The muscle-bone unit in children and adults with Crohn's disease

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Abstract

Background. Crohn's disease (CD) is associated with multifactorial insult to the muscle-bone unit. The independent and combined effects of chronic inflammation, undernutrition, and exposure to glucocorticoids adversely affect bone and muscle mass accrual and maintenance in paediatric and adult CD, respectively.

Aim. The overall aims of this thesis were to assess the muscle-bone unit in children and adults with CD and investigate the potential role for exercise to improve musculoskeletal outcomes. High-resolution MRI was used to comprehensively assess muscle-bone outcomes in paediatric and young adult with childhood onset CD managed with contemporary treatment strategies. Associations between CD and IBD with adverse musculoskeletal outcomes, in particular the risk of falls, were explored in a large population-based cohort of middle-aged and older adults using data from the UK Biobank research study. Subsequently, to explore the potential utility of exercise in CD and IBD, a systematic review was performed. Finally, the feasibility of jumping based exercise in CD was explored via an online survey followed by a short-term jumping-based exercise intervention in paediatric CD.

Results. The primary results highlight persistent muscle-bone deficits in CD across different age groups, despite currently well controlled disease and contemporary management. High-resolution MRI revealed deficits in trabecular microarchitecture in paediatric but not young adult CD. Paediatric and young adult with childhood onset CD were both associated with poor muscle function, and young adults had around 20% lower muscle area, compared to healthy populations. Older adults with CD also displayed higher likelihood of pathological muscle weakness and falls compared to age-matched controls. Systematic review revealed little evidence on the utility of exercise for managing muscle-bone deficits in CD, although some studies identified a benefit of exercise to muscle function and bone mass and mild improvements in HRQoL. Particularly, the evidence for exercise in young or paediatric CD was almost non-existent. The feasibility survey suggested jumping exercise to be an acceptable and feasible intervention in adolescents and young adults with CD, with most respondents stating a willingness to exercise and intentions to participate in future exercise

research. In feasibility study of jumping exercise, the recruitment rate was lower than expected from survey. Still, jumping exercise appeared feasible as the small number who participated achieved high adherence to the protocol.

Conclusions. This research provides valuable insight into the muscle-bone unit in CD across different age groups. Even in well controlled disease and under contemporary management, CD remains associated with adverse musculoskeletal outcomes, including low trabecular bone volume in paediatrics and low muscle mass in young adults. Poor muscle function was characteristic in these cohorts and was linked to increased risk of falls in early old adults with CD. These data further highlight the adverse muscle-bone unit in CD and emphasise the need for effective non-pharmacological strategies to address this. Preliminary data suggests exercise to be a feasible strategy for targeting muscle-bone outcomes in CD and future research should investigate this further.

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Publications

Steell L, Johnston BA, Dewantoro D, Foster JE, Gaya DR, Macdonald J, McMillan M, Russell RK, Seenan JP, Ahmed SF, Gray SR & Wong SC. Muscle deficits with normal bone microarchitecture and geometry in young adults with well-controlled childhood onset Crohn's disease. *European Journal of Gastroenterology & Hepatology* (2020), 32(12): 1497-1506. doi: 10.1097/MEG.0000000000001838.

Abstracts

Steell L, Johnston BA, Dewantoro D, Foster JE, Gaya DR, Macdonald J, McMillan M, Russell RK, Seenan JP, Ahmed SF, Gray SR & Wong SC. High-resolution MRI assessment of the muscle-fat-bone unit in young adults with childhood onset Crohn's disease. *Proceedings from the 52nd annual meeting of the European Society for Paediatric Gastroenterology, Hepataology and Nutrition (ESPGHAN), Glasgow, 2019. Journal of Paediatric Gastroenterology and Nutrition* (2019). 68: 603-604. doi: 10.1097/MPG.0000000000002403. *[Poster presentation]*

Steell L, Johnston BA, Dewantoro D, Foster JE, Gaya DR, Macdonald J, McMillan M, Russell RK, Seenan JP, Ahmed SF, Gray SR, Wong SC. High-resolution MRI assessment of the muscle-fat-bone unit in young adults with childhood onset Crohn's disease. *Proceedings from the 9th International Conference on Children's Bone Health (ICCBH), Salzburg, 2019. Bone Abstracts* (2019). 7: P100. doi: 10.1530/boneabs.7.P100. *[Poster presentation*]

Steell L, Gaya DR, Macdonald J, Russell RK, Seenan JP, Wong SC & Gray SR. High-impact exercise to improve musculoskeletal outcomes in Crohn's disease: a feasibility questionnaire. *Proceedings from the 9th International Conference on Children's Bone Health (ICCBH), Salzburg, 2019. Bone Abstracts* (2019). 7: P185. doi: 10.1530/boneabs.7.P185. *[Oral & Poster presentation]*

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This thesis is dedicated to my fiancée, Ruth. For everything you do, thank you.

Author's Declaration

I declare, except where reference is made to the contribution of others, that all work presented in this thesis was performed entirely by myself and has not been submitted for any other degree at the University of Glasgow, or any other institution.

Mr Lewis Steell

I certify that, except where reference is made to the contribution of others, the work reported in this thesis has been performed entirely by Mr Lewis Steell and during the period of study he has fulfilled the conditions of ordinance and regulations governing the Degree of Doctor of Philosophy, University of Glasgow.

Dr Stuart R Gray

Definitions/Abbreviations

Abbreviation	Definition
^{1}H-MRS	Proton magnetic resonance spectroscopy
aBMD	Areal bone mineral density
Anti-TNF-α	Anti- tumour necrosis factor alpha
appBVTV	Apparent bone volume/total volume ratio
appTbTh	Apparent trabecular thickness
appTbN	Apparent trabecular number
appTbSp	Apparent trabecular separation
B_0	External magnetic field
BMA	Bone marrow adiposity
BMAD	Bone mineral apparent density
BMC	Bone mineral content
BMI	Body mass index
BAP	Bone-specific alkaline phosphatase
BUA	Broadband attenuation
CD	Crohn's disease
CP	Cerebral palsy
CT	Computed tomography
CTX-1	Crosslinked telopeptide of type 1 collagen
DMD	Duchenne muscular dystrophy
DXA	Dual energy X-ray absorptiometry
EEN	Exclusive enteral nutrition
Endo. Circ	Endosteal circumference
FAP	Fibro adipogenic progenitors
FF%	Fat fraction percentage
FM	Fat mass
FoV	Field of view
FRAX	Fracture risk algorithm
GC	Glucocorticoids
GH	Growth hormone
HR	Hazard ratio
HRQoL	Health-related quality of life
IBD	Inflammatory bowel disease
IGF-1	Insulin like growth factor 1
IGFBP	Insulin like growth factor binding protein
IL-	Interleukin
IPAQ	International physical activity questionnaire
Kg	Kilograms
kN	Kilonewtons
kW	Kilowatts
LS	Lumbar spine
LM	Lean mass
M1LH	Multiple one-legged hopping
MAPK	Mitogen activated protein kinase
M-CFS	Macrophage colony stimulating factor
M-CSA	Muscle cross sectional area
mm	Millimetres
MRI	Magnetic resonance imaging

MSC	Mesenchymal stem cells
MVPA	Moderate to vigorous physical activity
N	Newtons
ND	Non-dominant
NMV	Net magnetic vector
OC	Osteocalcin
OPG	Osteoprotegerin
OR	Odds ratio
PA	Physical activity
PCDAI	Paediatric Crohn's disease activity index
Peri. Circ	Periosteal circumference
PPARγ	Peroxisome proliferator activated receptor gamma
pQCT	Peripheral quantitative computed tomography
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
RANKL	Receptor Activator of Nuclear Factor-kappa B Ligand
RF	Radio frequency
RHC	Royal hospital for children
RMA	Residual muscle cross sectional area
Runx2	Runt related transcription factor 2
ROI	Region of interest
RT	Resistance training
S2LJ	Single two-legged jump
SDS	Standard deviation scores
SNR	Signal to noise ratio
SoS	Speed of sound
SSI	Strength strain index
T1	Longitudinal magnetisation
T2	Transverse magnetisation
TB	Total body
TH	Total hip
TE	Echo time
T2D	Type 2 diabetes mellitus
TBLH	Total body less head
TR	Time to repetition
TSE	Turbo spin echo
UC	Ulcerative colitis
μCT	Micro computed tomography
vBMD	Volumetric bone mineral density
VF	Vertebral fracture
W	Watts
wPCDAI	Weighted paediatric Crohn's disease activity index
95% CI	95% Confidence interval

1 Introduction

1.1 Crohn's Disease

1.1.1 Overview

Crohn's disease (CD) is a chronic immune related disorder characterised by cyclical remitting and relapsing inflammation of the gastrointestinal (GI) tract. CD, alongside ulcerative colitis (UC), comprise the two main sub-types of inflammatory bowel disease (IBD). IBD is classified as either CD or UC based on endoscopic, histological, and radiological investigations, in line with accompanying clinical context. While CD and UC have some overlapping clinical characteristics, they have distinct differences which facilitate differential diagnoses and appropriate clinical response. Continuous inflammation of the mucosa and sub-mucosa that is confined to the colon is confirmatory of UC [1]. Transmural inflammation localized to any region of the GI tract from mouth to anus, together with distinct characteristics such as cobblestone appearance, skip lesions, stricturing, or fistulae, are suggestive of CD [1,2]. A third sub-type of IBD known as IBD-unclassified (IBDU) may be diagnosed based on pathology that is indeterminable as CD or UC. Classification of CD on the bases of age at diagnosis, disease location, and disease behaviour, is conducted according to the Paris disease classification [3].

1.1.2 Aetiology and pathogenesis

The aetiology of CD is complex, and the mechanisms not fully understood. Consensual understanding of CD pathogenesis suggests a major role and interaction between genetic and environmental factors, leading to intestinal dysbiosis [2]. Genetic influence on CD pathogenesis is recognised by common familial aggregation [4] and the identification of multiple susceptibility loci [5]. Most of the disease risk, however, is thought to be through exposure to specific environmental risk factors. A recent umbrella review of meta-analyses reported positive associations between onset of CD and multiple environmental risk factors. These included smoking, antibiotic exposure, vitamin D deficiency, and physical inactivity [6]. The adverse associations between smoking [7] and antibiotic exposure [8] with CD onset could be linked to negative alterations in gut microbiota.

Conversely, PA may promote positive changes in the microbiome [9] and is associated with reduced circulating pro-inflammatory cytokines [10,11], which could potentially confer protective effects against the onset of CD. Direct causal links between these factors and risk of CD, however, have not been established.

Disease pathogenesis occurs when epithelial barrier function becomes degraded, allowing the translocation of luminal antigens into the intestinal wall [2]. This triggers immune cell activation and expression of pro-inflammatory cytokines [12]. Anti-inflammatory and regulatory mechanisms are inhibited in CD, resulting in an inability to adequately resolve acute inflammatory episodes. Activated immune cells thus become dysregulated and produce excessive levels of pro-inflammatory cytokines, leading to chronic inflammation and tissue destruction [12]. Cytokines play a key role in CD pathogenesis and have been identified as contributory to intestinal inflammation, including tumour necrosis factor-α (TNF-α), interleukin (IL) -1β and IL-6 [13]. Furthermore, abundant pro-inflammatory cytokines also influence systemic effects and extra-intestinal manifestations of disease, such as musculoskeletal insult and growth failure in paediatric patients. The specific actions of pro-inflammatory cytokines on bone and muscle are discussed in Section 1.4.2.1.

1.1.3 Epidemiology

The epidemiology of CD lends further insight into the influence of genetic and environmental factors on disease pathogenesis. Disease prevalence is highest in industrialised regions such as Europe (250 - 320 per 100,000 in Norway, UK, & Germany) and North America (~300 per 100,000 in Canada) [14,15]. Recent estimates suggest a population prevalence of approximately 0.8% for IBD in Scotland, which is projected to increase to ~1.2% within the next decade [16]. This increasing prevalence is due to low mortality and stable disease incidence rates, as observed in other high-income countries. Low-income countries have experienced a rapid increase in IBD incidence that parallels their rate of industrialisation and adoption of a ‘westernised’ lifestyle. For example, Brazil and Taiwan experienced annual percentage increase in CD incidence of +11% and +4%, respectively, across approximately two decades (1990 - 2010) [14]. The emergence of IBD as a global disease highlights the need for cost-effective management strategies that will help alleviate the burden of disease independently of, or in conjunction with,

costly pharmacological therapies. The peak age of diagnosis occurs between the second and fourth decade of life, with a further spike in incidence between 50 to 60 years [2]. Approximately 25% of all new cases of CD are diagnosed in the paediatric age (age <17 years) with a peak age of onset between 10-14 years, concordant with a critical period of growth and pubertal development [17].

1.1.4 Clinical characteristics

Presenting symptoms of CD can vary widely, however the classic triad of symptoms are unsolicited weight loss, abdominal pain, and chronic diarrhoea [18]. These symptoms alone are not definitive of CD and are often mistaken as infective pathology. The onset of CD is insidious, resulting in prolonged period of symptoms prior to diagnosis. Diagnostic delay in CD has been associated with poorer disease outcomes, including increased need for surgery in adults [19], and more complicated disease and associated growth disturbance in paediatric patients [20]. In children with CD, active inflammation can disrupt growth and pubertal development, and these can often be presenting clinical features prior to the onset of any gastrointestinal symptoms. Extra-intestinal manifestations are very common in CD, occurring in up to 50% of patients [21]. These can be present at diagnosis or can develop later in the disease course. The most common secondary complications include arthralgia, osteopenia/osteoporosis, cutaneous involvement, and growth disturbance and pubertal delay in children [21].

1.1.5 Assessment of disease activity

Ongoing assessment of disease activity is a critical component in CD management and is conducted through various subjective and objective measures. In clinical practice, patients are routinely assessed using the validated Crohn's disease activity index (CDAI; aged ≥18 years) [22] or its paediatric equivalent (PCDAI; aged <18 years) [23]. These provide an assessment of disease activity based on seven-day history of symptoms, a physical examination, biochemical parameters, and extra-intestinal manifestations. Disease activity is categorised as clinical remission (CDAI <150; PCDAI <12.5), mildly active (150 – 220; 12.5 – 40), moderately active (221 – 450; >40 – 57.5) or severe (>450; >57.5). A mathematically weighted PCDAI (wPCDAI) was developed that better discriminates between disease activity categories than the original and is a more feasible tool in clinical practice [24]. The

threshold for a clinically meaningful response in CDAI has been reported as a reduction >100 points, whereas for the wPCDAI it is defined as a reduction of >17.5 points. These indexes have an important role in clinical practice as they provide rapid feedback on current symptoms. Despite this, the subjectivity of reporting means they correlate poorly with objective measures of disease such as endoscopy [25]. However, endoscopic assessment is highly invasive and administered only when disease reassessment is clinically indicated.

Faecal calprotectin has recently emerged as a useful objective marker of disease activity in CD for both diagnostic and monitoring purposes [26]. Inflammatory insult to the intestines causes leakage of neutrophils into the lumen, of which calprotectin accounts for ~60% of the cytosolic protein [27]. Faecal calprotectin can detect subclinical inflammation not picked up by CDAI [27] and can be used to predict response to therapy. Resultantly, calprotectin assessment has become routine in clinical practice and is used in conjunction with disease indexes to navigate clinical management.

1.1.6 Disease management

Management of CD prioritises effective symptom control through two core phases of treatment. At diagnosis, or during active disease flare, induction therapy is used to rapidly treat active inflammation and achieve remission [28]. Maintenance therapy is then introduced to sustain remission for as long as possible, avoiding subsequent disease flares. Beyond symptom control, therapeutic goals are to improve intestinal healing, optimise nutritional status, and optimise growth and development in paediatric patients [29]. Choice of pharmacological therapy is directed by national and international guidelines, dependant on disease location and phenotype [29]. Response to therapy varies widely among patients and should be tailored to the individual. Individuals who fail to respond to conventional therapies may advance to surgical intervention.

Historically, glucocorticoids (GC) have been first line induction therapy for severe CD. GC are highly effective, achieving a therapeutic response in up to 80% of patients [30,31]. Despite high levels of clinical response, GC therapy is associated with a significant side-effect profile including mood disturbances, weight gain, and increased cardiovascular risk [32]. Particularly, GC also have profound and

devastating effects in bone and muscle [33]. The specific actions of GC in bone and muscle are highlighted in section 1.4.2.3. Briefly, they are associated with rapid and sustained bone loss [34], catabolism and fat infiltration of skeletal muscle [35], and contribute to growth and pubertal delays in children [36,37]. The systemic toxicity of GC led to incorporation of GC free remission as a primary treatment outcome in CD research and clinical management. GC are not suitable as maintenance therapy and their use is closely monitored and tapered as quickly as is clinically viable to reduce risk of adverse effects.

Exclusive enteral nutrition (EEN) has become preferred first line induction therapy for paediatric CD patients in the UK and other regions [29]. This therapy consists of an eight-week, exclusively liquid diet and requires ongoing dietetic support to promote adherence. EEN has similarly high efficacy compared to GC, with up to 80% achieving clinical remission [38]. EEN, however, has few adverse side-effects and is associated with greater mucosal healing [39] and improved nutritional profile [40] compared to GC. Adults with CD are not routinely treated with EEN as it was associated with significantly lower likelihood of clinical remission compared to GC in meta-analysis (Pooled OR: 0.65 [95% CI: 0.52, 0.82]) [41]. These data were almost certainly influenced by non-adherence to therapy, with up to 40% of participants in some studies withdrawing from EEN due to issues with palatability. The restrictive nature of EEN means it is not a viable maintenance therapy, although research is ongoing to replicate the nutritional profile of EEN in a solid food-based diet. Preliminary data are promising, showing improved intestinal inflammation, good palatability, and positive influence on the gut microbiome [42].

The advent of cytokine-targeting biological therapies has transformed care in CD and IBD. Antibodies targeting tumour necrosis factor alpha (TNF-α) and other cytokines have become cornerstone treatments in IBD. Early use of anti-TNF-α biologics in paediatric CD was around 15-20%, whereas now their use is seen in >50% of patients [43]. Biologics are used as both induction and maintenance therapy in CD, often with concurrent immunosuppression from thiopurines or methotrexate [28,29,44]. Combination therapy has been shown to improve drug levels while reducing formation of antibodies, thus delaying loss of response [45]. Early introduction of anti-TNF-α has been associated with higher steroid- and surgery-free remission in children at one year, and lower rates of osteoporosis, reduced

risk of bowel stenosis, and fewer healthcare consultations in adults [46]. In paediatric patients, anti-TNF-α are associated with improvements in linear growth and biomarkers of muscle-bone [47-49], and reduced resection rates [50], although there is limited data on their long-term effects. Older adults with CD are less likely to be prescribed anti-TNF-α due to increased risk of opportunistic infections and issues with polypharmacy [51].

There is currently a lack of data regarding the utility of non-pharmacological interventions in the management of CD. Interventions that can be implemented alongside pharmacological therapy warrant investigation as a way of potentially alleviating the high healthcare burden that is associated with CD.

1.2 The muscle-bone unit

1.2.1 Overview

Bone and skeletal muscle are intricately linked across the lifespan, evidenced by their closely paralleled development, maintenance, and subsequent degradation. The mechanical relationship between bone and muscle is long established; however, bone and muscle have been also observed as important reciprocal regulators of one another through numerous crosstalk mechanisms. From this knowledge, the concept of a combined 'muscle-bone unit' has been developed, whereby their inherently linked physiology suggests one cannot be considered independently of the other. This section will briefly summarise the independent anatomy and functions of bone and skeletal muscle and review the mechanical and physiological relationships that underpin the muscle-bone unit. Adiposity of skeletal muscle and bone marrow will be reviewed, and regulators of the muscle-bone unit across the lifespan will also be discussed. Finally, the topic of osteoporosis will be introduced.

1.2.2 Bone

1.2.2.1 Bone: function and anatomy

Bone tissue is highly dynamic and serves multiple physiological functions. The skeleton provides physical apparatus for movement and protection of organs, contributes to haematopoiesis, regulates mineral homeostasis, and conducts important metabolic and endocrine functions.

Bone is a composite material with structures hierarchically arranged from micro- to macroscopic level. Approximately 25% and 65% of bone mass is organic and inorganic material, respectively; with the remaining ~10% being water [52]. The organic phase is predominantly (90%) type I collagen and non-collagenous proteins (10%). The inorganic phase is composed primarily of impure hydroxyapatite crystals. Microscopically, bone is comprised of an abundant network of type I collagen fibrils strengthened by the mineralised hydroxyapatite. These material properties provide the mechanical foundation of bone strength and any abnormalities in organic or inorganic structure can have marked effects on skeletal integrity [52].

Bone has two macrostructural compartments: cortical bone and trabecular bone. Cortical bone is a compact structure that constitutes the outer shell of most bones and the diaphyseal shaft of long bones. Cortical bone is characterised by the appearance of concentric lamellar bone organised as osteons around a central Haversian canal, through which vascular and neural networks travel (Figure 1-1). Cortical bone accounts for approximately 80% of total bone mass and has low porosity, thus providing great stiffness and high resistance to stress but low resistance to strain [53].

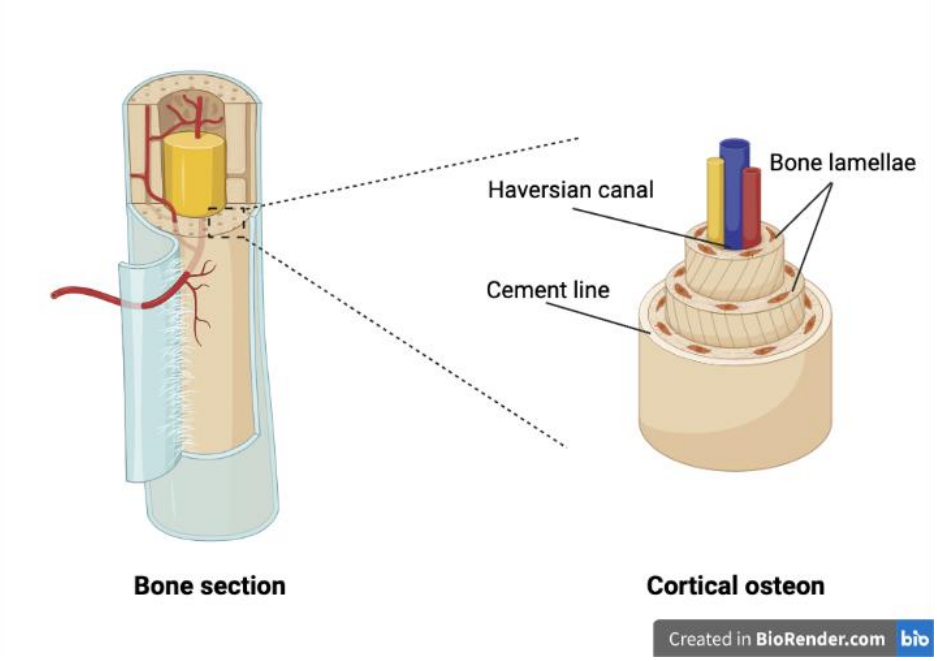

Figure 1-1 Structural arrangement of cortical bone.
Cortical bone structure is comprised of a network of osteons, each of which contain several layers of concentric lamellar bone formed around a central haversian canal. Created with BioRender.com.

Trabecular bone, conversely, is a highly cancellous structure which occupies the metaphysis of long bones, plus the vertebrae and ribs. Trabecular bone is comprised of a lattice microarchitecture of plate and rod like struts anywhere from 50-800μm thick (Figure 1-2). The high porosity and interconnected architecture of trabecular bone provides better elasticity compared to cortical bone [53]. This improves resistance to strain and the ability to distribute mechanical loading effectively. Additionally, trabeculae preferentially orientate along the axis from which they experience the greatest mechanical strains (Figure 1-2), highlighting their mechanical efficiency and biomechanical sensitivity [54,55].

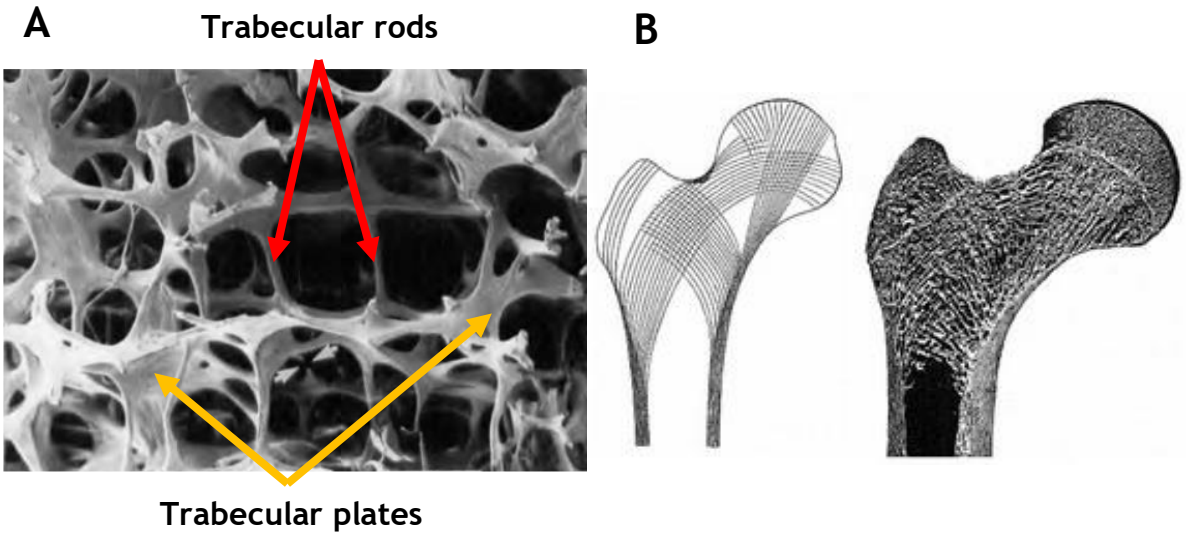

Figure 1-2 Trabecular bone structure.
Trabecular bone structure within the (A) vertebrae and (B) proximal femur. (A) Trabecular plates are thicker and more mechanically efficient compared to trabecular rods [Adapted from Mosekilde [56]]. (B) The organised orientation of trabeculae parallel to the axis of greatest mechanical strain is clear within the proximal femur [Adapted from Wolff [55]].

1.2.2.2 Bone cells

Bone cells, namely osteoblasts, osteoclasts, and osteocytes, each have distinct functions within bone.

Osteoblasts are bone forming cells derived from the same bone marrow mesenchymal stem cells (MSCs) as adipocytes. MSCs are committed to osteoblast lineage under direction of key transcription factors such as *Runx2* [57]. Mature osteoblasts make organic bone matrix (osteoid) by secreting large volumes of type I collagen together with other non-collagenous proteins [58]. These cells have a short lifespan and undergo apoptosis after cessation of bone formation. Some, however, become embedded in the mineralised matrix and terminally differentiate into osteocytes. Osteoblasts partly regulate bone turnover through the relative expression of receptor activator of nuclear factor κB ligand (RANKL) and osteoprotegerin (OPG), which promote and inhibit osteoclastogenesis, respectively [59].

Osteoclasts are multinucleated bone resorbing cells derived from the monocyte-macrophage lineage (Figure 1-3). Pre-osteoclasts differentiate and fuse together under the influence of RANKL and macrophage colony stimulating factor 1 (M-CSF) [60]. These are the only cells capable of resorbing bone and do so by secreting

protons which acidify the hydroxyapatite in bone. Osteoclasts undergo apoptosis after bone resorption; however, their lifespan can be prolonged in the presence of GC excess or elevated pro-inflammatory cytokines [34,61].

Osteocytes are the predominant cells in bone, accounting for 90 - 95% of all skeletal cells in adults [62]. These cells are terminally differentiated osteoblasts that have become embedded in the mineralised matrix. Osteocytes develop a dendritic morphology which creates a neural like network in bone through which signalling molecules can be transmitted (Figure 1-3). Osteocytes are the mechanosensory cells within bone and are regarded as the master regulators of bone turnover, expressing various molecules, and activating signalling pathways in the context or absence of mechanical loading [63]. Mechanical loading downregulates osteocyte derived production of the osteoblast inhibitor sclerostin and increases production of the decoy RANKL receptor OPG, resulting in net formation activity [64]. Conversely, mechanical disuse is associated with increased osteocyte expression of sclerostin and RANKL, and reduced OPG expression, leading to net bone resorption activity [64].

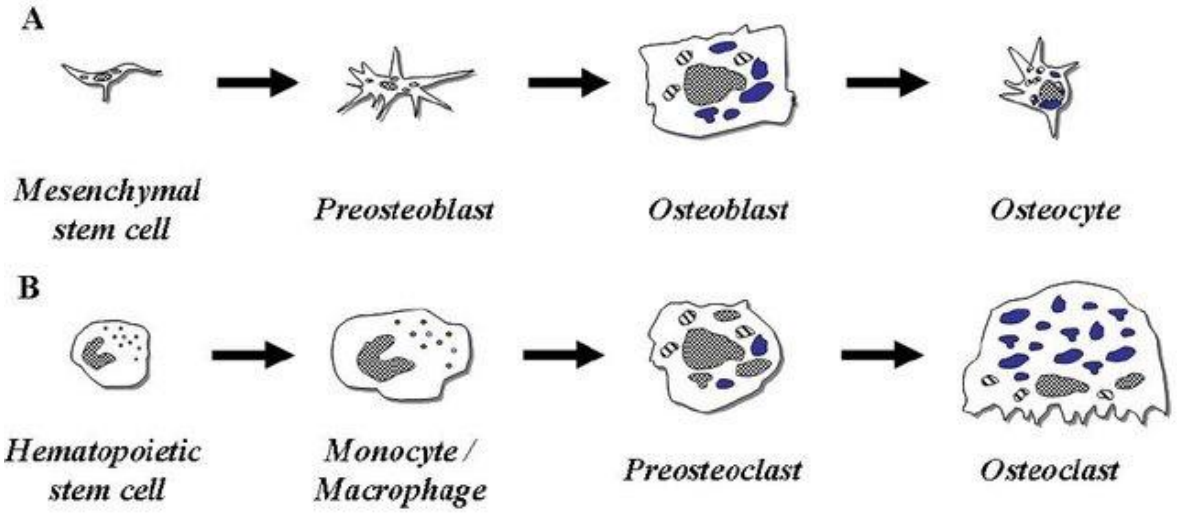

Figure 1-3 Lineage of osteoblasts, osteoclasts, and osteocytes.
Illustration of bone cell lineage. MSCs differentiate into pre-osteoblasts under direction of *Runx2* and some eventually terminally differentiate into osteocytes. Osteocytes develop their dendritic morphology post-differentiation to create a neural network and facilitate transfer of signalling molecules. Osteoclasts are from monocyte-macrophage lineage and differentiate under direction of RANKL and M-CSF into large, multinucleated cells.

1.2.2.3 Bone growth and modelling

Osteogenesis begins at around week seven of embryonic development and continues throughout the lifespan. Bone modelling refers to the independent and uncoupled processes of osteoblastic bone formation (i.e. 'formation modelling') or osteoclastic bone resorption (i.e. 'resorption modelling'), each of which are critical during bone growth. Longitudinal growth occurs at the epiphyseal growth plates in the long bones of the axial and appendicular skeleton via endochondral ossification. The growth plate is delineated into morphologically distinct zones that are classified based on their primary cellular processes (Figure 1-4). Chondrocytes rapidly proliferate and hypertrophy, which creates longitudinal growth in the bone. The chondrocytes then signal adjacent blood vessels to begin vascularisation of the area before undergoing apoptosis and releasing vesicles that promote mineralisation of the extracellular matrix. Finally, bone tissue is formed at the zone of ossification by newly recruited osteoblasts [65]. As bones grow longitudinally, formation modelling creates new metaphyseal trabeculae while older trabeculae undergo resorption modelling. This resorption modelling is necessary to remove trabeculae from the expanding diaphyseal shaft and create greater capacity for marrow storage. In humans, epiphyseal growth plates eventually fuse and become ossified, shortly after which linear growth ceases.

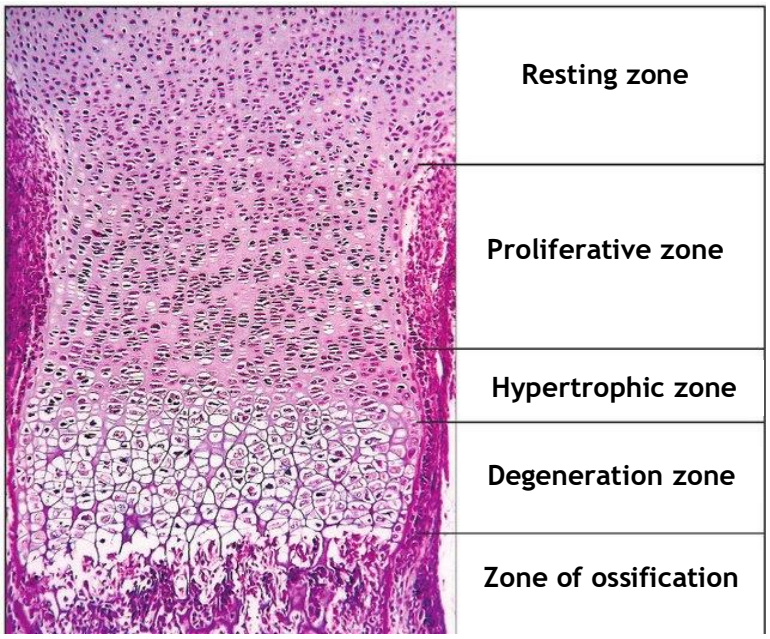

Figure 1-4 Histology of the epiphyseal growth plate.
(Adapted from Klepacz et al [66])

To maintain mechanical integrity, bones must increase their width as they increase their length. Failure of bones to grow radially proportionate to length would increase susceptibility to bending stresses and ultimately fracture [67]. Radial growth occurs via intramembranous ossification and is carefully coordinated to maintain the mechanical strength of bone. At the diaphysis, osteoblasts add bone tissue to the inner layer of the periosteum, known as periosteal apposition. Concurrently, endocortical resorption modelling takes place to increase the diameter of the marrow cavity [68] (Figure 1-5). This has the overall effect of increasing the diaphyseal width. Periosteal apposition also exceeds endocortical resorption, thus increasing cortical thickness and strength [69]. At the metaphysis, endocortical apposition modelling and periosteal resorption modelling occur, ultimately reducing the diameter of the metaphysis to fit the expanding diaphysis (Figure 1-5). Radial growth occurs much more slowly than longitudinal growth, especially during periods of rapid linear growth. This may create a transient mechanical deficit that is thought to contribute to the increased incidence of fractures observed in early pubertal children [70]. Unlike longitudinal growth, periosteal apposition continues throughout the adult lifespan, although at a much-reduced rate. Longitudinal and radial growth and their effects on bone strength are regulated by several physiological and mechanical factors (discussed in section 1.2.5).

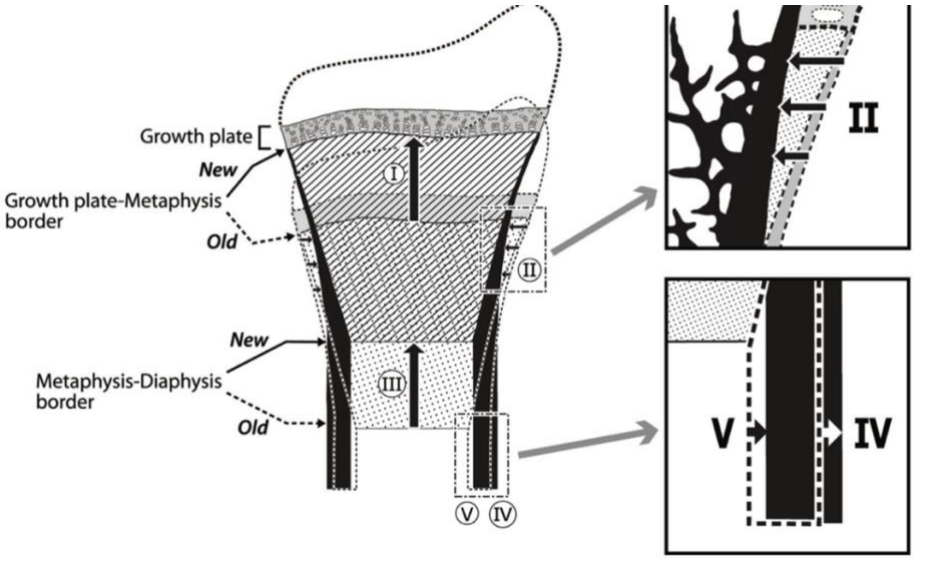

Figure 1-5 Bone growth and modelling at the diaphysis and metaphysis.
(I) Longitudinal growth occurs via endochondral ossification. (II) Periosteal resorption occurs to fit the old metaphysis with the diaphysis (metaphyseal in-wasting). (III) Central metaphyseal resorption modelling occurs to create space for marrow storage in the newly formed diaphysis. (IV & V) Periosteal apposition and endocortical resorption modelling occur simultaneously to expand the medullary cavity [From Rauch, 2012 [71]].

1.2.2.4 Bone remodelling

Bone remodelling relates to the coordinated and coupled activity of osteoblasts and osteoclasts at a single site, whereby old bone is resorbed and replaced by new tissue. The remodelling cycle occurs within a bone multicellular unit (BMU), controlled by the RANKL/OPG system and other factors [59]. Remodelling occurs in five distinct phases (Figure 1-6). First, osteoclast precursors are recruited, then differentiate and fuse to become mature multinucleated osteoclasts (*Activation* phase). Osteoclasts then bind to the bone surface and begin actively dissolving mineral and detaching collagen fragments (*Resorption* phase). Resorption eventually ceases and osteoblasts are recruited to begin bone formation under the direction of several growth factors including IGF-1 and transforming growth factor beta (TGF-β) [72] (*Reversal* phase). Osteoblasts subsequently lay new type I collagen rich organic matrix within the resorption cavity for the deposit of inorganic hydroxyapatite (*Formation* phase). Finally, bone formation ceases, and the bone surface is covered with bone lining cells (*Quiescent* phase). In healthy individuals a typical bone remodelling cycle is thought to take approximately 3-4 months, although mineralisation of newly formed matrix continues for several months thereafter [73].

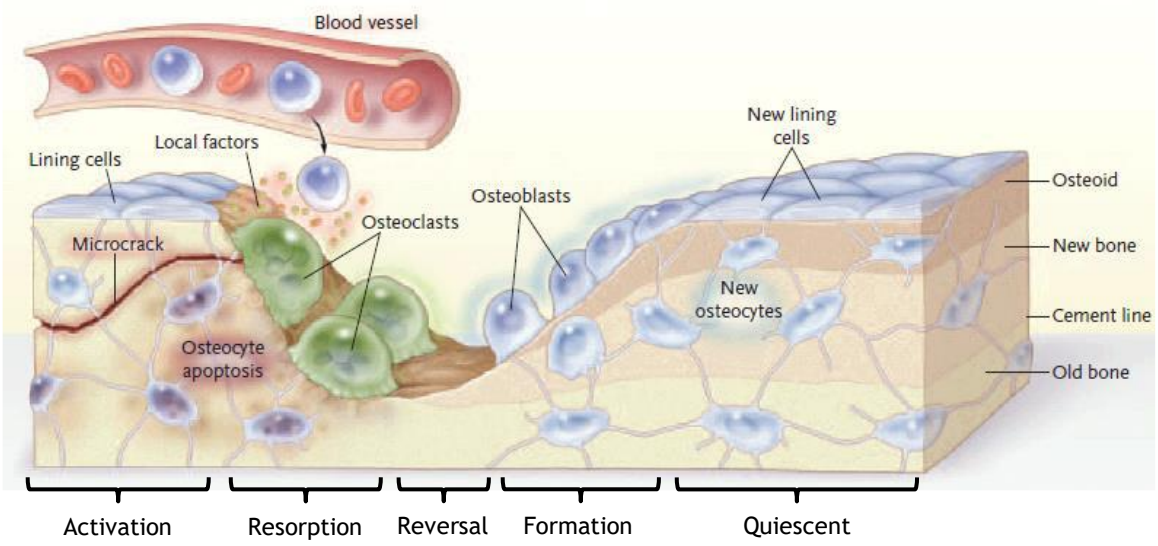

Figure 1-6 Bone remodelling cycle.
Illustration of sequential bone remodelling cycle in trabeculae. Osteocytes sense microdamage in canaliculi and signal osteoblast lineage cells to express RANKL and M-CSF to activate osteoclastogenesis (activation). Activated osteoclasts bind to and resorb the bone matrix (resorption). Osteoclasts undergo apoptosis and osteoblast precursors are recruited by growth factors (reversal). Precursors mature into active osteoblasts and synthesize new bone matrix (formation) which become mineralised over the proceeding several months (quiescent). [Adapted from Seeman and Delmas, 2006 [72]].

Bone remodelling is independent of skeletal growth and occurs across the entire lifespan. It is a critical mechanism for the maintenance and repair of bone mass, as well as mineral homeostasis. Targeted bone remodelling occurs in response to local events such as osteocyte apoptosis or microdamage [74]. Stochastic remodelling is linked to calcium homeostasis [74]. Remodelling activity is tightly regulated to ensure that resorption and formation activity are closely coupled. In growing children, remodelling is balanced towards slightly increased formation activity and thus net bone formation. In older adults the opposite is true, where resorption exceeds formation resulting in net bone loss [72]. Disordered bone remodelling can be pathological and lead to metabolic bone diseases such as osteoporosis. Bone remodelling is highly sensitive and influenced by various hormones, growth factors, and cytokines, as well as disease status and the mechanical environment. These regulators are discussed in section 1.2.5.

1.2.2.5 Bone turnover markers

The protracted timescale for an entire bone remodelling cycle makes short term investigation of material changes in bone difficult to assess. Assessing bone turnover through biochemical markers has become a useful method of assessing metabolic activity in bone. Bone formation activity can be assessed via bone specific alkaline phosphatase (BAP) or osteocalcin (OC). BAP is expressed by osteoblasts in very high quantities during the formation phase of bone remodelling and is regarded as a sensitive marker of formation activity [58,75]. OC is the most abundant non-collagenous protein in bone. OC primarily binds with calcium in bone after post-transcriptional carboxylation and may represent activity of mature osteoblasts [76]. The preferred marker of bone resorption activity is C-terminal cross-linked telopeptide of type I collagen (CTX-1). CTX-1 contains collagen fragments and is highly specific to bone as osteoclasts do not participate in degradation of other bodily sources of collagen [77]. Markers of bone turnover display large circadian variations and are sensitive to nutritional status and other factors. In children with delayed growth and puberty, like those with chronic diseases such as CD, it may not be acceptable to simply compare values with age and gender matched controls.

1.2.3 Bone marrow

1.2.3.1 Overview

Bone marrow is a multicellular tissue occupying approximately 80% of the medullary cavity in bone. Bone marrow consists of mainly red haematopoietic tissue in neonates that experiences rapid infiltration of adipocytes during childhood. This conversion to yellow, fatty tissue continues more gradually across adulthood [78]. As the primary site of haematopoiesis, red marrow is preserved in adults mainly within the axial skeleton (including the proximal femur), while yellow marrow accumulates in the appendicular skeleton in a centripetal manner [79]. Bone marrow is the common origin of both haematopoietic stem cells (HSCs) and pluripotent MSCs.

1.2.3.2 Mesenchymal stem cells (MSCs)

Pluripotent MSCs have the capacity to proliferate in undifferentiated form prior to their commitment into tissue specific progenitors. In response to signalling factors and/or gene expression, MSCs may commit to several different cell lineages, including osteogenic and adipogenic cells. Differentiation to osteoblast or adipocyte lineage depends on two vital transcription factors, *Runx2* and peroxisome proliferator-activated receptor γ (*PPARγ*), respectively. *Runx2* and *PPARγ* are reciprocally regulated and the upregulation of one tends to occur with downregulation of the other [80]. Evidence suggests that MSC commitment to osteogenic lineage occurs at the expense of adipogenesis and *vice versa* [81].

1.2.3.3 Bone marrow adiposity

The reciprocal regulation of osteoblast and adipocyte progenitors has led to increased exploration of the role of bone marrow adiposity (BMA) in skeletal health. MSCs are thought to develop an increased propensity for adipogenic differentiation under various conditions include aging and inflammation. This shift is thought to be one of the main factors associated with BMA accumulation [79]. The physiology of BMA is not entirely understood and may depend on its location and relative composition. Indeed, the metabolic function and genetic expression of BMA has been found to differ depending on its location [78]. This may be due to the relative haematopoietic capacity of proximal bone marrow in comparison to distal

fatty marrow [82]. BMA has been positively associated with bone mineral content (BMC) in growing children, suggesting a potential role in skeletal development [83]. Another study, however, found a negative relationship between BMA and cortical bone in children after adjusting for weight and body fat [84]. The association between BMA and BMC is therefore likely mediated by weight or total body adiposity and may represent a negative marker of bone health when adjusted for these factors in children [85]. In healthy adolescents and young adults, BMA of the lumbar spine is estimated at between 10-30% [86]. BMA is generally higher in males until menopausal age, after which rapid accumulation occurs in females, potentially due to the loss of oestrogen. The utility of BMA as a potential marker of skeletal fragility has been demonstrated by its negative associations with volumetric bone mineral density (vBMD) and trabecular bone volume in both children and adults [85,87]. Furthermore, BMA is elevated in conditions with associated skeletal morbidity, such as obesity, anorexia nervosa (AN), type 2 diabetes mellitus (T2D), and endogenous GC excess [88-90]. The paradoxical relationship between BMA and adiposity observed in AN suggest that BMA is metabolically distinct from other fat depots. It is currently unclear whether BMA plays a role in inflammatory mediated bone loss or whether its accumulation is altered in chronic inflammatory diseases such as CD.

1.2.4 Skeletal muscle

1.2.4.1 Skeletal muscle: function and anatomy

Skeletal muscle is a highly plastic tissue that contributes to many homeostatic functions. The primary mechanical function of muscle is to generate force for movement. Physiologically, skeletal muscle contributes to basal metabolic rate and maintenance of core temperature [91], is the primary site of glucose storage and metabolism [92] and stores amino acids [93]. Skeletal muscle has also been identified as an endocrine organ through its release of so called 'myokines', which exert autocrine, paracrine, and endocrine functions – including within bone [94,95].

Accounting for approximately 40% of total body mass, skeletal muscle is the most abundant tissue in humans. Muscle tissue is primarily composed of water (~75%) and protein (~20%) with a small amount of carbohydrates, lipids, and minerals. The size and orientation of individual skeletal muscles is dependent on their innate mechanical function. Anatomically, however, skeletal muscles share a common underlying structure. Muscles are arranged hierarchically into subunits of muscle cells (known as myofibers) and layers of connective tissue. This arrangement allows selective activation of specific regions or myofibers within a single muscle [96]. The connective tissue layers are rich in collagen that is ultimately entwined with the collagen in the muscle tendons. This facilitates near frictionless muscle contraction while maintaining the mechanical integrity of myofibers under large contractile forces.

Myofibers are multinucleated cells with a long, cylindrical morphology (~10-100μm diameter, ~1-30cm length). During development, these cells form through the fusion of myoblasts. Myofibers have a surrounding plasma membrane ('sarcolemma') and contain several cellular organelles including mitochondria; a T tubule system responsible for conduction of action potentials to the interior of the cell; and sarcoplasmic reticulum responsible for the storage, release, and reuptake of calcium ions post-activation [96]. Myofibers are comprised of many myofibrils, each of which contain millions of myofilaments (proteins) [97] (Figure 1-7). These myofilaments are highly organized into successive units known sarcomeres, which form the basic contractile unit of skeletal muscle. Sarcomeres are arranged into alternating thick (myosin) and thin (actin) filaments that are

anchored between cytoskeletal structures known as Z-lines. Sarcomeres arrange consecutively along the length of myofibrils. Sarcomeres generate force via excitation-contraction coupling in what is known as the sliding filament theory (Figure 1-8).

Skeletal muscle also contains specialized myogenic precursors known as satellite cells, located between the sarcolemma and basal lamina of terminally differentiated myofibers. Satellite cells are the adult stem cells of skeletal muscle and play a critical role in the plasticity and regenerative capacity of muscle tissue [98]. Under the influence of myogenic factors, satellite cells fuse to damaged myofibers for repair, or fuse to each other to form new myofibers [99]. The pool of available satellite cells reduces with age, leading to poorer muscle regeneration in older adults [100].

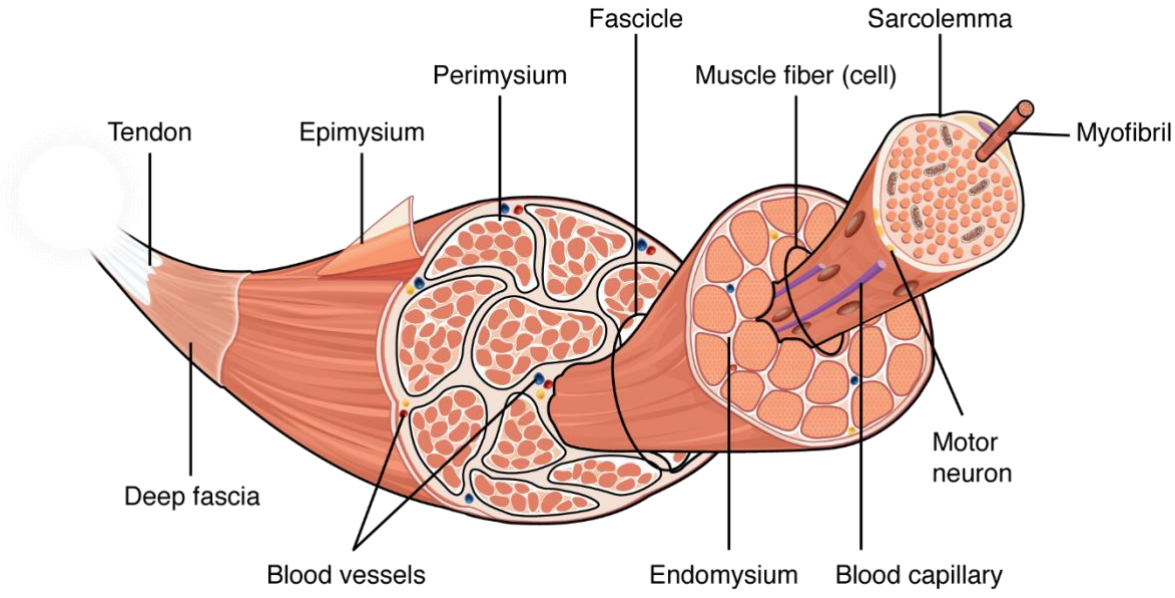

Figure 1-7 Anatomy of skeletal muscle.
Illustration of the detailed macro and microstructure of skeletal muscle. (From Biga et al [101])

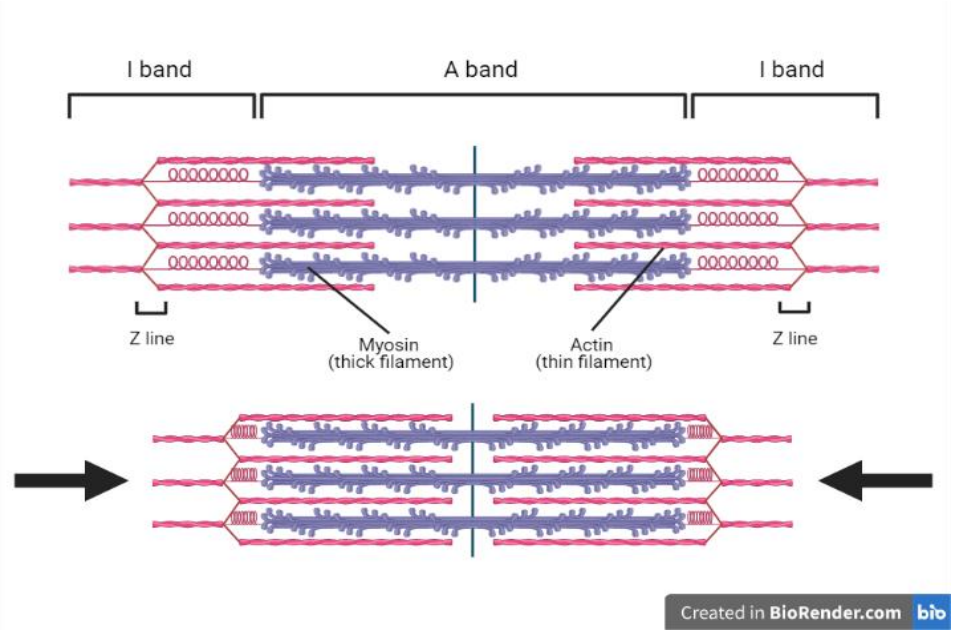

Figure 1-8 Sliding filament theory of muscular contraction in a sarcomere.
Illustration of the sliding filament theory of muscular contraction in a single sarcomere. Action potentials are received at the neuromuscular junction and transported into the myofiber through the T tubules. The following chain of molecular events results in the release of calcium ions into the sarcoplasm. These ultimately bind to troponin, altering the troponin-tropomyosin complex and exposing binding sites on actin filaments. Hydrolyzed myosin heads then bind to the actin filaments, creating new cross-bridges. Myosin releases its energy which pulls the actin filaments toward the centre of the sarcomere. During contraction, the A-band remains the same width, while the I-bands become smaller. This process happens concurrently in many thousands of consecutive sarcomeres, resulting in muscular contraction. (Created in BioRender.com).

