 Exclusion Criteria

- <18 years old
- On parenteral nutrition or tube feed

- Systemic steroid therapy, including prednisolone, budesonide, and Clipper within 3 months of study.
- Intestinal failure
- Stoma
- Any other reason, in the investigators' opinion, might make the patient unsuitable for the study.
- All subjects without a confirmed diagnosis of Crohn's disease in the opinion of the investigators

#### 4.3.4 Study Assessments

##### 4.3.4.1 Screening

The protocol for screening was designed to minimise the number and length of study visits to the hospital due to clinical restrictions during the COVID-19 pandemic. The pre-screening, screening and study questionnaires were done remotely with a postal invite followed by a self-screening questionnaire and consent via the MyMedicalRecord (MMR) electronic portal. Following an approach via post and return of an expression of interest, study subjects were sent a full patient information sheet with adequate time to consider the study (see appendix B2 Patient Information Sheet). Consent was recorded via a specially designed screening page via the secure online portal (MMR), which served as the data entry point for the study questionnaires. For subjects unable to access a computer/device, paper consent (via post, with confirmation at the visit) and paper questionnaires were completed.

Clinical remission was confirmed using the modified Harvey Bradshaw Index (HBI) <5, and biochemical remission was confirmed using a faecal calprotectin <250µg/g (the latter being an accepted surrogate for endoscopic remission) (78). The screening was valid for 3 months.

##### 4.3.4.2 Study Subject Characteristics

Demographics such as age and gender were added to the dataset, Self-reported micronutrient use was recorded as a potential confounder to micronutrient blood tests. In addition, medical notes were reviewed by the study doctors to establish the duration of CD and the number of

previous resection surgeries, which are both factors that may determine the accumulated tissue injury or reduction in absorptive capacity from disease onset.

The study subjects' disease phenotype was also collected and described using the Montreal Classification, a classification system developed to subgroup common genetic and pathophysiological phenotypes (79). Briefly, the Montreal classifications groups subjects by the age of CD onset (<15,15-40 or over 40), prior location of gastrointestinal tract CD involvement (Upper GI, ileal, ileocolonic or colonic) and prior disease behaviour (grouped by inflammatory, stricturing or penetrating, with or without previous perianal involvement).

### 4.3.5 Nutritional and Disease Assessments

#### 4.3.5.1 Assessment of Intake

##### 4.3.5.1.1 Food Diary

The dietary intake was assessed to capture the micronutrient adequacy of the habitual diet. A 7-day food record was chosen over a food frequency questionnaire to aim for the best possible quantitative estimate of macronutrient and micronutrient intake (80). To reduce the impact of day-to-day variability on estimated intake, subjects recorded seven consecutive days from which average daily intake was calculated. The study subjects in INTICO-1 expressed difficulty entering the data into myfood24. The study dietitian, therefore, reviewed the usability and database information of dietary collection tools and chose the Libro app from Nutritics. Briefly, Nutritics is a cloud-based database of the nutrition content of over 1 million foodstuffs.

In the week before assessment 1, subjects were asked to record all food and drink intake using the Libro app from Nutritics ®. Subjects enter each day's food intake immediately or at the end of each day by entering the food and then matching it with the chosen food or recipe component on a database, adding the amount. At the study visit, subjects were asked if they had managed to enter everything by the study team. Subjects were asked to continue their usual supplements and record them with the components listed on the label each day in their food diary. This was checked for accuracy by the study dietitians (VK and CW), who asked directly which supplements were taken at the study visit and added any that had not been recorded.

Subjects who could not access or use an app were asked to record paper food diaries, and the study team entered these diaries retrospectively via the Nutritics website.

The cloud-based software then calculated the mean daily intake for each subject. Extracted data by the research team provided a list of the foods recorded, the size of portions, and the mean daily intake of micronutrients (see samples food diary in Appendix C4).

To ensure the integrity of the data, the study dietitian VK reviewed the extracted data for each subject to correct any potential data errors (e.g., implausibly large or small portion sizes) and remove any food diaries which appeared to be incomplete. Average daily micronutrient data was calculated and expressed (where available) as a percentage of UK reference nutritional intake (RNI) and compared to lower reference nutritional intake (LRNI) (81).

Each nutrient was then recorded as either below LRNI ("below") or above LRNI, but below EAR ("between") and above RNI ("above"). To assess overall dietary quality, the number of nutrients that subjects failed to meet their LRNI was calculated as a numerical study variable. The nutrients that were evaluated in the analysis of intake are recorded in Table 22.

Table 22: Dietary micronutrients whose intakes were estimated by the Libro dietary analysis software and compared to LRNI and RNI from the 7-day food diary

| **Hydrosoluble Vitamins** | **Mineral** |
|---|---|
| Thiamine | Calcium |
| Riboflavin | Copper |
| Pyridoxine | Iodine |
| Folate | Iron |
| Cobalamin | Magnesium |
| Vitamin C | Manganese |
| **Liposoluble Vitamins** | Potassium |
| Vitamin A | Selenium |
| Vitamin D | Sodium |
| Vitamin E | Zinc |

#### 4.3.5.1.2 Inflammatory Bowel Disease Nutritional Assessment Tool: i-NAT

In addition to subjects completing a food diary, symptoms that might impact on intake (Dietary Impact Factors) and clinical features of an altered nutritional state were assessed using a novel patient-reported questionnaire developed by the study dietitian Catherine Westoby (CBW) (accepted for presentation at ECCO 2025- citation awaited). This assessment tool sought to formalise a comprehensive dietetic assessment for the study participants. The tool asked questions on preceding weight loss and other potential contributory factors to an impaired intake, specifically 1) a reduced appetite (using questions from the SNAQ score), 2) symptoms after eating in the previous 2 weeks, and 3) whether subjects avoided specific food or food groups (299). The tool contained a section of direct questions on potential aspects of IBD which may contribute to an altered nutritional state (previous surgery, known strictures)) and signs of nutritional deficiency (salt craving, poor wound healing, alopecia, and night blindness). This assessment tool generated multiple scores: a total score for appetite, the whole questionnaire score, and had specific questions on causes and consequences of an altered nutritional state against which other markers of nutritional state or CD outcome could be compared. Details of the tool and the scoring of the questions are described in the Appendices C5 and C6.

#### 4.3.5.2 Assessment of Blood Micronutrients

At the main study visit, samples were collected to analyse circulating micronutrient concentration, nutritionally sensitive metabolites, and haematology. The presence of C-reactive protein measured systemic inflammation. Intestinal inflammation was checked at screening using faecal calprotectin. Unless otherwise stated, samples for iron status, CRP, haematology, and routine biochemistry were analysed through the accredited pathology service at University Hospital Southampton. Extended micronutrient analyses and redox markers were assessed using collaborators at the Nestlé Institute for Health Sciences in Lausanne. A list of the biochemical tests of micronutrient status is recorded in Table 23.

Table 23: Blood biochemistry analyses at the main study visit

| **Analyte (UHS)** | **Analyte (NIHS) Liposoluble Vitamins** | |
|---|---|---|
| **Hb and Iron Indices** | Vitamin K1 | |
| Haemoglobin | 25-Hyroxy vitamin D3 | |
| Ferritin | α-Tocopherol | |
| Transferrin Saturation | β-Cryptoxanthin | |
| Soluble Transferrin Receptor Index | Lutein | |
| Serum Inflammatory Markers | Zeaxanthin | |
| C-reactive protein (CRP) | **Analyte (NIHS) Trace Elements** | |
| **Analyte (NIHS) Hydrosoluble Vitamins** | Bromine | Selenium |
| Nicotinamide | Barium | Magnesium |
| Nicotinic acid | Calcium | Copper |
| Thiamine | Caesium | Sulphur |
| Riboflavin | Iron | Potassium |
| Pantothenic Acid | Aluminium | Iodine |
| 5-methyl tetrahydrofolate (5-mTHF) | Rubidium | Manganese |
| Pyridoxic Acid | Molybdenum | Zinc |

#### 4.3.5.2.1 UHS Micronutrient analyses

Iron status was assessed using ferritin and the log soluble transferrin receptor index ratio (82). Ferritin was measured in serum using a two-step immunoenzymatic (“sandwich”) assay via the Tri-level Multichem IA Immunoassay, Technopath Product No. IA 310X. Soluble transferrin receptor was measured in plasma using a two-step immunoenzymatic (“sandwich”) assay with the Beckman Coulter Access sTfR, Access sTfR QC2 and QC3 Product No. B11057.

#### 4.3.5.2.2 Nestlé Institute of Health Sciences Analyses

Samples were also aliquoted for analysis in Nestlé Institute for Health Sciences laboratories for extended micronutrient profiles comprising extended hydrosoluble vitamins, liposoluble vitamins, trace elements, and fatty acids.

**Hydrosoluble Vitamins**

The analytical method for quantitating water-soluble vitamins in plasma samples consisted of a fully automated sample preparation followed by an Ultra Performance Liquid Chromatography Tandem Mass Spectrometry analysis (UPLC-MS/MS). Calibration Curve and QCs are prepared by automatically diluting standards working solutions into Milli-Q Water (Merck®, DE) in polypropylene tubes, then spiked into surrogate and plasma matrices. Into a 96 well plate, 100 µL of Calibration Curve, QCs and plasma are transferred, then 10 µL of an Ascorbic Acid (AsC) and DL-Dithiothreitol (DTT) solution in water, 10 µL of Internal Standards Working Solution and 100 µL of a Trichloroacetic acid (TCA) 7.5% solution in water are added. The plate is vortexed for 10 min at 1200 RPM at room temperature, then centrifuged at 2000 g for 10 min at room temperature. After centrifugation, 120 µL of supernatant is transferred to an AcroPrep Advance 96 Filter plate 0.2 µm supor membrane (PALL®, USA), then filtered by centrifugation at 2000 g for 15 min at room temp. The elution plate is sealed with an aluminium seal and injected into the UPLC-MS/MS for analysis. Chromatographic separation is performed using an Acquity UPLC System (Waters®, USA), 6µl is injected into an ACE Excel 2µm C18-PFP 100x2.1 ID analytical column (ACE®, SCT) using Milli-Q Water (Merck®, DE) containing 5% Acetic acid and 0.2% Heptafluorobutyric acid (HFBA) and Acetonitrile as mobile phases. Heptafluorobutyric acid is used as an ion-pairing agent to increase analyte retention. Mass spectrometric analysis and detection are performed using specific MRM for analytes and their related labelled internal standards on a Xevo TQ-XS (Waters®, USA), equipped with Electrospray Ionization source in positive mode and Argon as collision gas.

**Liposoluble Vitamins**

The analytical method for quantitating fat-soluble vitamins and carotenoids in plasma samples consists of a fully automated sample preparation followed by supercritical fluid chromatography coupled to a Mass Spectrometry analysis (UHP-SFC-MS/MS). Briefly, the Calibration Curve and QCs are prepared by automatic dilutions of standard working solutions

into Ethanol containing Butylated Hydroxytoluene (BHT) in glass tubes, then spiked into plasma matrices. Into glass tubes, 200 µL of Calibration Curve, QCs and plasmas are transferred, then 20 µL of Internal Standards Working, 10 µL of Internal Standards Working Solution and 300 µL Isopropanol/water 1/1 v/v are added, then mixed by aspirating/dispensing cycle to disrupt the protein binding. Finally, 100 µL Milli-Q Water (Merck®, DE) is added and then mixed by aspirating/dispensing cycle before being loaded into two ISOLUTE® SLE+ Supported Liquid Extraction Products (Biotage®, SE). A positive pressure of nitrogen is used to initiate impregnation, elution of samples is performed using two-time 1 mL of Hept/Isop 9/1 v/v containing 350 mg/L BHT, then three-time 1 mL of Heptan containing 350 mg/L BHT. The extracted volume of solvent is finally dried using a Centrifugal Vacuum Concentrator coupled with a CentriVap -84° C Cold Traps (Labconco®, USA) at 60°C for 25 min, then 6 °C for 15 min. The dry extract is resuspended by an addition of 200 µL of Hept/Isop 7/3 v/v containing 350 mg/L BHT, mixed by aspirating/dispensing, and then transferred into a glass vial before being injected into the SFC-MS/MS for analysis. Chromatographic separation is performed using an Acquity UPC2 System (Waters®, USA), 2µl is injected into a Viridis HSS C18 SB Column 3.0x100 1.8 um (Waters®, USA) using CO2 5.5 quality and MeOH containing 1.10 g/L of ammonium formate and 0.94 % of Milli-Q Water (Merck®, DE) as mobile phases. A mixture of Hept/Isop 7/3 v/v is used as a make-up solvent to promote the introduction of the flow into the Electrospray Ionization source. Mass spectrometric analysis and detection are performed using specific MRM for analytes and their related labelled internal standard on a Xevo TQ-XS (Waters®, USA), equipped with Electrospray Ionization source in positive mode and Argon as collision gas. Integration of chromatographic peaks is performed using Masslynx software (Waters®, USA). Raw data are uploaded to a LIMS system, “SLIMS” (Agilent®, USA), to be quantified using an “R” script embedded in the SLIMS server. A calibration curve is built using response (ratio of analyte area and internal standard area) and theoretical concentration of each calibration point, then weighted (1/X2). QCs and samples are quantified using the calibration curve and reported in ng/ml. The measured chemical entities encompass: All-trans Retinal (CAS Number: 116-31-4), Vitamin K1 (CAS Number: 84-80-0), α-Tocopherol (CAS Number: 10191-41-0), β-Tocopherol (CAS Number: 16698-35-4), γ-Tocopherol (CAS Number: 54-28-4), δ-Tocopherol (CAS Number: 119-13-1), Retinol (CAS Number: 68-26-8), α-Tocotrienol (CAS Number: 58864-81-6), γ-Tocotrienol (CAS Number: 14101-61-2), δ-Tocotrienol (CAS Number: 25612-59-3), β- Carotene (CAS Number: 7235-40-7), 25-Hydroxyvitamin D2 (CAS Number: 21343-40-8),

25Hydroxyvitamin D3 (CAS Number: 19356-17-3), β-Cryptoxanthin (CAS Number: 472-70-8), Lutein (CAS Number: 127-40-2), Zeaxanthin (CAS Number: 144-68-3).

**Minerals**

Mass spectrometric analysis and detection were performed using specific ions or multiple reaction parameters for analytes and related internal standards, including No Gas, Helium and Oxygen mode reaction gas, on an ICP-QQQ 8900 (Agilent®, USA). Counts are reported using MassHunter software (Agilent®, USA), and then raw data are uploaded to a LIMS system "SLims" (Agilent®, USA) to be quantified using a flow. Slims calculates the accuracy of each level based on theoretical values. The curve is forced by zero, and there is no ponderation weight. QCs and samples are quantified using the calibration curve and reported in ng/ml. The measured chemical entities encompass Aluminium (Al), Arsenic (As), Barium (Ba), Bromine (Br), Cadmium (Cd), Caesium (Cs), Calcium (Ca), Copper (Cu), Iodine (I), Iron (Fe), Lead (Pb), Magnesium (Mg) Manganese (Mn), Molybdenum (Mo), Phosphorus (P), Potassium (K), Rubidium (Rb), Selenium (Se), Strontium (Sr), Sulphur (S), Tin (Sn), Vanadium (V) and Zinc (Zn).

#### 4.3.5.3 Assessment of Form- Bio-electrical impedance analysis

Body form was assessed using multifrequency segmental bioelectrical impedance analysis (BIA). BIA was chosen as it is non-invasive and fast. BIA allows fat and lean mass estimates and provides raw impedance data correlated to nutritional state and disease outcome. The device used was the SECA mBCA 515 (medical Body Composition Analyser) with associated SECA BIA analysis software. Analysis software compared study data to age and sex-matched height-corrected measurements of healthy German blood donors to allow expression as standard deviation or "Z" scores (83). The BIA measurements of interest to the study can broadly be divided into 1) measured, i.e. raw impedance data; 2) derived, i.e. estimated body compartments using manufacturer-provided equations; and 3) Standardised; derived measurements corrected for height or converted to Z-scores for age and sex. Measurements were taken using standardised protocols from experts in nutritional assessment in the biomedical research facility.

Table 24: Bioelectrical Impedance Analysis measures and calculated data

| **Measured** | **Reported** | **Standardised** |
|---|---|---|
| R200 /R5, Phase angle 50KHz | Fat-Free Mass, Fat Mass, Total Body Water (TBW), Extra-cellular water (ECW) Visceral Adipose Tissue,<br>Skeletal Muscle mass | Fat-Free Mass Index (FFMI)<br>Fat-Free Mass Index (FFMI) "Z" score<br>Fat Mass Index (FMI)<br>Fat Mass "Z" score<br>Phase angle "Z" score |

#### 4.3.5.4 Assessment of function - Health-related Quality of life

Health-related quality of life (HR-QOL) was captured at each assessment using the fatigue-specific (FACIT-F), generic (short form 36(SF36) and IBD-specific (IBD Control) patient-reported outcome measures (PROMs), which are described below.

##### 4.3.5.4.1 FACIT-F

A key observation of INTICO-1 was the presence of individuals with excessive fatigue (36% reported fatigue on IBD Control PROM) and the reported change in this symptom with the dietetic intervention. A fatigue-specific PROM was chosen as the primary outcome variable. There is no established gold standard fatigue PROM for individuals with IBD. One fatigue-specific PROM was assessed using the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F). FACIT-F consists of 13 Likert-graded questions about the degree of fatigue symptoms and functional impairment, marked 1-5, with 52 representing no fatigue and lower scores denoting more significant fatigue. This validated score was initially developed to capture and quantify the degree of fatigue symptoms in individuals receiving cancer therapy, has internal consistency and can identify individuals with anaemia and poor performance status (84). FACIT-F has previously been validated in the IBD population (85). It has also been used in the IBD populations, with reported cut-offs for Fatigue (≤43) and severe fatigue (<30), respectively (86, 87).

FACIT-F is easy to administer, has a defined minimal clinically meaningful change, and a unidimensional score is easy to analyse. It has been used in large longitudinal studies of fatigue, and its responsiveness has been used in published measurements of the impact of IBD therapies on fatigue symptoms. It was this greater responsiveness and the shorter number of questions which the study team chose to feel that FACIT-F would be more suitable for the study as it was deemed less burdensome and more able to capture any changes over the cohort part of the study 12-month assessment. See sample FACIT-F appendix C7.

##### 4.3.5.4.2 Short Form 36 (SF-36)

The SF36, developed as part of the Medical Outcomes Surgery, assesses health-related quality of life in various medical conditions and interventions (88, 89). It comprises 36 questions with answers scored as grades 0-100 combined to calculate 8 scores. The SF36 scores explored in this study were for vitality (SF36VT), physical functioning (SF36PF), Physical limitation (RP) and

general health, which are generated from a mean of between 4 and 10 questions. SF36-PF and SF36-VT, generated from 10 and 4-question responses, respectively, have been reported as a measure of fatigue in IBD studies and were the composite scores of interests (90). The SF36 scores were converted to Z-scores using a healthy matched UK population (91). A sample of the questions, together with the scoring of each question and domain, is included in Appendix B5 SF-36 QUESTIONNAIRE (Fatigue).

#### 4.3.5.5 Disease Activity Assessments

Disease activity was assessed first at the screening step to ensure clinical (Harvey-Bradshaw Index <5) and biochemical (faecal calprotectin <250µg/g) remission. At each subsequent visit, disease activity was checked using serum C-reactive protein and faecal calprotectin as biomarkers of inflammation and the Harvey Bradshaw Index and IBD-Control (self-completed) as clinical disease scores.

### 4.3.6 The Nutritional State of the Population - Existing Screening Tools and Criteria to Identify Subjects with Malnutrition

Commonly used nutritional screening and assessment tools from current dietetic practice were applied retrospectively with the study data to consider whether any patients in whom the subsequent nutritional assessments (of intake, body composition, and biochemistry) found an altered nutritional state would have been screened for and diagnosed under the current practice. The tools described below require one or more assessments of body mass index (BMI), weight loss, lean body mass, and recent intake; data were available for 191 subjects.

#### 4.3.6.1 Nutritional Screening Tools

In UK dietetic practice, individuals at risk of malnutrition, such as or likely to require a nutritional intervention, are identified using the **Malnutrition Universal Screening Tool (MUST**) (300) (301). This adds a score for reduced Body Mass Index (BMI), percentage weight loss in the previous 3 months and whether is likely to be no nutritional intake for longer than 5 days. A score of 1 identifies medium risk, and 2 or more identifies high risk of malnutrition. . The score has been correlated to disease activity in IBD length of stay and disease complications in general inpatients (302) (303).

In current practice, subjects with a MUST score of ≥1 would undergo further nutritional assessment and be considered for intervention. The BMI was calculated using the height and weight measurements from the study visit.

Other nutritional screening tools have been used in dietetic practice and have published data in IBD patients, such as the **nutrition risk index (NRI),** the **Nutritional Risk Screening 2002 (NRS-2002), Malnutrition Inflammation Risk Tool (MIRT**) and more recently an IBD-specific tool, the **Saskatchewan IBD–Nutrition Risk (SaskIBD-NR Tool)** (228, 304)

These tools and their components and how they were applied to the INTICO-2 cohort are described below and in Table 25.

The **Nutritional Risk Index** generates a score from the multiple of the serum albumin and a multiple of the proportion of current weight divided by the stable weight 6 months ago. It classifies subjects to no risk of malnutrition or mild, moderate, or high (233).

The **NRS-2002** predicts response to nutritional interventions and scores someone as being at nutritional risk if they have one or more of a BMI <20.5kg/$m^2$, weight loss in the previous 3 months, reduced dietary intake in the last 7 days, or are a current intensive care patient(235). For the INTICO-2 data, BMI at the assessment visit and the response to a direct question regarding weight loss in the INAT PROM, (together with a recorded food diary below 50% of estimated requirements) were used to calculate NRS-2002.

