PROTEIN NUTRITION IN PAEDIATRIC CROHN'S DISEASE

By

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THESIS ABSTRACT

Altered nutritional status is well documented in Crohn's disease patients, which may lead to low muscle mass and deteriorated function (sarcopenia) and adverse disease outcomes. Although a variety of experimental methods have assessed muscle mass and function in paediatric CD patients, there is no conclusive consensus on the definition of sarcopenia in those patients. However, most of the studies in the literature have showed reduction in muscle mass (MM), lean mass (LM) and fat-free mass (FFM) in paediatric CD compared with healthy controls or reference data.

Historically, underweight was frequently linked with paediatric CD. However, recent evidence suggests a shift towards adiposity rather than underweight being prevalent in paediatric CD. Our knowledge of the relationship between adiposity and adverse disease outcomes in children with CD is limited. In addition, clinical characteristics of CD patients may vary depending on age at diagnosis. Moreover, adult CD patients exhibit altered nutritional status and eating behaviour that impact negatively on habitual dietary protein intake, protein metabolism and muscle mass. However, these relationships have never been investigated in paediatric CD.

This PhD thesis used the gold standard technique, magnetic resonance imaging (MRI), to assess leg muscle volume in children with stable CD disease and compared it with matched healthy subjects. These findings were linked with dietary protein intake, fasting plasma amino acids (AAs) and eating behaviour traits. In addition, the MRI technique was used to compare psoas muscle cross sectional area (PCSA) between paediatric with active CD versus those with inactive CD. However, conclusive findings were limited due to small sample size in both studies.

This thesis highlighted the need for standardisation of body composition and sarcopenia terminology, as well as the valid use of assessment tools in adequately powered populations with appropriately matched comparators, to establish a clear definition of sarcopenia and assess its prevalence in paediatric IBD. In addition, it revealed that adiposity is linked to poor clinical outcomes in a long-term follow-up cohort of paediatric-onset CD, and that the age at disease onset is also associated with negative

clinical outcomes. Moreover, this thesis revealed comparable dietary protein and energy intake, and eating behaviour traits between stable CD paediatric patients and matched healthy controls, which may explain the lack of differences in leg muscle volume and handgrip strength (indices of sarcopenia) and circulating AAs between groups. Furthermore, there were no significant differences in PCSA assessed by MRI between active and inactive paediatric CD; however, age and gender were associated with muscle mass. These findings might have been affected by disease duration and further disease burden that need to be taken into consideration in future studies. Data collection in this thesis was significantly impacted by the COVID pandemic. Hence, most of the studies performed were either retrospective in nature or cross-sectional studies which require further prospective cohort studies with larger sample size to enhance generalisability and confirm findings.

Therefore, the aims of this thesis were to a) systematically evaluate changes in skeletal muscle mass and function in paediatric IBD, b) investigate the relationship between adiposity and age of disease onset with adverse clinical outcomes in paediatric patients with CD, c) assess protein intake, circulating AAs and skeletal muscle mass and evaluate eating behaviour in paediatric CD and age, sex and BMI-matched healthy controls and d) describe the differences in PCSA assessed by MRI between active and inactive paediatric CD and examine the impact of age, gender, disease activity, and other disease-related variables on muscle mass.

This research integrated several approaches to assess the impact of nutritional status, early age of disease onset on disease outcomes, and muscle size in paediatric CD patients. In addition, it compared protein intake, eating behaviour between children with stable CD and healthy matched peers to offer a holistic understanding of their impact on muscle mass and strength (as an index of sarcopenia) and circulating amino acids profiles in paediatric CD. While the studies presented in this thesis did not reveal significant differences in certain aspects of dietary protein intake, muscle size and strength, they did highlight several areas requiring further investigation. The complicated nature of disease burden and activity, alongside paediatric growth, necessitates further research to better understand the definition, management and prevention of sarcopenia in paediatric CD patients.

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PUBLICATIONS

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DECLARATION

I hereby affirm that this dissertation and its contents are my own work, except where otherwise indicated. This thesis has not been previously submitted to this or any other university for a degree.

Bayan Aljilani

31st July 2024

ABBREVIATIONS

(AAs) Amino acids

(ASMI) Appendicular skeletal muscle index

(anti-TNF) Anti-tumor necrosis factor

(BIA) Bioelectrical impedance analysis

(BMI) Body mass index

(BCAA) Branched chain amino acid

(CRP) C-reactive protein

(CEBQ) Child Eating Behaviour questionnaire

(CTFEQr-17) Child three factor eating questionnaire-17

(CT) Computed Tomography

(CD) Crohn's Disease

(DEXA) Dual energy x-ray absorptiometry

(EAAs) Essential amino acids

(EEN) Exclusive Enteral Nutrition

(EIMs) Extra-intestinal manifestations

(FC) Faecal calprotectin

(FFM) Fat free mass

(GIT) Gastrointestinal tract

(HS) Handgrip strength

(HRA) Health Research Authority

(HC) Healthy controls

(IBD) Inflammatory bowel diseases

(LM) Lean mass

(LM) Lean mass

(MRE) Magnetic resonance enterography

(MRI) Magnetic Resonance Imaging

(MM) Muscle mass

(NIHR) National Institute for Health Research

(non-EAAs) Non-essential amino acids

(NREC) Nottingham Research Ethics Committee

(5-ASA) Oral 5-aminosalicylates

(PCDAI) Paediatric Crohn's Disease Activity Index

(PUCAI) Paediatric Ulcerative Colitis Activity Index

(p-QCT) Peripheral quantitative computerized tomography

(PAI) Psoas area index

(PCSA) Psoas cross-sectional area

(TNF-α) Tumor necrosis factor-alpha

(UC) Ulcerative Colitis

(VAT) Visceral adipose tissue

1. GENERAL INTRODUCTION

1.1. INTRODUCTION

Inflammatory bowel diseases (IBD) as shown in figure 1.1 (1) in the majority consist of Crohn's Disease (CD) and Ulcerative Colitis (UC), both being idiopathic lifelong diseases associated with inflammation of the gastrointestinal tract (GIT) (2, 3). The difference between CD and UC is that the latter is not transmural, which means it affects only the innermost lining of the intestinal wall (the mucosa) and does not extend through the entire thickness of the bowel wall. In contrast, CD can be transmural, affecting all layers of the bowel wall.

CD as shown in figure 1.2 (2) is characterized by its ability to affect any part of the GIT with patchy and transmural inflammation and it may be described by location (upper GI, colonic, ileocolic and terminal ileum), or by disease behaviour (inflammatory, stricturing or fistulising) (4). These variables have been combined in the Montreal and Paris classifications as shown in Table 1.1 (5). In addition, there is a third type of IBD that may affect up to 10% of children known as indeterminate colitis (IC) which involves a colonic disease location but having features of both UC and CD following clinical, radiological, endoscopic and pathologic examinations.

Twenty five percent of IBD occurs during childhood or adolescence with both genders equally affected (6, 7). The highest annual prevalence of IBD were reported in Europe (23 per 100,000), in North America (15.2 per 100,000) and in Asia/the Middle East and Oceania (11.4 per 100,000) (8). Additionally, recent evidence suggests that the population prevalence percentage exceeds 0.3% in many countries in Europe, Oceania and North America (9). In another systematic review, 16 of 22 studies in CD (72.7%) and 15 of 18 studies in UC (83.3%) reported stable or decreasing incidence of IBD in North America and Europe (10). The paediatric prevalence of IBD in United State of America (USA) has overall increased by 133%, from (33.0 per 100,000) in 2007 to (77.0 per 100,000) in 2016 and CD with CD being twice as prevalent as UC (45.9 vs 21.6 per 100,000) (9). Since 1990, the incidence has been growing in newly industrialized countries in South America, Asia and Africa (12). In the United Kingdom (UK), the annual incidence of IBD in children is predicted to be 5.2 per 100,000 (60% CD and 28% UC and 12% IC) and more common in the male gender (4). The increasing incidence of this condition combined with its incurability implies a significant healthcare cost. The annual

cost per patient is estimated for treating UC patients could reach £3,084 and for treating CD patients could reach £6,156 (11).

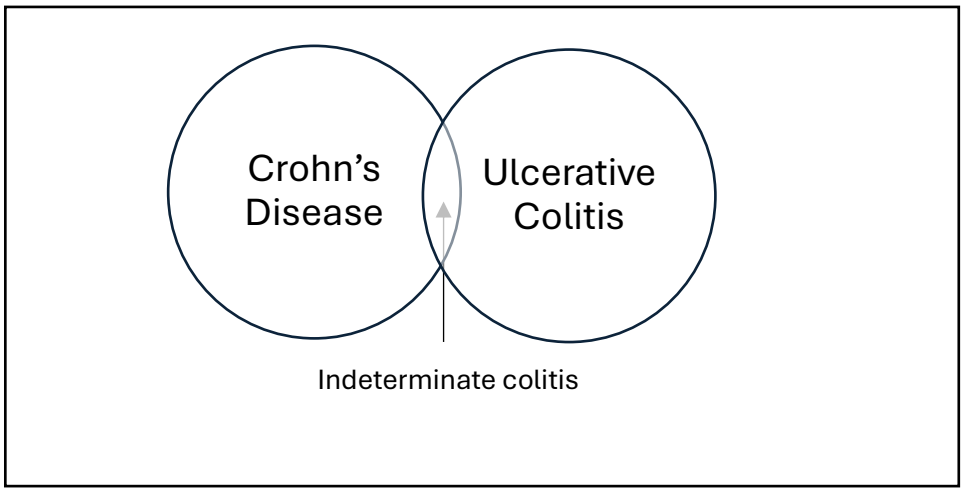

Figure 1.1. Inflammatory bowel disease subsets (1).

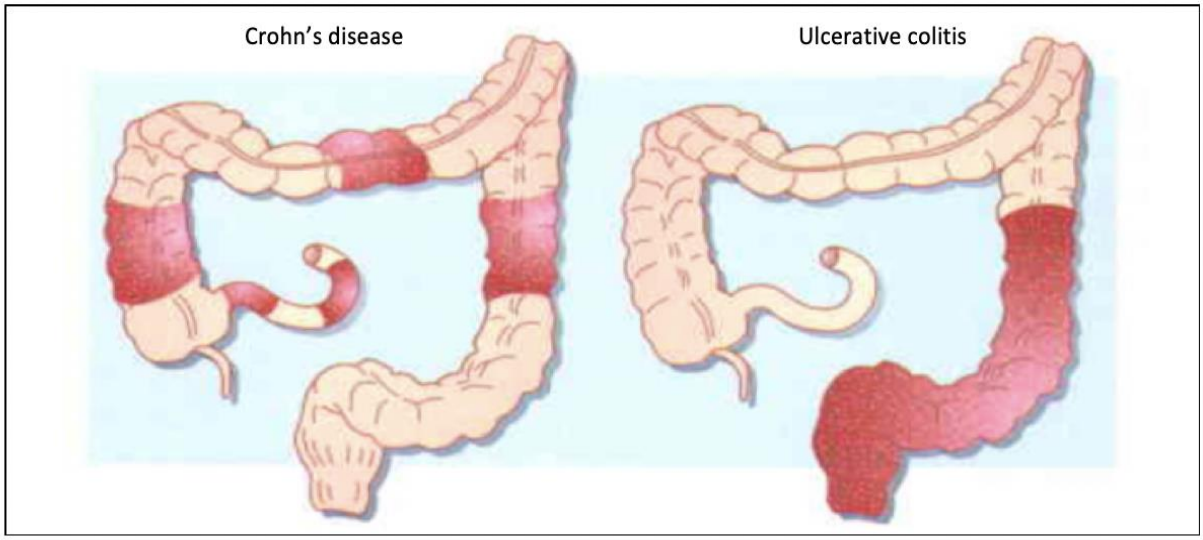

Figure 1.2. The difference in the affected areas between the Crohn's disease and Ulcerative colitis adapted from (2).

Table 1.1. Summary of Montreal and Paris classifications of CD		
	Montreal Classification	**Paris Classification**
Diagnosis disease at age of (A)	A1: below 16 years A2: from 17-40 years A3: over 40 years	A1a: 0-<10 years A1b: 10-<17 years A2: 17-40 years A3: >40 years
Site of disease (L)	L1: terminal ileal/ limited caecal disease L2: colonic L3: ileocolonic L4: upper gastrointestinal disease	L1: distal 1/3 ileum/ limited caecal disease L2: colonic L3: ileocolonic L4a: upper disease proximal to ligament of Treitz* L4b: upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum*
Disease Behavior (B)	B1: non-stricturing or nonpenetrating B2: stricturing B3: penetrating p: perianal disease modifier	B1: non-stricturing or non-penetrating B2: stricturing B3: penetrating B2B3: both penetrating and stricturing disease, either at the same or different times p: perianal disease modifier
Growth	-	G0: no evidence of growth delay G1: growth delay

*In both the Montreal and Paris Classification systems L4 and L4a/L4b may coexist with L1, L2, L3, respectively. B1 - Nonstricturing, nonpenetrating disease: uncomplicated inflammatory disease without evidence of stricturing or penetrating disease. B2 - Stricturing disease: the occurrence of constant luminal narrowing demonstrated by radiologic, endoscopic, or surgical examination combined with prestenotic dilation and/or obstructive signs or symptoms but without evidence of penetrating disease. B3 - Penetrating disease: the occurrence of bowel perforation, intraabdominal fistulas, inflammatory masses and/or abscesses at any time in the course of the disease, and not secondary postoperative intra-abdominal complication (excludes isolated perianal or rectovaginal fistulae). B2B3 – Stricturing and penetrating disease: the presence of both B2 and B3 phenotypes in the same patient, either at the same moment in time, or separately over a period of time.

The underlying cause of IBD is unknown. The onset of IBD in childhood is usually related with implications on growth retardation and delayed development (12, 13). Typical symptoms involve decreased appetite, abdominal pain, weight loss and bloody diarrhoea (6). Some children may develop severe malnutrition to World Health Organization (WHO) criteria as result of weight loss and growth failure (14, 15). Existing data show that malnutrition affects a large quantity of patients with IBD, especially patients with CD where the prevalence of this complication is potentially commoner (according 65–75%) than in UC (18–62%) (16). Additionally, the prevalence of malnutrition in paediatric population seems to be greater in CD patients than in UC patients (17, 18). Both diseases could cause life-threatening implications because of nutrient malabsorption as consequence of structural changes and inflammation in GIT due to disease development and malnutrition (19).

1.2. PATHOPHYSIOLOGY OF IBD

The aetiology of IBD is not completely well defined but it likely involves an interaction between the immune system in a genetically susceptible individual exposed to environmental factors. The abnormal mucosal immune response is related to a compromised epithelial barrier function leading to bacterial translocation and further perpetuation of the inflammatory process. The diversity in the genetic mutations among patients may elucidate the diversities in the onset, location, aggressiveness, complications and responsiveness to various therapies as seen in the clinical setting (20, 21). CD and its disease type is characterized by many single nucleotide polymorphisms in NOD2/CARD15, ATG16L and IL23R genes (22). Combination of these gene mutations have also been associated with CD (23). Although NOD2/CARD15 mutation is associated with stricturing ileal disease (24-28), an early development of penetrating or stricturing in CD is affected by disease location and activity and cigarette smoking but not by NOD2/CARD15 genotype (29).

In IBD the factors perpetuating the inflammatory and immune responses are enhanced and/or the regulatory mechanisms are disturbed, leading to an unregulated inflammatory response, followed by tissue fibrosis and destruction. The clinical course of the disease may be mild and episodic or severe and constant (2). Diet, food contaminants and microbes may be considered as environmental factors and could produce a large quantity of potential antigens that trigger relapse of IBD. In addition, the type and composition of the resident microflora can be influenced by dietary factors, such as lipids, that impact on the intensity of the inflammatory response (2).

1.3. SYMPTOMS OF IBD

The symptoms of IBD may vary between patients depending on the disease location. In UC the predominant symptoms are rectal bleeding and diarrhoea while in CD, abdominal pain and diarrhoea are the most commonly reported symptoms. Fatigue is a common symptom in both diseases.

Crohn's patients may develop complications such as strictures and fistulas (30) with symptomatology affected by such disease complications and their location in the GIT. CD patients with a main colonic distribution of the disease tend to present mainly with

chronic diarrhoea and rectal bleeding, while patients with small bowel disease can present with predominant obstructive symptoms, abdominal pain and a change in bowel habit (31).

1.4. DIAGNOSTIC CRITERIA

The European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHN) have created and developed a consensus protocol for investigation to ensure all affected children receive optimal care (32). The main modalities used involve biochemical and haematological laboratory investigations supported by endoscopic, histological and radiological investigations (4). Laboratory analyses usually include a full blood count (FBC), liver function tests (especially albumin), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Low values of haemoglobin and serum albumin with increased inflammatory markers (CRP, ESR and platelets) are predictive of IBD (4). Serological tests are applied rarely in clinical practice as their sensitivity range between 60-80%, although the *anti-Saccharomyces cerevisiae* antibody is associated regularly with CD and the *perinuclear anti-neutrophil cytoplasmic* antibody with UC (4).

Stool cultures are undertaken to exclude infectious diarrhoea from the differential diagnoses of IBD. Faecal calprotectin (FC) is a simple non-invasive stool test for a granulocyte protein shed into faeces by the inflamed bowel that has been used in recent years as a marker of disease activity and relapse to avoid more invasive investigations (33). A meta-analysis estimated it has 80-87% sensitivity and 68-82% specificity for clinical (34) and endoscopic activity (35), 78% sensitivity and 73% specificity to predict relapse, and it may be more reliable in UC than CD (33).

Generally, upper and lower GI endoscopies are advisable with biopsies to document the histological diagnosis and extent of disease. If classical histological features are not observed, disease distribution may aid in the diagnosis. Appropriate treatment can be provided when tissue diagnosis and disease distribution are confirmed. Histological evidence of CD in the upper GI tract can be found in up to 30% of cases even in the absence of upper GI symptoms (2). Unlike adults, more than 90% of children with UC have a pancolitis, making full colonoscopy preferable. Sigmoidoscopy is a safer option especially in severe UC where the risk of bowel perforation is higher. Ileocolonoscopy is

the preferred procedure for suspected IBD to confirm the disease and its extent (31). Abdominal radiography assessment is crucial to use in patients with severe colitis to exclude toxic megacolon as a complication or intestinal obstruction in structuring CD. Ultrasound (sonography) is a non-invasive sensitive procedure that may diagnose abscesses or free fluid in the peritoneum and can be used to assess disease activity and location in CD (4, 36). Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) may help in evaluating the activity and complications of disease and both can assess the abdomen and pelvis which requires luminal distension (37). MRI has similar diagnostic accuracy to CT, but MRI has a major advantage of not imparting ionizing radiation, while the CT has considerable limitation because of the radiation exposure (37). MRI is considered the golden standard imaging modality for assessing patients with CD who require many follow-up examinations (38). However, CT is more widely used in the acute setting.

1.5. MEDICAL MANAGEMENT

1.5.1. Pharmaceutical treatment

A number of therapies can be used for the treatment of IBD and various factors should be taken into account when choosing the appropriate therapy such as severity, location of the disease and the goal of therapy (maintenance or induction of remission) (39). Current medical management guidelines for paediatric patients including variety of therapies for induction and maintenance of remission (40).

Corticosteroids (steroids) can be beneficial for patients with moderate-to-severe IBD symptoms and can help to reduce the inflammatory burden and associated symptoms. Corticosteroids may be given orally and are normally administrated for disease relapses. Corticosteroids should be used only short term to avoid secondary complications from the medication if used long term. Patients with mild IBD may be treated with oral 5-aminosalicylates (5-ASA) formulations, such as mesalamine and sulfasalazine, which have both immunomodulatory and anti-inflammatory properties (41).

Immunomodulatory drugs, such as methotrexate and thiopurines, are used to maintain remission and in patient's refractory to 5-ASA therapies. Patients with high disease burden or who are intolerant or refractory to immunomodulatory agents can be treated

with biologics to induce and maintain remission such as anti-TNF drugs (Infliximab, golimumab and adalimumab), anti-integrin α4β7 (Vedolizumab), anti-Interleukin 12 and 23 (Ustekinumab), anti-interleukin 23 (Risankizumab, mirikizumab) and Janus Kinase inhibitors (Upadacitinib, Tofacitinib and Filgotinib) (40, 42, 43). It is best practice to undertake a clinical assessment of disease activity using one or a number of the above-mentioned modalities in a timely manner and whenever patients have symptoms.

1.5.2. Surgical treatment

For patients with refractory UC or in CD with a resistant inflammatory phenotype or with complicated disease (stenosis or internal fistula) that have failed to respond to medical therapy, a multidisciplinary discussion with the surgical team is usually required to advise regarding the appropriateness of an intestinal resection. In CD, over half (50.8%) of the patients experience a fibrotic or penetrating disease complication after 20 years (44) with an associated 10-year surgical risk of 46.6% (45). CD with perianal involvement is broadly managed in a similar fashion using the same agents with the important difference that in perianal CD it is of paramount importance to drain pelvic sepsis before instituting any medical therapy (46). For UC patients with severe refractory disease who failed to respond to aggressive medical treatment, surgery is a possible solution. A colectomy in the first five years from diagnosis with UC be will needed in 8%-26% of children (47). However, intestinal resection is not a curative option; it can induce remission of localized CD disease and should be followed by initiation or optimization of maintenance therapy (48).

1.5.3. Nutritional treatment

Exclusive Enteral Nutrition (EEN) has shown a similar impact as a corticosteroid therapy in inducing remission phase in children with CD (49-51). Moreover, EEN has demonstrated a positive effect on mucosal healing when used in combination with corticosteroid therapy rather than corticosteroid therapy alone during CD active phase (52). Parenteral Enteral Nutrition (PEN) is used as a maintenance therapy along with drugs in special conditions (48). Powell-Tuck et al. (53) found that whole body protein turnover were positively correlated with severity of disease but not with parenteral or enteral feeding in 19 undernourished adult IBD patients (84% CD), while Thomas et al.

(54) found that treatment by either steroids (n=4) or an elemental diet (n=6) can induce remission and reduce protein turnover in 10 CD paediatric patients with active disease.

Recently, prebiotics and probiotic have shown plausible use because of their ability to alter both intestinal microbiota and enhance the immunologic response (42). Many other dietary approaches have been discussed as potential adjuvant maintenance therapy for IBD, such as anti-inflammatory diet, low FODMAP diet, Paleolithic diet and specific carbohydrate diet, but none of them are supported by strong clinical evidence to date.

1.6. COMPLICATIONS AND NUTRITIONAL STATUS OF IBD

CD patients could develop complications such as intestinal obstruction because of strictures or internal fistula, thereby necessitating a surgical procedure to improve their quality of life such as ostomy creation, bowel resection, fistulotomy, stricturoplasty or abscess drainage (either perirectal or intra-abdominal) (55). UC Patients with an ileal pouch-anal anastomosis may suffer from pouchitis (56). However, most patients respond to antibiotics (57, 58).

Growth failure is one of the main complications and more common in CD than in UC as consequence of delayed skeletal maturation which leads to suboptimal linear growth and sometimes delayed puberty. The underlying aetiology is diverse and may include the inflammatory nature of the disease, malabsorption, chronic use of corticosteroids (56) and a change in eating behaviour (59). Large numbers of patients with IBD are affected by malnutrition with an estimation of 65–75% in CD and 18–62% in UC (16). Specifically in paediatric cohorts, a higher prevalence of malnutrition is observed in CD rather than UC (17, 18). Moreover, malnutrition is a predominant cause of growth failure in children and it may be caused by abnormal gastrointestinal function (16). The consequence of malnutrition and/or malabsorption and chronic inflammation may lead to sarcopenia and hence a low muscle mass which negatively effects quality of life and is strongly linked with fatigue (60). Furthermore, increased risk of postoperative complications in IBD patients are associated with increased incidence of sarcopenia (61, 62), but improving perioperative nutrition management may reduce this risk (63).

Underweight and malnutrition was frequently seen in paediatric with IBD, although a recent shift toward overweight and obesity has been presenting in children with IBD (64,

65). Although literature on obesity in children with IBD is limited, Long et al. (66) indicated high percentages of overweight or obese in CD by 20% and UC by 30% . Obesity could be as result of the alterations in the intestinal immune system and suspected dysbiosis in the pathophysiology of IBD (67, 68). Obesity is associated with proinflammatory state which triggered by adipocyte hypertrophy (69-71). Adipose tissue particularly visceral adipose tissue (VAT) has been shown to be high in adult and paediatric with CD and has been examined for its potential role in disease development and severe disease progression (72).

Although obesity is becoming a significant issue among adults with immune-mediated diseases like IBD, its impact on IBD clinical outcomes remains conflicting. Obesity in adult with IBD have associated with increased disease activity, higher morbidity, frequent perianal complications, earlier time to loss of response to anti-TNF treatment , and frequent hospitalisation (73). In addition, obese CD adults have higher risk of perioperative morbidity compared with normal weight group (32% vs. 22%) (74). Adult obese patients with CD and overweight patients with UC are at higher risk of disease flare-up compared with patients of normal body weight (75), although this association between obesity and poor IBD outcomes is not universal (76-79). Literature on the impact of obesity in children with IBD on clinical disease outcomes is even more limited and this is a still an area where further research is needed (66, 80-84).

As patients with GIT disorders are at higher risk of eating disorders, IBD patients may present with abnormal eating patterns irrespective of disease activity (85, 86). Around 75% of patients with IBD report a decrease in appetite during active phase of the disease behaviour (85). Up to 37% of CD patients exhibit irregular eating behaviours (87). Appetite and satiety consist of homeostatic and hedonic interactions. The homeostatic regulation of food intake is primarily governed by the enteroendocrine-gut-brain axis, while additional neuronal factors involve emotional and environmental factors form the hedonic regulation of appetite (88). Thereby, the homeostatic and hedonic balance may be affected by disordered eating.

Research on eating behaviour in adult patients with IBD is sparse with no literature in IBD paediatric population. In adults with IBD, dietary restriction (59, 89, 90) and a higher occurrence of disordered eating behaviour traits has been observed (59, 91-93).

Therefore, it is crucial to understand the role of appetitive behaviours and their associations with dietary intake and weight in paediatric patients with IBD by using validated tools with predefined terminology as they are affected as well by growth and puberty beside to disease activity.

1.7. SARCOPENIA

The first description of sarcopenia was made in 1989 by Rosenberg as a loss of lean muscle mass associated with a loss of muscle strength and a risk of physical disability and poor quality of life (94). The pathophysiological mechanism implicated in the aetiology of sarcopenia may include malnutrition, low muscle protein synthesis, high proteolysis and immobility (95, 96). The prevalence of sarcopenia among adult IBD patients is higher in CD (59.0%) than in UC (27.3%) when compared to a control healthy population (8.3%) and is a potential independent predictor for developing major postoperative complications (97).

Moreover, the higher prevalence of sarcopenia in CD seems to be in male patients (59, 97). However, several radiological measurements and different cutoff values have been used to define sarcopenia; thus, contributing to a lack of high-quality data (98). When sarcopenia is diagnosed by appendicular skeletal muscle index (ASMI), the incidence of sarcopenia in CD is found to be 12% (16), with grip strength being a better predictor of low ASMI than BMI (99). Patients at risk of sarcopenia may be identified by gender, age, serum albumin and body weight, which are all strongly associated with muscle volume in IBD (99). In a paediatric population, loss of lean mass was reported in 93.6% of patients with CD and in 47.7% of patients with UC in comparison with a healthy control group (100). However, the research of sarcopenia in pediatric population is also hampered by lack of longitudinal data, limited number of outcomes-based research and low study quality (101). In addition, studies related to body composition in paediatric patients with IBD are less documented (101, 102).

The aetiology of sarcopenia is very diverse and may include cytokine dysregulation, reduction in anabolic hormone production, a neurodegenerative state and an attenuation in protein intake and exercise.

1.7.1. Protein metabolism and sarcopenia

Proteostasis is the equilibrium between muscle protein synthesis and protein degradation during steady state (102). Apart from ingestion of dietary protein, muscle hypertrophy pathways involving insulin, growth hormone (GH) and insulin growth factor 1 (IGF-1) (16, 103) are pivotal. Under normal circumstances, dietary amino acids and insulin secretion promote muscle hypertrophy by activating the mammalian target of rapamycin (mTOR) pathway and reducing protein degradation by suppressing the activation of ubiquitin ligands (103-108). Muscle growth is blunted following protein ingestion because of low insulin sensitivity in older rather than younger individuals (109, 110). The two main proteolytic pathways in skeletal muscle are the autophagy-lysosome and the ubiquitin–proteasome system (UPS), while the calcium-dependent calpain and caspase systems are two additional systems responsible for proteolysis (111-113).

Muscle protein turnover is affected by several factors such as mechanical unloading, growth factors (myostatin), hormones (glucocorticoid), inflammatory cytokines, oxidative stress, metabolic stress and nutrient availability (glucose and amino acids) (114). Muscle atrophy occurs in response to a potential decrease in plasma and muscle IGF1 secondary to inflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), leading to GH resistance in muscles and liver, mTOR pathway dysregulation, ubiquitin ligands activation and altered enzyme expression involved in protein breakdown, in particular MUSA1, MuRF1 and MAFbx/Atrogin-1 (115-117). Evidence has indicated that overexpression of MAFbx and MuRF1 is essential in the development of muscle atrophy (118).

Chronic inflammation, is associated with an increase in protein breakdown and a concomitant decrease in myofibrillar proteins, which result in impairment of muscular contraction and loss of muscle mass (16). During the inflammatory process, muscle protein breakdown involves a coordinated network of proteolytic pathways regulated by inflammatory cytokines and hormones, which stimulate protein breakdown and reduce synthesis. Recent studies showed that cellular growth pathways are dysregulated in chronic inflammatory diseases particularly the autophagy pathways and the UPS (119-121).