1.2.4.2 Muscle adiposity

Infiltration of skeletal muscle with adipocytes is a common hallmark of aging [102] and neuromuscular disease [103]. Accumulation of adipocytes within muscle is positively associated with insulin resistance, which impairs the capacity for protein synthesis, ultimately leading to poor muscle mass and function [104]. Muscle adiposity also expresses pro-inflammatory adipokines that could contribute to systemic inflammation and is associated with reduced bone strength in children and adults [105].

Several mechanisms may contribute to the accumulation of adipocytes in skeletal muscle. In aging or chronic disease, chronically elevated pro-inflammatory cytokines may reduce the regenerative capacity of skeletal muscle. Excessive TNF-α has been found to epigenetically alter muscle satellite cells via p38 mitogen activated protein kinase (MAPK) signalling, inhibiting myogenesis [106]. A second population of stem cells located within muscle known as fibro/adipogenic progenitors (FAPs) can differentiate into adipocytes under conditions of injury or

GC excess [107]. Sex steroid deficiency has been implicated in muscle fat accumulation in both men with prostate cancer undergoing androgen deprivation therapy [108] and ovariectomised rodents [109]. The effects of sex steroid deficiency due to delayed puberty on muscle fat accumulation in children has not been established but appears biologically plausible. Disuse is also strongly associated with muscle fat infiltration as has been established in populations with immobility secondary to neuromuscular disease [103]. Conversely, exercise intervention can effectively decrease muscle fat accumulation in older adults [102]. The long-term implications of muscle fat infiltration and its contribution to systemic inflammation in CD is currently unknown; however, increased muscle fat has been associated with longer post-operative hospital stay in IBD patients [110].

1.2.5 Regulators of the muscle-bone unit

1.2.5.1 The mechanical muscle-bone unit

Mechanical loading is a critical regulator of bone mass. Bone tissue is sensitive to its biomechanical environment and adapts accordingly to mechanical loading or disuse. This mechanical relationship is well described by Frost's Mechanostat theory [111]. Bone adaptation occurs secondary to mechanical loading to optimize bone mass and geometry in relation to functional demands. Bone experiences varying types of deformation when placed under mechanical loading from compressive, tensile and shear strains from muscle contraction and external forces. The mechanostat exists to ensure deformation remains within an acceptable physiological range to avoid spontaneous fracture, while meeting functional demands.

The magnitude of strain experienced within bone from mechanical loading is perhaps the most important regulator of skeletal mass. The mechanostat mechanism functions as a non-linear continuum between the magnitude of strain and the resorptive, regenerative or formative activity within bone [53]. A minimum effective strain is required to maintain current bone mass [112]. If habitual mechanical loading falls below the minimum effective strain threshold, then osteoclast mediated bone resorption becomes upregulated to remove excess tissue [113]. In the absence of mechanical loading, bone loss occurs rapidly in adults and bone fails to develop in children, leading to a markedly increased risk of

skeletal fragility. This has been clearly evidenced in studies of hindlimb suspension in animals [114] and in children with neuromuscular disorders associated with impaired mobility such as cerebral palsy [115] and Duchenne muscular dystrophy (DMD) [116]. Mechanical loading within the normal physiological range results in maintenance of bone mass and structure, with similar levels of bone formation and resorption. Chronic exposure to mild supraphysiological mechanical loading, such as that experienced during resistance training or impact exercise, signals upregulation of bone formation activity which can lead to long term structural adaptations and improvements in bone strength [117-119]. Adaptations to mechanical loading include increases in cortical thickness, cortical and trabecular vBMD, and trabecular bone volume. For example, the dominant arm of elite youth tennis players had 40% [± 12%] greater cortical bone cross sectional area of the humerus compared to the contralateral limb [120], highlighting the large site-specific effects of repeated exposure to various high-magnitude strains. Skeletal gains made through exercise can also confer a lifelong benefit to bone strength, as highlighted by the persistence of elevated cortical bone mass in the throwing arm of retired baseball players [121]. The mechanostat model is highly dependent on other, non-mechanical influences such as nutrition, hormonal, and disease status and cannot override the contribution of these factors. For example, the loss of bone protective estrogen in post-menopausal women causes rapid bone loss despite no changes in their habitual loading patterns [122].

In growing children, increasing body mass and skeletal muscle mass results in higher magnitude of habitual loading of the skeleton from contracting muscle and gravitational forces. The increasing mechanical loads are sensed by mechanoreceptive osteocytes which subsequently signal osteoblasts to upregulate bone formation in areas where new bone is required to meet mechanical demands. This was nicely illustrated in a study of adolescents in whom peak velocity of lean body mass accretion was temporally followed by peak velocity in BMC accretion approximately 0.5 and 0.4 years later in girls and boys, respectively [123]. As stated, this mechanism is sensitive to other factors including pubertal development and disease status, which have specific implications in the growing child. Children who fail to accrue muscle mass secondary to chronic disease may subsequently fail to accrue bone mass due to inadequate mechanical loading. Peak bone mass is one of the most important predictors of skeletal fragility later in life, therefore poor

muscle and bone accretion during growth may predispose these individuals to increased risk of sarcopenia, osteoporosis, and fractures as adults.

1.2.5.2 Biochemical interaction between bone and muscle

Muscle and bone express myokines and osteokines, respectively, that exert endocrine functions in the opposing tissue. Myostatin, the first myokine identified, is a potent negative regulator of skeletal muscle mass that is upregulated during periods of chronic inflammation and GC excess [124]. In bone, myostatin promotes resorption, potentially through its actions in osteocytes, in which it has been demonstrated to upregulate expression of sclerostin and RANKL [125]. Myostatin has also been found to inhibit bone regeneration after injury, whereas its inhibition promoted improved bone healing in models of T2D [126].

Exercise induced IL-6 expression in skeletal muscle stimulates osteoclastogenesis by inducing RANKL expression in osteoblasts and osteocytes [127]. This IL-6 mediated bone resorption pathway facilitates physiological adaptation to exercise, rather than acting as a pathological mechanism [128]. Resorption releases uncarboxylated osteocalcin from the bone matrix, an osteokine with endocrine functions in skeletal muscle and other tissues [129]. Osteocalcin increases insulin sensitivity and mediates nutrient uptake into myofibers in a dose dependent manner [128]. Osteocalcin may also partly regulate muscle mass, as osteocalcin null mice displayed reduced muscle mass and exogenous osteocalcin administration increased muscle in older animals [130]. This is indicative of a muscle-bone biological crosstalk mechanism that moderates the physiological responses to exercise.

Muscle and bone specific expression of growth factors such as IGF-1 may have regulatory effects in the opposing tissue. Muscle derived IGF-1 is expressed after exercise and stimulates bone formation in local osteoblasts [131]. Local skeletal IGF-1 expression may also have anabolic effects in adjacent muscle, although to date no studies have assessed this.

1.2.5.3 Regulators of bone and muscle

The reciprocal regulatory relationship between bone and muscle is accompanied by their individual and combined regulation via systemic, local, and environmental factors. Bone and muscle development are primarily regulated by genetics, with

an estimated heritability of 50-80% for peak bone and muscle mass [132,133]. The remaining variability in bone and muscle development and maintenance are regulated by physiological and environmental factors.

1.2.5.3.1 Puberty

Puberty is a critical period for the accrual of bone and muscle mass [134]. Bone mass approximately doubles between the pre-pubertal and young adult age while true volumetric density is relatively unchanged. By late adolescence, approximately 90% of peak adult bone mass will be attained. The effects of puberty on bone and muscle are mainly moderated by a surge in sex steroid production [135]. Increased oestrogen expression is pivotal for the maturation and mineralisation of bone in both boys and girls during puberty [136], more so than testosterone. In early puberty, oestrogen may reduce the remodelling threshold of the bones mechanostat mechanisms, requiring lower mechanical loading to induce skeletal adaptation [137]. This potentially explains the more rapid accrual of bone mass in girls in the pre-pubertal years in comparison to boys, despite similar loading patterns. Testosterone expression upregulates androgen receptors in growth plate osteoblasts [138]; however, the bone promoting effects of testosterone may also be mediated by greater mechanical strain due to increasing muscle mass. Sex steroids also play an important role in bone and muscle maintenance during adulthood, and their eventual declines are associated with significant loss in both tissues [136]. Children with delayed onset or progression of puberty may fail to accrue adequate bone and muscle mass as a direct result of sex steroid deficiency, leading to persistent musculoskeletal deficits in adulthood.

1.2.5.3.2 GH/IGF-1 axis

The GH/IGF-1 axis is one of the primary regulators of linear skeletal growth and muscle accrual during childhood and puberty [139]. GH and IGF-1 promote bone growth through endocrine effects and local action at the growth plate. GH supports proliferation of osteoblasts and chondrocytes and triggers bone formation in mature osteoblasts [140,141]. GH also induces local IGF-1 expression and action at the growth plate, which stimulates linear growth [139,142]. In skeletal muscle, the anabolic actions of GH are mediated by systemic and local IGF-1, which stimulate protein synthesis and reduces protein degradation [143]. During puberty, the surge

in sex steroid production increases spontaneous GH secretion which further upregulates systemic and local IGF-1 production [144], leading to accelerated growth of bone and muscle. Children and adults with chronic inflammatory disease may display abnormalities in GH/IGF-1 axis including relative GH insufficiency or GH/IGF-1 resistance, which can inhibit anabolic processes in muscle and bone [145].

1.2.5.3.3 Nutrition

Nutritional status plays a central role in the development and maintenance of bone and muscle. Mechanisms affected by poor nutritional status include bone mineralisation, muscle protein synthesis, and GH/IGF-1 axis. The skeleton stores almost the entire bodily reserve of calcium, which is critical for accrual of bone mass and bone mineralisation [146]. Calcium also acts as a signalling molecule and is essential for healthy muscular contraction. Calcium is not produced endogenously and is entirely obtained through nutritional consumption. As such, poor intake is associated with low bone mass in children and adults [147]. Poor nutrition also adversely affects the GH/IGF-1 axis, inhibiting bone and muscle development [148]. In human studies, poor nutritional status is associated with increased systemic GH, potentially due to downregulation of hepatic GH receptors [148,149]. The onset of puberty is linked to nutritional status through the actions of leptin, an adipocyte-derived hormone that is reduced in the context of undernutrition [150]. Chronic undernutrition and low adiposity stores are therefore thought to be key regulators of delayed puberty and growth failure in children with chronic inflammatory disease.

1.2.5.3.4 Physical activity & exercise

The importance of PA as a regulator of muscle and bone is largely mediated by the influence of mechanical loading on these tissues (discussed in section 1.2.5.1). Observational data highlights that children and adults who participate in greater levels of moderate to vigorous PA (MVPA) have favourable bone mass compared to active and inactive peers [151]. Individuals who participate in high-impact activities such as gymnastics are routinely reported as having higher bone mass than inactive controls or other active cohorts [152-155]. As mentioned, skeletal adaptations are highly site-specific, as demonstrated by the large within-person variability in bone mass between dominant and non-dominant limbs of elite level athletes [120,121]. The

long-term benefits of exercise induced gains in bone mass highlight the potential for exercise as an effective strategy for augmenting muscle-bone development and reducing risk of future musculoskeletal morbidity. The enhanced mechanosensitivity of bone in children and young people may provide an optimal window for the application of strategies to improve bone mass and strength via PA and exercise. Jumping based exercise interventions generating ground reaction forces in the range of three to five times bodyweight have been demonstrated to effectively increase bone mass in paediatric cohorts [117-119]. Independently of gains in aBMD, chronic exposure to highly strenuous activity (i.e. military training) can lead to structural adaptations and improved bone strength in young women [156]. Even in older adults, maintenance of a physically active lifestyle is associated with an attenuation in the rate of bone loss [157].

Similarly, in skeletal muscle, PA and exercise are associated with better muscle mass, quality, and function across all ages [158,159]. The structural and functional adaptations within skeletal muscle are highly dependent on the mode and intensity of PA and exercise. Resistance exercise is highly effective for inducing muscle hypertrophy and strength gains, primarily through its actions in stimulating muscle protein synthesis [160,161]. A recent systematic review also suggested that plyometric training may be as effective at inducing gains in muscle mass when compared to traditional resistance training in previously untrained individuals, although this requires further investigation [162]. Aside from improvements in muscle mass, PA and exercise also indirectly stimulate lipolysis via expression of myokines which inhibits the accumulation of muscle fat infiltration and maintains muscle quality [94].

1.2.6 Osteoporosis

Osteoporosis is a systemic disorder characterised by low bone mass and deterioration of bone microarchitecture (i.e. bone quality) which leads to compromised skeletal strength and increased risk of fracture [163]. Osteoporosis is primarily an age-related disease of abnormal bone metabolism, whereby excessive bone resorption results in net bone loss [164]. Secondary osteoporosis relates to bone loss that occurs because of other factors, such as chronic inflammatory disease, GC exposure, or impaired mobility [165]. Secondary osteoporosis can occur at any age and can be caused by inadequate skeletal development in children, rather than bone loss per se [166].

In adults, a clinical diagnosis of osteoporosis is based on DXA derived measurements of areal bone mineral density (aBMD). As an age-related disease, osteoporosis is typically diagnosed in older adults, in whom DXA aBMD T-score is strongly predictive of fracture risk. A clinical diagnosis of osteoporosis is confirmed by a DXA aBMD T-score < -2.5 SDS, compared to a healthy young adult. This threshold is used for the initiation of bone protective therapies such as bisphosphonates or anti-RANKL antibodies [164]. Osteopenia, low bone mass that is not as pronounced as in osteoporosis, is characterised by an aBMD T-score of > -2.5 < -1.0 SDS in adults. In these individuals, the calculation of fracture risk algorithm (FRAX) score may be used to guide clinical management of bone. This tool uses several demographic and environmental risk factors to calculate the ten-year probability of a major osteoporotic fracture in adults aged >40 years [167]. At present, there is no robust data linking aBMD with fracture risk in young adults, as fracture risk is generally very low. This means, however, that there is currently a lack of clinical guidelines for the management of osteoporosis in this cohort, including young adults who may have aBMD deficits secondary to chronic disease.

In children, DXA aBMD is also not a useful predictor of fracture risk and paediatric osteoporosis is not clinically diagnosed by densitometry alone. Fractures occur in up to 50% of children [166], therefore the identification of fractures resultant of intrinsic skeletal fragility rather than typical childhood activities is necessary [168]. The fracture centric approach to the clinical diagnosis of osteoporosis in paediatrics also considers the type of trauma required for fracture, with low trauma fracture or fragility fracture defined as fracture from standing, sitting or

no faster than walking pace. Paediatric osteoporosis can be diagnosed in a child with ≥1 nontraumatic vertebral compression fracture (VF), independently of aBMD assessment, as nontraumatic VF are associated with skeletal pathology [168]. Alternatively, osteoporosis can be diagnosed in a child with an age- and body size-adjusted aBMD Z-score < -2.0 SDS plus a clinical history of long bone fractures (≥2 by age 10 years or ≥3 by age 19 years) [168]. These guidelines seek to avoid overdiagnosis of skeletal fragility in otherwise healthy children who happen to experience multiple fractures. On the other hand, the lack of clinical context or mechanistic insight into long bone fractures in the updated ISCD guidelines may underdiagnose skeletal fragility in children with underlying associated risk factors or pathology [166]. For example, children with CD, who have risk factors including chronic inflammation, nutritional deficits, and GC exposure, may benefit from a comprehensive skeletal evaluation after the occurrence of one long bone low trauma fracture. Yet, under current guidelines, this is not recommended; potentially delaying the identification of skeletal fragility until the occurrence of further fractures.

These clinical definitions of osteoporosis in the elderly and young are developed to target those at the highest risk of fragility fractures, or who have already sustained fragility fractures, for treatment with bone protective therapies. Whilst such definitions may be useful in clinical practice to limit unnecessary bone protective therapies, these definitions and DXA densitometry-based evaluation do not provide detailed information on skeletal abnormalities. Subsequently, more advanced imaging methods are required for the detailed assessment of bone in populations who do not meet these criteria.

1.3 Muscle-bone assessment methods

1.3.1 Overview

Collective evaluation of bone and muscle as an integrated unit *in vivo* is vital to our understanding of their close relationship across the lifespan in both health and disease. Currently, micro-computed tomography (μCT) and histomorphometry of bone biopsy is the 'gold standard' skeletal assessment. This is a highly invasive procedure that requires general anaesthetic in children and cannot concurrently evaluate muscle. Several non-invasive imaging methods for the assessment of the muscle-bone unit have been developed. Quantitative ultrasound (QUS), dual x-ray absorptiometry (DXA), peripheral quantitative computed tomography (pQCT) and advances in high-resolution magnetic resonance imaging (MRI) and spectroscopy (MRS) each have value in muscle-bone assessment. Several simple functional assessments are also available that may complement the use of imaging in assessing the muscle-bone unit. The application, suitability, and limitations of these methods for assessing muscle-bone will be discussed in the subsequent sections.

1.3.2 Muscle-bone imaging

1.3.2.1 Dual energy x-ray absorptiometry (DXA)

1.3.2.1.1 Overview

Dual energy x-ray absorptiometry (DXA) is the most widely used method of assessing bone health in both clinical and research settings. DXA scanners are widely available and thus routinely employed for assessment of body composition and bone monitoring. Indeed, DXA is the current 'gold standard' for clinical assessment of skeletal health and DXA derived areal bone mineral density (aBMD) is the primary outcome used in the assessment of osteoporosis [164]. DXA can also be implemented for the assessment of total body or appendicular muscle mass [169].

1.3.2.1.2 Technology

DXA is used to assess body composition based on a three-compartment model – bone mineral content (BMC), fat mass (FM) and lean mass (LM). During scanning, the individual lies in a supine position on the bed. In modern scanners the x-ray

source generates a narrow fan beam containing high and low energy photons that are passed through the individual in the posterior-anterior direction towards a scanning arm containing a scintillation detector. Tissue dependent photon attenuation occurs with high density materials (i.e. bone) allowing fewer photons through than low density materials (i.e. lean and fat mass). The ratio of attenuation coefficient between the two energy peaks is subsequently calculated for each tissue compartment and algorithmically computed into appropriate values for BMC, FM and LM.

1.3.2.1.3 Strengths and limitations

The advantages of DXA include quick scan time, low cost, and wide availability at medical facilities. Due to the large-scale application of DXA over recent decades, there are vast reference datasets available for paediatric and adult populations, thus allowing like-for-like comparisons - important due to the variability in bone and body composition based on age, sex, ethnicity, and other factors [170]. Additionally, reproducibility of aBMD and lean mass measurements are high (CVs ~1 to 3%), allowing small changes to be assessed with confidence. Skeletal muscle assessed by DXA also correlates strongly with the gold standard methods of MRI ($r > 0.8$) and CT ($r > 0.9$) in cross-sectional studies [171,172].

Despite the above, the technique also has several known limitations. Although in a relatively small dose per scan, DXA involves exposure to ionizing radiation. This may become problematic for people who require routine scanning and thus repeated radiation exposure, including children with chronic diseases associated with poor bone development or in those treated with chronic GC. DXA is also unable to delineate cortical and trabecular bone, each of which are important determinants of bone strength that can be independently affected by pathology. Indeed, only approximately 60-70% of bone strength variability is accounted for by DXA, meaning it fails to capture important contributors to overall mechanical strength. DXA alone is insensitive at identifying most individuals who would benefit from bone protective therapy or intervention as the greatest incidence of fractures occur in people who do not meet the diagnostic threshold for osteoporosis [173]. Instead, most fractures occur in individuals with low bone mass and other associated clinical risk factors as many more people fall into this category [173].

DXA measures are also inherently linked to bone size. DXA uses a two-dimensional projection of bone area to calculate BMC (g) and aBMD (g/cm^2) in a three-dimensional structure, without accounting for bone thickness in the anterior-posterior direction. This does not accurately reflective volumetric BMD ([mg hydroxyapatite/cm^3]) and results in systematic underestimation of bone density in children with small bones secondary to skeletal delay or pathology, as has been observed in CD [174]. Several methods of adjustment have been suggested including for bone area [175], bone age [176], and lean mass [177] alleviate concerns of underestimation. Bone mineral apparent density of the lumbar spine (LS BMAD) can also be calculated using the vertebral width based on an assumed cylindrical volume of the vertebra and is used as a surrogate measure of vBMD. LS BMAD is the preferred adjustment method used in local clinical practice.

1.3.2.2 Quantitative computed tomography (QCT)

1.3.2.2.1 Overview

Advances in quantitative computed tomography (QCT) techniques have facilitated improved measurement of skeletal density, structure, and strength in comparison with DXA. Peripheral QCT (pQCT) scanners allow the measurement of bone, muscle, and fat at the distal limbs at a fraction of the cost or radiation burden of traditional whole-body CT scanning [169].

1.3.2.2.2 Technology

Like DXA, pQCT technology is based on the physics of photoelectric absorption. The scanner contains a rotating gantry that houses an x-ray tube and adjacent detector. During scanning, the individual's limb is fixed within the rotating gantry to aid immobilisation. As the gantry rotates, the x-ray tube emits a fan beam of photons through the individual's limb and towards the detector. Tissue dependent x-ray attenuation allows the independent assessment of bone, muscle, and fat at the region of interest using an in-plane voxel size of 200µm – 800µm [178]. Attenuation data are reconstructed into a single two-dimensional tomographic slice for subsequent analyses. The assessment of trabecular bone occurs at the metaphysis, whereas cortical bone and muscle and fat cross-sectional area are measured at the diaphyseal shaft [179].

1.3.2.2.3 Strengths and limitations

Unlike DXA, pQCT independently evaluates trabecular and cortical bone compartments, providing a true vBMD for each that is independent of bone size. Bone size and geometry can also be quantified and estimated mechanical properties calculated from pQCT [179]. Skeletal muscle area assessed by pQCT correlates almost perfectly (r = 0.97) with the gold standard MRI when a strong image filter is applied [180]. Additionally, pQCT can assess intermuscular fat distribution, providing insight into overall limb composition and muscle quality, not available via DXA [169,179]. Mid-thigh measures of skeletal muscle adiposity also correlated very closely between pQCT and MRI (r^2 = 0.82) [181], highlighting the validity of this method for multicompartment assessment.

Standard pQCT resolution precludes direct assessment of trabecular microarchitecture; however, newer high-resolution pQCT (HR-pQCT) scanners have improved spatial resolution (82μm) that can more accurately quantify these minute structures. HR-pQCT outcomes associate closely with micro-CT analyses of bone biopsies [182]. A primary limitation of pQCT techniques is the inability to scan more proximal anatomical locations due to the relatively small gantry size and scanner setup. Availability of scanners is also low, and they are highly expensive, especially for HR-pQCT. Furthermore, the various scanning protocols in pQCT make comparisons between studies difficult.

1.3.2.3 Quantitative ultrasound

1.3.2.3.1 Overview

Quantitative ultrasound (QUS) provides a useful alternative to conventional imaging methods for the assessment of bone and muscle. QUS provides outcomes associated with bone mineralisation and trabecular structure and can also provide detailed visualisation of muscle thickness, area, fascicle length and pennation angle. Currently, the only accepted measurement site for assessing bone health using QUS is the heel. QUS is not a currently accepted method of assessing muscle mass clinically, although cross sectionally QUS measures of muscle thickness have correlated well with MRI muscle area (r = 0.82) [183]. In the absence of DXA imaging, heel QUS may be used in conjunction with other risk factors for the initiation of pharmacological intervention [184].

1.3.2.3.2 Technology

Ultrasound waves are mechanical vibrations that are altered in shape, intensity and speed by the medium through which they are travelling. QUS assesses the interaction and propagation of these waves across bone tissue [179]. Parameters derived from QUS are therefore based on physical interactions between ultrasound waves and bone tissue. The signal velocity and attenuation are dependent on the characteristics of bone tissue and allow deduction of the mechanical properties and structural anisotropy of bone including density, trabecular structure, and elasticity [185]. Velocity is measured as speed of sound (SoS [m/s]) and attenuation measured by broadband ultrasound attenuation (BUA [dB/MHz]). Variations of scanning technique exist, depending on the device used. Nevertheless, fundamentally, a scan consists of a transmitter producing ultrasound waves through the calcaneus towards a receiver located on the other side. A coupling medium is used, either gel or water immersion, to ensure correct coupling of the wave into the skin.

1.3.2.3.3 Strengths and limitations

The low-cost, quickness, and portability of QUS measurements are the primary advantages, particularly for use in neonates or population-based research where the application of DXA or other imaging may be impractical [186,187]. Despite this, QUS is hampered by many limitations. Measurement is limited to superficial sites and the only currently validated measurement site in osteoporosis management is the calcaneus. Inter-operator variability can be very high and differing technology and calibration methods precludes accurate comparisons between devices [179]. The utility of QUS for fracture prediction is controversial, although validated devices have been found to predict fracture in post-menopausal women and elderly men, independent of DXA aBMD [184]. While QUS measures of muscle mass show good correlation with reference methods, the lack of standardised procedures strongly limits comparisons.

1.3.2.4 Magnetic resonance imaging

1.3.2.4.1 Overview

Magnetic resonance imaging (MRI) has emerged as a useful tool for comprehensively assessing the musculoskeletal apparatus. Advances in high-field (≥3-Tesla) scanners have facilitated high-resolution imaging of bone at the macro- and microarchitectural level. MRI is also the gold standard imaging method for assessment of skeletal muscle. Previous research has found MRI assessment of trabecular microarchitecture to be sensitive at identifying individuals with prior fragility fractures who do not meet DXA aBMD criteria of osteoporosis [188]. Despite this, MRI is not a currently accepted clinical method of assessing skeletal fragility as the field is still in its relative infancy.

1.3.2.4.2 Technology

MRI physics is founded on the magnetic properties of atomic nuclei. Hydrogen nuclei, together with others that contain odd numbers of protons and neutrons, produce a small magnetic field due to the spinning of their positive magnetic moment around an axis. Hydrogen constitutes most tissue within the human body and generates the greatest magnetic signal, hence its use in MRI. During MRI, when a patient enters the scanner the magnetic moments of the hydrogen nuclei align parallel or anti-parallel with the external magnetic field (B_0), with a slightly larger number aligning parallel as this is a lower energy state (Figure 1-9) [189].

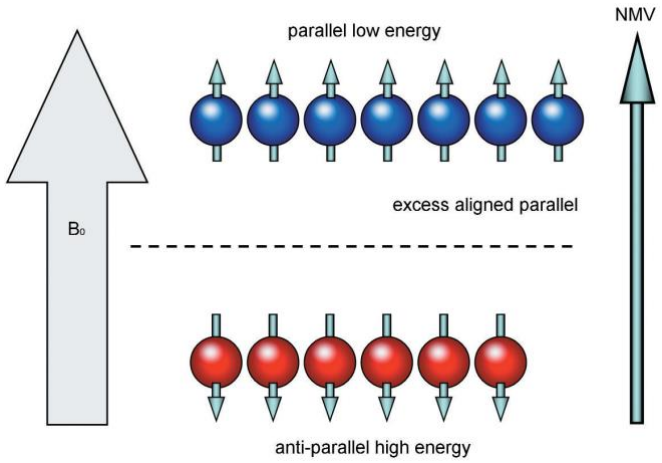

Figure 1-9 Alignment of magnetic moments relative to external magnetic field.
A slightly higher number of magnetic moments align parallel with B_0 as this is a lower energy state. This creates a net magnetic vector along the same direction as B_0. NMV – net magnetic vector; B_0 – external magnetic field. (From Westbrook [2018] [190])

The magnetic moments of these nuclei spin around the axis of B_0 at a frequency known as the *Larmor Frequency*, which is directly proportional to scanner field strength. The individual is then exposed to radio frequency (RF) pulses at precisely the value of the Larmor frequency. This causes *excitation* of the hydrogen nuclei as they absorb energy from the emitted RF pulses - known as *resonance*. Low energy nuclei begin to join the high-energy group and re-align anti-parallel to B_0. When enough energy is applied this shifts the net magnetic vector away from B_0 towards the transverse plane (Figure 1-10). Resonance also causes the magnetic moments of nuclei to move in phase with each other, meaning they spin at the same position on the precessional path around B_0 as each other [190]. The MR signal is generated when in phase transverse magnetisation crosses a receiver coil.

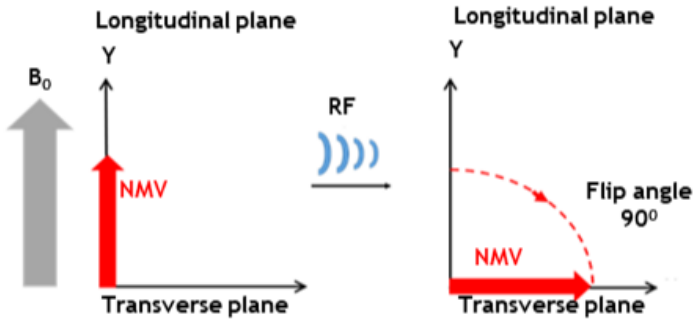

Figure 1-10 Excitation and flip-angle generation.
The NMV moves towards the transverse plane when enough energy is supplied by the RF as more moments align anti-parallel to B_0.

After removing the RF pulse the net magnetic vector returns to alignment with B_0 - causing magnetization in the longitudinal and transverse planes to increase (*T1 recovery*) and decrease (*T2 decay*), respectively. *T1 recovery* occurs when nuclei release their energy into the surrounding environment (*spin lattice relaxation*), whereas *T2 decay* occurs when the magnetic fields of adjacent nuclei interact with each other (*spin-spin relaxation*). These phenomena are both exponential processes with their *relaxation times* measured as the time taken for 63% of longitudinal magnetization to recover and 63% of transverse magnetization to be lost for *T1* and *T2*, respectively (Figure 1-11 A & B). The different compositions of body tissues mean that they have differing *T1* and *T2 relaxation times*. Each tissue therefore generates a different signal intensity at any given time, allowing clear distinction on the generated MRI image. Water, due to its high inherent energy

state, does not freely release energy to the surrounding lattice and has long *T1* and *T2 relaxation times*. Conversely, fat, due to its low energy state and tightly packed lipid molecules, has shorter *T1* and *T2 relaxation times*. These times are shorter again in muscle tissue [190] (Figure 1-11 C & D).

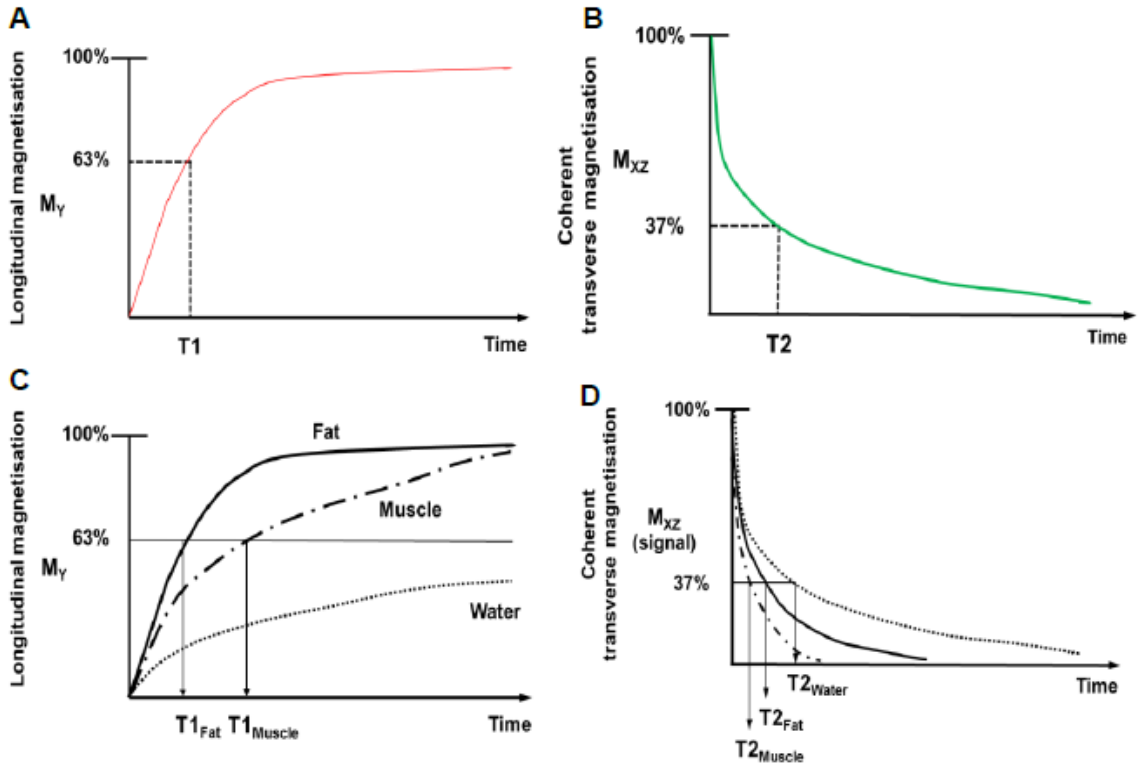

Figure 1-11 T1 and T2 relaxation times.
Illustration of exponential relaxation times for (A) T1 and (B) T2 magnetisation. Relaxation times differ depending on the specific tissue (C & D). (Adapted from Westbrook & Ridgway [2010] [191])

A *pulse sequence* constitutes carefully designed patterns of RF pulses designed to achieve the desired image contrast and quality. The time between two RF pulses (*repetition time* [*TR*]) dictates the level of T1 relaxation allowed prior to the next excitation. The time between applying the RF pulse to the peak signal induced in the receiver coil (*echo time* [*TE*]) determines the amount of T2 relaxation allowed prior to reading the signal. The MRI signal is retrieved by a receiver coil from the energy released during relaxation. To transform this signal into a comprehendible three-dimensional image a field of view (FOV) must be placed over the desired anatomical location for imaging. This FOV is divided into individual volumetric pixels known as voxels, which, together with slice thickness, determines the spatial resolution of the image.

The excellent contrast generation between tissues in MRI depends on both intrinsic and extrinsic factors. Intrinsic factors such as the T1 or T2 relaxation time of a tissue cannot be changed, whereas extrinsic factors can, such as pulse sequence parameters. Images are 'weighted', meaning the pulse sequence parameters are designed so that either T1 or T2 predominates over the other. T1-weighted images have a short TR and are characterised by hyperintense (bright) signal in fat and hypointense (dark) signal in water. T2-weighted images are the reverse, fat is hypointense and water hyperintense. In bone imaging, contrast is generated between the hypointense signal of bone and the hyperintense signal of adjacent fatty marrow, muscle, and subcutaneous fat.

Several factors influence the quality of MRI images, including spatial resolution, signal to noise ratio (SNR) and scan time. Increased spatial resolution improves image quality by allowing accurate differentiation between minute structures, whereas in large voxels signal intensities are averaged creating partial volume effects. SNR increases proportionately with scanner field strength but is negatively associated with spatial resolution. Scan time should be kept as short as possible to reduce movement artefacts that may negatively affect image quality, however high-resolution requires longer scan times. These factors must be carefully considered based on the tissue and structures being imaged to optimise image quality.

1.3.2.4.3 Strengths and limitations

The advantages of MRI over other imaging techniques when assessing muscle and bone are manifold. These include widespread availability at medical research facilities, lack of radiation exposure, and ability to scan more proximal anatomical locations compared to pQCT. The excellent tissue contrast that can be generated by MRI makes it the gold standard imaging method for assessment of skeletal muscle and fat [169]. The utility of MRI in assessing multiple facets of bone, muscle, and adiposity from a single series of imaging is another key advantage. The improved SNR and spatial resolution of high-field scanners has facilitated the study of trabecular bone microarchitecture, with results that correlate highly with micro-CT of bone biopsy (r^2 = 0.82 for trabecular bone volume) [192]. Three-dimensional reconstructions can be generated from a series of contiguous scans, allowing analysis of trabecular anisotropy as well as simulated stress testing [193].

Interscan repeatability in our centre for trabecular parameters was high with CVs of <3.5% for all outcomes [194]. Single slice MRI at the mid-thigh has been reported as an acceptable surrogate for total body skeletal muscle [195] and the assessment of intramuscular fat infiltration provides insight into muscle quality that is not achievable with pQCT or DXA.

Nevertheless, several limitations of MRI must also be considered. MRI scanning is expensive, requires specialist operators, and is time consuming in comparison to DXA or pQCT. The currently available spatial resolution of MRI (~100µm – 200µm) precludes accurate delineation of individual trabeculae, which is achievable for larger trabeculae using the improved resolution of HR-pQCT (82µm). MRI-derived trabecular microarchitecture measurements are therefore subject to partial volume effects, although have been found to perform equally as well as HR-pQCT in this regard [196]. Current scan times required to assess trabecular microarchitecture are relatively long (~9-12 minutes), resulting in increased susceptibility for movement artefacts. Even very slight movement can influence image quality due to the relative size of the structures being assessed, although the application of immobilisation methods or image filtering may alleviate this. Finally, no standardised MRI protocols for the assessment of bone exist, meaning custom pulse sequences and regions of interest are implemented, limiting comparisons between centres.

1.3.3 Magnetic resonance spectroscopy (MRS)

1.3.3.1.1 Overview

Magnetic resonance spectroscopy (MRS) allows the additional assessment of metabolites of interest in conjunction with MRI. Proton MRS (^{1}H-MRS) is regarded as the gold standard for quantification of BMA [197]. Recently, BMA has emerged as an indicator of skeletal health and its assessment in conjunction with muscle-bone MRI provides comprehensive data not available using other imaging techniques. BMA quantification is typically conducted in the lumbar spine, pelvis, and hip.

1.3.3.1.2 Technology

^{1}H-MRS is also founded on the magnetic properties of hydrogen atomic nuclei. When assessing BMA, point-resolved spectroscopy (PRESS) or stimulated echo

acquisition mode (STEAM) pulse sequences are used to measure the abundance of hydrogen nuclei within different elements of tissue (i.e. water and fat). This generates adiposity spectra within the region of interest. This spectrum plots signal intensity against chemical shift, measured in unit parts per million (ppm) (Figure 1-12). Chemical shift is caused by the shielding of an atoms electrons which results in identical nuclei resonating at different frequencies depending on their environment. In ^{1}H-MRS, the signal is separated into a lipid and water peak, from which the area under the curve is used to quantify the water-fat composition of the marrow and calculate BMA fat fraction [198].

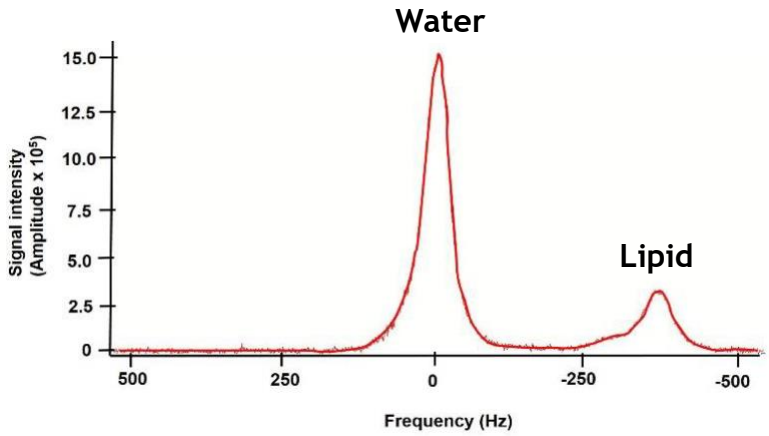

Figure 1-12 ^{1}H-MRS of the lumbar spine.
Representative water and lipid peaks from single voxel ^{1}H-MRS.

1.3.3.1.3 Strengths and limitations

The use of ^{1}H-MRS has many advantages, particularly when used in conjunction with MRI assessment of bone and muscle. These techniques provide the best overall assessment of bone, muscle, and marrow fat from a single series of scans. ^{1}H-MRS is the gold standard non-invasive assessment of BMA and closely correlates with marrow adiposity measured by biopsy [199]. Repeat imaging variability has been reported as <2% highlighting the reproducibility of the technique [200]. The primary limitations of ^{1}H-MRS are the lack of available reference data and careful consideration is required when selecting reference groups as controls, as BMA is influenced by numerous factors including age, sex, and hormonal and nutritional status [78].

1.3.4 Muscle-bone functional assessments

1.3.4.1 Jumping mechanography

1.3.4.1.1 Overview

Jumping mechanography has emerged as a useful tool for the functional assessment of the muscle-bone unit in both health and disease. This tool benefits from providing dynamic assessment of several aspects neuromuscular function. Functional assessments can be implemented in a range of populations, from children and adults with chronic diseases to athletic populations.

1.3.4.1.2 Technology

Jumping mechanography utilises a battery of functional tests of the lower limbs, using a portable force plate (Leonardo Mechanograph Ground Reaction Force Plate, Novotec Medical GmbH, Pforzheim, Germany). This force plate contains eight strain gauge force sensors, used to measure the vertical ground reaction forces (GRFs) applied during various physical tests. Force sensors sample at a frequency of 800Hz and data is transferred and analysed in specialist software (Leonardo Mechanography v4.4 Research Edition Software, Novotec Medical GmbH, Pforzheim, Germany). The two most common assessments performed are the single two-legged jump (S2LJ) and multiple one-legged hopping (M1LH) tests. Other tests simulating activities of daily living like the timed-up-and-go test can be implemented in older populations. S2LJ is used to assess dynamic function and anaerobic power of the lower limbs through a single countermovement jump for maximum height. Force plate data is sampled and analysed providing outputs for multiple aspects of neuromuscular function and jump performance. The primary outcomes assessed from S2LJ are maximum power (kilowatts [kW]) and maximum power relative to body mass (watts per kilogram [W/kg]) generated during the take-off phase, and maximum jump height achieved. M1LH is designed as an assessment of the functional muscle-bone unit by aiming to achieve the maximum possible GRF to the tibia during stiff-legged hopping. The primary outcomes of this test are the maximum applicable force (kN) and the maximum force as a multiple of bodyweight experienced during the landing phase of a single hop.

1.3.4.1.3 Strengths and limitations

Jumping mechanography benefits from providing dynamic assessments of the muscle-bone unit that provide insight into several aspects of neuromuscular function. Mechanography provides insight into the site-specific muscle-bone relationship that is not available from standard isometric dynamometry. The tests are accessible and representative of habitual movement of children and adolescents meaning they can be easily implemented without any learning effect. Further, the portability of the device means the tests can be applied in research or clinical settings, particularly compared to methods such as isokinetic dynamometry. Test-retest reliability is good in healthy children and adults, with CVs of approximately 5% for S2LJ and 4% for M1LH parameters, respectively [201].

A potential limitation of mechanography, applicable to most muscle assessments, is a reliance on participant motivation to achieve accurate results. Participants are asked to perform maximal effort physical tests and low motivation may confound results. Inter-operator reliability may vary depending on how tests are introduced and described to participants. For example, during M1LH, asking participants to hop as quickly as possible and as high as possible have both been reported, each producing markedly different results [202,203]. Standardisation of test technique is required to ensure results can be appropriately compared between studies and with the relevant reference data. Jumping mechanography may not be suitable for individuals with impaired mobility or those with neuromuscular disease, although it has been safely implemented in both children with osteogenesis imperfecta [204] and elderly adults [205], highlighting its safety.

1.3.4.2 Grip strength

1.3.4.2.1 Overview

Maximum isometric grip force, generally termed ‘grip strength’, is the most widely applied method of muscle function assessment. Grip strength provides a rapid assessment of isometric muscle force that has been reported as a useful prognostic indicator of morbidity and mortality [206].

1.3.4.2.2 Technology

Grip strength is assessed using a handheld analogue or digital dynamometer. The Jamar hydraulic dynamometer is most widely used and generally considered the gold standard for comparison. This very simple test involves holding the dynamometer in one hand and performing a single, maximal effort contraction of the musculature of the hand and forearm. Dynamometers are adaptable to ensure optimum positioning of the hand that may facilitate a maximum effort. The resultant output is a measure of force (Newtons [N]) that is generally converted to kilograms (kg) prior to reporting.

1.3.4.2.3 Strengths and limitations

Grip strength dynamometry is cheap can be very easily implemented in research or clinical practice settings. Test-retest reliability of grip dynamometry is very good [207] and grip strength is considered a reasonable marker of overall muscle function. Potential limitations include the dependence of grip strength on growth characteristics such as height and bodyweight. This limits the use of grip strength among children unless suitably age, sex, and size-adjusted Z-scores are available. The variability in techniques employed precludes direct comparisons between studies as different dynamometers, hand dominance, or posture may produce systematically different results. Finally, grip strength is a static assessment that does not represent typical muscular function. Nevertheless, grip strength is a reasonable indicator of muscle function that may be useful in the rapid assessment of strength between different adult populations.

1.4 Muscle-bone health in Crohn's disease

1.4.1 Overview

As discussed, bone and muscle are tightly regulated by many mechanical and non-mechanical factors. Chronic inflammatory diseases such as CD present several challenges to pathways involved in the growth and maintenance of bone and muscle. This section will present a brief overview of prior research into bone and muscle health in paediatric and adult CD, followed by discussion of the potential pathogenic factors associated with muscle-bone deficits in these populations.

1.4.2 Pathophysiology of muscle-bone deficits in Crohn's disease

The pathophysiology of muscle-bone deficit in CD is multifactorial and may be attributed to specific physiological actions of disease, lifestyle factors associated with disease, or adverse side effects of therapy.

1.4.2.1 Pro-inflammatory cytokines

Pro-inflammatory cytokines perpetuate gastrointestinal inflammation and tissue destruction in CD while also regulating some extra-intestinal manifestations and systemic effects of disease - including within bone and muscle. Pathological inflammation leads to the disordered development or loss of bone and muscle in children and adults with CD, respectively. The effects of chronic inflammation on bone and muscle in CD may predispose these patients to increased risk of adverse musculoskeletal events such as falls, fractures, and osteoporosis.

The role of inflammation in pathogenesis of skeletal deficits in CD has been highlighted by reduced bone mass in newly diagnosed cohorts of treatment naïve CD patients [208,209]. The serum of treatment naïve CD patients was also found to inhibit osteoblast function *in vitro*, indicating a biochemical component of CD that negatively regulates bone formation - most likely due to the pro-inflammatory milieu [208,210]. Several cytokines known to be elevated in CD such as TNF-α, IL-1ß, and IL-6 inhibit osteoblast function and promote osteoclastogenesis [61,211-213]. TNF-α may be the master regulator of bone loss in CD as it promotes expression of RANKL from osteogenic cells and independently interacts with RANKL as a potent stimulator of osteoclastogenesis, via the nuclear factor kappa B (NF-κB) signalling

pathway [214,215]. The decoy RANK receptor, OPG, is upregulated in an apparent attempt to maintain bone homeostasis in CD; however, this mechanism appears insufficient to reverse RANKL and TNF-α mediated bone resorption [216]. During a disease flare, CD patients were found to have elevated serum RANKL compared to active UC and controls, highlighting the influence of ongoing inflammation on this pathway [213]. The influence of TNF-α on bone turnover in CD is evidenced by the rapid improvement in bone formation markers in individuals treated with TNFα neutralizing antibodies [49,217]. Despite improved markers of bone turnover, however, the long-term effects of anti-TNFα therapy on bone mass are unclear. Children with CD treated with up to 12 months of anti-TNF therapy had persistent deficits in bone and muscle [49,217], despite mild improvements [49]. Whether long term treatment with anti-TNF-α or other cytokine targeting therapies is associated with improvements in skeletal structure and normalisation of BMD in paediatric CD remains unclear. The current data suggests that suppression of TNF-α may only facilitate skeletal improvements and other factors must be involved.

TNF-α and IL-6 are also key regulators of muscle loss in CD, a pathway shared with aging. Circulating TNF-α and IL-6 are increased in healthy elderly adults and are negatively associated with muscle mass and function [218]. These cytokines are elevated in the serum of CD [210,211] and have been found by some [219], but not others [220], to correlate with clinical disease activity. While cytokines play a key role in muscle homeostasis and regeneration, in the context of dysregulated immune function as that in CD, they mediate and contribute to skeletal muscle loss. TNF-α and IL-1ß mediated activation of NF-κB accelerate protein degradation [221] and decrease expression of pro-myogenic factor, MyoD [222]. TNF-α may also inhibit the regenerative capacity of satellite cells through epigenetic silencing of the Notch 1 gene [223]. These pathways can lead to inflammatory muscle loss in CD with reduced muscle mass and function. A prospective study of 19 adults with active CD treated with anti-TNF-α induction therapy observed a significant increase in quadriceps muscle volume at week 25, accompanied by reduced serum IL-6 levels, suggesting suppression of cytokines by anti-TNF-α facilitates the reversal of inflammatory muscle loss in CD [224]. In paediatric CD, anti-TNF-α and EEN have both been associated with short term improvements in biomarkers but not complete normalisation of muscle deficits [49,225].

1.4.2.2 Nutritional deficit

The aetiology of nutritional deficit in CD is multifactorial. Rapid weight loss is typically a key presenting characteristic of CD both at diagnosis and during relapse, indicative of nutritional deficiency. Severe malnutrition, arbitrarily classified as a BMI Z-score < -2 SDS, was observed in approximately 30% of paediatric CD patients at diagnosis in one prospective study, reducing to 15% at long term follow up [226]. Other studies have estimated malnutrition to affect 60 - 80% of patients at diagnosis [227-229].

Individuals with CD often develop food aversions or may follow exclusionary diets to prevent exacerbation of symptoms [230], leading to inadequate nutritional intake. Pro-inflammatory cytokines also directly interfere with appetite hormones and reduce hunger [231]. Dietary intake has been reported as lower than nutritional recommendations in both active and inactive disease [232-234]. During active disease, protein turnover may be increased, thus compounding nutritional deficit.

Malabsorption of nutrients such as vitamin D and calcium may also adversely affect bone and muscle health in CD. Vitamin D deficiency has been reported in up to 60% of children and adults with CD [235-237]. Still, no direct link between vitamin D status and musculoskeletal outcomes have been established in CD. Low calcium intake, however, was recently associated with reduced femoral neck aBMD in a cohort of young adults with CD, although around half of this cohort had active disease at time of assessment which may have contributed to bone loss [238].

Contrary to the typical low weight and BMI observed in CD, a large proportion of patients now present with overweight and obesity, in line with the increasing prevalence of obesity in the general population. This creates a separate issue of nutrition that may contribute to bone and muscle defects. The prevalence of obesity among adults with CD is estimated at 20-50% [239,240]. Obesity is associated with poor bone microarchitecture relative to bodyweight [241] and increased BMA and muscle fat infiltration [242], risk factors for poor muscle function and skeletal fragility. In the absence of obesity, however, CD remains associated with increased visceral adiposity or ‘creeping fat’. This visceral adiposity expresses pro-inflammatory adipokines such as TNF-α, IL-1ß, and IL-6 that may perpetuate systemic inflammation and contribute to bone and muscle loss in CD [12].