The **Malnutrition Inflammation Risk Tool (MIRT)** score was developed using baseline and 6-month follow-up data in 55 IBD patients to identify patients at risk of disease complications (231). Like MUST, the MIRT score assigns a score for weight loss and reduced BMI but combines it with a score for an elevation of CRP (>10mg/dL). The total score and each of the three contributory components were correlated to a higher risk of disease complications and a composite score of disease progression at 6 months, with (MIRT >3 having a relative risk of 4 for adverse outcomes).

The **Saskatchewan IBD–Nutrition Risk (SaskIBD-NR Tool)** recognises the limited utility screening for nutritional risk by BMI in IBD cohorts taken from populations likely to include obese subjects (228). It was designed by dietitians and assigns a score for 1) weight loss, 2) gastrointestinal symptoms in the previous two weeks, 3) poor intake due to reduced appetites and 4) food group restriction. The total score from these four domains is more sensitive and specific for identifying a diagnosis of malnutrition from a subsequent blinded comprehensive dietitian nutritional assessment than the BMI-based score generated from MUST.

The components of these nutritional screening scores and how each was considered and applied to the INTICO 2 cohort are described overleaf in Table 25.

Table 25: Nutritional Screening Scores previously used in IBD populations and how they were considered and applied (if possible) to the cohort

| **Screening Tool** | **Components** | **Applied to the INTICO2 Data** |
|---|---|---|
| MUST (301) | Weight loss in previous 3-6 months<br>BMI<br>Acute Illness and no intake for 5 days | Using INAT Question 1 (Yes/No) Q2 (amount and duration)<br>Using Study Recorded BMI<br>Not applicable to the study |
| Nutritional Risk Index (NRI) (233) | Serum Albumin<br>Weight<br>Percentage weight loss | As the "usual weight" was not recorded, this was not applied to the cohort. |
| Nutritional Risk Score 2002 (NRS-2002) (235) | BMI <20.5<br>Weight Loss within 3 months<br>Reduced Dietary intake in the last week<br>ICU Patient | Using Study Recorded BMI<br>Using INAT Question 1 (Yes/No) Q2 (amount and duration)<br>Using Food diary (<50% calculated requirements)<br>This component didn't apply |
| Malnutrition Inflammation Risk Tool (MIRT) (231) | BMI<br>Weight Loss in previous 6 months<br>CRP | Using Study Recorded BMI<br>Using INAT Question 1<br>Using Assessment blood test |
| Saskatchewan IBD–Nutrition Risk (SaskIBD-NR Tool) (228) | Nausea / Vomiting /diarrhoea / poor appetite in previous 2 weeks<br>Unintentional weight loss in the previous month<br>Amount of weight loss<br>Eating poorly due to decreased appetite<br>Restricting food groups | Subjects were not directly asked about weight loss in 1 month<br>Or, whether they were eating poorly due to a decreased appetite nor was it recorded, so SaskIBD could not be applied |
| IBD Nutritional Screening tool (NS-IBD) (236) | BMI<br>Unintended weight loss in previous 6 months<br>Chronic Diarrhoea or ileostomy<br>Other GI symptoms<br>Previous Surgery | Using Study Recorded BMI |

### 4.3.7 Statistical Analysis

Data analysis was performed according to the statistical analysis plan. Statistical analysis was performed in R version 3.4.4 using Tidyverse, SkimR, TableOne, GGPlot2, and GGPUBR.

Analysis of data was performed using a stepwise approach. First, simple descriptive statistics were used. Results were presented as mean ± standard deviation (SD) for normally distributed variables and median and interquartile range (IQR) otherwise.

Distribution was assessed using distribution graphs (histograms).

Continuous variables were assessed for normality, and differences between those with and without fatigue were evaluated for significance using a two-sample t-test (parametric) and a Wilcoxon rank-sum test (non-parametric), respectively. Categoric variables were compared between those with and without fatigue using a Chi-squared test. 95% CIs were calculated using the adjusted Wald method. P values for the differences between groups and linear trends across the groups were analysed, taking $p<0.05$ as a threshold of significance. Further, if two variables were expected to present linear relationship, Pearson (presented as 'r') and Spearman (presented as 'Rho') correlation analyses were undertaken to examine associations between normally and non-normally distributed variables, respectively. After assessing for confounding variables to include in the model, the relationship and significance between micronutrient blood tests and FACIT-F score was assessed using binary logistic regression for fatigue / non-fatigued and as a continuous variable using multiple linear regression.

## 4.4 Results

### 4.4.1 Study Recruitment and Screening

Of the 1386 potentially eligible subjects approached, 336 (24%) contacted the study team to express an interest in doing the study. Of these, 238 underwent screening for study eligibility, 27 failed screening, 21 for elevated FCP, 3 for elevated HBI and one for each of the following reasons: PR bleeding, suspected short bowel syndrome and uncertain diagnosis of Crohn's.

201 subjects underwent a study visit, with 3 removed by the investigator (SS and MM) after a review of the study documentation due to errors in the insecure diagnosis (1), being on glucocorticosteroids (1) and a combination of incomplete PROMS due to suspected dementia and bloods measures after bowel prep (1). This left 198 subjects completing the study visit, of which 195 consented to the 12-month study follow-up. 195 subjects completed the PROMS to be added to the dataset. The consort diagram for the study in displayed in Figure 34

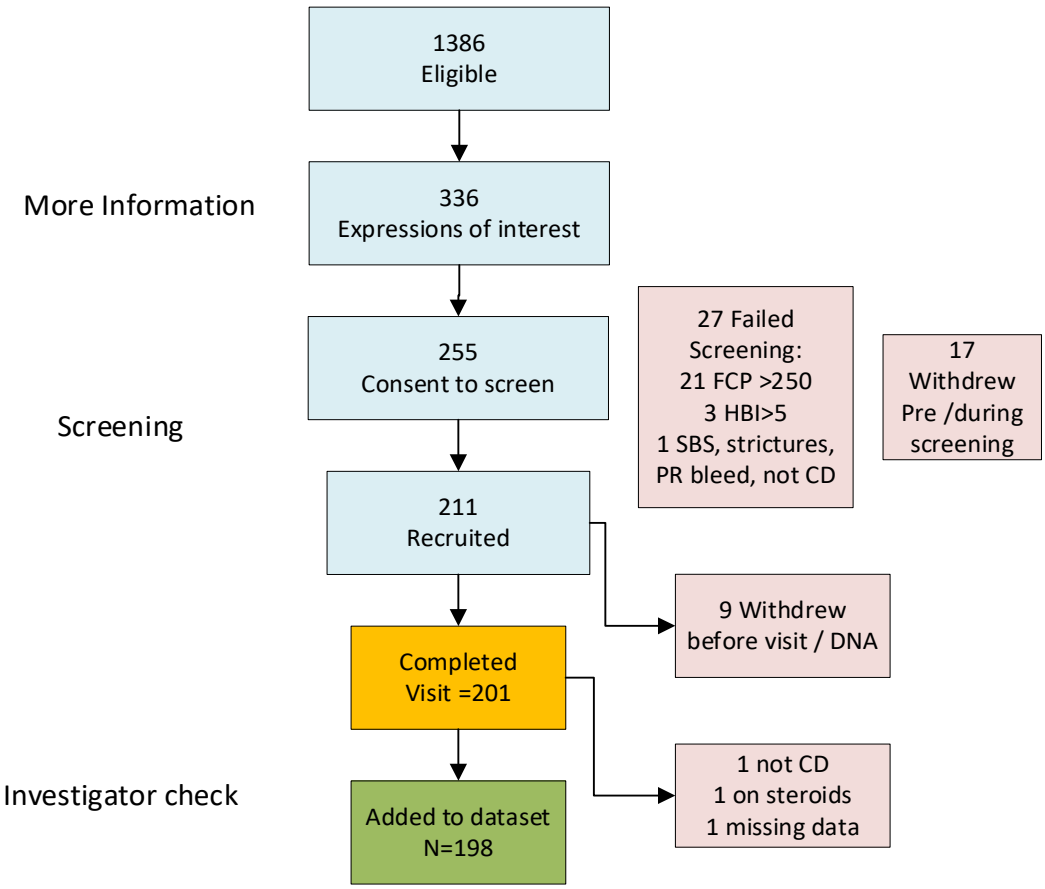


Figure 34: INTICO-2 Consort diagram for Screening and Recruitment

### 4.4.2 INTICO 2 Study Subject Characteristics

The characteristics, disease phenotype, treatment, and disease activity markers of 198 study subjects are described below in Table 26. Using criteria previously used to define severe Crohn's disease, subjects "Any Previous or Current Feature of severe disease" using the criteria previously described, specifically if their electronic record showed evidence for one or more of previous stricture, fistula, CD surgery or biological, extensive small bowel disease (>40cm documented) or were biologics experienced (305).

The cohort tended to be older, with the median age of study subjects being 52, and just over half of them were female. Characteristics of note were that 46/198 (23%) had undergone previous resection surgery for their Crohn's disease and 82/198 (41%) were on an advanced therapy (e.g. monoclonal antibody treatment) when entering the study. 118/198 (60%) had documented evidence of previous or current severe disease phenotype.

Table 26: INTICO-2 cohort study subject characteristics

| **Participant characteristics (n=198)** | |
|---|---|
| Age (mean (range)) | 52.3 (20-88) |
| Gender Male (%) | 97 (49) |
| Disease Duration (mean (range)) | 10 (1-61) |
| **Montreal** | |
| Subtype Age = A1 (%) | 12 (6.1) |
| Subtype Age = A2 (%) | 98(49.5) |
| Subtype Age = A3 (%) | 88 (44.4) |
| Subtype Location = L1 (%) | 65 (32.8) |
| Subtype Location = L1/L4 (%) | 3(1.5) |
| Subtype Location = L2 (%) | 59 (29.8) |
| Subtype Location = L3 (%) | 70 (35.4) |
| Subtype Location = L3/L4 (%) | 1(0.5) |
| Subtype Behaviour (%) | |

| | |
|---|---|
| B1 | 135 (68.2) |
| B2 | 46 (23.2) |
| B3 | 17 (18.7) |
| **Participant characteristics (n=198)** | |
| **Previous resection surgery** (%) | |
| 0 | 152 (76.8) |
| 1 | 37 (18.7) |
| 2 | 9 (4.5) |
| **Any Previous or Current Feature of Severe Crohn's** (Stricture/ Fistula/ CD Surgery/ previous biologic) | 112(61) |
| **Vitamin supplementation** | |
| Multivitamin | 82 (42.4) |
| Vitamin D | 76 (38.4) |
| B12 injection | 58 (29.3) |
| **IBD Medication** (%) | |
| Biologic | 82 (41.4) |
| Immunomodulator | 70 (35.4) |
| Harvey Bradshaw Index (median [range]) | 1.00 [0.00, 4.00] |
| Calprotectin (µg/L) (median [range]) | 34.5 [3.8, 248] |
| CRP (mg/L) (median [range]) | 2.00 [1.00, 22.00] |

### 4.4.3 Primary Outcome Measure: Fatigue

Of the 198 patients, 195 completed the FACIT-F PROM. The median FACIT-F score was 40 (IQR 17), and there was a left skew in the data (i.e. a larger number of study subjects had high scores, suggesting less fatigue). Severe fatigue, defined as a FACIT-F score <30, was present among 50/195 (26%) study subjects. 75/195 (38%) of study subjects had a FACIT-F score of 31-43 (mild fatigue). The distribution of FACIT-F score and cut-offs for mild and severe fatigue are expressed below in Figure 35. Women tended to be more tired than men, with a median (IQR) FACIT-F score of 39 (17) versus 42 (16) in men (Wilcoxon rank-sum test p 0.025).

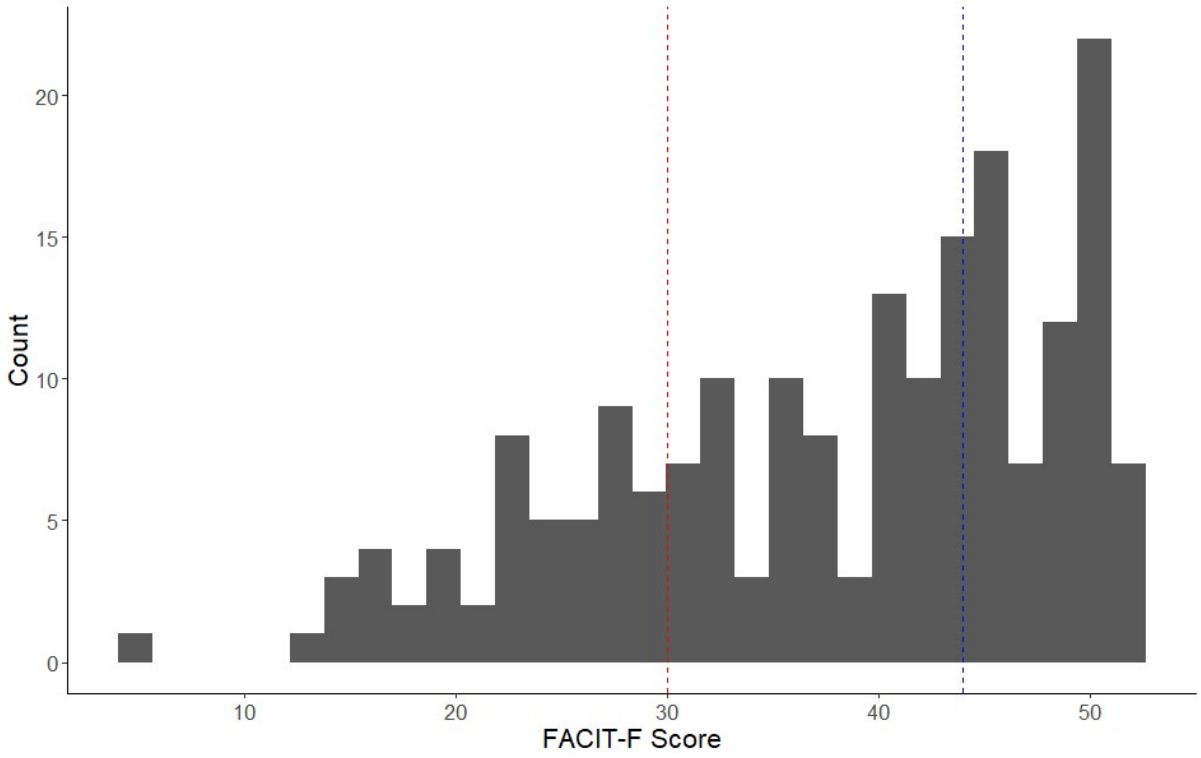


Figure 35: FACIT-F score histogram of study subjects.

Score out of 57, with a lower score denoting greater fatigue. The red line indicates the cut-off for severe fatigue (<30) and the blue line indicates the cut-off for any fatigue (44).

#### 4.4.3.1 Consistency of FACIT-F with other PROMS

The different PROMs used to assess fatigue were consistent in their findings. A strong and statistically significant positive correlation (*r*0.79, p 2.2-16) was observed between the FACIT-F score and SF-36 VT (see Figure 36). The answers to the IBD Control PROM fatigue question, "In the past 2 weeks did you often feel lacking in energy or fatigue (more than half the time?" were also consistent with the FACIT-F score, with those who answered "Yes" having a median FACIT F score in the range for severe fatigue that was statistically significantly lower than those who said "not sure" or "no" (29, 40 and 40 respectively p <0.00001; see Figure 37). FACIT-F also showed a weaker but statistically correlation with the SF-36 measures of performance status, SF36-Role Limit physical and SF36-Physical Function (r0.64, P 2.2e-16 and 0.54 P<6.8e-16), respectively.

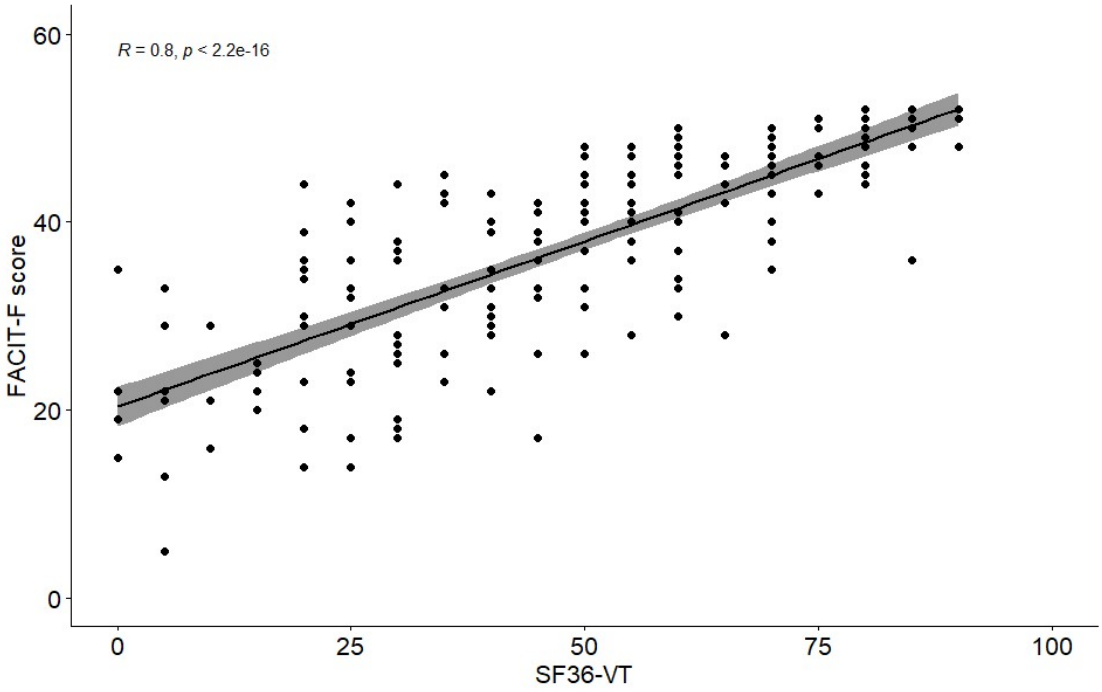


Figure 36: Scatter plot with correlation line the PROMs to capture fatigue; FACIT-F (primary outcome, score 0-57) vs SF36 Vitality score (score 0-100) (Pearson's Rank Correlation R 0.8, p <2.2e-16)

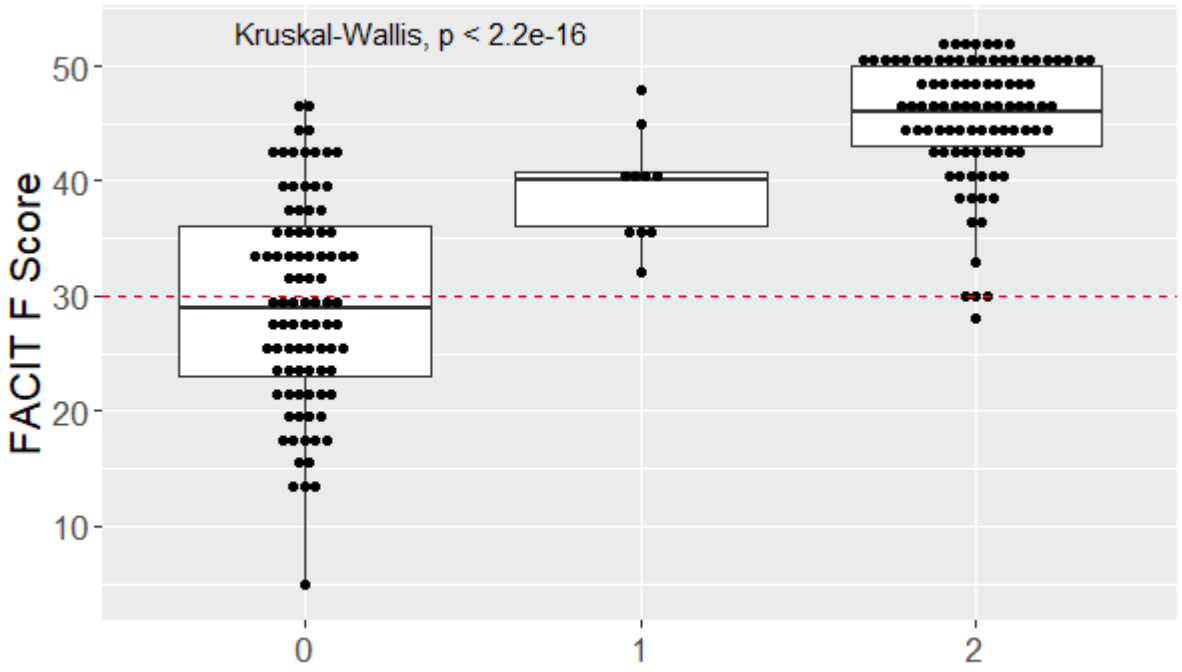


Figure 37: FACIT-F score vs response to fatigue question in IBD Control PROM

(0=" Yes" to tired more than 50% of the time, 1="not sure", 2="no") Median of three groups compared with Kruskal-Wallis H test

#### 4.4.3.2 Study Subject Characteristics Correlations to Severe Fatigue

The cohort study demographics were compared between those who did and didn't have severe fatigue (as classified by a FACIT-F score <30) (see Table 27).

Age, Montreal subtype, features of a severe CD phenotype, treatment group, gender and faecal calprotectin did not differ between the groups.

There was a statistically significant difference in the C-reactive protein (CRP) and Harvey Bradshaw Index (HBI) between the two groups, with a higher CRP and higher HBI in those with severe fatigue.