It is as yet unclear what the role of UPS is in the development of age-related sarcopenia (103). The two UPS-dependent skeletal muscle ligases involved in proteolysis are the E3-ubiquitin ligases MuRF-1 and MAFbx (122). Nevertheless, inconsistent data indicate decrease (123), no change (124, 125) or increase (126-129) in MuRF-1 and MAFbx expression in muscle tissue from older cohorts. Moreover, the main UPS peptidase activities have been noted to be decreased (130) or unchanged (126, 131). These conflicting findings suggest that calpain and autophagy pathways may play a more dominant role in the development of sarcopenia (103). Furthermore, evidence shows that with age there is a decline in autophagy function (132-134) leading to polyubiquitin protein accumulation and then destruction (103). Autophagy is found in all eukaryotic cells and involves a catabolic process that includes degradation of cytoplasmic components via the set of lysosomal and autophagic digestion (135). As a result, the current literature indicates that protein degradation in sarcopenia is mainly regulated by the autophagy and calpain pathways and not by the UPS pathway alone (103).

In chronic inflammation rather than age-related muscle loss the pathophysiology involved in the development of sarcopenia may be different. A significant activation of myostatin, a member of TGF-beta family, is observed (16), leading to sarcomeric protein degradation via the synthesis of MuRF1 and atrogin-1 through the upregulation of the transcription factors Smad2/3 and the suppression of AKT signaling (136, 137). Additionally, acute phase proteins and cytokines such as IL-6, TNF- α and IFN-gamma may have a direct role in muscle wasting by interfering with the myogenic program and activating the transcription factor NF-kB and the ubiquitin proteasome system. Experimental models showed that TNF- α can impair proliferation and differentiation of muscle stem cells by acting on NF-kB (138). Circulatory levels of TNF-α and IL-6 have been shown to be negatively correlated to muscle mass and strength in older individuals (139).

1.7.2. Sarcopenia assessment

1.7.2.1. Measures of skeletal muscle mass and imaging

As there is no standard criterion for assessing sarcopenia in all ages, diagnosis of sarcopenia should involve the use of correct predefined terminology with appropriate method. Lean mass (LM) and Fat free mass (FFM) have been used interchangeably,

although each one refers to different body compartment. LM is more correctly related to lean soft tissue (LST), which encompasses body water, total body protein, carbohydrates, non-fat lipids, and soft tissue minerals, excluding the fat and bone mineral compartments, while FFM consist of LST and bone mineral compartments (140). Appendicular skeletal muscle (ASM) refers to the amount of LST in the arms and legs which primarily consists of muscle (141, 142), while skeletal muscle index (SMI) measures LM relative to height or weight (143). SMI is commonly defined either as appendicular skeletal muscle mass divided by height squared (measured in kg/m^2) or as skeletal muscle mass divided by body mass multiplied by 100 (a unitless index) (144). Some authors distinguish these as appendicular lean mass/$height^2$ and SMI (145). Figure 1.3 demonstrates the difference in definition of muscle body compartments. Many tools have been used in the assessment of sarcopenia such as magnetic resonance imaging (MRI), computed tomography (CT) and dual energy x-ray absorptiometry (DEXA), but some of them have a limited use in clinical practice. Several studies used DEXA in assessing muscle mass due to its low cost and availability; although MRI is the golden standard method because of its high resolution and safe nature. However, it is time-consuming and expensive, reducing its routine use in standard clinical practice. Due to the limitations related to all modalities there is no standard radiological measure of lean muscle mass (63). The advantages and disadvantages of each modality in assessing body composition compartments are demonstrated in Table 1.2.

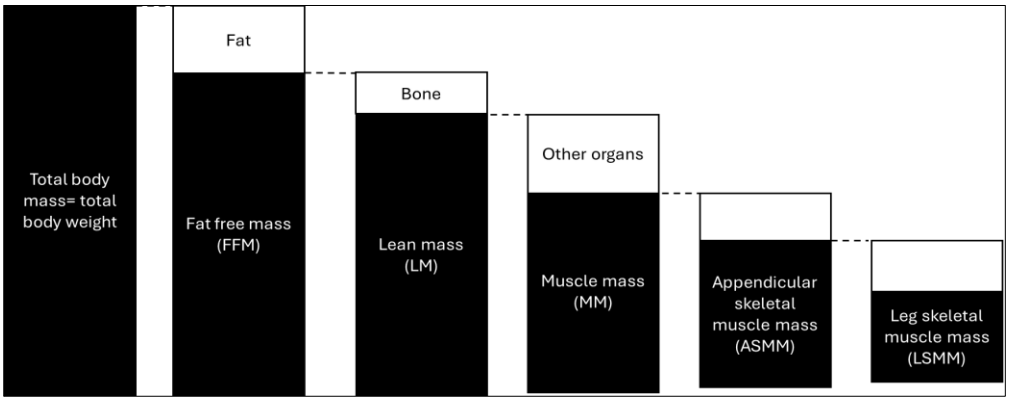

Figure 1.3. The difference between muscle body compartments.

Table 1.2. Methods to assess skeletal muscle mass and muscle imaging			
Measures	**Body composition analysis**	**Advantages**	**Disadvantages**
Creatinine excretion	Estimation of MM (146).	• Measure directly related to total body muscle mass. • Low cost. • Widely available.	• There are variations in creatinine excretion depend on physical activity and diet, infection status, different phase of menstrual cycles (146)
Whole-body potassium	Estimation of FFM and BCM, assuming potassium mainly distributed into the intracellular compartment with a relatively constant concentration within the BCM and FFM compartment (147-150), and hence estimation of FM (151, 152).	• Low cost. • Widely available.	• There are differences in the ratios of FFM depends on sex and age (153)
Skinfold thickness	Estimation of subcutaneous adipose tissue (154-156)	• Low cost. • Minimal maintenance. • Portable. • Results immediately available. • Does not require highly trained personnel.	• Not precise and accurate for very thin or very fat people (157-159) • Not accurate estimation of present percentage of body fat as it measures only fat under the skin (157-161)
Bioelectrical impedance analysis (BIA)	Estimate TBW and FFM (155, 162, 163); FM is obtained indirectly using the difference between weight and FFM (164, 165).	• Low cost. • Minimal maintenance. • Portable. • Results immediately available. • Does not require highly trained personnel.	• BIA may overestimate FFM and underestimate FM in morbid obese (166) • BIA affected by hydration status, considering that acute inflammation or hypoalbuminemia, caused by malnutrition, is known to cause edema (167, 168).
Dual energy X-ray absorptiometry (DEXA)	Measure FM, LM, bone mass and TBW (151, 152, 156, 157, 163, 169, 170).	• Low cost. • Widely available. • Sensitive and accurate method (171, 172). • Does not require highly trained personnel.	• Underestimate FM in lean individuals (173-177) and obese (178). • Various softwares by same or various manufacturers (148) • Affected by soft tissue hydration status (179). • Cannot be repeated due to exposure to ionizing radiation (169).
Computed tomography (CT)	Measure adipose tissue, MM, FFM, bone mass (178, 180).	• Cross-sectional measurement of lean and fat mass areas in a specific part of the body • Assessment of muscle quality (171).	• Radiation exposure (148, 150, 181-183). • Available in highly specialised settings and require technical skills (157, 184, 185). • Not applicable at bedside or morbid obese people BMI> 34 kg/m^2(157, 184). • Expensive (157, 185)

Magnetic resonance imaging (MRI)	Measure FFM, MM, adipose tissue and various organs (liver, kidneys, heart, spleen, pancreas) (148, 155, 163, 178, 184, 186-189).	• High resolution (171). • Cross-sectional measurement of lean and fat mass areas in a specific part of the body • Assessment of muscle quality (171).	• Morbid obese people BMI >40 kg/m^2 cannot be fit inside the field-of-view (178, 184). • Available in highly specialized settings and require technical skills (184, 190, 191). • Expensive (178, 184, 190, 191).

FFM= fat-free mass, MM= muscle mass, LM= lean mass, BCM= body cell mass, FM= fat mass consists of approximately 80% adipose tissue, which is primarily composed of triglycerides lipid (165), while adipose tissue is a connective tissue consist of adipocytes, collagenous and elastic fibers, fibroblasts, and capillaries with approximately 79% fat, 3% protein and 18% water (184, 192).

1.7.2.2. Measure of muscle strength

Muscle strength may be measured with an easy-to-handle and low-cost technique, using a handgrip strength (HS) test with a standard dynamometer (96). HS is a sensitive predictor of body composition and has been correlated with lean mass (99). Several studies used handgrip strength to assess muscle function in IBD patients. Lu et al (193) demonstrated a reduction in handgrip strength in CD patients (63% active and 37% remission) compared with controls and it was correlated significantly with body cell mass (BCM) measured by BIA. Similarly, a reduction in handgrip strength was found in paediatric CD patients with mostly mild or moderate disease associated with reduced muscle CSA measured by p-QCT compared with matched reference data (194). However, a reduction in muscle strength was observed in adult CD patients in remission versus controls with comparable in lean mass between groups (195, 196). This suggests that both the quality and quantity of muscle mass deficits can adversely affect function in CD.

Davies et al. (197) reported no difference in both dominant and non-dominant muscle strength between paediatric CD with active disease and healthy controls with comparable findings as well in LM and appendicular skeletal mass index except in FFM which was significantly reduced in CD group.

1.7.3. Nutritional status with sarcopenia

The main nutritional issues that affect 20-85% of IBD patients are macronutrient deficiencies with protein-calorie malnutrition during the active phase of the disease (198) and micronutrient deficiencies during the remission phase (199).

Many studies show that vitamin D deficiency is highly prevalent in paediatric and adult patients with IBD (13, 200, 201). Low muscle mass in children has been related to suboptimal vitamin D status but no association has been shown between vitamin D status and sarcopenia prevalence in IBD children (202). Some studies revealed that skeletal muscle vitamin D receptor protein is associated with elevation of IL-6 protein content in aged human skeletal muscle and activation of the NF-κB cascade, which may increase the risk for developing sarcopenia (203, 204). Although findings from various studies are inconsistent, vitamin D deficiency is associated with muscle weakness, movement imbalance, poor physical performance and sarcopenia (205-210). Randomised controlled trials indicated an improvement in muscle strength and physical performance with vitamin D supplementations in older people (211-214). This may be because the genomic consequences of vitamin D in the muscle cell lead to change in mRNA concentrations and in *de novo* protein synthesis, whereas the non-genomic consequences involve protein kinase C activation and calcium release into the cytosol for muscle contraction (215).

As mentioned above, protein turnover is essential for muscle metabolism and the balance between anabolism and catabolism is important for preserving skeletal muscle mass. The mTOR pathway is a crucial anabolic pathway that is positively controlled by the essential branched chain amino acid (BCAA) leucine (216). Increasing serum leucine concentrations generate a positive amino acid balance within the skeletal muscle and maintain a strong anabolic response (216). Studies with protein supplementation, either in the form of essential amino acids or whole protein, have documented improvements in LM over the intervention periods among healthy elderly population (216-220). In adult IBD patients, Fiorindi et al. (221) conducted a 14 month prospective study including 45 CD and 16 UC patients to assess personalised nutrition regimens in preoperative phase with a protein target 1.2-1.5 gram per kilogram of ideal body weight. A significant improvement in FFM was reported after 103 days compared to baseline measurements. In paediatric CD, Motail et al. (222) demonstrated that whole body protein turnover, weight and FFM were increased post nutritional supplementation by nasogastric tube overnight for 8-10 hours for 7 months, which increased daily dietary protein and energy intakes by 40%, in 6 CD paediatric patients. This might be as these patients had growth

failure, which may lead to the normalisation of nutritional status rather than the normalisation of disease activity (223).

Several studies reveal a positive relationship between protein intake and preservation of muscle mass (224-226). A 12-week intervention in CD children using elemental diet showed partial improvement in muscle cross-sectional area with no further change at 52 weeks, despite patients being in remission (194). Similarly in adults with CD, Zhao et al (227) conducted a 4 weeks EN intervention introduced to 61 CD patients irrespective of disease status and without any concomitant dugs and classified into three groups based on their CD activity index after EN administration, group A (n= 21) were active patients who turned to remission, group B (n= 19) were active patients and remained active and group C (n= 21) were in remission state before and after EN. The findings showed that skeletal muscle mass improved significantly in group A without any change in the other groups, this suggests that the normalisation of muscle mass might be related to normalisation of disease activity than to the effects of nutritional intervention but EN can significantly reduce inflammatory response in active CD patients.

Low muscle mass may still persist in the CD remission phase (228), even though protein intake is within the normal recommended limits. Furthermore, adequate high quality protein intake, especially leucine is effective in enhancing muscle mass (229). Whole-body protein synthesis improves with feeding and contributes positively to enhance muscle mass. However, anabolic resistance occurs with aging because of a reduction in skeletal muscle anabolic sensitivity to protein feeding. Anabolic resistance may be influenced by the rate of dietary protein digestion, amino acid absorption, availability of plasma amino acid, the capacity of myofibrillar protein synthesis and hormonal response (230).

A study in hospitalized patients with CD showed that 50% were in a negative nitrogen (protein) balance and up to 75% were malnourished (231). Similarly, a significant reduction in daily protein intake in active CD patients has been observed when compared to age- and BMI-matched healthy controls (HC) [CD, 70.3± 6.1g/day vs. HC, 92.6± 7.8g/day, p=0.03] (59). Similarly, in paediatric CD protein intake was significantly lower especially in males when compared to HC individuals [CD:75 ± 5g/day vs. HC:105 ± 15g/day, respectively, p=0.026] (197). A negative postprandial skeletal muscle protein

balance was demonstrated in paediatric CD, specifically of a male gender (p=0.049) with associated low protein intake (p=0.026), high muscle fatigue (p=0.014) and low appendicular muscle mass (p=0.034) (197). Despite the inverse relationship between habitual protein intake and muscle mass in paediatric patients with CD, there is a paucity of studies investigating the effect of chronic protein supplementation on protein turnover and muscle mass in these patients.

1.7.4. Physical activity with sarcopenia

Physical activity may also play a protective role in sarcopenia and the interaction between exercise and diet could be more effective than protein supplementation alone (232). Apart from enhancing muscle strength (212), regular physical activity could have an anti-inflammatory activity (233). A randomised controlled crossover trial examined the effect of moderate-intensity combined aerobic and resistance exercise training (RET) three times per week over 8-weeks in 17 adult IBD patients (234). By the end of 8-weeks, a significant increment in lean mass was reported in the exercise group only ($P<0.001$).

Though exercise and protein supplementation could be of potential benefit in IBD-related sarcopenia, most studies in older adults have failed to show additional benefits of protein supplementation with prolonged RET on skeletal muscle mass (235, 236). The implications of long-term combined exercise and high protein intake on sarcopenia are not clear (237). Further investigation is required to explore the effects of quantity, intensity and timing of exercise with and without protein supplementation as an anti-sarcopenic intervention specifically in patients with CD.

1.8 SUMMARY

In summary, alteration in muscle mass has been observed in paediatric IBD patients, especially in CD, although inaccurate terminology has been used with a wide variety of assessment tools making it difficult to draw any conclusions. Nutritional supplementation with adequate protein has an impact either on disease activity and muscle mass. Further studies are needed to assess muscle growth by assessing body muscle compartments with muscle function. Although CD usually linked with malnutrition and underweight, the obesogenic state is proinflammatory and this relationship has not been explored in paediatric IBD.

1.9 AIMS

The main aim of this thesis was to investigate muscle mass and function in paediatric CD patients by a) systematically appraise literature and evaluate changes in skeletal muscle mass and function in paediatric IBD, focusing on CD population, b) assessing the association of obesity and age of disease onset on clinical outcomes in paediatric CD patients, c) evaluating protein intake and circulating amino acids (AAs) in relation to muscle mass and examine their eating behaviour and compare this with healthy non-IBD controls, and d) investigating the relationship between muscle mass and age, gender, disease activity, and other disease-related variables in CD paediatric patients.

Firstly, a systematic review was undertaken (chapter 3) to understand and evaluate muscle mass and function using standardised terminology of body-muscle compartments and assessment tools in published studies on paediatric populations with IBD, focusing predominantly on CD (238). The review also assessed factors that can affect muscle mass such as nutrition supplements and physical activity.

Secondly, a retrospective study was conducted (chapter 4) using a UK nation-wide cohort to evaluate the prevalence of adiposity in paediatric-onset CD and examine its relationship with disease outcomes (239).

Thirdly, a pilot study was undertaken (chapter 5) in paediatric patients with stable CD and age-, sex- and BMI-matched healthy controls to compare their dietary protein intake, circulating AAs and eating behaviour.

Finally, a cross-sectional study was undertaken (chapter 6) to investigate the relationship between muscle mass and disease activity in paediatric CD using MRI images already acquired in the NHS for standard care reasons.

2. SYSTEMATIC REVIEW: SARCOPENIA IN PAEDIATRIC INFLAMMATORY BOWEL DISEASE

Please note that this chapter has been previously published under the same title (238).

2.1. INTRODUCTION

Weight loss and growth failure are prevalent problems in paediatric inflammatory bowel disease (IBD) (65, 240, 241). Weight loss has been described in 70% of children with Crohn's disease (CD) and in 34% with ulcerative colitis (UC) (242) with growth failure described in 40% of children with CD and in 10% with UC (243). As a result, changes in body composition are commonly reported in both in paediatric IBD and are often accompanied by alterations in muscle mass (MM) related compartments (lean mass; LM and fat free mass; FFM) when compared with healthy control populations (244). Specifically, loss of LM in paediatric cohorts has been reported to be as high as 93.6% in CD and 48% in UC (100). Aetiologies for the alterations in body composition may be diverse but can include low-calorie intake, nutrient malabsorption, elevated levels of inflammatory cytokines, surgeries and concomitant pharmacotherapies (244).

Maintaining skeletal muscle mass is a tightly controlled proteostasis between muscle protein synthesis and muscle protein breakdown (102). Muscle tissue from patients with active CD shows a significant decrease in expression of muscle hypertrophy signalling proteins with no apparent change in the expression of atrophy signaling (245). These molecular changes may, at least partly, underpin the reduction in LM previously observed in patients with CD (2). Failure to maintain LM can result in sarcopenia, first described in 1989 by Rosenberg as the loss of skeletal muscle mass and strength associated with physical disability and poor quality of life (94). The prevalence of sarcopenia among adults with IBD is higher in CD (59%) than in UC (27%) when compared to a control healthy population (8.3%) (97) based on criteria of the International Consensus on Sarcopenia, which defined sarcopenia as a skeletal muscle index two standard deviations below the norm for young, healthy adults (246). However, several methodologies and diverse definitions have been used to define sarcopenia; thus, contributing to a lack of high-quality data and appropriate definitions (98). Furthermore, research in paediatric sarcopenia is hindered by lack of longitudinal data, limited number of outcomes-based research and low study quality (101). In addition, studies investigating skeletal muscle composition and function in paediatric IBD are sparse (101, 102) and interventional studies are lacking. Furthermore, one aspect of the published

literature that is frequently overlooked, is the variety of experimental techniques employed to assess body composition and the inconsistent use of related terminology. For example, the terms LM and FFM (and often MM) are frequently used interchangeably in the literature due to the lack of understanding that they refer to separate body composition compartments, which makes it difficult to align specific research outcome measures with differences in body composition between paediatric IBD patients and healthy control populations.

Exploring the presence and aetiology of sarcopenia in paediatric IBD is important in informing the design of interventions and treatments aiming to reverse MM loss and fatigue and their associated long-term adverse outcomes. The aim of this review of literature is to systematically describe changes in skeletal muscle function and mass and related body compartments in paediatric IBD, and in doing so appraise relevant interventions aiming to reverse those changes, identify gaps in the literature and improve the design of future interventional studies.

2.2. METHODOLOGY

2.2.1 Systematic review of the literature

A comprehensive search was carried out in the Medline, Embase, Cochrane library central for registered control trials and Web of Science on the 20th January 2023 to identify relevant studies using keywords from inflammatory bowel disease and skeletal muscle mass including terms related to muscle compartments. In addition, a manual searching process was used to find relevant studies in the reference lists of all included studies.

2.2.2. Criteria for inclusion and exclusion

The study designs included in this review were randomised controlled trials, prospective, or concurrent cohort and cross-sectional studies pertaining to children aged ≤ 18 years who have IBD confirmed through histology irrespective of their sex and race. Editorials, opinion papers, literature reviews and any studies not in the English language were excluded.

2.2.3. Search strategy

The full search strategy is described below in Table 2.1 and a more details of search strategy in each database are provided in **Appendix 1– Supplementary Table 1.1**. This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (247). The protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO) (https://www.crd.york.ac.uk/prospero/) with registration number CRD42020196776 on 25/08/2020.

Table 2.1. Search Strategy used in the systematic review.

#1- IBD	1. "inflammatory bowel disease" or "IBD" [MeSH terms] 2. "colon" or "small bowel" [MeSH terms] 3. "ileitis" [MeSH terms] 4. "jejunitis" [MeSH terms] 5. "colitis" [MeSH terms]
#2- Sarcopenia	6. "sarcopenia" [MeSH terms] 7. "myopenia" [MeSH terms]

	8. “muscular atrophy” [MeSH terms] 9. “myosteatosis” [MeSH terms] 10. “dynapenia” [MeSH terms]
#3- Age	11. “baby” or “babies” [MeSH terms] 12. “infant” [MeSH terms] 13. “paediatric” [MeSH terms] 14. “child” [MeSH terms] 15. “adolescent” [MeSH terms] 16. “teenager” [MeSH terms] 17. “youth” [MeSH terms]
Sum	18. #1 or #2 or #3 or #4 or #5 19. #6 or #7 or #8 or #9 or #10 20. #11 or #12 or #13 or #14 or #15 or #16 or #17 21. #18 and #19 and #20

2.2.4. Data extraction and quality assessment

The selected studies were initially screened for eligibility by two authors (BAA and SJR). The abstracts were reviewed and those eligible were included for full text review. The full manuscripts were independently assessed (BAA and SJR) as per the inclusion criteria. Any disagreements were resolved by discussion and consensus with the other authors (KT and GWM).

The data extracted includes specific details about population demographics, context, culture, geographical location, study methods and muscle compartments. Data pertaining to disease phenotype as classified by the Paris classification (5) were included. All papers were read in full, but only data reflecting the aims of the review were extracted.

2.2.5. Risk of Bias

Bias was assessed through the Joanna Briggs Institute (JBI) critical appraisal tool (248) (**Appendix 1– Supplementary Tables 1.2, 1.3, 1.4, 1.5 and 1.6**). This was assessed independently by two authors (BAA and SJR) while any disagreements were resolved by consensus with co-authors (KT and GWM).

2.3. RESULTS

The literature search produced 422 manuscripts. After removing duplicates, 239 manuscripts were chosen for screening. After abstract and full manuscript screening, 12 manuscripts were selected to be included in this review and 2 further manuscripts were added from reference lists from published literature. The selection process of the chosen studies is demonstrated in the Prisma diagram in **figure 2.1** and the main characteristics of the studies included in this review are summarised in **Appendix 1– Supplementary Table 1.7**.

A systematic review was conducted because of the inconsistent findings, varying methodologies, populations, and outcome measures reported in the literature, which made it unfeasible to statistically combine the results in a meta-analysis. Initially, a meta-analysis was planned to provide a quantitative synthesis of the findings. However, upon reviewing the studies, substantial heterogeneity was identified in the following areas:

- **Study populations:** Differences in demographic profiles, disease severity, medical therapies and comorbid conditions.
- **Intervention and exposure definitions:** Variability in the assessment and reporting of nutritional intake, physical activity, and muscle mass.
- **Outcome measures:** Inconsistent use of metrics and definitions for key outcome muscle mass related body compartments and disease activity.

These discrepancies precluded the meaningful pooling of data, as it would not meet the assumptions required for a meta-analysis. Instead, a narrative synthesis approach was adopted, summarising and interpreting findings in a way that accounts for the diversity across studies.

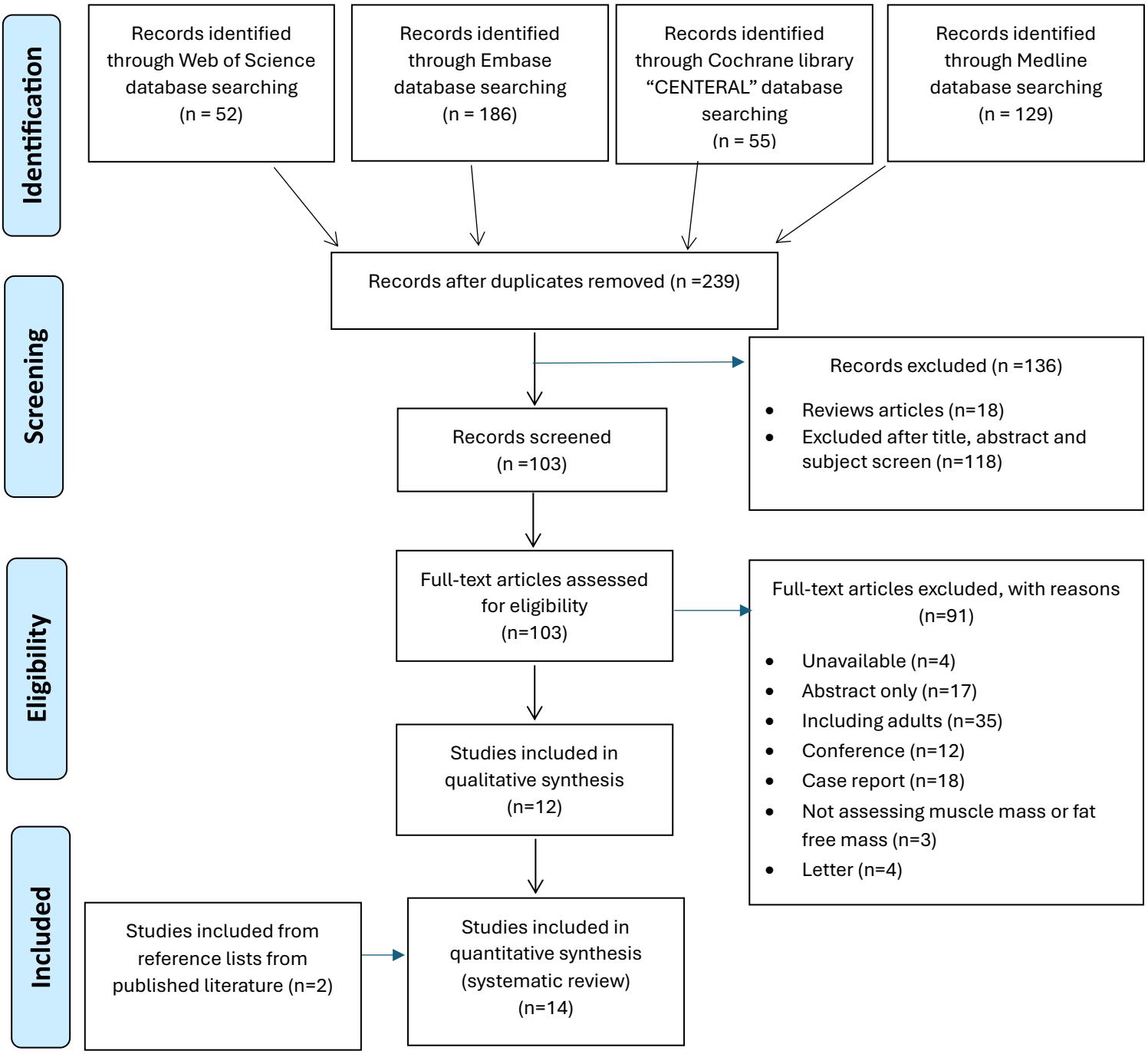

Figure 2.1. Flow chart of studies selection process.

2.3.1. Sample characteristics

The 14 studies involved in the review were published between 1982-2021. Ten studies were observational in design, while of the four interventional studies included, three studies were non-randomised and one had a randomised design. Studies were undertaken in the UK (17, 197, 249), Canada (13, 250-252), the United States of America (253), Germany (194, 254), Poland (255) , Australia (256), Croatia (257) and Israel (258). The total number of patients in those studies was 580 with IBD.

The participants' age range was 5-18 years, with a predominant male gender (58%) and only a single study (13) did not report the gender split. The majority (n=439) of the participants studied had CD (76%), 139 had UC (24%), 2 had unclassified IBD (0.3%) and 160 were healthy volunteers (22%). The concomitant medication reported were anti-tumour necrosis alpha therapies (n=39), corticosteroids (n=133), 5-amino salicylic acid (n=110), sulfasalazine (n=7), immunosuppressant agents (n=69), antibiotic therapies (n=3), anti-diarrheal agents (n=3) and proton pump inhibitors (n=2). Nutritional therapy was prescribed for 4 weeks in 12 participants and ≥ 4 weeks in 2 participants. Vitamin D and calcium supplementation were used in 3 participants. Only one study (258) assessed the relationship between drug therapy and muscle mass by using MRI to evaluate the psoas area index (PAI). An intestinal resection was performed in 11 participants, with the indication described as stricturing disease in 3 participants, penetrating disease in 2 participants and a colectomy and ileostomy formation in 1 participant.

2.3.2. Study quality and risk of bias

According to JBI critical appraisal tools, 9 studies (13, 194, 249-251, 254, 256-258) were considered as of good quality with their total score being ≥75% with the other 5 studies (17, 197, 252, 253, 255) having a total score of <75% with study subjects, setting and confounding factors not identified or described in detail. The grades and total scores are demonstrated in **Appendix 1– Supplementary Tables 1.2, 1.3, 1.4, 1.5** and **1.6**. In terms of comparability between diseased cohorts and healthy controls, one study used an unmatched unhealthy group (258), 6 studies (17, 249, 250, 254, 256, 258) used historical reference data unmatched to their own with 7 studies (13, 17, 249, 253, 254, 256, 257) recruited a healthy volunteer cohort adjusted for confounding factors such as age,

gender, height and puberty status. Findings were matched for age and sex based on control populations and presented as z-score in 6 studies (13, 194, 197, 251, 253, 255).