1.4.2.3 Glucocorticoids (GC)

GC are routinely administered as first line induction therapy for moderate to severe CD in adult and paediatric patients, although EEN is now preferred first line therapy for paediatrics. Despite the highly effective immunosuppressing properties of GC, they are associated with profound and devastating effects on musculoskeletal health. Chronic GC exposure can lead to muscle atrophy, GC induced osteoporosis (GIO), and a markedly increased risk of fractures [33,35,36]. In children, GC exposure can lead to delayed pubertal development and inhibition of linear growth [36,243].

In adults, GC induced bone and muscle loss occurs in a rapid, dose-dependent manner. GC induce preferential loss of metabolically active trabecular bone [244], highlighted by the high incidence of vertebral fractures in GC treated populations. Primarily, GC induced bone loss occurs via protracted reduction of bone formation, as GC inhibit osteoblast function [33]. They have also been observed to induce osteoblast and osteocyte apoptosis [245]. An initial increase in bone resorption is also observed as GC stimulates increased expression of RANKL from osteoblastic cells while downregulating OPG expression [246]. Additionally, GC reduce the capacity of mature osteoblasts to synthesize type I collagen, leading to a reduced volume of bone matrix for mineralisation [247]. Extra-skeletal effects of GC include impaired intestinal absorption of calcium and phosphate, impaired vitamin D metabolism, and increased renal calcium excretion, which may lead to secondary hyperparathyroidism [33].

In children, GC directly inhibit growth via peripheral and central mechanisms. GC cause local GH/IGF-1 insensitivity at the growth plate by disrupting GH/IGF-1 receptors in chondrocytes [144]. They also inhibit IGF-1 signalling which impedes proliferation and upregulates apoptosis in chondrocytes [248,249]. Chronic exposure to GC in children can also contribute to delayed puberty via hypogonadotropic hypogonadism due to suppression of gonadotrophic releasing hormone (GnRH) [250]. The use of GC in paediatric patients is therefore avoided if possible, or very closely monitored and tapered as quickly as is clinically viable to minimise developmental disturbance.

The catabolic effects of GC within muscle may also negatively influence bone. The muscle wasting properties of GC can be observed as little as 7 days after initiation of therapy [251]. GC inhibit protein synthesis, potentially via reductions in circulating and muscle IGF-1, which typically promotes activation of protein synthesis pathways. Myofibers also experience increased protein degradation via upregulation of atrophy related genes including muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx) [252,253], leading to muscle weakness. These GC induced muscle mass and function deficits may increase the risk of musculoskeletal morbidity, including sarcopenia, falls, and fractures.

The effects of GC, independent of inflammation, on musculoskeletal health in CD are difficult to establish. Active disease is an indication for GC therapy that is itself known to disrupt bone and muscle as discussed above (Section 1.4.2.1). In a prospective study of newly diagnosed paediatric CD who received subsequent GC therapy, cortical vBMD was normal whereas cortical area and trabecular vBMD were reduced at baseline [37]. At follow up, those with lower exposure to GC and inflammation had the greatest improvements in cortical area and linear growth, although they remained low compared to reference values. In a recent cross-sectional study of young adults with CD, all individuals who were currently on GC therapy (n = 14) had low aBMD assessed by DXA, while the majority (87%) not on GC had normal aBMD [238].

1.4.2.4 Pubertal delay

The insidious onset of CD in children typically manifests in the pre- or early pubertal age and can significantly delay the timing of puberty. Delayed pubertal development has extensive implications for muscle-bone health. The pubertal growth spurt is a critical period of muscle and bone accrual, and any disturbance can result in poor linear growth and sub-optimal bone and muscle accretion. Studies have used various maturational markers to assess pubertal delay, including bone age [254], age at menarche [255], and age at pubertal growth spurt [256]. Age at initiation of pubertal growth spurt was delayed by a median 0.8 (1.7, -0.4) and 0.5 (2.1, -1.5) years in girls and boys, respectively, compared to healthy controls [256]. Radiographic bone age was delayed by 1.3 and 0.7 years in girls and boys, respectively, compared to reference values [254]. In the menarcheal age cohort, median age at menarche was delayed by nearly two years in CD [255].

Nutritional deficits and inflammatory cytokines are implicated in the pathogenesis of pubertal delay in CD. Chronic undernutrition is associated with reduced fat mass and low circulating levels of leptin [257], which is an essential hormone for the advent of puberty. Animal models, however, have suggested that reduced leptin is not the sole regulator of delayed puberty in CD and that pro-inflammatory cytokines have a role, likely through their inhibitory actions on gonadotropin secretion [258,259]. This hypothesis is supported by a recent study in children with active CD who were treated with anti-TNF-α induction therapy. Pro-inflammatory cytokines were reduced and circulating sex hormones and gonadotropins improved, independently of body composition [260]. The role of inflammation in pubertal delay is also supported by evidence of pubertal progression after intestinal resection in refractory active disease [261]. While contemporary therapies have reduced the prevalence and severity of pubertal delay in paediatric CD, children with diagnostic delays or who fail to respond to current therapies remain at risk. Whether maturation delay shortens the proposed window of opportunity for skeletal growth is unclear. One study reported that continued linear growth past the expected timeframe was common in young people with CD and bone age delay, potentially due to delayed fusion of epiphyseal growth plates [262]. Expected epiphyseal fusion, however, was estimated based on chronological age in this study and bone age was not directly evaluated [262]. Nevertheless, these authors suggested that if inflammation can be well controlled, young people with CD may experience an extended period growth that could facilitate recovery of any skeletal deficits encountered earlier in the disease course.

1.4.2.5 GH/IGF-1 abnormalities

In paediatric CD, failure of linear growth is a concern that often precedes gastrointestinal symptoms, potentially caused by abnormalities in the GH/IGF-1 axis. Studies have generally reported normal levels of GH secretion with reduced IGF-1 and IGFBPs in CD, although a range of different abnormalities in GH/IGF-1 have been observed [263] Systemic inflammation inhibits multiple aspects of the GH/IGF-1 axis [264]. Pro-inflammatory cytokines such as IL-6 and TNF-α reduce IGF-1 through inhibition of GH signal transduction [265] in hepatocytes. IL-6 also increases proteolysis of IGFBP3, which inhibits IGF-1/IGFBP3 complex formation and increases IGF-1 clearance [266]. Anti-TNF-α therapy has been shown to improve IGF-1 in murine models of colitis [267] and adults with CD [268]. In two prospective

studies in paediatric CD, anti-TNF-α therapy was associated with a rapid improvement in IGF-1 Z-score after 10 weeks in one study (-0.4 SDS vs -1.0 SDS, $p < 0.001$) [48] but no change in another [217], although IGF-1 Z-scores were normal at baseline in the latter. Both studies reported persistent muscle-bone deficits at 12 months follow up, with serum IGF-1 levels comparable to baseline despite sustained improvements in disease activity [48,217]. Muscle mass and function deficits were also associated with reduced capacity for muscle protein synthesis in young adults with CD, potentially linked to reduced IGF-1 activation of hypertrophy pathways [269].

1.4.2.6 Physical inactivity

The important role of PA as a regulator of bone and muscle mass through mechanical and molecular pathways is clear (Section 1.2.5). Observational data highlight low levels of habitual PA and exercise in self-reported [270-272] and accelerometry [273,274] studies. Only 6% of adolescents with IBD achieved the recommended guidelines of 60 minutes per day of MVPA, while >50% had BMD Z-scores of < -1 SDS [273]. In the same study, MVPA was positively associated with BMD independently of pubertal status and BMI. Physical inactivity could potentially contribute to the systemic effects of CD independently of the lack of mechanical loading. The expression of myokines from skeletal muscle is downregulated during periods of inactivity. As myokines perform several anti-inflammatory functions, their absence leads to a pro-inflammatory state. This, plus increased accumulation of adipocytes within inactive muscle, could perpetuate local and systemic inflammation in CD. It has been suggested that low PA may contribute a physical component to the chronic fatigue observed in individuals with CD/IBD [274].

1.4.3 Bone health in CD

As discussed, the potentially adverse effects of CD and its associated risk factors on bone are multifactorial. A summary of major imaging studies assessing bone mass, geometry, and microarchitecture in paediatric and adult CD is available in Table 1-1. Imaging studies in paediatric CD have reported more consistent findings than those in adults.

The currently understood skeletal phenotype in paediatric CD is characterised by deficits in cortical bone geometry with low aBMD and trabecular vBMD but maintained cortical vBMD (Table 1-1). Abnormalities are evident at diagnosis in paediatric CD, with deficits in trabecular vBMD [275-277], cortical thickness [209,275-277], and muscle CSA [225,275-277] observed in pQCT studies. These highlight the role of uncontrolled inflammation on muscle-bone outcomes in paediatric patients which can lead to growth arrest or pubertal delay, often characterised by short stature [209,225,276,277]. Adverse skeletal outcomes have been demonstrated independently of linear growth or pubertal delay in studies that reported low DXA and pQCT Z-scores in CD when adjusted for height [277,278], bone size [174,276], or bone age delay [279,280]. Prospective studies have failed to report normalisation of bone deficits after long term follow up in paediatric CD patients [276,280] or in those treated with EEN [225] or anti-TNF-α [49,217,281], suggesting factors other than disease control are important. These results could also suggest that current methods of evaluating disease severity may not be sensitive enough to detect milder degrees of chronic inflammation. Currently, little is known regarding microarchitectural parameters in paediatric CD, as no *in vivo* high-resolution imaging studies have been performed exclusively in this population. One study analysed trans-iliac biopsies in a small cohort of newly diagnosed CD patients (n = 20) and found maintained trabecular bone volume despite cortical deficits, inhibited bone turnover activity, and low BMAD [209].

In adults with CD, imaging studies of bone health have been almost exclusively conducted using DXA, excluding two HR-pQCT studies [282,283]. The skeletal phenotype in adults with CD is less clear, as some conflicting data exists between studies. Additionally, several studies report data for CD and UC collectively under the umbrella of IBD, despite suggestions bone health is more adversely impacted in CD than UC. Nonetheless, the skeletal phenotype in adults with CD appears to be one of low aBMD, particularly at the lumbar spine and hip regions [284-287]. This was further highlighted in a recent meta-analysis that reported mean difference in aBMD Z-scores of -0.52 (95%CI: -0.71, -0.32) and -0.45 (95%CI: -0.62, -0.29) at the lumbar spine and femoral neck, respectively, in adults with IBD [288]. One population-based study reported a higher likelihood of osteoporosis in IBD compared to healthy age and sex matched controls (OR: 1.47 [95%CI: 1.2, 1.78]) [287]. In studies that report no overall group deficits in aBMD T-scores or Z-scores,

the prevalence of osteopenia remains high at up to 35% [286,289,290]; highlighting how the diagnosis of CD itself may not be the primary determining factor of low bone mass. Imaging assessment of skeletal macro and microstructure is less common in adults with CD. Two HR-pQCT studies reported deficits in trabecular microarchitecture and vBMD, and cortical geometry, in young [283] and middle-aged [282] adults with IBD. Most published studies represent patients of a previous era in terms of disease management and imaging technology. Further high-resolution imaging studies are required to understand the micro and macrostructural phenotype of bone in contemporary CD, especially those of childhood onset disease.

Table 1-1 Summary of major bone imaging studies in paediatric and adult CD

Author (yr) Country	N Age (years)	Study design	Methods	GC History	Bone / Muscle findings	Comments
Paediatric Studies						
Altowati (2018) [217] Scotland	19 CD 14.9 (11.2, 17.2)	Prospective cohort	pQCT ND Radius & Tibia DXA TB & LS (Lunar Prodigy)	47% GC at baseline. 11% at follow up.	↓ Trabecular vBMD ↔ Cortical vBMD ↓ Cortical Thickness & Muscle CSA (Radius only) ↓ TB & LS aBMD	No improvement in bone or muscle outcomes after 12 months anti-TNF-α. Linear growth improved in subgroup w/ growth potential. IGF-1 improved only in those with low levels at baseline.
Ward (2017) [277] Canada	73 CD 13.9 (7, 17.7)	Prospective cross-sectional	pQCT Left Tibia DXA TB & LS (Lunar Prodigy) VF radiograph	64% induction GC	↓ Trabecular vBMD ↑ Endo & ↓ Peri Circ. ↓ Cortical thickness & Muscle CSA ↓ LS aBMD, BMAD & TB BMC 1 VF	Incident cohort <35 days of initial therapy. Moderate-to-severe disease in 90%. Jumping mechanography muscle function low vs reference data (age- and sex-adjusted Z-scores).
Maratova (2017) [278] Czechia	70 IBD (53 CD) 14.2 (IQR 12.7, 16.1)	Prospective cross-sectional	pQCT ND Tibia Lateral radiograph VF	8/53 CD w/ GC <12 months	↓ Trabecular vBMD ↓ Cortical Thickness ↑ Cortical vBMD & SSI 1 VF; ↓ vertebral height in 27%.	Height adjusted pQCT Z-scores. Remission or mild disease. Jumping mechanography muscle function assessments were not different to reference data (sex and height adjusted).
Griffin (2015) [49] USA	74 CD 14 (5, 21)	Prospective cohort	pQCT Left Tibia	32% GC at baseline. 7% at follow up.	↓ Trabecular vBMD ↔ Cortical vBMD (base) ↓ Muscle CSA ↑ Endo & ↓ Peri Circ.	Infliximab induction cohort. 50% severe disease at baseline. Trabecular vBMD improved but still low at 12 months. Cortical vBMD low at follow up, endo circ. Improved. Improvements in younger and those with best short-term response to therapy.
Pichler (2015) [281] Austria	18 CD 14.4 (5.3, 19.1)	Retrospective	DXA LS (Lunar Prodigy)	61% GC at baseline.	↓ LS aBMD & BMAD	DXA aBMD Z-score not adjusted for body size. No change in aBMD or BMAD after one-year adalimumab. 14/18 were on infliximab prior to adalimumab.
Laakso (2012) [279] Finland	80 IBD (28 CD) 14.9 (5.1, 20.1)	Prospective cross-sectional	DXA TB, LS, TH & VF (Hologic)	81% ever GC. 30% current GC.	↓ TB & LS aBMD ↔ TH aBMD Abnormal vertebrae in 9 IBD (total 16 VF)	All IBD vs controls, although no differences between CD and UC. DXA Z-scores adjusted for chronological age and bone age. Huge variation in disease duration & cumulative GC.

Author (yr) Country	N Age (years)	Study design	Methods	GC History	Bone / Muscle findings	Comments
Werkstetter (2013) [225] Germany	10 CD 13.7 (10.6, 17.7)	Prospective cohorts	pQCT ND Radius	GC naïve. No GC during follow up.	↔ Trabecular & cortical vBMD ↓ Muscle CSA After 12 weeks: Trabecular vBMD & Muscle CSA ↑, cortical vBMD ↓. No further changes at 52 weeks.	Incident cohort. Height SDS low. Height adjusted pQCT Z-scores. Short term improvements in bone turnover and IGF-1, no further change at 52 weeks.
Ward (2010) [209] Canada	20 CD 14.7 (8.4, 17.7)	Prospective cross-sectional	Iliac biopsy histomorphometry DXA TB & LS (Lunar Prodigy) VF Radiograph	GC naïve	↔ Trabecular bone volume ↑ Trabecular number ↓ Cortical thickness ↓ LS aBMD & BMAD ↓ TB lean mass & BMC No evidence of VF	Incident cohort. Bone age ↑ vs CA. DXA BMC & lean mass Z-scores adjusted for height. BMC:lean mass ratio normal for height. Conflicting biopsy data – osteoclast surface was low but eroded surface high.
Bechtold (2010) [275] Germany	143 IBD (98 CD) I: 13 ± 3.3 P: 14.7 ± 3.4	Prospective cross-sectional	pQCT ND Radius	Incident: GC naïve Prevalent: Not reported	↓ Trabecular vBMD ↑ Cortical vBMD ↓ Bone & Muscle CSA	All IBD vs controls, CD not reported separately. Muscle CSA SDS lower in newly diagnosed vs prevalent disease. GC history did not influence results.
Dubner (2009) [276] USA	78 CD 12.7 ± 2.8	Prospective cohort	pQCT Left Tibia	62% GC, 0 to 6 months. 31% GC, 6 to 12 months	↓ Trabecular vBMD ↔ Cortical vBMD ↓ Muscle & Fat CSA ↑ Endo & ↓ Peri Circ. ↓ Section modulus (Zp)	pQCT geometry Z-scores adjusted for tibia length. Incident cohort <2 weeks of diagnosis. 84% with growth failure. Mild improvements after 12 months follow up but trabecular, muscle & Zp still low.
Sylvester (2007) [280] USA	58 CD 13 ± 3	Prospective cohort	DXA TB & LS (Lunar DPX)	56% GC during follow up.	↓ TB & LS aBMD. No change in Z-scores at 2 years follow up.	Incident cohort. Bone age delay ~1 year in CD. DXA adjusted for bone age. Elevated serum IL-6 associated with low aBMD in CD. TB aBMD positively correlated with IGF-1.
Burnham (2004) [174] USA	104 CD 15.4 ± 4.3	Prospective cross-sectional	DXA TB (Hologic)	90% previous GC. 11% current GC (enemas)	↓ TB BMC in sex, height and puberty adjusted models. ↔ TB BMC when + adjustment for lean mass.	DXA adjusted for bone size. Log-transformed models for BMC relative to height. Age & pubertal status of CD and controls different. Low BMI Z-score associated with low bone mass. GC history not correlated with growth or bone outcomes.

Author (yr) Country	N Age (years)	Study design	Methods	GC History	Bone / Muscle findings	Comments
Gupta (2004) [291] Canada	82 CD 11.8 ± 2.6	Prospective cohort	DXA LS (Hologic)	Not reported	↓ LS BMD at baseline No change in Z-scores at follow up of between 3 – 9 years (n = 25)	Incident cohort. Age adjusted DXA Z-scores. Not adjusted for body size. Locally recruited controls also had slightly low Z-scores vs reference data
Adult Studies						
Pepe (2018) [283] Switzerland	102 IBD (75 CD) 23.1 ± 5.8	Prospective cross-sectional	HR-pQCT ND Distal Radius & Tibia DXA LS, FN, PF & Radius, VF	17% current GC.	↓ aBMD at all sites in IBD vs control ↓ Total & Trabecular vBMD ↓ TbN & Cortical thickness at Tibia. ↑ TbSp at Radius & Tibia 5 VF	Includes paediatric & adult patients but data not presented separately. Fractured IBD had lower total and trabecular vBMD & TbTh, and higher TbSp & Tb inhomogeneity vs non-fractured.
Sigurdsson (2017) [292] Sweden	94 IBD (29 CD) 21.8 (18.3, 27.7)	Prospective cross-sectional	DXA TB, LS, FN, SMI (Lunar DPX)	93% current or previous GC. Unclear.	↓ TB, LS, FN aBMD & SMI Z-scores in male CD (n=20) ↔ TB, LS, FN aBMD & SMI Z-scores in female CD (n=9)	Childhood onset cohort. DXA not adjusted for body size. Males lower height & weight compared to controls. Higher myopenia (24% vs 15%) & myopenic-obesity (9% vs 2%) in IBD vs controls. Myopenia & myopenic-obesity associated with low aBMD at all sites.
Haschka (2016) [282] Germany	98 IBD (59 CD) 42.8 (IQR 30, 54)	Prospective cross-sectional	HR-pQCT Dominant Ultra-distal Radius	12% current GC.	↓ Total, Trabecular & Cortical vBMD ↓ Trabecular BV & TbTh ↓ Cortical thickness & area	Poorer vBMD and structural outcomes in CD vs UC. 72% current biologics use. 45% current/previous smokers. 54% history of chronic high-dose GC. Low BMD, female sex, and lack of remission associated with low cortical area.
Targownik (2013) [287] Canada	1230 IBD (719 CD) 49 (IQR 38, 57)	Retrospective	DXA LS, FN, TH (Lunar DPX/Prodigy)	23% GC @ DXA	↓ aBMD T-scores at all sites ↓ aBMD T-scores vs controls (n=44500) in adj. regression.	Increased risk of osteoporosis for CD vs controls in adjusted regression (OR: 1.47 [1.2, 1.78]). aBMD T-scores negatively associated with GC at all sites.
Leslie (2008) [285] Canada	101 IBD (56 CD) 46.9 ± 15.5	Prospective cohort	DXA TB, LS & TH (Hologic)	60% prior GC. Current GC not reported.	↓ LS & TH aBMD T-scores ↔ TB aBMD T-scores ↔ TB, LS & TH aBMD Z-scores	Mean LS aBMD T-score at lower end of normal (-0.76 ± 1.2). Young IBD (aged <50) lower BMD Z-scores vs older IBD (>50). Men lower Z-scores vs women at LS.
Bernstein (2003) [289] Canada	70 IBD (58 CD) 33 ± 7.4	Prospective cross-sectional	DXA TB, LS, FN & TH (Lunar Prodigy)	82% previous GC for >1 month	↔ aBMD T-scores at all sites 25/70 (36%) osteopenic T-scores at ≥1 site.	All premenopausal women. 'Early onset' IBD (diagnosed <20 years). CD & UC presented together. Manufacturer reference data. Low BMD associated with recent amenorrhea and low bodyweight.

Author (yr) Country	N Age (years)	Study design	Methods	GC History	Bone / Muscle findings	Comments
Schoon (2000) [286] Netherlands	68 IBD (24 CD) 29.7 ± 10.4	Prospective cross-sectional	DXA TB, LS & TH (Lunar DPX)	33% current GC.	↔ aBMD Z-scores at all sites 18/68 (26%) osteopenic T-scores at ≥1 site.	Incident cohort <6 months from diagnosis. Mean T-scores not reported. 12.5% active disease at time of DXA. Local controls for Z-scores (n=68).
Robinson (1998) [290] England	117 CD 40.6 ± 13.3	Prospective cross-sectional	DXA LS, PF (Lunar DPX)	22% current GC. 86% ever GC.	↔ aBMD Z-scores at all sites 34/117 (29%) osteopenic, 14/117 (12%) osteoporotic [Z-scores]	DXA age- and sex-adjusted Z-scores (Not T-score). aBMD negative association with GC history.
Ghosh (1994) [284] Scotland	30 IBD (15 CD) 24 (14, 83)	Prospective cohort	DXA LS, Right Arm (Hologic QDR)	47% started GC.	↓ LS & Arm aBMD Z-scores in CD	Incident cohort <3 weeks of diagnosis. Age- and sex-adjusted Z-scores from manufacturer. No change in Z-scores after 12 months.

CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis; N, number; GC, glucocorticoids; aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; DXA, dual energy x-ray absorptiometry; pQCT, peripheral quantitative computed tomography; BV, bone volume; TbN, trabecular number; TbSp, trabecular separation; endo., endosteal; peri., periosteal; circ., circumference; TbTh, trabecular thickness; TB, total body; TH, total hip; LS, lumbar spine; PF, proximal femur; FN, femoral neck; ND, non-dominant; ↑, high; ↓, low; ↔, not different.

1.4.4 Muscle health in CD

Despite sharing common risk factors, the assessment of muscle mass and particularly muscle function have received notably less attention than bone health in children and adults with CD. Recently, investigations of muscle in CD have increased as the relationship of muscle with bone and clinical outcomes has become more apparent. Many adult bone imaging studies in CD failed to report muscle outcomes (Table 1-1). Others have employed arbitrary definitions of sarcopenia, primarily based on muscle mass, in their assessment of skeletal muscle in CD. Low muscle mass was observed in 51% and 58% of CD patients at diagnosis [293] and during an acute flare [294], respectively, when assessed by abdominal CT. Similarly, using DXA, 60% of CD patients in remission were observed to have low muscle mass, 91% of which also had osteopenic BMD T-scores [295]. Combined low muscle mass and function was also associated with six-fold higher likelihood (OR: 6.3) of osteopenia in another cohort [296], highlighting the close relationship between muscle and bone intact in individuals with CD. Muscle function may be low in adults with CD compared to controls even when matched by BMI [297-299]; however, BMI can be reassuringly normal in the context of low muscle mass in CD [292]. Poor muscle function is associated with subjective fatigue [298], again suggesting that muscle fatigue might contribute to general fatigue in adults with CD [274]. Other studies have noted an association between low muscle mass and clinical outcomes in CD, including early failure of anti-TNF-α therapy [300] and post-surgical morbidity [294], although these were retrospective in design and require validation in prospective assessment.

The related development of muscle and bone during growth may explain the more consistent reporting of muscle outcomes in primarily bone focused research in paediatric CD. Studies using pQCT have observed significant deficits in muscle CSA at the tibia and radius in children with newly diagnosed [225,275-277] and established [49,217,275] CD. Newly diagnosed CD was associated with lower muscle CSA Z-scores *vs* prevalent disease in one study, suggesting recent inflammatory activity may be a key contributor to muscle deficits [275]. Deficits in lean and muscle mass were evident when adjusted for height [209,225,276] although the ratio of BMC to lean mass was maintained, highlighting no discordance in the muscle-bone relationship [209]. As with bone, muscle deficits have been found to improve slightly, but remain low, after long term follow up [49,217,225,276]. Two studies reported on dynamic muscle

function in CD using jumping mechanography, with conflicting results. No deficits were found for muscle function in a cohort of adolescents with mild prevalent IBD (N = 70, 53 CD) [278], whereas newly diagnosed CD (n = 73) was associated with significantly reduced muscle power compared to reference data [277]. The influence of active disease is an obvious confounder in the comparison between these studies, however they also used different methods of mechanography testing, compared to different reference datasets, and only one used Z-scores that were adjusted to body size [278]. Others using hand dynamometry have reported low age and sex-adjusted Z-scores for grip strength in CD, which is also likely to have been confounded by poor growth in these cohorts [225,275]. It is currently unclear if muscle function deficit in paediatric CD is a product of purely reduced muscle mass, or whether intrinsic deficits in muscle function exist that may be targeted for future interventions.

1.5 The potential role for exercise in CD

1.5.1 Overview

PA and exercise are critical determinants of physical and psychological wellbeing across the lifespan. A clear dose-dependent relationship exists between PA and the risk of morbidity and mortality from several chronic disease, with higher levels conferring the greatest protection. The utility of exercise as an adjunctive therapy in chronic disease is also well established. Despite this, current evidence for the role of PA and exercise in the management of CD and IBD is lacking. This section will discuss the potential mechanisms by which PA and exercise may alleviate the burden of IBD and its associated symptoms and briefly discuss research into the role of exercise in experimental models of IBD.

1.5.2 Exercise in CD: potential beneficial mechanisms

1.5.2.1 Inflammation

Exercise upregulates expression of skeletal muscle myokines that exert several anti-inflammatory functions [301]. These anti-inflammatory properties have potential to alleviate chronic low-grade inflammation, which remains elevated in CD even during periods of remission. The primary myokine expressed during exercise is IL-6, the expression of which is dose-dependently related to exercise intensity and duration [302]. Typically, elevated IL-6 is associated with pro-inflammatory mechanisms, and contributes to bone, muscle, and intestinal tissue destruction in CD. Muscle expressed IL-6, however, has direct and indirect anti-inflammatory actions. It stimulates expression of anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1ra), which inhibit the actions and expression of TNF-α and IL-1β [303]. Myocyte expressed IL-6 also induces lipolysis and increases systemic fat oxidation [94], indirectly contributing to reduced inflammation. Regular exercise can also inhibit the infiltration of macrophages into adipocytes [304], which are known to be abundant in the creeping fat of individuals with IBD and contributors to intestinal and systemic inflammation. Exercise has also been shown to downregulate the surface expression of toll like receptors (TLRs) in monocytes, effectively desensitising them to pro-inflammatory stimulus and helping generate an anti-inflammatory phenotype [301].

The cumulative anti-inflammatory effects of individual bouts of PA or exercise leads to long term reduction in low-grade inflammation. This has been highlighted in studies reporting inverse associations between habitual PA and inflammatory markers in both adolescents [305,306] and adults [307]. The association between IL-6 and systemic inflammation in CD suggests an exercise induced spike in circulating levels could potentially exacerbate disease; however, the demonstrable anti-inflammatory actions of muscle derived IL-6 suggest any exacerbation of disease is unlikely.

1.5.2.2 Fatigue

Fatigue is one of the most reported clinical problems in CD. Up to 50% of individuals with CD in clinical remission and 80% of those with active disease report chronic fatigue [308]. The anti-inflammatory effects of exercise may play a role in reduction of fatigue in CD, as inflammation has been implicated in the pathogenesis of fatigue in IBD [308]. Reduced PA and exercise capacity have been reported in a small study (n = 10) of fatigued compared to non-fatigued individuals with IBD [274], suggesting poor muscle function may be a contributor to overall fatigue. Similarly, severe fatigue was more prevalent among CD patients who self-reported being physically inactive compared to active peers (34% vs 19%) [272]. PA and fatigue likely exist in a negative feedback mechanism in CD, whereby a lack of activity leads to poorer physical function and increased feelings of fatigue, subsequently leading to less activity. The potential for exercise in combating fatigue in CD remains relatively unexplored and fatigue is commonly cited as one of the primary barriers to exercise participation in CD and IBD [271,272,309]. On the other hand, much research has been conducted into the effects of exercise in cancer related fatigue, which is also thought to be at least partly mediated by elevated circulating pro-inflammatory cytokines [310]. Exercise has been demonstrated as an effective intervention in the treatment of cancer related fatigue, both during and post treatment [311]. The mechanisms involved may therefore be beneficial to individuals with CD and this should be included as a primary outcome in future research, owing to the high fatigue burden in these patients.

1.5.2.3 Psychological wellbeing

The many facets and unpredictability of CD can lead to poor health related quality of life (HRQoL) and psychological wellbeing. The prevalence of anxiety and depression in adults with IBD is approximately double that of the healthy population [312]. Additionally, in adolescents with IBD, disturbances in physical maturation contribute to poor HRQoL. The association between PA and psychological wellbeing has been reported in health [313,314] and other inflammatory diseases [315]. The mechanisms that mediate the effects of PA on improved psychological wellbeing are not clear but may occur secondary to improved physical function, self-efficacy, or inflammation. The available data in CD and IBD is limited to self-reported studies. One study found self-reported PA to be negatively associated with depression after adjustment for potential confounders [272]. Another reported no associations between walking or MVPA with mental wellbeing [316]. These studies employed the same PA assessment tool but neither used an IBD-specific assessment of HRQoL, therefore failing to report on disease specific aspects of psychological wellbeing. Studies with directly measured PA and disease specific indices of HRQoL are needed to understand the potential role for PA and exercise in the psychological wellbeing of individuals with CD and IBD.

1.5.2.4 Bone

The potential benefits of PA and exercise on bone mass and architecture are mode and intensity dependent. As discussed, primary adaptations in bone occur due to its mechanosensitivity to external loading patterns (section 1.2.5.1). Skeletal adaptation to PA or exercise is therefore highly dependent on the type and chronicity of the activity. For example, significantly greater aBMD, bone CSA, and bone section modulus have been observed in women participating in high-impact (e.g., volleyball, football, sprinting) compared to low or non-impact (e.g., cycling, swimming) sports after adjusting for age, height, and weight [152]. High-impact loading exercise is an effective strategy for improving bone mass in school-aged children [317] and pre-menopausal women [318]. Self-reported bone loading activities during adolescence and young adulthood also positively associated with HR-pQCT measured cortical geometry and trabecular microarchitecture outcomes in young adults [319]. Studies of young women undergoing chronic strenuous activities (i.e. military training) have also reported 3-5% increases in micro and macrostructural

parameters of the tibia, independently of changes in aBMD [156]. However, the extremely high volumes associated with this training was also associated with a threefold increased risk of stress fractures, suggesting the optimal intensity for skeletal adaptation is likely to be lower.

Observational data suggest low-impact activities such as walking are most popular among individuals with CD and IBD [272,309], however these are likely to be ineffective for improving bone mass. One study assessed the effects of 12 months of daily low magnitude mechanical stimulation in children with CD and low tibia vBMD, observing no benefit to DXA or pQCT measured bone parameters [320]. The low strain magnitude may have been insufficient to stimulate bone adaptation in children with normal ambulation, suggesting higher mechanical loads are required. Agreeably, a recent observational study noted a positive association between accelerometer measured MVPA and bone mass in adolescents with IBD [273]. Contemporary disease management improves bone metabolism in CD but does not appear to normalise bone mass deficits. The promotion of impact-based activities may stimulate greater bone mass accretion and bone strength in paediatric and adult CD, respectively, and warrants investigation as an adjunctive intervention.

1.5.2.5 Muscle

The skeletal muscle wasting in CD may be counteracted by PA and exercise. Particularly, resistance training promotes muscle hypertrophy by activation of muscle protein synthesis pathways [160,161], which were found to be impaired in a cohort of young adults with CD [269]. This impaired activation of the IGF-1/Akt muscle protein synthesis pathway was associated with lower muscle mass and habitual PA. The rate of protein turnover is increased during active CD and can be alleviated by effective disease control [321,322]. However, therapies may alleviate protein degradation while concurrently reducing protein synthesis, with minimal effect on net protein balance. This may partly explain the persistence of muscle mass deficits in remissive CD [295,299]. For example, paediatric CD patients treated with EEN or anti-TNF-α therapy experience modest short-term improvements in muscle mass that parallel the induction of disease remission, but muscle mass deficits persist after long term follow up [49,217,225]. The addition of exercise as an adjunct to pharmacological therapy may promote muscle hypertrophy in the

context of reduced inflammation. Habitual PA and exercise may also indirectly reduce proteolysis in muscle through anti-inflammatory mechanisms that ultimately inhibit the actions and expression of pro-inflammatory cytokines like TNF-α [94]. The association between low muscle mass and poor clinical outcomes in CD suggests that increasing muscle could lead to improved disease status and help alleviate other complications such as fatigue or osteopenia.

1.5.3 Exercise in CD: summary

This section has highlighted the potential ways in which exercise may be an effective strategy for counteracting the secondary complications of CD, including musculoskeletal deficits. Currently, no disease specific guidelines exist for PA and exercise in CD or IBD, and the overall utility of exercise as a management tool in IBD is unknown. In Chapter 5, a systematic review of the current literature was performed to assess the potential role of exercise in both children and adults with IBD.

1.6 Rationale summary and aims of the present work

The muscle-bone unit is highly dynamic, and its development and maintenance are tightly regulated by multiple intrinsic and extrinsic factors. Here, it has been discussed how Crohn's disease is associated with multifactorial insult to the muscle-bone unit, including chronic inflammation, nutritional deficit, and prolonged exposure to GC. These factors directly and indirectly inhibit bone and muscle accrual in paediatric CD and can cause accelerated bone and muscle degradation in adult CD. Subsequently, individuals with CD may be at increased risk of musculoskeletal morbidity and adverse events such as falls and fractures. Advanced imaging techniques allow high-resolution assessment of the musculoskeletal apparatus that was previously unavailable and has not been extensively carried out in paediatric or young adults with CD. These detailed assessments may identify specific facets of the musculoskeletal apparatus that can be targeted in future interventions. Further, despite extensive knowledge of the positive effects of exercise on bone and muscle health, the potential role for exercise in alleviating musculoskeletal burden in CD remains relatively unexplored. Therefore, the overall aims of this study were to characterise the muscle-bone unit in young people with CD and to explore the potential role that exercise may play in the management of any associated muscle-bone deficits.

The specific aims of each chapter are as follows:

Chapter 2: MRI assessment of the muscle-bone unit in paediatric Crohn's disease. Aim – Investigate the muscle-bone unit in paediatric CD using high-resolution MRI imaging, MRS, and functional assessment of muscle-bone.

Chapter 3: MRI assessment of the muscle-bone unit in young adults with childhood onset Crohn's disease. Aim - Investigate the muscle-bone unit in paediatric CD using high-resolution MRI imaging, MRS, and functional and biochemical assessment of muscle-bone.

Chapter 4: 3 Associations between IBD and adverse musculoskeletal outcomes in middle-aged and older adults. Aim – Investigate the associations between a diagnosis of IBD, and its subtypes CD and UC, and musculoskeletal outcomes

including falls and fractures using data from a large, population-based cohort study.

Chapter 5: Systematic review of exercise in inflammatory bowel disease. Aim – Assess the available evidence for the potential role of exercise in the management of IBD and its associated complications.

Chapter 6: Feasibility of jumping based exercise as complementary therapy in adolescents and adults with Crohn's disease: a questionnaire study. Aim – Investigate the attitudes of adolescents and adults towards the exploration of jumping based exercise as an adjunctive therapy for the treatment of muscle-bone deficits in CD.

Chapter 7: Feasibility of jumping based exercise in children and adolescents with Crohn's disease. Aim – Assess the feasibility and safety of a short-term, jumping based exercise intervention in children and adolescents with CD.

2 MRI assessment of the muscle-bone unit in paediatric Crohn's disease

2.1 Abstract

Background. Paediatric CD is associated with multiple risk factors for adverse muscle-bone development. Prior studies have reported deficits in bone and muscle mass but evidence regarding bone microstructure is lacking, particularly in cohorts managed with contemporary therapies.

Aim. Investigate the muscle-bone unit in a cohort of paediatric CD in comparison with healthy age- and sex-matched controls.

Methods. High-resolution MRI was used to image trabecular microarchitecture, cortical geometry, and skeletal muscle area and adiposity at the distal femur in CD and controls. Bone marrow adiposity of the lumbar spine (L3) was measured using MRS. Muscle function was assessed using grip strength dynamometry and jumping mechanography in CD and compared to previously published normative datasets. A sub-cohort of CD participants also underwent full body DXA scans to measure TBLH-BMC and lumbar spine BMAD.

Results. Twenty-one children and adolescents (11 female) with CD with median age 14.5 (10.6, 16.4) years and 21 age- and sex-matched controls were included. Median age at CD diagnosis was 8 (5, 14) years. Sixteen of the 21 CD participants were in clinical remission and five had mildly active disease. Paediatric CD was associated with lower trabecular bone volume (0.55 [0.44, 0.62] vs 0.58 [0.53, 0.62], p = 0.003) and higher trabecular separation (0.24 mm [0.21, 0.39] vs 0.22 mm [0.20, 0.28], p = 0.002) than controls. No differences were observed between CD and controls for MRI assessed cortical geometry, or muscle area and adiposity; or for BMA assessed by MRS. Compared to previously published normative data, jumping mechanography Z-scores adjusted for age and weight, respectively, were consistently lower than zero (all p<0.05). In the sub-cohort with available DXA (n = 14), LS BMAD (Z-score: -0.6 [-2.1, 1.1], p = 0.01) was lower than zero but TBLH-BMC (Z-score: -0.1 [-0.9, 2.2], p = 0.49) was not. In CD, trabecular bone volume was higher in those with no prior exposure to GC. Self-reported PA was positively associated with trabecular bone volume (r = 0.57, p = 0.01) and negatively

associated with muscle fat fraction ($r = -0.73$, $p < 0.001$) and BMA ($r = -0.63$, $p = 0.002$) in CD.

Conclusions. Paediatric CD was associated with deficits in trabecular bone volume and separation compared to healthy controls, despite well controlled disease. These deficits appeared to be associated with prior GC exposure or disease severity. No differences were observed between CD and control groups for cortical geometry, muscle mass and adiposity, or BMA. CD was, however, associated with low muscle function compared to published reference data. Self-reported PA was also associated with several musculoskeletal outcomes in CD, suggesting it may play a role in any observed deficits.

2.2 Introduction

The onset of CD in childhood has adverse implications for bone and muscle development. Risk factors for poor bone and muscle in paediatric CD include chronic elevation of circulating pro-inflammatory cytokines, exposure to GC therapy, and nutritional deficits. These factors cause systemic and local disruptions in mechanisms of bone and muscle development, including GH/IGF-1 abnormalities [144], impaired bone formation [208,247], muscle catabolism, and delayed puberty [254,255]. Disturbed pubertal development of bone and muscle in CD predisposes these patients to poor peak muscle and bone mass [323]. In turn, these individuals are at increased risk of future adverse musculoskeletal events. This was highlighted in a recent population-based study that reported an elevated risk of hip fracture in adults with paediatric onset CD (HR: 1.7 [95% CI: 1.2, 2.5]) [324]. Assessing the muscle-bone unit in paediatric CD may identify deficits that can be targeted to augment development of bone and muscle mass.

Previous pQCT studies have identified deficits in muscle, cortical bone geometry, and trabecular vBMD, both in previously treated [49,217] and newly diagnosed paediatric CD patients [276,277]. These studies were primarily conducted in patients with active disease, potentially highlighting the adverse effects of underlying inflammation on bone and muscle. However, muscle-bone deficits persisted at 12-24 months follow up despite successful induction and maintenance of remission in most patients [49,217,276]. These studies may represent slightly older treatment paradigms as treatment practises have changed in the last 5-10 years. Little research has been conducted in cohorts with established paediatric CD under contemporary management. Additionally, no studies have employed high-resolution imaging in paediatric CD, meaning detailed evaluations of bone and muscle microstructure are lacking.

Advances in high-resolution MRI have facilitated its use as an assessment tool for bone microstructure [193], in addition to its known capacity as the gold standard imaging assessment of muscle area. Furthermore, when combined with MRS, the additional capacity to assess adiposity provides a comprehensive assessment of bone, muscle, and fat not possible with other imaging methods. BMA is an emerging marker of skeletal health that can be measured by proton MRS. Osteoblast and marrow adipocyte precursors share a common origin in

mesenchymal stem cells and differentiation is competitive between tissue specific lineages [80]. BMA has been found to be elevated in several populations with associated skeletal deficits and may negatively associate with bone mass [88,90]. To date, the role of BMA in skeletal health of paediatric CD has not been investigated in any published study. This study therefore aimed to provide a comprehensive assessment of the muscle-bone unit in paediatric CD using high-resolution MRI and MRS, in comparison to healthy age and sex matched controls.

2.3 Methods

2.3.1 Study population and design

This study adopted a cross-sectional, case control design. From September 2018 to October 2020, children diagnosed with CD who were managed by the paediatric gastroenterology team at the Royal Hospital for Children (RHC), Glasgow, were recruited. Study activity was suspended for a period of approximately 6 months during the period of March to September 2020 due to Covid-19. Potential recruits were identified by the gastroenterology team at RHC and contacted by mail or signposted to a member of the research team at outpatient clinic appointments. A total of 89 individuals were invited and 21 consented to participate in this study. Inclusion criteria were a confirmed diagnosis of CD at least 12 months prior to study involvement, a current age of between 8 and 17 years (inclusive), and no changes in medication for four weeks prior to the study (excluding dosage optimisations). Exclusion criteria were any significant comorbidity affecting musculoskeletal health, the use of medications known to affect bone health (excluding GC for the treatment of CD), and any contraindication to MRI. Prior to enrolment, all participants provided written informed consent or assent, and their respective legal guardian also provided written consent. This study was approved by the South Yorkshire Research Ethics Committee (REC Ref: 18/YH/0229).

2.3.2 Data collection

Disease specific data including age at CD diagnosis, current medication use, surgical history, and history of GC exposure were self-reported and validated in participants' electronic medical records. Disease phenotype at diagnosis according to the Paris classification [3] was also verified in participants' medical records. Disease activity was calculated according to the weighted paediatric Crohn's disease activity index (wPCDAI) [24] and categorised as in remission (<12.5), mildly active (12.5 – 40), moderately active (42.5 – 57.5), or severe (>57.5). Height was measured to the nearest 0.1mm using a wall mounted stadiometer and weight was measured to the nearest 0.1kg. BMI (kg/m^2) was calculated using height and weight measurements. Height, weight, and BMI were transformed into age- and sex-adjusted Z-scores according to UK population reference data. Pubertal status was self-assessed by participants and categorised according to

Tanner. For analysis, Tanner stages were categorised as pre-pubertal (Stage 1), early pubertal (Stages 2 & 3), and late pubertal (Stages 4 & 5). Physical activity was self-reported using age-appropriate questionnaires: the physical activity questionnaire for older children (PAQ-C) (age 8 – 14 years) [325] and the physical activity questionnaire for adolescents (PAQ-A) (age ≥14 years) [326]. These PA questionnaires were used to facilitate comparisons with PA data previously collected in the control cohort.

2.3.3 MRI & MRS Imaging

2.3.3.1 Overview

All MRI images were acquired using the same 3-Tesla MRI scanner (Prisma, Siemens, Erlangen, Germany) at the Glasgow Clinical Research Imaging Facility. Images were obtained using an 18-channel anterior array coil and 32-channel spine array. Participants attended a single MRI scanning session where all imaging was obtained over approximately 70 minutes.

2.3.3.2 MRI assessment of trabecular microarchitecture

2.3.3.2.1 Image acquisition

Localiser scans were obtained to measure the length of the right femur – calculated as the distance between the superior aspect of the femoral head and the distal aspect of the medial femoral condyle. To aid mobilisation and minimise movement artefact, padding was placed around the right leg. Trabecular microarchitecture images were obtained from the distal metaphysis, measured as 15% from the distal aspect of the femur. A series of twenty axial images with resolution of 0.2 x 0.2 x 0.4mm were obtained using a constructive interference in the steady state (CISS) pulse sequence (Table 2-1).

Table 2-1 MRI pulse sequence parameters for trabecular and cortical imaging

	Trabecular Microarchitecture	*Cortical Geometry*
Sequence Type	CISS	T1-weighted TSE
Resolution (mm)	0.2 x 0.2 x 0.4	0.4 x 0.4 x 2
TE (ms)	5.68	11
TR (ms)	12.62	650
Flip Angle (°)	50	150
No. of Averages	4	1
FoV (mm^2)	100 x 100	140 x 140
Matrix	448 x 448	320 x 240
Number of Slices	20	20
Bandwidth (Hz/pixel)	228	240
Scan Time (mins:secs)	9:24	1:05

CISS – constructive interference in the steady state; TSE – turbo spin echo; TE – echo time; ms – milliseconds; TR – repetition time; FoV – field of view; mm – millimetres; Hz – hertz; mins:secs – minutes:seconds

2.3.3.2.2 Image pre-processing

MRI images were analysed in MATLAB (MathWorks Inc., San Mateo, CA) software using code developed in-house by colleagues in NHS GGC medical physics. All MRI image analyses presented in this thesis were completed by the primary researcher (LS). Prior to analyses, trabecular images were pre-processed using a sharpening filter to improve the contrast between hyper- and hypo-intense tissue (i.e. marrow and bone). This involves subtracting a blurred version of each image from the original – known as 'unsharp masking'. While this improves contrast, the filter does not compensate for blurring due to movement during the scan. Images were visually inspected for gross motion artefact and these were excluded from the analysis. The images were further processed through a low pass filter to correct for bone marrow inhomogeneity, which is a common occurrence particularly in paediatric bone and may erroneously lead to marrow being assigned as bone.

2.3.3.2.3 Trabecular analysis

The central slice within the image (slice 10) was used for analyses in all but one participant. A proximal slice (slice 19) was used for this participant due to artefact on other images. Images were analysed in triplicate and the mean value used for subsequent analyses. Trabecular images were of sufficient quality for analyses in 41/42 participants (1 CD – excessive motion artefact). Inter-observer repeatability was assessed using a subset of 30 images, each also analysed in triplicate. Intra- and inter-observer repeatability were high with coefficient of variations (CVs) of <1% and <2% for all trabecular parameters, respectively.

A manually defined region of interest (ROI) was created by drawing a boundary at the endocortical surface (interface between trabecular and cortical bone). Another ROI equating to 50% of the total trabecular region was created by manually selecting the centre of the trabecular ROI. The central region was used for all subsequent trabecular analyses (Figure 2-1).

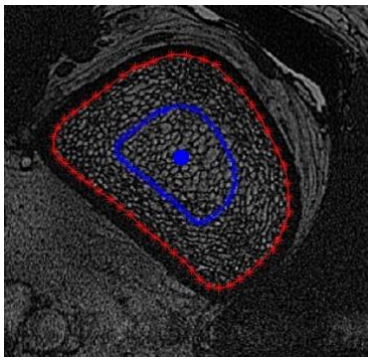

Figure 2-1 Example image of trabecular microarchitecture analysis at the distal femur.
The red line indicates the endocortical boundary between trabecular and cortical regions. Blue line represents the central 50% of the trabecular region used for subsequent analyses.

Finally, prior to quantitative measurement of trabecular outcomes, images were segmented into bone and marrow phases. A fuzzy c-means algorithm was implemented to binarize the images before subsequent designation of individual pixels as 'bone' (value of 1) if they contained a membership grade within the bone cluster higher than the required threshold. All other pixels were designated as 'marrow' (value of 0). Binarized images are displayed with bone in green and marrow in white and superimposed onto the original image for visual assessment of binarization quality (Figure 2-2)

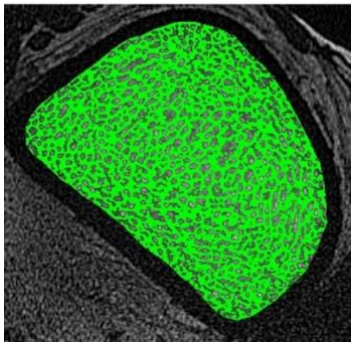

Figure 2-2 MRI image of trabecular microarchitecture binarized into bone and marrow phases.
Images were binarized into bone and marrow phases in order to designate pixels to the bone or marrow grade. The binarized image contains only pixels within the designated ROI and illustrates bone as green and marrow as white. This image is then superimposed onto the original image for quality assessment purposes.

‘Apparent’ values were calculated for trabecular microarchitecture parameters as the voxel size of 0.2 x 0.2 0.4mm is insufficient for accurate measurement of smaller trabeculae. These ‘apparent’ values also account for potential partial volume effects, a currently known limitation of MRI in assessment of trabecular microarchitecture due to limited spatial resolution.

2.3.3.2.4 Trabecular parameters

Apparent bone volume to total volume ratio (appBV/TV)

A measure of the volume of trabecular bone within the medullary cavity and primary outcome for trabecular microarchitecture. AppBV/TV is calculated as the ratio of bone pixels to the total number of pixels within the region of interest (ROI). The following equation is used:

$$appBV\ /\ TV = n\_bone/n\_total$$

Apparent trabecular thickness (appTbTh)

The Mean Intercept Length (MIL) at a given angle θ was calculated by the number of trabeculae-marrow boundaries that intercept a set of parallel rays oriented at an angle θ to the image.

The image was masked by a grid of parallel lines, each one pixel thick and separated by ten pixels, so only sections of the image contained within the lines were considered and the masked area is set to zero. Each grid line was examined pixel by pixel to ascertain the number of times the pixel designation changed from bone to marrow (1 to 0) or marrow to bone (0 to 1), defined as PL (θ). The number of bone pixels (PP) were also counted, and the MIL was calculated with the following equation:

$$MIL(\theta) = 2 \cdot \frac{P_P}{P_L(\theta)}$$

The MIL was calculated in steps of 10° through 0° to 360°, and apparent trabecular thickness (in mm) was computed by:

$$appTbTh\ (mm) = 0 \cdot 5\ \times mean\left(MIL(\theta)_{\vartheta=0,360}\right) \times R$$

Where R is pixel resolution in mm.

Apparent trabecular number (appTbN)

Apparent trabecular number (/mm) was calculated as follows:

$$appTbN\ (/mm) = \frac{appBV\ /\ TV}{appTbTh}$$

Apparent trabecular spacing (appTbSp)

Apparent trabecular spacing (mm) was calculated as follows:

$$appTbSp\ (mm) = \frac{1}{appTbN} - appTbTh$$

2.3.3.3 MRI assessment of cortical geometry

2.3.3.3.1 Image acquisition

Cortical geometry images were obtained from the same region (15% distal femur) as the trabecular microarchitecture parameters using a T1-weighted turbo spin echo sequence (parameters in Table 2-1). A series of twenty axial images were obtained. Due to differing image resolutions between trabecular and cortical images (slice thickness 0.4mm vs 2mm, respectively), scan series were conducted so that the 10th slice of each series were co-located. As such, the 10th slice of each series was used for subsequent analyses to provide estimates of microarchitecture and geometry at approximately the same region. Intra- and inter-observer repeatability were assessed using a subset of 30 images, each also analysed in triplicate. Mean CVs for intra- and inter-observer repeatability were <3% for all cortical parameters.

2.3.3.3.2 Cortical analysis

Analyses were performed using MATLAB. Manually determined boundaries were created for the endosteal and periosteal circumferences and processed through a

Savitzky-Golay filter for smoothing, before being superimposed on the original image to determine the cortical ROI (Figure 2-3).