To address confounding from the general well-being question of the Harvey Bradshaw Index, this was removed from the total HBI score (for the 184 subjects where the breakdown was available) from the electronic site file, generating a new numerical variable "HBIG".

The HBIG remained higher among those with severe fatigue than those without.

Table 27: Study Cohort Characteristics, stratified by those with and without severe Fatigue (FACIT-F <30)

| | level | Not Severe fatigue (FACIT-F ≥30) | Severe fatigue (FACIT-F <30) | p |
|---|---|---|---|---|
| n | | 141 | 50 | |
| Age (median [range]) | | 53 [21, 88] | 52 [20, 84] | 0.674 |
| Gender (%) | Female | 68 (48) | 31 (62.0) | 0.131 |
| | Male | 73 (52) | 19 (38.0) | |
| Disease Duration (median [range]) | | 9[1, 54] | 7.50 [0, 51] | 0.342 |
| MONTREAL A (%) | A1 | 8 (6) | 4 (8) | 0.31 |
| | A2 | 74 (53) | 20 (40) | |
| | A3 | 59 (42) | 26 (52) | |
| MONTREAL L (%) | L1 | 47 (33) | 16 (32) | 0.501 |
| | L1/L4 | 0 (0) | 1 (2) | |
| | L1L4 | 1 (1) | 1 (2) | |
| | L2 | 39 (28) | 16 (32.0) | |
| | L3 | 53 (38) | 16 (32.0) | |
| | L3L4 | 1 (1) | 0 (0) | |
| MONTREAL B (%) | B1 | 93 (66) | 37 (74) | 0.532 |
| | B2 | 34 (24) | 10 (20) | |
| | B3 | 14 (10) | 3 (6) | |
| Any Previous or Current Feature of Severe Crohn's (Stricture/ Fistula/ CD Surgery/ previous biologic) | no | 59 (42) | 19 (38) | 0.758 |
| | yes | 82 (58) | 31 (62) | |
| Previous IBD Resection surgery (%) | 0 | 106 (75) | 40 (80) | 0.788 |
| | 1 | 28 (20) | 8 (16) | |
| | 2 | 7 (5) | 2 (4) | |
| Biologic (%) | N | 89 (63.1) | 24 (48) | 0.089 |
| | Y | 52 (36.9) | 26 (52) | |
| Immunomodulator (%) | | 0( 0.0) | 1 (2) | 0.211 |
| | N | 89 (63.1) | 33 (66) | |

| | | | | |
|---|---|---|---|---|
| | Y | 52 (36.9) | 16 (32) | |
| Harvey Bradshaw Index (median [range]) | | 1 [0, 4] | 2.00 [0, 4] | <0.001 |
| Calprotectin (median [range]) | | 35 [3.8, 237] | 31 [3., 248] | 0.876 |
| CRP mg/L (median [range]) | | 2 [1, 22] | 3 [1, 17] | 0.015 |
| HBIG (median [range]) | | 0 [0, 4] | 1 [0, 3] | 0.002 |

#### 4.4.3.3 Discussion of Fatigue Measurement

The fatigue PROMS data, which included the primary study outcome, FACIT-F, defined just over a quarter of subjects as having severe fatigue. A further 38% fulfilled the criteria for mild fatigue. This supports the hypothesis that there were individuals in the CD remission population suffering from symptoms of fatigue. This percentage of individuals with severe fatigue is similar to that of another published cohort, validating the usage of FACIT-F in IBD but lower than a study in which more than a third of subjects were recorded as having active disease (17) (16). Overall, the cohort had a lower mean FACIT-F score (37) than published data from the healthy adult populations (43), suggesting that when considered against population norms, our population tended to be more tired (306).

The FACIT-F PROM was chosen as a fatigue-specific PROM for INTICO-2 after the SF36-VT score identified excessive fatigue in the INTICO-1 population. The SF-36-VT score, which has published data as a measure of fatigue in IBD, correlated strongly with the FACIT-F score, suggesting that they were consistent measures of fatigue for the study subjects. The SF-36-VT was similar in the INTICO-1 and INTICO-2 cohorts, suggesting that the fatigue signals seen in INTICO-2 were present in the larger cohort. Taken together, this data supports the view that excessive fatigue, as measured by FACIT-F is present in the CD remission cohort.

#### 4.4.3.4 Disease Features and Severe Fatigue

When the cohort characteristics were compared between those who did and didn't have severe fatigue (FACIT-F<30), those with fatigue had a higher clinical activity score and CRP, suggesting that systemic inflammation and persistent disease symptoms are implicated in excess fatigue in CD remission.

Biological therapies have been found to release fatigue but are also given to those with a more aggressive disease phenotype, which may offset one another with respect to correlation to fatigue.

### 4.4.4 The Nutritional State of the INTICO-2 Population

The first section of the results described the disease phenotype and fatigue PROMs of the INTICO-2 population. The nutritional status of the cohort will now be described.

The study data and cohort will then be considered in the context of current screening and assessment tools.

### 4.4.5 Nutritional State – INTAKE

Energy and nutrient intake were assessed using a food diary in the 7 days preceding the study visit and the INAT questionnaire at the study visit, which asked directly about factors that may impact habitual intake, referred to as “dietary impact factors”.

#### 4.4.5.1 Evidence of factors that may determine INTAKE: INAT Questionnaire

There was evidence of factors that may adversely affect the habitual intake of the study subjects captured by the INAT questionnaire.

##### 4.4.5.1.1 Appetite and Food Avoidance

An impaired appetite (defined as a SNAQ score <14) was present in 30% (57/192) of the subjects (299). 35% of the cohort reported at least one food restriction. The most common avoidances were dairy, wheat, red meat, pulses, garlic and onion.

##### 4.4.5.1.2 Food-related symptoms

Subjects were asked directly about symptoms after eating in the previous month. Food-related symptoms were common, with abdominal pain, distension and diarrhoea the most frequently reported among the cohort.

The number and percentage of subjects reporting food-related symptoms are described in Table 28.

Table 28: Food-related Symptoms in the Previous Month- as reported by the direct question in the INAT

| **Symptoms Present after Eating** | **N (%)** |
|---|---|
| Nausea | 49 (25) |
| Vomiting | 8 (4) |
| Diarrhoea | 70 (36) |
| Abdominal Pain | 84 (44) |
| Distension | 80 (42) |
| A Feeling of Food Getting Stuck | 18 (9) |

#### 4.4.5.2 Food Diary Analysis to Assess Nutritional State

##### 4.4.5.2.1 Macronutrient Intake

Dietary analysis data was available for 195 subjects. Following a review of the entries by the study dietitians, two subjects' results were removed from the dataset as they were deemed too incomplete to reliably assess the adequacy of intake.

The median daily recorded energy and macronutrient intake of the 193 subjects included in the dietary data is displayed below in Table 29.

Recorded energy intake was also expressed as a multiple of the estimated basal metabolic rate (BMR), or physical activity level (PAL). BMR was predicted using the Henry equation (307). The median (range) PAL of the cohort was 1.18 (0.33-1.95).

Six subjects recorded an intake below 70% of their estimated BMR, and as this was a cause of concern to the research team for adequacy of diet and accuracy of study data, they underwent a further review by one of the study dietitians (VK and CW).

This review first involved a detailed review of the diary to check for obvious omissions (e.g. missing days and implausible portion sizes. Those with a confirmed recorded intake of below 70% of their BMR were then reviewed on the electronic health record and had a follow-up phone call with the study dietitian. Two of these subjects were obese by BMI and aiming to lose weight, one had significant food avoidance due to concerns about previous strictures. The notes on each of these subjects are recorded in the appendix (see Appendix C8).

The median protein intake was 74 (29-193) g/day but was below the recommended intake of 0.75g/Kg in 18% (36/195) of subjects.

Table 29: Macronutrient intake from Food diary analysis of 195 INTICO2 participants

| **Nutrient (unit) (LRNI)** | **Median (RANGE)** |
|---|---|
| Energy intake (kcal) | 1836 (850-3571) |
| Energy Intake / measured BMR (PAL) | 1.18(0.33-1.95) |
| Protein (g) | 74(29-192) |
| Protein intake (g/Kg) (0.75) | 0.97 (0.33-1.95) |
| Carbohydrate (g) | 195(25-472) |
| Fat (g) | 71 (27-472) |
| Fibre (g) (30) | 18 (7-144) |

#### 4.4.5.2.2 Micronutrient adequacy of diet

41% (80/195) of the cohort took oral micronutrient supplements, which subjects were advised to continue and record during the 7-day food diary.

There were multiple nutrients for which subjects recorded a dietary intake that failed to meet the LRNI (see Table 30). When the micronutrient supplements were added to the intake, apparent inadequacies of intake were fewer (18% less), but remained common and often multiple, with a mean of 6 (SD 4) micronutrients below the LRNI per subject. The commonest inadequacies of intake post-inclusion of supplements became Copper (73%), Selenium (68%), Vitamin D (60%), Folate (52%) and Potassium (49%).

To demonstrate patterns of dietary inadequacies by subjects and nutrient, a heat map with mean daily intake for each micronutrient as a percentage of RNI (row) for each of the study subjects (column) is can be seen in Figure 38 F). This demonstrate that there were nutrients for which inadequacies of intake were common and that some individuals in the cohort were low across multiple nutrient intakes. The effect of the inclusion of supplements on the adequacy of intake vs LRNI can be visualised in Figure 39 and Figure 40 and , respectively (red denotes when the mean daily intake failed to meet LRNI_.

The median daily intake of each micronutrient before and after the inclusion of the supplement is displayed in Table 30.

Table 30: Mean Daily Micronutrient (Median for cohort) and Comparison to LRNI as estimated by Nutritics with and without usual supplements

| **Nutrient (LRNI)** | **Daily Intake from Diet alone** | **Number (%) with intake below LRNI from Diet alone** | **Daily Intake from Diet + supplement** | **Number (%) with intake below LRNI from Diet + supplement** |
|---|---|---|---|---|
| Vitamin A (RE 250 | 444 [28- 3937] | 40 (21) | 559[28- 3,937] | 34 (17) |
| Thiamine B1 (0.23mg/1000kcal) | 1.1 [0.3-8.0] | 0 | 1.2 [0.3-101.8] | 0 |
| Riboflavin B2 (0.8mg) | 1.2 [0.3- 18.9] | 46 (24) | 1.4 [0.3-18.9] | 38 (20) |
| Niacin B3 (4.4mg/1000kcal) | 23.1 [0.7- 61.8] | 0 | 27.0 [0.7-80.0] | 0 |
| Pantothenate B5 (5mg) | 3.8 [0.3-51.4] | 0 | 4.4 [0.3-51.4] | 0 |
| Pyridoxic acid B6 (11µg/g protein) | 1.3 [0.2- 6.4] | 59 (30) | 1.6 [0.4-140.7] | 44 (22) |
| Folate B9 (200µg) | 155 [7-961] | 137 (70) | 195 [7- 9,434] | 102 (52) |
| Hydroxocobalamin B12 (1µg) | 3.1 [0.3- 18.7] | 8 (4) | 3.8 [0.3-1,002] | 6 (3) |
| Vitamin C (10mg) | 53 [5-242] | 4 (2) | 64 [5- 3,151] | 4 (2) |
| Vitamin D (10µg) | 1.9 [0.00-11.3] | 190 (97) | 5.9 [0.2-126.8] | 116 (60) |
| Vitamin E (3mg) | 5.2 [0.7-13.0] | 27 (14) | 6.5 [0.7-55.3] | 21 (11) |
| Vitamin K (128µg) | 26 [0-349] | 0 | 27 [0- 349] | 0 |

| Nutrient (LRNI) | Daily Intake from Diet alone | Number (%) with intake below LRNI from Diet alone | Daily Intake from Diet + supplement | Number (%) with intake below LRNI from Diet + supplement |
|---|---|---|---|---|
| Calcium (400mg) | 570 [35-1,529] | 46(24) | 648 [35-3,418] | 39 (20) |
| Copper (1.2mg) | 0.8 [0.0-135.8] | 162(83) | 0.8 [0.1-1,000.6] | 142 (73) |
| Iodine (70µg) | 78 [3-325] | 82 (42) | 86 [3-425] | 69 (35) |
| Iron (Men all ages, women over 50: 4.7mg) (women 18-50: 8.0mg) | 6.9 [1.2-62.7] | 53 (27) | 8.4 [1.2-203.1] | 43 (22) |
| Magnesium (150mg) | 188 [10-634] | 77 (40) | 205 [10-1,217] | 67 (34) |
| Manganese (1.4mg) | 2.1 [0.1-10.2] | 46 (24) | 2.3 [0.1-102.6] | 40 (21) |
| Phosphorous (550mg) | 798 [37- 1,946] | 0 | 798 [37-2,041] | 0 |
| Potassium (2000mg) | 1,965 [382-5,826] | 98 (50) | 2,012 [383-5,827] | 96 (49) |
| Selenium (40µg) | 28.7 [0.5-73.8] | 142 (73) | 30.4 [0.5-128.9] | 132 (68) |
| Sodium (575mg) | 2,055 [774-5,373] | 0 | 2,055 [774, 6,321] | 0 |
| Zinc (Men: 4.5mg, women: 4.0mg) | 5.8 [0.3-48] | 55 (28) | 6.3 [0.3, 53.5] | 44 (23) |
| **Total number of nutrients with intake below LRNI in the cohort** | | 1266 | | 1037 |

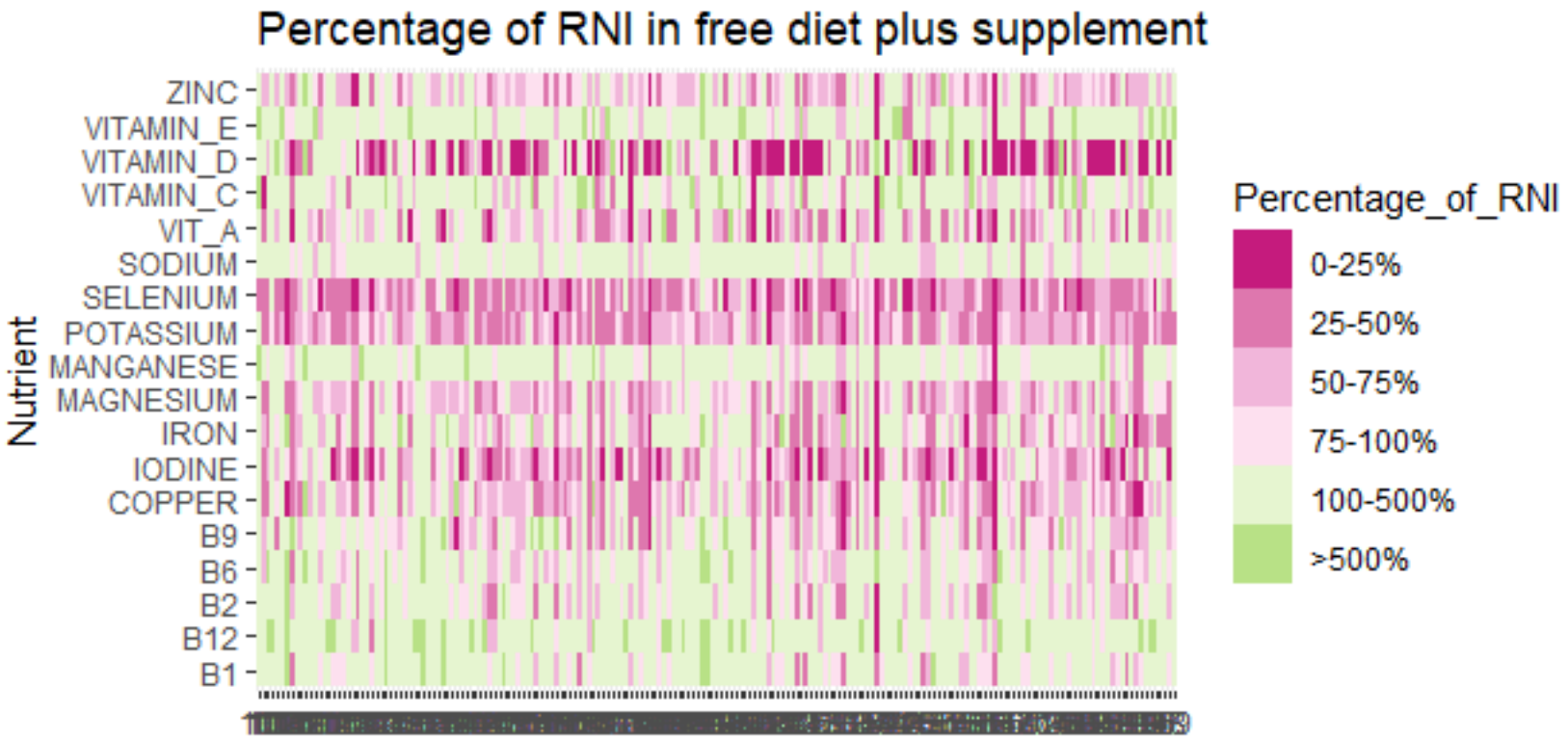


Figure 38: Micronutrient intake including supplements by subject and nutrient as a percentage of reference nutritional intake (RNI) from the 7-day food diary –

green represents >100%, pink <100%, with darker pink denoting a lower percentage of R

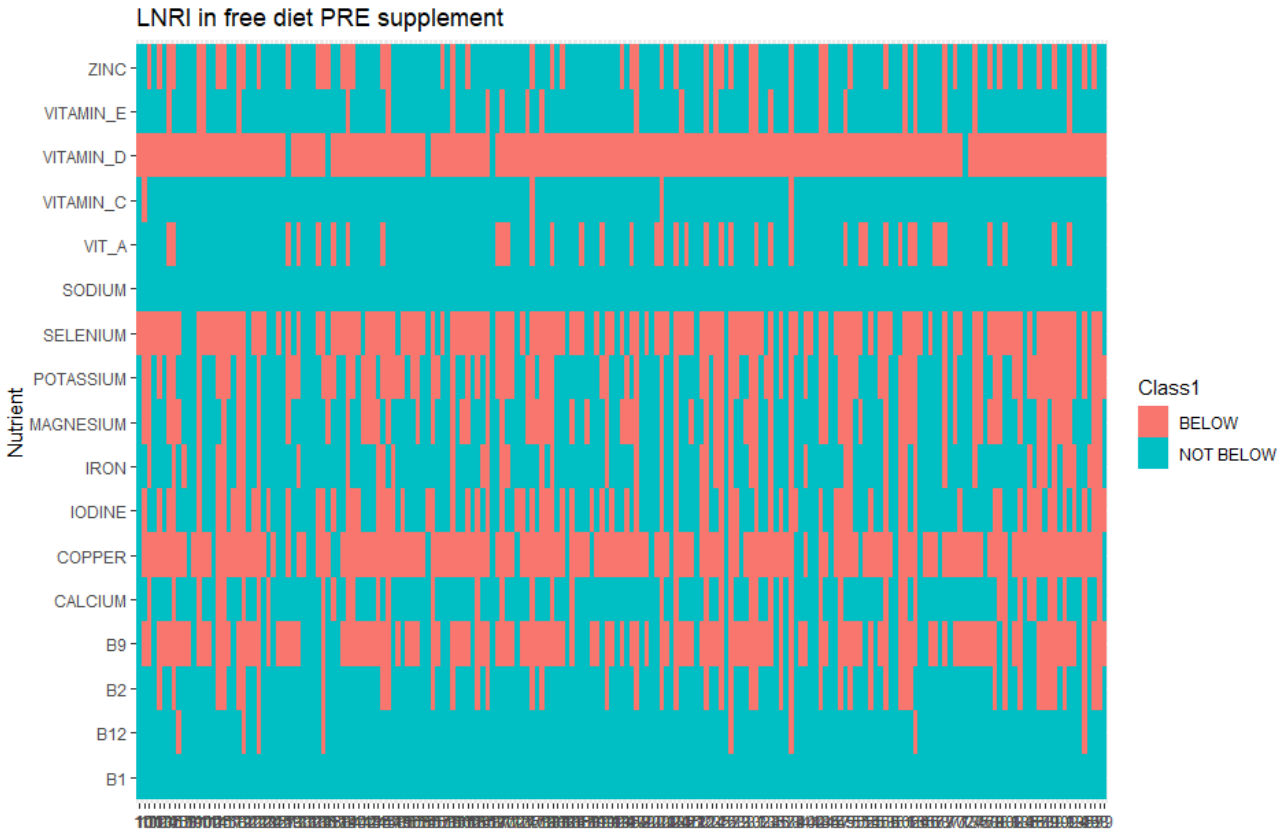


Figure 39: Micronutrient intake below LRNI from diet alone

Red<LRNI, Blue>LRNI. Each column represents a subject, each row the micronutrient

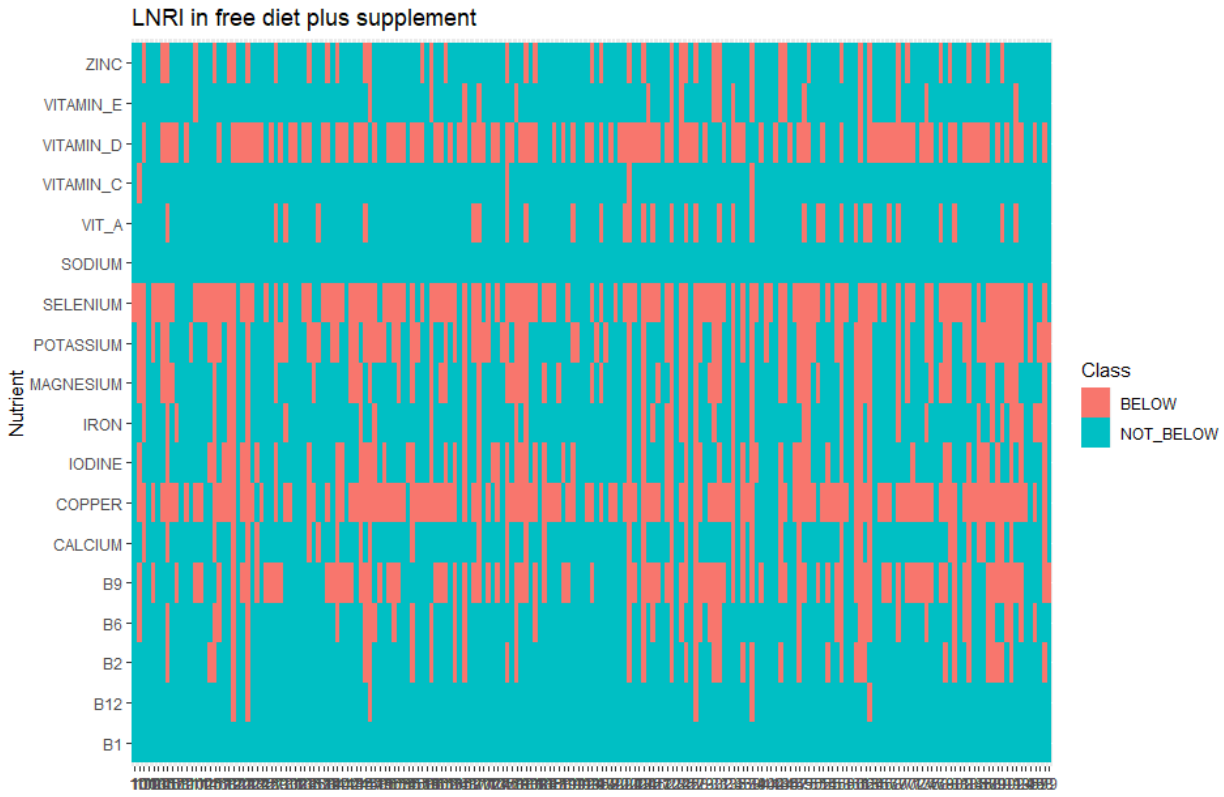


Figure 40: Micronutrient intake below LRNI from diet PLUS supplements

Red<LRNI, Blue>LRNI. Each column represents a subject, each row the micronutrient

#### 4.4.5.3 INAT dietary impact factors as a determinant of micronutrient adequacy of diet

The INAT questions on 1) impaired appetite, 2) restriction or avoidance of foods, and 3) food-related gastrointestinal symptoms appeared to be related to inadequacies of micronutrient intake.