2.3.3. Disease duration and activity

Disease duration was only reported in four studies (253-255, 257). Motil et al. (253) recruited CD patients with disease duration of 2-5 years, whereas Werkstetter et al., (254) recruited newly diagnosed patients with CD and UC with a disease duration of 2 months or less (CD=23, UC=7). Wiech et al., (255) recruited newly diagnosed patients with CD and UC (CD=10, UCC=10), with less than one-year (CD=3, UC=4) and more than one-year (CD=12, UC=14) disease duration, whereas Trivic et al. (257) recruited CD patients with mean disease duration 48.2± 8.1 months and UC patients with mean disease duration 50.6± 11.6 months. Only two studies reported exact disease duration of their participants (253, 257).

Disease activity status was assessed in 11 studies with different measures such as Paediatric Crohn's Disease Activity Index (PCDAI), Paediatric Ulcerative Colitis Activity Index (PUCAI) and C-reactive protein (CRP) (13, 17, 194, 197, 250-252, 254-256, 258). Most studies recruited patients with a wide variety of disease activity states ranging from deep remission to severely active disease (see **Table 2.2** for details) with only a single study objectively reporting disease activity through biomarker assessment (197). One study (258) assessed the relationship between muscle mass and disease activity and indicated that IBD patients in remission had significant higher median psoas area index (PAI; calculated as the average psoas area divided by body surface area) measured by MRI [median PAI (mm^2/m^2)= 411, range 312; 519] when compared with those in mild [median PAI (cm^2/m^2)= 312, range 223; 330, p=0.001], moderate [median PAI (mm^2/m^2)= 292, range 234; 365, P<0.001] and severe [median PAI (mm^2/m^2)= 278, range 185; 332, p=0.003] disease activity.

2.3.4. Assessment of muscle composition and function

The most common methodology used to assess body composition was dual energy x-ray absorptiometry (DEXA) (n=222) (13, 197, 251, 256, 257). Other methodologies used were peripheral quantitative computerized tomography (p-QCT) (n=113) (194, 250, 254), bioelectrical impedance analysis (BIA) (n=73) (252, 255), magnetic resonance imaging

(MRI) (n=111) (249, 258) and skinfold thickness (n=55) (17). Motil et al., (253) used whole-body potassium and urinary creatinine excretion. The main muscle composition findings from those studies are summarised in **Table 2.2** and the main muscle function findings are summarised in **Table 2.3**.

Table 2.2. Muscle composition findings from the studies included in this review						
Authors	**Parameter measured**	**Methods**	**Median (min; max) or mean±S.D in CD and UC vs. controls**			
			IBD	CD	UC	Controls
Lean mass						
Mager et al. (13)	LM/height^2 (kg/m^2)	DEXA	14.7±16.3	12.6± 2.2	19.3± 28.6	-
	LM-height^2 (z-score)		-0.7±1.6	−1.0± 1.2**↓	0.0± 2.2	AMRF
	Appendicular LM/height^2 (kg/m^2)		13.5±5.6	12.8± 5.5	8.3± 12.9	-
	Appendicular, LM-height^2 (z-score)		-0.9±1.8	−1.3±1.2*↓	− 0.3 ± 2.5	AMRF
Ward et al. (251)	LM for age and gender (z-score)	DEXA	-2.0 ± 0.9**↓	-	-	AMRF
	LM for height (z-score)		-1.0 ± 0.9**↓	-	-	AMRF
Brookes et al. (256)	LM for height (z-score)	DEXA	-	-1.1±1.1**↓		AMRF
	LM for height, adjusted for bone age (z-score)		-	-1±1.1**↓	-	AMRF
Ward et al. (250)	LM (z-score)	DEXA	-	-2.5 ±1.1**↓	-	RF
	LM for height (z-score)		-	-2.3±1.0**↓	-	RF
Davis et al. (197)	LM (kg)	DEXA	-	39.3±1.5	-	46.0±4.5
	Appendicular LM (kg)		-	18.7±0.8	-	22.9±2.6
	ASMI (kg/m^2)		-	6.8±0.2	-	7.6±0.4
Trivic et al. (259)	LM (%)	DEXA	-	64.8±4.3**↓	68.2±2.5	-
	LM (z-score)		-	-1.6±0.3	-2.0±0.4	AMRF
Muscle mass						
Motil et al. (253)	MM (kg)	urinary creatinine excretion	-	21.8±0.6*↓	-	33.8±4.3
Werkstetter et al. (254)	Muscle CSA at baseline (z-score)	p-QCT	-1.7 (-3.3; -0.3)]	-	-	RF
	Muscle CSA for height at baseline (z-score)		-1.5 (-4.9; 0.2)	-	-	RF
	Δ of the M-CSA between baseline and final measurements (z-score)		+0.8**↑ (-0.8; 2.9)	-	-	RF
	Δ of the Muscle CSA for height between baseline and final measurements (z-score)		+1.2**↑ (-0.3; 3.9)	-	-	RF
Werkstetter et al. (194)	Muscle CSA ^{height} at baseline (z-score)	p-QCT	-	-2.5**↓ (-3.5; -1.0)	-	AMRF
Ward et al. (250)	Muscle CSA (z-score)	Tibia p-QCT	-	-1.5±1.1**↓	-	RF
Ashton et al. (249)	combined psoas CSA (cm^2)	MRI	-	15.1 (6.8; 24.3)	-	-
	psoas area (z-score)		-	-2.1 (-0.4; -3.4)	-	RF
Atlan et al. (258)	PAI (mm^2/m^2)	MRI	326 (259; 418)**↓	-	-	528 (439; 615)
			-	326 (267; 418)	326 (201; 437)	-
Fat free mass						
Motil et al. (253)	FFM (kg)	whole body potassium	-	29.9±1.7*↓	-	42.7±4.3

	Upper arm muscle circumference (mm)	skinfold thickness	-	18.5±1.2**↓	-	22.9±3.3
Khoshoo et al. (252)	FFM (kg)	BIA	-	30.5± 7.7	-	-
Wiskin et al. (17)	upper arm muscle area (z-score)	Triceps skinfold and mid-upper arm circumference	-	-1.3**↓ (-1.8; -0.5)	-	RF
			-	-	-0.6 (-1.4; -0.2	RF
Mager et al. (13)	FFM (%)	DEXA	73.4±6.1	74.1±5.9	72.3±6.8	-
Wiech et al. (255)	FFM (kg)	BIA	-	-	38.1±12.9	39.2±13.4
			-	35.0±12.0*↓	-	42.9±13.5
Davis et al. (197)	FFM (z-score)	DEXA	-	-0.8± 0.2*↓	-	0.1± 0.4
Trivic et al. (259)	FFM (%)	DEXA	-	65.8±2.3	71.6±2.6	-

IBD= Inflammatory bowel diseases (CD+UC), CD group= Crohn's disease group, UC group= Ulcerative colitis group, HC group= Healthy control group, LM= Lean mass, MM= muscle mass, FFM= Fat free mass, SMM=Skeletal muscle mass, ASMI= Appendicular skeletal muscle index PAI= Psoas area index, Muscle CSA^{height}= Muscle cross sectional area per height, DEXA= Dual energy x-ray absorptiometry, p-QCT= Peripheral quantitative computerized tomography, BIA= Bioelectrical impedance analysis, MRI= Magnetic resonance imaging, RF=reference group, AMRF=age-matched reference group, Δ= difference,*=P≤0.05 statistically significant, **=P≤0.01, ↑/↓= increment or reduction between study groups.

2.3.4.1. Lean mass (LM)

LM measured by DEXA was reported as an outcome variable in 7 studies (total n=380, CD=303, UC=75) (13, 197, 250, 251, 256, 259). Half of these studies indicated that children with IBD had lower LM than healthy groups (197, 250, 251, 256). These changes were mainly predominant in CD, with only 4 studies including UC patients (13, 250, 251, 257). Ward et al. (251) observed significantly lower mean z-score LM for age and gender (-2.0±0.9, P<0.001) and mean z-score of LM for height (-1.0±0.9, P<0.001) in IBD groups when compared to controls. In accordance, Ward et al. (250) reported significantly lower mean z-scores of LM for age and gender (-2.5±1.1, P<0.0001) and mean z-scores LM for height (-2.3±1.0, P<0.0001) in CD group than a healthy reference population. Similarly, Brookes et al. (256) reported a significant reduction in the mean z score of LM for height (-1.1±1.1, P<0.001), even after adjusting for bone age (-1.0±1.1, P<0.001) in CD group than a healthy reference population.

Davies et al. (197) showed a trend towards a difference between CD and a healthy control group in LM (39.3±1.5 kg vs. 46.0±4.5 kg, p=0.08), appendicular LM (18.7±0.8 kg vs. 22.9±2.6 kg, p=0.06) and appendicular skeletal muscle index (ASMI) (6.8±0.2kg/m^2 vs. 7.6±0.4 kg/m^2, p=0.05), respectively. Trivic et al. (257) showed a significant difference in percentage LM relative to whole body weight in CD group when compared to UC (65±4% vs. 68±3%, p=0.001) but they did not include a comparator control group in their study. However, there was no difference in LM z-scores between groups (-1.6±0.3 vs. -2.0±0.4, p=0.5). In contrast, Mager et al. (13) demonstrated a significant difference between CD and UC patients in the mean z-score of LM for height (CD=−1.0±1.2 vs. UC=0.0±2.2, p=0.01) and the mean z-score of appendicular LM for height (CD=−1.3±1.2 vs. UC=− 0.3 ± 2.5, p=0.03), but not in the LM/height^2 (12.6± 2.2 kg/m^2 vs. 19.3±29.0 kg/m^2, p=0.9) nor in the appendicular LM/height^2 (12.8± 5.5 kg/m^2 vs. 8.3± 13.0 kg/m^2, p=0.2).

2.3.4.2. Muscle mass (MM)

One study (n=6, CD=6, UC=0) estimated whole body MM by urinary creatinine excretion (253). This study used a small sample size of CD, (n=6) and reported a lower mean MM in the CD group when compared with a healthy control group (21.8±0.6kg vs. 33.8±4.3kg, P<0.05, respectively).

Muscle cross sectional area (M-CSA) was assessed by p-QCT (which uses forearm or tibia as regions of interest in computed tomography (100) in 3 studies (194, 250, 254) and by MRI in 2 studies (249) (n=123, CD=116, UC=7). In a prospective longitudinal study (254) in a newly diagnosed IBD cohort, the z-score M-CSA was lower at baseline (median -1.7, range -3.3; -0.3) than a healthy reference group, even after correction for height (median -1.5, range -4.9; 0.2). At the end of follow-up (median interval 2.4 years), the IBD M-CSA z-score M-CSA was significantly improved (median +0.8, range -0.8; 2.9, P<0.01), even after correction for height (median +1.2, range -0.3; 3.9). In this mixed IBD cohort, nutritional therapy was only prescribed to CD patients for <4 weeks and this was complimented with standard licensed pharmacological therapies. The design of the study precluded further evaluation of the effectiveness of the intervention and whether MM may normalize independently of disease activity. Similarly, mean z-score M-CSA measured at the tibia by pQCT in the study by Ward et al. (250) was significantly lower in CD when compared to a healthy reference population [-1.5±1.1, range (-3.9; 1.2), P<0.01]. This was also true even after adjusting for confounders (194).

MRI was used in two recent studies (249, 258). Ashton et al. (249) assessed combined (left and right) psoas CSA (median 15 cm^2, range 7; 24 cm^2) in a CD group with these measures shown to be significantly lower than the comparative healthy norm assessed using computer tomography scans (median z-score -2.1, range -0.4; -3.4). Atlan et al. (258) found the median PAI in IBD group to be significantly lower than the control group (IBD= 326 mm^2/m^2, range 259; 418 vs. Controls= 528 mm^2/m^2, range 439; 615, P<0.001), while it was comparable between CD and UC groups (CD= 326 mm^2/m^2, range 267; 418 vs. UC= 326 mm^2/m^2, range 201; 437, p=0.8).

2.3.4.3. Fat free mass (FFM)

FFM was measured as a direct outcome in 6 studies (n=197, CD=143, UC=79) (13, 197, 252, 253, 255, 257) and indirect outcome in 2 studies (n=61, CD=43, UC=18) (17, 253). CD patients have been shown to have significantly less FFM (kg) when compared to healthy control groups when either whole body potassium (29.9±1.7 kg vs. 42.7±4.3 kg, P<0.05, respectively) (253) and BIA (35.0±12.0 kg vs. 42.9±14.0 kg, p=0.03, respectively) (255) measures were used. In contrast, no difference in FFM was observed between UC and healthy control groups though it is noted that the UC patients were in remission (255).

In two studies (13, 257), no significant difference was observed in the mean %FFM between CD and UC patients (CD= 66±2% vs. UC=72±3%, p=0.1 and CD=74±6% vs. UC=72±7, p=0.2, respectively). A single study (n=55, CD=37, UC=18) estimated FFM using upper-arm muscle area (UMA) z-score as measured by combining triceps skinfold and mid-upper arm circumference. Both IBD types showed negative UMA z-scores (CD=-1.3, range -1.8; -0.5 and UC= -0.59, range -1.36; -0.15) when compared to a healthy reference range (17). Similarly, Motil et al., (253) (n=6, CD=6, UC=0) estimated muscle size using skinfold thickness for upper arm muscle and reported significant decrease in CD when compared to healthy controls (CD=19±1 mm vs. healthy controls=23±3 mm, P<0.01, respectively).

2.3.4.4. Muscle function

When compared to a reference population (**Table 2.3**), a significant attenuation in hand-grip strength was described in a single CD study (194) (n=10, CD=10, UC=0) (median score -1.7kg, range -2.8; 0.8, p=0.02). However, such a difference was not replicated by Davies et al. (197) who observed comparable handgrip strength between CD and healthy control groups in both dominant arm (25.6±1.5 vs. 23.8±1.3 kg/kg forearm LM, respectively) and non-dominant arm (24.3±1.4 vs. 23.9±1.1 kg/kg forearm LM, respectively). Similarly, peak jump power was similar between CD and a healthy reference group (20).

Table 2.3. Muscle function findings from the studies included in this review

Study	Muscle function measures	CD	Controls
Werkstetter et al. (194)	Handgrip strength z-score	-1.7↓* (-2.8; 0.8)	AMRF
Ward et al. (250)	Peak Jump power of muscle CSA z-score	0.8 (0.5; 1.1)	RF
Davis et al. (197)	Handgrip dynamometer (kg/kg forearm LM)	Dom 25.6±1.5	Dom 23.8±1.3
		Non-dom 24.3±1.4	Non-dom 23.9±1.1

CD group= Crohn's disease group, Muscle CSA= Muscle cross sectional area, Dom= dominant arm, Non-dom= non dominant arm, RF=reference group, AMRF=age-matched reference group.

2.3.5. Nutrition and muscle composition

Two prospective studies (194, 252) and one retrospective study (258) have investigated the effect of a nutritional intervention on body composition in patients with CD (n=67,

CD=67, UC=0) presented in **Table 2.4**. Investigators either used exclusive enteral nutrition (EEN) or oral nutritional supplementation (194, 252, 253, 258). Motil et al. provided 1500 ml of Osmolite or Ensure via nasogastric tube (NGT) to their CD patients overnight for 8-10 hours for 7 months, which increased daily dietary protein and energy intakes by 40% and showed comparable height gains in the CD and control groups post supplementation, whereas weight gain was significantly higher in the CD group (1.2±0.3kg vs. 0.4±0.2kg, P<0.01).

Werkstetter et al. (194) administered exclusive Modulen formula (Modulen®; Nestlé, Frankfurt, Germany) via oral consumption or NGT to CD patients based on the energy requirements for ideal body weight for height over 8 weeks with the volume decreasing gradually over the last 2-4 weeks until transition to a normal diet. The PCDAI, median z-score of forearm M-CSA^{height} (baseline z-score vs. week 12= 1.0, range 0.6; 1.8, p=0.002) and handgrip strength (baseline z-score vs. week 12= 0.7, range -1.0; 2.8, p=0.07) improved after supplementation compared to baseline data. Only 10 patients were on immunosuppressants and 9 patients were on 5-ASA but no patients were exposed to corticosteroids or biological therapies during follow-up. Similarly, Khoshoo et al. (252) used Peptamen and Vital HN formulas with target energy intake 170% of resting energy expenditure (REE) for 3 weeks in a crossover design in CD patients and found both FFM (measured using BIA) (after 3 weeks= 34.7±7.7 kg and after 6 weeks= 37.5±6.4 kg vs. pretreatment= 30.5±7.7 kg, p<0.05) and triceps skinfold thickness (after 3 weeks= 10±3 mm and after 6 weeks= 13±3 mm vs. pretreatment= 8±3 mm, p<0.05) significantly improved after the feeding treatment compared with pretreatment. Atlan et al. (258) reported that in CD patients treated with EEN as induction therapy there was no correlation between EEN and PAI.

2.3.6. Physical activity and muscle mass

Trivic et al. (257) (n=40, CD=20, UC=18, IBD-U=2) was the only cross-sectional study that measured physical activity (PA) in IBD patients using fitbit charge 2 (Fitbit Inc., USA) as presented in **Table 2.4** and showed average time spent in moderate to vigorous PA was 45.7±8.2 min/day in this cohort. A significant positive correlation was observed between time spent in moderate to vigorous PA and LM z-score measured by DEXA (p=0.03).

Table 2.4. Effects of nutritional and physical activity interventions on muscle composition and function

Authors	Study design	Nutritional intervention	Physical activity	Muscle measurement		IBD	CD
Khoshoo et al. (252)	3 weeks randomized crossover intervention	Peptamen and vital HN The target energy intake was 170% of REE	-	FFM (kg)	BIA		pretreatment= 30.5± 7.7*↓ vs. after 3 weeks= 34.7±7.7 vs. after 6 weeks= 37.5± 6.4
Werkstetter et al. (194)	52 weeks non-randomised intervention	Exclusive Modulen formula via oral or NGT for 12 weeks	-	Muscle CSA ^{height} z-score	p-QCT		Δ Baseline vs. week 12= (1.0**↑, range 0.6; 1.8) Δ week 12 vs. week 52= (-0.1, range -0.7; 0.8)
				Handgrip strength z-score			Δ Baseline vs. week 12= (+0.7, range -1.0; 2.8) Δ week 12 vs. week 52= (+0.4, range -1.6; 2.7)
Atlan et al. (258)	Retrospective cohort study of 11 years	-	62.3% of CD treated with EEN for induction	PAI	MRI	-	No correlation between EEN and PAI
Trivic et al. (259)	Cross sectional cohort	-	PA measured by fitbit charge 2 with average time spent in moderate to vigorous PA 45.7±8.2 min/day	Correlation between the time spent in moderate to vigorous PA (min/day) and LM z-score	DEXA	Unstandardized coefficient 114.9 vs. standardized coefficient 0.4; p=0.03	-

IBD= Inflammatory bowel diseases (CD+UC), CD group= Crohn's disease group, UC group= Ulcerative colitis group, ♂= Male, ♀= Female, LM= Lean mass, MM= muscle mass, FFM= Fat free mass, SMM=Skeletal muscle mass, Muscle CSA= Muscle cross sectional area, Muscle CSA^{height}= Muscle cross sectional area per height, , PAI= Psoas area index, DEXA= Dual energy x-ray absorptiometry, p-QCT= Peripheral quantitative computerized tomography, BIA= Bioelectrical impedance analysis, MRI= Magnetic resonance imaging, EEN= exclusive enteral nutrition, NGT= Nasogastric tube, EEN= exclusive enteral nutrition, NGT= Nasogastric tube, Δ= the difference,*=P≤0.05 statistically significant, **=P≤0.01 statistically significant.

2.4. DISCUSSION

This systematic review describes sarcopenia by studying the alterations in MM-related compartments and their function and evaluates interventions aimed at normalising MM in paediatric IBD. In addition, it appraises relevant interventions that impact on muscle aiming to reverse those changes to identify the gaps in the literature and improve the design of future interventional studies.

Most (13, 17, 194, 197, 249-251, 253, 255, 256, 258) but not all (252, 254, 257) studies show a considerable reduction in MM-related compartments in IBD paediatric cohorts but of the 3 studies that have assessed muscle function only one showed a reduction in muscle strength when compared to healthy volunteer groups. Our results are similar to previous systematic reviews on body composition in paediatric (100) and adults (98) patients with IBD. However, Thangarajah et al. (100) included adult patients making their findings less representative of a paediatric population. Moreover in that review, the diverse measures used to quantify MM and possible interventions used to normalize it were not covered. When compared to healthy volunteer data sets, a significant decrease in MM-related compartments persists even with normalization of disease activity in CD (260) and UC (261), and across IBD populations (13, 194, 197, 251, 254-256).

Worse disease activity is associated with a lower muscle mass (13, 17, 197, 250, 256) and normalization of disease activity is associated with an improved muscle mass, albeit possibly as a result of nutritional therapy (194, 252). One study (258) assessed the relationship between muscle mass and disease activity and indicated that IBD patients in remission had significant higher median PAI measured by MRI when compared with those in mild, moderate or severe disease state. Moreover, they also found that patients treated with biological therapy tended to have higher PAI than patients treated with immunosuppressant agents. This may be related to the fact that biological agents are associated with higher induction rates (262) and probably a higher rate of normalisation in MM (263). Corticosteroids are commonly used as induction therapy in IBD. Indeed, 23% of patients in the studies included in this review were treated with glucocorticoids to induce clinical remission. Corticosteroids promote muscle degradation and alter fat-related body composition (264). Indeed, decreased LM and increased FM were documented in patients with cachectic diseases treated by long-term corticosteroid

therapy (265), which promotes sarcopenic obesity characterised by low MM and physical weakness and increased bodyweight (266). However, none of the studies included in this review had systematically examined the effect of drug management on disease activity and MM-related compartments in IBD patients, and this should be an important topic for future investigations. Interestingly, FFM in CD (253) was reduced when compared to a healthy reference population (17) or healthy control group (255). These observations were not replicated in UC cohorts that were in remission, once again underlining the strong symbiosis between muscle compartments and disease activity, a relationship that is possibly independent of disease type. In addition, it is essential to consider confounding factors that could affect body composition especially in paediatric populations, such as ethnicity, gender, age, pubertal status, and height as these factors can be responsible for variations in body compartments (267-269), with height being directly linked to bone age and puberty (270, 271).

There is a limited number of published studies investigating the effect of long-term nutritional therapies on body composition and muscle function in IBD paediatric populations, which makes it difficult to draw appropriate conclusions about their efficacy. Indeed, only three prospective studies (194, 252, 253) have measured the impact of nutrition on body composition in CD patients and showed improvement on disease activity, LM and FFM. Exclusive nutritional intake of the specific IBD polymeric formula Modulen® showed a significant improvement in muscle function using handgrip strength along with an improvement in muscle cross-sectional area (194). Similarly, semi-elemental formula used for 3 weeks in a crossover design in CD patients found that FFM significantly improved after the feeding compared with baseline measures when assessed using BIA (252). Modulen® is 100% casein-based and contains essential (e.g. leucine and lysine) and non-essential (e.g. glutamic acid) amino acids which have anti-inflammatory properties leading to an improvement in disease activity and intestinal mucosal integrity (272) and thereby improving LM (273, 274).

A recent study indicates that newly diagnosed IBD patients have high prevalence of malnutrition and micronutrient deficiencies which can impact upon disease progression. Hence, it is crucial routine screening for malnutrition using high sensitivity screening tools such as the Malnutrition Universal Screening Tool (MUST) and

Saskatchewan IBD Nutrition Risk Tool (SaskIBD-NR) is used to identify those who are at risk of malnutrition (275). In addition, there are no specific dietary requirements to be followed during the remission phase; all IBD patients in remission should receive consultation by a specialist dietician as part of the multidisciplinary approach required to improve their nutritional status and prevent malnutrition and nutrition-related disorders (276).

This review identified several limitations and heterogenous measures within the studies covered making it difficult to draw appropriate conclusions. We have included some studies that involved paediatric populations with participants aged up to 18 years old (13, 197, 255), but the age of 18 is typically considered as an adult not a paediatric patient (277). Studies to date had relatively small sample sizes populated by patients with diverse disease characteristics. Some studies did not recruit a healthy volunteer cohort, so all comparisons relied on historic data sets obtained from different healthy populations (17, 249, 250, 254, 256) or unmatched controls (258). By far, the biggest limitation relates to the methodologies used to measure body compartments. In some studies, the measured outcomes did not always align with the terminology used and/or the correct outcomes of the methods applied. For example, the terms LM and FFM (and often MM) are frequently used interchangeably in the literature due to the lack of understanding that they refer to separate body composition compartments. Indeed, LM and MM (typically assessed by imaging techniques including DEXA, MRI, CT or ultrasound) have been reported through measures of whole body potassium and creatinine excretion (253). However, whole body potassium is typically used to estimate FFM and not LM or MM (278). MM itself has been reported as an outcome of BIA measurements (255). However, BIA is used to estimate FFM and indirectly FM but not MM (140, 279). In addition, some studies have used anthropometric measurements (such as BMI, skinfold thickness and mid-upper arm circumference) to estimate FFM or (incorrectly) MM (17, 253) but these measurements lack sufficient accuracy and sensitivity (140, 280). DEXA can assess LM directly and could also estimate FFM if bone mineral density (BMD) is measured concurrently (152), but this has not always been the case (197). These observations unfortunately may limit the impact of these studies and the frequent interchangeable use of LM, FFM and MM make it difficult to comprehend

and compare data from different studies. Furthermore, bias assessment identified that some studies did not adjust for potential confounders such as age, gender, pubertal status, ethnicity and height (249, 250, 252, 254, 256, 258). Collectively, these heterogeneities restrict the direct comparison of key findings between outcomes of different studies, which is further compounded by the fact that no studies attempted to use predefined criteria for the definition of sarcopenia (possibly due to a lack of consensus on relevant cut-off points among IBD paediatrics) precluding the assessment of its prevalence in these populations. Therefore, it is important that future studies are designed to reduce risk of bias by identifying and describing in detail subject characteristics, methodologies and confounding factors.

In conclusion, standardization of body composition and sarcopenia terminology along with valid use of assessment tools in adequately powered populations with appropriately matched comparators are key essentials in future studies. Despite current limitations in the published literature, it appears that LM, FFM and MM were significantly reduced in children with IBD. Readouts for body compartments and muscle function should be available with follow up longitudinal data urgently needed to assess the effect of muscle growth, IBD therapies, surgical intervention, nutritional therapies and disease phenotype on these outcomes. Adequate dietary protein supplementation over an adequate period combined with concomitant physical activity may improve MM and its function. However, studies using interventions designed to have minimal attrition are needed before such therapies can be used as standard care across worldwide health care systems.

3. ASSOCIATION BETWEEN BODY MASS INDEX AND AGE OF DISEASE ONSET WITH CLINICAL OUTCOMES IN PAEDIATRIC-ONSET CROHN'S DISEASE (CD): A UK NATION-WIDE ANALYSES USING THE NIHR-IBD BIORESOURCE

Please note that this chapter has been previously published under the same title (239).

3.1. INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, relapsing and remitting inflammatory condition affecting the gastrointestinal tract and has significant health implications (281). IBD is predominantly sub-divided into Crohn's disease (CD) and ulcerative colitis (UC), which are characterised by diverse epidemiological, pathogenic and clinical characteristics (282). The incidence rates of IBD have increased rapidly in developed countries, predominantly in northern Europe, the United Kingdom (UK), and North America (283). Specifically in the paediatric population, the incidence rate of inflammatory bowel disease is 10.54/100,000/year and is higher in males (10.84/100,000/year) compared with females (6.69/100,000/year) (249). Although the peak incidence of CD occurs in young adulthood, 25% of disease onset occurs during childhood (7). Adiposity rates have also increased significantly in the developed countries, especially in the UK (284, 285). Nearly 26% of children in England aged 2–15 years are overweight or obese (286). Globally, around 39 million children under the age of 5 have been reported as overweight or obese in 2020 (287). The incidence and prevalence of IBD is also raising worldwide in parallel with the obesity epidemic (288).

Historically, an underweight or malnourished nutritional status was frequently linked to paediatric IBD. However, recent observations suggest a paradigm shift with more overweight rather than underweight children presenting with IBD (64). Indeed, an overweight or obese status is observed in nearly one in five children with CD (66). The association between obesity and chronic inflammation has been extensively studied in non-IBD populations (289, 290). Obesity is associated with a low grade inflammatory state (291, 292), which may be triggered by adipocyte hypertrophy and consequent secretion of pro-inflammatory markers, including interleukin (IL)-6, IL-8, IL-1β, C-reactive protein (CRP) (293), tumor necrosis factor-α (TNF-α) (294) and monocyte chemoattractant factor (295). Obesity related metabolic disorders are linked to higher volumes of visceral adipose tissue (VAT) (296). This increase in VAT is commonly observed in both adult and pediatric patients with CD and has been studied as a potential factor contributing to the development and progression of the disease (72).

Although obesity has been linked to more severe clinical outcomes in adult patients with CD (73, 74, 297), some studies showed no or weak relationship between obesity and adverse clinical outcomes (76, 77). The existing literature on the association between obesity and adverse clinical outcomes in paediatric-onset populations is also limited and conflicting (66, 80-84). Most studies included small cohorts (81-83, 298), used subjective outcomes to assess disease activity (84), have been undertaken in an inpatient setting where any association between obesity and clinical outcomes may be falsely amplified (80), or used alternative definitions of obesity (299).

Depending on age of diagnosis, CD patients may have different clinical disease outcomes (300, 301). Natural history studies in paediatric patients suggest that CD diagnosed during childhood presents with a different disease phenotype compared to cases diagnosed in adulthood (302). The development of CD adverse outcomes has shown to be similar in older patients compared with younger patients (303); however, other studies showed that older CD patients were less likely to be hospitalised (304) and less complicated disease (300). The available research on the relationship between age of disease onset and adverse clinical outcomes in paediatric-onset populations remains limited and conflicting (300, 302, 305, 306). Most of the studies included different age groups, including those with adult-onset disease >18 years old (300, 302), shorter follow-up duration (300, 305) and limited information exists on the impact of drug agents in paediatric disease onset (302, 305). As a result, further research is needed to investigate the association between age of disease onset and clinical outcomes in paediatric-onset CD patients.