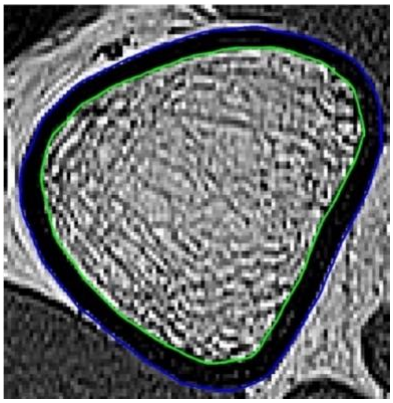

Figure 2-3 Example image of cortical geometry analysis at the distal femur.
Boundaries were manually drawn round the endosteal (green) and periosteal (blue) circumferences and superimposed onto the original image.

2.3.3.3.3 Cortical parameters

Cortical area (mm^2)

The pixels contained between the endosteal and periosteal circumferences were designated as cortical bone, therefore the cortical area ($A_{cortical}$ [mm^2]) was calculated using the number of cortical pixels ($n_{cortical}$) and pixel resolution (R [mm]) in the following equation:

$$A_{cortical} = n_{cortical} \times R^2$$

Endosteal and periosteal circumference (mm)

Endosteal and periosteal circumferences (C [mm]) were calculated from the manually derived boundaries. The sum of distances between adjacent points on the respective boundaries were calculated using the following equation:

$$C = \left(\left(\sum_{i=1}^{n-1} \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2} \right) + \sqrt{(x_1 - x_n)^2 + (y_1 - y_n)^2} \right) \times R$$

Where n is the number of points manually defined and R is the pixel resolution.

Mean cortical thickness (mm)

Mean cortical thickness ($t_{cortical}$) was calculated using a circular model as previously described by Johnston [327]. This method uses the endosteal and periosteal circumferences to calculate mean thickness within the following equation:

$$t_{cortical} = \frac{C_{peri} - C_{endo}}{2\pi}$$

2.3.3.4 MRI assessment of muscle

2.3.3.4.1 Image acquisition

Muscle images were obtained at 33% distal femur to provide adequate coverage of the main muscle groups of the upper leg. For assessment of muscle cross-sectional area (M-CSA [mm^2]) and fat fraction (FF [%], an indicator of fat infiltration) a series of twenty axial images were obtained using a six-point VIBE Dixon pulse sequence (Table 2-2).

Table 2-2 MRI pulse sequence parameters for muscle and bone marrow adiposity imaging.

	Muscle Area & Fat Fraction	*Bone Marrow Adiposity*
Sequence Type	6-point VIBE DIXON	PRESS
Resolution (mm)	1.1 x 1.1 x 2	20 x 20 x 20
TE (ms)	1.54, 2.97, 4.40, 5.83, 7.26, 8.69	30
TR (ms)	10.7	2000
Flip Angle (°)	4	90
No. of Averages	6	80
FoV (mm^2)	204 x 204	-
Matrix	192 x 192	-
Number of Slices	20	-
Bandwidth (Hz/pixel)	1090	1200
Scan Time (mins:secs)	3:00	2:50

PRESS – point resolved spectroscopy; TE – echo time; ms – milliseconds; TR – repetition time; FoV – field of view; mm – millimetres; Hz – hertz; mins:secs – minutes:seconds

2.3.3.4.2 Muscle area and adiposity analysis

For the assessment of cross-sectional area and fat infiltration, ROIs were manually determined around ten muscles of the thigh (Figure 2-4). M-CSA was defined as the sum of all individual muscle ROIs. Mean FF for each muscle was calculated as the proportion of fat to water signal within the respective ROI, and overall mean muscle FF (the sum of all mean FF values divided by number of muscle groups analysed) was used for subsequent analyses. To provide a surrogate measure of

functional muscle area, residual muscle area (RMA [mm^2]) was calculated as the total M-CSA adjusted for FF using the following equation:

$$RMA = \frac{M\text{-}CSA \times (100 - FF)}{100\%}$$

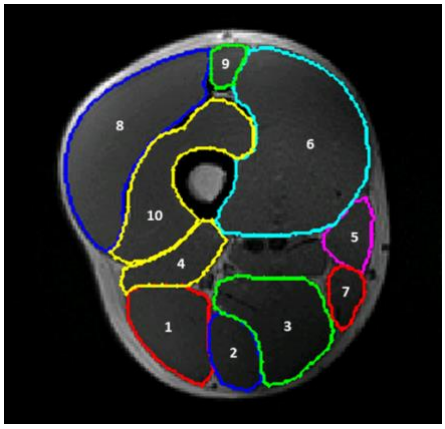

Figure 2-4 Example image of muscle analysis and individual ROIs.
Ten individual muscles were manually segmented, and total area and mean fat fraction calculated. 1 – biceps femoris long head; 2 – semitendinosus; 3 – semimembranosus; 4 – biceps femoris short head; 5 – sartorius; 6 -vastus medialis; 7 – gracilis; 8 – vastus lateralis; 9 rectus femoris; 10 – vastus intermedius.

2.3.3.5 MRS assessment of BMA

2.3.3.5.1 Image acquisition

Proton (^{1}H-) MRS was performed at the lumbar spine (L3) for assessment of vertebral bone marrow adiposity (BMA) using a 32-channel spine array. Lumbar spine was selected as the ROI as this has been successfully implemented in previous research. A point resolved spectroscopy (PRESS) pulse sequence with no water suppression was used to obtain spectra from the vertebral body of L3 (parameters in Table 2-2). Due to a sequencing error during initial scanning, comparable MRS data were only available in 23 CD and 27 control participants.

2.3.3.5.2 BMA analysis

MRS data were analysed within the Java-based magnetic resonance user interface software (jMRUI) [328] where spectra were fitted in the time domain using a least-squares algorithm [329]. BMA (FF %) was calculated with the following equation, where the lipid-to-water ratio was obtained from assessment of the area under the curve of the water (A_{water}) and lipid (A_{lipids}) peaks, respectively [330]:

$$BMA\ FF(\%) = \frac{A_{Lipids}}{A_{Lipids} + A_{Water}} \times 100$$

2.3.4 DXA

All CD participants were invited to a clinical DXA scan to assess aBMD. Fourteen CD participants underwent clinical DXA scans within four months following their study visit. These scans were included in this study to assess potential associations between MRI and DXA outcomes. All DXA scans were performed by the same densitometrist (SS) using a Lunar iDXA densitometer (GE Medical Systems, WI, USA) and analysed using encore software (Encore version 13.5, CA, USA). Participants' height and weight were measured prior to being positioned supine on the scanner. Once positioned, the scintillator arm moved in a cranio-caudal direction. The total scan time was approximately 15 minutes per participant. Data were collected for total body less head bone mineral content (TBLH-BMC; g) and lumbar spine bone mineral apparent density (BMAD; g/cm^3). Values were converted into age, sex, and ethnicity adjusted Z-scores based on UK reference data [170].

2.3.5 Muscle function

2.3.5.1 Mechanography

Lower limb muscle function was measured using a Leonardo Mechanograph Ground Reaction Force Plate (Novotec Medical GmbH, Pforzheim, Germany). This device comprises a force plate with eight strain gauge force sensors, used to measure the vertical ground reaction forces applied during various physical tests. Force sensors sampled at a frequency of 800 Hz and data transferred to a connected laptop, where they were subsequently analysed in specialist software (Leonardo Mechanography GRFP Research Edition v4.4b01.50). Prior to assessment the tests were verbally explained and physically demonstrated to the participant. Participants' bodyweight was measured during a two second stabilisation period after stepping onto the platform. All mechanography data were converted into separate sex-specific Z-scores adjusted for age and weight, respectively, using the LMS methods with previously published reference data [202]. The two functional tests used were the single two-legged jump (S2LJ; hereafter 'jumping test') and multiple one-legged hops (M1LH; hereafter 'hopping test') tests.

2.3.5.1.1 Jumping test (S2LJ)

The jumping test protocol consisted of a maximum effort vertical countermovement jump with freely moving arms, with the aim of achieving maximum height. Countermovement jumping is influenced by several factors including muscle power, technique, balance, and coordination. This test is designed to provide a more complex assessment of muscle function than is possible with isometric techniques. Each participant performed three maximum effort jump attempts and the result with the highest jump height was used in subsequent analyses. Outcomes of interest from jumping were maximum power (P_{max} [kW]) and power relative to body mass (Rel. P_{max} [W/kg]), recorded during the acceleration phase of the jump. Data were available in 20/21 CD participants (one participant – hardware error).

2.3.5.1.2 Hopping test (M1LH)

The hopping test protocol consisted of repeatedly hopping on the dominant foot, with the aim of achieving maximum ground reaction force. This test measures the maximal force to which the tibia is exposed and is applied as an assessment of the functional muscle-bone unit. Participants were instructed to hop as high as possible on the forefoot, while keeping their leg stiff and without allowing the heel to touch the ground between repetitions. All tests were performed using the dominant foot and each participant completed three attempts. The individual hop with the highest recorded ground reaction force was used for subsequent analyses. Outcomes of interest from hopping were maximum ground reaction force (F_{max} [kN]) and force as multiples of bodyweight (F_{max}*BW). Data were available in 17/21 CD participants (two refused, two hardware error).

2.3.5.2 Grip strength

Upper limb muscle function was assessed by maximal isometric grip strength using a digital handheld dynamometer (Takei 5401 Digital Hand Grip Dynamometer, Takei, Japan). All tests were performed in a seated position using the dominant hand, with the elbow flexed at approximately 90 degrees and resting on an arm rest. The participants were instructed to squeeze the dynamometer as hard as they could for a period of two to three seconds, with verbal encouragement. Three attempts were made and the test with the highest value was used in subsequent

analyses. The control group did not undertake grip strength assessment; therefore, the CD group data were transformed into age- and sex-specific Z-scores using the LMS method with previously published UK reference data for comparison [331].

2.3.6 Biochemical analyses

All CD participants were requested to provide a blood sample for assessment of clinical biochemistry and markers of bone turnover and inflammation. Non-fasted blood samples were drawn by a research nurse at the time of study visit or were collected during routine blood monitoring if this was conducted ±4 weeks of the study visit. Blood samples were available for 19/21 participants (1 refused, 1 failed). Clinical samples were sent directly to the local NHS accredited laboratory for analyses of the following: 25-OHD, PTH, Alk. Phosphatase, calcium, albumin, ESR, and CRP. Research samples were centrifuged, aliquoted, and supernatant stored at -80°C. Research bloods were to be assessed for markers of bone turnover, GH/IGF-1, and inflammatory cytokines, however these analyses were not conducted as access to laboratory facilities were restricted due to Covid-19. Participants were also asked to submit a stool sample for assessment of faecal calprotectin. If a sample was not submitted, a clinical sample from ±12 weeks was used for analyses if available. Faecal calprotectin data were available in 14/21 CD participants.

2.3.7 Control group

Data from 21 age- and sex-matched controls were used for comparison of MRI & MRS parameters, anthropometrics, fracture history, and lifestyle factors (i.e. use of Vit D supplements and self-reported PA). These participants were part of a study within the research group using identical MRI & MRS methods to establish reference ranges for muscle, bone, and BMA in healthy children and young adults. Control participants were selected based on the closest match to participants in the current study by age and all were matched ±1 year and ±1 self-assessed Tanner stage of puberty. As part of that study, controls underwent physical measurements including height, weight, and BMI, and self-assessed pubertal status according to Tanner. Use of vitamin D or calcium supplements and history of fractures were self-reported and habitual PA was self-reported using the same age-appropriate

questionnaires [325,326]. These participants did not provide blood samples or conduct assessments of muscle function. All MRI and MRS images of control participants were re-analysed by the primary researcher (LS) using the same methods as the CD group.

2.3.8 Study power

Study power was calculated based on data from a study of trabecular microarchitecture in healthy children within the research group (unpublished). Mean trabecular bone volume was 0.571 ± 0.028 in the first ten healthy controls. The minimum clinically meaningful difference in trabecular bone volume is currently unknown, so an arbitrary difference of 5% was selected. To detect a 5% lower trabecular bone volume in CD compared to controls, with a power of 0.8 and $\alpha<0.05$, a sample size of 15 per group was required.

2.3.9 Statistical Analyses

Continuous data are presented as median (range) and categorical data as frequency (%) unless otherwise stated. Data were visualised and assessed for normal distribution using Shapiro-Wilk tests. Between group comparisons for continuous variables were made using t-tests or Mann Whitney U tests for parametric and non-parametric continuous variables, respectively. Categorical variables were compared using chi-squared tests. Cortical geometry parameters were also compared using multiple linear regression models adjusted for height. Age and sex-adjusted Z-scores for anthropometry, mechanography, and DXA outcomes were compared to respective reference data using single sample t-tests or Wilcoxon signed rank test for parametric and non-parametric data, respectively. Associations between MRI, DXA, and disease outcomes were analysed using Pearson or Spearman correlation analyses for parametric and non-parametric data, respectively. Statistical analyses were performed using SPSS (Version 24; IBM Corp, Armonk, NY) and GraphPad Prism (Version 9; GraphPad Software, San Diego, CA).

2.4 Results

2.4.1 Clinical characteristics

Twenty-one children and adolescents with CD with median age 14.5 (10.6, 16.4) years and 21 age- and sex-matched controls were included in this study. There were no differences in height, weight or BMI Z-scores between CD and controls and both groups were not different from reference data (Table 2-3). Groups did not differ by self-assessed Tanner stage of puberty or age at menarche in females (Table 2-3). Six CD participants were currently taking vitamin D supplements compared to none of the control group ($p = 0.01$). Self-reported history of fractures was not different between groups. One CD participant with previous exposure to prolonged GC therapy reported multiple fractures, although the location of these could not be confirmed due to management in another health board. All other self-reported fractures were associated with trauma and there were no participants with history of vertebral fracture. No differences were observed between groups for self-reported PA.

In CD, the median age at diagnosis was 8.9 (5.1, 14.2) years and median disease duration 3.6 (1.1, 8.0) years. Panenteric (42.9%) and ileocolonic (28.6%) disease were the most common disease locations at diagnosis, according to Paris disease classification. Anti-TNF-α biologics (76.2%) were the most common current medication followed by thiopurines (61.9%) and methotrexate (23.8%). One participant was currently on Ustekinumab monotherapy, and one was on a course of budesonide enemas. No other current GC use was self-reported. The median self-reported number of previous GC courses was 1 (0, 4) and three participants had received GC within the previous 12 months. At the time of study 15/21 had disease in remission and 6/21 had mildly active disease according to wPCDAI. For clinical biochemistry, median serum 25-OHD was 49 nmol/l (17, 106) and only 2/18 samples were 25-OHD deficient (<25 nmol/l). Median serum PTH was 5.1 pmol/l (2.6, 12.36) and 6/18 samples were above the upper healthy reference range (>7.5 pmol/l). Median faecal calprotectin (n = 14) was 152 ug/g (30, 1316), five of which were above the criteria for clinically relevant intestinal inflammation (>250 ug/g).

Table 2-3 Cohort characteristics of paediatric CD and controls

	Crohn's Disease (n = 21)	Controls (n = 21)	p-value
Cohort Characteristics			
Sex (f/m)	11/10	11/10	
Age (years)	14.5 (10.6, 16.4)	13.4 (10.5, 17.0)	0.58
Height (cm)	158.5 (137.4, 180.3)	158.0 (137.0, 176.0)	0.92
Height Z-score (SDS)	-0.01 (-1.73, 1.91)	0.04 (-1.7, 2.36)	0.5
Weight (kg)	48.8 (32.2, 79.4)	45.9 (29.9, 86.2)	0.99
Weight Z-score (SDS)	-0.01 (-1.33, 2.21)	0.42 (-1.33, 2.34)	0.79
BMI (kg/m^2)	19.5 (16.2, 28.2)	19.0 (12.9, 30.6)	0.84
BMI Z-score (SDS)	0.22 (-1.45, 2.60)	0.16 (-2.83, 2.57)	0.68
Tanner stage	3 (1, 5)	3 (1, 5)	0.58
Pubertal Stage, n (%)			
Pre-pubertal (Tanner 1)	1 (4.8)	2 (9.5)	
Early pubertal (Tanner 2/3)	13 (61.9)	8 (38.1)	
Late/post-pubertal (Tanner 4/5)	7 (33.3)	11 (52.4)	
Clinical Characteristics			
Vitamin D / Calcium Supplements, n (%)	6 (28.6)	0 (0)	0.01
Reached menarche, n (%)	6 (54.5)	6 (54.5)	1.0
Age at menarche (years)	12 (11, 15)	12 (10, 14)	0.51
Previous Fracture, n (%)	7 (33.3)	5 (23.8)	0.49
PA Score	2.3 (1.1, 3.7)	2.7 (1, 3.7)	0.46
Age at diagnosis (years)	8 (5, 14)		
Disease duration (years)	3.6 (1.1, 8.0)		
Disease location, n (%) [c]			
L1: Ileal	0 (0)		
L2: Colonic	2 (9.5)		
L3: Ileocolonic	6 (28.6)		
L4a/L4b/L4ab: Upper GI disease	2 (9.5)		
L3L4: Panenteric	9 (42.9)		
Disease Activity			
wPCDAI Score	0 (0, 25)		
wPCDAI activity category, n (%)			
Remission	16 (76.2)		
Mildly active	5 (23.8)		
Moderate or severe	0 (0)		
Medications, n (%)			
Anti-TNF-α	16 (76.2)		
Thiopurines	13 (61.9)		
Methotrexate	5 (23.8)		
Glucocorticoids	1 (4.8)		
Ustekinumab	1 (4.8)		
Previous GC courses	1 (0, 4)		
GC ever, n (%)	13 (61.9)		
Recent GC (<12 months), n (%)	4 (19)		
Previous CD Surgery, n (%)	4 (19)		

f/m – female/male; cm – centimetres; kg – kilograms; m – metres; wPCDAI – weight paediatric Crohn's disease activity index; TNF – tumour necrosis factor; anti-TNFα – infliximab/adalimumab; CD – Crohn's disease. Continuous variables displayed as median (range). [a] t-test; [b] Mann Whitney U test. [c] Disease location and behaviour at diagnosis according to Paris classification of IBD. L4 or P disease location can be coexistent with L1/L2/L3.

2.4.2 MRI assessment of bone, muscle, and BMA

2.4.2.1 Trabecular microarchitecture

Trabecular bone volume was lower (0.55 [0.44, 0.62] vs 0.58 [0.53, 0.62], p = 0.003) and trabecular separation higher (0.24 mm [0.21, 0.39] vs 0.22 mm [0.20, 0.28], p = 0.002) in CD compared to controls (Figure 2-5 A & D). The difference in trabecular separation remained significant after removing the outlier (p = 0.01). There were no between group differences for trabecular number or thickness (Figure 2-5).

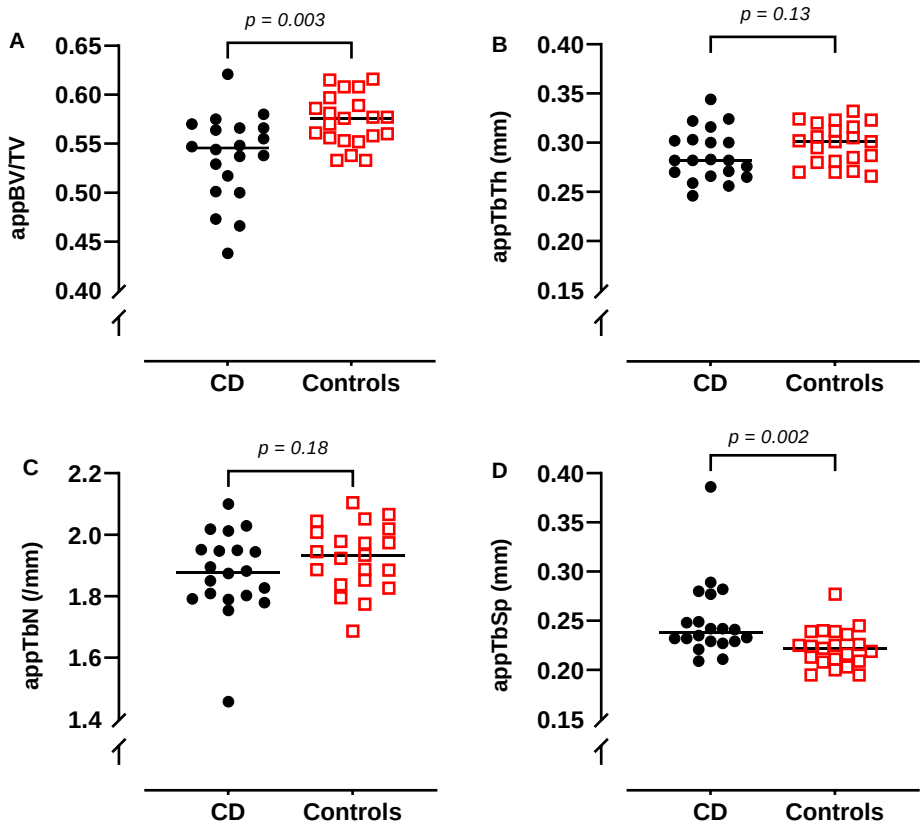

Figure 2-5 Trabecular microarchitecture parameters in paediatric CD and controls.
Individual dot plots comparing trabecular microarchitecture parameters at 15% distal femur in CD and controls. Between group differences assessed by t-tests (A, B, C) or Mann Whitney test (D). Black circles – CD; red squares – controls. N = 40 (20 CD, 20 control). A – appBV/TV; B – appTbTh; C – appTbN; D – appTbSp. AppBV/TV – apparent bone volume fraction; appTbTh – apparent trabecular thickness; appTbN – apparent trabecular number; appTbSp – apparent trabecular separation. Horizontal line represents median.

2.4.2.2 Cortical geometry

Median cortical area was 258.9 mm^2 (199.3, 391.9) and 268.9 mm^2 (215.4, 320.5) in CD and controls, respectively (p = 0.98) (Figure 2-6 A). Median cortical thickness was 2.39 mm (1.81, 2.94) and 2.5 mm (2.01, 2.82) in CD and controls, respectively (p = 0.34) (Figure 2-6 B). No differences were observed between CD and controls for endosteal or periosteal circumferences (Figure 2-6 C & D). In regression analyses adjusted for sex and height, there were no differences between CD and controls for any cortical geometry parameters (Table 2-4).

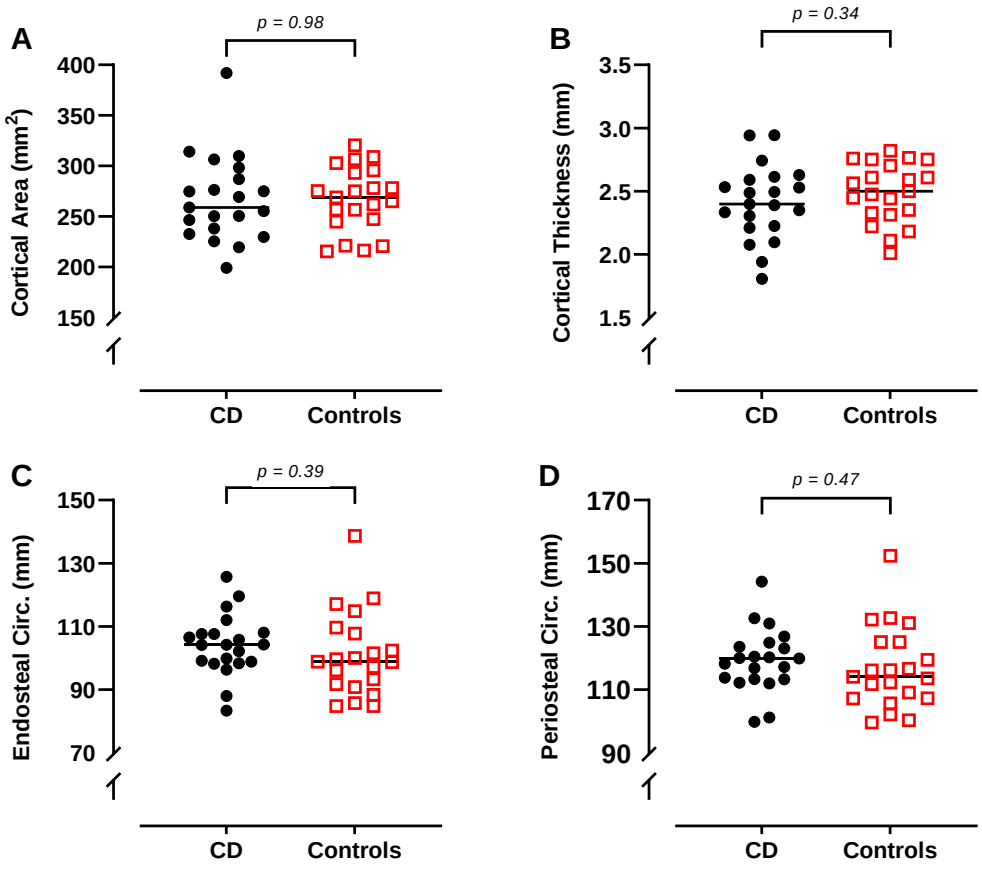

Figure 2-6 Cortical geometry in paediatric CD and controls.
Individual dot plots comparing cortical geometry parameters at 15% distal femur between paediatric CD and controls. Between group difference assessed by t-tests. Black circles – CD; red squares – controls. A – Cortical Area; B – Cortical Thickness; C – Endosteal Circumference; D – Periosteal Circumference. Horizontal line represents median.

Table 2-4 Multiple regression analyses of cortical geometry parameters at distal femur.

Cortical Geometry	B (95% CI)	Std. ß	Model R^2	p-value
Cortical Area (mm^2)	0.98 (-13.4, 15.3)	.138	0.64	0.89
Cortical Thickness (mm)	-0.08 (-0.23, 0.08)	-.145	0.11	0.33
Endosteal Circumference (mm)	3.34 (-2.31, 9.0)	.146	0.43	0.24
Periosteal Circumference (mm)	2.86 (-2.32, 8.04)	.127	0.51	0.27

B – Unstandardized Beta; Std β – Standardized Beta; mm – millimetres. Models are adjusted for height and sex. Controls used as reference group in each model.

2.4.2.3 Muscle area & fat infiltration

Median muscle CSA was 5170 mm^2 (4350, 9473) and 5792 mm^2 (4221, 9524) in CD and controls, respectively (p = 0.42) (Figure 2-7 A). Muscle fat fraction was not different between CD and controls (4.1 % [0.4, 8.2] vs 3.5 % [0.4, 9.2], p = 0.74) (Figure 2-7 B). After subtracting muscle fat fraction, median RMA was 4998 mm^2 (4082, 9439) and 5604 mm^2 (4121, 8652) in CD and controls, respectively (p = 0.32) (Figure 2-7 C). In regression analyses adjusted for height, RMA was not different between CD and controls (Unstandardised ß: -288 mm^2 [95% CI: -923, 347], p = 0.37).

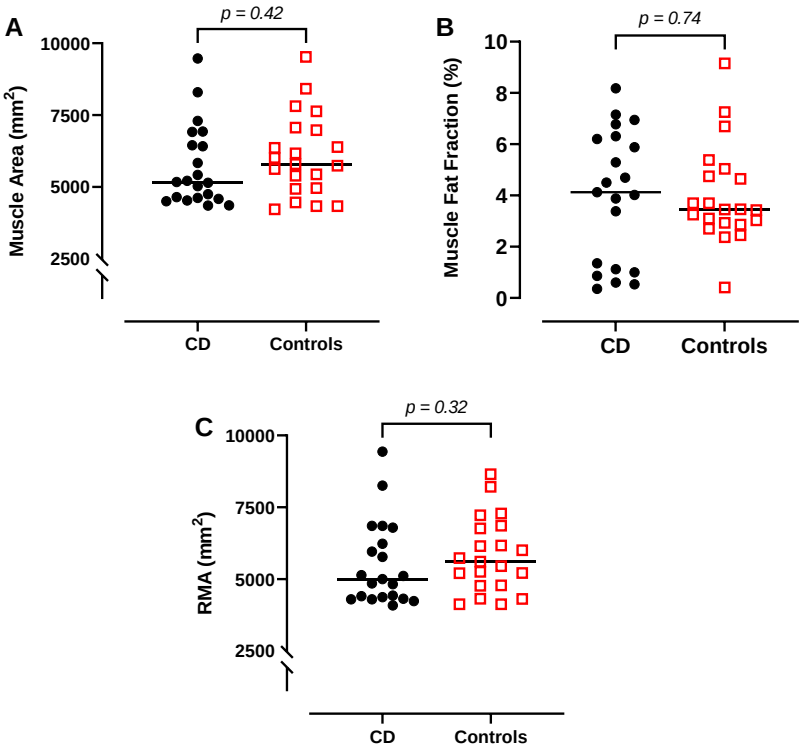

Figure 2-7 Muscle area and adiposity in paediatric CD and controls.
Individual dot plots comparing muscle area and adiposity at 33% distal femur between paediatric CD and controls. Between group differences assessed by Mann Whitney tests. Black circles – CD; red squares – controls. A – muscle cross sectional area; B – muscle fat fraction; C – residual muscle area. Horizontal line represents median. FF – fat fraction; RMA – residual muscle area; mm – millimetres.

2.4.2.4 Lumbar spine BMA

Median BMA was 22.3 % (12.0, 51.1) and 21.1 % (10.0, 35.8) in CD and controls, respectively (p = 0.58) (Figure 2-8).

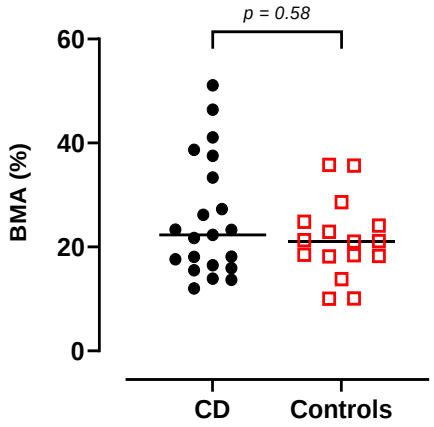

Figure 2-8 Bone marrow adiposity in paediatric CD and controls.
Individual dot plots comparing bone marrow adiposity of the lumbar spine between paediatric CD and controls. Between group difference assessed by Mann Whitney U test. Black circles – CD; red squares – controls. n = 36 (21 CD, 16 controls). Horizontal line represents median. BMA – bone marrow adiposity.

2.4.3 Muscle function

Assessment of the muscle-bone unit using jumping mechanography in CD produced Z-scores consistently lower than reference data (Figure 2-9). From the jumping test (n = 20), median Z-scores for absolute muscle power were -0.74 (-1.99, 1.17) (p = 0.02) and -1.13 (-2.78, 1.99) (p = 0.02) when adjusted for age and weight, respectively (Figure 2-9 A). Similarly, median Z-scores for muscle power relative to body mass were -1.08 (-4.20, 1.08) (p < 0.001) and -1.11 (-3.03, 0.99) (p = 0.001) when adjusted for age and weight, respectively (Figure 2-9 A). From the hopping test (n = 17), median Z-scores for absolute maximum force generated were -0.55 (-2.68, 0.45) (p = 0.004) and -1.46 (-3.39, 0.64) (p < 0.001) when adjusted for age and weight, respectively (Figure 2-9 B). Median Z-scores for maximum force generated as a multiple of bodyweight were -2.08 (-4.03, 0.21) (p < 0.001) and -1.83 (-3.58. 0.19) (p < 0.001) when adjusted for age and weight, respectively (Figure 2-9 B). For upper limb muscle function, median grip strength was 20.1 kg (12.0, 37.1) (Figure 2-9 C). When converted into age and sex specific values, median grip strength Z-score was -0.42 (-2.62, 1.00), which was not different to the reference population (Figure 2-9 D) (p = 0.18).

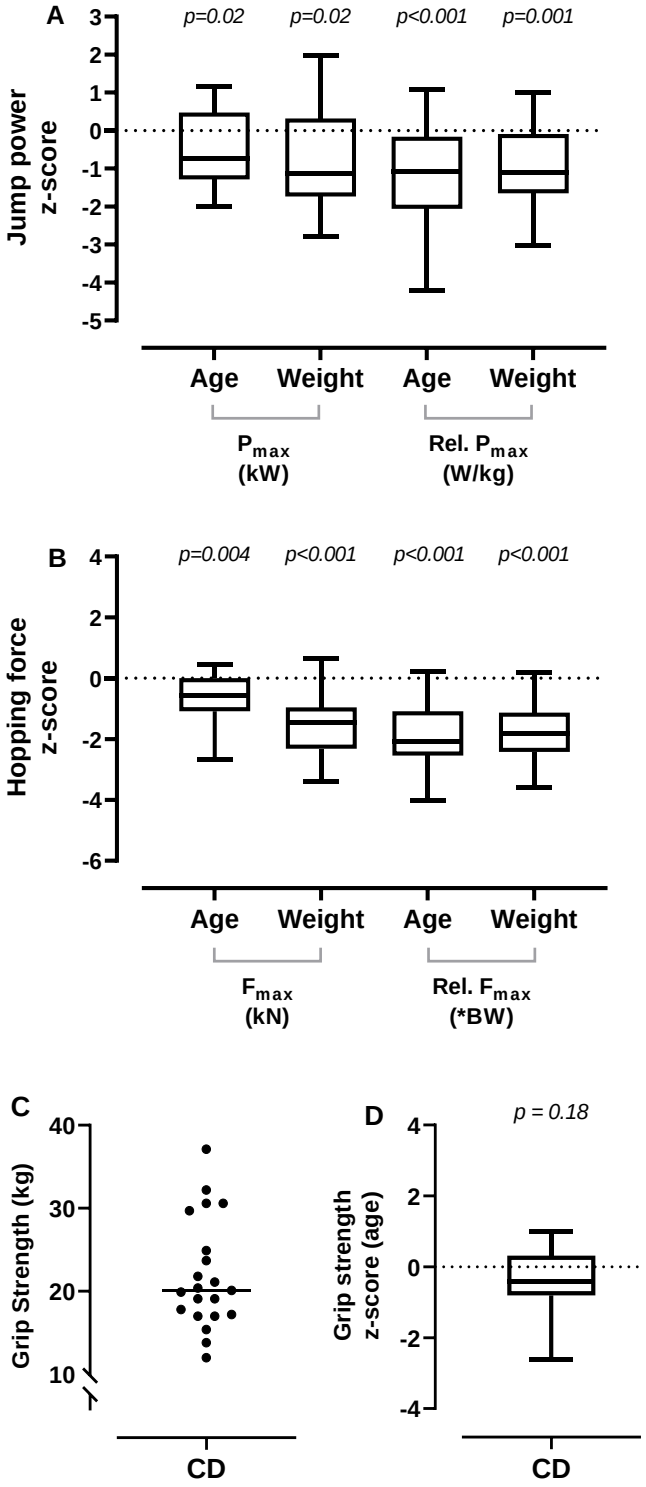

Figure 2-9 Muscle-bone unit assessments of the upper and lower limbs in paediatric CD. Boxplots and individual dot plot displaying assessment of muscle function and the muscle-bone unit in paediatric CD. A – Muscle power and relative power Z-scores assessed by jumping (mechanography); B – maximum force and force as a multiple of bodyweight Z-scores assessed by hopping (mechanography); C – maximum isometric grip strength; D – maximum grip strength Z-scores. Sex-specific age- and weight-adjusted Z-scores calculated using the LMS method with reference data for jumping mechanography (A & B) from Sumnik et al. [202]. Age- and sex-specific Z-scores calculated using LMS method with reference data for grip strength from Dodds et al. [331]. Z-scores were compared to zero using one sample Wilcoxon tests. A – n = 20; B – n = 17; C & D – n = 21.

2.4.4 DXA

In those with CD and available DXA (n = 14), LS BMAD was lower than reference data, but TBLH-BMC was not (Figure 2-10). Median LS BMAD was -0.6 (-2.1, 1.1) (p = 0.01). Median TBLH-BMC was -0.1 (-0.9, 2.2) (p = 0.49). One participant had LS BMAD Z-score < -2.0 SDS and 6/14 had LS BMAD Z-score < -1.0 SDS. No participants had TBLH-BMC Z-scores < -1.0 SDS (Figure 2-10).

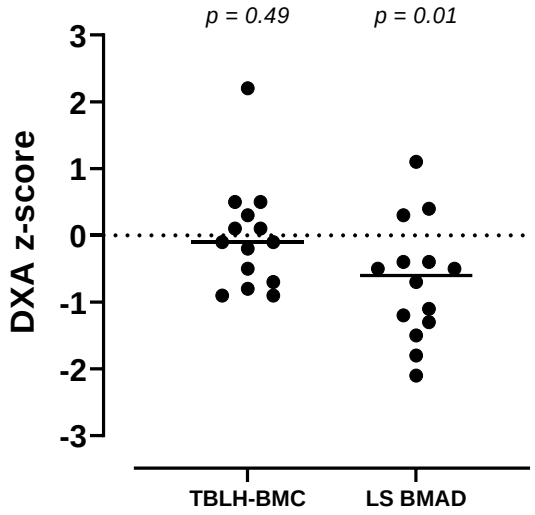

Figure 2-10 Assessment of bone using DXA in paediatric CD.
Individual dot plot displaying TBLH-BMC and BMAD at the total body and lumbar spine in paediatric CD. n = 14. Age-, sex-, and ethnicity-specific Z-scores were calculated using the LMS methods from UK reference data (Crabtree et al. [170]).

2.4.5 Associations between bone, muscle, and adiposity

2.4.5.1 Trabecular bone

Trabecular bone volume did not associate with absolute weight (r = 0.09, p = 0.7) or BMI (r = 0.17, p = 0.47) but was positively associated with weight Z-score (r = 0.46, p = 0.04) in CD. No such associations were observed in controls. No associations were observed between trabecular microarchitecture parameters and RMA in CD or controls (Figure 2-11). Also, there was no evidence of any associations between trabecular microarchitecture parameters and DXA measures of LS BMAD or TBLH-BMC. Trabecular bone volume was, however, positively associated with self-reported PA in CD (r = 0.57, p = 0.01) but not controls (r = -0.04, p = 0.88).

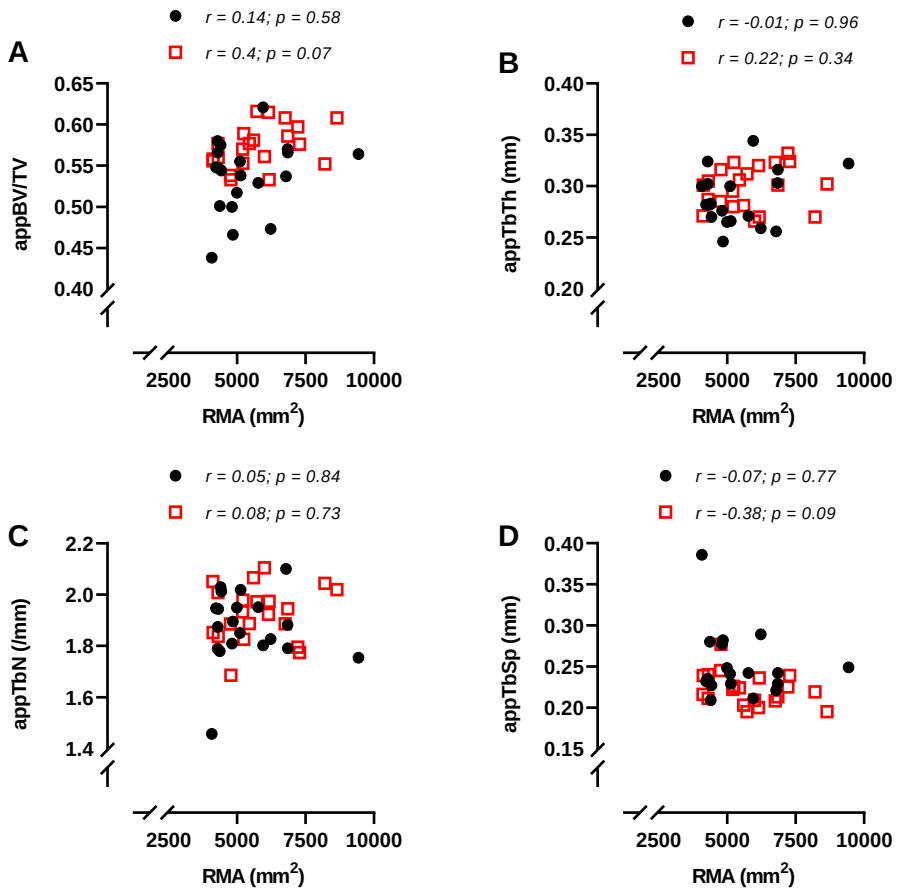

Figure 2-11 Associations between RMA and trabecular microarchitecture in paediatric CD and controls.
Scatterplots displaying associations between RMA and trabecular microarchitecture in paediatric CD and controls. A – appBVTV; B – appTbTh; C – appTbN; D – appTbSp. Black circles – CD, red squares – controls. Associations assessed using Spearman rank correlation analyses for non-parametric data. AppBV/TV = apparent trabecular bone volume; appTbTh – apparent trabecular thickness; appTbN – apparent trabecular number; appTbSp – apparent trabecular spacing. BMI – body mass index; mm – millimetre.

2.4.5.2 Cortical bone

Cortical area was strongly positively associated with height in both CD ($r = 0.84$, $p < 0.001$) and controls ($r = 0.73$, $p < 0.001$). Conversely, cortical thickness was positively associated with height in CD ($r = 0.66$, $p = 0.001$) but not controls ($r = -0.01$, $p = 0.97$). Cortical area, but not cortical thickness, was positively associated with RMA in CD and controls (Figure 2-12). In multiple regression analyses adjusted for sex and height, cortical area was positively associated with RMA in CD only (Table 2-5). Cortical geometry was not associated with DXA measures of TBLH-BMC or LS BMAD, nor was it associated with self-reported PA.

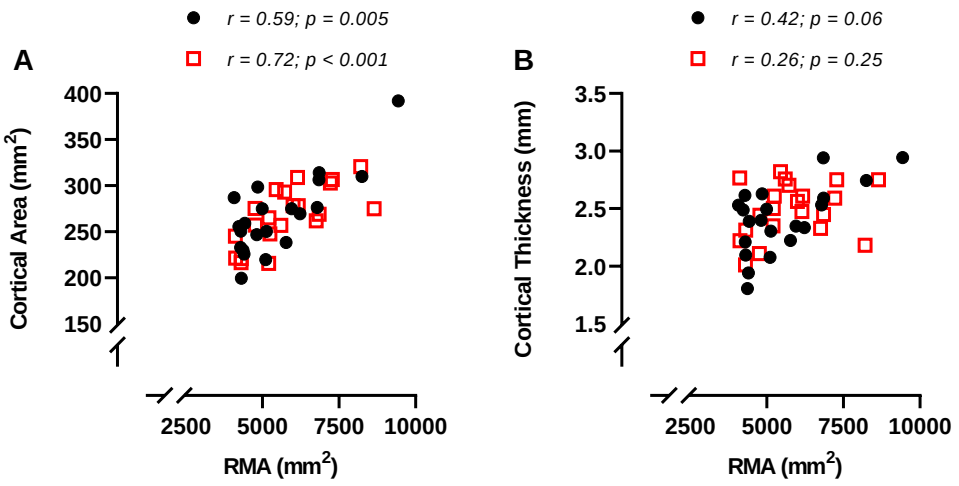

Figure 2-12 Associations between RMA and cortical geometry in paediatric CD and controls. Scatterplots displaying associations between RMA and cortical geometry. A – cortical area; B – cortical thickness. Black circles – CD, red squares – controls. Associations assessed using Spearman rank correlation analyses for non-parametric data. mm – millimetres.

Table 2-5 Multiple regression analyses of residual muscle area and cortical geometry in paediatric CD and controls.

	Crohn's Disease (n = 21)			
Outcome	**B (95% CI)**	**Std. β**	**Model R^2**	**p-value**
Cortical Area (mm^2)	0.01 (0.004, 0.02)	.437	0.82	0.01
Cortical Thickness (mm)	6.0 $x10^{-5}$ (0.00, 0.00)	.296	0.42	0.25
	Controls (n = 21)			
Outcome	**B (95% CI)**	**Std. β**	**Model R^2**	**p-value**
Cortical Area (mm^2)	0.01 (-0.00, 0.02)	.341	0.59	0.16
Cortical Thickness (mm)	1.1 $x10^{-4}$ (0.00, 0.00)	.610	0.17	0.09

Multiple linear regression analyses of residual muscle area and cortical geometry in CD and controls, respectively. RMA is the predictor for each model. All models adjusted for sex and height. B – unstandardized beta coefficient; Std. β – standardized beta coefficient; mm – millimetre.

2.4.5.3 Muscle area, adiposity, and function

Muscle fat fraction also negatively associated with all measured muscle function Z-scores (Table 2-6). In CD, self-reported PA was positively associated with RMA in regression analyses adjusted for sex and height (Unstandardised β: 1022 mm^2 [95%CI: 450, 1594], p = 0.002). PA was also strongly negatively associated with muscle fat fraction in CD (r = -0.73, p < 0.001), and positively associated with Z-scores for grip strength, muscle power and relative power, and relative force in CD (Table 2-6).

Table 2-6 Associations between muscle fat, PA, and muscle function in paediatric CD.

	Grip Z-score (age) [a]	P_{max} Z-score (weight) [b]	Rel. P_{max} Z-score (weight) [b]	F_{max} Z-score (weight) [c]	Rel. F_{max} Z-score (weight) [c]
Muscle fat fraction (%)	-0.57 (0.01)	-0.82 (<0.001)	-0.88 (<0.001)	-0.61 (0.01)	-0.74 (0.001)
PA Score	0.51 (0.02)	0.61 (0.004)	0.63 (0.003)	0.39 (0.13)	0.52 (0.03)

Spearman rank correlations for associations between muscle fat fraction and self-reported PA with muscle function Z-scores in paediatric CD. [a] n = 21; [b] n = 20; [c] n = 17. Parentheses represent p-values.

2.4.5.4 Bone marrow adiposity

Lumbar spine BMA was negatively associated with trabecular bone volume in CD (r = -0.57, p = 0.01) but not controls (r = 0.22, p = 0.36). No other associations were observed between BMA and trabecular microarchitecture or cortical geometry parameters in CD or controls. BMA also did not associate with DXA-derived measures of TBLH-BMC or BMAD in CD (n = 14). Additionally, BMA was negatively associated with self-reported PA in CD (r = -0.63, p = 0.002 [n = 20]) but not controls (r = 0.09, p = 0.76 [n = 16]).

2.4.6 Associations between prior GC exposure and bone, muscle, and adiposity outcomes

2.4.6.1 Trabecular and cortical bone

Trabecular bone volume and trabecular thickness were higher in CD participants with no previous exposure to GC therapy (Figure 2-13 A & B). The number of previous courses of GC therapy was also negatively associated with MRI-derived trabecular bone volume (r = -0.47, p = 0.04) and trabecular thickness (r = -0.58, p = 0.01). Previous GC exposure was not associated with cortical geometry (Figure 2-13 C & D).

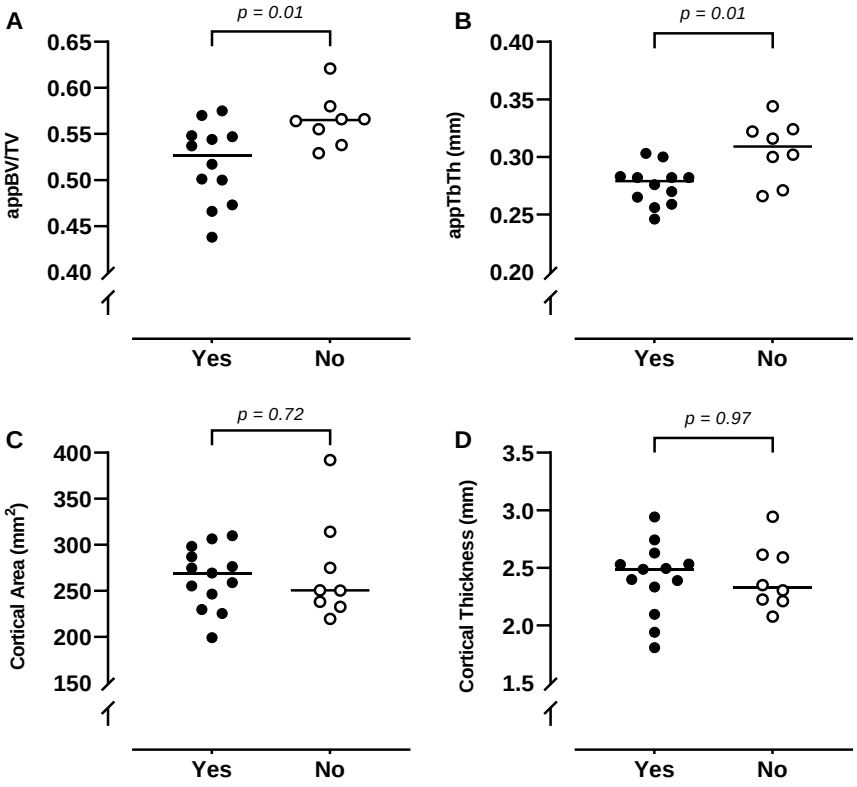

Figure 2-13 Exposure to GC and trabecular and cortical bone in paediatric CD.
Individual dot plots comparing trabecular bone volume (A), trabecular thickness (B), cortical area (C), and cortical thickness (D) in paediatric CD with and without previous exposure to GC therapy. Closed circles – previously exposed to GC; open circles – no previous GC exposure. Between group differences assessed using t-tests.

2.4.6.2 Muscle area and adiposity

RMA was not associated with prior GC exposure in regression analyses adjusted for height and sex (β = -105 mm^2 [95% CI: -626, 414], p = 0.74). Muscle fat fraction also was not associated with previous GC courses (r = 0.36, p = 0.11) and did not differ between CD participants with and without prior GC exposure (p = 0.11). No associations were observed between prior GC exposure and any muscle function Z-scores.

2.5 Discussion

This study used novel methods of combined MRI and MRS together with a battery of functional tests to assess the muscle-bone unit in paediatric CD compared to healthy controls. High-resolution MRI revealed deficits in trabecular microarchitecture in CD compared to controls, while no differences in cortical bone geometry or muscle area and adiposity were observed. Muscle mass and fat infiltration did not differ between CD and controls. Paediatric CD was, however, consistently associated with low Z-scores for muscle function parameters compared to previously published data.

Despite paediatric CD being commonly associated with abnormalities in skeletal development, trabecular microarchitecture has not been well characterised in this population. In this study, paediatric CD was associated with lower trabecular bone volume and higher trabecular separation at the distal femur compared to healthy controls. Similarly, one study found low lumbar spine trabecular bone score in paediatric CD when compared to healthy controls and paediatric UC [332]. That study corroborates the low trabecular bone volume and LS BMAD observed here. Conversely, newly diagnosed paediatric IBD was associated with normal trabecular bone volume at the iliac crest in another study using bone biopsy histomorphometry (n = 20; 17 CD) [209]. In that study, the inclusion of some UC participants may have attenuated any association between CD and trabecular outcomes. However, in histomorphometric analysis, bone turnover appeared to be almost entirely suppressed, which may have explained the lack of trabecular deficit. Inflammation has been suggested as a primary regulator of bone loss in paediatric CD/IBD, yet trabecular deficits were observed in the current study despite currently quiescent disease activity. Systemic and local inflammation were not measured in the current study and the relative contribution of these to skeletal outcomes in paediatric CD requires assessment in prospective trials.

Another potential contributor to bone abnormalities in paediatric CD is exposure to GC, which can have devastating effects on the growing skeleton. Previous GC exposure may have moderated the trabecular deficits observed in this cohort. Those with CD and no prior GC exposure were observed to have higher trabecular bone volume and trabecular thickness compared to previously GC-treated peers. Self-reported number of previous GC courses was also negatively associated with

trabecular bone volume (r = -0.47) and trabecular thickness (r = -0.58) in CD. These correlations may underestimate the true association between GC exposure and trabecular microarchitecture as, in several instances, one self-reported course of GC represented prolonged exposure of between several months up to approximately two years. Unfortunately, insufficient prescription data were available to calculate cumulative past GC exposure in this cohort. Nevertheless, these results indicate that any prior exposure to GC in paediatric CD may lead to systemic trabecular deficits. In paediatric patients with potential for skeletal growth, GC-induced bone deficits can be transient and reversible after cessation of therapy [36]. However, few participants in the current study reported recent GC, suggesting any adverse effects of GC were incurred earlier in the disease course and have subsequently failed to improve. This is despite ongoing management with contemporary therapies, highlighting the role of other factors in skeletal recovery. However, CD is a model of a disease where the underlying insult is generally not removed and low-grade chronic inflammation is likely to be present on an ongoing basis. Alternatively, other factors that were not studied such as nutritional status may have regulated trabecular deficits in the current cohort. Further high-resolution imaging studies are required to characterise trabecular bone in paediatric CD and elucidate key clinical factors associated with trabecular deficits.