A statistically significant but weak negative correlation existed between the SNAQ appetite score and the number of nutrients below the LRNI (*r* -0.29, *p*=3.6e-05) (see Figure 41).

The total INAT score generated for the 6 questions on food-related gastrointestinal symptoms (whether subjects had nausea, vomiting, diarrhoea, pain, distension and a feeling of food getting stuck after eating (a higher score for the absence of each symptom)) was also negatively correlated to the number of nutrients below the LRNI (r -0.25, p =0.00042) (see Figure 42). When SNAQ and food-related gastrointestinal symptoms were combined, the correlation was stronger (see Figure 43).

Finally, a composite score of "dietary impact factors" generated by reporting the exclusion of foods, the number of food-related symptoms and impaired appetites (as assessed by the SNAQ) showed the strongest correlation (*R* 0.32) to having a greater number of nutrients below the LRNI from the food diary analysis (see Figure 44).

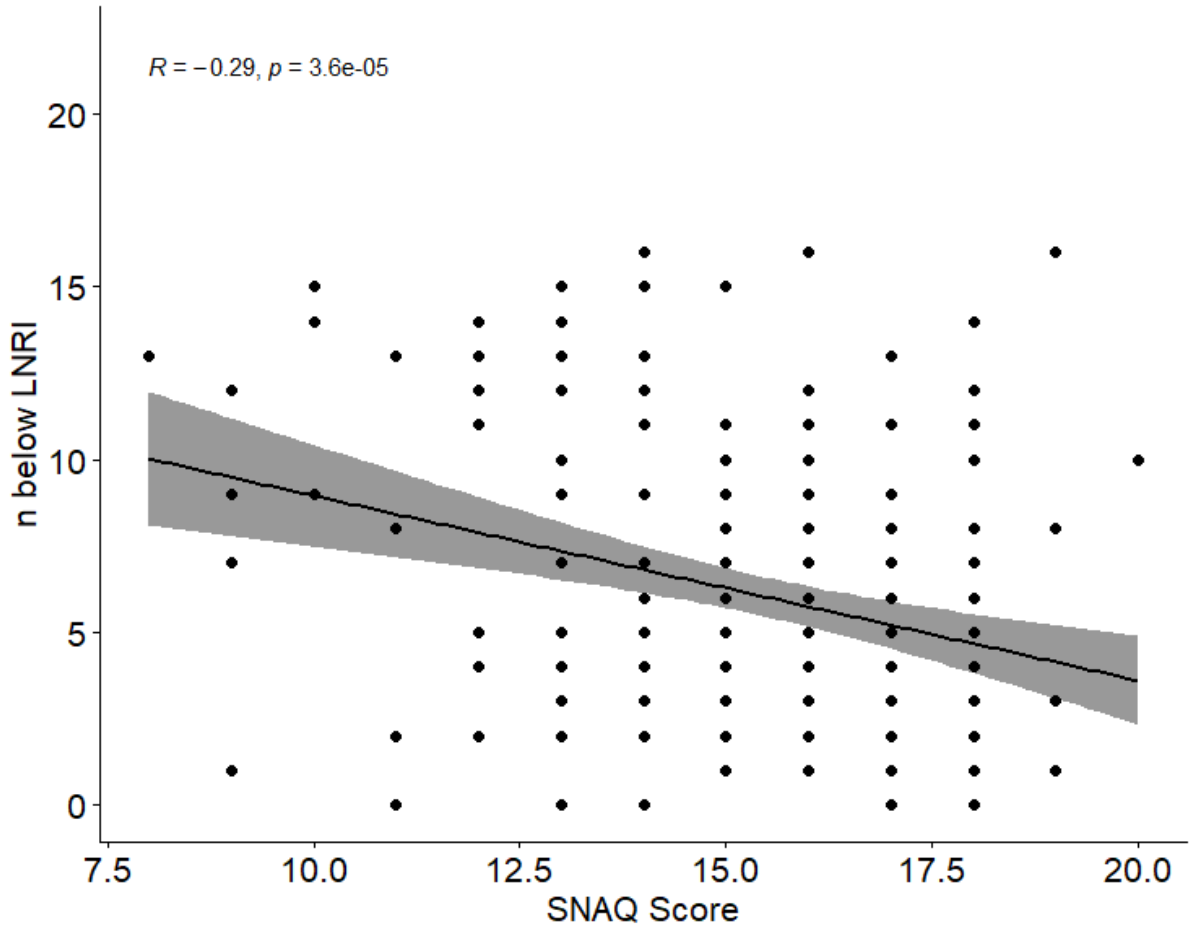


Figure 41: Micronutrient adequacy of recorded daily intake vsSNAQ Score

(a lower SNAQ score denotes reduced appetite, <14 considered a poor appetite) micronutrient adequacy assessed by the number of nutrients per subject that fell below the LRNI)

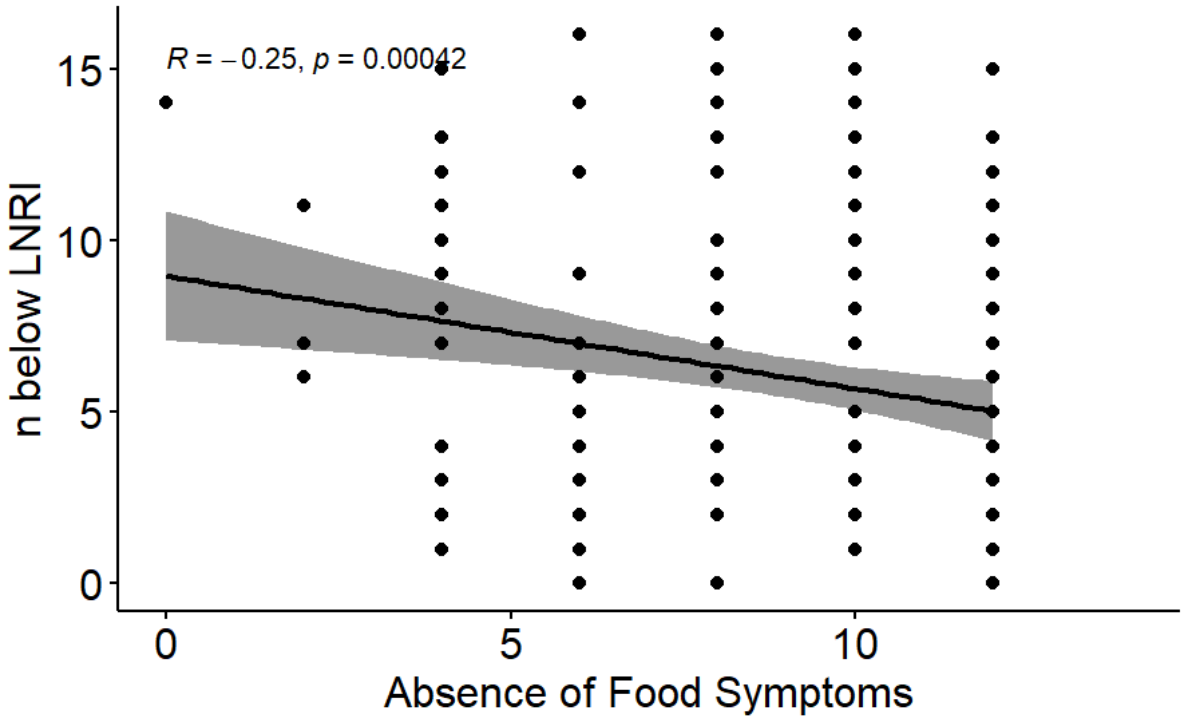


Figure 42: Micronutrient adequacy of the diet Vs The absence of food symptoms

lower food symptom score for those with one or more of Nausea, Vomiting, diarrhoea, pain or distension after food in the preceding 7 days)

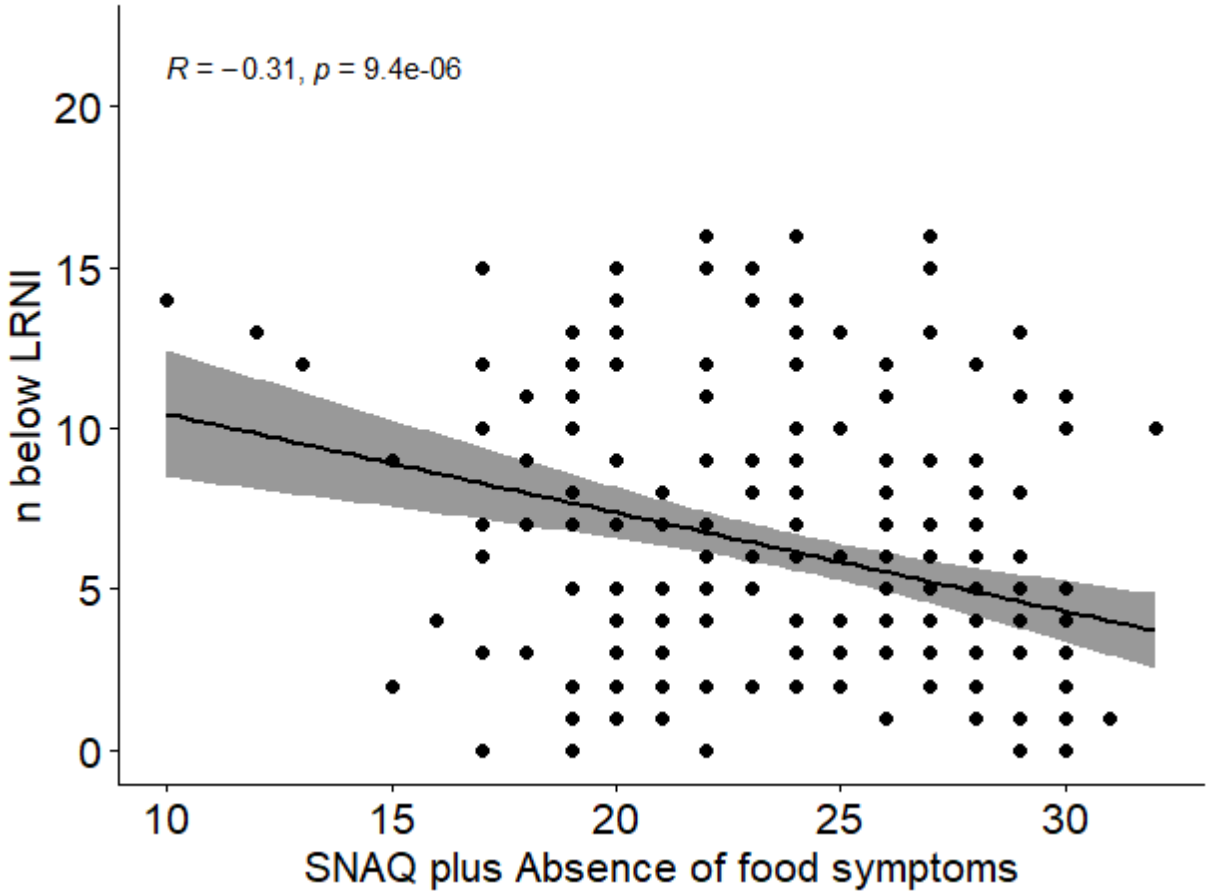


Figure 43: Number of micronutrient below LRNI vs the SNAQ score plus absence of gastrointestinal symptoms reported after eating

the greater the number of food-related symptoms and the more impaired appetite, the lower the score

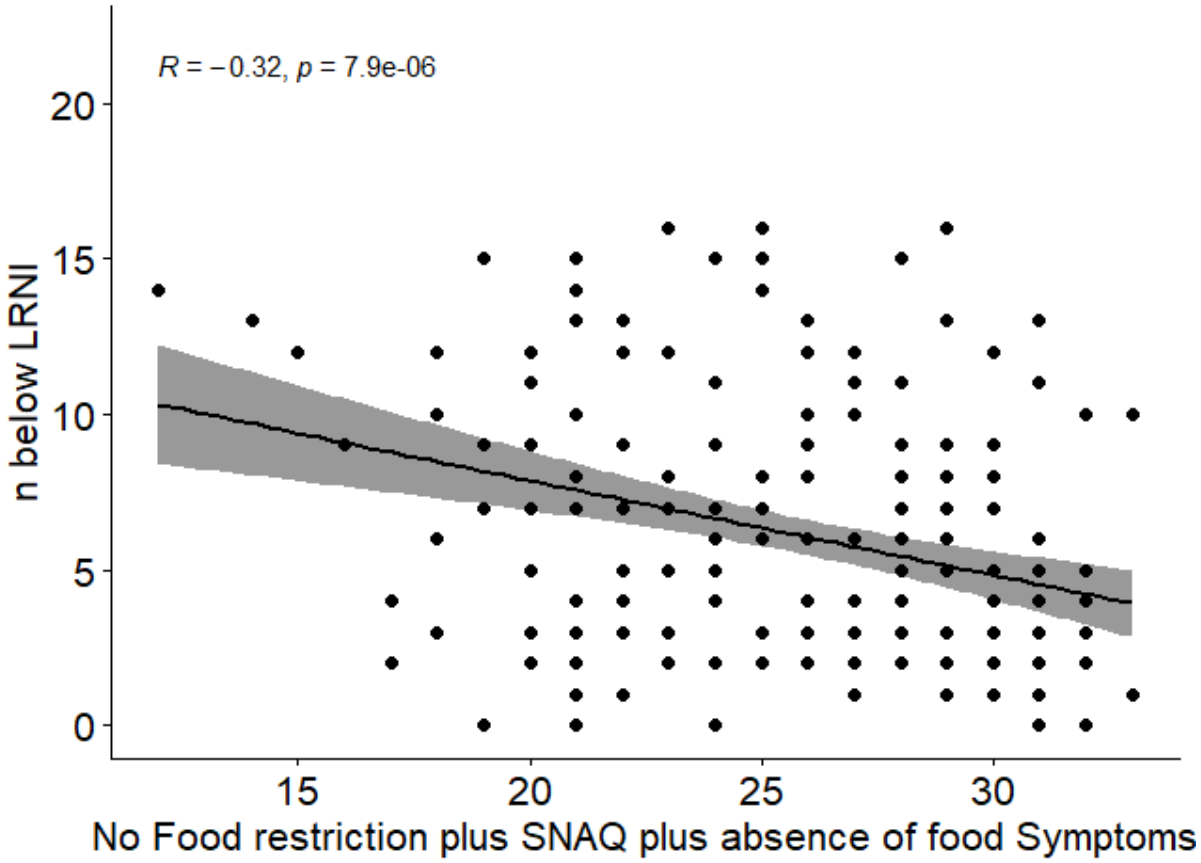


Figure 44: Composite Score for all Dietary impact factors captured by the INAT questionnaire vs n of nutrients below LRNI :
Composite score from 1) whether the subjects avoided one or more foods, 2) SNAQ score and 3) food-related gastrointestinal symptoms, (lower score reflecting a greater number of dietary impact factors) plotted against micronutrient adequacy of diet as assessed by the number of nutrients below the LRNI

#### 4.4.5.4 An Impaired Nutritional state as assessed by INTAKE vs FACIT-F

The nutritional state, as considered from the intake perspective (micronutrient adequacy of diet and dietary impact factors), was compared to the FACIT-F score. Correlation analysis assessed whether those with a greater number of micronutrient inadequacies (marked by the number of

nutrients below LRNI) and the greatest number of dietary impact factors (marked by the negatively marked INAT questions, described below) had a greater degree of fatigue (marked by a lower FACIT-F score)

There was a weak but statistically significant negative correlation between the number of nutrients below the LRNI and the FACIT-F score (*R* -0.17, *p* 0.015 see Figure 43)

The dietary impact factors showed a stronger relationship to the FACIT-F score, with the SNAQ score and food-related GI symptom score both positively correlated to the FACIT-F score. A composite score of the dietary impact factor questions of the INAT questions (SNAQ, food-related GI symptoms score and food avoidance) showed a moderate positive correlation to the total FACIT-F score.

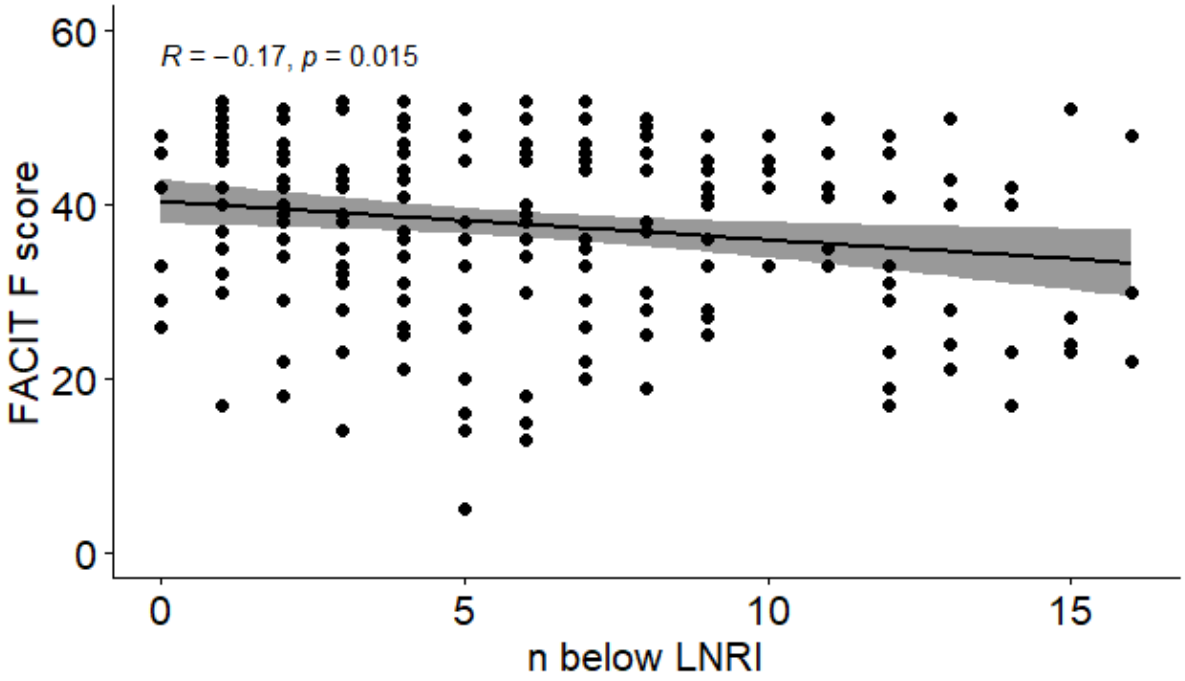


Figure 45: FACIT-F score versus the number of nutrients for which the study subject recorded an intake below the LRNI

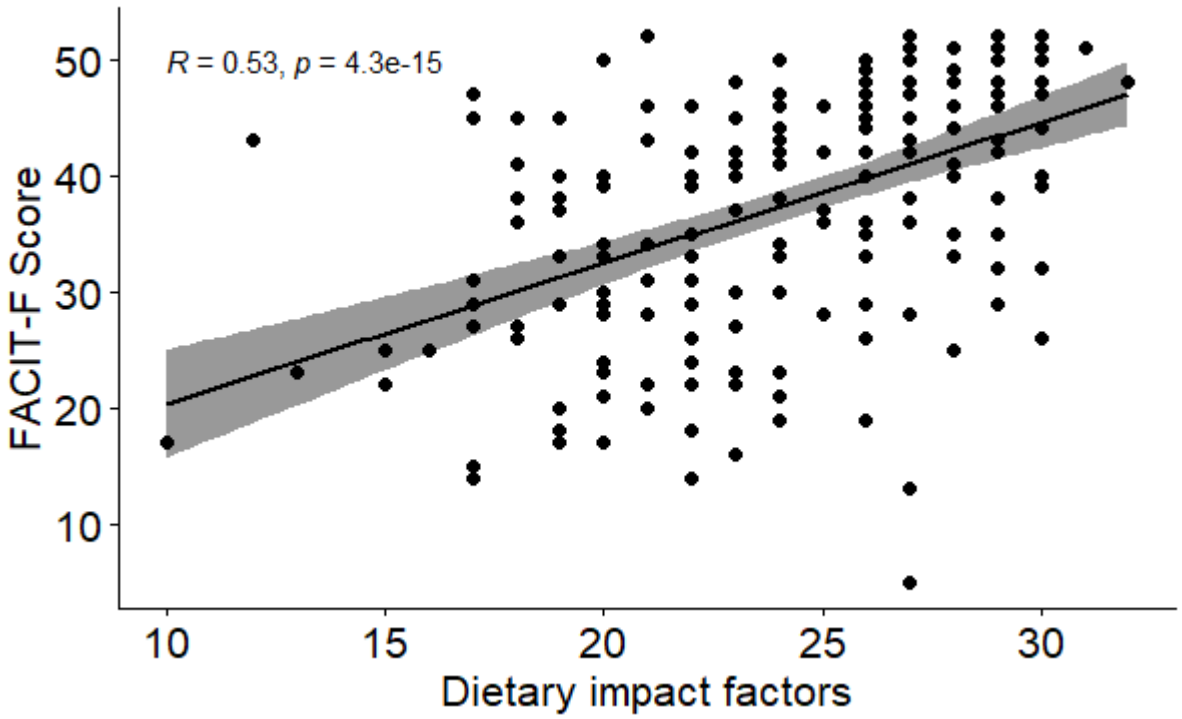


Figure 46: FACIT-F score plotted against a composite of dietary impact factor (DIF) questions of the INAT Questionnaire

DIF score generated by absence of food-related GI symptoms, SNAQ score and food restriction with a lower DIF score suggesting fewer dietary impact factors)

### 4.4.6 Nutritional State - FORM

Body composition data from anthropometry measurements and bioelectrical impedance analysis were available for 194 study participants and are presented below.