In the present study, the NIHR IBD BioResource was explored to undertake a UK-wide analyses to understand the relationship between a high BMI and CD outcomes as the primary study outcome in a cohort with paediatric onset of CD. We used standard BMI-based definitions of obesity in a large cohort of patients with CD and examined objective disease outcomes to investigate this relationship. Additionally, the association between clinical outcomes and the age of disease onset was explored as a secondary outcome.

3.2. METHODS

3.2.1. Characterisation of the cohort

Data were obtained from the National Institute for Health Research (NIHR) IBD BioResource- launched on 4th April 2016 as part of the UK BioResource that encompasses a large cohort of 'recallable' CD patients on whom clinical details were ascertained at enrolment (307). We conducted a retrospective study to assess the relationship between BMI and clinical outcomes in CD. BMI was used to divide the patient population into cohorts of normal weight (<25kg/m^2) and overweight and obesity (≥25 kg/m^2). The population was also stratified into three age groups to consider differences in sexual maturation and included: (A1 group) pre-puberty/early puberty (<11 years), (A2 group) puberty (11-14 years) and (A3 group) post-puberty (15-17 years).

All the participants signed an informed consent. Patients were excluded if they were ≥18 years at age of diagnosis or had missing data for key exposure and outcome variables. Those with UC or IBD-unclassified (IBDU) were also excluded. Patients aged ≤17 years at diagnosis of CD and who were between the age of 16 to 30 years at consent were included in the analyses. CD phenotype data, including disease duration and behaviour, hospitalisation, frequency of drug therapies, frequency of surgeries and extra-intestinal manifestations (EIMs), were ascertained at NIHR IBD BioResource enrolment.

3.2.2. Characterisation of the outcome variables

The key outcomes of interest were (a) hospitalisation, (b) surgery and evidence of disease progression, specifically disease behaviour, (c) usage of therapeutic drugs, and (d) presence of EIMs. Hospitalisation was defined as Yes or No with unknown assumed to be negative for a hospitalisation history. Disease behaviour was stratified into B1-inflammatory, B2-stenosing and B3-internal penetrating according to disease behaviour in Montreal classification (308). The surgical resections included colectomy and ileostomy, colectomy and ileo-anal pouch, defunctioning ileostomy, colostomy, drainage of intra-abdominal abscess, ileal or jejunal resection, ileal or jejunal stricturoplasty, ileocaecal resection, right hemicolectomy, partial colectomy, proctectomy, stricturoplasty, insertion of seton suture, drainage of perianal abscess, perianal fistula repair, closure of stoma and other. Drug therapies were classified into 4 groups:

immunosuppressants, biological drugs, corticosteroids, and 5-aminosalicylates. Immunosuppressants included thiopurines (azathioprine or mercaptopurine), methotrexate or ciclosporin. Biological drugs included infliximab, adalimumab, vedolizumab and ustekinumab. EIMs included primary sclerosing cholangitis, enteropathic arthritis, erythema nodosum, iritis/uveitis, orofacial granulomatosis, psoriasis and ankylosing spondylitis. Details relevant to EIMs and surgeries are presented in the **Appendix 2– Supplementary Table 2.1**.

3.2.3. Statistical analyses

Continuous variables were presented as medians (interquartile range) and categorical variables as frequencies and percentages. Chi-square test was used to compare categorical variables. Spearman rank correlation was used to test the associations between continuous variables. Significance was defined as $P \leq 0.05$ (two-tailed). Analyses were carried out using Microsoft Excel Worksheet for Windows (Version 15.0, Microsoft Corp., Redmond, WA, SA) and IBM SPSS for Windows (Version 28.0, IBM Corp., Armonk, NY, USA).

3.3. RESULTS

3.3.1. Cohort demographics

At data lock, on 11th March 2022, 17,020 patients were enrolled (**Figure 3.1**). After applying the inclusion and exclusion criteria described in Methods, 16,172 participants were removed. Participants with missing data on age at diagnosis (n=211) and at consent (n=21) were removed. Moreover, those who aged ≥18 years at diagnosis (n=13555) and those who aged >30 years at consent (n=1305) were excluded. The included participants with an age <18 years at IBD diagnosis and with a reported BMI were n=953. Additionally, 18 participants were removed due to having a diagnosis of IBD unclassified, 1 participant with UC and 78 participants because they had an unidentified disease behaviour. Eight participants were co-prescribed 2 or more biological agents concurrently and hence were removed from the cohort as it was felt these may be data entry errors and not representative of typical paediatric CD patients in the UK.

A total of 848 participants were included in the final analyses and their characteristics are displayed in **Table 3.1**. The cohort had a similar gender split (51.8% males and 48.2% females) and were of a predominant white or British white (88.8%) demographic, with a median age at diagnosis of 14 years (range 11; 16 years) and a median age at consent and inclusion in the NIHR BioResource of 24 years (range 20; 27 years). The median BMI at age of consent was 22.6 kg/m^2 (range 20.4; 25.6 kg/m^2). The majority of the participants (N= 605) had a BMI<25 kg/m^2 with 243 participants (29%) having a BMI≥25 kg/m^2 at inclusion in the NIHR IBD BioResource.

3.3.2. Clinical characteristics of the cohort

The majority (70.2%) of patients had an inflammatory disease behaviour, with the rest having stenosing (19.9%) or penetrating (9.9%) disease behaviour. The hospitalisation rate is approximately 0.062 per person-year over the 10-year disease duration. Almost half of the cohort had been exposed to one immunosuppressant (47.3%) or biological agent (59.0%), whereas the majority had not been exposed to 5-aminosalicylates (89.3%) or corticosteroids (93.4%). Most of the participants were naïve to surgery (61.1%) and never experienced EIMs (79.0%) (**Table 3.1**).

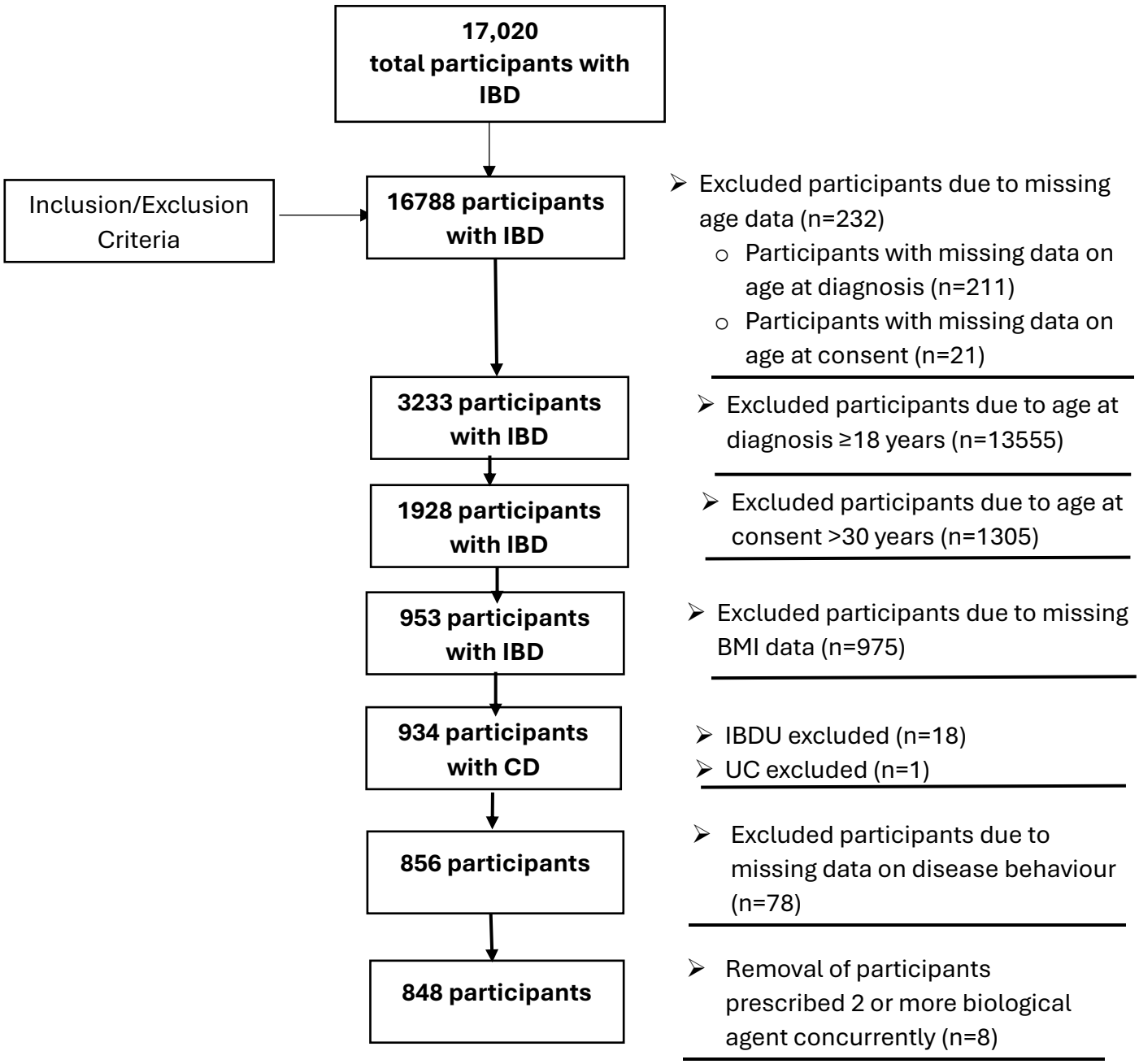

Figure 3.1. Consort diagram describing the selection of the final population sample included in the analyses. CD Crohn's disease, BMI body mass index, IBDU Inflammatory bowel disease unclassified.

Table 3.1. Descriptive characteristics of the whole study population and stratified by sex.			
	All Participants	**Males**	**Females**
N (%)	848	439 (51.8%)	409 (48.2%)
Age at consent (years)	24 (20; 27)	24 (20; 26)	24 (20; 27)
Age at diagnosis (years)	14 (11; 16)	14 (12; 16)	14 (11; 16)
BMI (kg/m^2)	22.6 (20.4; 25.6)	22.3 (20.2; 25.2)	22.8 (20.5; 26.1)
BMI groups			
<25 kg/m^2	605 (71.3%)	325 (74%)	280 (68.5%)
≥25 kg/m^2	243 (28.7%)	114 (26%)	129 (31.5%)
Ethnicity			
Not stated	34 (4%)	19 (4.3%)	15 (3.7%)
White or British White	753 (88.8%)	380 (86.6%)	373 (91.2%)
Asian or Asian British	31 (3.7%)	19 (4.3%)	12 (2.9%)
Black or Black British	<10 (0.9%)	<10 (1.6%)	<5 (0.2%)
Mixed	17 (2%)	10 (2.3%)	7 (1.7%)
Other ethnic	<10 (0.6%)	<5 (0.9%)	<5 (0.2%)
Smoke			
None smoker	656 (77.4%)	335 (76.3%)	321 (78.5%)
Past smoker	104 (12.3%)	54 (12.3%)	50 (12.2%)
Current smoker	88 (10.4%)	50 (11.4%)	38 (9.3%)
Alcohol intake			
No	274 (32.3%)	141 (32.1%)	133 (32.5%)
Yes	574 (67.7%)	298 (67.9%)	276 (67.5%)
Diet preference			
None	715 (84.3%)	387 (88.2%)	328 (80.2%)
Vegetarian	28 (3.3%)	7 (1.6%)	21 (5.1%)
Pescatarian	18 (2.1%)	4 (0.9%)	14 (3.4%)
Vegan	9 (1.1%)	3 (0.7%)	6 (1.5%)
Others	78 (9.2%)	38 (8.7%)	40 (9.8%)
Disease duration (years)	10 (6; 14)	10 (6; 13)	10 (7; 14)
Disease behaviour			

B1-inflammatory	595 (70.2%)	318 (72.4%)	277 (67.7%)
B2-stenosing	169 (19.9%)	84 (19.1%)	85 (20.8%)
B3-internal penetrating	84 (9.9%)	37 (8.4%)	47 (11.5%)
Hospatilisation			
No	321 (37.9%)	159 (36.2%)	162 (39.6%)
Yes	527 (62.1%)	280 (63.8%)	247 (60.4%)
Hospatilisation rate (person-year)	0.062	0.064	0.061
Total current Immunosuppressant agent usage			
0	444 (52.4%)	220 (50.1%)	224 (54.8%)
1	401 (47.3%)	217 (49.4%)	184 (45%)
2	3 (0.4%)	2 (0.5%)	1 (0.2)
Total current biological agent usage			
0	348 (41%)	168 (38.3%)	180 (44%)
1	500 (59%)	271 (61.7%)	229 (56%)
Current corticosteroid usage			
No	792 (93.4%)	407 (92.7%)	385 (94.1%)
Yes	56 (6.6%)	32 (7.3%)	24 (5.9%)
Total current mesalazine usage			
No	757 (89.3%)	391 (89.1%)	366 (89.5%)
Yes	91 (10.7%)	48 (10.9%)	43 (10.5%)
Total surgeries			
No history of surgery	518 (61.1%)	262 (59.7%)	256 (62.6%)
History of 1 resection	209 (24.6%)	107 (24.4%)	102 (24.9%)
History of 2 or more resections	121 (14.3%)	70 (15.9%)	51 (12.5%)
Total EIMs			
No EIMs	670 (79%)	366 (83.4%)	304 (74.3%)
1 EIM	125 (14.7%)	57 (13%)	68 (16.6%)
≥2 EIMs	53 (6.3%)	16 (3.6%)	37 (9%)

N, number of cases, BMI, body mass index, EIMs, extra-intestinal manifestations. Continuous variables were described as median (inter-quartile range). Categorical variables were described by reporting absolute frequency.

3.3.3. Outcome analyses

Chi-square test showed no significant difference in the key outcomes was observed between patients with a low or high BMI except in the prevalence of EIMs. There was no difference in hospitalisation, surgery, disease behaviour and medication usage between patients with a low or high BMI (**Table 3.2**). More participants with high BMI experienced 1 EIM than those with a low BMI (BMI≥25 = 18.5% vs. BMI<25 = 13.2%, p=0.05). Presence of ≥2 EIMs was also more common in patients with a higher BMI (BMI≥25 = 7.8% vs. BMI<25 = 5.6%, p=0.05). There was also a trend for higher use of corticosteroids in the lower BMI group (BMI<25 = 7.6% vs. BMI≥25 = 4.1%; p=0.06).

Chi-square test also indicated that earlier age at diagnosis was associated with significantly (p=0.01) higher rate of stenosing (25.6%) and penetrating (15.4%) disease behaviour in the A1 (<11 years) group compared with both the A2 (11-14 years) group (stenosing: 20.4% and penetrating: 8.0%) and A3 (15-17 years) group (stenosing: 17.0% and penetrating: 9.2%). Participants with an early age at diagnosis (<11 years) experienced significantly more hospitalisations (76.3%) than those diagnosed at a later age (15-17 years = 57.3%; p<0.001). No significant difference was found in medication usage, prevalence of EIMs and surgeries across age groups. Interestingly, the use of corticosteroids tended to be higher (p=0.06) in those diagnosed at a later age (15-17 years = 8.7%) when compared with those diagnosed at an earlier age (<11 years = 3.1% and 11-14 years = 3.2%) (**Table 3.2**). A weak negative spearman correlation was observed between BMI and corticosteroid use (ρ=-0.09, p=0.01, 95% CI [-0.16, -0.02]), whereas a weak positive spearman correlation was observed between age at diagnosis and corticosterids use (ρ=0.08, p=0.03, 95% CI [0.01, 0.14]) (**Table 3.3**).

Table 3.2. Clinical outcomes in patients with Crohn's disease stratified by BMI and age at diagnosis.							
	BMI <25	**BMI ≥25**	**p value**	**Age at diagnosis <11 years A1 group**	**Age at diagnosis 11-14 years A2 group**	**Age at diagnosis 15-17 years A3 group**	**p value**
Age at diagnosis	(N=605)	(N=243)	0.7	-	-	-	-
<11 years	19.1%	16.9%					
11-14 years	38.8%	40.7%					
15-17 years	42.1%	42.4%					
BMI	-	-	-	(N=156)	(N=334)	(N=358)	
<25				73.7%	70.4%	71.2%	0.7
≥25				26.3%	29.6%	28.8%	
Disease behaviour	(N=605)	(N=243)		(N=156)	(N=334)	(N=358)	
B1-inflammatory	71.3%	67.5%	0.3	59.0%	71.6%	73.8%	0.01
B2-stenosing	19.8%	20.2%		25.6%	20.4%	17.0%	
B3-internal penetrating	8.9%	12.3%		15.4%	8.0%	9.2%	
Hospitalisation	(N=605)	(N=243)		(N=156)	(N=334)	(N=358)	
No	38.2%	37%	0.8	23.7%	39.2%	42.7%	<0.001
Yes	61.8%	63%		76.3%	60.8%	57.3%	
Immunosuppressants	(N=605)	(N=243)		(N=156)	(N=334)	(N=362)	
0	51.9%	53.5%	0.9	51.92%	47.9%	56.6%	0.2
1	47.8%	46.1%		47.44%	51.8%	43.1%	
≥2	0.3%	0.4%		0.64	0.3%	0.3%	
Biological drug	(N=605)	(N=243)		(N=156)	(N=334)	(N=358)	
0	39.7%	44.4%	0.2	39.7%	41.3%	41.3%	0.9
1	60.3%	55.6%		60.3%	58.7%	58.7%	
Corticosteroids	(N=605)	(N=246)		(N=159)	(N=156)	(N=358)	
0	92.4%	95.9%	0.06	96.9%	96.8%	91.3%	0.06
1	7.6%	4.1%		3.1%	3.2%	8.7%	

Mesalazine	(N=605)	(N=243)		(N=156)	(N=334)	(N=358)	
0	89.6%	88.5%	0.6	86.5%	90.4%	89.4%	0.4
1	10.4%	11.5%		13.5%	9.6%	10.6%	
Total surgeries	(N=596)	(N=243)		(N=156)	(N=334)	(N=358)	
0	61.6%	58.4%	0.2	52.6%	62.6%	63.4%	0.2
1	23.3%	28.8%		28.8%	23.4%	24%	
≥2	15.1%	12.8%		18.6%	14%	12.6%	
Total EIMs	(N=605)	(N=243)		(N=156)	(N=334)	(N=358)	
0	81.2%	73.7%	0.05	73.7%	77.5%	82.7%	0.1
1	13.2%	18.5%		18.6%	16.5%	11.5%	
≥2	5.6%	7.8%		7.7%	6.0%	5.8%	

N, number of cases in each group, BMI, body mass index, EIMs, extra-intestinal manifestations. Categorical variables were described by reporting percentage frequency. Chi-Square Test was used to evaluate between-group differences for categorical variables.

Table 3.3. Associations between BMI, age of diagnosis and clinical outcomes in the whole population of patients with Crohn's Disease						
	BMI (kg/m²)			**Age at diagnosis (years)**		
	ρ	**p value**	**95% CI [lower, upper]**	**ρ**	**p value**	**95% CI [lower, upper]**
Age at diagnosis (years)	0.003	0.92	-0.07, 0.07	-	-	-
BMI (kg/m²)	-	-	-	0.003	0.92	
Immunosuppressants	-0.05	0.12	-0.12, 0.02	-0.05	0.13	-0.12, 0.02
Biological drugs	-0.05	0.16	-0.12, 0.02	-0.003	0.93	-0.07, 0.07
Corticosteroids	-0.09	0.01	-0.16, -0.02	0.08	0.03	0.01, 0.14
Mesalazine	0.004	0.91	-0.07, 0.07	-0.018	0.61	-0.09, 0.05
Total surgeries	0.01	0.85	-0.06, 0.08	-0.06	0.06	-0.14, 0.00
Total EIMs	0.06	0.10	-0.01, 0.13	-0.06	0.09	-0.13, 0.01

Number of participants = 848; BMI, body mass index. EIMs, extra-intestinal manifestations. **ρ**= Spearman rank correlation was used to test the associations between variables. Statistically significant difference (p≤0.05). 95% CI= 95% confedince interval [lower, upper].

3.4. DISCUSSION

This study investigated whether a higher BMI was related to worse outcomes in paediatric-onset CD based on measures of hospitalisation, surgery, disease behaviour, biologic use and the frequency of EIMs. It also explored the association between age of disease onset and these clinical outcomes. The findings revealed that patients with higher BMI experienced more EIMs and a negative correlation between BMI and corticosteroid usage was observed. This may reflect the negative effect of disease activity and hence corticosteroid use on body weight, with anorexia and sarcopenia (reduced muscle mass) being typical signs of chronic disease activity. It was also shown that CD patients diagnosed in the pre-pubertal stage (<11 years) experienced a high prevalence of stenosing and penetrating disease and hospitalisations. A younger age at diagnosis was also positively correlated with corticosteroid use.

The effects of excess adiposity on disease severity and development of disease complications in paediatric CD is still unclear. Previous studies reported no correlation between the obesogenic state and disease severity (66, 80, 82, 83), surgery (66, 80-82) or hospitalisation rates (80, 82, 309) among paediatric IBD patients. However, a positive association between a high BMI and adverse IBD outcomes has been observed in some studies showing an increase in surgical risk (309) and rate of disease exacerbation (81, 83, 84), therapy failure (83) and hospitalisation (81, 83) in paediatric IBD populations with a high BMI. However, it must be noted that most of those studies (81, 83, 84) defined high BMI by ≥85th percentile except for Von Graffenried et al. (309), who defined high BMI by ≥90th percentile.

In the adult IBD literature, some studies have shown the incidence of hospitalisation (73), and CD-related surgery (297) is significantly higher in patients with a high BMI. However, the inverse has also been reported with a decrease in surgical and hospitalisation and biologic usage observed in patients with a high BMI (77). Results from the Swiss Inflammatory Bowel Disease cohort using a multivariate regression model based on datasets from 3075 patients, showed that obesity was negatively associated with disease remission in CD (odds ratio 0.61, 95% confidence interval 0.40-0.92, p=0.02), but not UC (310). Another study by the same group in a paediatric population showed no relationship between obesity and disease progression as defined by a clinically complicated disease

phenotype (309). In contrast, prior IBD-related surgery was associated with overweight and obesity in a cohort of 1598 children with CD (14). However, the present study found no association between obesity and a complicated disease behaviour. It is important to note that previous studies defined obesity based on the World Health Organization (WHO) child growth chart standards (299), or defined their at-risk population as one with a BMI ≥85th percentile (14) or BMI >30kg/m^2 (36), while we employed a more conservative approach of defining our at-risk population as one with a BMI of >25 kg/m^2.

Concomitant medication usage is a surrogate of disease severity that may be utilised along with hospitalisation and surgery. We observed no association between 5-aminosalicylic acid, immunosuppressants, biologic use and BMI. Similarly, no association between BMI and 5-aminosalicylic acid (84), immunosuppressants (66, 83, 84) and biologic (66, 82, 84, 298, 309, 311, 312) use has been observed in the literature with only a single, albeit small study showing an association between a high BMI (>75th percentile) and both disease exacerbation and the need of biologic therapy (83). The results from the present study indicated no positive association between high BMI and corticosteroids use which is in accordance with the literature (66, 82-84). Interestingly, we observed a weak negative correlation between BMI and corticosteroid usage probably indicating that a cohort with more active disease used corticosteroids more often, with chronic active disease being inversely related to body weight due to a change in eating behaviour (59), sarcopenia and protein-losing enteropathy. However, weight gain and fluid retention are the most common reported adverse outcomes of long-term use of steroids (313, 314), though this treatment strategy is rarely used (315) and goes against published guidelines (316). Interestingly, a positive correlation was observed between corticosteroid use and age at diagnosis. This probably reflects the practice of using enteral nutrition to induce remission in younger cohorts and corticosteroids in older cohorts (317).

In the present study, young Crohn's disease patients with a high BMI also experienced significantly more EIMs with this observation holding true even in patients experiencing two or more of these manifestations. This may corroborate our hypothesis that the obesogenic state is pro-inflammatory as the incidence of some extra-intestinal

manifestations such as arthritis and aphthous ulcerations are intimately linked with intestinal inflammatory activity (309, 318).

CD patients diagnosed in a pre-pubertal age (<11yrs), experienced a higher incidence of stricturing disease and hospitalisation when compared to an older age at diagnosis. These findings reflect previous observations showing that in paediatric CD, a stenosing or penetrating disease behaviour doubled during the follow-up period from 29% at diagnosis to 59%, reaching a relative plateau after 9–10 years of follow-up (306). Our results are consistent with Polito et al. (319) in which an earlier age of diagnosis correlated with severe disease behaviour, although they compared an age group that was <20 years old with an older group that was >40 years old.

The present study has some limitations. The selection of data was influenced by selection bias and omitted variable bias, as we excluded participants without recorded BMI data, which may impact both the internal and external validity of the findings as the sample may not fully represent the broader population of CD patients. Although the CD patients were included in this study based on their age at diagnosis (≤17 years), the relevant data sets of BMI and clinical outcomes were recorded at the age of consent to their inclusion in the BioResource database. As the outcome data was obtained at the age of consent, it is subject to recall bias, which may subsequently affect the reliability of the study's outcomes. In addition, the outcome data is susceptible to reverse causation as hospitalisations that occurred first are likely to have affected BMI measurements taken later due to their medical conditions, potentially confounding the association between BMI and health outcomes. However, as our study primarily employed a cross-sectional design, with BMI measured at a single time point (enrollment), we acknowledge that this design limits our ability to fully assess causality. Due to the cross-sectional nature of this database, BMI at diagnosis and its change over time until enrolment in the database was not available. Nevertheless, the approach in the present study allowed us to interrogate the association between BMI and objective disease outcomes in patients with paediatric-onset disease over a considerable follow-up period until early adulthood. The retrospective follow-up period enabled the calculation of hospitalisation rates using a person-years approach, with BMI measured at the end of this period upon enrolment. While this approach accounts for variations in

follow-up time, it cannot fully eliminate potential biases arising from differences in disease monitoring or treatment approaches over time. Additionally, the lack of prospective control limits the ability to fully adjust for confounding factors such as disease severity at different time points. Nevertheless, it provides valuable insights into long-term disease burden.

In this study, all analyses were conducted unadjusted due to the lack of significant correlations, as adjusting for multiple factors would have further reduced the statistical power and was unlikely to yield additional meaningful insights given the observed weak associations. In addition, the Tanner staging was not addressed, and therefore the pubertal and pre- or post-pubertal stages were based solely on the age of the child. Finally, the definition of a high BMI in this study was >25kg/m^2. A more at-risk population would have been one with a higher BMI of >30kg/m^2, but a lack of patients within that range in the database precluded this sub-analyses.

In conclusion, our findings indicate associations between both a high BMI and an early age of disease onset with worsened disease outcomes. Specifically, a high BMI is linked to a higher presence of EIMs, while an early age at diagnosis correlates with a higher prevalence of stricturing disease behaviour and increased hospitalisations. However, due to the cross-sectional nature of our study, these results do not imply causality our findings, but are hypothesis-generating showing association and warrant further investigation, as the timing of BMI measurement and adverse outcomes suggests that the adverse outcomes may have influenced BMI rather than BMI being the cause of those outcomes. Future prospective cohort studies in paediatric patients with CD are required to investigate the causal link between adiposity and CD clinical outcomes to improve the prediction of disease severity, response to therapy and corticosteroids dependency, hospitalisation rate, risk of EIMs and need for surgery.

4. INVESTIGATING PROTEIN NUTRITION IN PAEDIATRIC CROHN'S DISEASE

4.1. INTRODUCTION

Up to 60% of adult Crohn's disease (CD) patients have clinical evidence of low muscle mass even when in clinical remission (320) indicating the poorly reversible nature of this chronic phenomenon. Muscle mass is maintained through the daily balance of muscle protein synthesis (MPS) and muscle protein breakdown (MPB), with the essential amino acid (EAA) components of a meal and muscle contraction being the primary stimulators of MPS.

The majority (20-85%) of Inflammatory Bowel Disease (IBD) patients suffer from nutritional deficiencies with protein-calorie malnutrition common when in an active phase of the disease (198) and micronutrient deficiencies a recognisable problem in remission (199). In CD, up to 75% of hospitalised patients are malnourished and as many as 50% are in negative nitrogen balance (231). Adult patients with active CD ingest considerably less daily protein intake than age- BMI- matched healthy controls (CD, 70.3± 6.1g; HC, 92.6±7.8g, p= 0.03) (59). Similar observations may be true for children with active CD, where protein intake is lower with 79± 5g/day reported in CD and 90±10g/day reported in HC (197), though these findings were not statistically significant.

The essential amino acid (EAA) components of a protein-meal are crucial for the stimulation of MPS and it has been shown of all the EAA, leucine plays a key role in driving MPS. Low serum levels EAA/leucine have been reported in adult CD (321) but their role in the aetiology of sarcopenia in paediatric CD is unknown. Further, how CD affects the protein digestion or absorption and how this contributes to low EAA/leucine remains unclear. Moreover, a reduction in circulating EAAs (leucine, lysine and valine), semi-EAAs (arginine and tryptophan) and non-EAA (serine) were reported in CD subjects compared to controls (322). Low serum levels of tryptophan and histidine have been shown in patients with CD compared with healthy individuals (321). A previous study showed that valine, methionine, leucine, histidine, glutamine and tryptophan were significantly correlated negatively with CD disease activity index in adults which reflects degree of inflammation (323). These observations suggest that alterations in plasma AAs is associated with CD; however, this has never been investigated in paediatric CD compared with healthy peers.