The negative effects of GC exposure and inflammation are both moderated by the severity of underlying disease, which may serve as one of, if not the most important factor in the pathogenesis of musculoskeletal deficits in CD. Severe disease phenotypes are associated with greater inflammatory burden and increased risk of extraintestinal manifestations of disease. To compound these effects, severe disease is primarily treated with prolonged exposure to high dose GC, which, as discussed, have devastating effects in the growing skeleton [243,247,248]. In this cross-sectional study of children with established CD, current disease activity was low, and no longitudinal assessment of disease course was conducted. Nevertheless, it should be noted that the five CD participants with the poorest trabecular bone volume fraction scores were all noted to have severe disease at time of diagnosis that was treated with protracted courses of GC. Similar observations have been made in children with incident rheumatic diseases, where initial disease severity and subsequent cumulative GC exposure are

predictors of vertebral fracture, an extreme indicator of trabecular bone pathology [166]. To assess the true associations between initial disease severity and clinical management with musculoskeletal outcomes in paediatric CD would require large scale, multi centre prospective studies with long term imaging follow up from onset of disease to several years post-diagnosis.

Despite deficits in trabecular bone, no differences in cortical bone geometry at the distal femur were observed between CD and controls in the current study. This contrasts previous pQCT studies that have consistently observed low cortical area and thickness in paediatric CD, in both newly diagnosed [209,276] and previously treated patients [49,275,278]. Cortical bone deficits in those studies may have been partly attributable to delayed growth, as height Z-scores were generally below zero, unlike the current study. Additionally, previous studies observed improved linear growth and cortical in children with CD treated with anti-TNF-α therapy [47,49]. The common use of anti-TNF-α in the current cohort may therefore have contributed to normal growth and cortical bone development. It cannot, however, be ruled out that cortical deficits may exist in paediatric CD that were not detected in this study. The heterogeneity of cortical parameters in growing children make it difficult to interpret these data in such a small cohort without access to suitable age, sex, and preferably height-adjusted reference values. This study represents the only evaluation of cortical geometry of the distal femur in paediatric CD to date and future trials are necessary to validate these results. Further analyses of the mechanical properties of the femoral cortex would provide further insight into cortical strength in this cohort.

A sub-cohort of CD participants underwent total body and lumbar spine DXA scans (n = 14). LS BMAD, but not TBLH-BMC, Z-scores were lower than reference values, with 6/14 displaying low BMAD Z-score (< -1.0 SDS). This agrees with the MRI findings of deficits in trabecular but not in cortical bone, as the lumbar spine is a trabecular rich region, whereas TBLH-BMC is generally representative of cortical bone mass. These results add to the existing studies reporting low trabecular vBMD in paediatric CD [49,217,275-277,333,334]. The present data highlight the persistence of skeletal concerns in children with CD, despite contemporary management. Although deficits in both trabecular microarchitecture and LS BMAD were observed, no associations between these or any other DXA or MRI parameters were

noted. Similarly, a lack of association between DXA and MRI outcomes have been reported in children [335] with type 1 diabetes mellitus, demonstrating that trabecular assessment by MRI provides additional information not available via densitometry. The lack of association may also be due to different anatomical locations and mechanical loading patterns between the femur and lumbar spine. MRI assessment of trabecular microarchitecture may offer an alternative outcome in the assessment of skeletal health in paediatric CD and its utility as a prognostic indicator of bone status should be investigated further.

Risk factors for skeletal disorder in paediatric CD also negatively influence muscle mass and function. Prior imaging studies have reported low muscle mass in paediatric CD that persists after long-term follow up [276,280] or treatment with anti-TNF-α therapy [49,217]. In the current study, no differences were observed in muscle area of the mid-thigh between paediatric CD and controls. Improving underlying disease status may partially improve muscle mass deficits in CD and the quiescent disease activity of the current cohort may have facilitated regular muscle development under contemporary management. However, other non-clinical factors such as nutrition and PA are also integral to muscle growth. Self-reported PA was positively associated with RMA in CD, in regression analyses adjusted for height and sex (ß: 1022 mm^2 [95%CI: 450, 1594], p = 0.002). The inclusion of several highly active participants in the current study may have skewed the RMA findings towards a higher muscle mass than would be expected for the paediatric CD cohort in general. These data suggest higher levels of PA and exercise may be associated with greater muscle mass development in CD and muscle targeting interventions should be investigated.

Independently of muscle mass, infiltration of skeletal muscle by adipocytes can lead to impaired muscle quality and function. This study quantified skeletal muscle fat in paediatric CD for the first time, finding no differences compared to healthy controls. Muscle fat fraction is strongly regulated by mechanical stimuli and PA, highlighted by high levels of fat infiltration in skeletal muscle in populations with impaired mobility [103]. In accordance, a strong negative association between self-reported PA and muscle fat fraction (r = -0.73) was observed in the current CD cohort. Muscle adipocytes express pro-inflammatory adipokines that may contribute to systemic inflammation in CD, although this has

not been investigated. Further studies are required to elucidate any association between CD and muscle fat infiltration and its potential role in systemic inflammation.

This comparison of paediatric CD and healthy controls found no differences in skeletal muscle area or fatty infiltration. In contrast, assessments of muscle function compared to previously published reference data produce some conflicting results. Firstly, grip strength Z-scores, adjusted for age and sex, were not different in CD compared to reference data from UK children [331]. Conversely, sex specific Z-scores for jumping mechanography, adjusted separately for age and weight, respectively, were consistently low in CD compared to reference data [202]. Low grip strength has been previously reported in paediatric CD in cohorts with concurrent muscle mass deficits [217,225]. Mechanography Z-scores, on the other hand, were reported as normal in a prior cohort of paediatric IBD (n = 70; 53 CD), although muscle mass was not reported [278]. The discordance between the current results and those of Maratova and colleagues [278] may be due to their adjustment of mechanography Z-scores for height rather than weight, owing to short stature in their CD cohort. Power and force Z-scores correlate very closely with weight in growing children and weight-adjusted Z-scores are thought to be preferable due to the wide variability in physical characteristics in children of the same age [202]. Poor dynamic muscle function in paediatric CD after adjustment for weight, accounting for muscle mass, suggests a potential mechanism of dysfunction independent of muscle size. PA and muscle fat were positively and negatively associated with muscle function, respectively. Whether other disease specific factors contribute to muscle function requires further investigation.

Muscle and bone development are closely paralleled during growth. One previous MRI study in typically developing children (age 6 - 12 years) reported strong associations between muscle volume at the mid-thigh with trabecular ($r = 0.81$) and cortical ($r = 0.96$) bone volume at distal femur [336]. In the current study, RMA positively associated with cortical area but not trabecular bone volume in CD. The lack of association between muscle and trabecular bone may be due to small sample size in this study, or possibly represent the discordance between bone and muscle during the adolescent growth spurt. While muscle mass per se was not associated with trabecular bone, the potential importance of mechanical loading

for trabecular development was evidenced by the positive association between self-reported PA (r = 0.57) with trabecular bone volume. There is currently a lack of published data tracking the relationships between trabecular microarchitecture, muscle mass, and PA across the adolescent age in both health and disease and this warrants investigation in future prospective trials.

BMA has emerged as a novel marker of bone health yet its association with skeletal health in inflammatory diseases is not well established. In this study, BMA did not differ between paediatric CD and controls. A negative association between BMA and trabecular bone volume was observed in CD but not in controls, as has been found in other diseases with associated skeletal morbidity [90,337,338]. BMA is metabolically distinct to other adipose depots but was associated with muscle fat fraction in CD. Mechanical loading and exercise partly regulate BMA and a negative association between BMA and PA was found in CD (r = -0.63). It can be speculated that the negative associations between BMA and muscle fat fraction is most likely moderated by their respective associations with PA. Any disease associated mechanisms of BMA accrual in CD are not evident from the current study. Mechanistic studies are required to evaluate any contribution of BMA to bone loss in both CD and other inflammatory conditions.

This study has some important limitations. Due to the cross-sectional nature of these analyses, no assessment of cause and effect is possible. For example, similar adverse associations were observed between low self-reported PA and previous GC exposure with trabecular bone volume. There is biological plausibility for the effects of both PA and GC in bone, but the available data does not permit an assessment of which, if any, contributed to skeletal deficits. Furthermore, other factors such as nutritional status or clinical biomarkers that were not measured may be involved. It can be speculated, however, that the combined effects of severe disease at baseline with subsequent high dose GC exposure, followed by a lack of habitual PA even when disease is brought into remission, may create a sub-optimal environment for trabecular development that led to the observed deficits in some participants. As previously mentioned, heterogeneity in physical development between children of the same age limits assessment of skeletal muscle or cortical bone outcomes on a one-to-one matched basis and access to a much larger control group would be needed to assess these with adequate

statistical power. There may be underlying biochemical differences in bone turnover, GH/IGF-1, or inflammatory markers that were unable to be investigated due to restricted availability of laboratory facilities and these will be analysed in the future. The study had a small sample size but remained adequately powered to detect differences in trabecular bone volume which was the primary outcome.

The reproducibility of MRI for assessing trabecular microarchitecture has been demonstrated in previous studies and mean inter-observer CVs of <2% were found for all current analyses. However, the use of MRI has some important limitations. Primarily, MRI scans are expensive and require specialist input for both pulse sequence design and scanning. The high-resolution and signal-to-noise ratio required to assess trabecular bone results in long scan times (approx. 9 minutes) that are highly susceptible to movement artefacts. No objective grading system exists for trabecular image quality, and this was subjectively assessed prior to analyses. Most scans were of sufficient quality for analysis, but it remains possible that some will have at least minor movement artefact which could have affected the results. Despite these points, MRI has many strengths including being the gold standard imaging modality for skeletal muscle assessment. Additionally, the combination of MRI and MRS enabled a comprehensive evaluation of the muscle-bone unit that is not possible with other techniques, like HR pQCT.

In conclusion, this study identified trabecular microarchitecture deficits at the distal femur in a cohort of paediatric CD with currently well-controlled disease. Trabecular bone in CD appeared moderated by prior exposure to GC therapy. Cortical geometry and muscle area were not different between groups, although muscle function in CD was low compared to reference data. Self-reported PA was associated with several musculoskeletal outcomes in CD, including trabecular bone volume, muscle mass and fat infiltration, and BMA. Future work should address the utility of targeted exercise interventions as an adjunct to improve the observed musculoskeletal deficits in paediatric CD.

3 MRI assessment of the muscle-bone unit in young adults with childhood onset Crohn's disease

3.1 Abstract

Background. Long term musculoskeletal outcomes in adults with childhood onset CD are currently unknown, especially in those managed with contemporary therapies.

Aim. Investigate the muscle-bone unit in young adults with childhood onset CD in comparison with healthy age- and sex-matched controls.

Methods. Cross-sectional, case-control study using 3 Tesla-MRI and MRS to assess trabecular microarchitecture and cortical geometry at 15% distal femur, muscle cross sectional area and adiposity at 33% distal femur and BMA at lumbar spine. Muscle function was assessed by grip strength dynamometry and jumping mechanography. Biochemical assessment of the muscle-bone unit, GH axis and inflammatory markers were measured in blood.

Results. Twenty-seven adults (15 female) with CD with median age 23.2 (18.0, 36.1) years were compared with 29 controls (15 females) with median age 22.7 (18.2, 37.0). Median age at CD diagnosis was 12 (6, 17) years. Twenty of the 27 CD participants were in clinical remission and seven had mildly active disease. Trabecular microarchitecture and cortical geometry parameters were not different between CD and controls. Median muscle cross sectional area was 6863 mm^2 (3404, 10667) and 8684 mm^2 (5084, 13768) in CD and controls, respectively ($p = 0.01$). Median muscle FF% was 5.3% (0.6, 8.9) and 4.1% (0.5, 9.2) in CD and controls, respectively ($p = 0.07$). In multiple regression analyses, CD had significantly lower grip strength (-4.7 kg [95% CI: -7.2, -2.2], $p < 0.001$) and lower muscle power relative to body mass (-5.0 W/kg [95% CI: -8.8, -1.2], $p = 0.01$) compared with controls. Median BMA fat fraction was 31.0% (9.5, 58.8) and 29.5% (11.0, 47.1) in CD and controls, respectively ($p = 0.644$). Markers of bone and muscle metabolism were similar between groups, although serum OC was higher in CD ($p = 0.002$). Pro-inflammatory cytokines and IGFBP-2 were elevated in CD.

Disease activity was negatively associated with trabecular bone volume ($r = -0.40$, $p = 0.04$) and muscle cross sectional area ($r = -0.41$, $p = 0.03$).

Conclusions. Young adults with well controlled CD do not display any deficits in trabecular microarchitecture or cortical geometry of the distal femur, as assessed by high-resolution MRI. Despite well controlled disease, however, childhood onset CD was associated with significantly lower muscle mass and function, and current disease activity was negatively associated with muscle-bone outcomes. Strategies to improve muscle mass and function warrant investigation to mitigate the future risk of musculoskeletal morbidity.

3.2 Background

In Chapter 2, assessment of the muscle-bone unit in paediatric CD revealed deficits in trabecular microarchitecture and skeletal muscle function. This study, along with previously published research [217], highlights the persistence of musculoskeletal problems in children with CD, even in those managed with contemporary therapies. Few studies, however, have assessed long term musculoskeletal outcomes in young adults diagnosed with CD in childhood. It therefore remains unknown whether muscle-bone deficits persist into adulthood.

Adolescents with IBD (n = 47; CD = 17) were observed to have deteriorating aBMD after five years of follow up into early adulthood in one historic study [323]. Young adults with IBD (n = 102; CD =75) were found to have 10% reduced trabecular vBMD compared with healthy controls together with lower trabecular number and increased trabecular separation, although this cohort contained both childhood and adult-onset disease [283]. Exposure to GC was higher in these cohorts relative to current treatment paradigms and few patients were treated with anti-TNFα therapy, which are being increasingly used in the long-term management of CD. Anti-TNFα therapy rapidly improves biomarkers of bone formation and IGF-1 in paediatric CD [49,339]. However, persistent muscle-bone deficits in vBMD are observed after up to 12 months of treatment [49,217]. Whether long term management with contemporary therapies may facilitate resolution of muscle-bone deficits incurred early in the disease course is not clear.

The aim of this study was, therefore, to perform high-resolution MRI imaging, biochemical, and functional assessment of the muscle-bone unit in young adults with childhood onset CD compared to healthy age- and sex-matched controls.

3.3 Methods

3.3.1 Study Population and Design

Between January 2017 and January 2019 adults with childhood onset CD were recruited from across NHS Greater Glasgow & Clyde. Eligible individuals were contacted by their gastroenterology consultant or signposted to the research team at outpatient clinic appointments. The operation of this study was taken over by the primary researcher (LS) in October 2017, at which point 31 participants had already been recruited by research nurses at the CRF. A total of 97 individuals with CD were invited and 27 volunteered and consented to participate in this study. Twenty-nine healthy age- and sex-matched controls were recruited from study advertisements at the local university hospital campus and through associates of CD participants. Eligibility criteria for the CD group were as follows: aged 18 – 40 years (inclusive), CD diagnosed in the paediatric age (≤17 years), diagnosed ≥12 months prior to study involvement, stable medication for >4 weeks prior to study involvement. Exclusion criteria were contraindication to MRI, significant comorbidity affecting muscle-bone, pregnancy, medications known to affect bone (excluding those used exclusively to treat CD [e.g. glucocorticoids]). Each CD participant was age- and sex-matched to at least one healthy control (± 1 year). The study was approved by the North of Scotland NHS Research Ethics Committee (REC Ref: 16/NS/0060), and all participants provided written informed consent prior to participation.

3.3.2 Data collection

Health and lifestyle data were collected including self-reported physical activity (IPAQ long form [340]), use of calcium/vitamin D supplements, fracture history, current medication use, comorbidities and age at menarche and oral contraceptive use (females only). Height was measured to the nearest mm and weight measured to the nearest 0.1 kg. Age at diagnosis, history of CD related surgery, disease location according to Paris classification [3] and self-reported number of glucocorticoid courses in the paediatric age were collected from the CD group and, where possible, verified in the medical history. Disease activity was assessed using the validated Crohn's Disease Activity Index (CDAI) [22] with participants classed as severe disease (>451 CDAI score), moderate disease (221 –

450), mild disease (150 – 220) or clinical remission (0 – 149). All study participants were also requested to provide a stool sample for assessment of faecal calprotectin.

3.3.3 MRI & MRS Imaging

3.3.3.1 Overview

MRI & MRS imaging methods were conducted as described in Chapter 2. These will be only briefly discussed here. The same 3-Tesla MRI scanner was used (Prisma, Siemens, Erlangen, Germany). Imaging lasted approximately 70 minutes per participant.

3.3.3.2 MRI assessment of trabecular microarchitecture

As previously described, a series of 20 axial images were acquired at 15% distal femur using a CISS pulse sequence. The central slice of the image (slice 10) was used for analyses in all but two participants, where this was not possible due to gross motion artefact. Trabecular images were of sufficient quality for analysis in 54 participants for this study (n = 26 CD; n = 28 controls). The trabecular ROI was manually defined and the central region, equating to 50% of total trabecular ROI, was used for all subsequent analyses. Trabecular outcomes of interest were appBVTV, appTbTh (mm), appTbN (/mm), appTbSp (mm).

3.3.3.3 MRI assessment of cortical geometry

A series of 20 axial images were acquired also at 15% distal femur using a T1-weighted turbo spin echo sequence. The central slice of the image (slice 10) was also used for cortical analyses as these were co-located with trabecular slice 10. Cortical images were of sufficient quality for analysis in all participants. Cortical geometry outcomes of interest were cortical area (mm^2), cortical thickness (mm), endosteal circumference (mm), and periosteal circumference (mm).

3.3.3.4 MRI assessment of muscle

A series of 20 axial images were acquired at 33% distal femur using a six-point VIBE Dixon pulse sequence. The central slice of the image deck was used for all analyses. Muscle images were of sufficient quality for analysis in all participants.

Manual ROIs were drawn around ten muscle groups of the thigh, as described in Section 2.3.3.4. Muscle outcomes of interest were M-CSA (mm^2), fat infiltration (FF%), and RMA (mm^2).

3.3.3.5 MRS assessment of BMA

^{1}H-MRS was performed at L3 for assessment of BMA using a PRESS pulse sequence with no water suppression. Due to an initial sequencing error, BMA data were available for 50 participants (CD, n=23; controls, n=27). BMA (%) was calculated using area under the curve of the respective lipid and water peaks on the spectra.

3.3.4 Muscle function assessment

3.3.4.1 Grip strength

Maximal isometric grip strength (kg) of the dominant hand was measured using a digital handheld dynamometer (Takei 5401 Digital Hand Grip Dynamometer, Takei, Japan). Participants were instructed to stand with their elbow flexed at 90°, holding the dynamometer with their hand in a neutral position and ensuring their elbow was not in contact with the trunk. After a countdown the participants were instructed to squeeze the dynamometer as hard as possible for 2-3 seconds, with verbal encouragement. This was repeated three times and the highest value was taken as maximal grip strength for subsequent analysis.

3.3.4.2 Jumping mechanography

Lower limb muscle function was assessed using jumping mechanography as previously described (Section 2.3.5.1). In this study, the jumping test (S2LJ) only was performed. Briefly, participants were asked to perform a maximum effort countermovement jump with freely moving arms. This was repeated three times and the jump with the highest height was used for subsequent analysis. Due to hardware issues, mechanography data were only available in 37 participants (19 CD, 18 controls). Mechanography outcomes of interest from the jumping test were power (W) and power relative to body mass (W/kg).

Measured outcomes were absolute power (kilowatts [kW]), relative power (power/body mass [W/kg]), absolute force (kilonewton [kN]), relative force (force/bodyweight [*BW]) and jump height (cm).

3.3.5 Biochemical analyses

Participants arrived fasted from the previous evening and fasted blood samples were drawn between 0900 – 1000h by a research nurse. Research samples were centrifuged and aliquoted then stored at -80°C until subsequent analysis. Blood samples were available for all 27 CD participants and 28/29 controls (one refused).

3.3.5.1 Markers of bone turnover

Bone turnover activity was assessed through measurement of multiple biomarkers in bloods samples. Bone specific alkaline phosphatase (BSAP) and osteocalcin (OC) were measured as markers of bone formation and sclerostin and c-terminal telopeptide of type I collagen (CTX-1) were measured as markers of bone resorption in serum. BSAP was measured using immunoenzymatic assay (Ostase BAP, Immunodiagnostic Systems, Boldon, UK; intra-assay CV 10.8%). OC was measured by enzyme-linked immunosorbent assay (ELISA) (N-MID®, Immunodiagnostic Systems, Boldon, UK; intra-assay CV 7%). Sclerostin was measured by ELISA (TECO® Sclerostin, TECO Medical AG, Sissach, Switzerland; Intra-assay variance 5%). CTX-1 was measured by ELISA (Serum CrossLaps®, Immunodiagnostic Systems, Boldon, UK; intra-assay CV 2.3%). Serum 25-hydroxyvitamin D (25-OHD) was measured in serum by liquid chromatography-tandem mass spectrometry using an in-house method (Intra-assay CV <10%).

3.3.5.2 Growth hormone / Insulin-like growth factor 1 axis

Insulin-like growth factor 1 (IGF-1) and its binding proteins (IGFBP-) 2 and 3, and acid labile subunit (ALS) were measured in serum using ELISA (Mediagnost, Reutlingen, Germany; intra-assay CVs 6.9%, <1%, <1%, 3.2%, respectively). Serum myostatin was measured by ELISA (Cusabio, Hubei Province, China; intra-assay CV 13.4%).

3.3.5.3 Markers of inflammation

Measurement of serum pro-inflammatory cytokines was conducted on a Luminex based Bio-Plex multi array system using a Bio-Plex Pro Human Cytokine assay (Bio-Rad, California, USA). This assay measured serum TNF-α, IFN-γ, IL-6, and IL-8. Serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and faecal calprotectin (FC) were measured by the local NHS accredited laboratory.

3.3.6 Sample size calculation

Statistical power of this study was inferred post hoc using data from the first ten healthy controls. Mean trabecular bone volume in these ten control participants was 0.553 ± 0.018. Based on these data, the study was powered to detect a 3% difference in trabecular bone volume in CD vs controls (power of 0.8 and $\alpha < 0.05$) with a sample size of 19 per group.

3.3.7 Statistical Analyses

Continuous data are presented as median (range) and categorical data as frequency (%) unless otherwise stated. Data were initially visualised then assessed for normality using Shapiro-Wilk tests. Between group comparisons of continuous variables were made using t-tests and Mann Whitney U tests for parametric and non-parametric data, respectively. Categorical variables were compared using chi-square tests. Due to the reliance of cortical geometry parameters on physical size, comparisons between groups were made using multiple regression analyses adjusted for sex and height. Comparisons of mechanography outcomes were also made using multiple regressions, adjusted for age and sex, as these data were available in unmatched participants. Associations between continuous variables were assessed using Pearson or Spearman rank correlation analyses for parametric and non-parametric data, respectively. Statistical analyses were performed using SPSS (Version 24; IBM Corp, Armonk, NY) and GraphPad Prism (Version 9; GraphPad Software, San Diego, CA).

3.4 Results

3.4.1 Clinical characteristics

Twenty-seven adults (15 female) with CD with median age 23.2 (18.0, 36.1) years and 29 (15 female) healthy controls with median age 22.7 (18.2, 37.0) years participated in this study. Median BMI was 21.1 kg/m2 (16.1, 34.4) and 23.5 kg/m^2 (18.2, 28.6) in CD and controls, respectively (p = 0.03). Self-reported PA and use of vitamin D/calcium supplements were similar between groups (Table 3-1). Forty-four percent and 31% of CD and control participants, respectively, reported a history of fracture (p = 0.3). All fractures were in long bones with associated history of trauma and no participants had history of vertebral fracture. None of the participants reported current or previous cigarette smoking.

Median age at diagnosis of CD was 12 (6, 17) years and median disease duration was 9 (3, 22) years. The most common disease location at diagnosis was ileocolonic (59%) or isolated colonic (33%), according to the Paris classification. Twenty-nine percent had peri-anal involvement at diagnosis. At the time of study visit 74% (20/27) were in clinical remission and 26% (7/27) had mildly active disease according to CDAI. Current medications were most commonly thiopurines (63%) and anti-TNFα antibodies (48%) and only one participant was currently on GC therapy. Median self-reported courses of oral GC in the paediatric age were 1 (0, 8) and 4/27 had received GC therapy within the previous 12 months. Two-thirds of participants were currently, or had previously been, treated with anti-TNFα therapy.

Table 3-1 Clinical characteristics in CD and controls

	Crohn's Disease (n = 27)	Controls (n = 29)	p-value
Demographics & Lifestyle			
Sex (f/m)	15/12	15/14	-
Age (years)	23.2 (18.0, 36.1)	22.7 (18.2, 37.0)	-
Height (cm)	168.3 (154.0, 192.0)	172.0 (156.0, 193.0)	0.25 [a]
Weight (kg)	61.5 (38.2, 104.1)	69.4 (49.8, 93.3)	0.06 [a]
BMI (kg/m^2)	21.1 (16.1, 34.4)	23.5 (18.2, 28.6)	0.03 [b]
Physical Activity (MET-mins/week)	2953 (585, 10392)	3373 (603, 12798)	0.77 [b]
Calcium/Vitamin D Supplements, n (%)	7 (25.9)	5 (17.2)	0.39
Oral Contraceptives, n (%)	3 (20.0)	5 (33.3)	0.41
Clinical Characteristics			
Age at menarche (years)	13 (11, 15)	12 (10, 15)	0.07 [a]
Previous Fracture, n (%)	12 (44.4)	9 (31.0)	0.3
Age at diagnosis (years)	12 (6, 17)	-	
Disease duration (years)	9 (3, 22)	-	
Disease Location, n (%) [c]			
L1: Distal Ileum	2 (7.4)	-	
L2: Colonic	9 (33.3)	-	
L3: Ileocolonic	16 (59.3)	-	
L4a/L4b/L4ab: Upper disease	0/4/4 (29.6)	-	
P: Peri-anal disease	8 (29.6)	-	
Disease Activity			
CDAI Score	63 (0, 217)	-	
Medications, n (%)			
Thiopurines	17 (63.0)	-	
Anti-TNFα	13 (48.1)	-	
Methotrexate	2 (7.4)	-	
Glucocorticoids	1 (3.7)	-	
Vedolizumab	2 (7.4)	-	
5-ASA	3 (11.1)	-	
Number CD related surgeries, n (%)		-	
None	17 (63.0)	-	
One	6 (22.2)	-	
Two	1 (3.7)	-	
Three	3 (11.1)	-	
Recent GC (<12 months), n (%)	4 (14.8)	-	
No. GC courses <18 years, n (%)	1 (0, 8)	-	
Current/previous anti-TNFα, n (%)	18 (66.7)	-	

f/m – female/male; cm – centimetres; kg – kilograms; m – metres; MET – metabolic equivalent of task; CDAI – Crohn's disease activity index; L4a – upper disease involvement proximal to the ligament of Treitz; L4b – upper disease involvement distal to the ligament of Treitz but proximal to the distal ileum; L4ab – combination of L4a and L4b; TNF – tumour necrosis factor; anti-TNFα – infliximab/adalimumab; CD – Crohn's disease; biologic use – previous or current anti-TNFα therapy. Continuous variables displayed as median (range). [a] t-test; [b] Mann Whitney U test. [c] Disease location according to Paris classification of IBD. L4 or P disease location can be coexistent with L1/L2/L3.

3.4.2 MRI assessment of bone, muscle, and BMA

3.4.2.1 Trabecular microarchitecture

No between group differences were observed for any of the MRI-derived measures of trabecular microarchitecture (Figure 3-1).

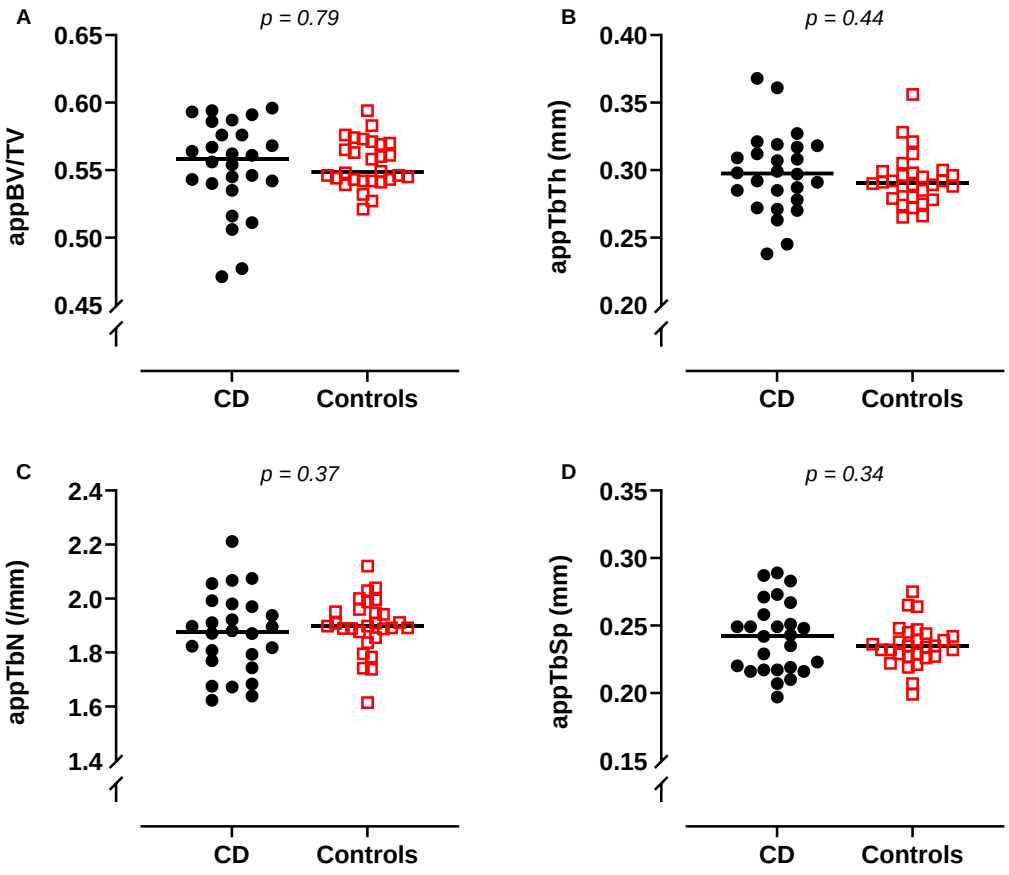

Figure 3-1 Comparison of trabecular microarchitecture at distal femur between CD and controls.
Individual dot plots comparing trabecular microarchitecture parameters at 15% distal femur between CD and controls. Between group difference assessed by t-test (A, C, D) or Mann Whitney U test (B). Back circles – CD; red squares – controls. N = 54 (26 CD; 28 controls). A – appBV/TV; B – appTbTh; C – appTbN; D – appTbSp. AppBV/TV – apparent bone volume fraction; appTbTh – apparent trabecular thickness; appTbN – apparent trabecular number; appTbSp – apparent trabecular separation. Horizontal line represents median.

3.4.2.2 Cortical geometry

No differences were observed between groups for cortical area or cortical thickness (Figure 3-2 A & B). Endosteal and periosteal circumferences were both lower in the CD group compared with controls (p = 0.04 and p = 0.03, respectively) (Figure 3-2 C & D). However, in multiple regression analysis adjusted for sex and

height, there were no between group differences for any cortical geometry parameters (Table 3-2).

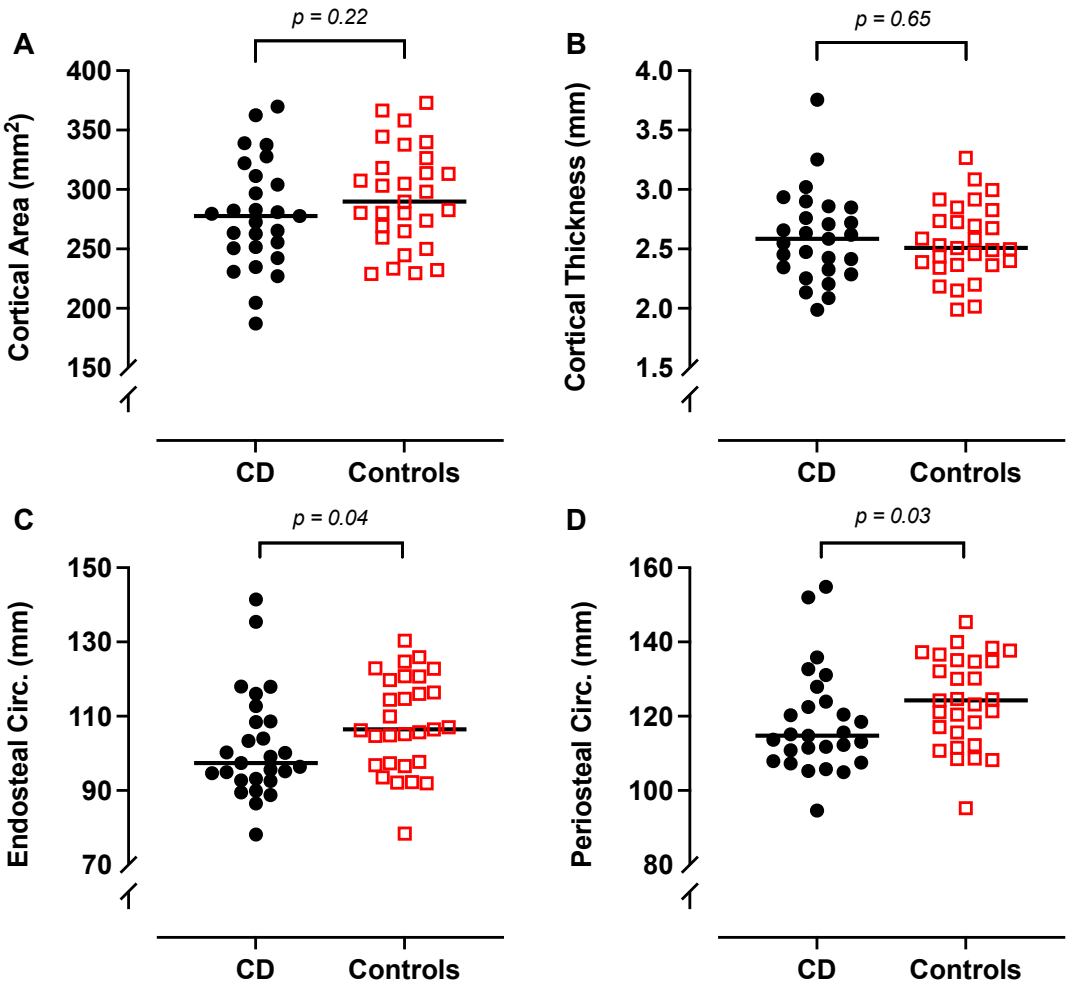

Figure 3-2 Comparison of cortical geometry at distal femur between CD and controls. Individual dot plots comparing cortical geometry parameters at 15% distal femur between CD and controls. Between group difference assessed by t-tests (A, B) or Mann Whitney U test (C, D). Back circles – CD; red squares – controls. A – Cortical Area; B – Cortical Thickness; C – Endosteal Circumference; D – Periosteal Circumference. Horizontal line represents median. * denotes significant difference between groups.

Table 3-2 Multiple regression analyses of cortical geometry parameters at distal femur.

	B (95% CI)	Std. β	Model R^2	p-value
Cortical Geometry				
Cortical Area (mm^2)	-7.10 (-23.04, 8.84)	-.082	0.58	0.38
Cortical Thickness (mm)	0.05 (-0.13, 0.23)	.068	0.14	0.61
Endosteal Circumference (mm)	-3.49 (-9.34, 2.36)	-.127	0.43	0.24
Periosteal Circumference (mm)	-3.07 (-8.28, 2.14)	-.117	0.51	0.24

B – Unstandardized Beta; Std β – Standardized Beta; mm – millimetres. Models are adjusted for sex and height. Controls used as reference group.

3.4.2.3 Muscle adiposity and cross-sectional area

Median muscle FF% was 5.3% (0.6, 8.9) and 4.1% (0.5, 9.2) in CD and controls, respectively (p = 0.07) (Figure 3-3 A). M-CSA was lower in CD compared with controls (p = 0.01) (Figure 3-3 B). After accounting for muscle FF%, median RMA was 6497 mm^2 (3215, 10203) and 8367 mm^2 (4874, 13102) in CD and controls, respectively (p = 0.004) (Figure 3-3 C). Adjusting for sex, height, and weight, multiple regression analyses demonstrated that CD was associated with 1096 mm^2 (95% CI: 483, 1709) lower RMA than controls (Model R^2 = 0.75, p = 0.001).

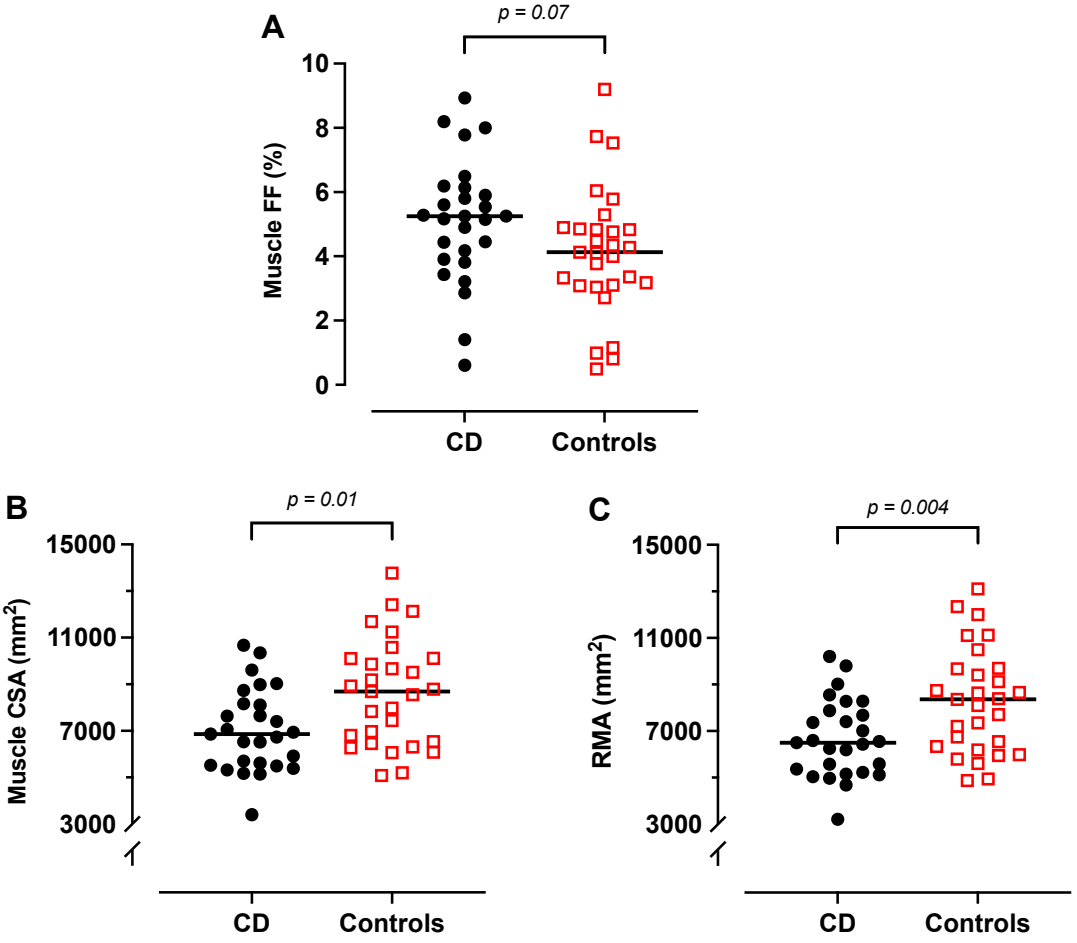

Figure 3-3 Comparison of muscle adiposity and cross-sectional area at the distal femur in CD and controls.
Individual dot plots comparing muscle adiposity and area at 33% distal femur between CD and controls. Between group differences assessed by t-tests. Back circles – CD; red squares – controls. A – muscle fat fraction; B – muscle cross sectional area; C – residual muscle area. Horizontal line represents median. FF – fat fraction; M-CSA – muscle cross sectional area; RMA – residual muscle area; mm – millimetres. * p<0.05

3.4.2.4 Bone marrow adiposity

No differences were observed in lumbar spine BMA between CD and controls (31.0% [9.5, 58.8] vs 29.5% [11.0, 47.1], respectively; p = 0.64) (Figure 3-4).

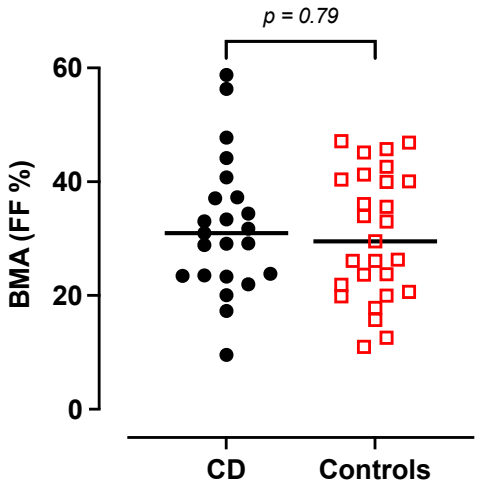

Figure 3-4 Comparison of bone marrow adiposity at the lumber spine between CD and controls.
Individual dot plots comparing bone marrow adiposity of the lumbar spine between CD and controls. Between group difference assessed by Mann Whitney U test. Back circles – CD; red squares – controls. n = 50 (23 CD, 27 controls). Horizontal line represents median. BMA – bone marrow adiposity; FF – fat fraction.

3.4.3 Muscle function

3.4.3.1 Maximum isometric grip strength

Grip strength was significantly lower in CD compared with controls (26.3 kg [17.0, 41.8] vs 32.6 kg [22.1, 52.5], respectively; p = 0.02) (Figure 3-5). Multiple regression analyses adjusted for sex, height and weight indicated CD was associated with 4.7 kg (95% CI: 2.2, 7.2) lower grip strength than healthy controls (Model R^2 = 0.73; p < 0.001). Further adjustment for RMA attenuated this association but CD remained associated with 2.4 kg (95% CI: 0.0, 4.8) lower grip strength (Model R^2 = 0.8; p = 0.05).

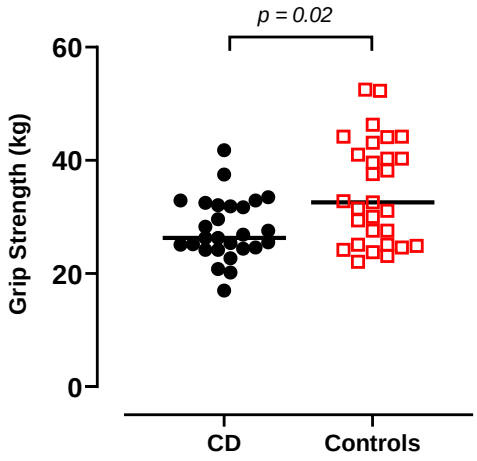

Figure 3-5 Comparison of maximal isometric grip strength between CD and controls.
Individual dot plots comparing maximal isometric grip strength between CD and controls. Between group difference assessed by Mann Whitney U test. Black circles – CD; red squares – controls. Horizontal line represents median. kg – kilograms. * denotes significant difference between groups.

3.4.3.2 Jumping mechanography

Mechanography data were available in 37 participants (19 CD, 18 controls). Cohort characteristics of these participants are available in Table 3-3. There were no differences between the two groups for any characteristics other than grip strength, which was lower in CD. In unadjusted mechanography outcomes, no difference in power and force output were seen between control and CD (Figure 3-6). In multiple regression analysis adjusted for age and sex, CD was associated with lower absolute and relative muscle power, and lower absolute force (Table 3-4).

Table 3-3 Cohort characteristics of participants with available mechanography data comparing CD and controls.

	Crohn's Disease (n = 19)	Controls (n = 18)	p-value
Demographics & Lifestyle			
Sex (f/m)	11/8	9/9	
Age (years)	22.6 (18.0, 29.1)	23.1 (18.2, 37.0)	0.21
Height (cm)	169.0 (154.0, 193.0)	174.1 (157.0, 193.0)	0.13
Weight (kg)	61.8 (38.2, 104.1)	67.1 (55.2, 85.3)	0.23
BMI (kg/m^2)	21.8 (16.1, 34.4)	23.3 (18.8, 28.2)	0.34
Physical Activity (MET-mins/week)	5328 (585, 10392)	4351 (680, 9000)	0.51
Residual muscle area (mm^2)	7017 (3216, 10203)	7998 (4945, 12007)	0.08
Grip strength (kg)	27.6 (17.0, 41.8)	32.7 (22.1, 52.5)	0.03*

f/m – female/male; cm – centimetres; kg – kilograms; BMI – body mass index; m – metres; MET – metabolic equivalent of task; mins – minutes; mm – millimetres. * Denotes significant between group difference.

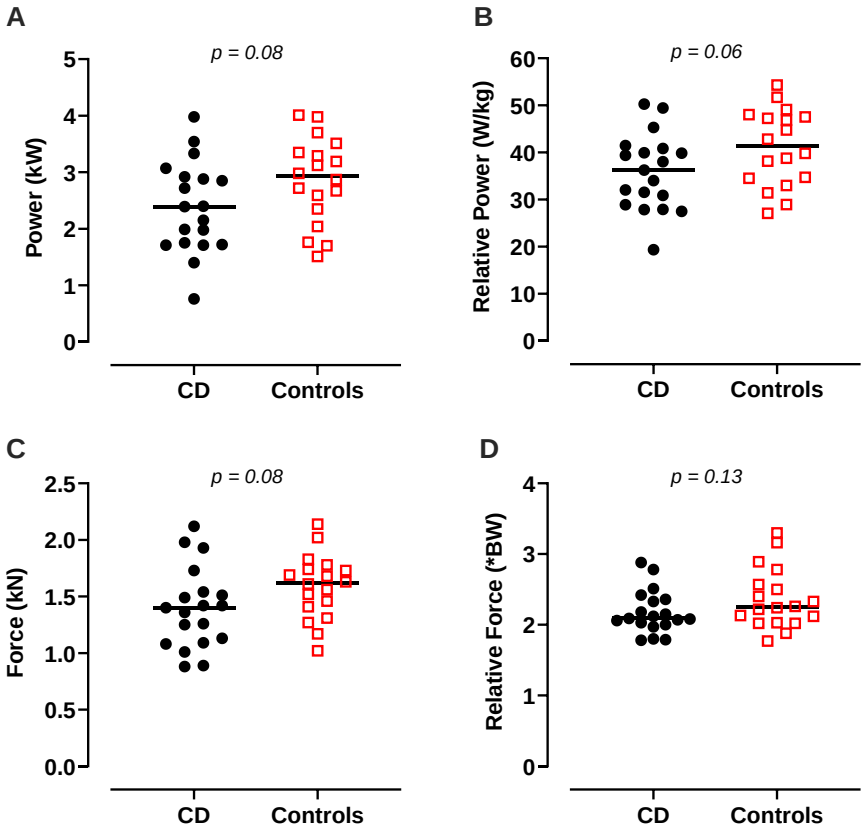

Figure 3-6 Comparison of lower leg muscle function parameters measured by jumping mechanography between CD and controls.
Individual dot plots comparing lower leg muscle function parameters between CD and controls. Between group differences assessed by t-tests. Black circles – CD; red squares – controls. N = 37 (19 CD, 18 controls). Horizontal line represents median. kW - kilowatts; W/kg – watts per kilogram body mass; kN – kilonewtons; *BW – force as multiples of bodyweight. * denotes significant difference between groups.

Table 3-4 Multiple regression analyses of jumping mechanography parameters between CD and controls.

Mechanography outcome	B (95% CI)	Std. β	Model R^2	p-value
Power (kW)	-0.45 (-0.80, -0.10)	-.282	0.65	0.01
Relative power (W/kg)	-5.0 (-8.8, -1.2)	-.302	0.6	0.01
Force (kN)	-0.21 (-0.41, 0.00)	-.317	0.28	0.05
Relative force (*BW)	-0.22 (-0.48, 0.35)	-.300	0.1	0.09

N = 37 (19 CD, 18 Controls). kW - kilowatts; W/kg – watts per kilogram body mass; kN – kilonewton; *BW – force as multiples of bodyweight. Models adjusted for age and sex. Control group used as reference.

3.4.4 Biomarkers of bone, muscle, and inflammation

3.4.4.1 Markers of muscle-bone and GH axis

There were no between group differences observed in BSAP, however median OC was higher in CD compared to controls (23.7 ng/ml [13.3, 57.2] vs 19.5 ng/ml [9.8, 35.8]; p = 0.01) (Figure 3-7 B). Bone resorption marker CTX-1 and sclerostin were not different between CD and controls (Figure 3-7 C-D & Table 3-5). Median 25-OHD was 51 nmol/l (14, 142) and 41 nmol/l (16, 101) in CD and controls, respectively (p = 0.76). No difference was observed in 25-OHD after adjustment for seasonal variation. Nineteen percent of CD participants and 11% of controls had a deficient serum 25-OHD level (<25 nmol/l). PTH was increased in CD compared to controls (p = 0.03) with 9/27 samples above the upper limit of normal (>7.5 pmol/l) (Table 3-5). Serum IGF-1 was not different between groups, nor were IGFBP3, ALS or myostatin (Table 3-5).

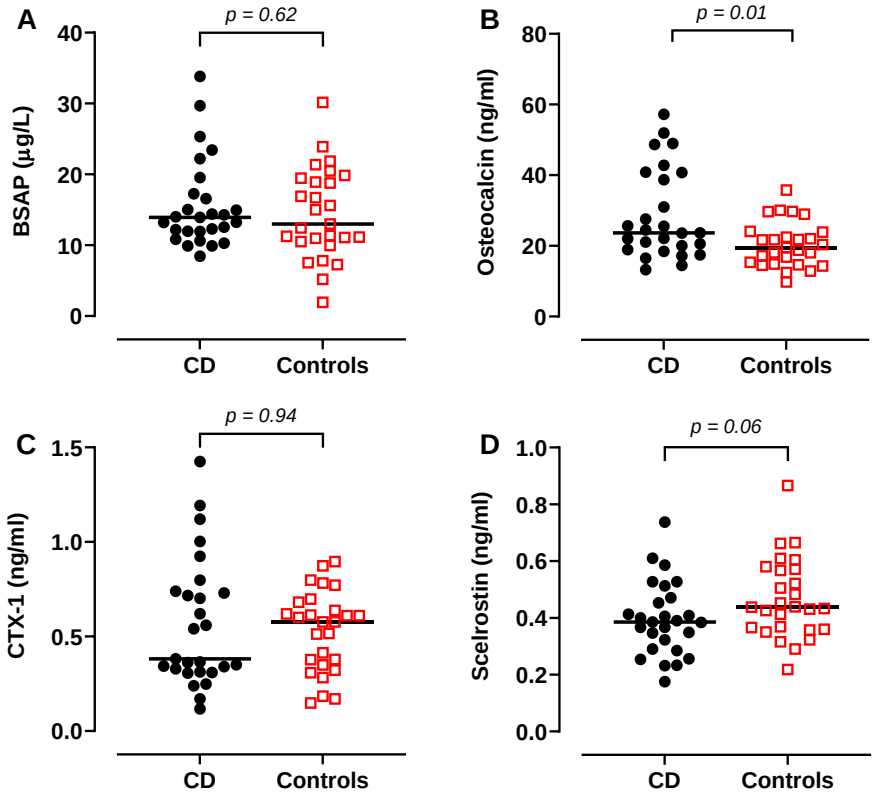

Figure 3-7 Comparison of serum bone turnover markers between CD and controls.
Individual dot plots comparing circulating bone turnover markers between CD and controls. Between group differences assessed by Mann Whitney U test (A, B, C) or t-test (D). Black circles – CD; red squares – controls. N = 54 (27 CD; 27 controls). Horizontal line represents median. BSAP – bone specific alkaline phosphatase; CTX-1 – C-terminal telopeptide of Type I collagen; µg – micrograms; ng – nanograms; ml - millilitre; L - litre.