Table 31: Body Composition of the INTICO-2 cohort

| | | **Overall** |
|---|---|---|
| n | | **194** |
| BMI group (%) | Underweight (<20kg/m$^2$) | 1 (0.5) |
| | Normal Weight (20-25kg/m$^2$) | 75 (38.7) |
| | Overweight (25-30kg/m$^2$) | 71 (36.6) |
| | Obese (≥30kg/m$^2$) | 47 (24.2) |
| Weight (median [range]) | | 76.03 [46.9, 135.6] |
| Height (mean (SD)) | | 170.0 (9.43) |
| Body Mass Index (kg/m$^2$) (median [range]) | | 26.1 [18.0, 49.0] |
| Waist Circumference (cm) (median [range]) | | 91 [55, 150] |
| Fat-Free Mass Index (kg/m$^2$) (median [range]) | | 17.3 [11.6, 23.2] |
| Fat-Free Mass Index “Z” score (mean (SD)) | | -0.52 (1.39) |
| Fat Mass Index (kg/m$^2$) (median [range]) | | 9.2 [1.8, 27.9] |
| Fat Mass Index “Z” score (mean (SD)) | | 0.79 (1.38) |
| Skeletal Muscle Mass (Kg) (median [range]) | | 23.8 [11.2, 38.6] |
| Phase Angle (median [range]) | | 5.08 [3.3, 7.2] |
| Phase Angle “Z” score (mean (SD)) | | -0.25 (1.15) |

#### 4.4.6.1 Body Mass Index

The Median Body Mass Index (BMI) was in the overweight range at 26.1 kg/m$^2$ (18.0-48.9). 61% of the cohort had a BMI over 25, with 24% in the obese range (>30kg/m$^2$), and 37% were overweight by BMI. The prevalence of overweight and obesity is comparable to that of the UK population (26% and 38%, respectively) (308).

#### 4.4.6.2 Body Composition of the cohort - in comparison to age and sex-matched controls

The Fat Mass Index Z scores (FMIz) and Fat-Free Mass Index Z score (FFMIz) represent adiposity and lean mass expressed per metre height and standardised for age and sex from healthy controls. The relationship between FMIz and FFMIz is shown in Figure 45 where the values for each individual are plotted, and the limits of the normal distribution are indicated (+/- 2 SD).

The resultant plot demonstrates shows that i) 67% (131/194) of the subjects fall within the limits of normal distribution ii) none of the cohort were overtly wasted (low FMIz <-2 and low FFMIz <-2), and iii) few were obese by fat mass and fat-free mass (8/194) high FFMiz and high FMIz). A significant proportion of the cohort had either a low FFMiz for a normal FMIz (14%) or a high FMIz for a normal FFMIz (14%). In other words, subjects tended to lack lean mass relative to their adiposity.

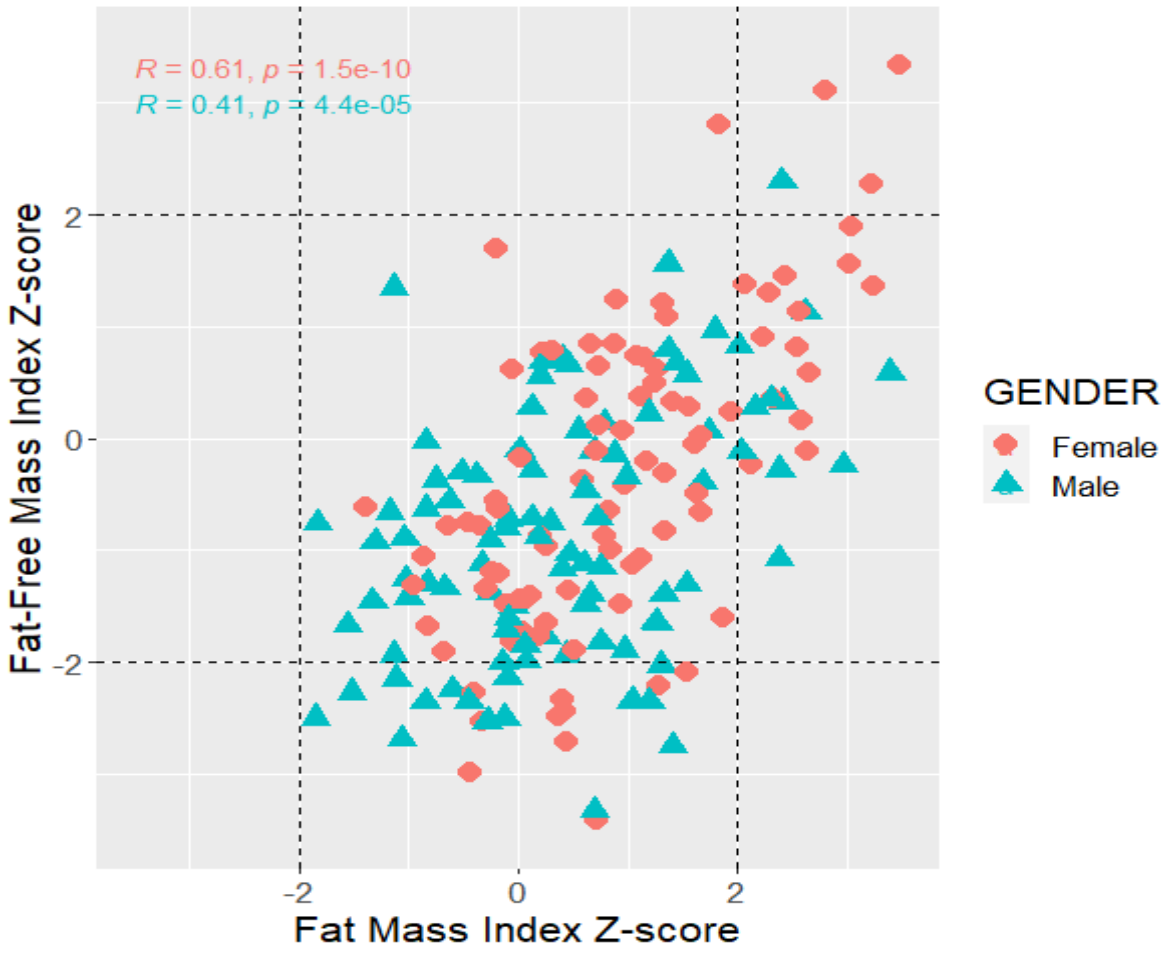


Figure 47: Fat-Free Mass Index Z score vs Fat Mass Index Z score by subject.

A score FFMIz score <0 (bottom half of figure) shows a reduced lean mass and a score <-2 shows a lean mass below the 5th percentile for age, gender, and sex. An FMIz score >0 (right-hand showed excessive adiposity

#### 4.4.6.3 Excessive adiposity vs Body Mass Index in the cohort

There was excessive adiposity in the cohort, with 14/97 men and 21/97 women having a Fat Mass Index "Z" score (FMIz) of >2. An elevated BMI over 30kg/$m^2$ was 100% sensitive and 92% specific for identifying these subjects.

There was a strong positive correlation between BMI and FMIz, particularly in both men and women (correlation strongest) (*R* 0.91 and *R 0*.95 respectively, *p* <2.2e-16 see Figure 48).

#### 4.4.6.4 Reduced Lean Mass vs Body Mass Index in the cohort

16/97 men and 11/97 women had evidence of a reduced lean mass with a Fat-Free Mass "Z" score (FFMIz). The correlation between FFMIz and BMI was weaker, particularly in men (*r* 0.84 (F), *r* 0.75 (M), p <2.2e-16 see Figure 49).

A reduced BMI under 20kg/$m^2$ was a specific but not sensitive marker for individuals having a low FFMIz score (19% and 98%, respectively).

Of the 27 with an FFMIz ≤-2, 59% had a BMI between 20-25kg/$m^2$, and 22% were overweight by BMI. A low BMI would have identified just 19% (5/27) of those with FFMIz.

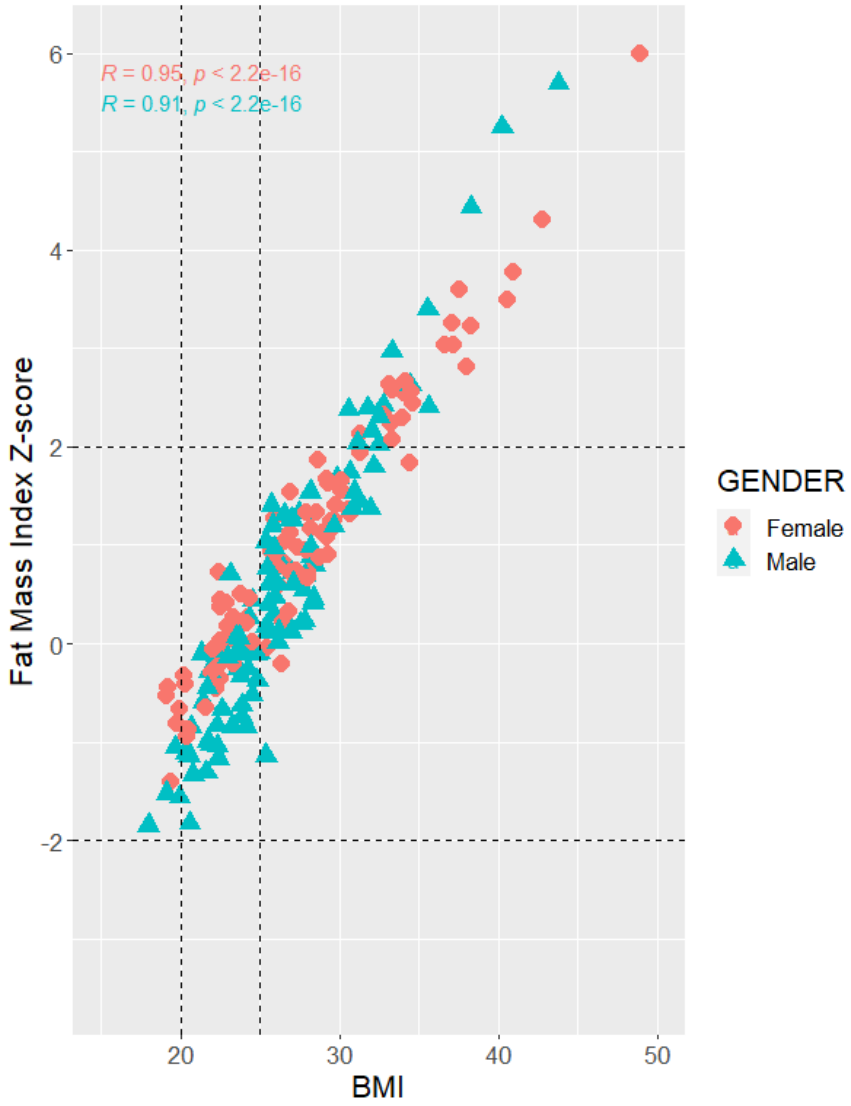


Figure 48: Fat Mass Index Z score vs Body Mass Index by subject.

Subjects to the right-hand side of the right vertical line (>25) were overweight by BMI, demonstrating a strong correlation. Vertical lines denote BMI 20 and 25 with those to the right overweight or obese by BMI, horizontal lines represent +/- 2 standard deviations, with those above 2 being classified as having excessive adipose


Figure 49 Fat-free Mass Index Z-score by subject plotted against BMI

Plot demonstrating moderate to strong correlation. Vertical lines denote BMI 20 and 25 with those to the right overweight or obese by BMI, horizontal lines represent +/- 2 standard deviations, with those below -2 being classified as having a low lean mass

#### 4.4.6.5 BIA–derived Markers of Nutritional State

The Phase Angle was expressed as a standardised score (SD Score or Z Score) using age and sex-matched data. The Phase Angle Z score tended to be low (a low phase angle is consistent in other disease areas with poor nutritional state / adverse outcomes), with the mean Phase angle Z-score of the cohort -0.25 and more values (84) below the 50$^{th}$ centile (i.e. below the average for the reference population) than above it (64).

Twelve Subjects had a phase angle SD score below -2 (approx. 5$^{th}$ centile), and 46 had an SD below ≤ -1 (approx. 15$^{th}$ centile).

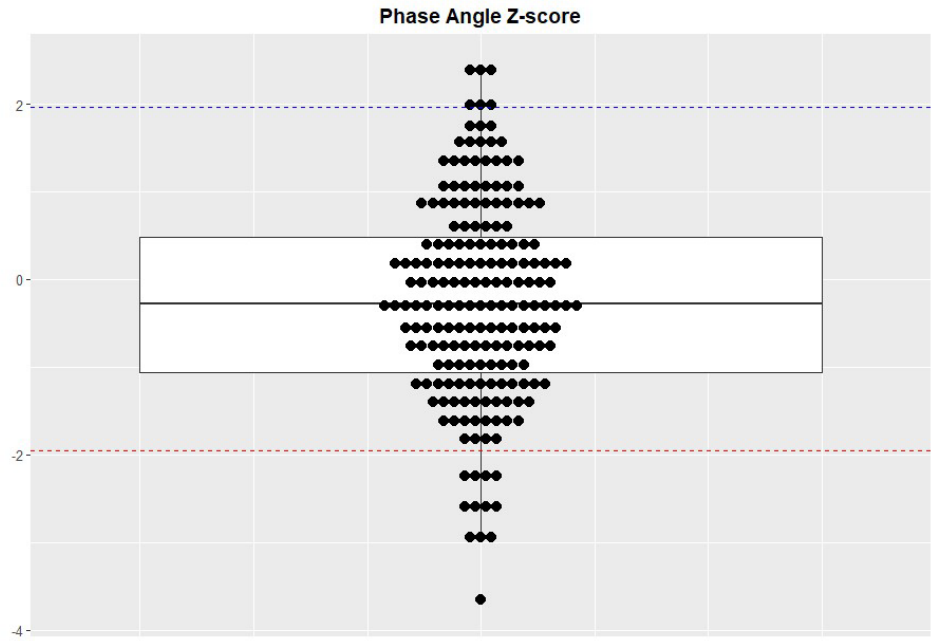


Figure 50 Phase Angle Z-score of study subjects with mean.

Horizontal dashed red line represents 5th centile and dashed blue line represents 95th centile

#### 4.4.6.6 Nutritional State – FORM and fatigue

The body composition and impedance measures of those who did or did not report severe fatigue are shown in Table 32: Body Composition and BIA data for those with and without severe fatigue (As defined by FACIT-F +/-30, with p value denoting significance of difference in means or medians assessed using either paired t-test (parametric) or Wilcoxon rank-sum (non-parametric)..

When those with severe fatigue (FACIT-F<30) were compared to the rest of the cohort, they had a higher Body Mass Index, (27.0 vs 25.8 p 0.44), higher fat-mass index Z-score (1.3 vs 0.6 *p* 0.001); markers of adiposity such as waist circumference, absolute fat mass, and visceral adipose tissue, were lower among those with severe fatigue.

There was no difference in the FFMIz or skeletal muscle mass (assessment of lean mass) between those with and without fatigue. The Phase angle "Z"-score was significantly lower among those with fatigue than those without (-0.6 vs -0.15 *p* 0.018).

Table 32: Body Composition and BIA data for those with and without severe fatigue

Fatigue, as defined by FACIT-F +/-30, with p value denoting significance of difference in means or medians assessed using either paired t-test (parametric) or Wilcoxon rank-sum (non-parametric).

| | **Not Severe fatigue FACIT-F ≥30** | **Severe fatigue FACIT-F<30** | **p** |
|---|---|---|---|
| | 138 | 49 | |
| **Weight (median [range])** | 74.9 [47.0, 135.6] | 78.1 [46.9, 126.1] | 0.149 |
| **Height (mean (SD))** | 170.6 (9.2) | 168.0 (9.6) | 0.09 |
| **Body Mass Index (kg/m$^2$) (median [range])** | 25.8 [18.0, 43.8] | 27.01 [19.35, 48.93] | 0.017 |
| **Waist Circumference (cm) (median [range])** | 89.0 [55.0, 150.0] | 96.0 [69.0, 132.0] | 0.004 |
| **Fat-Free Mass Index (kg/m$^2$) (median [range])** | 17.4 [11.6, 23.2] | 17.1 [12.6, 23.2] | 0.761 |
| **Fat-Free Mass Index "Z" score (mean (SD))** | -0.6 (1.3) | -0.29 (1.59) | 0.146 |
| **Fat Mass Index (kg/m$^2$) (median [range])** | 8.30 [1.8, 22.4] | 11.5 [1.9, 27.9] | 0.001 |
| **Fat Mass Index "Z" score (mean (SD))** | 0.6 (1.3) | 1.32 (1.55) | 0.001 |
| **Skeletal Muscle Mass (Kg) (median [range])** | 23.7 [11.2, 38.6] | 22.3 [13.0, 35.4] | 0.464 |
| **Absolute Fat Mass** | 24.5 [5.8, 63.7] | 31.5 [4.9, 71.8] | 0.001 |
| **Phase Angle (median [range])** | 5.1 [3.4, 7.2] | 5.0 [3.3, 6.4] | 0.101 |
| **Phase Angle "Z" score (mean (SD))** | -0.15 (1.08) | -0.60 (1.25) | 0.018 |

### 4.4.7 Micronutrient Status

#### 4.4.7.1 Iron Indices and Anaemia

Of the 197 subjects with a blood haemoglobin result,14 were anaemic (defined as haemoglobin <130g/L in males or <120g/L in females). Six showed evidence of iron deficiency (defined as one or more of; a serum Ferritin concentration of <11µg/L in women, or <24µg/L in men, or by a transferrin saturation (<16%), or a soluble transferrin receptor index >14); 24 subjects had evidence of iron deficiency (using the above indices) without anaemia.

#### 4.4.7.2 Serum and Plasma Markers of Micronutrient Status

Of the 197 subjects with a blood haemoglobin result,14 were anaemic (defined as haemoglobin <130g/L in males or <120g/L in females). Six showed evidence of iron deficiency (defined one or more of a serum Ferritin concentration of <11µg/L in women or <24µg/L in men or by a transferrin saturation (<16%) or a soluble transferrin receptor index >14); 24 subjects had evidence of iron deficiency (using the above indices) without anaemia.

The micronutrient biochemistry was analysed through collaboration with the Nestlé Institute of Health Sciences in Lausanne. At the time of writing this thesis, the laboratory was unable to generate reference ranges for the analytes measured. This precluded statements on the prevalence of individuals with blood tests compatible with risk of deficiency or insufficiency in the population.

The micronutrient data will therefore be explored by comparison of the results from each study subject against the rest of the cohort and as a continuous variable against study outcomes.

11 Subjects with elevated serum CRP (>10mg/L), due to its confounding estimates of micronutrient status, will be removed from micronutrient analyses.

#### 4.4.7.3 Hydrosoluble Vitamins

The following vitamers; Nicotinic acid (3), Pyridoxamine (0), Pyridoxine (3) and Folic acid (6) there were insufficient subjects with quantifiable values to include in the analyses.

Adequate data for classification by quartiles was available for Nicotinamide, Thiamine, Riboflavin, Pantothenic Acid, 5 methyl tetrahydrofolate, Pyridoxic Acid and Pyridoxal. The analytic reports for the hydrosoluble vitamins are described in Appendix C.