Patients with CD are at greater risk of nutritional deficiency, malnutrition, low body mass index (BMI), and low bone mineral density, myopenia and more recently obesity and sarcopenia (324-326). It is important to understand the impact of eating behaviour disorders on the nutritional status in patients with CD. Only one previous study has reported altered eating behaviour traits such as lower positive mood, lower craving control with greater craving for sweet and savoury in control of eating questionnaire, higher binge eating scales and higher emotional and external eating in Dutch eating behaviour questionnaire in adults with active CD compared with healthy subjects (59), but this has never been examined before in paediatric CD.

This study aims to assess protein nutrition in paediatric CD compared to age-, sex-, and BMI-matched healthy controls (HC). BMI matching was used to control for the confounding effects of body size and composition when comparing dietary intake, eating behaviour and muscle volume between groups.

The primary focus is to evaluate protein nutrition in paediatric CD by accurately measuring protein intake, as well as fasting plasma EAAs and non-EAAs. It also aims to associate these findings to leg muscle volume measured through MRI and to understand more about eating behaviour between CD and matched age- sex- and BMI healthy volunteer groups.

4.2. MATERIAL AND METHODS

4.2.1. Study setting and population

We aimed to recruit male and female, stable CD outpatients aged between 12-17 years old and BMI <30 kg/m^2 with confirmed diagnosis by endoscopy and histology at least 6 months prior to enrolment, no change in medication in the last 3 months (including corticosteroids) and no CD-related surgical intervention in the last 6 months. Exclusion criteria were as follows: a UC diagnoses, indeterminate colitis, microscopic colitis or short-bowel syndrome, any patients having serious underlying disease other than CD that, in the opinion of the investigator, may interfere with the subject's ability to participate fully in the study, any patients having contraindications for MRI scanning e.g. pacemaker, any patients having dairy intolerance/milk protein allergy or those who were non-English speakers. Paediatric CD patients were recruited from Nottingham University Hospitals Trust and Royal Derby Hospital. Patients were screened based on medical inclusion criteria and then referred to the study by their consultant physician. Age-, gender-, and BMI-matched healthy controls (HC) were recruited through various methods. Advertisements were placed across Nottingham University Hospitals and Nottingham University campuses, and direct emails were sent to members of the School of Medicine, School of Life Sciences, and School of Biosciences, as well as to official emails of local secondary schools. Social media advertisements targeting local parents were also posted, and relatives and friends of the recruited CD patients were invited to participate.

Study briefings and informed consent (parental consent and participant assent for those aged 12–15 years, and participant consent for those aged 16 years and older) were obtained by a member of the research team via a phone call with the guardian. A follow-up call was then made to allow participants to raise any inquiries before agreeing to participate and to arrange the study visit. It was made clear that the feeding visit is optional and they have the right to withdraw at any time. None of the subjects had taken part in another study within or prior 3 months. Prior to participation, participants filled out a general health questionnaire and a brief medical screening to meet ensure study eligibility. This study was gained a favourable ethical opinion by the Health Research Authority (HRA) and Health and Care Research Wales (HCRW) (21/SC/0285) on the 22nd

December 2021 with the study conforming to the recognised standards of the Declaration of Helsinki. This study was reviewed by the NIHR BRC Patient and Public Involvement panel before submission to the Research Ethics Committee and HRA. The panel provided valuable feedback on the study's methodology, as well as on the patient information and consent documentation. Medical cover was available for the duration of all study visits and full experimental records were kept.

4.2.2. Measurements

The primary outcome was the average of detailed 3-day protein intake. Secondary outcomes were the average of detailed 3-day calorie intake and dietary macronutrients composition, fasting plasma AAs, eating behaviour questionnaires, muscle mass and the association between protein intake and outcomes such as amino acid levels in circulation and muscle mass.

Muscle mass was assessed by leg muscle volume (L) using 3T MRI, muscle function was assessed by peak forearm muscle strength (kg) and handgrip strength index, defined as peak forearm muscle strength (kg) per weight (kg).

4.2.3. Study protocol.

Participants arrived at the laboratory in early morning, following an overnight fast for at least 6 hours and having abstained from strenuous exercise for the previous 48 hours. A written and verbal information were given to the participants and their parents to ensure their understanding of the procedures. Upon arrival a signed consent form was taken from the participants and their guardian for participants <16 years and from participants only when ≥16 years of age.

After consent, a fasting blood samples were taken. Then, participants completed two questionnaires: child Eating Behaviour Questionnaire, (CEBQ) and the child Tree-Factor Eating Questionnaire-17 (CTFEQr-17). 24hr dietary intake for the previous day was filled through online Intake24 questionnaire. Height and weight were measured and BMI was calculated. Following that, forearm strength of the dominant hand was measured via maximal voluntary contraction using a handgrip dynamometer (MIE Medical Research Ltd., Leeds, UK). Participants performed three maximal voluntary static contractions, with a one-minute rest between each measurement, using their dominant arm. The peak

contraction was taken as maximal handgrip isometric strength (kg). Each test was assessed and recorded by the same trained operator. Participants were acquainted with the protocol, and trained operators (AN and BAA) provided instructions and took measurements for all participants (327).

4.2.4. Main techniques

4.2.4.1 Blood sampling

During the screening visit, a superficial vein was used in all participants for blood sampling. For some of the CD patients, blood was obtained from the same cannula used for the infusion of anti-tumor necrosis factor alpha (TNF-α) medication later on that day. Blood samples were taken by the research nurse or trained health care assistant from all participants who had not been previously screened for another study in the last 3 months.

4.2.4.2 Blood analyses

The blood samples were analysed for full blood count, urea and electrolytes, liver function tests, and C-reactive protein (CRP). These tests were carried out to exclude the potential presence of anaemia, acute phase, abnormal kidney or liver function tests that may affect the study outcomes. All blood samples taken were labelled, sealed and delivered to the pathology lab by the investigator. The blood results were reviewed by the study principal supervisor or research nurse. Participants with normal blood results were deemed eligible and were invited to attend the study visits.

During the first visit of the study blood samples were also obtained for the analyses of plasma AAs. All samples were centrifuged for 15 min at 3,000g at 4oC in the David Greenfield Human Physiology Laboratories, School of Life Sciences, and then stored in 1ml aliquots at -80 °C for future analyses as per HTA guidance.

Plasma separated from Becton Dickinson (BD) Vacutainer® Tube Lithium Heparin (green top) treated blood was stored at -80 ˚C until analyses. Plasma AAs analysed by Dr Sarir Sarmad, a senior technician in the School of Life Sciences, using hydrophilic interaction liquid chromatography coupled to high resolution mass spectrometry (LC-MS) as previously described by Tsintzas et al. (328). Briefly, plasma samples (100 μL) were vigorously vortexed for 5 min following the addition of 500 μL of isopropanol (containing

an appropriate amount of internal standard)/ 1 mol/L KH2PO4 buffer (1:1 vol:vol), and then for a further 5 min following the addition of 500 µL of acetonitrile. Samples were then centrifuged for 20 min at 14,000 x g at 4°C. The supernatant was removed and evaporated to dryness under vacuum centrifuge and samples were subsequently resuspended in 100 µL of methanol: water (1:1 vol:vol) for LC-MS analyses. Absolute metabolite quantification was achieved using an isotopically (uniformly labelled ^{13}C) internal standard method.

C-reactive protein serum was measured using ELISA at the Department of Clinical Chemistry, Queen's Medical Centre, Nottingham, either during the initial screening process or from first visit blood samples.

4.2.4.3 Assessment of habitual dietary intake and anthropometrics

Participants were asked to record their routine dietary intake over three days (one weekend day and two weekdays) using the Intake24 questionnaire (329). Participants completed the first day of online food diary during their first study visit, with the remaining two days completed remotely via email. Detailed instructions were provided, and the diaries were checked remotely to ensure accuracy and submission. Follow-up calls and messages were made to participants who did not complete their food intake diaries. To minimize potential inaccuracies, the single operator (BAA) analysed all the diaries. The mean energy in KJ and carbohydrate, fat and protein in grams were calculated to estimated total macronutrients intake. Anthropometrics were recorded by a single investigator using a standard protocol and BMI for age z-score was calculated using WHO Growth Reference data for 5-19 year olds (330).

4.2.4.4 Assessment of eating behaviour traits

Eating behaviour was assessed by CEBQ which is a 35-item parent-report questionnaire measuring appetitive behaviors in children (331) and CTFEQr-17 is a self-reported scale based on the restraint (restriction of food intake to control overweight), disinhibition (tendency to overeat opportunistically) and hunger (responsiveness to internal hunger sensations) (332).

4.2.4.4.1. Child Eating Behaviour questionnaire (CEBQ)

Parents completed the CEBQ, which consists of four food avoidance sub-scales and four food approach sub-scales. The food avoidance sub-scales indicate a high internal satiety and avoidance of eating which are Satiety Responsiveness (SR, 5 items), Slowness in Eating (SE, 4 items), Emotional Undereating (EUE, 4 items), Food Fussiness (FF, 6 items). The food approach sub-scales indicate a high responsiveness to food which are Food Responsiveness (FR, 5 items), Enjoyment of Food (EF, 4 items), Desire to Drink (DD, 3 items), and Emotional Overeating (EOE, 4 items). Parents answer each question using a 5-point Likert scale (Never, Rarely, Sometimes, Often, Always) with answers scored from 1 to 5 respectively. CEBQ is demonstrated in **Appendix 3– Supplementary Questionnaire 3.1**.

4.2.4.4.2 Child three factor eating questionnaire-17 (CTFEQr-17)

Participants filled out the CTFEQr-17 which was developed by Bryant et al. (332) to measure three dimensions of human eating behaviour: cognitive restraint of eating, disinhibition and hunger. The questionnaire consists of seventeen items, all used the Likert scale format, with response options as follows: 1 = completely false, 2 = mostly false, 3 = mostly true, and 4 = completely true. The seventeenth item employs a four-point Likert scale, with responses such as 'only during mealtimes,' 'sometimes between meals,' 'often between meals,' and 'almost always.' This scale is structured into three factors denoting cognitive restraint (CR), uncontrolled eating (UE), and emotional eating (EE). A higher score in any sub-category of the scale indicates a higher level of eating behavior associated with that specific sub-category. CTFEQr-7 is provided in **Appendix 3– Supplementary Questionnaire 3.2**.

4.2.4.5. Estimating leg muscle volume by using MRI

MRI scanning was carried out using a 3T scanner (GE Premier, General Electric, USA). The AIR Anterior array coil was used and placed over the participant's legs. The following scan protocol was used to measure muscle volume.

Images to calculate muscle volume were collected using a 3D Gradient Echo (GRE) LAVA flex sequence acquiring with maximum six stacks of 46 contiguous axial slices to cover leg with a slice thickness of 5.0 mm (0 gap) and 6-slices overlap between stacks. Data

was reconstructed with a 256 x 256 in plane matrix from a 500 x 500 mm^2 field of view with resulting in-plane resolution 1.9531 mm × 1.9531 mm. The parameters of the sequence were: flip angle 12, TR=3.736 ms, TE1=1.1 ms, TE2= 2.2 ms.

All subjects' images were load into the WEASIS DICOM viewer® platform (Roduit, N. Weasis DICOM viewer. Version 4.1.0. https://github.com/nroduit/Weasis) to determine whether any of the stacks have got the fat and water labelling incorrect for one of the legs.

Data were analysed using Matlab® (The Mathworks Inc, Cambridge, UK, https://uk.mathworks.com/) using custom software written by Dr Caroline Hoad. The segmented data were saved as part of the analyses and volumes of water and fat were measured from the segmented data across the whole leg. In case of water and fat mislabelling station, a fixing of mislabelling was used by drawing around the affected limb to correct it (much brighter than the grey background) as shown in figure 2.1. The used technique identifies water and fat in the MRI scans were segmented and measured with removing foot as shown in **figure 4.1**.

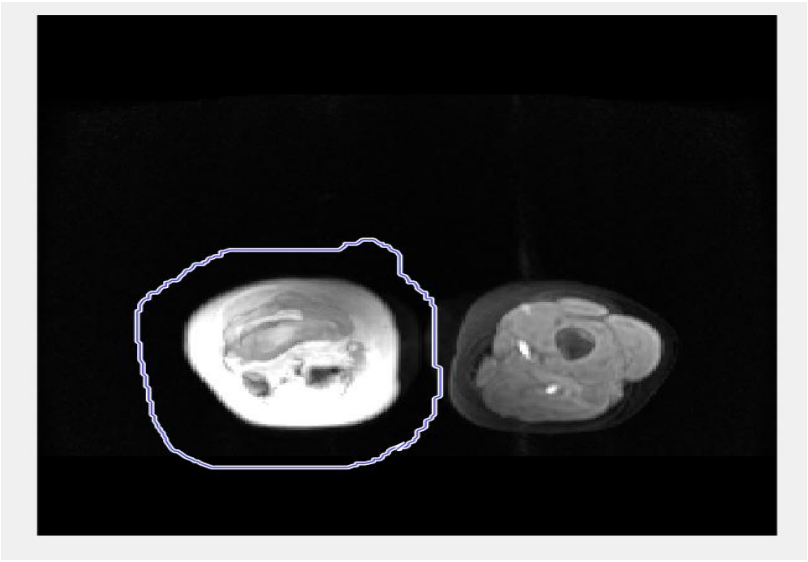

Figure 4.1. Water image of upper legs with labelling of RHS leg.

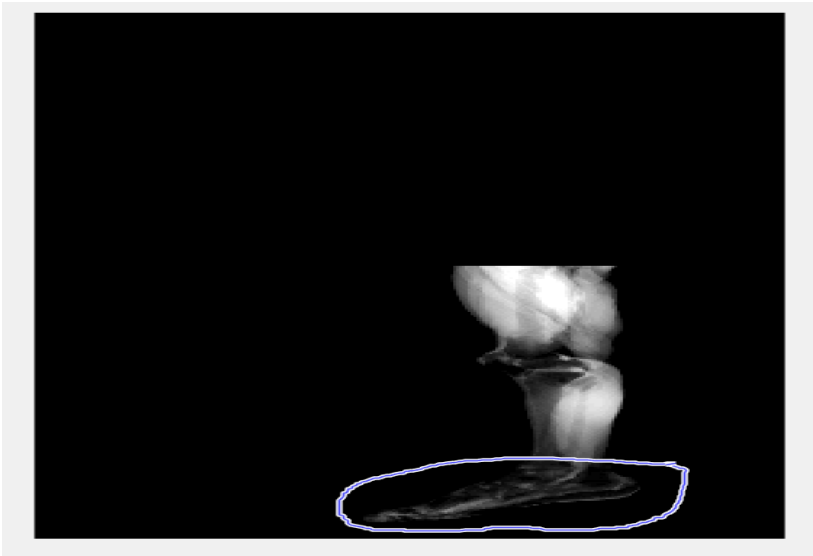

Figure 4.2. Foot was drawn around to

The analyses process to determine the leg muscle volume was made up of several steps. Firstly, any images that had their water and fat images mislabelled from the scanner algorithm were corrected using a simple drawing tool where the observer selected the mislabelled limb and the fat and water data for that limb were swapped (see figure). Following this, the data from the different stacks were merged to form a single data set for each image type. The skin around the leg was automatically removed using simple image processing techniques. Finally, a maximum intensity projection of the water image in the sagittal plane was displayed to the observer, and they manually removed the foot from the image. This was repeated for the other leg. The remaining data were then summed to give a total volume of all the leg muscle.

4.2.5. Sample size calculation

This pilot study serves as the baseline analysis for an ongoing investigation into protein nutrition and eating behaviour in paediatric CD. Importantly, the sample size calculation for this study was originally performed based on the 2nd visit (feeding visit) outcomes anticipated for the full, larger-scale study rather than the specific analysis presented here. The original sample size calculation aimed to detect a clinically relevant difference in protein intake and circulating AAs based on the expectation that significant differences between paediatric CD patients and HC would emerge in the larger sample. For instance,

the study targeted a meaningful difference in protein intake, as observed in Davies et al., (197) where healthy male controls had an intake of 105 ± 15 g/day compared to 75 ± 5 g/day for male CD patients ($p< 0.05$). Such differences in protein intake are clinically significant, as they could influence disease outcomes including MM as reflected in appendicular LM, with healthy controls showing LM values of 27.2 ± 3.6 kg compared to 20.6 ± 1.0 kg in CD patients ($p< 0.05$). These differences underscore the potential impacts on overall nutritional status and health outcomes in paediatric CD patients.

In the adult study by Wardle et al. (59), a significantly lower protein intake was observed in CD patients (n=30) compared to healthy controls (n= 31) [CD= 70.3 ± 6.1 g/day vs. HC= 92.6 ± 7.8 g/day; p= 0.03]. While in paediatrics, limited research exists on protein intake and amino acid levels in CD. We used a pilot paediatric study from our laboratory Davies et al. (197), which found no significant difference in protein intake between paediatric CD patients (n=20) and healthy controls (n= 9) [CD= 79 ± 5 g/day vs. HC = 90 ± 10 g/day; p= n.s.], LBM in CD was 46.0 ± 4.5 kg while in HC was 39.3 ± 1.5 kg. Mean postprandial arterialised plasma branched amino acid in male paediatric CD was 355 ± 21 µmol/L while in healthy controls was 321 ± 27 µmol/L.

Using a 1-sided t-test, alpha of 0.05 and power of 90%, we predict that we need 15 participants per arm of the study for our main outcome assessment (rounded up to 20) and 8 participants per arm for the MRI muscle mass assessment (rounded up to 10) and 10 participants per arm for the acute feeding study.

Due to time constraint, we recruited 11 CD with 11 matched HC for our main outcome assessment and 6 CD with 6 matched HC for the MRI muscle mass assessment. The analysis for the acute feeding study was not included in this thesis, as recruitment is still ongoing to reach the target sample size. Once recruitment is complete, all samples will be sent to Maastricht University for analysis, as they have specialised mass spectrometry for measuring both labelled and unlabelled plasma AAs.

4.2.6. Statistical analyses

Shapiro-Wilk test was used to determine the parametric or non-parametric nature of the data. Continuous parametric data has been examined using t-test and presented as mean ± standard error of the mean (SEM) and continuous non-parametric was analysed

using Mann-Whitney test and presented as median plus interquartile range (IQR) based on normality test. Categorical data were compared with a chi square test. Spearman rank correlation was used to test the association between continuous non-parametric variables, while Pearson correlations was used to test the association between continuous parametric variables. P value of ≤0.05 was considered significant. Data analyses were undertaken with IBM SPSS software for Windows (Version 28.0, IBM Corp., Armonk, NY, USA).

4.3. RESULTS

4.3.1. Demographic characteristics

A total of 36 participants were enrolled in the study, comprising 18 CD patients and 18 HC participants, out of the initially planned 20 CD and 20 HC. One healthy participant was excluded due to anaemia, leaving 35 participants eligible for the first study visit.

For the current analysis, 13 participants (6 HC and 7 CD) were excluded due to unmatched age, gender, or BMI. The remaining sample consisted of 22 participants (11 CD and 11 HC) matched for age, gender, and BMI. All participants completed assessments for protein intake (24-hour food diary), handgrip strength index, and eating behaviour questionnaires. Baseline AAs analysis was performed on 9 CD and 11 HC participants; two CD samples were not analysed due to laboratory requirements for processing larger batch sizes.

A sub-analysis using MRI was conducted on a subset of participants, specifically 6 CD and 6 HC, from the initial pool of 10 participants in each group. Recruitment remains ongoing to reach the target sample sizes in first and second visits.

Eleven CD participants (**figure 4.3**) with mean age of 15.18±1.4 years and BMI 21.06±3.73 kg/m^2 were recruited as shown in **table 4.1**. All CD participants were stable disease with no medication change in last 3 months. Four CD participants were active disease with mean CRP of 8.50±9.26mg/dl, mean FC of 836.28±577.33µg/g or had evidence of ulceration at MRE, while the other seven CD participants were in remission status with mean CRP of 1.50±1.22mg/dl, mean FC of 43.87±68.40µg/g or no evidence of ulceration at MRE. Seven CD participants were being treated with immunomodulators and biologics and two participants were taken mesalazine while the remaining three participants were naïve to drug therapies. None of the included participants had a history of surgical procedures. Eleven healthy control (HC) participants with mean age of 14.27±1.8 years and BMI 20.93±2.53 kg/m2 were recruited and matched to CD participants. Although there were no significant differences in anthropometrics between groups, 36.4% of CD patients had a BMI z-score of <-2.

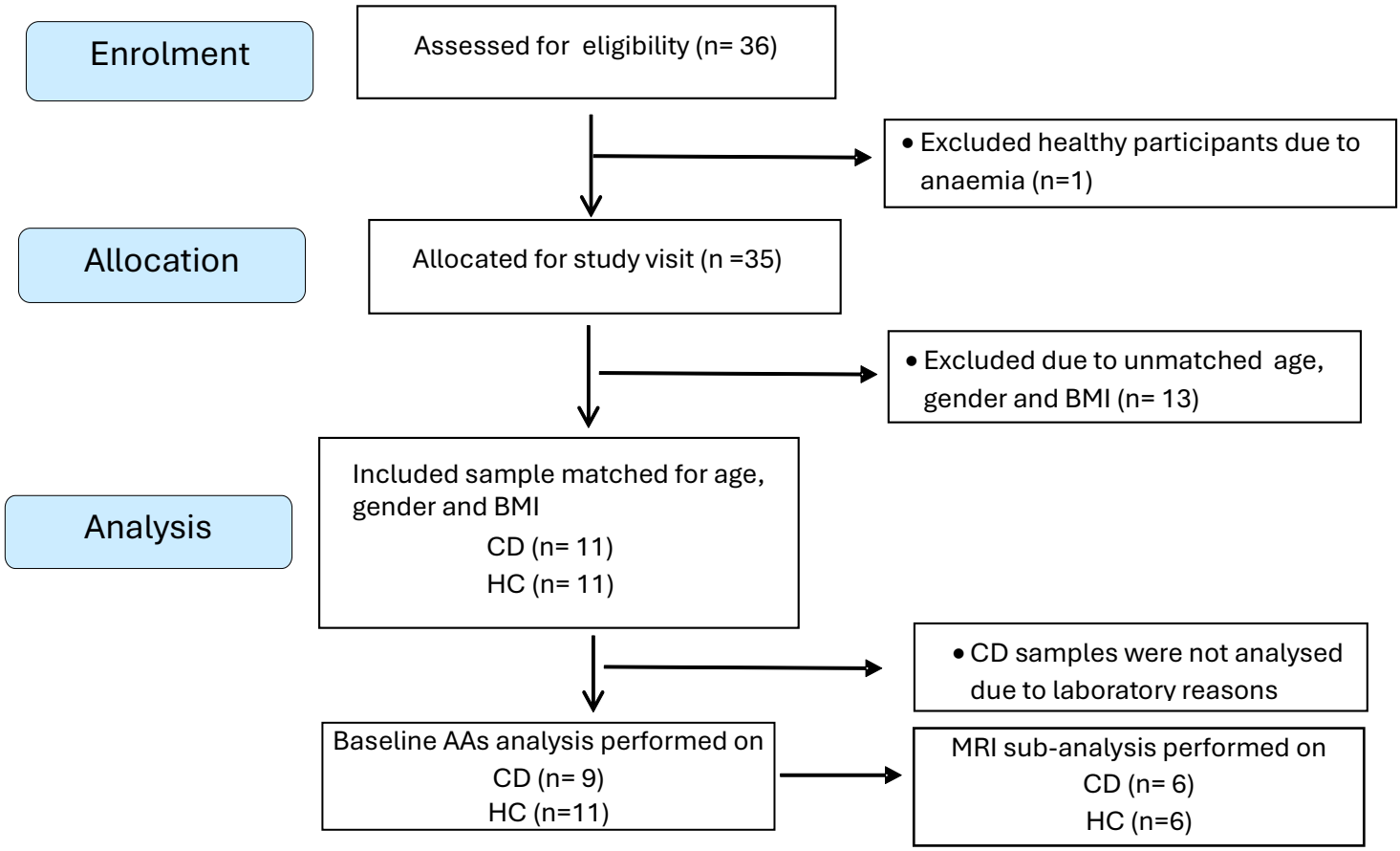

Figure 4.3. Consort flow diagram describing the selection of the final population sample included in the analyses. CD Crohn's disease, HC Healthy controls, BMI body mass index, AA Amino acids, MRI Magnetic resonance imaging.

Table 4.1. Descriptive characteristics of the whole study population and stratified by group status			
	CD (n=11)	**HC (n=11)**	**P value**
Gender			
Male	9 (81.8%)	9 (81.8%)	1.0
Female	2 (18.2%)	2 (18.2%)	
Ethinicity			
White	8 (72.7%)	5 (45.5%)	
Asian	2 (18.2%)	2 (18.2%)	
Black	0	0	0.3
Mix	0	0	
Other	1 (9.1%)	4 (36.4%)	
Age (years)	15.2±0.4	14.3±0.5	0.2
Height (cm)	168.0±3.2	166.7±3.4	0.8
Weight (kg)	60.0±4.5	58.01±2.4	0.7
BMI	21.1±1.1	20.93±0.8	0.9
BMI for age			
Underweight	1 (9.1%)	0	0.3
Healthy	7 (63.6%)	10 (90.9%)	
Overweight	1 (9.1%)	0	
Obese	2 (18.2%)	1 (9.1%)	
BMI for age (z-score)	-0.03±0.4	0.26±0.3	0.5
Handgrip strength (peak- kg)	27.6±2.4	28.5±2.6	0.8
Handgrip strength index	0.5±0.03	0.5±0.04	0.7
CD drugs			
None	3 (27.3%)	11 (100%)	0.006
Meslazine	1 (9.1%)	0	
Biologics	0	0	
Immunomodulators	0	0	
Meslazine+biloligics+immunomodulat	1 (9.1%)	0	
ors Immunomodulators+biologics	6 (54.5%)	0	
FC (µg/g)	332.0±154.5	0	n/a
CRP (mg/dl)	(n=10)	(n=6)	0.2
	4.3±2.1	1.0±0.0	

All values presented as means ± SEM except for categorical variables presented as absolute frequency (percentage) and z-score which was calculated BMI-for-age using WHO standards. CD= Crohn's disease, HC= Healthy controls. FC= Faecal calprotectin, CRP= C-reactive protein. n/a= Not applicable.

4.3.2. Macronutrients analyses

There was no significant difference in the mean intake of energy (KJ/day) (CD=8543.23±1188.01 KJ/day and HC=7650.98±748.29; p=0.5), protein (g/day) (CD=79.16±7.39 and HC= 77.89±10.36; p=0.4), fat (g/day) (CD=73.84±11.20 and HC=77.74±10.41; p=0.8) and CHO (g/day) (CD=277.5±43.87 and HC=239.84±19.91; p=0.9) intake between CD and HC groups. The self-reported 24-hour intake of energy and macronutrients for the CD and HC cohort are shown in **figure 4.4**.

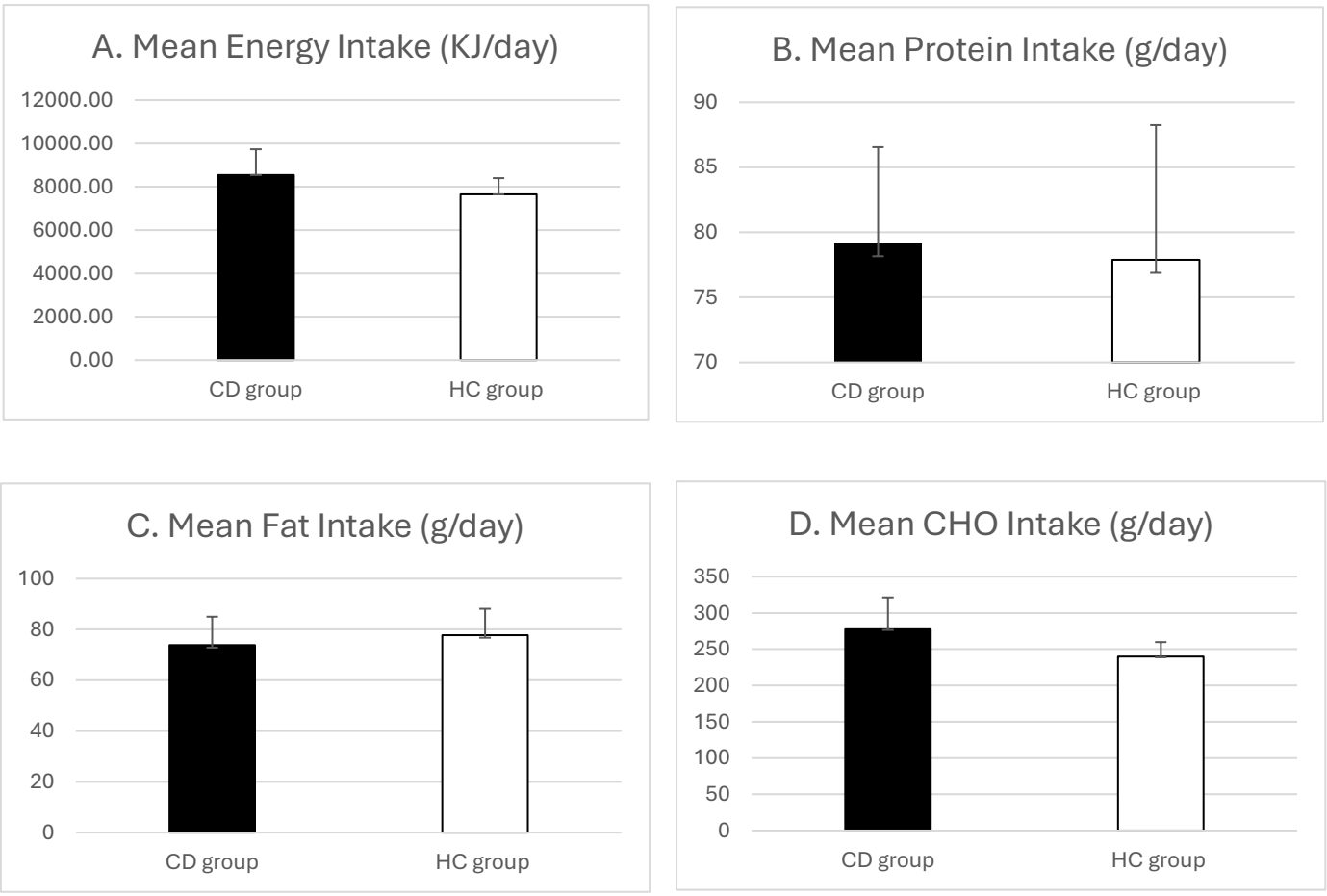

Figure 4.4. Macronutrients intake in CD vs. HC: (A) mean energy intake (KJ/day), (B) mean protein intake (g/day), (C) mean fat intake (g/day), (D) mean CHO intake (g/day). Values are means±SEM. Significant difference ($p \leq 0.05$) is marked. CD= Crohn's disease, HC= Healthy controls, CHO= Carbohydrate.