Table 3-5 Biochemical markers of bone, muscle, and inflammation in CD and controls.

Clinical biochemistry	Crohn's Disease (n = 27)	Controls (n = 28)	p-value
Albumin (g/l)	41 (32, 48)	42 (35, 47)	0.12
Faecal Calprotectin (µg/g) [a]	81 (30, 1800)	30 (30, 217)	0.05
ESR (mm/hour)	5 (1, 90)	2 (2, 8)	0.05
CRP (mg/l)	1 (0, 63)	0 (0, 9)	0.002
25-OHD (nmol/l)	51 (14, 142)	41 (16, 101)	0.76
PTH (pmol/l)	6.5 (2.8, 13.6)	5.4 (1.8, 9.4)	0.03
Alkaline Phosphatase (U/l)	70 (38, 124)	63 (23, 121)	0.11
Markers of bone turnover			
BSAP (µg/l)	13.9 (8.4, 33.8)	13.0 (1.9, 30.1)	0.62
Osteocalcin (ng/ml)	23.7 (13.3, 57.2)	19.5 (9.8, 35.8)	0.01
CTX-1 (ng/ml)	0.38 (0.12, 1.43)	0.58 (0.15, 0.90)	0.94
Sclerostin (ng/ml)	0.39 (0.18, 0.74)	0.44 (0.22, 0.87)	0.06
Markers of GH/IGF-1 axis			
IGF-1 (ng/ml)	298 (142, 493)	334 (235, 539)	0.14
IGFBP2 (ng/ml)	377 (107, 858)	334 (111, 634)	0.02
IGFBP3 (ng/ml)	4337 (2718, 5971)	4421 (3092, 6244)	0.99
ALS (ng/ml)	10802 (5484, 16348)	11770 (7566, 21974)	0.51
Pro-inflammatory cytokines			
TNFα (pg/ml)	10.53 (3.51, 31.59)	5.67 (2.27, 18.42)	0.01
IFN-γ (pg/ml)	2.48 (1.56, 6.56)	1.56 (0.36, 3.40)	<0.001
IL-6 (pg/ml) [b]	1.08 (0.36, 8.92)	1.38 (0.40, 5.72)	0.81
IL-8 (pg/ml) [c]	11.40 (3.40, 122.72)	8.34 (3.40, 15.60)	0.02
Myokines			
Myostatin (ng/ml)	2.77 (0.14, 21.95)	3.78 (0.09, 19.98)	0.27

25-OHD – 25 hydroxyvitamin D; PTH – parathyroid hormone; ESR – erythrocyte sedimentation rate; CRP – C reactive protein; BSAP – bone specific alkaline phosphatase; CTX-1 – C terminal telopeptide of Type 1 collagen; IGF – insulin like growth factor; IGFBP – IGF binding protein; ALS – acid labile subunit; IL - interleukin. Continuous variables displayed as median (range). * denotes significant difference between groups. [a] n = 24 (13 CD, 11 controls), [b] n = 15 (9 CD, 6 controls), [c] n = 39 (27 CD, 12 controls).

3.4.4.2 Markers of inflammation

Serum ESR and CRP were significantly higher in CD compared with controls. Serum albumin was not different between groups (Table 3-5). IGFBP-2 was elevated in CD compared to controls ($p = 0.02$) as were pro-inflammatory cytokines TNF-α, IFN-γ and IL-8 (all $p < 0.05$). Serum IL-6 was not different between groups (Table 3-5). Faecal calprotectin was higher in CD (n = 13) compared with controls (n = 11) ($p < 0.05$). Only three CD participants had a clinically relevant faecal calprotectin of >250 µg/g.

3.4.5 Associations between bone, muscle, and adiposity

3.4.5.1 Trabecular bone

Trabecular bone volume and trabecular thickness were positively associated with BMI in CD (r = 0.53, p = 0.01 and r = 0.64, p = 0.004, respectively), but not controls (r = 0.16, p = 0.42 and r = 0.03, p = 0.89, respectively). In CD, RMA was positively associated with trabecular bone volume and thickness but not separation or number (Figure 3-8). No associations between RMA and trabecular microarchitecture were observed in controls (Figure 3-8). Additionally, no associations were observed between trabecular parameters and self-reported PA. RMA remained associated with trabecular bone volume and thickness in regression analyses, after adjustment for sex and grip strength in CD (Table 3-6).

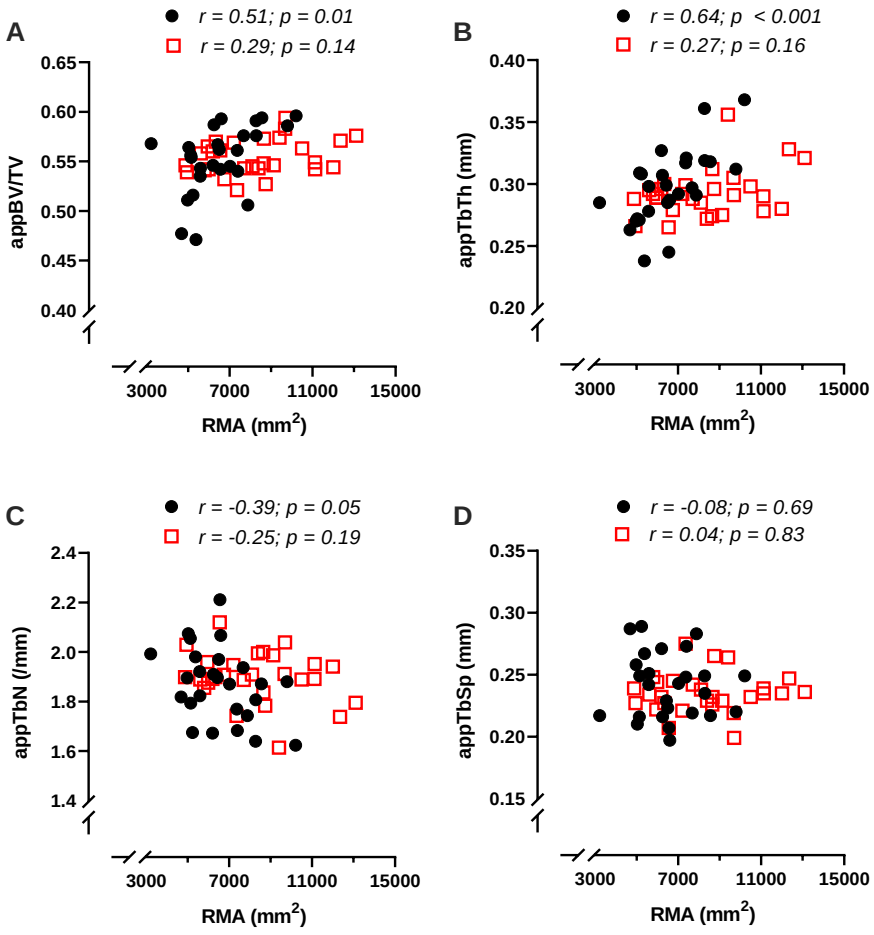

Figure 3-8 Associations between RMA and trabecular parameters in CD and controls. Scatterplots displaying associations between RMA and trabecular microarchitecture in paediatric CD and controls. Associations assessed using Spearman rank (A, C, D) or Pearson (B) correlation analyses, respectively. Black circles – CD; red squares – controls. A – RMA vs appBV/TV; B – RMA vs appTbTh; C – RMA vs appTbN; D – RMA vs appTbSp. AppBV/TV = apparent trabecular bone volume; appTbTh – apparent trabecular thickness; appTbN – apparent trabecular number; appTbSp – apparent trabecular spacing. RMA – residual muscle cross-sectional area; mm – millimetre.

Table 3-6 Multiple regression analyses of muscle area with trabecular parameters in CD and controls.

		Crohn's Disease			
Predictor	*Outcome*	**β (95% CI)**	**Std. β**	**Model R^2**	**p-value**
RMA (mm^2) [a]	appBV/TV	1.82 $x10^{-5}$ (0.00, 0.00)	.892	0.37	0.03
	appTbTh (mm)	1.17 $x10^{-5}$ (0.00, 0.00)	.647	0.56	0.06
		Controls			
		β (95% CI)	**Std. β**	**Model R^2**	**p-value**
RMA (mm^2) [a]	appBV/TV	3.92 $x10^{-6}$ (0.00, 0.00)	.505	0.18	0.13
	appTbTh (mm)	2.17 $x10^{-6}$ (0.00, 0.00)	.256	0.14	0.45

Multiple linear regression analyses of muscle function and selected trabecular parameters in CD and controls, respectively. Models adjusted for sex and grip strength. [a] – n = 54 (26 CD; 28 controls). appBV/TV – apparent trabecular volume; appTbTh – apparent trabecular thickness; appTbN – apparent trabecular number; appTbSp – apparent trabecular spacing; B – unstandardized beta coefficient; Std. β – standardized beta coefficient; kg – kilograms; kW – kilowatts; W/kg – watts per kilogram body mass.

3.4.5.2 Cortical bone

Cortical area was positively associated with RMA in both CD and controls, respectively (Figure 3-9). RMA positively associated with cortical thickness in the control but not CD group (Figure 3-9). In regression analyses adjusted for sex and height, RMA remained positively associated with cortical area in controls only (Table 3-7).

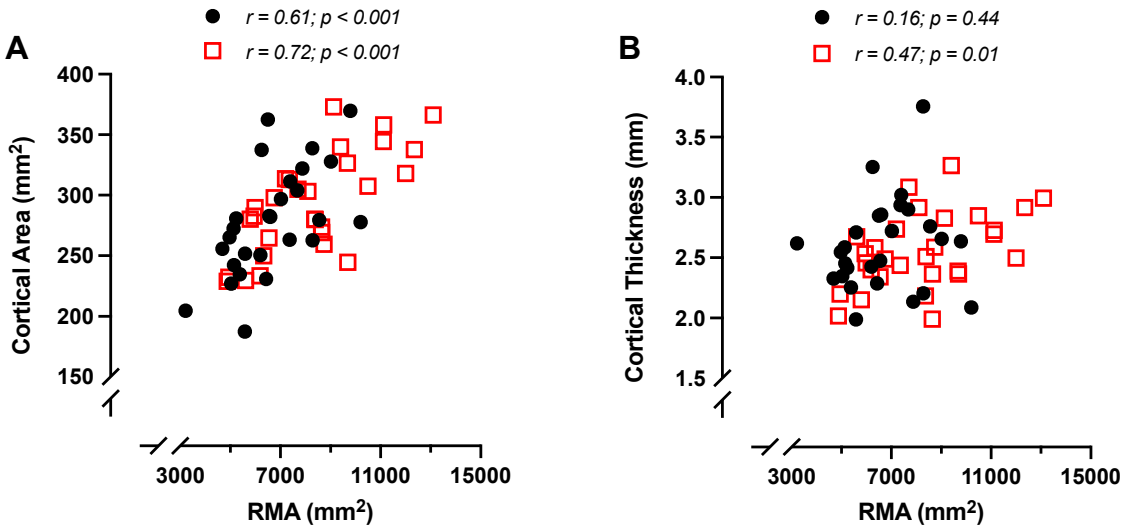

Figure 3-9 Associations between RMA and cortical geometry in CD and controls.
Spearman rank correlation analyses between residual muscle area and cortical geometry parameters in CD and controls. Black circles – CD; red squares – controls. Circ. – circumference; mm – millimetre; RMA – residual muscle area.

Table 3-7 Multiple regression analyses of residual muscle area and cortical geometry in CD and controls.

Crohn's Disease (n = 27)				
Outcome	**B (95% CI)**	**Std. ß**	**Model R^2**	**p-value**
Cortical Area (mm^2)	0.008 (-0.002, 0.017)	.285	0.64	0.1
Cortical Thickness (mm)	3.92 $x10^{-6}$ (0.00, 0.00)	.017	0.05	0.34
Controls (n = 29)				
Outcome	**B (95% CI)**	**Std. ß**	**Model R^2**	**p-value**
Cortical Area (mm^2)	0.007 (0.000, 0.013)	.368	0.64	0.04
Cortical Thickness (mm)	3.53 $x10^{-5}$ (0.00, 0.00)	.256	0.39	0.26

Multiple linear regression analyses of residual muscle area and cortical geometry in CD and controls, respectively. RMA is the predictor for each model. All models adjusted for sex and height. B – unstandardized beta coefficient; Std. β – standardized beta coefficient; mm – millimetre; circ. – circumference.

3.4.5.3 Bone marrow adiposity

There was no evidence of any associations between BMA and MRI derived measures of trabecular microarchitecture, cortical geometry, or muscle area and adiposity.

3.4.6 Associations between disease indices and bone, muscle & adiposity in CD

3.4.6.1 Trabecular and cortical bone

Current CDAI score was negatively associated with trabecular bone volume and thickness and positively associated with trabecular number (Table 3-8). Self-reported number of GC courses was negatively associated with trabecular bone volume and cortical area (Table 3-8). Number of CD related surgeries was negatively associated with trabecular thickness and positively associated with trabecular number.

Table 3-8 Associations between disease indices and trabecular microarchitecture and cortical geometry in CD.

	CDAI score	**GC history**	**No. of CD surgeries**
Trabecular Parameter [a]			
appBV/TV	-0.4 (0.05)	-0.42 (0.03)	-0.17 (0.42)
appTbTh	-0.56 (0.003)	-0.21 (0.31)	-0.58 (0.002)
appTbN	0.42 (0.04)	-0.01 (0.97)	0.47 (0.02)
appTbSp	0.0 (1.0)	0.34 (0.09)	-0.12 (0.57)
	CDAI score	**GC history**	**No. of CD surgeries**
Cortical Parameter [b]			
Cortical area	-0.05 (0.8)	-0.47 (0.01)	-0.37 (0.06)
Cortical Thickness	0.02 (0.91)	-0.37 (0.06)	-0.19 (0.33)

Spearman rank correlation analyses between markers of disease and trabecular microarchitecture and cortical geometry of the distal femur in CD only. Parentheses represent p-values. AppBV/TV – apparent bone volume fraction; appTbTh – apparent trabecular thickness; appTbN – apparent trabecular number; appTbSp – apparent trabecular spacing; CDAI – Crohn's disease activity index;

GC history – self-reported courses of glucocorticoids in the paediatric age (<18 years). [a] n = 26; [b] n = 27.

3.4.6.2 Muscle and adiposity

Muscle FF and BMA were not associated with any disease indices. RMA was negatively associated with current CDAI score, self-reported GC courses, and number of CD related surgeries (Table 3-9). Grip strength was negatively associated with number of CD related surgeries.

Table 3-9 Associations between disease indices and muscle and adiposity in CD.

	CDAI score	GC courses	No. of CD surgeries
Muscle Parameter			
RMA [a]	-0.41 (0.03)	-0.42 (0.03)	-0.44 (0.02)
Grip Strength [a]	-0.33 (0.09)	-0.3 (0.13)	-0.48 (0.01)
Mechanography Power [b]	-0.25 (0.31)	-0.37 (0.12)	-0.23 (0.36)
Mechanography Relative Power [b]	0.05 (0.84)	-0.27 (0.27)	-0.23 (0.35)
	CDAI score	**GC courses**	**No. of CD surgeries**
Adiposity Parameter			
Muscle FF [a]	0.03 (0.9)	-0.16 (0.44)	0 (1.0)
BMA [c]	0 (1.0)	-0.07 (0.75)	-0.27 (0.21)

Spearman rank correlation analyses between markers of disease and muscle and adiposity in CD only. Parentheses represent p-values. RMA – residual muscle area; FF – fat fraction; BMA – bone marrow adiposity; CDAI – Crohn's disease activity index; GC history – self-reported courses of glucocorticoids in the paediatric age (<18 years). [a] n = 27; [b] n = 19; [c] n = 23.

3.5 Discussion

This study comprehensively assessed the muscle-bone unit in young adults with well controlled childhood onset CD using high-resolution MRI and MRS. Detailed evaluation of trabecular microarchitecture and cortical geometry at the distal femur revealed no deficits in CD compared to healthy age and sex matched controls. Conversely, CD was associated with lower muscle mass and function compared to controls. No differences in biochemical markers of the muscle-bone unit were observed, although CD was associated with elevated circulating inflammatory cytokines. Disease activity was also negatively associated with trabecular bone volume and muscle.

In Chapter 2, paediatric CD was associated with deficits in trabecular bone volume compared to healthy controls. The current study, conversely, suggests young adults with well controlled childhood onset CD do not display deficits in trabecular microarchitecture. This study also contrasts with the few prior assessments of trabecular bone in adults with CD. In two studies using HR-pQCT, deficits in trabecular microarchitecture of the tibia and radius have been observed in cohorts of young (mean age 23.1 yrs; n = 102, 74% CD) [283] and middle aged (mean age 42.8 yrs; n = 59) [282] adults with CD. Another bone biopsy histomorphometry study found markedly reduced trabecular bone volume in quiescent CD compared to healthy controls [341], although this cohort had previously established osteopenia. Discrepant results for trabecular bone between studies may be related to several disease and non-disease related factors.

One likely explanation for the observed discrepancies may be prior GC exposure. The current cohort have been primarily managed in the era of contemporary management of paediatric CD, with EEN being preferred first line therapy. Resultantly, exposure to GC in the paediatric era was limited, with an average one course of GC reported. In contrast, around 20% of CD participants were currently being treated with GC in one study [283], and >50% of middle-aged CD participants had previous long-term exposure to high dose GC [282]. The deleterious effects of GC on trabecular bone have been well described [244] and potentially contributed to trabecular loss in those cohorts. Additionally, those studies consisted primarily of individuals with adult-onset CD which may have limited the ability of bone to recover from any GC induced deficits post-treatment. Despite

the limited exposure to GC in the current study, prior GC courses were negatively associated with trabecular bone volume. It is, however, worth noting that information regarding the timing, duration and cumulative dose of GC was unavailable, and it was not possible to determine whether those treated with GC earlier in the disease course or at a younger age were able to recover any incurred trabecular deficits. The inclusion of participants with greater exposure to GC or calculable cumulative GC exposure would allow for assessment of its association with trabecular bone. Nevertheless, GC are used sparingly in modern CD management and the current cohort represent this contemporary approach.

Aside from GC, the use of other therapies may also be associated with trabecular outcomes in CD. Long-term management with contemporary therapies, such as anti-TNF-α, has previously been found to improve markers of bone formation and IGF-1 [49,217,339] and linear growth [47,49]in paediatric CD. Markers of bone turnover were not different between CD and controls in the current study Two thirds of the current cohort had current or previous exposure to biologics which may have facilitated more normal trabecular development. Haschka et al. [282] also reported a high prevalence of current biologic therapy in their CD cohort (71%) despite observing deficits in trabecular microarchitecture. Indeed, the effects of anti-TNF-α in adult bone are less clear, with inconsistent reports for effects on BMD. Improvements in lumbar spine aBMD have been reported after anti-TNF-α treatment in adults CD [342]. However, anti-TNF-α has also been associated with increased rate of osteopenia and low trabecular bone score in other cohorts [343]. Any association between anti-TNF-α and poor skeletal outcomes in adults with CD is likely confounded by disease phenotype. Anti-TNF-α biologics are generally reserved for adults with disease that is refractory to conventional therapy and therefore considered to be more severe, which may have independently contributed to bone loss in those studies. The use of thiopurines, which are commonly prescribed alongside anti-TNF-α and were the most common current therapy in this study, may also be associated with skeletal outcomes. A recent study in murine colitis demonstrated a negative effect of azathioprine on skeletal outcomes [344], although this has not been demonstrated in humans. Further investigation is required into the long-term effects of contemporary treatment strategies on skeletal development in paediatric and young adult CD.

Another potential explanation for the difference in trabecular results between studies is the method of assessment used. While 3T-MRI is a reliable method for assessing trabecular microarchitecture in other clinical populations [194,335,345,346], it may fail to detect subtle differences in microarchitectural parameters due to resolution limitations. Bone biopsy histomorphometry is the gold standard for trabecular assessment, while HR-pQCT employs increased spatial resolution compared to MRI (82μm vs 200μm, respectively). It remains possible that deficits in trabecular bone may exist in CD beyond the current resolution capacity of 3T-MRI, although whether such minute differences would be clinically meaningful is unclear. Methods for assessing trabecular microarchitecture using high-resolution MRI are also in their relative infancy and as such there is no recognised standard between research groups, limiting comparisons between studies. Additional analyses of the current data using other methods such as topological analyses may have provided greater insight into the structural composition of trabecular bone in CD. Using those methods, high-resolution MRI at the proximal femur was reported as a sensitive tool for discriminating between individuals with and without fragility fracture that would not be identified by DXA [346]. Indeed, MRI-derived trabecular microarchitecture parameters do not associate with measures of bone density using DXA (Chapter 2) [335,345], highlighting the additional information attainable from MRI that cannot be achieved with DXA. Further work is required to determine if MRI assessment of trabecular microarchitecture at the distal femur may be a useful prognostic indicator of skeletal fragility.

Deficits in cortical thickness and area have been observed in paediatric CD [49,209,276,277,334] most likely due to inflammation induced relative increases in endocortical resorption. No differences were observed between CD and controls for any cortical parameters in the current study, after adjustment for sex and height, suggesting no cortical deficits in this cohort. Cortical geometry may improve after long term follow up in children with CD exposed to minimal GC doses and who achieve effective disease control [37]. Cortical section modulus – a function of cortical geometry and an estimate of bone strength – was negatively associated with cumulative GC dose in adolescents and young adults with CD [347] and decreased during follow up in newly diagnosed paediatric CD patients subsequently treated, primarily, with systemic GC [276]. These findings corroborate the negative association between previous GC exposure and cortical area observed

in the current cohort, although this association was lost after adjusting for height, further highlighting the importance of linear growth for cortical development. Anti-TNF-α therapy may also facilitate improvements in cortical geometry via increased linear growth and muscle accrual [48], although persistent cortical deficits have been reported at follow up, despite some improvement [49]. Long-term control of underlying disease mechanisms using contemporary therapies may facilitate improved skeletal development in children with CD, perhaps explaining the lack of cortical deficits in the current study.

Skeletal muscle deficits are a common complication of paediatric CD. A systematic review of body composition in paediatric CD reported lean mass deficits in >90% of all included CD participants, when compared with healthy reference populations [348]. Muscle mass deficits also persist in paediatric CD after long-term follow up [37,280] and up to 12 months of anti-TNF-α therapy [49,217], yet it was unknown whether such deficits remain into adulthood. In the current study, muscle area of the thigh was lower in CD compared to healthy controls. In regression analyses, RMA remained lower in CD compared to controls after adjusting for sex, height, and weight, indicating muscle mass relative to body size was also lower in CD. In young adults with childhood onset IBD, skeletal muscle index (SMI), assessed by DXA, was low in young men but not women with CD, compared to healthy controls [292]. Three retrospective studies of clinical CT and MRI scans at L3 level identified low skeletal muscle index (a proxy for sarcopenia) in 40 - 58% of adults with CD [293,294,349]. One study also reported higher likelihood of sarcopenia in those with active versus inactive disease (OR: 2.08 [95% CI: 1.03, 4,27]) [349]. Likewise, despite generally low disease activity in the current cohort, a negative association between RMA and current CDAI score was observed, suggesting poorer disease control may contribute to muscle deficits.

Chronic low-grade inflammation in CD may partly explain the muscle deficit observed, as circulating pro-inflammatory cytokines were elevated in comparison with controls. Chronically elevated cytokines induce increased muscle protein degradation and elevated TNF-α has been found to epigenetically alter skeletal muscle satellite cells which may inhibit their capacity for myogenesis [106]. In contrast, a previous study found low muscle CSA in CD despite lower circulating TNF-α than the control cohort [269]. Lower TNF-α may have been the product of

effective disease control or elevated TNF-α in the control cohort and highlights the importance of factors other than inflammatory cytokines in the moderation of muscle mass in CD. Molecular analysis revealed no differences between CD and controls in atrophy related gene expression, but a significantly reduced capacity for muscle protein synthesis in CD, likely due to low circulating IGF-1 [269,298]. Abnormalities in GH/IGF-1 have been previously reported in CD and suggested as a potential mechanism of muscle-bone deficit [145] and improvements in IGF-1 were predictive of increases in muscle mass in children with CD undertaking anti-TNF-α therapy [48]. Despite this, IGF-1 was not lower in CD compared to controls in the current study. Lean mass deficits may be associated with poorer disease outcomes, including higher need for hospitalisation and surgery [294] and early treatment failure [300]. It is therefore vital that the mechanism of muscle deficit in CD be further investigated and targeted in future interventions.

Muscle function assessed by grip strength and jumping mechanography were also lower in CD compared to controls, as was also observed in the paediatric cohort of Chapter 2. After adjusting for sex, height, weight, and RMA, CD remained associated with reduced grip strength, suggesting a potential mechanism for muscle weakness that is independent of muscle size. Grip strength is a useful clinical indicator and the observed deficit in this study is equivalent to that associated with up to 20% higher risk of morbidity and mortality in prospective study of older adults [206]. It can be speculated that muscle function deficits in young adulthood. Low handgrip strength has been previously reported in CD [296,297] but was no different from healthy controls when expressed relative to FFM in another cohort [299]. Improvements in muscle mass and function may therefore serve to improve clinical outcomes in CD but also reduce risk of morbidity and mortality from other diseases. The effects of CD on mechanisms underpinning muscle strength, independent of muscle mass, have not been elucidated and warrant further investigation in both pre-clinical and human studies.

The current study is the first to report jumping mechanography outcomes in adults with CD. Paediatric studies have reported normal [278] or low values (Chapter 2, [277]) compared to reference data. In one adult study, objectively measured muscle fatigue was worse in CD compared to controls, despite no differences in absolute muscle force [298]. In that cohort, poor muscle function was associated with lower

vitamin D and IGF-1. However, these did not differ between CD and controls in the current study. There is, however, a possibility that malabsorption and loss of nutrients may have contributed to the observed muscle deficits, especially in those with a history of surgical resection, which was negatively associated with muscle mass and function. While the current cohort may not fit a predefined definition of sarcopenia, muscle mass and function deficits in early adulthood may predispose these individuals to increased likelihood of sarcopenia and frailty at a younger age than their healthy counterparts if muscle targeting strategies are not employed.

Skeletal muscle fat infiltration progresses with age and may be accelerated by the primary or secondary effects of chronic disease. This study observed a non-significantly increased level of muscle fat in CD compared to healthy controls (5.3% [0.6, 8.9] vs 4.1% [0.5, 9.2]; p = 0.07). The association between CD and muscle fat infiltration has not been extensively studied. However, CD shares previously reported risk factors for muscle fat accumulation such as GC exposure [104], systemic inflammation [350], and low activity levels [351]. The long-term clinical implications of increased muscle fat in CD are unclear. Muscle fat was, however, associated with increased length of hospital stay and short-term readmission in those with CD undergoing surgical resection [110]. Older adults with rheumatoid arthritis were found to have comparable muscle fat to sex and BMI matched cohort aged 10 – 20 years older than themselves [352]. This suggests inflammatory disease may accelerate the accumulation of muscle fat in a similar manner to regular aging, which could ultimately lead to impaired muscle function and increased risk of fracture, independently of muscle mass [353]. Muscle adipocytes also express pro-inflammatory adipokines that may contribute to systemic inflammation in CD.

Bone marrow adiposity has emerged as a useful indicator of skeletal health, yet the role of BMA in bone health in CD remains unclear. In the current study there were no differences in BMA between CD and controls and BMA was not associated with any indices of disease. Furthermore, BMA was not associated with any trabecular microarchitecture parameters in CD. Only one previous study has assessed BMA in CD patients. Adults with CD in long-term, steroid-free remission had preserved BMA of the lumbar spine, despite low BMD in the lumbar spine and femoral neck in 19% and 47% of patients, respectively [354]. Similarly, in a dextran

sodium sulfate (DSS) induced model of colitis, BMA was unaffected despite a reduction in cortical and trabecular thickness [355]. Taken together, this and previous studies suggest no direct association between CD and BMA, although this still requires validation in future trials.

This study has some important limitations that must be considered. Primarily, the cross-sectional nature of the data precludes any assessment of causality. There are several potential mechanisms involved in musculoskeletal deficit in CD which cannot be extricated from the current data and require further investigation to optimise improvement strategies. As mentioned, high-resolution MRI is unable to accurately delineate trabecular structures due to resolution limitations and may have failed to detect subtle differences between groups. Furthermore, there is a lack of available reference data using the current MRI parameters therefore we are unable to comment on a comparison between CD and the wider population. Finally, this study did not provide a concurrent assessment of BMD and it remains possible that these patients may exhibit deficits in bone mass, which remains the largest contributor to skeletal fragility. Despite these limitations, this study provides important insight into the current muscle-bone status of a cohort of young adults with CD and provides the basis upon which future interventions or monitoring studies may be founded.

In summary, this study has comprehensively evaluated the muscle-bone unit of young adults specifically with childhood onset CD for the first time. In this cohort with generally well-controlled disease, young adults with CD displayed lower muscle mass and function but no abnormalities in trabecular or cortical bone of the distal femur. Biochemical markers of the muscle-bone unit did not reveal any evidence of abnormal bone or muscle metabolism and it is likely that the deficits observed in skeletal muscle are the product of multiple contributing disease and lifestyle associated factors. Deficits in muscle mass and function may augment the deleterious effects of CD on muscle-bone and interventions to improve these require future investigation.

4 Associations between IBD and adverse musculoskeletal outcomes in middle-aged and older adults.

4.1 Abstract

Background. The chronic effects of inflammatory bowel disease and ageing may contribute to an increased risk of adverse muscle-bone outcomes. Furthermore, the association between IBD and falls has never been studied.

Aim. To assess the likelihood of adverse musculoskeletal outcomes in middle aged and older adults with IBD and its subtypes CD and UC using data from a very large, population-based cohort study.

Methods. Cross-sectional analysis of UK Biobank baseline data. Associations between IBD, CD, and UC with likelihood of self-reported falls and fractures, and muscle weakness assessed by grip strength dynamometry, were assessed by multivariable logistic regression analyses. Association between disease and estimated BMD, measured by QUS, was assessed using multivariable linear regression. Exploratory analyses also investigated risk factors associated with muscle-bone outcomes in CD.

Results. A total of 401,850 participants were included in these analyses, of which 4619 had a history of IBD. In the subtype analyses, 1386 had CD and 3007 had UC. IBD was associated with an increased likelihood of a self-reported fall in both women (OR: 1.13 [95%CI: 1.03, 1.25]) and men (OR: 1.16 [95%CI: 1.03, 1.29]). Women with CD were the only group to have higher likelihood of a self-reported fracture (OR: 1.35 [95%CI: 1.09, 1.66]) or fragility fracture (OR: 1.35 [95%CI: 1.06, 1.73]) compared to controls. All disease subgroups were more likely to have muscle weakness compared to controls, with the strongest association observed in women with CD (OR: 1.55 [95%CI: 1.25, 1.92]). In CD, muscle weakness was associated with increased likelihood of a fall, and a previous fall was associated with increased likelihood of a fracture.

Conclusions. This study revealed an association between mild IBD and adverse muscle-bone outcomes, with women with CD displaying the most severe

phenotype. Men and women with CD were at higher risk of falls which is one of the primary contributors to morbidity and mortality in older adults.

4.2 Introduction

Chronic inflammatory disease is associated with adverse musculoskeletal outcomes, even in individuals who achieve clinical remission. In this work, both children and young adults with currently quiescent CD have been observed to have a variety of bone and muscle deficits. Failure to resolve these deficits may lead to increased lifelong risk of adverse musculoskeletal outcomes, such as falls and fractures.

Aging is associated with changes in the inflammasome that mimic some of the effects of inflammatory disease, such as chronic elevation of pro-inflammatory cytokines [218]. As such, IBD has been compared to something like an accelerated aging process, whereby inflammatory mediated tissue destruction leads to early declines in bone and muscle. It is therefore possible that those with IBD may be at increased risk of typically age-related adverse musculoskeletal events due to the combined effects of disease and aging. Falls and fractures are major contributors to morbidity and mortality and one of the leading causes of disability adjusted life years [356]. To date, no studies have assessed the risk of falls in older adults with IBD, while some have suggested an increased risk of hip fractures only in elderly IBD patients with prior GC exposure [324].

The aim of this study was to characterise bone and muscle outcomes and associated factors in middle aged and older adults with IBD in a large population-based cohort. The study investigated associations between a diagnosis of IBD and its subtypes CD and UC with falls, fractures, estimated BMD (eBMD), and muscle weakness.

4.3 Methods

4.3.1 Study design

UK Biobank is a prospective, population cohort study of middle-aged and older adults registered with the national health service (NHS) in England, Scotland, and Wales. Initially, approximately 9.2 million people were approached to participate in this study and 502,628 participants, aged 37 – 73 years, were recruited (5.5% response rate). Baseline assessments were conducted between April 2007 and December 2010 at one of 22 regional assessment centres. Baseline assessments involved a series of touch screen questionnaires, physical measurements, and biological sampling, followed by face-to-face interviews with trained research staff. Details of the protocol, including transcript for the touch screen questionnaires, can be found online (www.ukbiobank.ac.uk). UK Biobank is conducted under ethical approval from the North West Multi-Centre Research Ethics Committee (REC Ref: 11/NW/03820) and all participants provided written informed consent prior to enrolment in the study. This analysis of UK Biobank data was conducted under Application ID 7155.

4.3.2 Data collection

Detailed information was collected regarding sociodemographic factors (age, sex, ethnicity, postcode, associated area-based deprivation) lifestyle (smoking status, dietary intake, alcohol consumption, physical activity, sedentary behaviour), and medical history (prevalent comorbidities, current medications, surgical history, medical events [e.g., falls and fractures]). Age was calculated using date of birth and assessment date. Ethnicity was self-reported and categorised as: White, Black, South Asian, Chinese, mixed, or other. Area based socio-economic deprivation was derived from postcode using the Townsend deprivation index, with negative values indicating relative affluence and positive values representing greater deprivation [357]. Dietary intake was collected using a validated 24 h recall questionnaire, the Oxford WebQ [358]. PA was self-reported using the international physical activity questionnaire (IPAQ) [340] and expressed as the sum of walking, moderate, and vigorous PA over the previous seven days (MET-mins/week). Sedentary behaviour was derived from the sum of total self-reported time spent

watching televisions, using a computer, and driving in a typical day (hours). Smoking status was defined as 'current', 'previous', or 'never'.

Physical measurements were conducted by trained research nurses using standard procedures and calibrated equipment. Height was measured to the nearest 1 cm (Seca 202 height measure; Seca GmbH, Germany) and weight to the nearest 0.1 kg (Tanita BC-418MA, Tanita, Japan). Corresponding BMI was calculated and categorised according to WHO criteria: underweight <18.5, normal weight 18.5 – 24.9, overweight 25.0 – 29.9, and obese >30.0 kg/m^2. Body composition (body fat percentage, fat mass, fat free mass) was measured using bio-impedance analyses (Tanita BC-418MA). Waist circumference was measured to the nearest 0.1 cm to assess abdominal obesity: ≥ 102 cm in males and ≥ 88 cm in females. Grip strength was measured using a Jamar J00105 hydraulic hand dynamometer and expressed in kilograms. The mean grip strength value of right and left hands was used in analyses.

Bone mineral density (eBMD) was estimated using quantitative ultrasound, which has been validated as an acceptable tool for BMD measurement in large scale prospective studies and correlates highly with DXA derived measurements [187]. Following correct calibration and positioning, a Sahara bone sonometer was used to measure eBMD in the left and right calcaneus using the following equation:

$$BMD = 0.002592 \times (BUA + SOS) - 3.687\ g/cm^2$$

where SOS is the speed of sound (m/s) and BUA is broadband ultrasound attenuation (dH/MHz). Each bone sonometer underwent daily quality control checks using quality control phantoms for at least one hour prior to use. Absolute BMD results were converted into T-scores, equalling the number of standard deviations the participant's value differed from the mean value of a young adult of the same sex. eBMD was derived from the measurement of the left calcaneus (original protocol) or the mean value of measurements of both left and right calcaneus (updated protocol).

Medical history and prevalent comorbidities were self-reported at baseline. Participants were asked to self-report any diagnoses that had been previously made by a physician. Any self-reported diagnoses were confirmed during interview

with research staff and mapped to associated three-character ICD-10 (International classification of diseases, 10th edition) codes. Age at disease diagnosis was approximated using month and year of birth and the estimated date of disease diagnosis. Participants were asked to self-report history of previous surgeries and all procedures involving the lower GI tract were extracted and pooled into a single 'lower GI surgery' variable. Current medication use was also recorded during the interview by selecting the appropriate drug name from a pre-defined list or adding as free text where the medication was not listed. To retrieve and code medications used in the treatment of IBD for inclusion in analyses, the names of all licensed therapies for the treatment of IBD (including brand names) according to the British national formulary (www.bnf.nice.org.uk) were manually searched within the database. This medication list was then reviewed by three consultant gastroenterologists from NHS GGC for relevant omissions. The use of bisphosphonate medications was also retrieved. Participants provided consent for UK Biobank to link to their NHS clinical data, including hospital in-patient and primary care records. In-patient data were available via Hospital Episode Statistics (HES) from 1997 onwards for England, Scottish Morbidity Records (SMR01) from 1981 onwards for Scotland, and the Patient Episode Database for Wales (PEDW) from 1998 onwards for Wales. These data include all in-patient admissions and discharges and associated primary and secondary diagnostic ICD-10 codes. Primary care diagnosis data were available for approximately 45% (n = ~230,000) of the UK Biobank cohort at the time of analyses. Further information regarding the data linkage procedures can be found online (www.biobank.ac.uk).

4.3.3 Exposure

Exposures of interest in this study were a history of IBD or its subtypes (CD or UC) at time of baseline UK Biobank assessment. Disease history was considered present if participants had a relevant in-patient or primary care ICD-10 code in their medical records, or self-reported disease at baseline assessment. ICD-10 codes for CD and UC were K50 and K51, respectively. Where a participant had a recorded ICD-10 code for both CD and UC, this was cross-referenced with the self-reported diagnosis data. These participants were included in overall IBD analyses but excluded from subtype analyses unless they also self-reported either CD or UC at baseline assessment. Any participant who self-reported both CD and UC at baseline (n = 28) was excluded from subtype analyses.

4.3.4 Outcomes

In this cross-sectional analysis of UK Biobank baseline data, outcomes of interest were self-reported falls, fractures, and fragility fractures, estimated BMD, and muscle weakness. Self-reported falls and fractures were reported during touch-screen questionnaires. Participants were asked 'In the last year have you had any falls?'. The responses 'only one fall' and 'more than one fall' were grouped and recoded to create a binary answer of 'yes' or 'no' to this question. Participants were also asked 'Have you fractured/broken any bones in the last 5 years?', coded into a binary outcome 'yes' or 'no'. Those who self-reported a fracture were then asked, 'Did the fracture result from a simple fall (i.e. from standing height)?', which was used as a measure of fragility fractures. Those who responded 'yes' were coded as having experienced a fragility fracture. For these three questions, participants who responded 'do not know' or 'prefer not to answer' were excluded from subsequent analyses. Muscle weakness was defined as low grip strength according to the European Working Group on Sarcopenia in Older People (EWGSOP2) criteria of <16 kg for females and <27 kg for males [359]. Participants who were unable to perform grip strength measurements due to illness or disability were excluded from muscle weakness analyses.

4.3.5 Statistical Analyses

All baseline characteristics and subsequent analyses were stratified by sex. Cohort characteristics were summarised using descriptive statistics and expressed using median and interquartile range (IQR) for continuous variables and frequencies and percentages for categorical variables. Associations between IBD and its subtypes CD and UC with falls, fractures, muscle weakness and eBMD were explored using logistic and linear regression models. Regression models were designed with increasing adjustment for covariates as follows: model 0 was unadjusted; model 1 was minimally adjusted for sociodemographic factors (age, ethnicity, and socioeconomic deprivation); and model 2 was adjusted for model 1 plus known risk factors for musculoskeletal health (BMI, smoking status, and self-reported PA). A sensitivity analyses was then performed using a more rigorously defined cohort for CD and UC. In sensitivity analyses, anyone with a history of both CD and UC was excluded. Exploratory analyses of factors associated with falls, fractures, muscle weakness, and BMD were conducted in CD only using multiple logistic and

linear regression analyses adjusted for age and deprivation (where these were not the exposure of interest). All statistical analyses were performed using Stata v16.0 (Statacorp, TX, USA). Analyses were restricted to participants with available data for all covariates (n = 401 850).

4.4 Results

4.4.1 Cohort characteristics

A total of 401,850 participants were included in this study. The number of participants with data for each musculoskeletal outcomes of interest is available in Figure 4-1. A history of IBD was present in 4619 (1.2%) participants. After excluding those with a recorded episode of both CD and UC who did not also self-report disease, 1386 (0.3%) participants were included in the final CD cohort and 3007 (0.8%) in the final UC cohort (Figure 4-1).

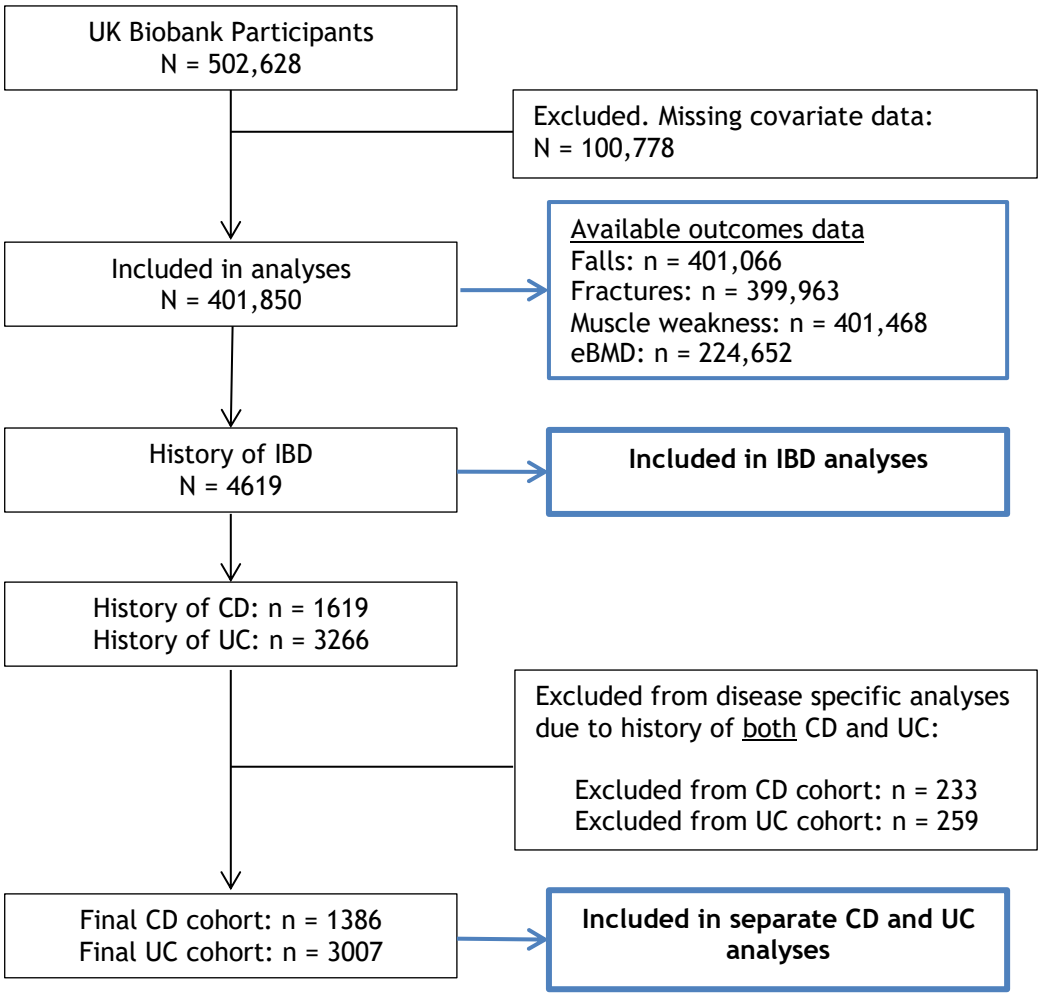

Figure 4-1 Consort diagram for participants included/excluded in the analyses.

Cohort characteristics stratified by disease category are available in Table 1 and Table 2 for women and men, respectively. Overall, those with CD were younger and those with UC older than the rest of the cohort. Both CD and UC had a higher proportion of white ethnicity than the rest of the cohort. In men, socioeconomic deprivation was higher in CD and lower in UC. Deprivation was similar in women with CD and UC compared to those without disease. Prevalence of current smoking was higher in CD and lower in UC compared to the rest of cohort. BMI was similar between groups in female participants but was lower in males with CD, as bodyweight was also lower. Prevalence of central obesity was also lower in men with CD. Grip strength was slightly lower in CD and UC, although differences were relatively small (difference in medians 0.5 – 2kg). Self-reported PA was lower in women with CD but no different in other disease groups compared to the cohort overall.

Those with CD had a lower average age at diagnosis compared to UC and men with UC had a higher proportion of diagnoses at age >40 years. Immunosuppressant use was higher in CD and 5-ASA use higher in UC. There were no differences in self-reported use of GC therapy between CD and UC, and only five participants with CD self-reported use of anti-TNF-α therapy. Over half of all CD (55.9%) and UC (51.2%) participants self-reported using no IBD-specific medications at all. Only a small proportion of CD (1.9%) and UC (2.1%) participants self-reported a history of lower GI surgery, which was similar to the rest of the cohort (2.2%).

Adverse musculoskeletal outcomes were more common in women than in men for both CD and UC, as in the rest of the cohort. Across both sexes, the prevalence of falls and fractures was higher in CD than UC. History of at least one fall in the previous 12 months was self-reported by 307 (22.2%) and 613 (20.4%) of those with CD and UC, respectively, compared to 76,386 (19.1%) of the rest of the cohort. Any fracture within the five years prior to baseline assessment was self-reported by 160 (11.6%) and 292 (9.8%) of CD and UC participants, respectively, compared to 34,520 (9.5%) of non-IBD participants. Fragility fractures were reported by 94 (6.8%) of CD, 186 (6.2%) of UC, and 21,639 (5.5%) of non-IBD participants. Fragility fractures, therefore, accounted for 58.8%, 63.7%, and 62.7% of all self-reported fractures in CD, UC, and non-IBD, respectively.

Table 4-1 Cohort characteristics of female participants with and without IBD, Crohn's disease, and ulcerative colitis.

	Non-IBD (n = 214,349)	IBD (n = 2390)	CD (n = 769)	UC (n = 1503)
Age (years)	57 (50, 63)	58 (51, 63)	58 (49, 63)	59 (51, 64)
White ethnicity, n (%)	203 370 (94.9)	2317 (97.0)	756 (98.3)	1448 (96.3)
Deprivation index	-2.18 (-3.66, 0.39)	-2.12 (-3.58, 0.57)	-2.25 (-3.63, 0.67)	-2.09 (-3.57, 0.42)
Height (cm)	163 (158, 167)	162 (158, 166)	162 (158, 166)	163 (158, 167)
Weight (kg)	68.5 (61.3, 77.6)	67.7 (60.6, 76.6)	67.4 (59.3, 75.3)	67.9 (61.2, 77.0)
BMI (kg/m²)	25.9 (23.3, 29.3)	25.7 (23.2, 29.1)	25.6 (23.0, 29.0)	25.7 (23.3, 29.1)
BMI categories, n (%)				
Underweight	1683 (0.8)	22 (0.9)	12 (1.6)	10 (0.7)
Normal	87 860 (41.0)	1004 (42.0)	324 (42.1)	630 (41.9)
Overweight	79 214 (36.9)	889 (37.2)	283 (36.8)	566 (37.7)
Obese	45 592 (21.3)	475 (19.9)	150 (19.5)	297 (19.8)
Waist circumference (cm)	82 (75, 91)	82 (75, 91)	83 (75, 91)	82 (75, 91)
Central obesity, n (%)	72 326 (33.8)	850 (35.6)	278 (36.2)	530 (35.3)
Bodyfat (%)	36.4 (31.6, 40.9)	36.5 (31.8, 41)	36.4 (31.7, 40.8)	36.5 (31.9, 41.0)
Physical activity (MET-mins/week)	1746 (792, 3480)	1640 (720, 3395)	1495 (693, 3393)	1680 (792, 3375)
Grip strength (kg)	23.5 (19.5, 28)	23 (19, 27)	23 (18.5, 27)	22.5 (19, 27)
Total energy intake (kcal)	1918 (1610, 2259)	1960 (1657, 2296)	1977 (1605, 2353)	1954 (1680, 2274)
Smoking status, n (%)				
Never	128 423 (59.9)	1255 (52.5)	365 (47.5)	818 (54.5)
Previous	67 951 (31.7)	897 (37.5)	276 (35.9)	586 (39.0)
Current	19 975 (8.4)	238 (10.0)	128 (16.6)	99 (6.6)
Musculoskeletal outcomes				
Fall, n (%)	47 882 (22.4)	594 (24.9)	193 (25.1)	373 (24.8)
Fracture, n (%)	21 812 (10.2)	281 (11.8)	104 (13.6)	160 (10.7)
Fragility fracture, n (%)	14 890 (7.0)	206 (8.7)	72 (9.4)	123 (8.2)
Muscle weakness, n (%)	20 292 (9.5)	299 (12.5)	103 (13.5)	180 (12.0)
Heel eBMD (T-score)	-0.64 (-1.3, 0.09)	-0.77 (-1.42, 0.01)	-0.87 (-1.47, -0.01)	-0.72 (-1.38, 0.06)
Disease & Medications				
Age at diagnosis (years)	-	-	36.2 (26.4, 48.8)	39.3 (29, 50.6)
Disease duration (years)	-	-	17.7 (7.3, 29.0)	15.4 (7.6, 27.9)
Diagnosis age category, n (%)				
<18 years	-	-	41 (5.3)	73 (4.9)
18 – 40 years	-	-	403 (52.5)	705 (46.9)
>40 years	-	-	324 (42.2)	724 (48.2)
Medications, n (%)				

	Non-IBD (n = 214,349)	IBD (n = 2390)	CD (n = 769)	UC (n = 1503)
Glucocorticoids	5465 (2.6)	184 (7.7)	64 (8.3)	112 (7.5)
Immunosuppressants	1460 (0.7)	260 (10.9)	117 (15.2)	136 (9.1)
5-Aminosalicylates	539 (0.3)	802 (33.6)	225 (29.3)	552 (36.7)
Antibiotics	175 (0.1)	8 (0.3)	3 (0.4)	4 (0.3)
Anti-TNF-α	63 (0.03)	4 (0.2)	4 (0.5)	0 (0)
No IBD-related medication	207 238 (96.7)	1346 (56.3)	439 (57.1)	825 (54.9)
Vitamin D supplements	4635 (2.2)	103 (4.3)	36 (4.7)	61 (4.1)
Bisphosphonates	5528 (2.6)	163 (6.8)	80 (10.4)	78 (5.2)
History of lower GI surgery	4770 (2.2)	51 (2.1)	18 (2.3)	29 (1.9)

All continuous variables are presented as median (IQR) and categorical variables as frequency (%).BMI – body mass index; cm – centimetres; kg – kilograms; MET – metabolic equivalent of task; mins – minutes; h – hours; immunosuppressants – azathioprine, mercaptopurine, cyclosporine, methotrexate; anti-TNF-α – anti-tumour necrosis factor alpha (adalimumab only); antibiotics – ciprofloxacin or metronidazole; GI – gastrointestinal; muscle weakness – grip strength <16kg for females and <27kg for males; eBMD – estimated bone mineral density

Table 4-2 Cohort characteristics of male participants with and without CD, Crohn's disease, and ulcerative colitis.