#### 4.4.7.4 Liposoluble Vitamins

For Vitamin K1, 83 subjects had a measurement that was below the level of quantification, so this was not included in the quartile analysis.

Adequate data for classification by quartiles was available for 25 OH Vitamin D, β-Cryptoxanthin, α- Tocopherol, Lutein and Zeaxanthin. The analytic reports for the liposoluble vitamins are described in Appendix C.

#### 4.4.7.5 Minerals

The following minerals: Cadmium (0), Iodine (103), Barium (1), Arsenic (37), Vanadium (0), Caesium (117), Molybdenum (132), Strontium (33), Tin (I), Lead (2) and Manganese (2) were not detected in sufficient concentration in serum to be included in further analyses.

Adequate data for classification by quartiles was available for Aluminium, Bromine, Calcium, Copper, Iron, Magnesium, Potassium, Rubidium, Selenium, Sulphur, and Zinc. The analytic reports for the minerals are described in Appendix C.

### 4.4.8 Micronutrients versus FACIT-F

A Wilcoxon Rank paired test compared whether the micronutrients different between those with a FACIT-F score above or below 30 (cut-off used to define severe fatigue) see Table 33: Micronutrient Status between subjects with and without Severe Fatigue, Wilcoxon signed rank. Five analytes were significantly lower in those reporting severe fatigue than those that did not. This persisted after the Bonferroni adjustment for the fat-soluble anti-oxidant vitamins Lutein and Beta-Crypotoxanthin.

Table 33: Micronutrient Status between subjects with and without Severe Fatigue,

Wilcoxon signed rank

| **Micronutrient Median [range]** | **Not Severe fatigue FACIT-F ≥30 Median [range]** | **Severe fatigue FACIT-F<30 Median [range]** | **P value (Wilcoxon rank) fatigue vs non fatigue** | **Adjusted P value Fatigue vs non-fatigue** |
|---|---|---|---|---|
| Lutein | 87 [17, 546] | 64 [10, 230] | 2.77E-0.5 | 0.0012 |
| Beta Cryptoxanthin | 65 [10,829] | 42[10,338] | 0.0006 | 0.0128 |
| 5-methyl tetrahydrofolate | 6.5 [1.1,40.7] | 4.8 [1.5,62.8] | 0.0076 | 0.0836 |
| Zeaxanthin | 23 [10,85] | 18 [10,91] | 0.0007 | 0.0836 |
| Selenium | 101 [60,347] | 92 [4,186 | 0.0211 | 0.1858 |

When multivariate logistic regression was performed controlling for adiposity and gender, just Lutein had a statistically significant correlation with the fatigue outcome, with a very weak positive correlation coefficient of 0.014.

When the FACIT-F score was considered as a continuous variable, with gender in the model and those with CRP > 10mg/dL removed, just Lutein had an adjusted statistically significant correlation. The micronutrients with a P value < 0.05 and their correlation coefficients are considered inTable 34.

Table 34: Multivariate Regression of Micronutrient Regression against severe / non-severe fatigue

Fatigue assessed by FACIT-F with correlation coefficients and p values, adjusted for gender with CRP >10 removed

| **Micronutrient** | **Estimate** | **Adjusted P value Fatigue vs non-fatigue** | **T Value** | **P Value** | **Adjust P Value** |
|---|---|---|---|---|---|
| Lutein | 0.0504 | 0.0120 | 4.2183 | 3.87E-05 | 0.001 |
| Nicotinamide | -0.1317 | 0.0444 | 2.9593 | 0.0035 | 0.0685 |
| Zeaxanthin | 0.1430 | 0.0495 | 2.8930 | 0.0043 | 0.0685 |
| Nicotinic Acid | -40.0614 | 16.0960 | 2.4489 | 0.0137 | 0.1645 |

### 4.4.9 Nutritional Screening applied to the INTICO-2 cohort

To contextualise the measures of the nutritional state of the INTICO-2 cohort against current practice, the next section will consider current malnutrition screening and assessment tools when applied to the cohort.

#### 4.4.9.1 Nutritional Screening Tools

##### 4.4.9.1.1 MUST

Of the 191 subjects who underwent body composition analysis, nine had a score of > 0 on the reduced BMI subcategory of MUST (BMI <20 kg/m$^2$). Of these, just 1/9 subjects had a BMI <18.5 kg/m$^2$ (which gives a score of 2) and were therefore high risk by BMI alone. Seven subjects in the cohort reported weight loss in the INAT; of these, two reported <5%, so they would not have scored > 0 on MUST. The remaining 5 subjects either did not specify how much weight they had lost (2) or reported a weight loss between 5-10% (3 subjects), specifying times between 2 months and 1 year. Assuming that weight was lost in 6 months and the two subjects who did not complete the question had lost 5-10% in 6 months, 5/191 subjects would have scored 1 on weight loss for MUST.

Of the 9/191 subjects with a BMI between 18.5 and 20 kg/m$^2$, just one reported 5-10% weight loss, so a combination of weight loss (+1) and BMI of 19.1 kg/m$^2$ (+1) defined another subject by MUST as being at high nutritional risk (≥2). The other 4 subjects reporting recent weight loss >5% were either normal BMI or obese (3 subjects in each BMI subcategory) (309).

For the INTICO-2 cohort, MUST defined only 2 of the 191 subjects (1%) as high risk for malnutrition, and 8 other subjects as intermediate risk. Therefore, applying MUST screening would have defined 5% of the cohort as being at nutritional risk.

#### 4.4.9.1.2 NRI

The NRI score required the patient's usual weight (defined as stable body weight for the last 6 months). Longitudinal weight data for the study subjects was not available. The NRI was not therefore applied to the cohort

#### 4.4.9.1.3 NRS-2002

Fourteen subjects had a BMI below the NRS cut-off of 20.5 kg/$m^2$, seven reported weight loss (1/7 with BMI <20.5 kg/$m^2$), and seven subjects recorded an intake of <50% of requirements. Applying the NRS-2002 identified 26 subjects (14%) at risk of malnutrition, including 10 subjects who were also identified as at risk by the MUST tool.

#### 4.4.9.1.4 MIRT

The MIRT score was applied to the INTICO-2 cohort, adding a score of 2 for an elevated serum CRP (defined as 5-50mg/L) for 37 subjects to the BMI and weight loss scores calculated for MUST did not identify any subjects as being at high risk for disease proregression (MIRT score >3).

#### 4.4.9.1.5 Saskatchewan IBD–Nutrition Risk (SaskIBD-NR) Tool

The Sask-IBD-NR assigns a score based on weight loss in the previous month. However, the study data did not capture whether the seven subjects who reported weight loss had lost weight in the last month, so this could not be applied to the cohort. Another component of the Sask-IBD NR score is whether subjects have a “poor intake due to reduced appetite”. While intake and appetites were captured separately by food diary and PROMS, respectively, the reasons for a reduced intake were not recorded in the cohort. Therefore, a study subject who may have deliberately reduced their intake to try and lose weight would not be differentiated from one with poor intake due to symptoms. Due to these two key components of the score not being captured, the Sask-IBD-NR tool could not be applied to the cohort.

#### 4.4.9.2 Combining Nutritional Screening Tools

Applying the nutritional screening tools of NRS-2002 and MUST, identified 14% (26/191) of the cohort as being at nutritional risk.

To demonstrate how these scoring systems related to the INTICO-2 cohort, a breakdown of how the individuals in the cohort were scored at risk and the number of individuals fulfilling each criterion is expressed below in Table 35

The NRS, by virtue of having a higher cut-off for BMI and not specifying weight loss, identified all the subjects who were identified by MUST, and an additional 16 through a combination of low intake (6) and BMI below 20.5 kg/m$^2$.

The MIRT score did not identify any subjects in the cohort as being at risk of disease complications, and the other tools (Sask IBD-NR and NRI) could not retrospectively be applied to the cohort.

Table 35: Nutritional Risk Score criteria applied to the INTICO-2 cohort

(n=191 for complete risk data)

| **Nutritional Risk Score Parameter** | **Number (%)_of subjects** |
|---|---|
| MUST BMI Score > 1 | 9 (5) |
| MUST Weight Loss Score | 5 (3) |
| Total MUST “at risk” (≥1) | 10 (5) |
| MUST “high risk” (≥2) | 2 (1) |
| NRS 2002 BMI >1 | 14 (7) |
| NRS low intake (<50% recorded) | 7(4) |
| NRS weight loss | 7(4) |
| At risk by NRS (any >1) | 26 (14) |
| At Risk by MUST or NRS 2002 | 26 (14) |

#### 4.4.9.3 Nutritional Assessment Tools

After identifying individuals at risk of malnutrition in usual clinical care, these subjects would be referred for a more comprehensive assessment for malnutrition. In UK care, this would typically be done by a dietitian using the subjective global assessment (SGA) (310).

Recently, from the international perspective, the global nutrition community introduced the Global Leadership Initiative on Malnutrition (GLIM) score, standardising the criteria, scoring and cut-offs to establish a diagnosis and grade the severity of malnutrition (287). The Global Leadership Initiative on Malnutrition (GLIM) was a 2016 initiative to gain consensus from national and international nutrition expert societies on defining and characterising malnutrition in a clinical setting. The panel then defined and characterised malnutrition using a combination of what they considered the most clinically relevant criteria, with a consensus that following screening using a tool such as MUST a subsequent diagnosis required both **aetiological** and **phenotypic** criteria, with the latter being used to grade the severity of malnutrition.

Aetiological criteria require a reason for malnutrition, specifically a factor such as 1) reduced food intake or 2) reduced assimilation, 3) disease burden or inflammation. For the phenotypic criteria, cut-offs were defined for malnutrition from literature in the subcategories of 1) percentage weight loss, 2) Body Mass Index (BMI) and 3) a validated measure to estimate lean mass such as Fat-Free Mass Index (FFMI). Percentage weight loss and body Mass Index were further subdivided into malnutrition and severe malnutrition.

The GLIM score has been found to be both sensitive and specific to the SGA in a large adult CD population (AUC = 084) (311). As the SGA does not have clear cut-offs or definitions, and the study dietitians were not blinded to the other assessments (such as the INAT score) and study assessments, the GLIM criteria were retrospectively applied to the INTICO2 cohort. The GLIM tool, its criteria, and cut-offs and how it was applied to those screened as being at nutritional risk in the cohort is described below.

The cut-offs for BMI, FFMI and weight loss to define and grade the severity of the phenotypic component of malnutrition are described in Table 36.

Table 36: GLIM phenotypic criteria thresholds for severity grading of malnutrition

(abbreviations mo= months, FFMI – Fat-Free Mass Index)

| | **Phenotypic Criteria** | | |
|---|---|---|---|
| | **Weight Loss %** | **Low body mass index (kg/ $m^2$)** | **Reduced Muscle Mass (FFMI kg/ $m^2$)** |
| Stage 1 / Moderate Malnutrition | 5-10% within past 6 mo, or 10-20% beyond 6 mo | <20 if <70,<br><22 if ≥70 | FFMI <15 (f)<br>FFMI <17 (m) |
| Stage 2 / Severe Malnutrition | >10% within the past 6 mo or 20% beyond 6 mo | <18.5 if <70,<br><20 if ≥70 | No definition |

#### 4.4.9.3.1 Applying GLIM to the INTICO-2 cohort.

#### 4.4.9.3.2 Aetiological GLIM criteria in the INTICO-2 cohort

The exclusion criteria should have precluded entry of individuals with a chronically reduced intake from the study. However, subjects could be identified from the study data as fulfilling the other definition of reduced intake defined by GLIM of <50% of energy requirements in the preceding 7 days (from analysis of food diaries compared to estimated daily energy requirement). Energy requirements were estimated using a HENRY equation (using values calculated by self-reported height and weight) multiplied by physical activity level (PAL) estimated by the researcher at screening (307). Excluding incomplete food diaries, 7-day intake data was assessed in 191 subjects. Subjects who recorded an average daily intake of <50% of estimated requirements (total energy estimated TEE) were, therefore, classified as fulfilling a GLIM aetiological criterion.

The other GLIM aetiological criteria of "reduced assimilation" due to GI symptoms or tissue injury may, to some degree, apply to any of the study subjects. However, as factors such as short bowel, clinically or biochemically active disease (HBI >4, FCP >250µg/g respectively) were exclusion criteria, "reduced assimilation" was not felt to apply securely as an aetiological criterion for malnutrition among the subjects. One GLIM aetiological criterion of the cohort was permitted, with the inclusion criteria (which did not include a maximal CRP) being "inflammatory burden". CRP is recognised as a relevant aetiological criterion for malnutrition, and a further GLIM consensus has been reached on cut-offs to define degrees of CRP elevation (3.0-9.9mg/L – mild, 10-50mg/L – "moderate" and >50- "severe") (312)

Therefore, the following GLIM aetiological criteria were applied to 191 of the INTICO2 cohort, regardless of the NRS 2002 or MUST.

1. A recorded food intake of less than 50% of requirements in the preceding 7 days or
2. Having evidence of increased inflammatory burden at the time of the nutritional assessment (defined as CRP ≥3mg/L)

The GLIM aetiological criterion of a reduced intake (recorded food diary that was <50% of estimated TEE for a 7-day food diary) identified 7 subjects, of which 3 were among the 84

subjects classified as having an elevated CRP. The GLIM aetiological criteria for CRP elevation as a measure of inflammatory burden and in the cohort are displayed in Table 37.

Table 37 GLIM- Aetiological Criteria - disease burden by CRP in the INTICO-2 cohort

| **GLIM- Aetiological Criteria** | **Number of subjects (%)** |
|---|---|
| Inflammatory Disease Burden (CRP (mg/L)) | |
| Normal (<3) | 112 (59) |
| Mild Inflammation (3.0-9.9) | 69 (36) |
| Moderate Inflammation (10-50) | 10 (5) |
| Severe Inflammation (>50) | 0 |
| Reduced Intake | |
| <50% of estimated TEE on food diary | 7 (4) |
| Any Aetiological Criteria | 83 (43) |

#### 4.4.9.3.3 Phenotypic GLIM criteria in the INTICO2 cohort

After applying a suitable screening tool and identifying an aetiological criterion for malnutrition, a phenotypic marker of an impaired nutritional state is required to diagnose malnutrition.

The NRS-2002 identified 16 additional subjects as being at risk of malnutrition than the MUST because of the higher cut-off for BMI and, to a lesser extent, the inclusion of a reduced intake.

Applying these BMI and weight loss-based scoring systems defined just 26 subjects (14% (26/191) of the cohort as being at risk of malnutrition. Under usual practice, therefore, the remainder of the cohort would not have undergone a formal assessment for malnutrition (e.g using GLIM or SGA).

To explore whether the GLIM grading was relevant to more subjects than just those identified by MUST and NRS-2002, the phenotypic GLIM criteria of one or more of 1) weight loss, 2) low BMI and 3) low FFMI were applied to the INTICO-2 cohort.

Five subjects reported 5-10% weight loss, thus fulfilling this GLIM phenotypic criterion for a non-severe phenotypic GLIM feature. Applying the BMI cut-offs (see Table 38), defined twelve (12/191 (6%) of subjects as having malnutrition by BMI, one of whom fulfilled the criteria for stage 2 / severe malnutrition. One of the 12 also reported weight loss. Of these 12 subjects with a low BMI (as defined by GLIM phenotypic criteria), 9 also had a reduced FFMI, as did 44 other subjects with a normal (33) or overweight range BMI (11). A total of 58/191 (30%) of the cohort thus fulfilled at least GLIM phenotypic criteria.

A Venn diagram summarising the subjects who fulfilled one or more GLIM phenotypic criteria in the subjects in the INTICO-2 cohort is shown overleaf in Figure 51

Table 38: GLIM Phenotypic criteria of weight loss and BIA Assessment of Lean Mass applied to the whole cohort regardless of nutritional risk screening

| **GLIM- Phenotypic Criteria** | **Number of subjects (%)** |
|---|---|
| No Phenotypic Criteria | 133 (70) |
| Low BMI- Stage 1 | 11 (4) |
| Low BMI Stage 2 | 1 (43) |
| Weight Loss (all 5-10%) | 5 |
| Low Lean Mass (FFMI <15kg/m2 women, <18kg/m2 men) | 53 |
| Any Phenotypic Criteria | 58 (30) |

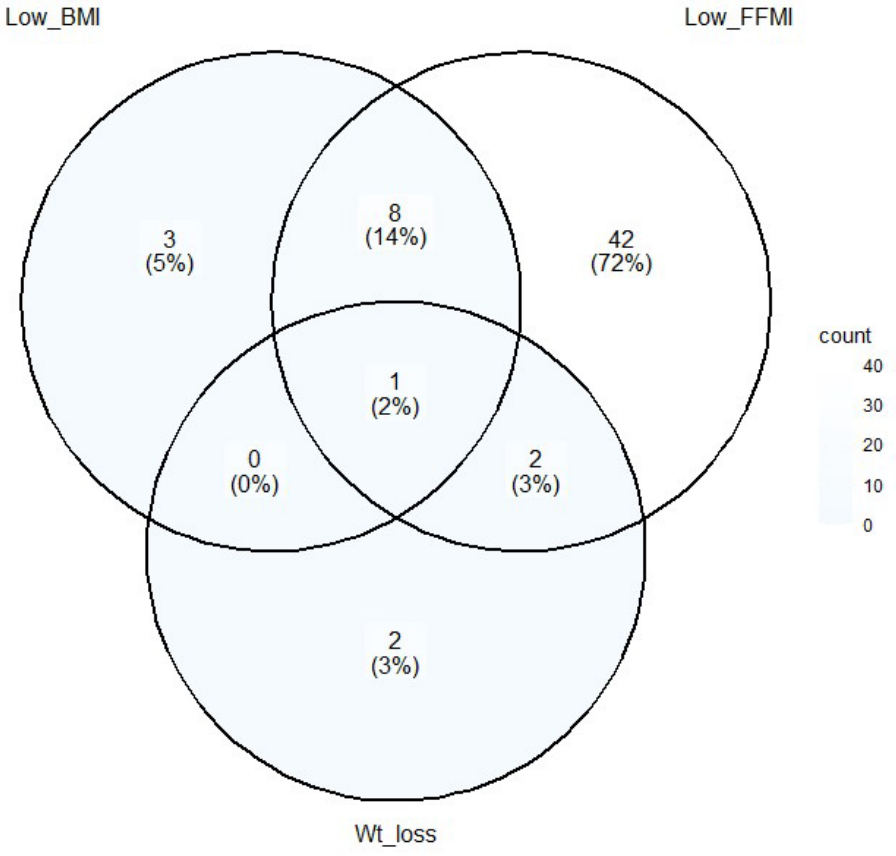


Figure 51: Phenotypic Criteria Positive Subjects in the Cohort

#### 4.4.9.3.4 Combined GLIM Aetiological and Phenotypic Criteria for the INTICO-2 Cohort

To fulfil the GLIM definition of malnutrition, subjects must fulfil an aetiological and phenotypic criterion. Applying the GLIM-specified aetiological and phenotypic definition to the 194 study subjects found 21 subjects to have both an aetiological (inflammatory burden as defined by CRP) and a phenotypic criterion (one or more of Weight loss, low BMI, low FFMI), as displayed in Figure 52 and Figure 53.