4.3.3. Plasma Amino acids analyses

There were no significant differences in AAs except in glutamic acid which was significantly higher in CD group than HC group (p=0.03) (**Table 4.2**). Moderate positive correlations were observed between protein intake (g/day) and both glutamine (µM/L) (r=0.45*, p=0.04) and asparagine (µM/L) (r=0.46*, p=0.04). See **Appendix 3– Supplementary Table 3.1** for all the correlations between protein intake and plasma AAs.

Table 4.2. Comparison of plasma AAs profiles stratified by group status

Plasma AAs	CD (n=9)	HC (n=11)	p value
Leucine (µM/L)	101.3±5.4	102.3±5.3	0.9
Isoleucin (µM/L)	68.1±5.0	67.9±4.8	1.0
Valine (µM/L)	175.9 (173.7, 196.4)	187.7 (169.5, 224.2)	0.6
Phenylalanine (µM/L)	15.4±0.6	17.0±0.9	0.2
Methionine (µM/L)	40.1±4.7	43.8±2.7	0.5
Tryptophan (µM/L)	65.4±2.2	71.0±2.5	0.1
Proline (µM/L)	94.9 (46.6, 116.4)	62.9 (52.5, 86.3)	0.5
Tyrosine (µM/L)	23.1±1.4	24.6±1.7	0.5
Glutamic Acid (µM/L)	32.1 (24.4, 80.8)	19.5 (16.0, 33.5)	0.03
Threonine (µM/L)	40.8 (30.3, 53.4)	38.2 (30.6, 41.5)	0.3
Aspartic Acid (µM/L)	1.1 (1.1, 2.3)	1.1 (0.6, 1.4)	0.3
Histidine (µM/L)	49.7±2.8	46.2±1.8	0.3
Alanine (µM/L)	286.8±23.7	247.2±12.6	0.1
Glutamine (µM/L)	435.9±13.3	429.4±12.3	0.7
Asparagine (µM/L)	26.9±1.2	29.8±1.3	0.1
Cysteine (µM/L)	17.9±1.4	16.5±1.4	0.5
Glycine (µM/L)	150.5±7.1	135.1±5.6	0.1
Serine (µM/L)	49.1±2.1	47.7±3.0	0.7
Lysine (µM/L)	15.6±1.0	16.4±1.3	0.7
Arginine (µM/L)	36.7±4.4	39.6±2.2	0.6
Total EAAs (µM/L)	588.4±25.3	596.2±28.3	0.8
Total non-EAAs (µM/L)	1204.2 (971.9, 1360.8)	1027.3 (983.2, 1185.5)	0.3

All values presented as mean±SEM except for non-parametric data which are presented as median (Inter-quartile range). Significant difference ($p \leq 0.05$) is marked. AAs= Amino acids, EAAs= Essential amino acids, non-EAAs= non-essential amnio acids.

4.3.4. Muscle mass and its function

There was no statistical difference in peak muscle strength (p=0.3) and muscle strength index (p=0.4) between CD group and HC group as well as Leg muscle volume (L) measured by MRI (p=0.7) as shown in **Table 4.3**.

A positive correlation was found between leg muscle volume and daily protein intake as shown in **figure 4.5**. It also identified an individual as an outlier, who was the only healthy participant classified as obese, with a BMI-for-age at the 97th percentile. Notably, we also had two CD participants who were similarly classified as obese and together they represent a segment of the population that should not be excluded from analysis.

Specifically, strong positive correlations were seen between leg muscle volume with-methionine (r=0.63*; p=0.03), proline (ρ=0.80**; p=0.002), alanine (ρ=0.66*; p=0.02), arginine (ρ=0.67*; p=0.02) and total non-EAAs (ρ= .71; p=0.01) and moderate positive correlation with glutamine (ρ=0.60*; p=0.04). See **Appendix 3– Supplementary Table 3.1** for all the correlations between leg muscle volume and plasma AAs.

Table 4.3. Comparison of muscle mass and its function stratified by group status

	CD (n=6)	HC (n=6)	p value
Handgrip strength (peak- kg)	25.9±3.3	31.0±2.9	0.3
Handgrip strength index	0.5±0.0	0.5±0.1	0.4
Leg muscle volume (L)	7.8±1.0	8.1±0.6	0.7

All continuous variables presented as means ± SEM. Statistically significant differences ($p \leq 0.05$). CD= Crohn's disease, HC= Healthy controls, Handgrip strength index= Handgrip peak strength (kg)/ weight (kg).

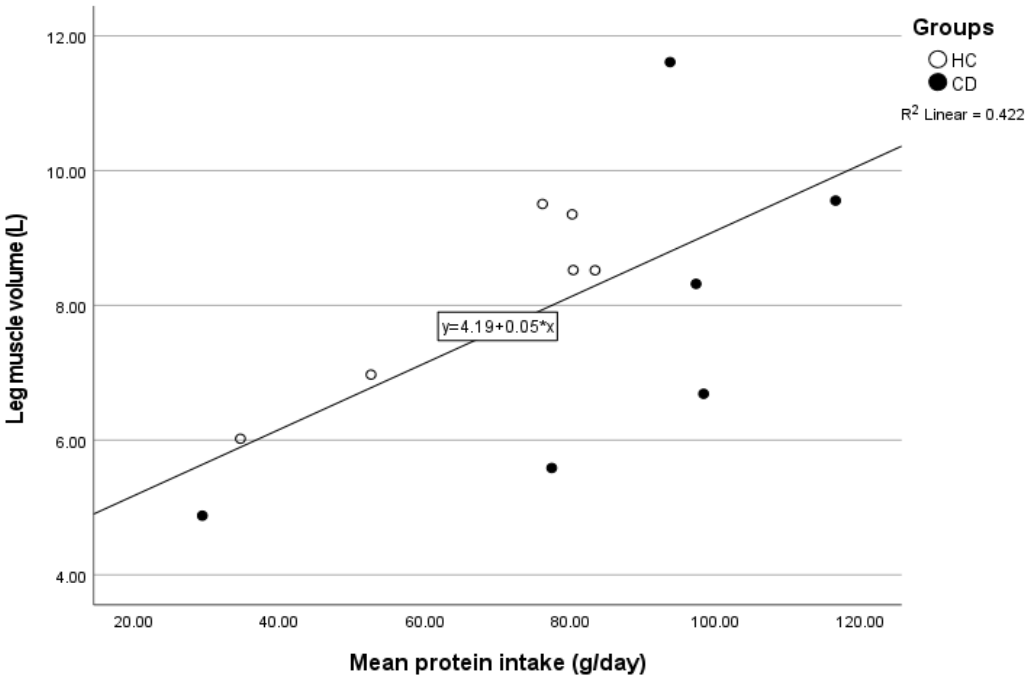

Figure 4.5. A strong positive correlation between protein intake and leg muscle volume in CD participants and healthy subjects. Pearson correlation coefficient (r)= 0.65, p=0.02.

4.3.5. Eating behaviour analyses

The outcome scores of psychometric eating behaviour questionnaires are shown in **table 4.4**. There was no significant difference between the cohorts in CEBQ food approach composite (p=0.2) nor its subscales: FR (p=1.0), EF (p=0. 8) and EOE (p=0.5) except in the DD which has a significant higher score in CD group than HC group (3. 5±0.7 vs. 2.4±0.8; p=0.01). The results also showed no significant difference in the cohorts in CEBQ food avoidance composite (p=0.5) nor its subscales: SR (p=0.7), EUE (p=0.3) and FF (p=0.3). In addition, there was no significant difference in CTFEQr-17 subscales between the cohorts UE= (p=0.4) and EE (p=0.3), and CR (p=0.6).

Interestingly, protein intake had strong negative correlation with CEBQ in SR (r=-0.47; p=0.03). Moreover, moderate negative correlations were also found between protein intake with CEBQ in SE (r=-0.43; p=0.05) and CTFEQr-17 in CR (r=-0.42; p=0.05). BMI z-score showed a moderate negative correlation with SE (r=-0.46; p=0.04). See **Supplementary Table 3.2** for all the associations between BMI and daily protein intake with eating behaviour traits in whole population.

Table 4.4. Eating behaviour traits in CD participants and age, sex and BMI-matched HC participants.			
	CD (n=11)	**HC (n=11)**	**p value**
CEBQ			
Food Approach composite	3.3±0.2	2.9±0.2	0.2
Food responsiveness (FR)	3.0±0.2	3.0±0.3	1.0
Enjoyment of food (EF)	4.2±0.2	4.1±0.2	0.8
Desire to drink (DD)	3.5±0.2	2.4±0.3	0.0
Emotional overeating (EOE)	2.7±0.4	2.4±0.3	0.5
Food Avoidance composite	2.7±0.3	2.5±0.2	0.5
Satiety responsiveness (SR)	2.5±0.3	2.4±0.2	0.7
Slowness in eating (SE)	2.5±0.4	2.3±0.3	0.8
Emotional undereating (EUE)	2. 5±0.3	2.9±0.3	0.3
Food fussiness (FF)	2.7±0.3	3.0±0.3	0.3
CTFEQr-17			
Uncontrolled eating (UE)	2.4±0.2	2.2±0.2	0.4
Cognitive restraint (CR)	1.6±0.2	1.7±0.2	0.6
Emotional eating (EE)	1.8±0.2	1.5±0.2	0.3

All continuous variables presented mean±SEM. Statistically significant differences ($p \leq 0.05$). CD= Crohn's disease, HC= Healthy controls, CEBQ= Child Eating Behaviour questionnaire, CTFEQr-17 = Child Tree-Factor Eating Questionnaire-17.

4.4. DISCUSSION

This study aimed to compare protein intake in paediatric CD versus healthy individuals matched for age, sex and BMI. It also aimed to link these findings with fasting plasma AAs, leg muscle volume measured by MRI and eating behaviour traits. The novel finding from the present study was an absence of a significant difference in protein intake and eating behaviour between the groups, which may explain the similar profile of fasting AAs and leg muscle volume. Adult patients with active CD ingest considerably less daily protein intake than age- BMI- matched healthy controls [CD, 70.3 g ± 6.1; HV, 92.6 g ± 7.8, p= 0.03] (59). In accordance a recent systematic review showed that adults with CD consumed significantly less protein than healthy non-IBD controls (333). Similarly a previous study on children with CD, 9 with active disease and 11 participants in deep remission, reported lower protein intake in CD than healthy subjects (79 ± 5g/day and 90 ± 10g/day) although though those findings were not statistically significant (197). The current study showed comparable protein intake in paediatric CD and healthy controls. This might be due to the small sample size, which was insufficient to reliably detect differences smaller than 11 g/day based on Davies et al. (197). Moreover, as all our included CD participants were in stable disease status. In addition, both Davies et al (197) and the current study showed that CD participants consumed considerably more protein than the recommended Reference Nutrient Intake (RNI) of 0.75 g of protein per kilogram of body weight per day (g/kg/d) (334). Furthermore, they consumed more protein (79g) than the average values (67g) from the National Diet and Nutrition Survey (NDNS, aged 11-18 years) (335). However, higher protein intake has a positive relationship with amino acid availability and protein balance because AAs stimulate protein synthesis and suppress muscle protein breakdown (336). Plasma AAs are influenced by protein intake, and the effect of the most recent meal is minimal, though animal studies have reported that chronic alteration of protein intake affect fasting AA concentrations (337). A recent study showed that plasma levels of essential (leucine, lysine, and valine), semi-essential (arginine and glutamine), and non-essential (serine) AAs were lower in adults CD subjects compared to control subjects (322). Our results did not show any significant differences in fasting plasma AAs between paediatric CD patients and heathy volunteers except in glutamic acid which was higher in CD than healthy subjects. However, there is

only one study that included paediatric IBD patients, with active disease status and in remission, that found that tryptophan and histidine were significantly lower compared with healthy controls (338). Although their results did not show significant difference in plasma histidine between the two phases of active disease and in remission, they showed that plasma tryptophan was significantly lower in patients with active IBD. These results agreed with Hisamatsu et al. (321), who stated that the plasma levels of tryptophan and histidine in adults with IBD were lower than in healthy controls, and were also lower in patients with active disease compared to those in remission. In addition, a study conducted by Nikolaus et al. (339) found that the plasma concentration of tryptophan was significantly lower in adults with IBD compared to the healthy group, and there was a significant relationship between disease activity and plasma tryptophan levels. Another study showed that a reduction in plasma level of tryptophan is associated with active CD (340). Chiba et al. (323) showed significant correlation between CD activity index scores in adults with CD and plasma levels of valine, methionine, leucine, histidine, and tryptophan. These AAs belong to the EAAs supplied by the diet and their lower levels in CD could be the result of insufficient protein intake, protein-losing enteropathy and malabsorption and metabolic disturbance caused by disease activity in chronic disease (323, 341). Our results showed moderate positive correlations between protein intake and both glutamine and asparagine concentrations, both of which are considered as semi-EAAs (342). Glutamine can become EAA in some clinical situations associated with protein wasting and stress (343). Glutamine and asparagine are associated with protein synthesis and proliferation in the absence of glutamine requires asparagine (342). Our results did not show difference in their plasma levels between the two groups. This might be because the CD participants in the current study were in stable disease with normal protein intake, when compared to non-CD controls.

Adequate protein intake is essential to maintain the balance between anabolism and catabolism thereby preserving muscle mass. Generally, an increase in protein intake, rather than administering specific amino acid supplements, is more effective in both stimulating muscle protein synthesis and reducing muscle protein breakdown (344). Our results showed comparable muscle volume measured by MRI and its strength measured by HS dynamometer between the CD and HC groups. This might be due to the stable

disease status of the CD participants, who have normal protein intake and normal eating behaviour compared with matched healthy subjects. These results are consistent with pilot study of Davies et al (197), who found comparable protein intake, lean muscle mass measured by dual energy x-ray absorptiometry and muscle strength measured by HS dynamometer between asymptomatic CD participants and matched healthy volunteers. Decreased muscle mass in paediatric CD with remission disease status is reported in some published research (228, 345-347). Reduced muscle strength has also been reported in paediatric CD in mild and remission disease status (194). However, these studies compared their results to different reference populations. In addition, given the small sample size (n = 6), it is important to highlight that the study was underpowered to detect differences of this magnitude. The minimum detectable difference in LM measured by DEXA was more than 6.7 kg and muscle strength was more than 1.1 kg/kg in Davies et al. study (197). However, our study specifically assessed leg muscle volume (L) using MRI. As a result, the current findings may not be fully comparable to those based on DEXA measurements, and further studies with larger sample sizes are needed to confirm these trends and their clinical significance. Future large cohort studies are needed to confirm our findings.

There are concerns regarding food intake in CD patients who experience unintentional weight loss (348). However, recent studies suggest a shift toward an overweight status presenting in paediatric CD (66). Only one study in adults CD has addressed the relationship between eating behaviour traits and nutritional status (59), while this has never been investigated in paediatric with CD. Abnormal nutritional status has always been associated with CD, but very limited data was found on detailed analyses of eating behaviour in CD patients compared with HC (59). As our results show no significant difference in the calorie and macronutrients intake between CD and healthy, there was no significant difference between CD and healthy in the CTFEQr-17 subscales as well as in CEBQ subscales except in DD which was higher in CD group. This subscale assesses the desire to drink without specifying beverage types (water or a caloric beverage) to illustrates associations with energy intake and consequently weight (349), although there were no differences in the energy intake nor in BMI between the groups. A moderate negative association was found between SE and BMI (z-score). This is supported by

several previous studies which found an expected positive correlation between excess body weight and faster eating rate among heathy paediatric (349-356) cohorts. Protein intake showed a negative correlation with SE and SR. This implies a higher protein intake is associated with less slowness in eating and lower satiety responsiveness. This might be because a higher protein intake stimulates the release of appetite-controlling hormones, such as peptide YY, glucagon-like peptide-1 and cholecystokinin, which may increase feelings of fullness as dietary protein takes longer time to digest than other nutrients and reduce hunger cues (357, 358). Our findings align with Mallan et al (359) in which SR and SE were inversely associated with energy intake of the lunch meal. Moreover, SE and SR are usually clustered together as shown in previous studies of CEBQ (331, 356). However, this novel finding needs further investigation on protein intake and meal duration with eating patterns to be validated. In addition, protein intake showed a moderate negative correlation with CTFEQr-17 subscale (CR). This suggests reduced restrained eating has been associated with increased caloric intake, including protein intake, and increased BMI (360).

This pilot study was novel in assessing the habitual protein intake, fasting plasma AAs and linking these finding with muscle volume measured by MRI and eating behaviour of paediatric CD compared with matched healthy subjects.However, several limitations should be acknowledged.

First, while BMI matching allows for direct comparison of dietary intake, eating behavior, and muscle volume between CD patients and HC by controlling for the confounding effects of body size and composition, it also introduces limitations. CD patients often have higher metabolic demands due to factors such as inflammation, increased energy expenditure, and impaired nutrient absorption. However, BMI matching assumes similar metabolic demands between groups, which may not be accurate. If CD patients consume the same amount of protein as HC but have higher nutritional requirements, their intake may appear adequate when it is insufficient relative to their needs. Additionally, CD patients with a BMI matched to HC may have different body compositions, such as lower lean muscle mass and higher fat mass, due to disease-related factors. BMI matching does not account for these differences, which could influence muscle health outcomes and lead to misinterpretation of the data. To improve

the accuracy of comparisons, future studies should consider incorporating additional matching criteria or covariates, such as lean mass index and disease activity markers. Metabolic assessments, including resting energy expenditure or protein turnover rates, could also help adjust dietary intake based on the specific needs of CD patients. It is crucial to interpret results with the understanding that BMI matching may obscure underlying differences in nutritional adequacy and metabolic demands unique to CD patients.

Second, the small number of enrolled participants may affect statistical validity and minimum detectable differences between the two groups. The reason for the small sample size was the inevitable delay to start the study because of the COVID-19 pandemic. Additionally, recruiting 35 participants (17 HC and 18 CD patients) took approximately 18 months, primarily due to participant availability and limited MRI booking slots. Recruitment was concentrated during school holidays and on days when parents could take time off work, leading to scheduling constraints. As a result, the recruitment timeline was extended, as potential participants were only available at certain times of the year. In future studies, adopting a more flexible recruitment schedule and optimizing MRI booking options may better accommodate participants' schedules. This experience underscores the importance of aligning study timelines with participant availability to create a more efficient and feasible recruitment process.

Third, due to small sample size, we could not examine the effect of disease burden in CD patients on eating behaviour traits. Additionally, since this study includes individuals identified as outliers, we could not exclude them, as they represent a segment of the population. Given the study's limited sample size and underpowered nature, including such individuals is crucial for obtaining a more representative understanding of the population.Fourth, the cross-sectional design does not allow the investigation whether dietary intake, plasma AAs and eating behaviour in CD patients change across disease activity states.

Fifth, 66.7% of CD participants treated with immunomodulators and biologics, both of which induce and maintain remission (361). The reason we chose to study a cohort with stable disease is because in a clinical setting, this would be the most common scenario

experienced by clinicians. Any patients with active disease would be promptly treated to clinical/endoscopic or radiological disease or in some clinical settings when this is not possible to stable disease where the clinician feels that any further clinical intervention is unwarranted. Our cohort was in a stable disease with some patients having mild active asymptomatic disease that did not warrant any further change in therapy and others being in remission. The study's underpowered design, due to the inability to recruit the target sample size, increases the risk of false-positive findings. Further validation in larger, more definitive studies to confirm any preliminary findings is needed.

The 24-h food intake recall has been used and validated in previous research (362). Participants could be affected by recall bias, which may impact the accuracy of the outcome. However, we conducted a 3-day assessment, including 2 working days and 1 weekend day. in addition, some biochemical and radiological measures of disease activity were obtained as part of routine standard care rather than for a study screening process. Consequently, there was a variable lag between the dates of these assessments and recruitment into this study, although none of the included patients changed their therapy after these investigations or before their recruitment.

In conclusion, this study provides preliminary data on protein intake, plasma AAs and eating behaviour traits in a paediatric cohort with CD. All of the CD participants were in stable disease, which was associated with similar protein intake, plasma AA concentrations, muscle mass and strength, and eating behaviour compared with matched healthy subjects. The small sample size and cross-sectional design of the study preclude firm conclusions to be made about causality and reliability relationships between parameters. Therefore, a future large statistically powered prospective cohort study is required to confirm these results and understand the effect of disease activity on plasma AAs and eating behaviour traits in paediatric CD compared with healthy controls.

5. Age and gender but not disease activity and disease duration are associated with muscle mass in routinely collected MRI data sets in paediatric Crohn's disease.

5.1. INTRODUCTION

Crohn's disease (CD) is an inflammatory bowel disease (IBD) of unknown aetiology which is characterized by chronic inflammation of the gastrointestinal tract and a progressive and complicated disease course. The incidence of CD in the western world, ranges from 0.3 to 12.7 cases per 100,000 individuals (10). Specifically in paediatric onset disease, CD is associated with weight loss, delayed growth, loss of muscle mass and malnutrition (241), so the ability to routinely measure muscle mass in paediatric CD is clinically important.

There is a challenge in assessing body composition in children due to the difficulty of implementing technology in regular clinical settings. Interpreting values is a further difficulty because of variations in age and the normal growth patterns and puberty during childhood. Various methods have been used to estimate muscle mass in paediatric IBD, involving additional technique assessments through dual-energy X-ray absorptiometry (DEXA) or computed tomography (CT) scans (320, 345, 347). Although these data produce valuable findings, the radiation exposure complicates their routine implementation as a standard assessment in children. Therefore, due to the chronic nature of CD patients who necessitate recurrent radiological examinations, a free ionizing radiation imaging modalities should be preferentially used (249, 258, 363, 364).

Magnetic resonance imaging provides an ideal platform to measure muscle mass. It is routinely used to assess disease activity in patients with IBD through magnetic resonance enterography (MRE). Psoas muscle mass, through either a combined psoas cross sectional area z-score (12) or through a median psoas area index (258) have been shown to be significantly smaller in IBD cohorts when compared to non-IBD controls, with no obvious differences observed between IBD subtypes (19). Though a difference between diseased and healthy groups is expected it is still unclear if muscle mass is as dynamic, acutely affected by the chronic inflammatory state with possible significant differences observed within patients across disease states. Low muscle mass was correlated with disease activity, therapy usage and colectomy in acute sever colitis patients (97). Therefore, assessing the effect of disease burden on muscle mass in CD is crucial to understand the bidirectional relationships between inflammatory disease active and muscle mass.

Although psoas area is subjectively commented upon by radiologists, no quantitative measures of psoas area volume exist (365). MRI scans are routinely used in paediatric IBD to assess small bowel disease activity and extent (40). We aimed to assess the psoas muscle cross sectional area in paediatric CD patients and compare this between those with active or inactive disease to better understand the dynamic nature of muscle mass and its possible role as a disease biomarker. Additionally, we examined the impact of age, gender, disease activity and other disease variables on muscle mass.

5.2. METHODS

5.2.1. Characterisation of the cohort

We conducted a cross-sectional analysis of children with CD to evaluate the association between chronic inflammation and muscle mass, using the psoas muscle cross sectional area (PCSA) (measured in cm^2) as an indicator of muscle mass. Clinical and demographic data, including disease activity (categorised as active or inactive based on MRI radiology reports), gender, age at the time of MRI, disease duration (in days), and current biologic use, were collected.

Anonymised datasets of all paediatric Crohn's disease patients who underwent MRE between 2016 and 2023, without any specific selection criteria applied. These datasets were obtained from routine NHS MREs acquired as standard care within Nottingham University Hospitals in order to assess disease activity in CD patients. The included participants were diagnosed with CD in line with the modified Porto criteria for the diagnosis of inflammatory bowel disease in paediatric patients based on the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (366).
Our primary analyses consisted of a PCSA comparison between patients with active and inactive disease. Secondary analyses consistent of an adjusted analyses to explore the relationship between PCSA and disease variables.

5.2.2. Measurement of the psoas muscle cross sectional area

The primary outcome was PCSA, which was analysed using 2 slices acquired at L3 from one MRI per patient. In patients having multiple MRIs we have used the earliest one in the series to analyses the PCSA. All anonymised MRI images were retrieved from NHS PACS system and were then uploaded to the General Electric Edison™ Datalogue™ Connect Suite (GE HealthCare, Centricity Universal Viewer Web Client, USA, https://uk.gecentricity360.com/#!/). The cross sectional area of psoas muscle was assessed by using Medical Imaging Processing, Analyses, and Visualization software (MIPAV, Center for Information Technology, National Institute of Health, Bethesda, Maryland, U.S, https://mipav.cit.nih.gov/) (367), using one of the following balanced turbo field echo (BTFE) MRI sequences: BTFE, BTFE_ax, BTFE_BH and Mobiview BTFE_ax). Within each image slice, a region of interest (ROI) was manually drawn around

the area of the right and left L3 vertebra segment of psoas muscle area (368, 369) as shown in **figure 5.1**. These measures were then combined to give the sum of the volumes measured from each image slice to give the total PCSA reported in cm^2.

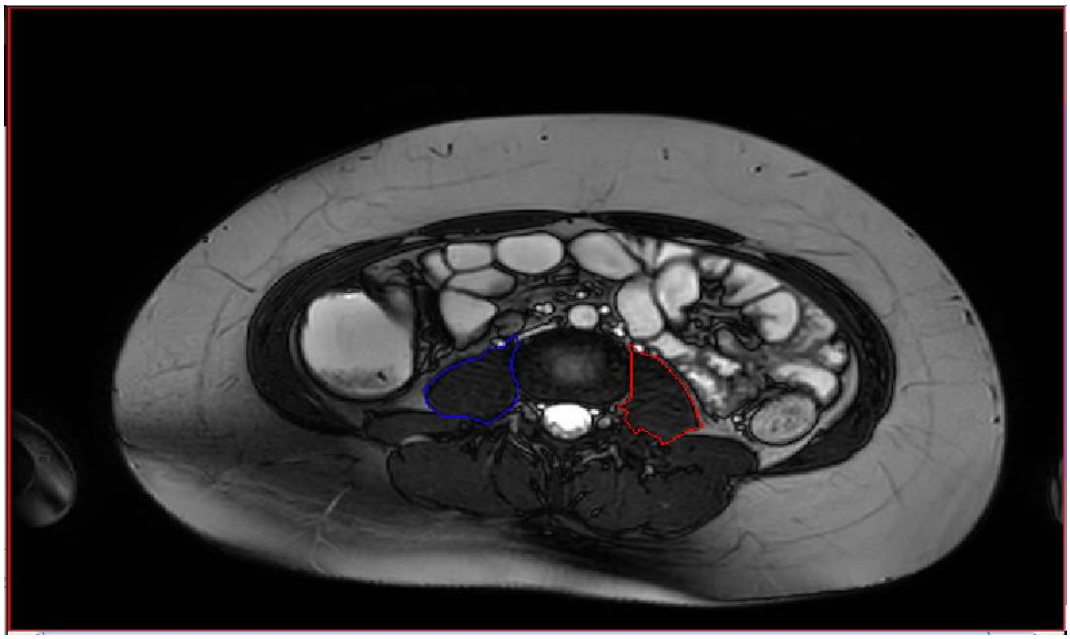

Figure 5.1. Psoas area measurement at the upper level of L3 magnetic resonance images by freehand region of interest.

5.2.3. Statistical analyses

The normality test was used to determine the parametric or non-parametric nature of the data. Continuous variables were presented as medians (interquartile range) and categorical variables as frequencies and percentages. Chi-square test was used to compare categorical variables while a Mann Whitney U test was used to compare continuous dependent variables and categorical independent variables. Bivariable regression was used to explore unadjusted relationships, separate linear regression models were performed between PCSA (dependent variable) and each independent variable.

A multiple linear regression model was then constructed to adjust for potential confounders, including disease activity, gender, age at MRI, disease duration, and current biologic use. Variance Inflation Factor (VIF) was calculated for all predictors to assess

multicollinearity. A VIF threshold of <5 was used to confirm the absence of significant collinearity.

All analyses were conducted using Python (version 3.11) with the stats models package. Statistical significance was defined as $p \leq 0.05$.

5.2.4. Ethical approval

This study was reviewed and given a favourable opinion by the NHS Healthy Research Authority (HRA) and Health and Care Research Wales (HCRW), IRAS project ID: 326627 and REC reference: 23/HRA/1908. on the 31st May 2023 with the study conforming to the recognised standards of the Declaration of Helsinki.

5.3. RESULTS

5.3.1. Cohort demographics

At data lock on 15th January 2024, 165 participant images were enrolled (**Figure 5.2**). After applying the inclusion and exclusion criteria, 58 participants were removed either due to duplicated participants or missing data. 107 participants of patients with CD were included with a small bowel or pelvic MRIs and an age <18 years.

Participant characteristics are displayed in **Table 5.1**. The cohort was predominantly of a male gender (57.9%). The median age was 13 years (11, 15). The median PCSA was 26.08 cm^2 (19.69, 34.19). The majority of patients (64.5%) had active disease and the median disease duration for the whole cohort was approximately 1 years. Most of the cohort had been exposed to immunosuppressant or advance therapy.