	Non-IBD (n = 182,882)	IBD (n = 2229)	CD (n = 617)	UC (n = 1504)
Age (years)	58 (50, 64)	60 (51, 64)	57 (50, 63)	60 (52, 64)
White ethnicity, n (%)	173 702 (95.0)	2152 (96.6)	599 (97.1)	1449 (96.3)
Deprivation index	-2.19 (-3.68, 0.49)	-2.18 (-3.63, 0.55)	-1.9 (-3.44, 1.14)	-2.34 (-3.73, 0.31)
Height (cm)	176 (171, 180)	175 (170, 180)	175 (170, 179)	175 (171, 180)
Weight (kg)	83.9 (76, 93.1)	82.4 (74.5, 91.2)	80.7 (73.5, 89.4)	83.0 (75, 91.8)
BMI (kg/m^2)	27.2 (24.9, 29.8)	26.9 (24.6, 29.4)	26.7 (24.2, 28.9)	27.0 (24.7, 29.6)
BMI categories, n (%)				
Underweight	399 (0.2)	10 (0.5)	5 (0.8)	5 (0.3)
Normal	47 242 (25.8)	626 (28.1)	190 (30.8)	411 (27.3)
Overweight	91 864 (50.1)	1136 (51.0)	315 (51.1)	766 (50.9)
Obese	43 557 (23.8)	457 (20.5)	107 (17.3)	322 (21.4)
Waist circumference (cm)	95 (89, 103)	95 (89, 102)	94 (86, 101)	95 (89, 103)
Central obesity, n (%)	52 102 (28.5)	602 (27.1)	145 (23.5)	424 (28.3)
Bodyfat (%)	25.1 (21.3, 28.8)	25 (21.2, 28.8)	24.3 (20.3, 28)	25.2 (21.5, 29)
Physical activity (MET-mins/week)	1960 (924, 4053)	1866 (840, 3954)	1839 (792, 4080)	1893 (855, 3884)
Grip strength (kg)	40 (34, 45)	39 (33, 44.5)	38 (33, 44)	39 (33, 44.5)
Total energy intake (kcal)	2230 (1865, 2646)	2296 (1922, 2755)	2276 (1947, 2823)	2296 (1911, 2720)
Smoking status, n (%)				
Never	90 547 (49.5)	945 (42.4)	246 (39.9)	640 (42.6)
Previous	70 568 (38.6)	1092 (49.0)	292 (47.3)	7665 (50.9)
Current	21 767 (11.9)	192 (8.6)	79 (12.8)	99 (6.6)
Musculoskeletal outcomes				
Fall, n (%)	27 838 (15.3)	379 (17.0)	114 (18.5)	240 (16.0)
Fracture, n (%)	15 708 (8.6)	200 (9.0)	56 (9.1)	132 (8.8)
Fragility fracture, n (%)	6659 (3.7)	92 (4.2)	22 (3.6)	63 (4.2)
Muscle weakness, n (%)	11 597 (6.4)	176 (7.9)	49 (8.0)	118 (7.9)
Heel eBMD (T-score)	-0.19 (-0.89, 0.6)	-0.33 (-1.06, 0.39)	-0.33 (-1.07, 0.42)	-0.31 (-1.06, 0.39)
Disease & Medications				
Age at diagnosis (years)	-	-	38.9 (27.1, 50.1)	43.1 (32.8, 53.5)
Disease duration (years)	-	-	15.8 (7.4, 27.3)	12.0 (6.0, 23.9)
Diagnosis age category, n (%)				
< 18 years	-	-	39 (6.3)	35 (2.3)
18 – 40 years	-	-	283 (45.9)	595 (39.6)
>40 years	-	-	295 (47.8)	873 (58.1)
Medications, n (%)				

	Non-IBD (n = 182,882)	IBD (n = 2229)	CD (n = 617)	UC (n = 1504)
Glucocorticoids	4501 (2.5)	227 (10.2)	67 (10.9)	148 (9.8)
Immunosuppressants	890 (0.5)	293 (13.1)	99 (16.1)	184 (12.2)
5-Aminosalicylates	380 (0.2)	867 (38.9)	196 (31.8)	644 (42.8)
Antibiotics	180 (0.1)	15 (0.7)	3 (0.5)	10 (0.7)
Anti-TNF-α	40 (0.02)	1 (0.04)	1 (0.2)	0 (0)
No IBD-specific medication	177 280 (96.9)	1118 (50.2)	336 (54.5)	713 (47.4)
Vitamin D supplements	843 (0.5)	60 (2.7)	27 (4.4)	31 (2.1)
Bisphosphonates	863 (0.5)	78 (3.5)	32 (5.2)	41 (2.7)
History of lower GI surgery	3903 (2.1)	44 (2.0)	9 (1.5)	33 (2.2)

All continuous variables are presented as median (IQR) and categorical variables as frequency (%).BMI – body mass index; cm – centimetres; kg – kilograms; MET – metabolic equivalent of task; mins – minutes; h – hours; Immunosuppressants – azathioprine, mercaptopurine, ciclosporin, methotrexate; anti-TNF-α – anti-tumour necrosis factor alpha (adalimumab only); anti-biotics – ciprofloxacin, metronidazole; GI – gastrointestinal; muscle weakness – grip strength <16kg for females and <27kg for males; eBMD – estimated bone mineral density

4.4.2 Associations of disease with falls

A history of IBD was associated with higher likelihood of a self-reported fall within the previous 12 months in both men and women (Table 4-3). The magnitude of these associations remained consistent across different levels of covariate adjustment. In separate analyses of CD and UC, male CD but not UC was associated with a higher likelihood of a fall. In fully adjusted models, male CD was associated with a 29% (95% CI: 5 to 58%) higher likelihood of a fall compared to men without IBD (Table 4-3). Women with UC also displayed a higher likelihood of a fall than women without IBD, whereas the association appeared positive but was not significant in women with CD (Table 4-3). Sensitivity analyses strengthened the association between CD and falls in women (OR: 1.21 (1.02, 1.44), p = 0.03) but did not alter any other results (Table 4-7).

Table 4-3 Association of disease status with history of self-reported falls by sex

		Female		Male	
		Odds Ratio (95%CI)	**p-value**	**Odds Ratio (95%CI)**	**p-value**
IBD	Model 0	1.15 (1.05, 1.26)	0.004	1.14 (1.02, 1.27)	0.02
	Model 1	1.13 (1.03, 1.24)	0.01	1.12 (1.01, 1.26)	0.04
	Model 2	1.13 (1.03, 1.25)	0.01	1.16 (1.03, 1.29)	0.01
CD	Model 0	1.16 (0.99, 1.37)	0.07	1.26 (1.03, 1.55)	0.03
	Model 1	1.17 (0.99, 1.38)	0.07	1.25 (1.02, 1.53)	0.04
	Model 2	1.16 (0.99, 1.37)	0.07	1.29 (1.05, 1.58)	0.02
UC	Model 0	1.15 (1.02, 1.29)	0.02	1.06 (0.92, 1.21)	0.44
	Model 1	1.12 (0.99, 1.26)	0.07	1.04 (0.91, 1.2)	0.56
	Model 2	1.13 (1.01, 1.28)	0.04	1.07 (0.93, 1.23)	0.33

Logistic regression assessing associations between IBD, CD, and UC with a history of self-reported falls within the previous 12 months. In all models, the reference group is participants without history of IBD. Model 0 – unadjusted; model 1 – adjusted for age, ethnicity, and socioeconomic deprivation; model 2 – adjusted for model 1 + BMI, smoking status, and PA.

4.4.3 Associations of disease with fractures

IBD was associated with a higher likelihood of self-reported fracture and fragility fracture in women but not men. Indeed, in separate analyses of CD and UC, male sex was not associated with likelihood of fracture or fragility fracture across any model (Table 4-4). Women with CD displayed a higher likelihood of self-reported fracture and fragility fracture, whereas women with UC did not. In fully adjusted models, female CD was associated with a 35% (95% CI: 9 to 66%) and 35% (95% CI: 6 to 73%) increased likelihood of fracture and fragility fracture, respectively (Table 4-4). When analyses of fragility fractures were restricted to those who self-reported any fracture, those with IBD were not at increased risk of a fragility fractures. Associations were not altered in sensitivity analyses (Table 4-7).

Table 4-4 Associations of disease status with self-reported history of fracture and fragility fracture by sex

		Female		Male	
		Odds Ratio (95%CI)	p-value	Odds Ratio (95%CI)	p-value
Any Fracture					
IBD	Model 0	1.18 (1.04, 1.33)	0.01	1.05 (0.91, 1.21)	0.53
	Model 1	1.15 (1.01, 1.3)	0.03	1.06 (0.92, 1.23)	0.4
	Model 2	1.14 (1.01, 1.3)	0.04	1.08 (0.93, 1.25)	0.32
CD	Model 0	1.38 (1.12, 1.7)	0.002	1.06 (0.8, 1.39)	0.68
	Model 1	1.37 (1.11, 1.69)	0.003	1.02 (0.78, 1.35)	0.86
	Model 2	1.35 (1.09, 1.66)	0.01	1.03 (0.78, 1.36)	0.83
UC	Model 0	1.05 (0.89, 1.24)	0.55	1.02 (0.85, 1.22)	0.81
	Model 1	1.02 (0.86, 1.2)	0.83	1.07 (0.89, 1.28)	0.49
	Model 2	1.02 (0.87, 1.21)	0.8	1.08 (0.9, 1.3)	0.4
Fragility Fracture					
IBD	Model 0	1.26 (1.09, 1.45)	0.002	1.14 (0.92, 1.4)	0.23
	Model 1	1.21 (1.05, 1.4)	0.01	1.13 (0.92, 1.39)	0.25
	Model 2	1.21 (1.05, 1.4)	0.01	1.15 (0.93, 1.42)	0.2
CD	Model 0	1.38 (1.08, 1.76)	0.01	0.97 (0.64, 1.49)	0.9
	Model 1	1.37 (1.07, 1.75)	0.01	0.95 (0.62, 1.46)	0.82
	Model 2	1.35 (1.06, 1.73)	0.02	0.96 (0.63, 1.47)	0.85
UC	Model 0	1.18 (0.99, 1.43)	0.07	1.16 (0.9, 1.49)	0.26
	Model 1	1.13 (0.94, 1.37)	0.18	1.16 (0.9, 1.49)	0.25
	Model 2	1.14 (0.95, 1.38)	0.16	1.18 (0.92, 1.53)	0.19

Logistic regression assessing associations between IBD, CD, and UC with a history of self-reported fractures within the previous five years. In all models, the reference group is participants without history of IBD. Model 0 – unadjusted; model 1 – adjusted for age, ethnicity, and socioeconomic deprivation; model 2 – adjusted for model 1 + BMI, smoking status, and PA.

4.4.4 Associations of disease status with eBMD

A history of IBD was associated with lower eBMD T-score in both men and women (Table 4-5). The associations appeared stronger in males, with an estimated deficit of -0.15 (95% CI: -0.23, -0.08) T-score compared to those without IBD after adjustment for covariates. In women, CD was associated with lower eBMD, but UC was not. The magnitude of associations remained similar across different levels of covariate adjustment (Model 2: -0.16 [95% CI: -0.26, -0.06]). In males, both CD and UC displayed similar negative associations with eBMD T-score of -0.14 (95%CI: -0.28, 0.00) and -0.16 (95%CI: -0.24, -0.07), respectively, in fully adjusted models (Table 4-5). Sensitivity analyses did not alter the direction or magnitude of any of the observed associations (Table 4-7).

Table 4-5 Associations of disease status with estimated BMD by sex

		Female		Male	
		β Coefficient (95%CI)	**p-value**	**β Coefficient (95%CI)**	**p-value**
eBMD T-score					
IBD [a]	Model 0	-0.11 (-0.16, -0.05)	<0.001	-0.2 (-0.27, -0.12)	<0.001
	Model 1	-0.09 (-0.15, -0.03)	0.002	-0.19 (-0.26, -0.11)	<0.001
	Model 2	-0.08 (-0.13, -0.02)	0.01	-0.15 (-0.23, -0.08)	<0.001
CD [b]	Model 0	-0.18 (-0.29, -0.08)	<0.001	-0.2 (-0.34, -0.06)	0.01
	Model 1	-0.18 (-0.28, -0.08)	<0.001	-0.19 (-0.33, -0.05)	0.01
	Model 2	-0.16 (-0.26, -0.06)	0.002	-0.14 (-0.28, 0.00)	0.05
UC [c]	Model 0	-0.07 (-0.14, 0.01)	0.08	-0.2 (-0.29, -0.1)	<0.001
	Model 1	-0.04 (-0.11, 0.03)	0.25	-0.18 (-0.27, -0.09)	<0.001
	Model 2	-0.04 (-0.11, 0.04)	0.32	-0.16 (-0.25, -0.07)	<0.001

Linear regression analyses for associations between IBD, CD, and UC with estimated bone mineral density. In all models, the reference group is participants without history of IBD. Model 0 – unadjusted; model 1 – adjusted for age, ethnicity, and socioeconomic deprivation; model 2 – adjusted for model 1 + BMI, smoking status, and PA. [a] n = 2604 (1330 female, 1274 male); [b] n = 796 (437 female, 359 male), [c] n = 1677 (819 female, 848 male).

4.4.5 Associations of disease with muscle weakness

Men and women with IBD displayed a significantly higher odds of muscle weakness compared to the rest of the cohort (Table 4-6). In disease specific analyses, men and women with both CD and UC had increased likelihood of muscle weakness. After adjustment for covariates, the association was strongest in women with CD, who displayed an estimated 55% (95%CI: 25 to 92%) higher likelihood of muscle weakness (Table 4-6). Women with UC had an estimated 24% (95%CI: 5 to 45%) higher odds of muscle weakness than women without IBD. Men with UC and CD both had increased odds of muscle weakness, although these associations were no longer significant after adjustment for covariates (Table 4-6). In sensitivity analyses, the association between CD and muscle weakness was stronger in men and slightly weaker in women but remained consistent in UC (Table 4-7).

Table 4-6 Associations of disease status with muscle weakness by sex

		Female		Male	
		Odds Ratio (95%CI)	**p-value**	**Odds Ratio (95%CI)**	**p-value**
IBD	Model 0	1.37 (1.21, 1.55)	<0.001	1.27 (1.08, 1.48)	0.003
	Model 1	1.33 (1.17, 1.5)	<0.001	1.23 (1.06, 1.45)	0.01
	Model 2	1.33 (1.18, 1.51)	<0.001	1.25 (1.07, 1.47)	0.01
CD	Model 0	1.48 (1.21, 1.83)	<0.001	1.28 (0.95, 1.71)	0.1
	Model 1	1.55 (1.25, 1.91)	<0.001	1.32 (0.98, 1.78)	0.07
	Model 2	1.55 (1.25, 1.92)	<0.001	1.33 (0.99, 1.79)	0.06
UC	Model 0	1.3 (1.11, 1.52)	0.001	1.26 (1.04, 1.52)	0.02
	Model 1	1.22 (1.04, 1.43)	0.01	1.2 (0.99, 1.46)	0.06
	Model 2	1.24 (1.05, 1.45)	0.01	1.21 (1.00, 1.47)	0.05

Binary logistic regression assessing associations between IBD, CD, and UC with muscle weakness. Muscle weakness is defined as low grip strength according to EWGSOP2 criteria: <16kg for women and <27kg for men. In all models, the reference group is participants without history of IBD. Model 0 – unadjusted; model 1 – adjusted for age, ethnicity, and socioeconomic deprivation; model 2 – adjusted for model 1 + BMI, smoking status, and PA.

Table 4-7 Sensitivity analyses for associations between disease status and falls, fractures, BMD, and muscle weakness by sex

		CD		UC	
		Odds Ratio (95%CI)	**p-value**	**Odds Ratio (95%CI)**	**p-value**
Falls	Females	1.21 (1.02, 1.44)	0.03	1.15 (1.02, 1.3)	0.03
	Males	1.25 (1.00, 1.56)	0.05	1.09 (0.95, 1.26)	0.24
Fractures	Females	1.33 (1.06, 1.65)	0.01	1.01 (0.85, 1.19)	0.94
	Males	1.01 (0.75, 1.35)	0.97	1.08 (0.9, 1.3)	0.43
Fragility fractures	Females	1.34 (1.03, 1.74)	0.03	1.14 (0.94, 1.37)	0.19
	Males	0.99 (0.63, 1.55)	0.97	1.2 (0.93, 1.55)	0.17
Muscle weakness	Females	1.39 (1.11, 1.76)	0.01	1.24 (1.06, 1.46)	0.01
	Males	1.42 (1.04, 1.93)	0.03	1.22 (1.00, 1.48)	0.05
		β Coefficient (95%CI)	**p-value**	**β Coefficient (95%CI)**	**p-value**
eBMD T-score	Females	-0.14 (-0.25, -0.04)	0.01	-0.04 (-0.11, 0.04)	0.32
	Males	-0.14 (-0.29, 0.01)	0.06	-0.15 (-0.24, -0.05)	0.002

Sensitivity analyses excluding all participants with a recorded episode of both CD and UC from disease specific regression models. N = 190 were excluded from the CD group and N = 165 were excluded from the UC group. All models presented are adjusted as in model 2 (age, ethnicity, deprivation, BMI, smoking status, and PA).

4.4.6 Exploratory analyses of factors associated with musculoskeletal outcomes in CD

4.4.6.1 Falls

Overall, women with CD had increased odds of a previous fall compared to men (OR: 1.52 [95% CI: 1.17, 1.98], p <0.001). Muscle weakness was associated with an estimated 90% [95% CI: 21 to 199%] increased likelihood of a fall in women with CD (Figure 4-2). A similar association was observed in men but with wider confidence intervals, likely due to a reduced number of male participants with muscle weakness and fall events overall (Figure 4-3). In women, overweight and obesity were positively associated with likelihood of a fall (Figure 4-2). In men, being a current smoker or from an area with more socioeconomic deprivation were both positively associated with likelihood of a fall (Figure 4-3). No associations were observed between age or age at disease diagnosis and self-reported falls in men or women with CD. Women who self-reported current use of 5-ASA medications had 45% [95%CI: 2 to 107%] increased likelihood of reporting a fall, but no other associations between medications and falls were observed.

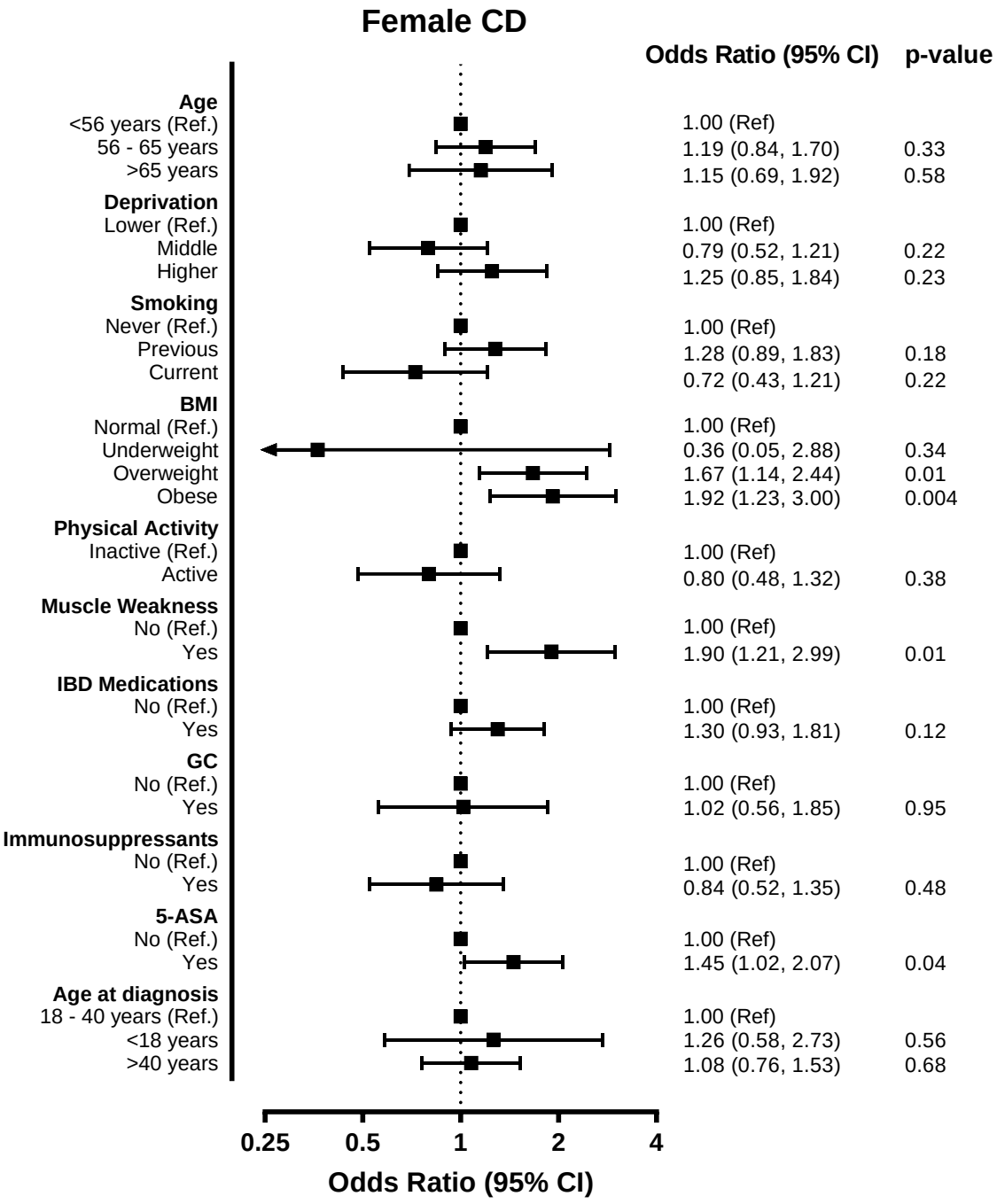

Figure 4-2 Factors associated with self-reported falls in women with CD
Exploratory analyses of factors associated with self-reported falls in women with CD. OR (95%CI) represents the odds of a fracture compared to the reference groups in the respective analysis. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates.

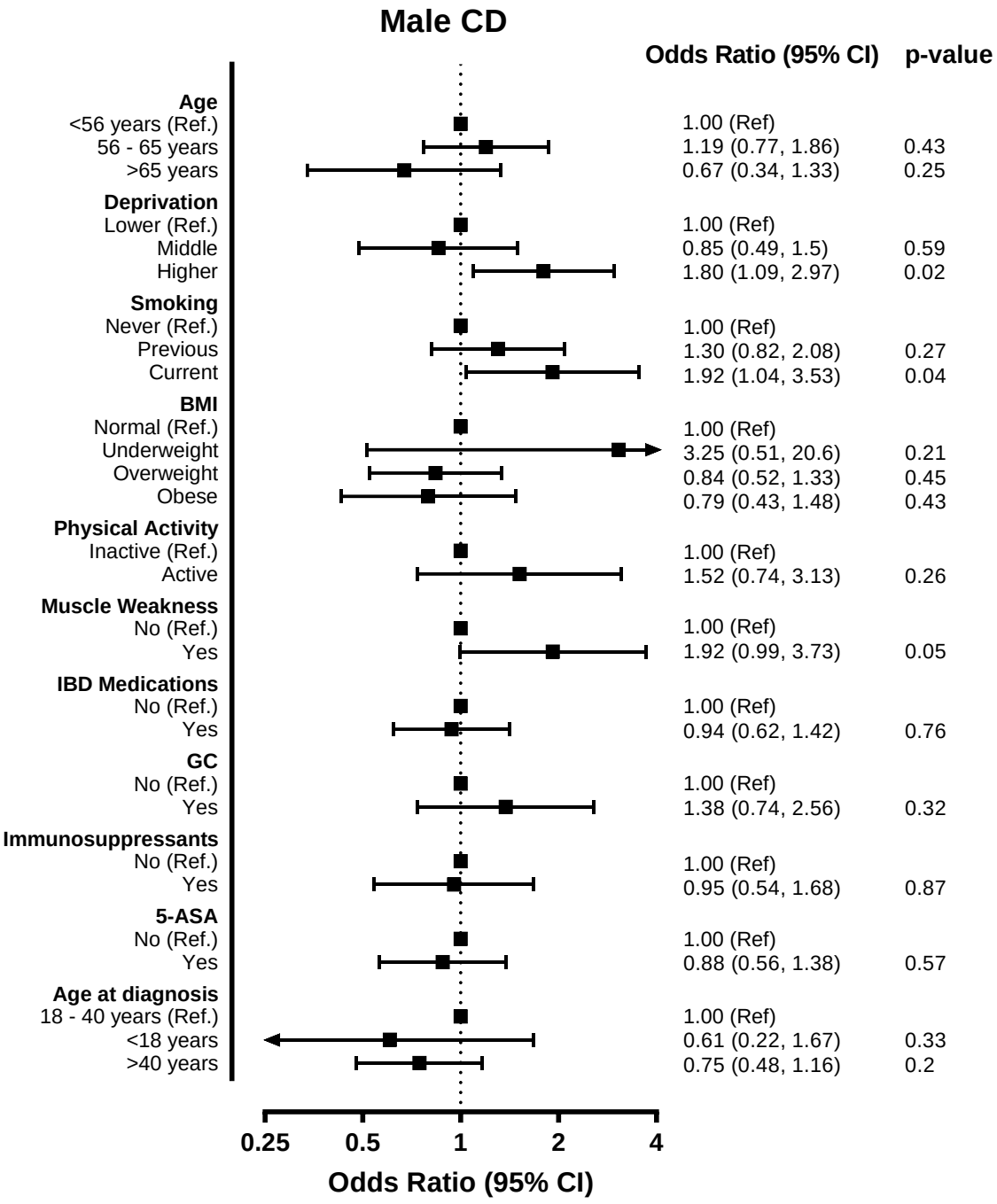

Figure 4-3 Factors associated with self-reported falls in men with CD
Exploratory analyses of factors associated with self-reported falls in men with CD. OR (95%CI) represents the odds of a fracture compared to the reference groups in the respective analysis. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates.

4.4.6.2 Fractures

Female sex was also associated with increased odds of a previous fracture compared to males in CD (OR: 1.59 [95% CI: 1.13, 2.24], p = 0.01). The factor most strongly associated with a previous fracture in both male and female CD was a self-reported fall within the previous 12 months (Figure 4-4 & Figure 4-5). Females who experienced a fall were an estimated three times more likely to have a self-reported fracture in the past five years (Figure 4-4). Males were almost four times as likely, although with much wider confidence intervals due to lower number of fractures overall (Figure 4-5). Women with low eBMD T-score were also an estimated 84% [95%CI: 3 to 231%] more likely to report a fracture than those with normal eBMD (Figure 4-4), whereas eBMD was not associated with fractures in male CD. Older age was positively associated with fractures in women but negatively with fractures in men with CD.

In similar analyses exploring factors associated with fragility fractures, of the factors analysed only older age (>65 years OR: 2.31 [95% CI: 1.22, 4.39], p = 0.01) and history of a fall within the previous 12 months (OR: 2.96 [95% CI: 1.79, 4.87], p <0.001) were associated with a fragility fracture in the previous five years in women with CD. In men with CD, only a previous fall within 12 months was positively associated with likelihood of a fragility fracture over the previous five years (OR: 3.8 [95% CI: 1.58, 9.17], p = 0.003).

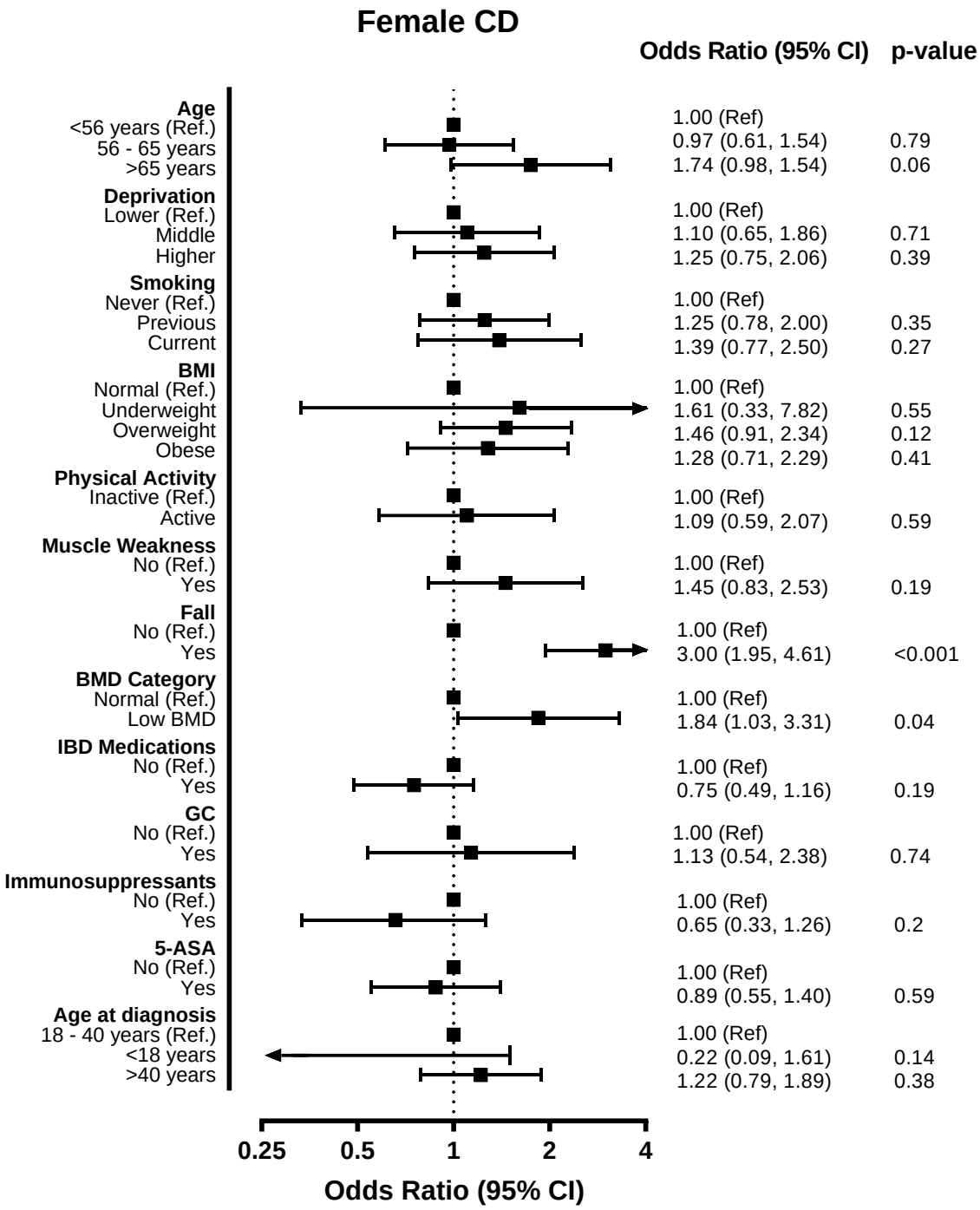

Figure 4-4 Factors associated with fractures in women with CD
Exploratory analyses of factors associated with self-reported fractures in women with CD. OR (95%CI) represents the odds of a fracture compared to the reference groups in the respective analysis. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates; low BMD – eBMD T-score < -1.0.

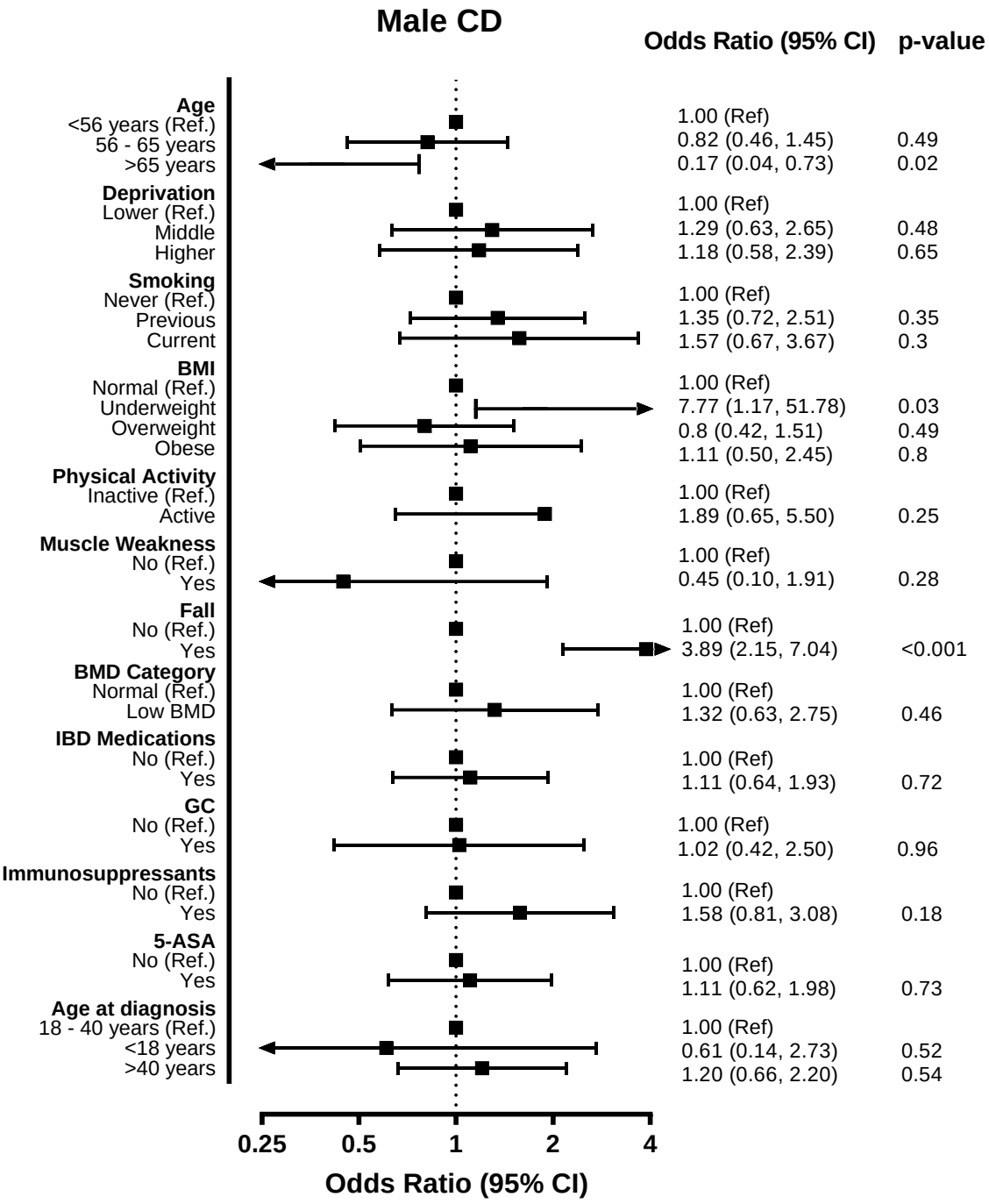

Figure 4-5 Factors associated with fractures in men with CD
Exploratory analyses of factors associated with self-reported fractures in men with CD. OR (95%CI) represents the odds of a fracture compared to the reference groups in the respective analysis. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates; low BMD – eBMD T-score < -1.0.

4.4.6.3 Muscle weakness

Women with CD were more likely to have muscle weakness compared to men (OR: 1.93 [95%CI: 1.34, 2.78], p <0.001). Compared to younger women with CD, those in both the middle and older age group had significantly higher odds of muscle weakness (Figure 4-6). Older age was also positively associated with muscle weakness in men with CD (Figure 4-7). Higher socioeconomic deprivation was positively associated with muscle weakness in women with CD, an association that appeared similar in men but was not statistically significant. Women with CD who were physically active, according to their IPAQ data, were an estimated 64% [95%CI: 34 to 80%] less likely to have muscle weakness than women who were inactive (Figure 4-6). In men, however, PA was not associated with muscle weakness.

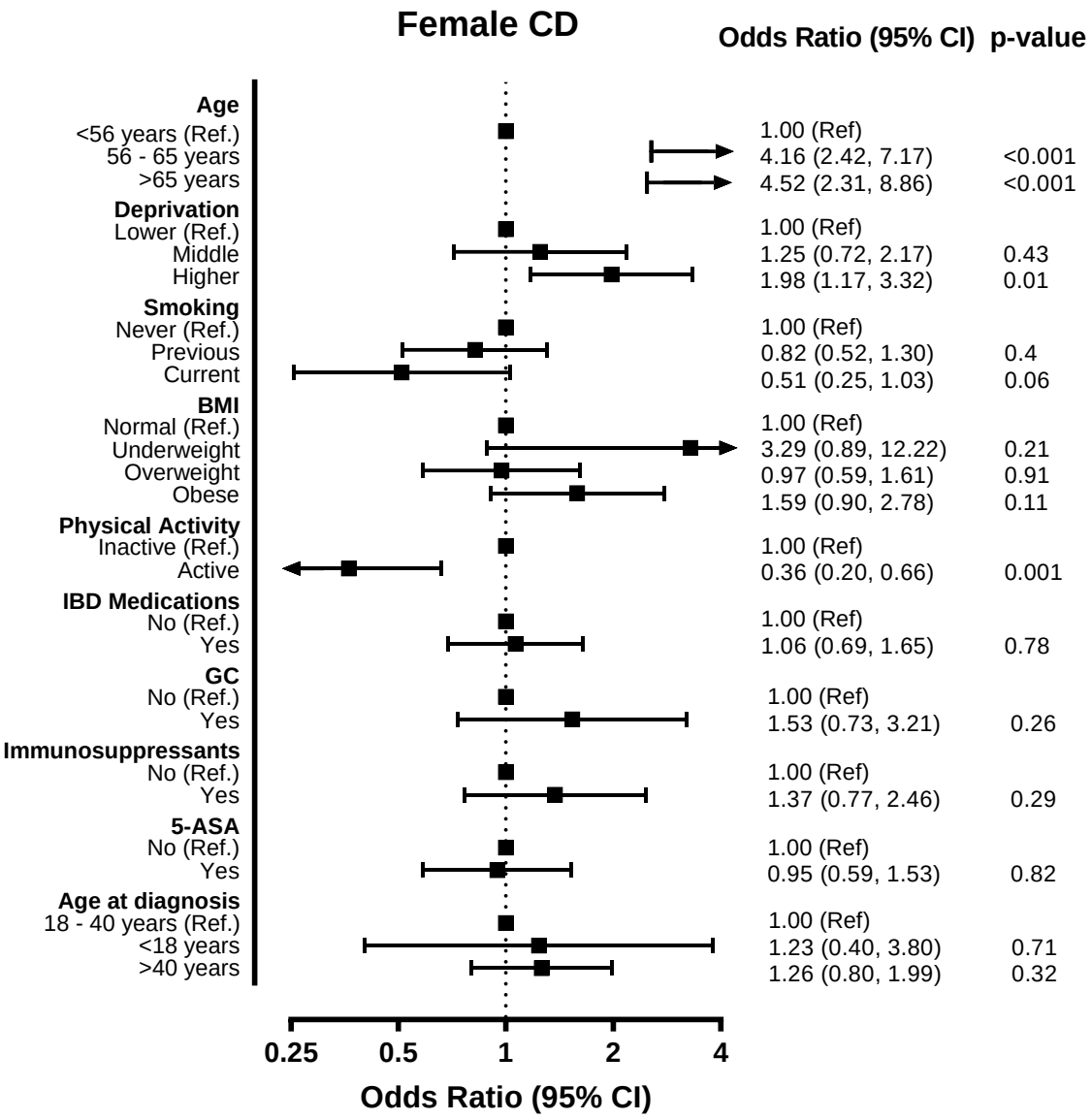

Figure 4-6 Factors associated with muscle weakness in women with CD
Exploratory analyses of factors associated with muscle weakness in women with CD. OR (95%CI) represents the odds of a fracture compared to the reference groups in the respective analysis. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates; low BMD – eBMD T-score < -1.0.

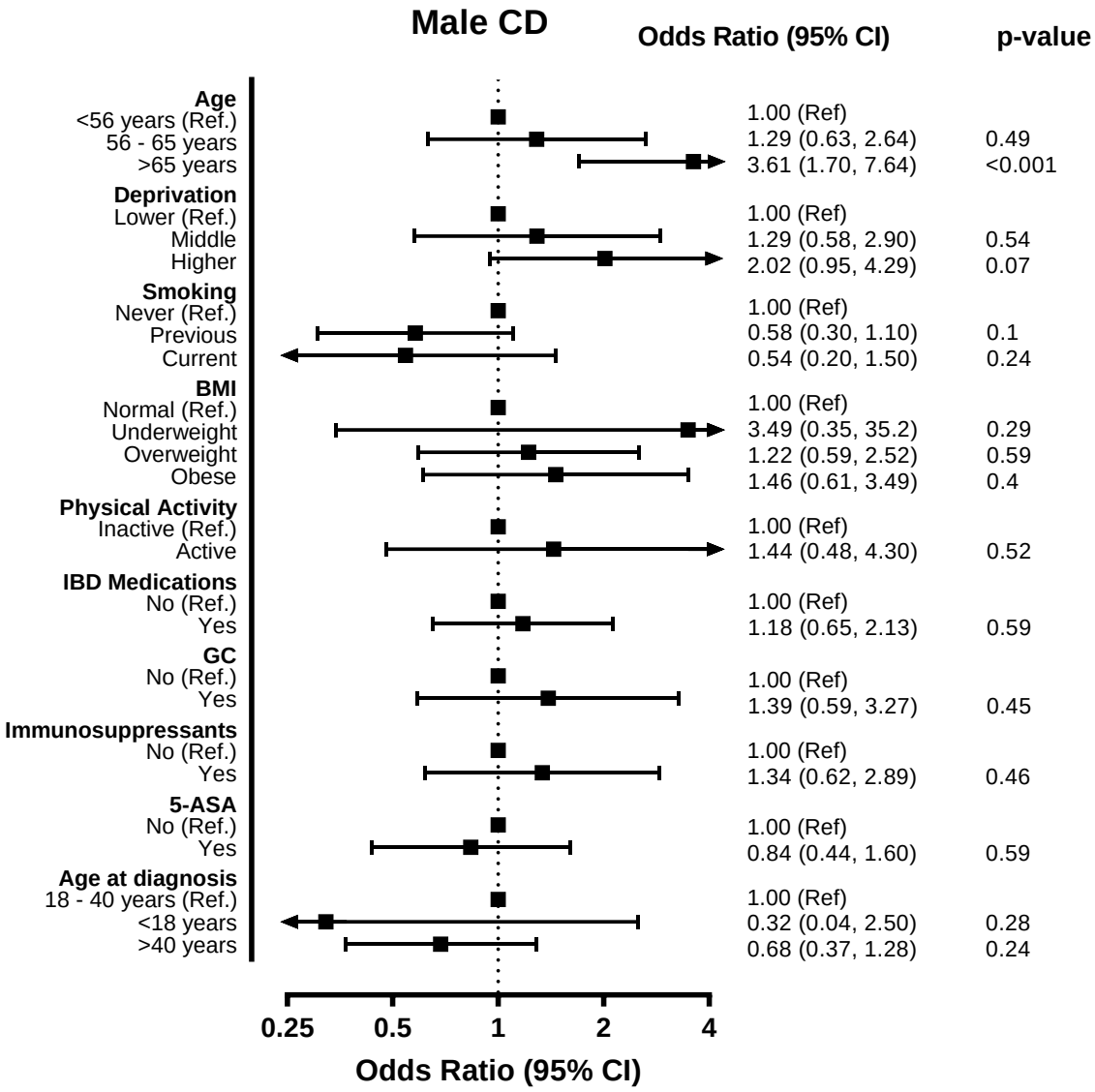

Figure 4-7 Factors associated with muscle weakness in men with CD
Exploratory analyses of factors associated with muscle weakness in men with CD. OR (95%CI) represents the odds of a fracture compared to the reference groups in the respective analysis. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates; low BMD – eBMD T-score < -1.0.

4.4.6.4 eBMD T-score

Older age and being a current smoker were associated with lower eBMD T-score in women but not men with CD (Figure 4-8). Underweight was associated with lower eBMD in both sexes, while overweight and obesity was associated with higher eBMD in men only, compared to normal weight reference groups (Figure 4-9). Current use of any IBD medication, and particularly the use of immunosuppressants was associated with lower eBMD T-score in men only (Figure 4-9). Current use of GC therapy and age at disease diagnosis were not associated with eBMD in either sex.

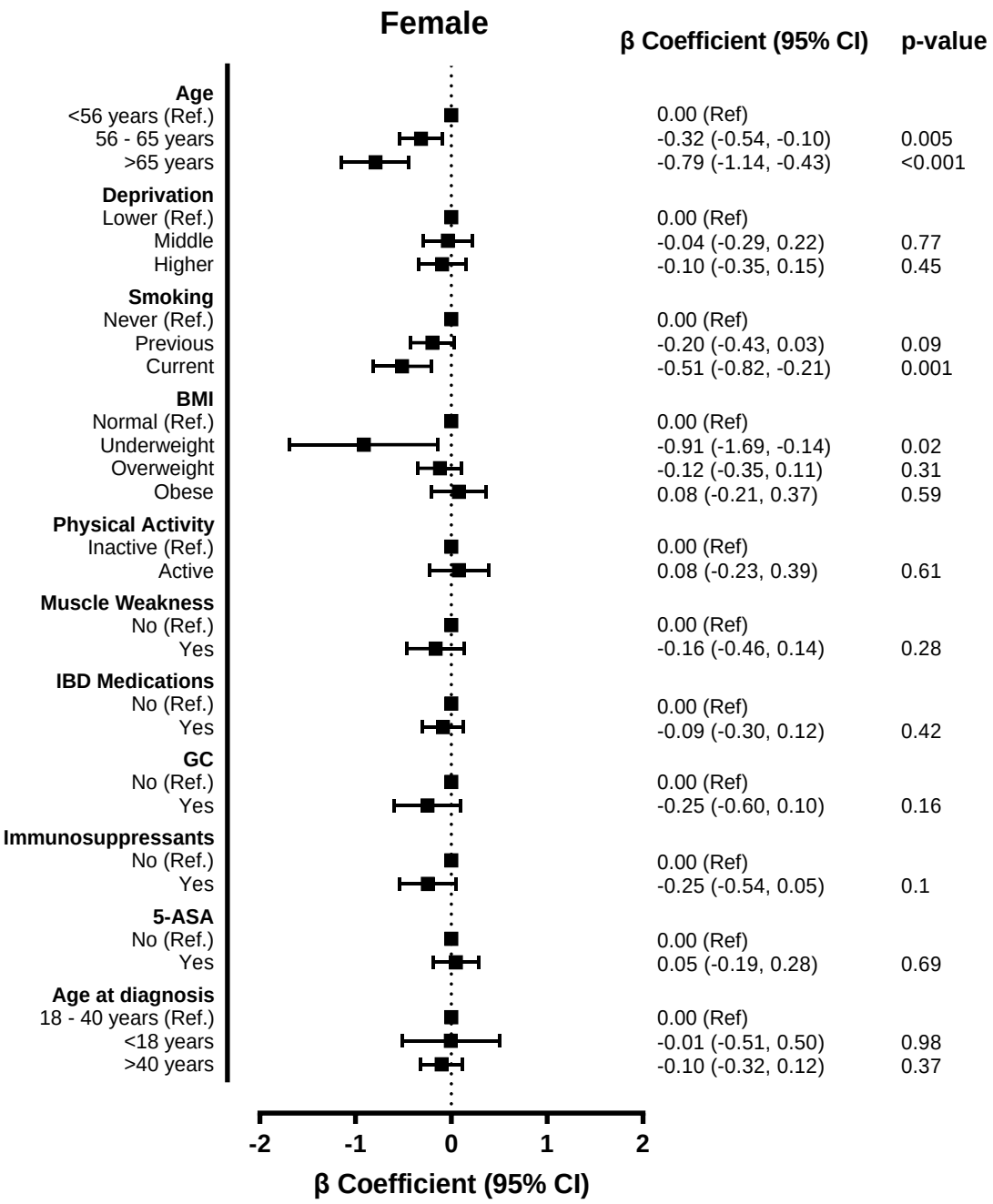

Figure 4-8 Factors associated with eBMD in women with CD
Exploratory analyses of factors associated with eBMD in women with CD. β (95% CI) represents the difference in eBMD T-score compared to the reference group in multiple regression for the respective analyses. All analyses were adjusted for age and deprivation (when not the factor of interest). N = 437. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates.

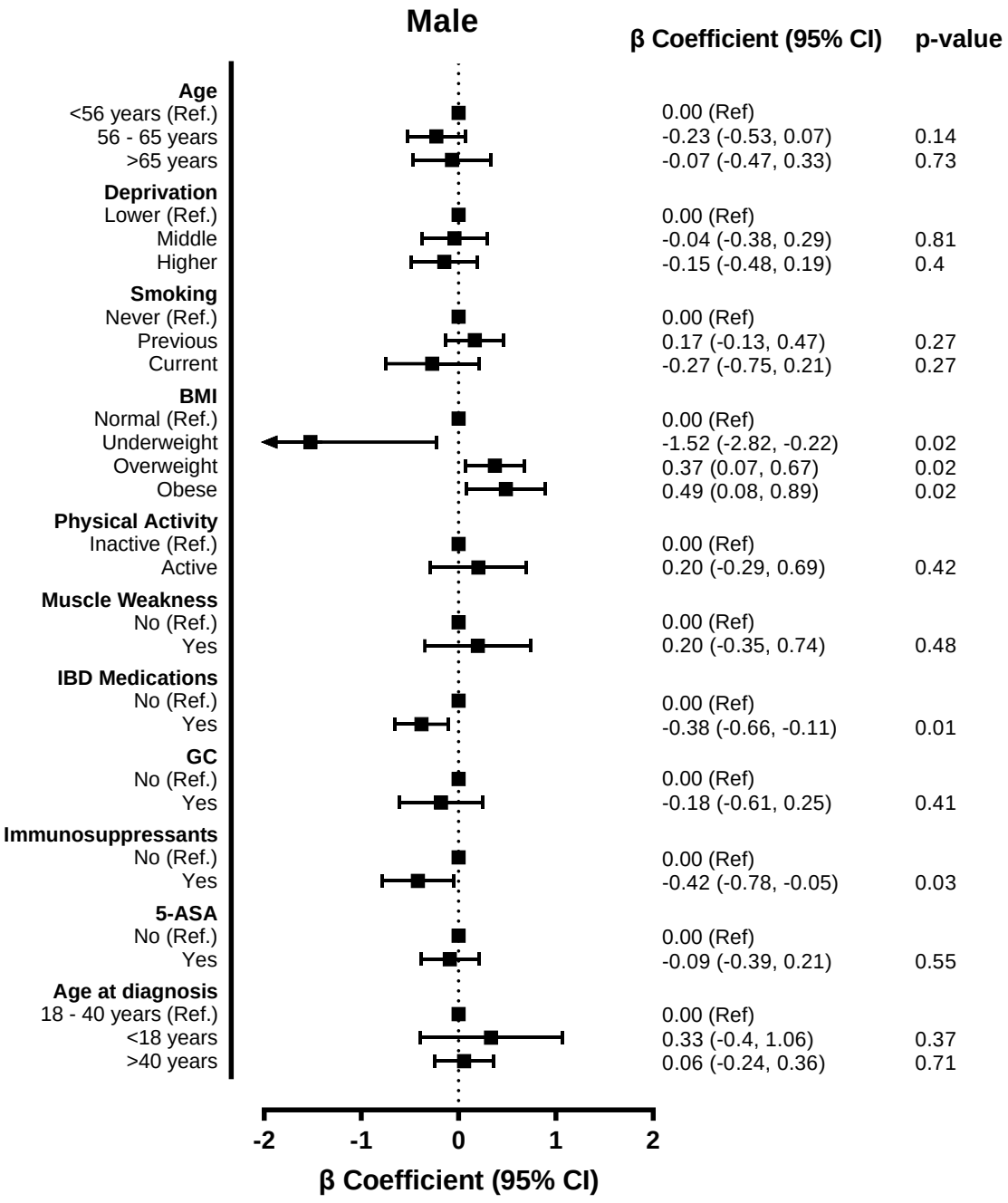

Figure 4-9 Factors associated with eBMD in men with CD
Exploratory analyses of factors associated with eBMD in men with CD. β (95% CI) represents the difference in eBMD T-score compared to the reference group in multiple regression for the respective analyses. All analyses were adjusted for age and deprivation (when not the factor of interest). N = 359. BMI – body mass index; GC – glucocorticoids; immunosuppressants – azathioprine, mercaptopurine, methotrexate, cyclosporin; 5-ASA – 5-aminosalicylates.