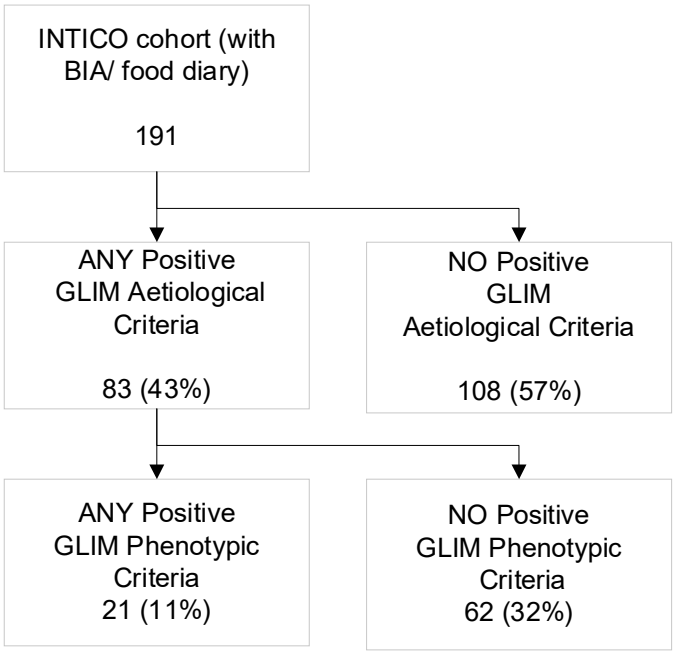


Figure 52: Consort Diagram of applying first the Aetiological and then Phenotypic Criteria for Malnutrition as defined by the GLIM consensus to the INTICO-2 cohort (with complete data,

(n=191)

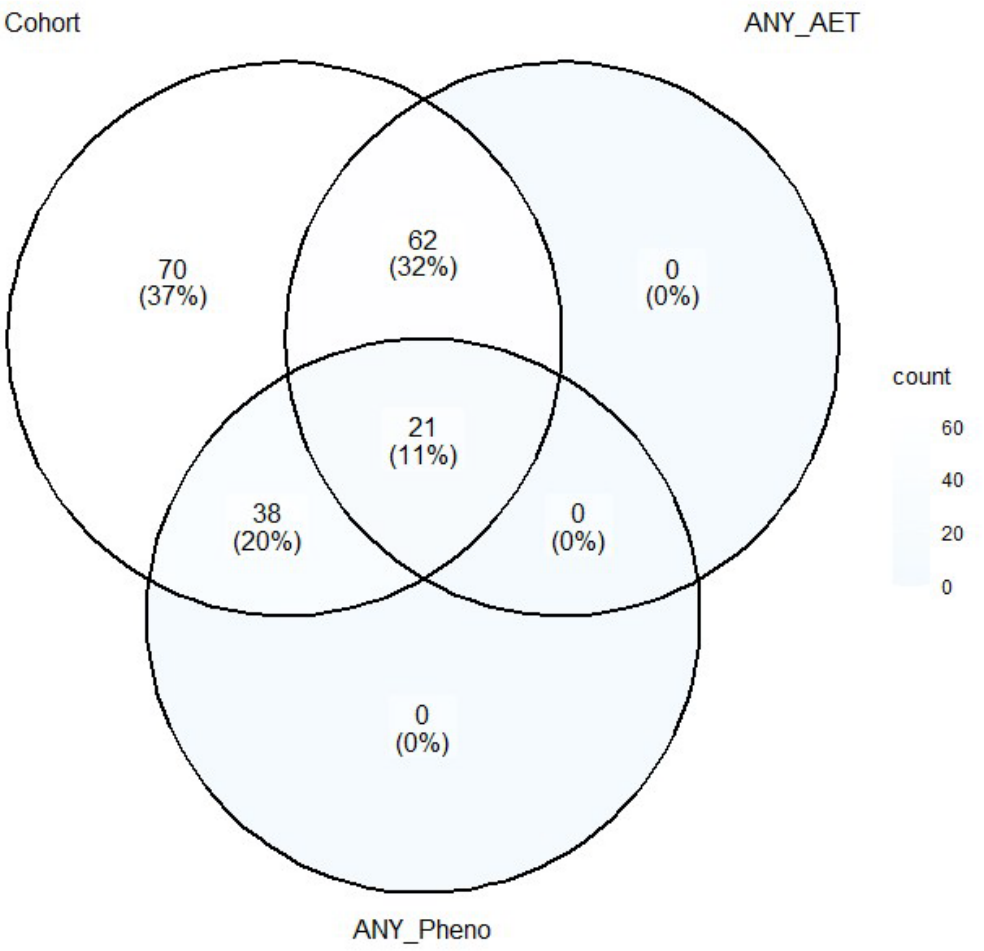


Figure 53: GLIM aetiological and phenotypic criteria Venn Diagram for INTICO-2 cohort

Aetiological Criteria applied 1) (intake <50%) or 2) High Inflammatory Burden (CRP <3) labelled "ANY_AET "and Any Phenotypic Criterion (1) self-reported weight loss, 2) low BMI or 3) low FFMI) labelled "ANY_Pheno"

#### 4.4.9.4 Applying the usual practice of screening (MUST or NRS 2002), then GLIM assessment to INTICO-2 cohort

The GLIM consensus of 2016 recommends that subjects undergo screening with a nutritional screening tool and, if screened to be at risk, the clinician further assesses using the GLIM aetiological and phenotypic criteria. Applying MUST and NRS-2002 criteria to the 26 subjects scored > 1 so would have been expected to undergo further assessment. Of these 26 subjects, just 3, who would have been identified by both MUST and NRS 2002, subsequently fulfilled GLIM aetiological and phenotypic criteria. Therefore, only 3 subjects (1%) in the INTICO-2 cohort would have been screened and then diagnosed as having malnutrition under existing care.

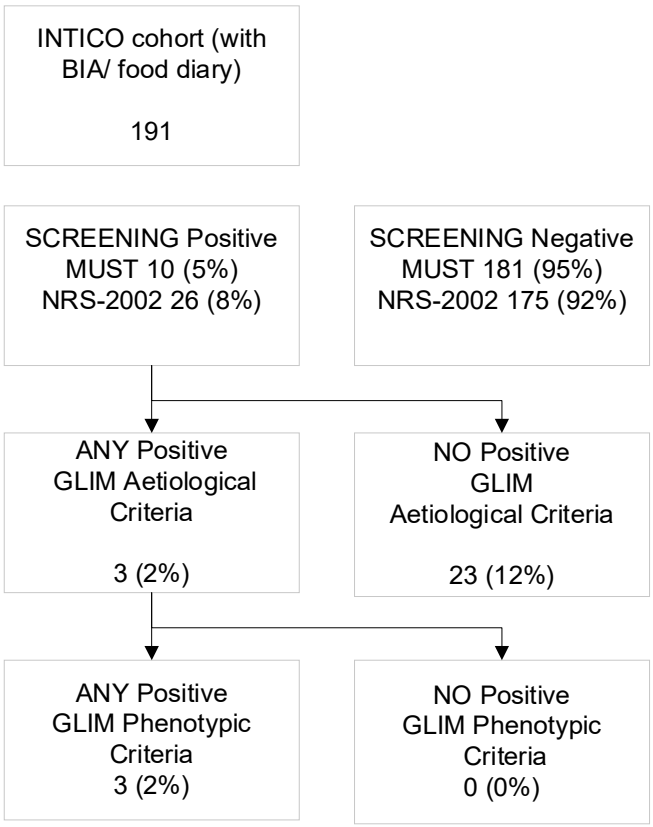


Figure 54: Sequential MUST/ NRS 2002 Screening then GLIM aetiological criteria and GLIM Phenotypic Criteria using study data

## 4.5 Discussion

The INTICO-2 has identified multiple previously undiagnosed nutritional problems in a well-characterised population of adults in confirmed CD remission. Excessive fatigue was common in this population, as reported in other CD cohorts, with 24% (50/195) of subjects recording a FACIT-F score below the level under which a subject is considered to have severe fatigue (95). The cohort had a lower median FACIT-F score than reported in healthy populations, suggesting that the PROM-captured fatigue among the cohort that was worse than that of the general population (96). These findings support the view that there are undiagnosed nutritional issues and unaddressed fatigue symptoms in adults with CD during what is defined as CD remission.

The PROM chosen to assess subjects' fatigue (FACIT-F) was consistent with other PROMs in this study. The sub-score of the SF-36 used to quantify fatigue, the SF-36 vitality score (SF36VT), had the best correlation with the fatigue PROM FACIT-F (r0.8, p2.2e-16) (90). The statistically significant albeit weaker correlation with other less fatigue-specific parts of the SF-36, the "physical functional" and "role limit physical", shows some consistency in reduced physical capacity and fatigue symptoms within the study subjects.

The study assessments of nutritional state have been taken from the intake, form, and micronutrient biochemistry perspectives. Assessments have generated a large data set of PROMS, comprehensive food diary analysis, biochemistry, and bioelectrical impedance analyses to demonstrate a range of previously unconsidered impairments of the nutritional state. The study data from these three interrelated perspectives supports the view that the nutritional state is impaired in CD remission populations.

The study has identified a significant burden of nutritional issues in the cohort, this suggests that the nutritional issues observed at the baseline (1$^{st}$) assessment of the dietary intervention study INTICO-1 are likely to represent the nutritional state across larger CD remission populations.

The study first considered and assessed the nutritional intake by directly questioning study subjects on dietary impact factors, specifically the number of GI symptoms after eating in the preceding month, whether specific foods were avoided and if subjects had an impaired appetite. Each of these dietary impact factors may put additional pressure on an individual's ability to maintain dietary quality and thus reduce the chance of meeting the minimal intakes of essential or conditionally essential micronutrients in a habitual diet. The study team analysed a

comprehensive food diary of subjects' habitual intake and demonstrated that these restrictors were correlated to the number of micronutrients below the LRNI.

Dietary impact factors were common in the cohort, with 30% having an impaired appetite and 35% of subjects avoiding a food or food group. More concerningly was the percentage of individuals who had reported gastrointestinal symptoms after eating in the previous month, with the most typical being abdominal pain, abdominal distension, and diarrhoea in 44%, 42%, and 36%, respectively. Abdominal pain has been correlated to poor appetite scores in IBD cohorts and is independently associated with subsequent weight loss (313). The food-related GI symptoms were likely to have had a meaningful impact on the nutritional state of the INTICO-2 cohort.

The comprehensive food diary analysis has demonstrated that, like in INTICO-1, there were many nutrients for which subjects had a dietary inadequacy. This was defined as an intake that fell below the LRNI, defined as the estimated requirements of the lowest 2.5% of the population (280). Those with an intake below LRNI are likely to have had a true inadequacy of intake. The commonest inadequacies of intake were Copper (73%), Selenium (68%), Vitamin D (60%), Folate (52%) and Potassium (49%).

Data was analysed with and without the addition of usual micronutrient supplements. This was done before and after food diary analysis and showed 18% fewer inadequacies in the cohort after adding supplements to the software. Supplements partially abrogated some of the dietary inadequacies in the cohort. Still, they may not have been taken by those who needed it most or been targeted to contain the elements for which habitual diet was inadequate. Overall, the data supports the usage of micronutrient supplements alongside diet to meet requirements but shows that self-administered supplements may not be taken by the patients who most need them.

What was notable from the data and heatmaps was that some subjects in the cohort had multiple micronutrients for which they failed to meet the LRNI from diet and (if taken) their micronutrient supplement. The INTICO-2 study subjects were not generally considered malnourished by BMI or with active disease, so they would not ordinarily undergo food diary analysis or assessment. Finding individuals in the population who have multiple dietary inadequacies could allow targeted dietetic counselling or micronutrient supplementation.

Under-reporting is common in dietary recall studies, as food intake tends to be reduced as a response to additional attention during dietary recalls (289, 314). For context, the recorded calorie intake of the INTICO-2 median1836 kcal (850-3571) is above that of the recent NDNS

study (1739kcal), suggesting that the under-reporting was not a particular problem for the INTICO-2 food diaries. The more plausible recorded energy intake in INTICO-2 than in INTICO-1 may be due to a more usable dietary recall platform.

Despite having a higher estimated calorie intake than the NDNS data, the average daily intake of the micronutrients captured in NDNS Folate, Calcium, Vitamin D and Iron was lower in the INTICO-2, supporting the view that the CD remission cohort has a relatively micronutrient-poor diet.

The patterns and prevalence of dietary inadequacies were, like that of the INTICO-1 dietary intervention study, supportive of the view that dietary inadequacies seen in this cohort were representative of the wider CD remission population rather than just those who came forward for a dietary intervention study.

The nutritional status of the INTICO-2 cohort was assessed and considered from the perspective of form through body mass index and bioelectrical impedance analysis (BIA). When considered from the perspective of BMI, this cohort tended to be overweight, and 61% of the cohort were overweight or obese, which is similar to the broader UK population(308). Only one subject in the cohort would have been classified as underweight by BMI (<18.5kg/$m^2$), with just eight others having a BMI below 20 (315). BMI-based nutritional risk scoring systems would not have considered most of the cohort at risk of malnutrition and eligible for further assessments.

With Bioelectrical impedance analysis (BIA), the analysis went beyond BMI and predicted subjects' body composition, specifically fat and fat-free (lean) mass. With the help of reference population data, these body compartments could be standardised for age, height, and sex, and data could be expressed as a "Z" score. A low lean mass has been correlated to various adverse outcomes and mortality (208). With the Fat-free Mass Index Z score (FFMIz), the study analysis could identify the range containing 95% of such subjects (+/- 1.96). 27/194 subjects were found to have an FFMIz below this (<-2) and, by this parameter, to have an impaired nutritional state. These were not subjects who would ordinarily undergo an assessment of relative lean mass in routine care. The usual screening tool for undernutrition by reduced body weight is the Body Mass Index (BMI). However, in the INTICO-2 cohort, this was not a sensitive marker for identifying a low lean mass. All but 5 of these subjects had a BMI over 20, so they would not have been identified as being at risk by BMI alone.

With BIA, the study data also identified those with a relative excess of adipose. This is of general relevance for health and may be of specific clinical relevance to the IBD population as excess adipose has been associated with adverse disease outcomes in IBD as well as more general

metabolic and cardiovascular disease and mortality (316, 317). Applying the cut-off of ≥2 for the Fat-Mass Index Z-score defined 35/194 subjects as having excess adipose.

BMI was strongly correlated to adiposity and was a highly sensitive and specific marker of those with high FMIz scores, suggesting that while BMI has limited utility when reduced in identifying those with low lean mass, this easily calculated parameter is an effective tool to identify those with excess adipose. Excessive adipose appeared to be related to the primary study outcome in the cohort; the 26% of subjects with a FACIT-F score <30 (in keeping with severe fatigue) had a significantly higher Fat-Mass Index "Z" score, suggesting that excess adipose was related to a poor-quality CD remission (1.32 vs. 0.6 *p* 0.001). It is unclear whether the adipose tissue is a mediator of fatigue or a feature of a process that leads to both fatigue and accumulation of adipose.

BIA measurements of study subjects also allowed more direct measurements without the need for regression equations to generate data used elsewhere in research and clinical practice to characterise the clinical and nutritional state, such as the phase angle (318). The Phase Angle reflects the relationship between resistance and reactance to a current of 50kHz (319). This parameter is believed to reflect body cell mass and tissue integrity, which both may be reduced as part of structural alterations in body form consistent with a poor nutritional and/ or clinical state (320). A low phase angle has been correlated to adverse outcomes in cancer surgery, an increased length of stay and higher mortality in ITU (290, 293, 318, 321). In the nutritional setting, it has been correlated to the multi-faceted subjective global assessment (SGA) and in the IBD setting, it has been found to be lower among those with active disease and to increase in response to biological therapy (291) (322). The thesis used data from age, BMI, and sex-matched populations to express Phase angle as a Z-score and demonstrate that the population tended to have a lower phase angle than matched controls, suggesting adverse structural nutritional or clinical state in the CD remission population. The corrected phase angle was relevant to the primary study outcome, as it was lower among those with excessive fatigue, suggesting that, like excessive adiposity, there may be a shared common underlying pathogenesis to both fatigue and this nutritional marker of body composition (-0.60 vs. -0.15 *p* 0.018)

The phase angle was impaired at the baseline INTICO-1 assessment and showed a statistically significant increase in response to the nutritional intervention. The fact that INTICO-2 replicated the finding of a low Phase angle Z-score in the larger cohort and correlation to fatigue supports its utility as a valuable marker of clinical and nutritional state in CD remission.

The following perspective from which nutritional status was assessed in CD was availability. This was assessed via a comprehensive panel of vitamers in the plasma and serum, within the costs of the study and with a small amount of blood. Unfortunately, the collaborating laboratory did not have data to generate a “normal range” for the blood micronutrient concentrations at the time of thesis submission. This thesis cannot comment on the prevalence of individuals with blood tests showing a risk of insufficiency or deficiency in the population. Work is ongoing with the laboratory to develop a validation cohort to address these issues. When this is available, some of the gaps in the literature on micronutrient status in CD remission identified by the systematic review chapter will be addressed.

While this thesis cannot make a statement on the percentage of individuals with blood tests that were in a range that put them at risk of clinical deficiency, regression analysis has explored whether there were analytes which were lower among those with a low FACIT-F. It has also considered the FACIT-F score as a continuous variable using regression analysis against the micronutrient measures.

Both methods consistently found that antioxidant vitamins were correlated with fatigue, with a weak correlation coefficient, although only Lutein remained statistically significant after a Bonferroni. The data supports the view that low serum Lutein was among the factors related to excessive fatigue in the cohort, suggesting that the micronutrient may be a factor in excessive fatigue. Lutein is a fat-soluble liposoluble vitamin among the carotenoids. It is not routinely measured as part of clinical care but has been reported to be lower among subjects with an elevated Crohn’s Disease Activity Index when compared to those without an elevated CDAI(323). Lutein intake has also been associated with a lower faecal blood and stool count in UC (324). The correlation between lutein and fatigue seen in the INTICO-2 cohort may reflect lutein being related to ongoing inflammation rather than contributing to fatigue. There is evidence for the role of lutein as a supplement to treat and prevent age-related macular degeneration, but none for IBD (325).

What is not known is whether the analytes measured were reflective of tissue availability of the nutrients and whether a limiting nutrient was relevant to the study outcome, FACIT-F fatigue. The comprehensive clinical chemistry micronutrient panel that was analysed in 24 subjects who completed INTICO-1 included several analytes (with validated ranges) which have known biological links to mitochondrial function, such as Vitamin A, B12, red cell B2 and red Cell B9 that were not available from the nestle laboratory. The finances available in this project did not allow for more comprehensive and validated micronutrient blood measurements to be run over

the larger cohort, which limited our understanding of the relationship between micronutrient status and study outcomes.

The study outcome to measure fatigue in CD remission was chosen as FACIT-F, which has been correlated to low mood, which may have confounded attempted correlations with nutritional state. In the study population, I found that those reporting a low mood because of their IBD or poor sleep had a lower FACIT-F score, which may have affected FACIT-F independently of nutrition. The original study protocol included cardiopulmonary exercise testing (CPET), which may have generated a more objective marker of functional and cellular capacity against which to compare nutrition than FACIT-F. This was approved and funded with guidance and collaboration from colleagues in Perioperative Medicine at UHS but was removed from the study protocol to allow the study to go ahead during the COVID-19 pandemic due to the unacceptable risk of aerosol generation.

Despite the considerable amount of effort and analytical time deployed to blood biochemistry, it was not possible to draw definitive conclusions from the micronutrient status in this study. The micronutrient blood measurements showed a weak correlation between one antioxidant fat-soluble vitamin and fatigue, showing that it was the most common factor related to fatigue. Blood biochemistry may not have accurately reflected measures of micronutrient status in the functional pools, and FACIT-F may not have been an accurate measure of muscle or cellular fatigue.

The demographics of the INTICO-2 cohort and the timing of the study must be considered alongside the observed changes in the nutritional state. The study was conducted during the COVID-19 pandemic, with visits occurring around national lockdowns. This was when there was a shift to home working, with limited permitted outdoor leisure activities. Data suggest that household energy intake tended to increase in the UK during this period, and the initial lockdowns were associated with an increased tendency to gain weight (326) (327). This may have led to fewer individuals being identified as at risk through BMI and weight-loss-based scoring systems. Longitudinal weight data was not available, but insight was gained from the INAT questionnaire, where the majority answered yes to recent weight gain. Many individuals with CD were advised to shield due to concerns around their immunosuppressant medications, meaning that recruitment may have been biased towards those who were least anxious about coming to the hospital for the study visit.

The study subjects recruited were most commonly (135/198, 68%) of a non-stricturing, non-fistulising disease phenotype. This is a similar percentage to that reported in 2019 from the UK

bioresource registry, suggesting that in this respect, this population is reflective of the broader UK IBD population (92). The INTICO-2 subjects recruited tended to be older, with a median age of 52, which may reflect the retired population being more able to take part in research visits (which were conducted on Monday to Friday). 41% of our cohort were on a biological medication at the time of study entry. While national consensus guidelines recommend when and in whom to use these medications, this relatively high percentage usage in a cohort with predominant inflammatory disease may not be typical of other centres. IBD physicians' medication choices can vary, so the high percentage usage of biologics in this cohort may not reflect other UK CD cohorts (93, 94).

The BMI-based nutritional screening tools, such as the widely used MUST score, identified just 13% (26/194) of the INITCO-2 population as being at nutritional risk. Therefore, further nutritional assessments such as the ones in the study, such as body composition analysis, food diary and micronutrient blood tests, would not have been done to assess most of the cohort. Applying MUST or NRS as a screening tool before a subsequent assessment against GLIM criteria would result in just 1% of the cohort being diagnosed with malnutrition. When applying GLIM, aetiological and phenotypic criteria without screening would have diagnosed 11% with malnutrition.

The lack of subjects screening to be at risk by MUST or NRS-2002 is likely a consequence of the high percentage of overweight and obesity in the population (61%), which makes a BMI-based risk assessment tool likely to identify subjects. Weight loss, another feature of MUST, was also rare in the population, which perhaps reflects that the subjects needed stable clinical and calprotectin remission as a study inclusion requirement.

While clinical practice advocates only applying the GLIM criteria to malnutrition in those with an elevated MUST score, the accepted aetiological and phenotypic criteria for this emerging consensus for malnutrition were used across the whole cohort. When these criteria were applied, it demonstrated aetiological factors such as an underappreciated inflammatory burden, to a lesser extent, a reduced energy intake and phenotypic criteria such as a low lean were common and found 10% of subjects to fulfil both aetiological and phenotypic GLIM criteria for malnutrition.

The limited utility of a BMI-based screening score in IBD populations, in which obesity and overweight are likely to be common, was recognised by the authors of the Sask-IBD- NR tool. They found better concordance with a subsequent comprehensive dietetic assessment IBD outpatients by including GI symptoms and food restriction.  Our data supports the utility of the

INAT dietary impact factors as a predictor of fatigue and micronutrient adequacy of diet, thus supporting the role of identifying dietary restrictions and food-related symptoms to screen CD remission populations for nutritional issues.

INTICO-2 was an in-depth characterisation of a large outpatient cohort in clinical and biochemical remission. It has shown that there are individuals with excessive fatigue and has found issues with dietary impact factors, inadequacies of micronutrient intake and altered body composition. Excessive adiposity, poor quality diet and, to a lesser extent, micronutrient biochemistry were correlated to excessive fatigue. The summary findings of both INTICO-1 and INTICO-2, their strengths and limitations are discussed in Chapter 5.

# Chapter 5 Summary, discussions and future work

## 5.1 Summary and Discussion

Crohn's disease is characterised by intestinal inflammation and its sequelae and is treated with medications to reduce intestinal inflammation to a state defined as remission. This thesis has found that the nutritional state can be impaired during remission, and some of the burden of residual symptoms, particularly excessive fatigue.