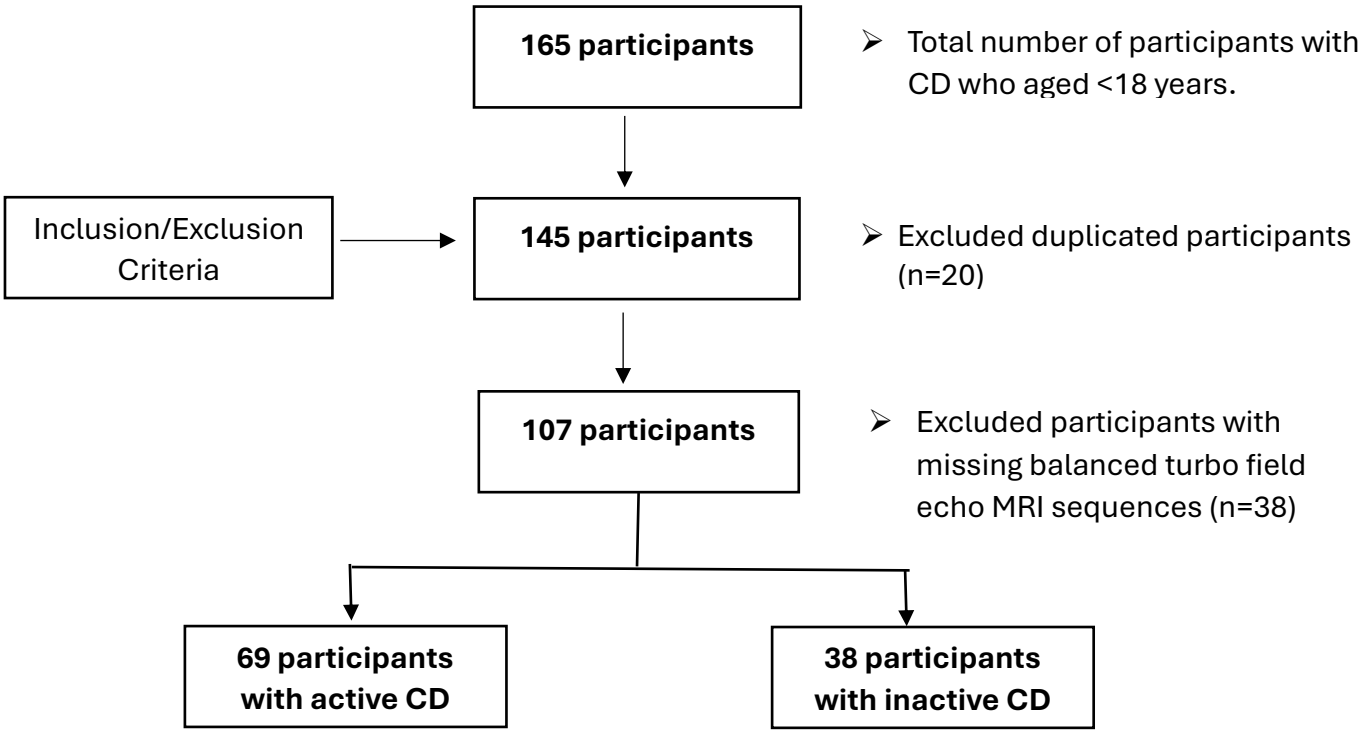

Figure 5.2. Consort diagram describing the selection of the final sample included in the analyses

Table 5.1. Descriptive characteristics of the whole study population and stratified by disease activity				
Index	**Whole cohort**	**Active**	**Inactive**	**p-value**
Disease Activity	107	69	38	-
Age at MRI	13.0 (11.0, 15.0)	13.0 (11.0, 15.0)	12.0 (10.25, 14.75)	0.52
PCSA (cm^2)	26.08 (19.69, 34.19)	25.06 (19.51, 33.99)	26.505 (20.62, 36.99)	0.372
Disease Duration (years)	0.966 (0.487, 3.072)	0.706 (0.331, 1.383)	3.064 (0.634, 4.152)	0.001
Gender				
Male	62 (57.9%)	44 (63.8%)	18 (47.4%)	0.15
Female	45 (42.1%)	25 (36.2%)	20 (52.6%)	
Disease Location				
L1	18 (16.8%)	14 (20.3%)	4 (10.5%)	0.195
L2	31 (29.0%)	20 (29.0%)	11 (28.9%)	
L3	26 (24.3%)	19 (27.5%)	7 (18.4%)	
L3, L4	26 (24.3%)	12 (17.4%)	14 (36.8%)	
L2, L4	6 (5.6%)	4 (5.8%)	2 (5.3%)	
Inflammatory				
Yes	106 (99.1%)	69 (100.0%)	37 (97.4%)	0.761
No	1 (0.9%)	0 (0.0%)	1 (2.6%)	
Stricturing				
Yes	13 (12.1%)	9 (13.0%)	4 (10.5%)	0.942
No	94 (87.9%)	60 (97.0%)	34 (89.5%)	
Penetrating				
Yes	11 (10.3%)	7 (10.1%)	4 (10.5%)	1
No	96 (89.7%)	62 (89.9%)	34 (89.5%)	
Current Steroid Use				
No	105 (98.1%)	68 (98.6%)	37 (97.4%)	1
Yes	2 (1.9%)	1 (1.4%)	1 (2.6%)	
Current Biologic Use				
Yes	91 (85.0%)	59 (85.5%)	32 (84.2%)	1
No	16 (15.0%)	10 (14.5%)	6 (15.8%)	
Previous Biologic Use				
No	82 (76.6%)	53 (76.8%)	29 (76.3%)	1
Yes	25 (23.4%)	16 (23.2%)	9 (23.7%)	
Current Immunotherapy use				
Thiopurines	74 (69.8%)	49 (72.1%)	25 (65.8%)	0.625
Methotrexate	4 (3.8%)	3 (4.4%)	1 (2.6%)	
No	28 (26.4%)	16 (23.5%)	12 (31.6%)	
Perianal				
No	97 (90.7%)	63 (91.3%)	34 (89.5%)	1

Yes	10 (9.3%)	6 (8.7%)	4 (10.5%)	
Surgeries				
No	100 (93.5%)	66 (95.7%)	34 (89.5%)	0.407
Yes	7 (6.5%)	3 (4.3%)	4 (10.5%)	
Comorbidities				
No	94 (87.9%)	63 (91.3%)	31 (81.6%)	0.244
Yes	13 (12.1%)	6 (8.7%)	7 (18.4%)	

N, number of cases in each group. PCSA, psoas cross sectional area. pCD, perianal CD. Non-parametric test results where values are median and interquartile range. Categorical variables were described by reporting percentage frequency. Chi-Square Test was used to evaluate between-group differences for categorical variables. Significant differences ($p \leq 0.05$) is marked.

5.3.2. Unadjusted comparison between psoas muscle area and clinical variables

In the unadjusted models, age at MRI ($\beta = 3.04$, $p < 0.000$) and gender ($\beta = -8.90$, $p = 0.008$) showed significant associations with PCSA. Age at MRI demonstrated a positive relationship with PCSA, indicating that older children had higher muscle mass. Similarly, gender revealed that males had higher PCSA compared to females. Other variables, including disease activity ($p = 0.833$), disease duration ($p = 0.532$), and current advanced therapy use ($p = 0.756$), were not significantly associated with PCSA in bivariable analyses (**Table 3.2**).

Table 5.2. Unadjusted analyses comparing disease and patient variables to PCSA.

Variable	Coef (β)	p-value	95% CI (Lower)	95% CI (Upper)
Disease Activity	-0.59	0.833	-6.14	4.96
Gender	-8.90	0.001	-14.00	-3.80
Age at MRI	3.04	<0.001	2.17	3.91
Disease Duration (Days)	0.00	0.532	-0.00	0.00
Current Biologic Use	1.17	0.756	-6.27	8.62

5.3.3. Multivariate regression shows an association between muscle area and gender and age but not disease activity.

After adjusting for all covariates, the multivariable model identified gender and age at MRI as significant predictors of PCSA. Gender ($\beta = -6.72$, $p = 0.004$). Males had a 6.72 cm^2 higher PCSA on average compared to females, after adjusting for other variables. This

suggests that gender plays a substantial role in muscle mass, with males exhibiting higher muscle mass relative to females in this cohort.

Age at MRI (β = 2.92, p < 0.000). For every 1-year increase in age, PCSA increased by approximately 2.92 cm^2 on average, adjusting for other covariates. This indicates that older children tend to have greater muscle mass, which aligns with expected growth and development patterns.

Other covariates, including disease activity (p = 0.220), disease duration (p = 0.489), and current biologic use (p = 0.942), did not show significant associations in the adjusted model **(Table 3.3)**. Variance Inflation Factor (VIF) values for all predictors were <1.3, indicating no evidence of multicollinearity.

Table 5.3. multivariate regression showing association between PCSA, gender and age but not disease activity measures.

Variable	Coef (β)	Std. Error	p-value	95% CI (Lower)	95% CI (Upper)
Disease Activity	-3.01	2.44	0.220	-7.84	1.83
Gender	-6.72	2.25	0.004	-11.19	-2.25
Age at MRI	2.92	0.45	<0.001	2.04	3.81
Disease Duration (Days)	-0.001	0.001	0.489	-0.004	0.002
Current Biologic Use	-0.22	3.05	0.942	-6.26	5.82

5.4. DISCUSSION

This study shows that conventional sequences taken from the routine practice in paediatric CD can be used to define the psoas cross-sectional area measurements by using magnetic resonance enterography (MRE). We did not find any difference in PCSA between patients with active or inactive disease. Indeed, on multivariate regression, only gender and age but not disease activity nor duration had any relationship with muscle mass as measured through PCSA.

Recent studies indicate that the cross sectional area of psoas muscle is considered as surrogate proxy marker of sarcopenia which can be easily and reliably assessed by heath care professionals using standard image viewing software (370, 371).

A reduction in muscle mass assessed by different measurements in paediatric CD was associated with worse disease activity (13, 17, 197, 250, 256), while normalization of disease activity may lead to improved muscle mass in paediatric with CD (194, 252). Atlan et al. (258) observed a significantly lower psoas area and psoas area index (PAI) as measured by MRI in paediatric IBD group when compared with control non-IBD group. In addition, they demonstrated a higher PAI assessed by MRI in IBD paediatric patients who were in remission status compared with those in mild, moderate or severe disease activity. In our work, we observed no difference in PCSA between patients with active and inactive disease. The two studies may be hard to compare though due to the difference in measuring psoas muscle area and disease activity.

We have undertaken a cross-sectional analysis between two groups who either had active or inactive disease at analyses. CD is a progressive disease with fluctuating states of disease activity which will certainly influence muscle mass. Muscle mass may also reflect the cumulative effects of past episodes of disease activity, which might not be fully captured by a snapshot of disease status at the time of assessment. It is important to note that the median disease duration in this cohort studies here was only 1 year which may not have been long enough to see a significant effect of disease activity or disease duration on muscle mass. It is more likely that a cleaner comparison with a non-IBD control group might have observed similar findings to the ones previously published. To this effect, serial measures of muscle mass together with measures of disease activity

need to be undertaken over time to better understand the relationship between chronic inflammation and muscle mass.

Previous work (249) failed to show a significant relationship between age at the time of MRI and PCSA in CD. This is disconcordant to our findings where age and gender but not disease activity and duration having the only significant relationships with muscle mass.

A larger psoas muscle area has been observed in patients exposed to biological rather than just immunosuppressive agents (258). The probable reason for this would be that biological therapy would induce a deeper state of remission which would be associated with a trophic state and has been linked with an increase in muscle volume in adults CD (262) and reduced whole body protein turnover in paediatric CD (263). Our results did not show any difference in PCSA between active and inactive CD patients, regardless of whether they had current or previous treatment with advanced therapy though it should be pointed out that the vast majority of our cohort was currently being treated with biological therapy, an observation that may nullify any comparison with non-exposed cohorts and also may explain the lack of a difference in PCSA in patients with active and inactive disease. Previous work has shown (262) that biological therapy significantly improved muscle volume and strength within 25 weeks of infliximab therapy even if disease status is not completely in remission. It is important to consider treatment duration when assessing muscle size and strength as sarcopenia is considered reversible with treatment targeting the underlying disease activity.

Furthermore, Atlan et al. (258) found that PAI was significantly lower in patients that presented with penetrating and ileocolonic IBD complications compared with disease limited to the colon. We did not observe any difference in PCSA by disease behaviour, which supports our observation of no observed interaction between muscle mass and disease activity and duration. It is important to note that the disease duration in our cohort was only 1 year hence any effect of disease behaviour with a relatively low prevalence of complicated disease.

Our study was novel in assessing the difference in PCSA between active and disease-inactive paediatric CD. This study may have some limitations. Firstly, this was a cross-sectional analysis that only allowed a comparison between patients with active and

inactive disease. A longitudinal study measuring serial PCSA across time in patients with routinely active and inactive disease might have been more beneficial. Secondly, muscle strength was not assessed and hence we could only define myopenia rather than sarcopenia where a measure of muscle function is needed. Thirdly, confounding factors such as BMI, hight and Tanner stages to assess pubertal status of the patients, corticosteroids, enteral nutrition and treatment duration have important role on muscle were not available. In addition, Muscle mass may be better understood as reflecting the cumulative impact of past episodes of disease activity rather than solely the disease activity status observed at the time of assessment. This highlights the importance of considering the long-term trajectory of the disease when interpreting muscle mass data. Finally, PCSA at L3 may have an accurate measure of total lean mass, although it is limited in the paediatric population due to a lack of reference data (190).

In conclusion, there was no significant difference in PCSA measured by MRI in paediatrics with active CD compared with paediatrics CD with inactive disease. Our cohort might have been hampered by the relative short disease duration and high biological usage. A large prospective cohort study is warranted to investigate the relationship between disease activity and PCSA as measured by MRI in children with CD. In addition, serial measures are needed to investigate the effect of chronic disease on muscle growth. Moreover, as disease assessment of small bowel inflammation in children with CD by using MRI scans is simple, feasible and routinely acquired, utilising these scans in assessing body composition would be highly recommended.

6. GENERAL DISCUSSION

The work in this PhD thesis aimed to understand protein nutrition and nutritional status in a paediatric population with CD at several levels. First, we aimed to appraise the relevant literature in a systematic way to understand the general definition of low muscle mass and deteriorated function (sarcopenia) and the impact of lifestyle interventions on these conditions in IBD paediatric populations (chapter 2). Second, specific hypotheses were tested in this thesis: A) Whether a high BMI is associated with adverse clinical outcomes in cross-sectional cohort of paediatric onset CD and the association between the age of the onset of disease and adverse clinical outcomes (chapter 3). B) Whether there is a difference in in eating behaviour and habitual protein intake between stable CD patients and matched healthy subjects, resulting in potential differences in plasma AAs, muscle mass and strength (chapter 4). C) Whether there is a difference in muscle mass in paediatric CD patients with active and inactive disease status as measured by MRI and the impact of age, gender, disease activity, and other disease-related variables on MM in CD paediatric patients (chapter 5).

In our systematic review (chapter 2), although most of the literature used different terminology and methods to define sarcopenia, several studies showed considerable reduction in MM-related compartments in CD paediatric cohorts (13, 194, 197, 250, 253, 255, 256, 259, 372) and one study among them showed a reduction in HS (194). On the contrary, other studies showed comparable findings in MM-related compartments between CD patients and different reference populations (13, 197, 249, 252, 258, 259), and one study among them showed comparable HS finding between CD and matched healthy controls (197). While some of these studies used accurate methods that produce valuable findings such as DEXA and CT scan (13, 194, 197, 256, 259, 320, 345, 347), the radiation exposure complicates its implementation as routine assessments in children. Therefore, non- ionizing radiation imaging method such as MRI, considered the gold standard, must be used due to the chronic nature of CD, which requires recurrent radiological examinations (249, 258, 363, 364). In this thesis, MRI was employed to investigate and compare MM in stable CD patients and matched healthy individuals (chapter 4) as well as in age- and sex matched active CD versus inactive disease status (chapter 5). To date, two studies (249, 258) have used MRI to assess MM in paediatric CD patients, in which Ashton et al. (249) compared CD patients versus a reference group,

while Atlan et al. (258) compared paediatric IBD patients versus a reference group, and CD patients versus UC patients. However, no previous studies have compared MM assessed by MRI in paediatric CD patients to age-and sex-matched healthy peers (chapter 4) or between active and inactive CD disease patients (chapter 5).

Recent observations have shown that nutritional status has been shifted with more overweight rather than underweight phenotypes being observed in IBD children (64). Specifically, nearly one in five children with CD is either overweight or obese (66). Our retrospective study (chapter 3) showed that higher BMI is associated with higher EIMs. This aligns with our hypothesis that the obesogenic state is associated with low grade inflammatory state (291, 292), which may be triggered by adipocyte hypertrophy and the subsequent release of pro-inflammatory markers (294, 295). This is linked with increased intestinal inflammatory activity and an increased incidence of some EIMs (309, 318). Our findings also showed a negative correlation between BMI and corticosteroid use. This may indicate the negative impact of disease activity and corticosteroid usage on body weight, with anorexia and sarcopenia being common symptoms of chronic disease activity. It is well known that corticosteroids can lead to muscle degradation and changes in fat distribution (264). In fact, decreased LM and increased FM have been observed in patients with cachectic diseases treated by long-term corticosteroid therapy (265), leading to sarcopenic obesity, which is characterised by low MM, physical weakness and increased body fat and weight (266). Unfortunately, we were not able to assess the impact of the use of corticosteroid and other drugs on MM in those with BMI≥25kg/m^2 or BMI<25kg/m^2 due to lack of relevant studies (chapter 3). Similarly, none of the studies included in the published systematic review (chapter 2) examined the effect of drug use on MM. Therefore, this is an important area for future research.

CD patients with earlier age at diagnosis (pre-puberty <11 years) experienced a higher incidence of stricturing disease and hospitalisations compared to those diagnosed at an older age (chapter 3). These findings are consistent with previous observations showing that the occurrence of stenosing or penetrating disease behaviour doubled during the follow-up period from 29% at diagnosis to 59%, reaching a relative plateau after 9–10 years of follow-up in paediatric CD (306). Our results also align with Polito et al. (319) in which an earlier age of diagnosis was associated with more severe disease behaviour,

although their comparison was made between patients younger than 20 years and those older than 40 years. In our cross-sectional study (chapter 5), we were able to assess the PCSA and age in pediatric patients with active disease compared to those with inactive disease status. Our findings showed that age and gender but not disease activity and duration having the only significant relationships with muscle mass. It is important to note that the disease duration in our cohort was only one year, which may explain the relatively low prevalence of complicated disease and the limited impact of disease behaviour on muscle mass. Gender analysis revealed that males had higher PCSA compared to females. This suggests that gender plays a substantial role in muscle mass, with males exhibiting greater muscle mass than females within this cohort. Age at MRI demonstrated a positive relationship with PCSA, indicating that older children had higher muscle mass. This contrasts the findings of Ashton et al (249) who did not find a correlation between age at MRI scan and PCSA in CD paediatric patients. Furthermore, our bioresource study (chapter 3) showed a positive correlation between younger age at diagnosis and corticosteroid usage. For this reason, serial measures of MM and disease must be taken over time when assessing MM between active versus inactive CD patients.

In our study (chapter 5), PCSA was measured manually using magnetic resonance imaging (MRI). While MRI is a highly precise and non-invasive imaging modality, manual measurements introduce variability due to the reliance on manually drawn regions of interest (ROI). This variability, inherent in observer-dependent methods, adds complexity to defining clinically meaningful thresholds for PCSA changes. Furthermore, PCSA represents only one component of skeletal muscle health, and its clinical implications may not align directly with total muscle mass or functional outcomes.

The absence of minimal clinically important differences thresholds for PCSA is compounded by the lack of literature addressing this issue in paediatric populations. While clinically significant changes in skeletal muscle mass have been explored in adults—often suggested to range between 5–10% of baseline values—such thresholds may not be directly applicable to children. paediatric populations exhibit unique growth and developmental patterns, necessitating age- and sex-specific considerations when interpreting muscle mass changes.

To improve the clinical utility of PCSA measurements, future studies should focus on Developing standardizing measurement protocol for assessing PCSA to minimize variability introduced by manual ROI delineation, establishing associations between changes in PCSA and relevant clinical outcomes and identifying thresholds for clinically meaningful changes that account for developmental differences in paediatric populations.

A recent study assessed dietary protein intake, FFM, LM and appendicular LM measured by DEXA and strength assessed by HS dynamometer in paediatric active CD patients compared with matched healthy children (197). They did not find significant differences in any of the outcomes measured between the groups, except in FFM (z-score) which was lower in CD than healthy individuals ($p=0.015$). Therefore, the present thesis assessed protein intake, leg muscle volume by MRI and hand strength by dynamometry in paediatric CD patients with stable disease compared with matched healthy controls. In accordance with the earlier study (197), our findings did not reveal any differences in habitual protein intake, leg muscle volume and hand strength between CD and healthy groups. This might be because in both studies habitual protein intake in paediatric CD was approximately 1.4 g per kilogram body weight per day, which is significantly higher than the intake stipulated by the Reference Nutrient Intake for healthy individuals of 0.75 per kilogram body weight per day (334). In addition, the minimum detectable difference in protein intake should be more than 11 g/day for a reliable result, which we could not achieve due to the study being underpowered. As we were unable to recruit the target sample size, it is important to emphasize that the small sample size limited the power of the study to detect meaningful differences. The minimum detectable difference in LM measured by DEXA was found to be more than 6.7 kg in the Davies et al. (197) study. However, our study specifically assessed leg muscle volume (L) using MRI, which may not be directly comparable to DEXA measurements (197) nor to previous studies (249, 258) which used psoas area measured by MRI in paediatric IBD compared to control or psoas area measured by MRI in paediatric CD group compared with reference population. Consequently, larger cohort studies are required to confirm these trends and their clinical significance.

Interestingly, a study on adult patients with CD showed that a 25% increase of the recommended protein intake is associated with increased LM (373). This may indicate that the habitual levels of dietary protein intake in peadiatric patients with CD may not be a limiting factor for muscle mass growth. Interestingly, we also found that leg muscle volume correlated positively with circulating levels of selected amino acids: methionine, proline, alanine, arginine, glutamine and total non-EAAs. However, our study had a small sample size and longitudinal studies with larger sample size are warranted.

The plasma AAs fasting profiles were also similar between CD and healthy controls, except for glutamic acid, which was higher in former group. Additionally, there was a strong positive correlation between protein intake and fasting glutamine and asparagine levels, both of which are considered as semi-EAAs (342). This may be because our CD population was in stable disease status with normal protein intake. Interestingly, Davies et al (197) found that children with active CD did not show significant difference in levels of postprandial arterialised branched chain AAs after ingestion of a standardised liquid meal compared with healthy peers. In contrast, a study on adults with CD showed significant correlations between CD activity index and circulating EAAs, in particular valine, methionine, leucine, histidine and tryptophan (323). These EAA supplied by the diet and their levels may be affected by low protein intake, protein-losing enteropathy and malabsorption, and metabolic disturbance caused by chronic disease activity (323, 341). Therefore, our future work will focus on a protein feeding study to investigate the digestibility of dietary protein by comparing the appearance in the circulation of arterialised amino acids originated from both oral consumption of isotopically labelled whole protein and isolated amino acids in paediatric CD with stable disease activity when compared with matched healthy volunteers. This will be analysed by using stable isotope tracer techniques, which enable accurate determination of protein digestibility (374). Upon ingestion of high-quality protein drink that includes intrinsically labelled AAs and intrinsically labelled algae protein, the appearance of these labelled proteins in the blood stream following digestion allows for accurate determination of protein digestibility (375).

The novel work in this thesis also evaluated and compared eating behaviour traits between children with stable CD and matched healthy controls. Despite the known

association between CD and abnormal nutritional status, there is limited research on the eating habits of CD patients compared to healthy individuals (59). Generally, our findings were comparable in both CTFEQr-17 and CEBQ subscales between stable CD children and matched healthy peers. In addition, we found a negative association between CEBQ (slowness in eating subscale) and BMI (z-score). This is corroborated by previous studies that observed excess body weight is associated positively with faster eating rate in healthy paediatric populations (349-356). Moreover, dietary protein intake showed negative correlations with CEBQ (satiety responsiveness and slowness in eating subscales) and CTFEQr-17 (cognitive restraint subscale). This might be because a higher protein intake stimulates the release of appetite-controlling hormones, such as peptide YY, glucagon-like peptide-1 and cholecystokinin, which collectively may increase feelings of fullness as dietary protein typically takes longer time to digest than other nutrients and was shown to reduce hunger cues (357, 358). Our findings align with Mallan et al (359) in which satiety responsiveness and slowness in eating were inversely associated with energy intake of the lunch meal. Additionally, satiety responsiveness and slowness in CEBQ subscales are usually clustered together as demonstrated in previous studies (331, 356). This suggests that a higher protein intake is associated with less slowness in eating and lower satiety responsiveness, as well as with reduced restrained eating (360). Therefore, a future study needs to investigate the relationship between protein intake and meal duration with eating patterns to be validated.

The limitations of each study were discussed in detail in the relevant chapters of this thesis. The general limitation of this PhD work was the relatively under powered, small sample sizes used which could not allow for comprehensive comparisons and limit the ability to detect differences and increase risk of random error. The retrospective study (chapter 3) and cross-sectional study (chapter 5) were based on data availability, while the prospective cohort study (chapter 4) presented challenges in recruiting children for the following reasons. First, COVID pandemic and government restrictions delayed the recruitment. Second, school commitments and working parents also limited study recruitment opportunities, as a legal guardian must accompany those who are under 16 years old. Third, we aimed to recruit stable CD participants, which limited our recruitment strategy due to the risk of recurrent disease flare-ups. In this thesis, some

findings were purely exploratory and hypothesis-generating which we acknowledge that some significant results may be false positives. Thus, future longitudinal studies with larger sample sizes are required to strengthen the current findings, enhance their generalisability and allow for more comprehensive comparisons between groups and sex-specific sub-analysis.

In addition, the cross-sectional designs (chapters 4 and 5) do not allow for assessing the impact of disease activity over-time on dietary protein intake, plasma AAs levels, and eating behaviours on stable CD participants when compared with healthy controls (chapter 4), as well as on PCSA in active versus inactive CD patients (chapter 5). Therefore, further prospective cohort studies are required to understand the changes in these outcomes according to disease activity and over-time.

Confounding factors such as Tanner stage classification were not addressed due to the retrospective nature of the studies (Chapters 3 and 5). As a result, pubertal and pre- or post-pubertal stages were determined based on the child's age rather than their actual puberty status. Considering Tanner stage is critical in paediatric studies to reduce assessment bias, as it affects outcomes such as weight and MM. Unadjusted confounders such as height (Chapters 3 and 5), weight (Chapter 5), puberty stage (Chapters 3, 4, and 5), and surgical history (Chapters 3, 4, and 5) were not accounted for due to limited data availability. These factors may have influenced muscle health and other clinical outcomes. Furthermore, the retrospective nature of the study limits the reliability and accuracy of disease activity assessments, as MRI reports alone may not fully capture the clinical condition of participants. The study design also involved several assumptions due to limited data, particularly regarding disease activity, MM, and dietary intake. These assumptions introduce uncertainty and may restrict the generalizability of the findings. Lastly, while whole-body muscle mass was assessed using MRI in Chapter 4, we were only able to analyse leg muscle volume due to the absence of arm muscle data for some participants.

The impact of small sample sizes and participant heterogeneity is a critical consideration in interpreting the findings of this thesis and in detecting differences between comparison groups. Across all included chapters, participants demonstrated variability in factors such as disease duration, treatment regimens (e.g., corticosteroids,

immunomodulators, and biologics), and nutritional status. These differences could significantly influence outcomes such as muscle mass, muscle strength, dietary intake, and eating behaviour. Although efforts were made to match participants based on age, sex, and BMI where possible, key factors like height, pubertal status, and surgical history were not adjusted for due to inconsistent data availability. This unaccounted heterogeneity likely contributed to variability in the results, complicating the detection of true associations.

The effect of random variation is amplified in studies with small sample sizes, making it challenging to identify significant differences. For example, while potential differences in muscle mass and dietary intake between Crohn's disease (CD) and healthy controls (HC) were explored in Chapter 4, the limited sample size may have precluded these differences from reaching statistical significance. This highlights the increased risk of a Type II error, where true differences exist but are not detected due to insufficient statistical power. Consequently, while the results of Chapter 4 suggest no significant differences between CD and HC groups, it is not possible to definitively conclude the absence of a true difference. Instead, the lack of significant findings may reflect the limited power of the study rather than the absence of biological or clinical differences. Moreover, the study may have been underpowered to detect subtle yet clinically meaningful differences.

Adjustments for confounding variables, such as height, puberty stage, or surgical history, could potentially provide clearer insights by accounting for their influences. However, with the current small sample size, adjusting for multiple variables would have further diminished statistical power and increased the risk of overfitting, potentially leading to unreliable results. In future studies, the inclusion of these adjustments will be critical to elucidate the relationships between variables and to strengthen the reliability of findings.

To address these limitations, future research should focus on recruiting larger, more homogeneous sample sizes or employing stratification based on critical variables such as disease severity, treatment type, or growth parameters. A prospective study design would offer the advantage of better control over confounding factors and enable longitudinal analysis of muscle health, dietary intake, and eating behaviour across time.

These approaches will enhance the capacity to detect meaningful differences and improve the generalisability of the findings.

This thesis highlights several areas for further exploration and the studies conducted can serve as a foundation or pilot for a larger, more comprehensive investigation into protein nutrition and eating behaviour in CD paediatric. Future research should be designed as a longitudinal, multi-centre study with a adequately powered larger sample sizes to enhance statistical power, necessary for detecting clinically meaningful differences, assuming the observed effect sizes. Such a study would also allow for a more detailed examination of potential differences between paediatric CD patients and healthy controls.