Previous literature, taken from an era in which diagnosis was often delayed and limited effective medical treatments were available, has established that Crohn's disease can impact the adequacy of intake and lead to changes in keeping with overt undernutrition. Existing nutritional care guidelines cite these previous studies, consider the needs of those with active disease, wasting and intestinal failure and advocate for screening tools that identify those with overt malnutrition. This thesis considered the nutritional state and CD remission in an era of effective treatments, increasing adiposity, metabolic disease, and earlier diagnosis.

The evidence on the nutritional state in adults with Crohn's disease in Chapter 1 was drawn from general IBD populations and cohorts with variable disease activity. Inflammatory bowel disease is a heterogeneous condition with distinct sub-types, different stages of disease, differing severity and different activity. The nutritional requirements, losses, food tolerance,

intestinal barrier function, appetite and absorptive capacity will vary throughout the disease course. Nutritional studies must acknowledge these issues and present the data of different IBD subtypes separately while also recognising the distinct nutritional issues of active, untreated disease versus those in remission. Such an evidence base can inform a more tailored approach to supporting the nutritional care of those with IBD.

The systematic review on the micronutrient status in CD remission required the data for adults with CD in remission (by a defined disease activity score) to be reported separately as an inclusion (Chapter 3) criterion. This focussed review of the literature found insufficient information about the likely prevalence of individuals likely to be at risk of deficiency in CD remission populations and the causes and consequences of micronutrient insufficiency.

The prior understanding identified a need to better characterise the nutritional state using two experimental approaches. Both studies looked at subjects in confirmed clinical and biochemical CD remission. Under usual care, they would not be considered for a change in medical therapy, to be at nutritional risk or to be prescribed nutritional interventions.

This thesis has focussed on better understanding nutritional care, not to induce remission or to address overt malnutrition, but rather to benefit those in remission on their treatment. The first approach was a time-limited trial of a nutritional intervention that would be considered nutritionally complete in clinical practice (INTICO-1; Chapter 3). The intervention, Modulen, is prescribed routinely to children and adults with CD to induce remission, was familiar to the patients in the study, and is readily available in clinical practice. This was not an attempt to demonstrate the efficacy of the commercial product in this application. Rather, it was a pragmatic approach to control the diet where adherence to a food-based dietary intervention would have been variable, and the challenge of personalising the diet to both individual needs and food choice, whilst maintaining constancy, would have been problematic without intensive dietetic contact. If the intervention were formulated appropriately, prescribed to meet the energy needs of each individual, and consumed, this approach would provide a uniform and consistent source of energy and nutrients in amounts sufficient to meet most patients' needs.

This initial hypothesis-generating study required clinically stable subjects not receiving dietetic care before the study to change their habitual diet to a liquid-only formula intake. The comprehensive characterisation revealed many unrecognised nutritional issues in this cohort. The baseline nutritional assessment identified inadequacies of dietary intake, altered micronutrient biochemistry, sarcopenia, excessive adiposity, altered bioelectrical properties of the body and poor health-related quality of life among the small cohort. When the intake was

changed for just 7 days, it led to profound changes in micronutrient biochemistry, objective positive changes in the bioelectrical impedance measurements, and, for some, a change in fatigue. The reporting of improvement in longstanding fatigue was an unexpected observation but an exciting one as it represents a novel treatment consideration of a common and debilitating feature of Crohn's that can persist throughout the disease course.

The changes in SF-36 physical functioning score or reported fatigue may have been due to a placebo effect, but there were objective beneficial changes in blood biochemistry and the bioelectrical impedance after the dietary interventions to refute this.

Measures of blood micronutrient biochemistry in INTICO-1 showed profound changes in response to the time-limited trial of the dietary intervention. This nutritional restitution is with previous work by Gerasimidis et al. in Glasgow that demonstrated changes in micronutrient pools on EEN, alongside a beneficial increment in lean but not fat mass in children (328). Thiamine supplementation has been shown to improve fatigue in IBD, and at least one of the subjects with this response post EEN had multiple baseline deficiencies in nutrients known to be mitochondrial co-factors (193, 329). Some of these blood tests were corrected by the EEN, so the change seen in fatigue for this subject and others may have been due to improved availability of previously limiting micronutrients. The improved bioavailability of previously limiting nutrients may also be the mechanism underlying the increase in phase angle, which is believed to be reflective of whole-body membrane structure and integrity.

In this first study, micronutrient status was determined in standard NHS biochemistry laboratories with ranges that allowed identifying those at risk of deficiency for a given nutrient. In this study, among the 21 subjects at baseline, 26 blood tests demonstrated biochemical risk for deficiency across the panel of micronutrients, with multiple inadequacies seen in 5 subjects, and one subject with 6 abnormal blood tests. This subject had some of these blood results corrected by the EEN and was among those who reported a marked improvement in their fatigue. The total number of blood tests below the laboratory fell to 14 after the EEN, showing that it can correct micronutrient biochemistry as an intervention. In an exploratory analysis of this small study, without a prior power calculation, I found that neither an increase in phase angle, nor SF36 Vitality score correlated to the number of baseline deficiencies, but this could be a type II error.

There is increasing use of EEN in adult medicine, and it is being trialled to downgrade inflammation and reduce disease complications in elective Crohn's surgery. There is an

emerging understanding of the mechanisms of EEN, which include a resolution of dysbiosis among EEN responders, but the specific mechanisms are unknown (330).

The observed increase in faecal calprotectin at day 7 in subjects who remained in clinical admission is a novel and unexpected finding. It challenges the view that EEN leads to a calprotectin fall and that an early high calprotectin represents deteriorating disease activity. Previous groups have shown a fall at 4 and 6 weeks, that is followed by a rebound upon a return to diet, but they did not measure day 7 calprotectin on EEN (331). It is not known whether the calprotectin would have eventually fallen in our subjects with an initial rise. It was hypothesised that the changes seen here may represent an increase in neutrophils in the bowel wall as part of healing and occur due to nutrient-driven beneficial immune restitution.

The second approach used the experience from INTICO-1 to conduct an observational study of nutritional state in a larger cohort of patients with CD in remission, whether this population had evidence of excessive fatigue, and whether the two were related (see The INTICO Cohort Study: INTICO-2).

The next study, INTICO-2 used the experience from the exploratory study to assess whether the impairments of nutritional state were present across the larger population, whether this population had evidence of excessive fatigue, and whether the two were related. Over a quarter of the INTICO-2 population was defined as having severe fatigue using agreed cut-offs for the FACIT-F PROM. This is consistent with other published cohorts of outpatient populations with Crohn's disease (16, 17). FACIT-F scores were lower than published population norms(306). The Harvey Bradshaw Index correlated to fatigue independently of the general well-being sub-score, suggesting it was partly due to residual disease activity. The SF-36 and IBD Control PROM were consistent with FACIT-F, with INTICO-1 and INTICO-2 finding subjects to have an impaired health-related quality of life during CD remission.

Many of the markers of an impaired nutritional state in INTICO-1 were also altered in INTICO-2. Analysis of the food diary found multiple inadequacies of micronutrient intake, which was only partially mitigated by micronutrient supplementation. The dietitian-designed questionnaire of clinical signs and symptoms supported the view that intake may have been impaired by dietary impact factors, which were widely reported in this cohort. The number of micronutrients consumed at intakes below the LRNI and the number of dietary impact factors correlated to excessive fatigue.

The body weight and weight history would not have identified these patients as at risk nutritionally. The body composition data estimated by BIA found that these patients in INTICO-2

tended to be overweight or obese and with a relative lack of lean body mass (sarcopenia) and excessive adiposity (sarcopenic obesity), as was seen in INTICO-1. The results show that 14% of subjects were below the 5$^{th}$ centile for lean mass when expressed as standardised scores for age and gender. A large multicentre nutritional observation study assessed body composition in outpatients with IBD in a population (190 with CD) with 26% with clinically active disease and found that adding a BIA-derived estimate of lean mass and SGA criteria increased their diagnosis of malnutrition to 17% of those with Crohn's in the cohort (212).

Body Mass Index is an easily accessible clinical tool. Whilst many participants had a normal Body Mass Index (BMI), this masked deficits in lean mass due to relative excess adiposity. A low BMI was not a sensitive marker for identifying a deficit of lean mass. Low lean mass has been associated with various adverse outcomes and is increasingly incorporated into malnutrition and nutritional risk definitions. Excessive adiposity was common in both study populations. An elevated BMI was found to be a more sensitive and specific marker of excessive adiposity. Adiposity is increasingly understood to be pro-inflammatory and may be a modulator of intestinal inflammation or a reduced response to medications (9, 215, 317). Excess adipose was common and correlated to worse fatigue in this treated patient population with biochemical and clinical remission. Low lean and excess adipose may be related to disease outcome, and prospective studies on IBD outpatients address this question (332).

Bioelectrical impedance analysis also found that the Phase Angle creduced in INTICO-1 and responsive to the nutritional intervention, was generally lower in the INTICO-2 cohort and was related to excessive fatigue. This measure, reflective of cellular mass, membrane capacity and integrity, is when reduced, increasingly associated with adverse clinical outcomes. It is a relatively simple, non-invasive test, so it may be a helpful adjunct to identify individuals at risk of nutritional deficits and measure the impact of nutritional interventions in CD (290).

The micronutrient data for INTICO-2 showed a weak correlation with the FACIT-F score for a limited number of analytes. When the reference ranges are available, the biochemistry results from the INTICO-2 cohort will be added to the literature on micronutrient blood tests in CD remission through publication. Although it was a sensitive and highly refined platform, it had not previously been used to characterise nutritional state in clinical studies or healthy individuals, and many of the analytes were below the limits of quantification. A parallel activity is ongoing, which will generate reference ranges for all analytes, but the results were not available at the time of writing the thesis. Therefore, the blood micronutrient biochemistry was restricted to within-group comparisons. These analyses showed a weak correlation between the FACIT-F score and several micronutrients. This questions the view that baseline blood measurements of

micronutrient status relate to fatigue. INTICO-2 did not include a time-limited trial of any nutritional intervention. It may be that time-limited trials and repeated measurements rather than single measurements are a better way to interrogate the nutritional sensitivity of an individual or process.

## 5.2 Strengths and Limitations

The strengths of the work described in this thesis are the deep multi-dimensional characterisation of the clinical and nutritional state and the inclusion of a study with a time-limited trial together with a large observational cohort. The three nutritional assessments over 21 days in INTICO-1, while challenging for the research team and participants, have potentially demonstrated some of the mechanisms behind Crohn's disease and its sequelae. There are published studies looking at the effect of EEN on enterocytes, endoscopic appearances, microbiome and faecal metabolome, but not with the same intense observation of multiple markers of nutritional state(134, 136, 333) and not in a cohort of patients in remission. This work, for the first time, suggests that a nutritional intervention may improve the quality of remission.

In INTICO-2, the nutritional state was assessed using a range of measurements at a single time point, and the baseline observations of INTICO-1 were confirmed across a large cohort. This included a comprehensive data set of dietary analyses, a questionnaire to explore the cause of any inadequacies, and standardised body composition and impedance data. The simultaneous measurement of nutritional status and PROMS has allowed the exploration of nutritional state and its clinical consequences.

There have been relatively recently published cohorts of the nutritional state in adults with Crohn's disease. A Spanish multicentre study that looked at body composition and dietary impact symptoms (but no dietary data) in 30 centres, including 190 subjects with Crohn's (26% active disease), was consistent with our findings in identifying sarcopenia and found that malnutrition (defined by one or more of low lean, low BMI or SGA) was related to food avoidance (212). A broadly cited study from the USA looked at nutritional state and nutritional risk scores in newly diagnosed individuals and found that a third of individuals fulfil MUST criteria for risk of malnutrition, which predicted ESPEN-defined malnutrition (230). Their study used retrospective clinician-requested blood micronutrient measurements, so they may have introduced bias towards those with the most significant concerns and overestimated prevalence. The cohort also had a higher incidence of active disease and did not assess HR-QOL or outcome.

The work has several major limitations. INTICO-1 was a small trial with no control arm. The study involved a screening visit, followed by 3 study visits in 21 days and a switch from habitual diet to exclusive enteral nutrition. This burdensome study protocol may have been more appealing to those most concerned about their preceding nutritional inadequacy and introduced a recruitment bias. The fatigue improvement may be partly due to the involvement in

the trial and placebo. The first trial did not have a fatigue-specific PROM. In addition, practical challenges with the myfood24 platform likely led to under-reporting of intake and thus over-estimated the degree of dietary inadequacies. Another limitation of the study is the dietary control delivered through an intervention known to downregulate intestinal inflammation by removing harmful elements from habitual diets or providing anti-inflammatory components. We cannot say the extent to which the beneficial changes were due to the downstream benefits of treating mild intestinal inflammation versus nutritional restitution.

INTICO-2 had several significant limitations. There was no assessment of depression symptoms and poor sleep, which are known factors in excessive fatigue, so this may have confounded any of the attempted correlations to markers of nutritional state. To allow remote recruitment during COVID-19, C-reactive protein (CRP) was removed as an exclusion criterion, but some individuals with an elevated CRP in the cohort remain. This may have been reflective of not being in remission and confounded the assessment of fatigue and measurement of nutritional state. CPET testing was not performed due to COVID restrictions at the time of performing the study but would have added a further dimension to the assessment of fatigue. In INTICO-2, due to the considerable cost of analysing extended micronutrient blood tests for 198 subjects in a clinical laboratory, the collaborating lab in Lausanne analysed the blood. At the time of writing, the limitations of this platform have precluded our ability to use the data to estimate the prevalence of those at risk of deficiency from blood tests. While awaiting data for matched populations to estimate laboratory ranges, the team at Glasgow have published a large cohort of micronutrient blood tests using the clinical laboratory used in INTICO-1. This study of IBD patients treated with biological medication, including 127 with Crohn's disease, has allowed a better estimate of the likely prevalence of deficiency across a comprehensive panel of clinically relevant analytes in the current treatment era (334).

The recruitment of INTICO-2 subjects was more structured than INTICO-1, with all pre-screened individuals invited from across the hospital outpatient population. Nevertheless, there may have been a bias towards those most interested in fatigue and/or nutrition (mentioned in the invitation letter) being the most likely to come forward as study volunteers. This study was completed during the COVID-19 pandemic, which may have affected the type of patients coming forward, their mood and fatigue, and their nutritional state. The cohort was 98% Caucasian and may have been biased towards those most able to come in for a study due to work, income and family commitments. Nutritional intake varies between countries and ethnicities, so the observations here may not be valid outside the UK. Whilst it was possible to compare the BIA data against device-specific reference values, there were no reference values

against which the food diary data and blood biochemistry results could be compared. Therefore, it is not possible to say whether all the changes seen were particular to Crohn's disease or issues across the wider population.

## 5.3 Future Work

The priority of future work should be to confirm the observed beneficial effects of nutritional intervention on fatigue seen in INTICO-1 in a study design with a control arm of usual care and adequate power to detect a clinically meaningful difference in the measurement of fatigue. The hypothesis to be tested would be that the nutritional intervention would improve dietary adequacy, reduce intestinal inflammation and reduce fatigue.

The design could be similar to INTICO-1 with multiple time measures before the interventions, after the interventional and after a washout. The primary outcome would be self-reported fatigue FACIT-F. Previous studies have shown that a 7–10-point improvement in FACIT-F score with an intervention is a meaningful within-person difference and clinically significant (335). Using previously published data, the number of necessary subjects was estimated under the assumption that the mean difference in improvement in FACIT-F of 7 points, with a typical standard deviation of 9 points, with a confidence level of 95% and 80% power, a bilateral test and a drop out of 10%, we estimate that we would require between 30-35 patients in each arm. Unfortunately, the FACIT-F score was not used in the interventional study (INTICO-1), so evidence-based statements on the potential effect size of a nutritional intervention on fatigue assessed by this metric cannot be made based on the work from this thesis.

The Crohn's Disease Exclusion Diet with or without partial Enteral Nutrition has similar mechanisms and efficacy to EEN, but is better tolerated, so may offer a more acceptable longer dietary intervention to test a dietary intervention as a measure to improve fatigue in subjects with CD (149, 150). The study may need consideration for stratified by baseline dietary impact factors, FACIT-F score, poor sleep, and depression score. For this study, secondary objective measures of fatigue, such as CPET anaerobic threshold, could be measured alongside markers of nutritional state, such as corrected phase angle and fat/lean mass. Replication of the findings of this study could demonstrate that nutritional interventions offer a novel treatment avenue for fatigue in Crohn's disease, something for which treatments have been limited.

As to exploring the mechanism underlying nutritional interventions, serum panels of inflammatory cytokines, microbiome or metabolome, micronutrient biochemistry, redox state markers, and barrier function measures could be incorporated into the study design with

appropriate expertise and collaborators. It is proposed that assessing disease inflammation, activity, and nutritional state should be considered alongside one another. This thesis has focussed on the nutritional state, but it is a false dichotomy as adequate nutrition is required for effective resolution of inflammation, restoration of barrier function and immune homeostasis.

In the immediate term, when the micronutrient platforms used in INTICO-2 can be compared against reference values from a healthy control population, it will be possible to further explore the likely adequacy or otherwise of micronutrient status, adding to the published literature on micronutrient status in Crohn's disease remission.

## 5.4 Implications for Practice

In an era of increasing obesity, with fewer patients having uncontrolled intestinal inflammation, issues such as overt undernutrition and intestinal failure are likely to be less prevalent in the outpatient Crohn's disease population. Dietetic care may shift to identifying and supporting those with excessive adiposity to improve the quality and balance of their dietary intake. Micronutrient adequacy of diet has been proposed as among the factors that regulate satiety; addressing dietary quality may improve energy balance and aid in this (336).

The food diary data was time-consuming to collect and analyse but revealed that poor-quality diets were common. Dietitians are a limited resource; self-screening with dietary impact questionnaires and electronically-supported food diary collection and analysis of those individuals would improve CD dietetic care. The observed dietary inadequacies in INTICO-1 resulted in the design and introduction of a questionnaire by an experienced dietitian to capture the dietary impact factors and symptoms of dietary restrictions for use in the clinical setting. This questionnaire was trialled in INTICO-2 and shown to be correlated with the adequacy of the diet and fatigue. Dietary counselling has been shown to improve Vitamin C intake and biochemistry in outpatients with Crohn's disease (337). Identifying and supporting those individuals with the greatest number of dietary inadequacies is a challenge that must be addressed to improve care. There is an increasing interest in the relationship between ultra-processed food and both IBD and obesity, with meta-analysis linking the latter. Ultra-processed food is of increasing interest to IBD patients (338). It may be a surrogate marker for micronutrient-poor, high-fat, high-sugar diets, likely to promote intestinal inflammation, dysbiosis and obesity (339). This was not the focus of this work, but it may interest IBD patients and dietitians, alongside supporting dietary quality.

The findings of this thesis highlight the limited utility of BMI-based screening for malnutrition and support the use of body composition analysis in the clinic to identify those with low lean mass, especially when present with excess body fat. While those with weight loss and low BMI need to be identified and directed to dietetic support, limiting nutritional care to only that group of patients is likely to deny nutritional assessment and care to a large proportion of the population. Identifying and supporting those with low lean mass may improve outcomes. Bioelectrical impedance is a quick, painless and non-invasive way to estimate body compartments. It can also provide additional information, such as the Phase Angle. Together, they may serve as markers of nutritional and clinical state and are used to identify nutritional risk and assess the response to nutritional or medical interventions.

In an environment of restricted dietetics capacity, nutritional care should be directed towards those that need it most, including patients with Crohn's who have active disease, undergoing surgery or with intestinal failure. This work has demonstrated a need for nutritional support in the remission population. Upskilling IBD teams in screening CD patients for nutritional deficiencies as part of routine care and patient self-screening via electronic portals could be used to identify subjects who may benefit from a comprehensive dietary analysis, dietetic counselling and advice on supplements. Those clinicians who deliver IBD services should know and acknowledge the limits of their nutritional understanding and work with specialist dietitians in the IBD service to improve nutritional care.

Assessing dietary impact factor questions in those patients in remission using questions on food avoidance, food-related symptoms and appetite predicted dietary inadequacies and fatigue so that it could be used as a more effective screening tool than MUST or NRS-2002. A self-screening tool for digital platforms incorporating IBD symptoms and dietary impact factors, such as the Inflammatory bowel disease self-screening tool (IBD-NST), would suit this setting (238). The experimental work in this thesis supports targeted food diaries, body composition analysis and time-limited trials of nutritional interventions but does not advocate for single timepoint blood biochemistry as a tool to aid clinical decision-making.

The era of CD treatments is changing to early top-down control of inflammation before tissue injury using measures of mucosal healing as a target for clinical trials. Nutritional care must reflect this change in targets with adequate control of inflammation, nutritional support aiming for an improved health-related quality of life, treatment of fatigue and minimising the risk of osteoporosis, vascular disease and obesity-related cancers, a target for better care.

## 5.5 Conclusions

This thesis sought to determine if nutritional state was impaired during Crohn's disease remission and whether it was a determinant of the quality of remission. Results have shown that nutritional state is impaired during Crohn's disease remission and that existing tools to screen for more comprehensive nutritional assessments and interventions fail to identify the vast majority of those in need. It has shown that despite these individuals being appropriately treated in other aspects of clinical care, this population may have a burden of residual inflammation and excessive fatigue. Treatment targets need to change to incorporate quality of life and fatigue as targets for clinicians.

This thesis has indicated that nutritional therapy may be an adjunct to other medical treatments and may offer a way to improve the quality of disease remission. Adequately powered and targeted nutritional intervention studies in CD remission populations will be needed to test this observation. This nutritional perspective on the needs of adults with Crohn's disease during their time with this lifelong condition may offer a new avenue for treating a persistent, common and often addressed symptom.

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