A longitudinal study is essential to comprehensively understand the impact of CD on nutritional status, dietary intake, and muscle health, critical measures should include muscle mass and strength, assessed through more precise imaging techniques and strength tests to accurately track muscle health; protein metabolism which evaluated through the measurement of circulating amino acids to capture metabolic changes associated with CD, eating behaviour which measured by using validated eating behaviour questionnaires and dietary intake which assessed by using detailed dietary assessments to examine the relationships between disease status, nutrient intake, eating behaviour and change of MM over time. By integrating these measures, the study would provide valuable insights into the complex interactions between disease status, nutrient intake, eating behavior, and changes in muscle mass over time. These findings could inform the development of tailored interventions and strategies to improve disease management and patient outcomes.

The results from this thesis suggest that continued research in this area could yield valuable insights, especially considering the potential impact of nutrition on disease outcomes and quality of life in paediatric CD. However, if future studies with larger cohorts still reveal minimal differences, this may indicate that factors other than dietary intake and protein metabolism play a more significant role in disease progression and muscle health in paediatric CD.

In summary, this PhD thesis included a systematic review, a bioresource retrospective study, one MRI-based analysis, and a prospective study on protein nutrition and eating behaviour in paediatric patients with CD. The utilization and integration of different approaches to assess nutritional status, the impact of early disease onset on disease outcomes, and muscle size, as well as evaluating habitual protein intake, circulating amino acids, eating behaviour, and measuring muscle mass, offer a holistic understanding of protein nutrition assessment in paediatric patients with CD. Although most of the work performed in this thesis did not reveal significant differences in selected outcomes between inactive paediatric CD patients and those with matched active CD or healthy controls, it has opened avenues for additional research to address the limitations identified and broaden our understanding of nutritional status, dietary intake, eating behaviour, and their impact on muscle mass and strength, as well as protein metabolism alongside disease burden and activity in paediatric CD.

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8. APPENDICES

APPENDIX 1– Supplementary Material For Systematic Review: Sarcopenia In Paediatric Inflammatory Bowel Disease

To increase the reference retrieval, each database was individually searched by applying the medical subject headings (MeSH) and free-text searching (376). Search terms were determined through the 'suggested search terms' and 'explode' selection. The Boolean operator 'OR' was used within each facet to maximise the searches and the operator 'AND' was used between facets to combine terms.

Supplementary Table 1.1. Terms used in search engine

Main keywords	Medline	Embase	WoS	Cochrane
#1 - IBD	1. ("inflammatory bowel disease" OR IBD).ti,ab.	1. ("inflammatory bowel disease" OR IBD).mp.	1. ("inflammatory bowel disease*" OR IBD)	1. inflammatory bowel disease* OR IBD
	2. exp Inflammatory Bowel Diseases/	2. exp Inflammatory Bowel Diseases/	-	-
	3. ((colon* OR "small bowel") adj3 inflam*).ti,ab.	3. ((colon* OR "small bowel") adj3 inflam*).mp.	2. ((colon* OR "small bowel") "near" (inflam*))	2. colon* inflam* OR "small bowel inflam*"
	4. (ileitis).ti,ab.	4. (ileitis).mp.	3.TS=ileitis	3. ileitis
	5. exp Ileitis/	5. exp Ileitis/	-	-
	6. (jejunitis).ti,ab.	6. (jejunitis).mp.	4.TS=jejunitis	4. jejunitis
	7. (colitis).ti,ab.	7. (colitis).mp.	5.TS=colitis	5. colitis
	8. exp Colitis/	8. exp Colitis/	-	
	9. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8	9. #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8	6. #1 OR #2 OR #3 OR #4 OR #5	6. #1 OR #2 OR #3 OR #4 OR #5
#2 - Sarcopenia	10. (Sarcopenia).ti,ab.	10. (Sarcopeni*).mp.	7. Sarcopeni*	7. Sarcopeni*
	11. exp Sarcopenia/	11. exp Sarcopenia/	-	-
	12. #10 OR #11	12. #10 OR #11	-	-
	13. (Myopenia).ti,ab.	13. (Myopeni*).mp.	8. Myopeni*	8. Myopeni*
	14. exp Muscular Atrophy/	14. exp Muscular Atrophy/	-	-
	15. (Myosteatosis).ti,ab.	15. (Myosteatos*).mp.	9. Myosteatos*	9. Myosteatos*
	16. (Dynapenia).ti,ab.	16. (Dynapeni*).mp.	10. Dynapeni*	10. Dynapeni*
	17. (muscle adj3 (atroph* or wast* or weak* or frail* or strength or mass or loss)).ti,ab.	17. (muscle adj3 (atroph* or wast* or weak* or frail* or strength or mass or loss)).mp.	11. ((muscle) NEAR/3 (atroph* OR wast* OR weak* OR frail OR strength OR mass OR loss))	11. (muscle NEAR/3 (atroph* OR wast* OR weak* OR frail* OR strength OR mass OR loss))
	18. #12 OR #13 OR #14 OR #15 OR #16 OR #17	18. #12 OR #13 OR #14 OR #15 OR #16 OR #17	12. #7 OR #8 OR #9 OR #10 OR #11	12. #7 OR #8 OR #9 OR #10 OR #11
#3- Age	~~19. (baby OR babies).ti,ab.~~	19. (baby OR babies).mp.	13. (baby OR babies)	13. baby OR babies
	~~20. (infan*).ti,ab.~~	20. (infan*).mp.	14. infan*	14. infan*
	~~21. (p?edatri*).ti,ab.~~	21. (p?edatri*).mp.	15. p$ediatri*	15. Paediatri* OR Pediatri*

	~~22. (child*).ti,ab.~~	22. (child*).mp.	16. child*	16. Child*
	~~23. (adolescen*).ti,ab.~~	23. (adolescen*).mp.	17. adolescen*	17. Adolescen*
	~~24. (teenager*).ti,ab.~~	24. (teenager*).mp.	18. teenager*	18. teenager*
	~~25. (youth*).ti,ab.~~	25. (youth*).mp.	19. youth*	19. youth*
	~~26. #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25~~	26. #19 OR #20 OR #21 OR #22 OR #23 OR #24 OR #25	20. #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19	20. #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19
Sum	27. #9 AND #18	27. #9 AND #18 AND #26	21. #6 AND #12 AND #20	21. #6 AND #12 AND #20
	28. #27 Limit to full text, human, all child (0-18) and English language	28. #27 Limit to full text, human and English language	22. #21 AND English language	22. #21 limit to trials

Supplementary Table 1.2. Cross-sectional studies grades and total scores based on JBI criteria

Author(s), year	Were the criteria for inclusion in the sample clearly defined	Were the study subjects and the setting described in detail?	Was the exposure measured in a valid and reliable way?	Were objective, standard criteria used for measurement of the condition?	Were confounder factors identified?	Were strategies to deal with confounding factors stated?	Were the outcomes measured in a valid and relaible way?	Was appropriate statistical analysis used?	Quality
Motil et al., (1982)	1	0	0	1	0	1	0	1	50%
Ward et al., (2009)	1	1	1	1	0	0	1	1	75%
Wiskin et al., (2010)	1	0	1	1	0	1	0	1	62.5%
Brookes et al., (2016)	1	1	1	1	0	1	1	1	87.5%
Ashton wt al., (2021)	1	1	1	1	0	1	1	0	75%
Ward et al., (2017)	1	1	1	1	0	0	1	1	75%
Mager et al., (2018)	1	0	1	1	0	1	1	1	75%
Trivic et al., (2021)	1	0	1	1	0	1	1	1	75%

Supplementary Table 1.3. Non-randomised studies grades and total scores based on JBI criteria

Author(s), year	Is it clear in the study what is the "cause" and what is the "effect"?	Were the participants included in any comparisons similar?	Was the participants included in any comparisons receiving similar tretment/care, other than the exposure or intervention of interest?	Was there a control group?	Were there multiple measurements of the outcome both pre and post the intervention/exposure?	Was follow up complete and if not, were differences between groups in terms of their follow up adequately and analyzed?	Were the outcomes of participants included in any comparisons measured in the same way?	Were outcomes measured in a relaible way?	Was appropriate statistical analysis used?	Quality
Davis et al., (2019)	1	0	1	1	0	0	1	1	1	66.7%
Werkstetter et al., (2013)	1	0	1	0	1	1	1	1	1	77.8%

Supplementary Table 1.4. Randomised studies grades and total scores based on JBI criteria

Author(s), year	Was true randomization used for assignment of participants to treatment groups?	Was allocation to treatment groups concealed?	Were treatment groups similar at the baseline?	Were participants blind to treatment assignment?	Were those delivering treatment blind to treatment assignment?	Were outcomes assessors blind to treatment assignment?	Were treatment groups treated identically other than the intervention of interest?	Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	Were participants analyzed in the groups to which they were randomized?	Were outcomes measured in the same way for treatment groups?	Were outcomes measured in a reliable way?	Was appropriate statistical analysis used?	Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	Quality
Khoshoo et al., (1996)	1	0	1	0	0	0	0	1	1	1	1	1	1	61.5%

Supplementary Table 1.5. Cohort study grades and total scores based on JBI criteria

Author(s), year	Were the two groups similar and recruited from the same population?	Were the exposures measured similarly to assign people	to both exposed and unexposed groups?	Was the exposure measured in a valid and reliable way?	Were confounding factors identified?	Were strategies to deal with confounding factors stated?	Were the groups/ participants free of the outcome at the start of the study (or at the moment of exposure)?	Were the outcomes measured in a valid and reliable way?	Was the follow up time reported and sufficient to be long enough for outcomes to occur?	Was follow up complete, and if not, were the reasons to loss to follow up described and explored?	Were strategies to address incomplete follow up utilized?	Was appropriate statistical analysis used?	Quality
Werkstetter et al., (2011)	1	1	1	1	1	1	0	1	1	1	0	1	83.3%
Atlan et al., (2021)	1	1	1	1	0	0	0	1	1	1	1	1	75%

Supplementary Table 1.6. Case control study grades and total scores based on JBI criteria

Author(s), year	Were the groups comperable other than the presence of disease in cases or the absence of disease incontrols?	Were cases and controls mtched appropriately?	Was same creteria used for identification of cases and controls?	Was exposure measured in the standard, valid and reliable way?	Was exposure measured in the same way for cases and controls?	Were confounding factors identified?	Were strategies to deal with confounding factors stated?	Were outcomes assessed in a standard, valid and reliable way for cases and controls?	Was the exposure period of interest long enough to be meaningful?	Was appropriate statistical analysis used?	Quality
Wiech et al., (2018)	1	1	1	0	1	0	0	1	1	1	70%

Supplementary Table 1.7. Summary of main characteristics of the studies included in this review

Authors (published year), country	Study duration & design	Study group	Control group	Age (years) mean ±S.D. or median (range)	BMI mean ±S.D. or median (range)	Disease duration years median (range)	Disease Activity PCDAI, PUCAI	Paris or / Montreal classification	Concurrent therapies No. of subjects	History of surgeries No. of subjects	Nutritional intervention	Physical activity
Motil et al., (1982)(253), USA	3-6 months intervention	6 ♂ CD only	5 age-matched healthy controls	15± 1.9	NR	2-5 years	-	-	Corticosteroids (n=4) 5-ASA (n=5) Anti-diarrheal agent (Diphenoxylate) (n=3)	-	1500 ml (Osmolite or Ensure) via NGT overnight for 8-10 hr for 7 months	-
Khoshoo et al., (1996)(252), Canada	3 weeks randomized crossover intervention	14 6 ♂ 8 ♀ CD only	-	13.7± 1.8	15.5± 3.1	-	Mean PCDAI= 52.1± 11.2	-	Corticosteroids (n=10) Metronidazole (n=7) 5-ASA (n=9) Sulfasalazine (n=3)	-	Peptamen and vital HN The target energy intake was 170% of REE	-
Ward et al., (2009)(251), Canada	Cross sectional cohort	20 12 ♂ 8 ♀ CD=17 UC=3	Age and gender-matched healthy reference values	Range (8.4 -17.7) years	NR	-	PCDAI: Mild= 6 Moderate to sever =1 PUCAI: Mild=1 Moderate=1 Sever=1	-	-	-	-	-
Wiskin et al., (2010)(17), UK	Cross sectional cohort	55 35 ♂ 20 ♀ CD=37 UC=18	Unmatched healthy reference values	13.7 (6.5;17.7)	-0.30 (-1.0; 0.7)	-	PCDAI Active CD 59%	-	-	-	-	-
Werkstetter et al., (2011)(254) , Germany	median interval of 2.4 years prospective longitudinal cohort study	30 19 ♂ 11 ♀ CD=23 UC=7	Healthy reference population	Range (6.9- 15.6) years	-1.1 (-3.6; 0.4)	≤ 2 months	PCDAI: Median 27.5= mild PUCAI: Median 25= mild	**CD=** L1 3, L2 3, L3 17, L4 10 B1 16, B2 4, B3 3, p 3 **UC=**	Nil (n=8) Corticosteroid (n=5) 5-ASA (n=16) Sulfasalazine (n=4) Azathioprine (n=6) Nutrition therapy given to CD only for 4 weeks (n=12)	-	-	-

								E1 0, E2 1, E3 6, S0 0, S1 2, S2 5, S3 0	≥ 4 weeks (n=2) Vitamin D and Ca supplementation (n=3)			
Werkstetter et al., (2013)(194), Germany	52 weeks non-randomised intervention	10 7 ♂ 3 ♀ CD only	age-matched reference population	13.7 (10.6;17.7)	-1.3 (-2; 0.2)	-	PCDAI Mild n=3 Moderate n=5 Sever n=2	-	Immunosuppressant (n=10) 5-ASA (n=9)	-	Exclusive Modulen formula via oral or NGT based on energy requirements for 8 weeks and then decreased gradually during 2-4 weeks until transition to full normal diet	-
Brookes et al., (2016)(256) , Australia	Cross sectional cohort	57 42 ♂ 15 ♀ CD only	Healthy reference population	13.6 ±2.2	18.1 ± 0.5	-	PCDAI for 54 subjects: Inactive 43% Mild 26% Moderate to severe 31%	-	-	-	-	-
Ward et al., (2017)(250), Canada	Cross sectional cohort	73 47 ♂ 26 ♀ CD only	Healthy population	13.9 years (7; 17.7) years	Mean z-score -1.0 SD (1.3)	-	PCDAI: Mild 9.6% Moderate to severe 90.4%	-	Nil (n=11) Corticosteroids (n=55) Anti-TNF (n=1) 5-ASA (n=6) Protein pump inhibitor (n=2)		Peptamen junior via NGT (n=1) All given Ca 135mg/day Vitamin D 199 IU/day	
Mager et al., (2018)(13) , Canada	Cross sectional cohort	85 44 ♂ 41 ♀ CD=58 UC=27	age and gender dependent value of SMM in healthy children	13.1 ± 2.9	18.2± 3.6	-	PCDAI: 60.3% subjects had mild PUCAI: 24.3% subjects had mild-moderate	**CD=** A1a 18%, A1b 81%, L1 23%, L2 21%, L3 56%, L4a 37%, L4b 5.4%, B1 70%, B2 18%, B2B3 5.4%, B37% P 31%, G1 39% **UC=** E2 14%, E3 16%, E4 71% S0 57%, S1 43%	-	-	-	-

Wiech et al., (2018)(255), Poland	Case control	59 34 ♂ 25 ♀ CD=25 UC=34	Age and gender-matched healthy control with same number of CD and UC groups	13.7 ± 3.3	19.2±3.6	New diagnosis CD 10 UC 16 Up to 1-year CD 3 UC 4 More than 1-year CD 12 UC 14	PCDAI/ PUCAI Remission CD 7 UC 12 Mild CD 5 UC 8 Moderate CD 9 UC 10 Sever CD 4 UC 4	**CD=** L1 1, L2 2, L3 3, L4b 1, L1/L4b 2, L1/L4b 6, L3/L4a 10 **UC=** P 27, L 3, E 4	CD: Immunosuppressants (n=12) 5-ASA (n=3) UC: Immunosuppressants (n=9) Corticosteroid (n=3) 5-ASA (n=6)	-	-	-
Davis et al., (2020)(197), the UK	2 hours intervention	20 11 ♂ 9 ♀ CD only	9 Age, gender and BMI-matched healthy volunteers	15.6± 0.5	20.6± 0.9	-	Faecal calprotectin and CRP Active (n=9) Deep remission (n=11)	**-**	5-ASA (n=11) Immunosuppressants (n=19) Anti-TNF (n=11)	Intestinal resection (n=5)	220 ml Ensure plus on study	Pedometer IPAQ
Ashton et al., (2021) (249), UK	Cross sectional cohort	10 9 ♂ 1♀ CD only	normal pediatric data from CT scan	Mean 14.6 (11.7-16.3) years	-	-	-	**-**	--	-	-	-
Atlan et al., (2021) (258), Israel	Retrospective cohort study of 11 years	101 43 ♂ 58♀ CD=69 UC=32	87 controls	15±3.3 years	CD= -0.4 [(-1.5)-0.6] UC= -0.1[(-0.8)-0.5]	-	PCDAI/ PUCAI Remission CD 29 UC 12 Mild CD 25 UC 9 Moderate/ severe CD 15 UC 11	**CD=** L1 29, L2 12, L3 28 **UC=** E1 3, E2 7, E3 4, E4 18	Corticosteroid (n=12) 5-ASA (n=45) Immunosuppressants (n=23) Biologic agents (n=27)	-	62% of CD treated with EEN for induction	-

Trivic et al., (2021) (257), Croatia	Cross sectional cohort	40 24 ♂ 16♀ CD=20 UC=18 IBD-U=2	Age and gender reference population	15.3± 0.4 years	z-score of CD= 0.2±1.3 z-score of UC= -0.26±0.94	CD= 48.2± 8.1 months UC= 50.5± 11.6 months	-	**CD=** L1 5, L2 2, L3 13, L4 11 **UC=** E1 & E2 8, E3 5, E4 26	Cumulative oral corticosteroid dose **CD=** 3.5± 5.1 mg/weight/ year **UC=** 12.5±19.3 mg/weight/ year	Intestinal resection= 6 Colectomy and ileostomy formation=1	-	PA measured by fitbit charge 2 with average time spent in moderate to vigorous PA 45.73±8.2 min/day

IBD= Inflammatory bowel diseases (CD+UC), IBD-U= Inflammatory bowel disease unclassified, CD group= Crohn's disease group, UC group= Ulcerative colitis group, ♂= Male, ♀= Female, LM= Lean mass, PCDAI= Paediatric Crohn's Disease Activity Index, PUCAI= Paediatric Ulcerative Colitis Activity Index, CRP= C-reactive protein, 5- ASA= 5- amino salicylic acid, Anti-TNF= anti-tumor necrosis factor, EEN= exclusive enteral nutrition, NGT= Nasogastric tube

APPENDIX 2– SUPPLEMENTARY MATERIAL FOR ASSOCIATION BETWEEN BODY MASS INDEX AND AGE OF DISEASE ONSET WITH CLINICAL OUTCOMES IN PAEDIATRIC-ONSET CROHN'S DISEASE (CD): A UK NATION-WIDE ANALYSES USING THE NIHR-IBD BIORESOURCE

Supplementary Table 2.1. Descriptive characteristics of immunosuppressants, biological exposure and total comorbidities in the whole study population (N= 848)

Outcomes	Number of participants
Immunosuppressants (N)	
Thiopurine	368
Methotrexate	37
Ciclosporin	2
Biological drug (N)	
Infliximab	244
Adalimumab	167
Vedolizumab	41
Ustekinumab	48
Corticosteroids (N)	
Oral steroids (prednisolone or budesonide)	56
IV steroids	0
Mesalazine (N)	91
Total surgeries (N)	
Colectomy and ileostomy	57
Colectomy and ileo-anal pouch	6
Defunctioning ileostomy colostomy	35
Drainage of intra-abdominal abscess	7
Ileal jejunal resection	59
Ileal jejunal stricturoplasty	8
Ileocaecal resection right hemicolectomy	127
Partial_colectomy	22
Proctectomy	9
Stricturoplasty	15
Insertion of seton suture	52
Drainage of perianal abscess	59
Perianal fistula repair	23
Closure of stoma	5
Other	9
Extra-intestinal manifestations (N)	
Primary Sclerosing Cholangitis	9
Enteropathic arthritis	50
Erythema Nodosum	34
Iritis / Uveitis	13
Orofacial Granulomatosis (Oral Crohn's)	37
Psoriasis	48
Ankylosing Spondylitis	7

APPENDIX 3– SUPPLEMENTARY MATERIAL FOR INVESTIGATING PROTEIN NUTRITION IN PAEDIATRIC CROHN'S DISEASE

Supplementary Questionnaire 3.1. The Child Eating Behaviour Questionnaire (CEBQ)

Please read the following statements and tick the boxes most appropriate to your child's eating behaviour.

	Never	Rarely	Some-times	Often	Always	
My child loves food	☐	☐	☐	☐	☐	EF
My child eats more when worried	☐	☐	☐	☐	☐	EOE
My child has a big appetite	☐	☐	☐	☐	☐	SR*
My child finishes his/her meal quickly	☐	☐	☐	☐	☐	SE*
My child is interested in food	☐	☐	☐	☐	☐	EF
My child is always asking for a drink	☐	☐	☐	☐	☐	DD
My child refuses new foods at first	☐	☐	☐	☐	☐	FF
My child eats slowly	☐	☐	☐	☐	☐	SE
My child eats less when angry	☐	☐	☐	☐	☐	EUE
My child enjoys tasting new foods	☐	☐	☐	☐	☐	FF*
My child eats less when s/he is tired	☐	☐	☐	☐	☐	EUE
My child is always asking for food	☐	☐	☐	☐	☐	FR
My child eats more when annoyed	☐	☐	☐	☐	☐	EOE
If allowed to, my child would eat too much	☐	☐	☐	☐	☐	FR
My child eats more when anxious	☐	☐	☐	☐	☐	EOE
My child enjoys a wide variety of foods	☐	☐	☐	☐	☐	FF*
My child leaves food on his/her plate at the end of a meal	☐	☐	☐	☐	☐	SR
My child takes more than 30 minutes to finish a meal	☐	☐	☐	☐	☐	SE

	Never	Rarely	Some -times	Often	Always	
Given the choice, my child would eat most of the time	☐	☐	☐	☐	☐	FR
My child looks forward to mealtimes	☐	☐	☐	☐	☐	EF
My child gets full before his/her meal is finished	☐	☐	☐	☐	☐	SR
My child enjoys eating	☐	☐	☐	☐	☐	EF
My child eats more when she is happy	☐	☐	☐	☐	☐	EUE
My child is difficult to please with meals	☐	☐	☐	☐	☐	FF
My child eats less when upset	☐	☐	☐	☐	☐	EUE
My child gets full up easily	☐	☐	☐	☐	☐	SR
My child eats more when s/he has nothing else to do	☐	☐	☐	☐	☐	EOE
Even if my child is full up s/he finds room to eat his/her favourite food	☐	☐	☐	☐	☐	FR
If given the chance, my child would drink continuously throughout the day	☐	☐	☐	☐	☐	DD
My child cannot eat a meal if s/he has had a snack just before	☐	☐	☐	☐	☐	SR
If given the chance, my child would always be having a drink	☐	☐	☐	☐	☐	DD
My child is interested in tasting food s/he hasn't tasted before	☐	☐	☐	☐	☐	FF*
My child decides that s/he doesn't like a food, even without tasting it	☐	☐	☐	☐	☐	FF
If given the chance, my child would always have food in his/her mouth	☐	☐	☐	☐	☐	FR
My child eats more and more slowly during the course of a meal	☐	☐	☐	☐	☐	SE

SCORING OF THE CEBQ (331).

(Never=1, Rarely=2, Sometimes=3, Often=4, Always=5)

Food responsiveness	=	item mean FR
Emotional over-eating	=	item mean EOE
Enjoyment of food	=	item mean EF
Desire to drink	=	item mean DD
Satiety responsiveness	=	item mean SR
Slowness in eating	=	item mean SE
Emotional under-eating	=	item mean EUE
Food fussiness	=	item mean FF

*Reversed items

Supplementary Questionnaire 3.2. The Child Three-Factor Eating Questionnaire-17 (CTFEQr-17)

Please read the following statements and tick the boxes most appropriate to your eating behaviour.

	Totally true	Mostly true	Mostly false	Totally false
I eat small portions of food to help control my weight.	☐	☐	☐	☐
I start to eat when I feel worried.	☐	☐	☐	☐
Sometimes when I start eating, it seems I can't stop.	☐	☐	☐	☐
When I am sad, I usually eat too much	☐	☐	☐	☐
I don't eat some kinds of food because they can make me fat.	☐	☐	☐	☐
When I am next to someone who is eating, I also feel like eating.	☐	☐	☐	☐
When I feel angry, I need to eat	☐	☐	☐	☐
I often get so hungry that I feel like I could eat loads of food without getting full.	☐	☐	☐	☐
When I am hungry, I feel like to have to eat all of the food on my plate in one go, without stopping	☐	☐	☐	☐
When I feel lonely, I make myself feel better by eating.	☐	☐	☐	☐
I eat less than I want at meal times to stop myself putting on weight.	☐	☐	☐	☐
When I smell or see my favourite food, I find it hard to stop myself from eating it, even if I've just finished a meal	☐	☐	☐	☐
I'm always hungry enough to eat at any time	☐	☐	☐	☐
If I feel nervous, I try to calm myself down by eating	☐	☐	☐	☐
When I see something that looks delicious, I get so hungry that I have to eat it right away.	☐	☐	☐	☐
When I feel really upset, I want to eat.	☐	☐	☐	☐

	Only at mealtimes	Sometimes between meals	Often between meals	Almost always
How often do you feel hungry?	☐	☐	☐	☐

SCORING OF THE CTFEQr-17

The items have been coded as in the original TFEQr21 (377).

Totally true =4, Mostly true=3, Mostly false=2, Totally false=1.

Only at mealtimes=1, Sometimes between meals=2, Often between meals=3, Almost always=4).

Supplementary Table 3.1. Association between protein intake, leg muscle volume and plasma AAs.

Plasma AAs	Protein intake (g/day)	Leg muscle volume (L)
Leucine (μM/L)	r=0.33 P=0.16	r=0.05 P=0.88
Iso-leucin (μM/L)	r=0.24 P=0.30	r=0.10 P=0.75
Valine (μM/L)	ρ=0.01 P= 0.97	ρ=0. P=0.80
Phenylalanine (μM/L)	r=0.27 P=0.25	r=0.05 p=0.89
Methionine (μM/L)	r=0.41 P=0.08	r=.63* p=0.03
Tryptophan (μM/L)	r=0.17 P=0.48	r=0.21 p=0.51
Proline (μM/L)	ρ=0.43 P=0.16	ρ=.80** P=0.002
Tyrosine (μM/L)	r=-0.03 p=0.90	r=0.35 p=0.26
Glutamic Acid (μM/L)	ρ=0.22 P= 0.48	ρ=0.34 P=0.29
Threonine (μM/L)	ρ=0.45 P=0.15	ρ=0.55 P=0.07
Aspartic Acid (μM/L)	ρ=0.43 P= 0.16	ρ=0.51 P=0.09
Histidine (μM/L)	r=0.35 P=0.13	r=0.46 p=0.14
Alanine (μM/L)	r=0.28 P=0.24	r=.687* p=0.014
Glutamine (μM/L)	r=.45* P=0.04	r=.60* p=0.04
Asparagine (μM/L)	r=.46* P=0.04	r=0.57 p=0.06
Cysteine (μM/L)	r=-0.03 P=0.91	r=0.46 p=0.13
Glycine (μM/L)	r=0.35 P=0.14	r=0.36 p=0.26
Serine (μM/L)	r=-0.02 P=0.95	r=0.12 p=0.70
Lysine (μM/L)	r=0.29 P=0.22	r=0.22 p=0.50
Arginine (μM/L)	r=0.22 P=0.36	r=.68* p=0.016
Total EAAs (μM/L)	r=0.39 P=0.09	r=0.35 p=0.26
Total non-EAAs (μM/L)	ρ=0.55 P= 0.06	ρ=0.71* P=0.01

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Pearson correlation (r) was used to test the associations between parametric variables, while Spearman rank correlation (ρ) was used to test the associations between non-parametric variables. AAs= Amino acids, EAAs= Essential amino acids, non-EAAs= Non-essential amnio acids.

Supplementary Table 3.2. Associations between BMI and protein intake and eating behaviour traits in whole population.

Eating Behaviour Traits	BMI (z-score)	Mean Protein Intake (g/day)
CEBQ		
Food approach composite	r=0.21 p=0.36	r=0.25 p=0.28
Food responsiveness	r=0.34 p=0.13	r=0.25 p=0.28
Emotional overeating	ρ=0.19 p=0.42	r=0.13 p=0.58
Enjoyment of food	r=0.37 p=0.10	r=0.28 p=0.22
Desire to drink	r=0.10 p=0.68	r=-0.16 p=0.48
Food avoidance composite	r=0.07 p=0.77	r=-0.19 p=0.42
Satiety responsiveness	r=-0.09 p=0.68	r=-0.47* p=0.03
Slowness in eating	r=-0.46* p=0.04	r=-0.43* p=0.05
Emotional undereating	r=-0.30 p=0.19	r=0.19 p=0.41
Food fussiness	r=0.40 p=0.08	r=-0.07 p=0.78
CTFEQr-17		
Uncontrolled eating	r=0.02 p=0.94	r=0.11 p=0.61
Cognitive restraint	r=0.02 p=0.93	r=-0.42* p=0.05
Emotional eating	r=-0.04 p=0.87	r=-0.19 p=0.39

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

CEBQ= Child Eating Behaviour questionnaire, CTFEQr-17 = Child Tree-Factor Eating Questionnaire-17, BMI= Body mass index. Pearson correlation (r) was used to test the associations between parametric variables.