4.5 Discussion

In this study, a history of IBD was associated with a range of adverse musculoskeletal outcomes in both men and women. The IBD cohort in this study most likely represent a group with mild disease, highlighted by low use of IBD medications and very low rates of GI surgery. This is the first study to investigate falls in IBD, reporting higher odds of a self-reported fall in both men and women with CD and women with UC. All disease groups were more likely to have muscle weakness, compared to non-IBD participants, and women with CD were the most likely to be affected. Deficits in eBMD T-scores were also observed in men and women with both CD and UC. Women with CD displayed an increased likelihood of a self-reported fracture or fragility fracture compared to those without a history of IBD. Despite the cohort of mild disease status, musculoskeletal deficits are observed in older men and women with IBD including clinically meaningful outcomes like falls and fragility fractures.

The chronic elevation of pro-inflammatory cytokines is one of the key regulators of bone and muscle loss in IBD, as previously discussed. Chronic low-grade inflammation, however, also partly moderates tissue degradation and age-related disease pathogenesis in healthy ageing [360]. Inflammatory-mediated ageing (termed 'inflammageing') and IBD share some common mechanisms. These include chronic activation of the NFκB pathway, elevated expression of pro-inflammatory cytokines, and excessive infiltration of M1 macrophages into adipocytes [360]. Individuals with IBD may therefore experience something akin to an accelerated aging process. Subsequently, they may be at increased risk of adverse musculoskeletal events such as falls and fractures at an earlier age compared to typically aging counterparts.

Falls are a major contributor to morbidity and mortality in aging adults and one of the leading causes of disability adjusted life years globally [356]. A key risk factor for falls in older adults is muscle weakness. The economic costs of muscle weakness were recently estimated at £2.5 billion per year in the UK [361]. Currently, no published studies have assessed muscle weakness and falls risk in older adults with IBD. Here, a history of IBD was associated with a higher likelihood of a recent fall and muscle weakness in both men and women, when compared to age matched, non-IBD peers. Only men with UC displayed no increased likelihood of a

fall, while all groups were at increased odds of having muscle weakness. Increased propensity for falls may be linked to the reduced functional capacity of the lower limbs that has been observed in CD cohorts in this thesis and previous research [269,299]. Small differences in grip strength, such as those observed in this study (median difference: 0.5 – 2kg), have also been found to mediate the risk of falls in older adults [362]. The likelihood of a recent fall was approximately 3% lower (OR: 0.97 [95%CI: 0.94, 0.99]) per 1kg increase in grip strength in a cohort of older adults (n = 808), adjusting for age and sex [362]. Agreeably, low grip strength was strongly associated with falls in CD in the current study. Future prospective studies should investigate the risk of falls in CD and associated clinical risk factors, including muscle weakness. This may be particularly important for younger CD cohorts, as they consistently display poor muscle function that may increase the risk of falls and associated morbidity in future.

Overall, women but not men with IBD displayed an increased likelihood of any fracture. This association was driven by a higher fracture risk in women with CD (OR: 1.35 [95% CI: 1.09, 1.66]) but not UC (OR: 1.03 [95% CI: 0.78, 1.36]). Women with CD also displayed an equally increased likelihood of fragility fracture. Recent studies have also reported significantly increased risk of fracture in older adults with IBD [324,363]. Bartko et al found women with CD were more than twice as likely to have a hip fracture (OR: 2.91, [95% CI 2.17, 3.89]) compared to age and sex matched controls, after adjustment for confounders including prior GC exposure [363]. No associations between current GC and fractures were observed in the current study. Whether cumulative GC exposure may have been associated with fractures is unclear as these data were not available. In exploratory analyses, only a previous fall and low eBMD were significant predictors of fractures in women with CD. While further investigations are required into the risk of falls in CD, targeting bone and muscle may moderate falls and associated fracture risk in CD.

Consistent with previous studies reporting low bone mass in IBD, eBMD T-scores were lower in men and women with CD, but also in men with UC, compared to controls. Disease severity and GC therapy are primary risk factors for low BMD in IBD. No association between GC and eBMD was observed, potentially due to the grouping of all GC therapies together, including systemic and topical treatments. In men with CD, immunosuppressant use was associated with lower eBMD. As

immunosuppressants are typically reserved for more active disease in older adults [364], this association was likely an indicator of disease severity rather than an effect of therapy. However, a negative effect of azathioprine has been recently observed on trabecular bone volume of murine models of colitis [344] and has previously been associated with an increased risk of fracture across multiple conditions including CD [365]. Further evaluation of medications and their association with musculoskeletal outcomes in older adults with IBD is needed.

A strength of this study was the ability to assess musculoskeletal outcomes in IBD compared to a very large control population. The UK Biobank, however, had a low response rate (5.5%) and is affected by healthy volunteer bias, including lower rates of morbidity and mortality compared to population data [366]. Nonetheless, the control population contained many participants with primary and secondary musculoskeletal disorders, therefore the observation of an adverse muscle-bone phenotype in IBD relative to age-matched peers is robust. The IBD cohort itself may not be immune to this healthy bias and, based on the clinical data available, appears to represent a group with uncomplicated disease. This is highlighted particularly low rates of GI surgery and non-use of IBD medications. The use of contemporary therapies such as anti-TNFα were very low, partly due to the time of the study but also as these were primarily licensed for use in severe disease. The use of anti-TNFα is now more commonplace among adults with IBD, although they are still used less often than in younger cohorts due to concerns of opportune infection [364]. These drugs have been associated with recovery of some bone and muscle in young cohorts of CD. Nevertheless, recent studies are required to evaluate the risk of adverse musculoskeletal outcomes in older adults under modern treatment regimens.

There are some important limitations to this study. The analyses relied on self-reported falls and fractures which may be subject to recall bias. Prior studies have observed up to 14% false positives for fractures [367] but under reporting of falls by around 30% when recorded retrospectively [368]. Linking analyses to clinically confirmed fractures via HES would be preferable. Heel eBMD was measured using QUS instead of DXA, which is the clinical gold standard for BMD. QUS does, however, correlate well with DXA and has been previously validated for use in epidemiological research [187]. A QUS eBMD T-score of <-1.0 SDS was used to define

low eBMD; however, QUS and DXA BMD T-scores are not equivalent and the precise cut off for low bone mass by QUS is not known. The cross-sectional nature of the study precludes an assessment of causality, although the findings corroborate previous reports of adverse musculoskeletal health in IBD. Analyses were not adjusted for use of bisphosphonates as there was no information on timing of initiation of bisphosphonates. However, bisphosphonate use was ten times more common in men (5.2% vs 0.5%) and four times more common in women (10.4 % vs 2.6%) with CD, compared to the non-IBD cohort.

The methods of case ascertainment are another limitation to the study. History of IBD was based on primary care and in-patient ICD-10 codes together with self-reported diagnoses data. As such, some individuals had a reported history of both CD and UC, potentially due to initial misclassification of disease or 'working diagnoses' in ICD codes. The accuracy of ICD codes has been previously improved by combination with other data such as prescriptions [369]. Self-reported diagnoses were used to moderate history of CD/UC. Where someone had a history of both CD and UC, they were included in analyses based on their self-reported diagnosis. Individuals who self-reported both CD and UC were excluded from subtype analyses. Additionally, sensitivity analyses excluding all individuals with a history of both CD and UC (n = 386) were conducted. These analyses did not alter the direction or magnitude of any of the observed associations, suggesting the cross-referencing of ICD codes with self-report may be a useful method of case ascertainment. Despite the known limitations, this study provides valuable information on adverse musculoskeletal outcomes in older adults with IBD and provides a precursor upon which more detailed research can be designed.

In conclusion, this study has for the first time quantified an increased risk of falls in adults with IBD in what appears to be a cohort with generally uncomplicated disease. Fracture risk in IBD was only elevated in women with CD, however all IBD subgroups displayed in increased likelihood of muscle weakness and low eBMD compared to non-IBD peers. These data further suggest increased risk of adverse musculoskeletal outcomes in even mild IBD, consistent with the previous chapters. Future studies should aim to characterise musculoskeletal outcomes and associated clinical risk factors in older adults with IBD and assess the efficacy of

musculoskeletal interventions in this cohort, particularly as the elderly IBD population continues to expand.

5 Systematic review of exercise in inflammatory bowel disease

5.1 Abstract

Background. Exercise may be a suitable adjunctive therapy for alleviating many of the secondary complications of IBD, yet little is known about its utility in practice.

Aim. Perform a systematic review to analyse the current evidence for the effects of exercise on disease symptoms and secondary complications of IBD in children and adults.

Methods. A systematic search of four online databases (EMBASE, Medline, Cochrane, and CINAHL) was conducted from their inception through April 2021. Any studies including specific physical activity or exercise interventions in individuals with IBD were included. Studies were assessed for methodological quality using a modified Downs-Black checklist.

Results. A total of 1362 articles were identified of which 17 were included in the final review. Thirteen studies were conducted in adults and four in paediatric populations. All studies included only participants with mild or inactive disease. A range of interventions and measured outcomes were implemented, making comparisons between studies difficult. One common theme was a small to moderate clinically meaningful improvement in HRQoL from exercise, as assessed by the IBDQ tool. Some evidence for improvements in physical function, BMD, and fatigue were also noted in individual studies. Very few exercise related adverse events and no exacerbations of disease symptoms due to exercise were reported across studies.

Conclusions. The current evidence for the utility of exercise in IBD is limited by the small number of underpowered, heterogenous published studies. There is very little evidence on the potential role for exercise in alleviating the musculoskeletal or inflammatory burden in CD, and future, adequately designed studies are needed to address these concerns.

5.2 Introduction

Exercise interventions are one of the most effective strategies for improving bone and muscle health, which are adversely affected across the lifespan in CD (Chapters 2, 3 & 4). Additionally, there is plausible rationale for the potential role of exercise for improving other secondary complications of CD, including fatigue, HRQoL, and chronic inflammation (Section 1.5.2). Currently, no disease specific exercise guidelines exist for individuals with CD and the general utility of exercise as a management tool for the complications of CD is unknown. Prior to designing future interventions assessing exercise utility in CD, it is important to synthesise the available evidence to establish what is currently known.

The aim of this study, therefore, was to perform a systematic review of the literature to assess the effects of exercise on primary and secondary complications of CD and IBD.

5.3 Methods

5.3.1 Search strategy

A systematic search of the MEDLINE, EMBASE, CINAHL and Cochrane Central Library databases was conducted using records from their inception through April 2021. Reference lists of potentially relevant articles and reviews were also searched manually. The original search strategy was devised within the MEDLINE database and subsequently adapted to each of the other databases, respectively (search strategy in Appendix 2). Due to the heterogeneity of exercise research in IBD, both randomised and non-randomised studies were included. Only eligible full texts that were available in English were considered for review.

5.3.2 Eligibility criteria

All randomised and non-randomised trials implementing a specific physical activity or exercise intervention in individuals with a diagnosis of inflammatory bowel disease (CD, UC, or IBDU) were included. Both paediatric and adult studies were eligible. Studies assessing both the acute and chronic effects of PA or exercise were eligible. A full description of the prescribed intervention was required and general information with regards to increasing PA were not included. Primary outcome measures included health related quality of life (HRQoL), immune and inflammatory response, disease activity, and body composition and musculoskeletal outcomes.

5.3.3 Data extraction

The titles and abstracts of retrieved articles were evaluated for eligibility. Abstracts that did not provide sufficient information for exclusion were retrieved for full-text analysis. After full text analysis, articles that were deemed eligible for inclusion had the following data extracted: title, first author, year of publication, study design, participant characteristics, sample size, intervention type, intervention length, measured outcomes, and main findings. A qualitative analysis was conducted to present the current body of evidence and identify future research needs. Results are presented by grouped outcomes associated with complications of CD. This systematic review was conducted in line with the

preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.

5.3.4 Assessment of quality

A modified version of the Downs-Black checklist was used to assess the methodological quality of studies included in this review [370]. This tool is designed to assess the methodological quality of both randomised and non-randomised studies against a 27-item checklist. The tool assesses quality within five sub-domains: reporting, external validity, internal validity (bias), internal validity (confounding) and study power. Study quality was assessed from a possible 28 points as follows: excellent (26 – 28), good (20 - 25), fair (15 – 19), poor (≤14). Quality assessment was conducted independently by the researcher (LS) and a second reviewer (JW). The second reviewer's assessment was conducted for purposes of quality control and was overall similar to the primary reviewer's assessment. Minor discrepancies were resolved by consensus.

5.4 Results: included studies

5.4.1 Description of studies

A total of 1362 potentially relevant articles were retrieved from electronic database searches and manual searches of relevant reference lists. A consort diagram of the number of retrieved articles and the selection process is available in Figure 5-1. After removing duplicates and scanning titles and abstracts, 52 articles were selected for full-text analysis. Seventeen articles were selected for inclusion in the final qualitative review and quality assessment.

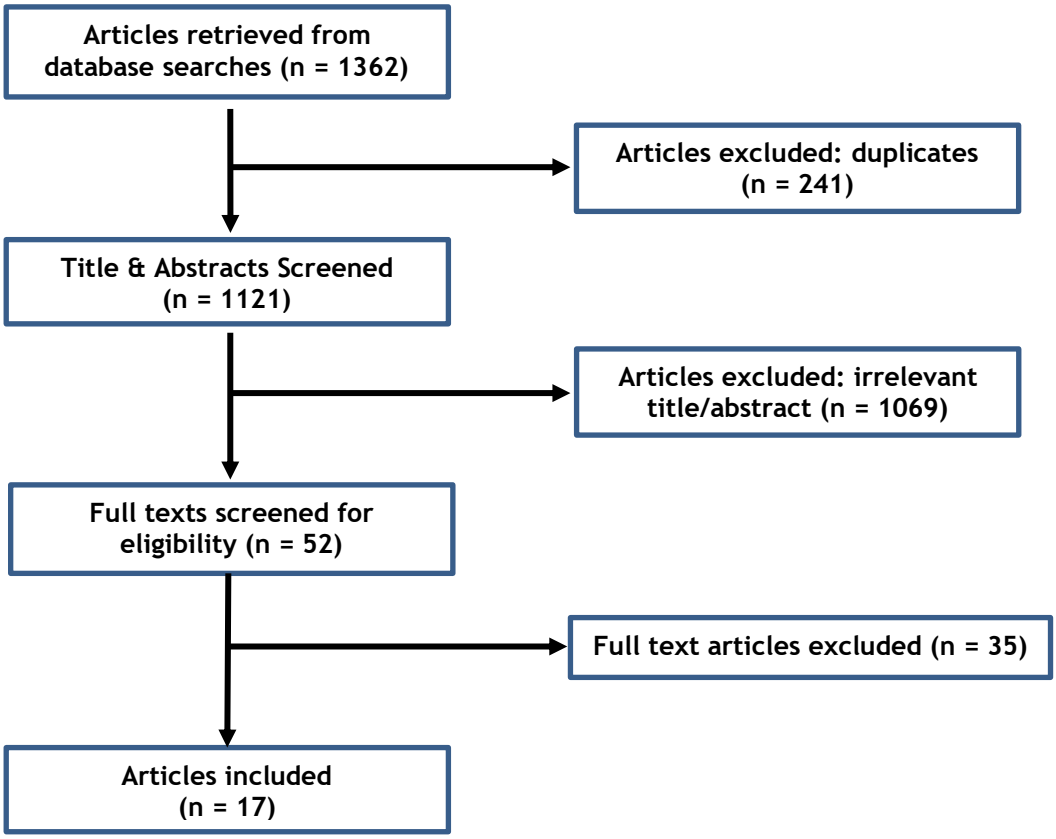

Figure 5-1 Consort diagram of search and study selection procedure.

5.4.2 Study characteristics

Of the seventeen articles included in the final review, 13 were conducted in adults [371-383] and four in paediatric populations [384-387]. A total of 623 participants with IBD (435 CD) were originally recruited across the 17 studies. Eight studies included individuals with both CD and UC, eight assessed only CD patients, and one assessed only UC patients.

Three studies investigated the acute effects of a single bout of exercise, while thirteen studies investigated the effects of PA or exercise interventions ranging in duration from eight weeks to 12 months. A variety of interventions were implemented including walking [377,378], running [376], cycling [382], yoga [371,381,384], active videogame play [385,386], resistance training [374], aerobic and resistance training [372,380,383], resistance and impact training [375], and core strengthening exercises [379]. Study characteristics and results are displayed in Table 5-1.

Table 5-1 Data extraction table for studies exploring the effects of PA and exercise in populations with IBD

Author (year)	Study Design	Cohort	Methods	Outcomes	Adherence & Adverse Events	Main Findings
Adult studies						
Cramer et al (2017)	RCT	UC [Clinical remission] N = 77 (IG: 29, CG 38). 58/77 female. Age: 45	12 weeks IG: 1 x 90 mins yoga per week + daily at home practice encouraged CG: Written self-care book	IBDQ RI	Mean 60.8% adherence. 4 SAEs (unrelated) [3 IG, 1 CG] 16 AEs [7 IG,9 CG]	IBDQ ↑ in IG not CG: 12 wk mean diff. 14.7 [95% CI: 2.4, 26.9], p = 0.02 24 wk mean diff. 16.4 [95% CI: 2.5, 30.3], p = 0.02 RI non-sig ↓ IG & ↑ CG: 24 wk mean diff. -1.2 [95% CI: -2.3, -0.1], p =0.03
Cronin et al (2019)	Randomised crossover trial	IBD [Clinical remission] N = 17 (IG: 15, CG: 8) 5/17 female Age: IG 33 [IQR 31, 36],	8 weeks IG: 3 per week progressive aerobic exercise & RT. CG: No intervention. Crossover after 8-weeks	Body comp. QoL Cytokines Faecal microbiome	Mean 87.5% No AE	Lean mass: IG +1.59kg [95%CI: 0.68, 2.69] vs CG -1.38 [95%CI: -2.45, 0.26], p = 0.003. Bodyfat %: IG -2.1 [95%CI: -2.15, -0.45] vs CG +0.1 [95%CI: -0.4, 1], p = 0.02. No change QoL, microbiome, cytokines.
de Souza Tajiri et al (2014)	Single arm prospective	IBD [Clinical remission] N = 19 10 CD, 9 UC All female Age unknown	8 weeks 2 x 20 mins progressive RT (leg extension) per week	Quadriceps strength Thigh circumference IBDQ	Adherence not reported AE: Not reported	Maximal isometric quadriceps strength ↑ ~40% (28.5 [± 5.6] to 39.0 [± 3.2] kg, p<0.001). No change thigh circumference. IBDQ ↑ 156.3 (± 29) to 180.5 (± 24) (p<0.001).
D'Inca et al (1999)	Case-control (acute)	CD [Remission] N = 6 (+ 6 HC) Age: CD 28.2 (±3.2)	Acute study 1 x 60 mins cycling at 60% VO_{2max}	Bowel symptoms Motility Immune cells Intestinal permeability	100% AE: Not reported	No change symptoms, motility, or permeability. Similar immune response vs healthy controls.
Klare et al (2015)	RCT	IBD [Remission/mild active] N = 36 [18 per group] 19 CD, 11 UC 26/36 female Age: IG 39.7 (± 14.7), CG 42.5 (± 13.9)	10 weeks IG: 3 x moderate intensity running per week CG: Maintain current activity <2 hrs per week	IBDQ CDAI / RI FC CRP Leukocytes	Mean 80% (24 [±4] of 30 sessions) 1 AE	IBDQ ↑ in IG (Mean change 28.3 [± 24.5], p = 0.001) and CG (mean change 14.5 [± 16.1], p = 0.004). No diff in IBDQ change between IG & CG No sig. change in RI/CDAI No group diff. in calprotectin, CRP or leukocytes.

Author (year)	Study Design	Cohort	Methods	Outcomes	Adherence & Adverse Events	Main Findings
Loudon et al (1999)	Single arm prospective	CD [Remission/mild active] N = 16 10/12 female Mean age: 38.3 (± 7.5)	12 weeks 3 x group or individual walking sessions per week. Progressive: 20 - 35 mins	IBDQ IBD stress index HBI CRF	Mean 97.5% (35.1 [± 3.6] sessions) AE: Not reported	IBDQ ↑ 172 [± 27] to 189 [± 12], p = 0.01 IBD stress index ↓ 29.2 [± 15.4] to 19.5 [± 10.8], p<0.001 HBI ↓ 5.9 [± 5.0] to 3.6 [± 3.1], p = 0.02 CRF ↑ 30.6 [± 4.7] to 32.4 [± 4.8], p = 0.001
Ng et al (2007)	RCT	CD [Remission/mild active] N = 32 (16 per group) Sex unknown Mean age: IG 40.6 [± 11.7], CG 37 [± 12.7]	12 weeks IG: 3 x 30 mins walking @ 60% HR_{max} CG: maintain habitual activity	IBDQ IBD-SI HBI	100% AE: Not reported	IBDQ ↑ (p<0.05; data not clearly reported). IBD-SI ↓ (p<0.05; data not clearly reported). HBI ↓ (p<0.05, data not clearly reported).
Robinson et al (1998)	RCT	CD [Remission/mild active] N = 117 (60 IG, 57 CG) 69/117 female Mean age: IG 40.1 [± 12.6], CG 41.2 [± 14.1]	12 months IG: ≥ 2 x dynamic core strengthening exercise sessions per week [10 per month min] CG: Not reported	aBMD LS & Hip	Adherence: 62% @ 3 months, 52% @ 12 months No AEs reported	Non-sig. ↑ aBMD at all sites. Fully compliant cohort sig. ↑ aBMD at greater trochanter vs controls (Mean diff. 4.67 [95%CI: 0.86, 8.48], p = 0.02).
Sharma et al (2015)	RCT	IBD [Clinical remission] N = 100 40 CD, 60 UC Sex & age not reported	8 weeks IG: Daily 1-hour yoga for 1 week, then unsupervised 1-hour daily yoga at home for remaining time. CG: Standard care	Clinical symptoms Anxiety sIL-2R ECP	Adherence: CD mean 81%, 56 (22, 100) mins/day UC mean 76%, 45 (20, 77) mins/day AE: Not reported	No. reporting arthralgia ↓ post-intervention in IG State (p = 0.01) and trait (p = 0.001) anxiety ↓ in UC but not CD (IG) No change in sIL-2R or ECP.
Tew et al (2019)	RCT	CD [Remission/mild active] N = 36 19 female Mean age: 36.9 [± 11.2]	12 weeks HIIT: Cycling 10 x 1-min @ 90% W_{peak}, 3 x per week MICT: Cycling 30 min @ 35% W_{peak}, 3 x per week CG: Standard care	Acceptability CDAI FC Body composition QoL Fatigue CRF	Adherence: HIIT mean 62% MICT mean 75%. Overall 68%. 3 AEs	Good acceptability of HIIT & MICT No formal statistics performed for other outcomes. Descriptive data suggests no clear changes in CDAI, FC, body composition, QoL or fatigue in HIIT or MICT. CRF appeared to ↑ slightly in HIIT (+2.4 ml/kg/min) but not MICT

Author (year)	Study Design	Cohort	Methods	Outcomes	Adherence & Adverse Events	Main Findings
Jones et al (2020)	RCT	CD [Remission/mild active] N = 47 32 female Mean age 49.3 [± 13]	6 months IG: 3 x 60 mins RT & impact exercise per week. CG: No intervention	aBMD LS & Hip Grip strength Chair stand test Isokinetic dynamometry IBDQ IBD-F	Adherence: Median 50/78 sessions [IQR 36, 59] 3 AEs	LS aBMD ↑ in IG vs CG (Mean diff. 3.8% [95% CI: 2.6, 5.1], p<0.001). No diff. in hip aBMD. All muscle function ↑ in IG vs CG. IBDQ ↑ @ 3 months (Mean diff. 17 [95% CI: 7, 26], p = 0.001) but no diff. at follow up. IBD-F ↓ in IG vs CG (Mean diff. -2 [95% CI: -4, -1], p = 0.004)
Seeger et al (2020)	Three arm, parallel pilot RCT	CD [Quiescent/mild active] N = 45 *RT group*: n = 15, 8 female, mean age 42 [± 13.1] years *ET group*: n = 17, 11 female, mean age 39.6 [± 12] years *Control*: n = 13, 9 female, mean age 43.7 [± 12] years	12 weeks RT group: 3 x 30-40mins bodyweight RT ET group: 3 x 30 mins @ 60-80% HR_{max} (gauged by RPE) Control: no intervention	Safety Feasibility CDAI Short IBDQ Quadriceps & grip strength	Adherence not reported. AE: Not reported	ET: 47% dropout; RT: 13% dropout. No change CDAI in any group. No change sIBDQ in ET or RT group. Quadriceps & grip strength ↑ in RT & ET (data not clearly reported)
Van Erp et al (2020)	Single arm prospective	IBD [Quiescent + severe fatigue] N = 25 21 CD, 2 UC, 1 IBDU 10 female Mean age: 45 [± 2.6] years	12 weeks 3 x 60 mins per week combined aerobic & RT Personalised intensity	CIS IBDQ Body comp. CRF Power (cycling)	Adherence: 22/25 completed >30/36 sessions AE: Not reported	CIS ↓ (mean diff. -38 [95%CI: -48, -28], p<0.001). IBDQ ↑ (mean diff. 20 [95%CI: 11, 29], p<0.001). Bodyfat % ↓ (mean diff. -1.8 % [95%CI: -2.8, -0.8], p = 0.002). Non-sig. ↑ in CRF & sig. ↑ max power (mean diff. 0.3 W/kg [95%CI: 0.1, 0.5], p = 0.002).
Paediatric studies						
Arruda et al (2018)	Single arm prospective	IBD N = 9 8 CD, 1 UC 8 female Mean age: 14.1 years	8 weeks Attend 1 x 60 mins yoga session in week 1, 3 & 8. Complete 3 x 30 mins yoga videos at home per week	PUCAI FC QoL	Adherence: 100% attended ≥2/3 sessions. 8/9 ≥1 home video per week. AE: Not reported	No change in PUCAI, FC or QoL.

Author (year)	Study Design	Cohort	Methods	Outcomes	Adherence & Adverse Events	Main Findings
Mählmann et al (2017)	Case-control	IBD [Remission/active] N = 22 12 CD, 7 UC, 3 IBDU *IBD-Remission:* n = 14; 7 CD, 4 UC, 3 IBDU; 6 female. *IBD-Active:* n = 7; 4 CD, 3UC; 4 female. *HC:* n = 23. 15 female.	8 weeks 5 x 30 mins active videogame play per week	QoL Sleep EEG Subjective sleep Habitual PA Exercise capacity	Adherence: IBD-AD 77.5%, IBD-RE 84.5%, HC 88.7% achieved 100% adherence AE: Not reported	No change QoL. Decreased sleep time in IBD-AD. Decreased REM sleep in IBD-RE & IBD-AD. No change subjective sleep. No change objective PA, self-reported PA reduced Increased exercise capacity in all groups (↑ 6MWT distance).
Legeret et al (2020)	Case-control (intervention & acute)	IBD [Remission/active] N = 21 11 CD, 9 UC, 1 IBDU *IBD-Remission:* n = 14; 8 CD, 5 UC, 1 IBDU; 8 female *IBD-Active:* n = 7; 3 CD, 4 UC; 2 female *HC:* n = 23; 15 female	8 weeks *Intervention*: 5 x 30 mins active videogame play per week *Acute response*: 6-minute walk test (before & after intervention)	*Intervention:* Circulating inflammatory markers *Acute:* Inflammatory response to 6MWT	Adherence: Not reported AE: Not reported	ESR and CRP ↓in IBD-AD & IBD-RE. Transient inflammatory response to acute exercise similar in all groups. Acute response to exercise did not change post-intervention.
Ploeger et al (2012)	Case-control crossover (acute)	CD [Clinical remission] N = 15 2 female Mean age: 14.5 [± 2.4] years HC: n = 15; 2 female; mean age 13.9 [± 2.2] years	Acute response to HIIT & MICT. HIIT: 6 sets of 4 x 15s cycling @ 100% W_{peak} MICT: 2 x 30mins cycling @ 50% W_{peak}	Immune cells Cytokines: TNF-α, IL-6, IL-17 Growth factors: GH, IGF-1	5 withdrawals pre-exercise 15 CD & HC completed both HIIT and MICT AE: Not reported	All immune cells ↑ during MICT but not HIIT in both groups. IL-6 ↑ during MICT in both groups, no cytokine change during HIIT. IL-6 return to baseline quicker in CD vs HC. (baseline higher in CD). IGF-1 ↓ at onset of HIIT and MICT in CD and return to baseline by mid-recovery. IGF-1 unchanged in HC. GH ↑ at onset of MICT in both groups and onset of HIIT in HC.

Key characteristics of studies included in this systematic review (n = 15). CD, Crohn's disease; UC, ulcerative colitis; IBDU, inflammatory bowel disease unclassified; IBD, inflammatory bowel disease; IG, intervention group; CG, control group; HC, healthy controls; RCT, randomised controlled trial; AE, adverse events; IQR, inter-quartile range; 95%CI, 95% confidence interval; diff., difference; sig., significant; ↑, increase; ↓, decrease; CDAI, Crohn's disease activity index; HBI, Harvey-Bradshaw index; PUCAI, paediatric ulcerative colitis activity index; RI, Rachmilewitz index; IBDQ, inflammatory bowel disease questionnaire; CIS, checklist individual strength; QoL, quality of life; FC, faecal calprotectin; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-, interleukin; TNF-α, tumour necrosis factor alpha; ECP, eosinophilic cationic protein; sIL-2R, soluble interleukin 2 receptor; GH, growth hormone; IGF-1, insulin-like growth factor 1; PA, physical activity; 6MWT, 6-minute walk test; RT, resistance training; ET, endurance training; HIIT, high intensity interval training; MICT, moderate intensity continuous training; CRF, cardiorespiratory fitness; VO_{2max}, maximal rate of oxygen consumption; EEG, electroencephalogram; W, watts; ml, millilitres; kg, kilograms;$W_{peak,}$ peak power output.

5.4.3 Results: Assessment of quality

Table 5-2 presents the quality assessment score for each of the included articles according to the modified Downs Black checklist. The median score was 15 (range 8, 23) from a possible 28 points. By this scoring system only two studies were rated as 'good' quality, nine were rated 'fair' and five studies were rated 'poor'. One feasibility study performed no formal statistical analyses and as such some Downs Black checklist items were not applicable. This study scored 15 from an available 21 points. All studies scored particularly poorly on external validity and the large number of pilot and feasibility trials meant that only 4/17 articles included an *a priori* power calculation.

Table 5-2 Assessment of study quality using modified Downs Black checklist.

	Downs-Black Checklist Category						
Author (year)	Reporting [x/11]	External Validity [x/3]	Internal Validity [Bias] [x/7]	Internal validity [confounding] [x/6]	Power Calculation [1]	Total Score [x/28]	Study Quality
Arruda (2018)	5	0	5	3	0	15	Fair
Cramer (2017)	11	0	6	5	1	23	Good
Cronin (2018)	9	0	5	2	1	17	Fair
De Souza Tajiri (2014)	5	0	2	1	0	8	Poor
D'Inca (1999)	9	0	5	2	0	16	Fair
Jones (2020)	11	0	6	4	1	22	Good
Klare (2015)	11	0	4	4	0	19	Fair
Legeret (2019)	8	0	5	1	0	14	Poor
Loudon (1999)	9	0	5	0	0	14	Poor
Mahlmann (2017)	8	0	5	1	0	14	Poor
Ng (2007)	6	0	5	4	0	15	Fair
Ploeger (2012)	8	0	5	2	0	15	Fair
Robinson (1998)	9	0	4	4	1	18	Fair
Seeger (2020)	6	0	3	3	0	12	Poor
Sharma (2015)	7	0	5	3	0	15	Fair
Tew (2019) *	10/10	0/3	2/4	3/4	n/a	15/21	n/a
Van Erp (2020)	10	0	5	3	0	18	Fair

Assessment of study quality using modified Downs Black checklist. Maximum available score was 28. Parentheses indicate score available per category. * No statistical analyses were performed in this study and some items from the checklist were not applicable.

5.4.4 Results: Adherence and safety of exercise in IBD

Adherence to exercise varied quite widely across the different intervention studies. Adherence tended to be lower in longer duration interventions that contained a large proportion of unsupervised exercise [371,375,379,382]. A mean adherence of 62% was reported in cycling [382], resistance training [375], and core strengthening [379] exercise, although adherence dropped to 52% by 12 months in the latter. Adherence to other interventions was good, with walking being the highest (97.5% [377] and 100% [378]). Three intervention studies failed to report on adherence [374,380,385]. Reporting of exercise safety was poor, with 11/17 studies not providing any data on adverse events. In studies that reported on safety, very few AEs occurred, highlighting the overall safety of various modes of exercise IBD. No intervention-related SAEs occurred in any study. All studies included exclusively individuals with disease in remission or mildly active, therefore the safety of exercise in moderate or severely active disease remains unknown.

5.4.5 Results: Effects of exercise on IBD complications

5.4.5.1 Disease activity and symptoms

The effects of PA and exercise on disease activity and symptoms were assessed in ten studies [371-373,376-378,380-382,384]. Disease activity was assessed using different validated clinical indexes including the CDAI [372,376-378,380,382], Harvey Bradshaw index (HBI), PCDAI [385], paediatric ulcerative colitis activity index (PUCAI) [384], Rachmilewitz index (RI) [371,376], and the simple colitis index (SCI) [372]. Two studies qualitatively assessed disease symptoms post-intervention [373,381]. Twelve weeks of thrice weekly low-intensity walking resulted in statistically significant reductions in mean HBI score in two separate studies of adults with CD [377,378]. Mean difference in HBI scores post-intervention were -2.3 ($p = 0.02$) [377] and -3.0 ($p < 0.05$) [378], respectively, reducing the overall mean HBI scores from ‘mildly active’ to ‘remission’ in both studies. Three studies of yoga in adolescents [384] and adults with IBD [371,381] reported no significant benefits to disease activity or bowel symptoms. Cramer et al did report a significant mean difference in RI score after long term follow up in favour of the yoga cohort, but only by -1.2 (95% CI: -2.5, -0.1; $p = 0.03$) points. Similarly, a single bout of moderate cycling exercise did not have any effects on intestinal symptoms in a small cohort of male CD ($n = 6$) [373].

Ten weeks of moderate intensity running [376] or eight weeks of progressive moderate intensity aerobic and resistance training [372] did not significantly alter disease activity scores in two cohorts of adults with quiescent IBD. Bodyweight resistance or endurance training also did not alter CDAI score in adults with CD [380]. While no formal statistical testing was completed, results from a pilot RCT of moderate or high intensity cycling in CD suggest no clear effects of either disease modality on CDAI [382]. It should be noted that all these cohorts included only participants in clinical remission or with mildly active disease and the low clinical disease activity scores reported at baseline reflected this. In one study that included participants with active disease, Legeret et al observed reductions in the PCDAI or PUCAI scores of all adolescent participants with active disease who undertook an eight-week active videogame play intervention (n = 7). Despite this, the pooled effect of intervention on disease status of the active and remission groups were not significant, as many in the remission group reported a baseline activity score of zero [385].

5.4.5.2 Immune & inflammatory markers

The acute inflammatory response to exercise was assessed in three studies [373,385,387] and the long-term effects of exercise on inflammatory markers were evaluated in six studies [372,376,381,382,384,385]. In adolescents with CD, Ploeger et al reported a single 60-minute bout of moderate intensity exercise (MICT), but not a shorter bout of high intensity exercise, significantly increased circulating immune cells in a manner similar to the healthy control group [387]. Exercise did not increase circulating TNF-α in CD, but IL-6 increased during MICT, peaking at 30-minutes post-exercise, before returning to baseline. The magnitude of increased IL-6 was greater in controls, but absolute peak levels were higher in CD as mean baseline levels were six times higher (approx. 6 pg/ml vs 1 pg/ml) [387]. A similar bout of cycling exercise did not alter immune function in young men with CD [373]. An acute sub-maximal walking test did not alter CRP or ESR in adolescents with IBD [385], suggesting no exercise induced increase in IL-6. After eight weeks of combined aerobic and resistance training in adults with quiescent IBD (n = 13), circulating pro-inflammatory cytokines IL-6, TNF-α and IL-8 were unchanged from baseline levels [372]. Faecal calprotectin (FC), a validated clinical marker of intestinal inflammation, was measured in only three studies [376,382,384]. Yoga did not alter FC in paediatric IBD, although baseline levels were not reported [384]. Twelve weeks of

moderate or high intensity cycling had no apparent effect on FC (baseline vs follow up: 45 ± 40 ug/g vs 63 ± 113 ug/g and 89 ± 72 vs 100 ± 113 ug/g, respectively), although no formal statistical testing was conducted [382]. Moderate intensity running resulted in a mean within group change of +186 ± 324 ug/g in the intervention group, yet this did not reach statistical significance (p = 0.06) [376]. Median (range) FC at follow up in this group was 141 (<30 – 1069) ug/g and the number of participants with potentially pathological FC (>250 ug/g) was not reported.

5.4.5.3 Quality of life & psychological wellbeing

HRQoL was assessed in ten studies of adult participants, eight of which applied the IBDQ tool [371,374-378,382,383]. In two paediatric studies, no benefits to HRQoL or psychological wellbeing were reported after a yoga [384] or active videogame play [386] intervention, respectively. Conversely, a yoga intervention improved mean IBDQ scores in adults with UC (143.5 ± 22.3 to 159.8 ± 32.2) resulting in a significant between group difference compared to controls, whose scores did not change from baseline (mean difference: 14.7 [95% CI: 2.4, 26.9], p = 0.02) [371]. A separate yoga intervention was found to reduce anxiety levels, but only in UC, not CD participants [381]. Klare et al observed improvements in IBDQ after 10 weeks of moderate intensity running (156.5 ± 35.0 to 184.9 ± 20.9, p = 0.001) with improvements in all sub-dimensions [376]. Surprisingly, the non-exercising control group also experienced improved IBDQ in this study (167.7 ± 31.1 to 182.2 ± 26.6, p = 0.004) and in all sub-dimensions excluding social function, meaning the change in IBDQ over time did not differ between groups [376]. Twelve weeks of walking was associated with a statistically significant improvement in IBDQ scores in adults with CD (172 ± 27 to 189 ±12, p = 0.01) [377]. A similar walking intervention also observed improvements in IBDQ although the absolute values were not reported [378]. Both walking studies also reported improvements in IBD stress index (IBD-SI) scores despite scores being relatively low at baseline (mean: 29.2 ± 15.4 to 19.5 ± 10.8 [377] and 31.4 to 18.7 [378], respectively. Scores range from 0 – 102). IBDQ scores improved after eight weeks of quadriceps focused resistance training in women with IBD (10 CD) and quadriceps weakness at baseline [374]. The age and disease status of these women was unknown, but IBDQ increased from 156.3 ± 29.0 to 180.5 ± 24.2 (p < 0.001) with significant improvements in all sub-dimensions. A combination of resistance training and aerobic exercise also improved IBDQ in a

cohort of middle-aged adults (n = 25; 21 CD) with mild IBD and severe fatigue at baseline [383]. Improvements in all sub-dimensions except bowel functioning were reported in this group, with overall mean increase in IBDQ of 20 (95% CI: 11, 29; p < 0.001). Conversely, combined resistance and impact training had no effect on IBDQ, although mean score was high at baseline (183 ± 23) [375].

5.4.5.4 Muscle-bone, body composition, and physical fitness

Few studies assessed the effects of PA or exercise intervention on physical outcomes in IBD. Combined aerobic and resistance training in sedentary adults with IBD resulted in a gain in DXA-derived lean mass (median 1.59 kg [IQR: 0.68, 2.69]) and reduction of fat mass (-1.52 kg [IQR: -2.32, -0.04]) and bodyfat percentage (-2.1 % [IQR: -2.15, -0.45]) [372]. Progressive resistance training was effective for improving quadriceps strength in women with IBD and quadriceps weakness (28.5 kg ± 5.6 to 39.0 kg ± 3.2, p < 0.001), despite no concurrent increase in thigh circumference [374]. Resistance and impact training also had large positive effects on several muscle function parameters including grip strength and upper and lower limb isokinetic strength, with improvements of up to 50% [375]. Both resistance and endurance training were associated with increased grip strength in CD although the absolute changes were not reported [380]. Estimated cardiorespiratory fitness was improved by a 12-week walking programme (VO_{2max} 30.6 [± 4.7] ml/kg/min to 32.4 [± 4.8] ml/kg/min, p = 0.001) [377] and appeared to be improved by high intensity, but not moderate intensity, cycling (mean increase 2.4 ml/kg/min) [382] in separate cohorts of CD. Adolescents with active (n = 7) and remissive (n = 14) IBD increased their submaximal 6MWT distance at follow up compared to control, which was reported as an improvement in exercise capacity [386].

Two studies investigated the effects of exercise on skeletal outcomes. Low impact core exercises improved aBMD at the hip in CD but only in participants who achieved full intervention adherence [379]. The aBMD improvement in exercise group was significantly greater than that observed in non-exercising controls (7.77 % ± 8.2 vs 3.1 % ± 5.83, p = 0.02). Another CD cohort had significantly greater LS aBMD compared to controls after six months of resistance and impact exercise (Mean diff. 3.8% [95% CI: 2.6, 5.1], p<0.001) [375]. In both studies, aBMD improvements were positively associated with number of exercise sessions completed.

5.4.5.5 Fatigue

Two directly addressed the effects of exercise on fatigue in IBD [375,383]. Twenty-five adults with clinically quiescent IBD (21 CD) who had severe fatigue at baseline (CIS Fatigue sub-scale score ≥35) were enrolled to participate in three supervised sessions of combined aerobic and resistance training per week for 12 weeks. In the 22 who completed the intervention, mean CIS-F sub-score reduced from 49 ± 4.7 to 29 ± 8.9 ($p < 0.001$)[383]. IBD-F score was significantly different between exercise and control group after resistance and impact training, although this was due to deteriorating score in controls rather than improvement in the exercise group (Mean diff. -2 [95% CI: -4, -1], $p = 0.004$) [375]. Objective and subjective sleep quality, potential contributors to fatigue in adolescent IBD, were assessed in one study but were not improved by an active videogame play intervention [386].

5.5 Discussion

The potential mechanisms by which PA and exercise could benefit individuals with IBD are multifactorial. Despite this, the currently available evidence for the role of exercise in the management of IBD has several limitations. In this review, assessment of study quality indicated only two studies to be of good quality and none were rated as excellent. The wide heterogeneity in terms of methodological approaches and measured outcomes makes between study comparisons difficult. Subsequently, a pooled review of the evidence is not possible for many of the outcomes. The multiple use of single arm cohort studies inhibits assessment of interventions versus control conditions and limits the statistical power of any reported associations. Exercise of various formats do, however, appear to be a safe and feasible intervention for individuals with IBD. Larger, more rigorously designed studies are required to further explore the potential benefits of exercise in the management of IBD and its associated complications.

A consistent finding across studies was mild improvements in HRQoL from exercise, as assessed by IBDQ [371,374,376-378,383]. The IBDQ has been validated as a reliable and responsive measure of HRQoL in IBD [388]. The mechanisms that mediate the effects of exercise on HRQoL in IBD are unclear but may be linked to improved physical function. Some improvement was reported in all sub-dimensions of the IBDQ in most studies [374,376,377,383], suggesting a multifactorial benefit. Debate regarding the

change in IBDQ required to reflect a clinically meaningful benefit exists, with different studies suggesting an improvement of 16 to 27 points [389,390]. Most studies therefore reported a change in IBDQ that likely reflected a small clinically meaningful improvement in HRQoL. The inclusion of only remissive or mildly active disease was reflected in baseline IBDQ scores, which were slightly lower than the accepted cut-off for remission [391]. Whether exercise may elicit a stronger benefit to HRQoL in those with active disease or with low baseline HRQoL should be investigated in future.

There were no clear effects for exercise on disease activity or symptoms. Exploration of the beneficial effects of exercise on disease activity was confounded by the inclusion of only mild or inactive disease, meaning baseline disease activity scores were low. The evidence does, however, indicate that exercise has no detrimental effect on disease activity in this population as no worsening of symptoms or markers of inflammation were observed in any study. The acute inflammatory response to exercise was not different between IBD and controls [373,385,387]. Critically, the upregulated expression of IL-6 induced by exercise was not associated with a concurrent increase in TNF-α, suggesting a similar inflammatory response to exercise in CD as is observed in healthy populations [94]. As the only study to date to assess the acute effects of exercise on cytokine response, further research using different modes and intensities of exercise are required to validate these findings. Also, whether exercise can help alleviate systemic inflammation in more active IBD remains unclear.

Exercise has vast potential for improving the musculoskeletal burden associated with IBD, yet few studies directly addressed this. Resistance and impact training induced large improvements in muscle function [375], while another much shorter quadriceps training programme also elicited 40% increases in strength with no concurrent increases in muscle mass [374]. These studies highlight the potential utility of exercise for overcoming functional deficits in IBD as muscle in quiescent IBD is clearly responsive to resistance exercise. The high prevalence of low bone mass in IBD, particularly CD, represents a clinical problem that could also potentially be alleviated by exercise. Resistance training with or without impact exercise improved aBMD in adults with CD in those with high levels of adherence. Strategies to promote adherence to exercise interventions are needed to augment

the potential physiological effects of exercise for improving muscle and bone in IBD. Exercise including impact-based activities have been shown to promote the greatest effects on bone mass and geometry [120,317] but are relatively unexplored in IBD, particularly in younger populations. Successfully improving bone and muscle mass in young populations is likely to confer long term benefits [121,392]. There is currently a paucity of exercise research in paediatric IBD, and interventions designed to alleviate the substantial musculoskeletal burden in this population should be conducted.

In conclusion, exercise appears to be safe and feasible for people with IBD. Despite being limited by small, heterogenous studies, there is evidence of small clinical improvements in HRQoL from exercise in adults with mild or inactive IBD. Many aspects regarding the potential role for exercise in the management of IBD and its secondary complications remain unanswered. The effects of exercise on systemic inflammation in IBD and the potential role of exercise for augmenting muscle-bone development in IBD are two key avenues of research that warrant investigation, as these have the potential to improve other aspects of disease.

6 Feasibility of jumping based exercise as complementary therapy in adolescents and adults with Crohn's disease: a questionnaire study

6.1 Abstract

Background. Exercise may be a useful and cost-effective adjunctive therapy for alleviating the secondary complications of CD, particularly muscle-bone deficits. There is currently limited evidence for the utility of exercise in young CD population and the acceptability of different exercise methods are unknown.

Aim. To assess the feasibility and acceptability of introducing high impact exercise as a therapeutic option for improving bone and muscle health in adolescents and adults with CD.

Methods. An online questionnaire was designed to survey the attitudes of adolescents and adults with CD towards the exploration of jumping based exercise as an adjunctive therapy in CD.

Results. Forty-eight (22 adolescents, 26 adults) CD patients with median (range) age 19 years (13, 40) responded. Overall disease control was good, and wellbeing was primarily 'very well' (51%), or 'slightly below average' (40%). Fifty-six percent said CD makes exercising difficult, primarily due to fatigue (78%), joint pain (56%) and abdominal pain (52%). Perceived benefits of exercise included improved mood (77%), improved strength and fitness (56%) and improved energy (31%). Exercise was reported to exacerbate fatigue and joint pain in 44% and 31% of respondents, respectively. Exercise had no negative effects in 38% of respondents. Sixty-five percent of respondents were willing to exercise three or more times per week, and 98% believed the opportunity to improve bone health was worth participating, three times per week, in high impact exercise. Attitudes towards exercise research participation were positive, with 83% stating they would be interested in taking part in future exercise studies.

Conclusions. Adolescents and young adults with CD displayed generally positive attitudes towards the exploration of jumping based exercise as a therapeutic

option. Most respondents stated a willingness to exercise several times per week for potential benefits to health and intentions to participate in future research studies, highlighting the acceptability of jumping based exercise. These data were used to inform the development of pilot exercise studies.

6.2 Introduction

The previous chapters have clearly demonstrated bone and muscle deficits among children and young adults with CD, even with well controlled disease and contemporary disease management. Other studies have shown that, despite improving underlying disease, contemporary therapies such as EEN [225] and anti-TNF-α [49,217] are associated with persistent muscle-bone deficits in paediatric CD at 12-months follow up. These data suggest that current therapies may be ineffective for resolving musculoskeletal deficits in CD. Muscle-bone deficits in young CD patients, if unresolved, predisposes these individuals to increased risk of adverse musculoskeletal events in adulthood. Despite this, little attention has been given to developing novel strategies to target muscle-bone deficits in CD in conjunction with pharmacological disease management.

Exercise has great potential as a treatment modality for targeting secondary complications of CD. However, at present, no evidence-based guidelines regarding PA and exercise exist for individuals with CD. The published exercise studies in CD are generally small and underpowered, with heterogeneous outcomes (Chapter 5). However, a mild positive benefit to HRQoL [374,376,377,383] and improvements in musculoskeletal outcomes [375,379,382] have been observed from different interventions. Additionally, no safety concerns or exacerbation of disease symptoms linked to exercise have been reported in any study to date. Importantly, the inflammatory response to even high intensity exercise does not appear to differ from healthy controls [387]. Despite this, observational data suggests habitual PA is low in CD [272] and that exercise participation and subjective feelings of physical fitness are reduced compared to pre-diagnosis [271,393]. These observations are likely mediated by multiple disease specific barriers to exercise participation that have been reported by individuals with CD [271,272,309]. There is, therefore, a need to evaluate easily accessible interventions that can be implemented with consideration to disease specific barriers.

Jumping based exercise offers a potentially cost-effective and accessible intervention that provides combined anabolic stimulus to counteract the muscle and bone deficits observed in CD. The acceptability of such high impact exercise is unknown among those with CD and requires evaluation prior to consideration therapeutically. This preliminary study, therefore, employed an electronic

questionnaire to evaluate the feasibility and acceptability of exploring high impact, jumping based exercise as a potential complementary therapy in adolescents and adults with CD.

6.3 Methods

6.3.1 Population

Patients attending routine gastroenterology outpatient clinic appointments across three hospitals within NHS Greater Glasgow & Clyde were approached to complete an electronic questionnaire. Potential respondents were identified by their clinical gastroenterology team and signposted to the researcher (LS) after outpatient appointments. Any individual with CD aged from 13 – 40 years was eligible to participate in this questionnaire. According to research governance, this anonymous questionnaire gathering opinions on the exploration of exercise as a potential complementary therapy in CD did not require ethical approval [394]. All questionnaires were completed voluntarily, and consent was implied by questionnaire completion, or by verbal confirmation of a parent/guardian prior to completion by adolescents.

6.3.2 Questionnaire

This questionnaire was designed to assess the feasibility and acceptability of exploring jumping based exercise as a complementary therapy in adolescents and adults with CD. Two versions of the questionnaire were developed for use in adolescents (aged 13 – 17 years) and adults (18 – 40 years), respectively. These questionnaires followed an identical format with only minor wording differences. The questionnaires were checked for content validity among adult and paediatric gastroenterologists and for readability among laypeople. The questionnaires were hosted on the online platform SurveyMonkey.

The questionnaire collected information regarding participants' age, disease status, and current exercise habits. They were asked if participating in exercise positively or negatively affected any of their disease symptoms and about their attitude and perceptions towards exercise participation. The perceived difficulty of exercise participation and intentions towards participation in future exercise research studies were assessed, based on a short video demonstrating a series of

jumping based exercises (https://www.youtube.com/watch?v=_uvyOJITB_Q). Most questions were multiple choice; two questions employed a visual analogue scale (coded from 0 to 100) when asking about recent disease control and perceived difficulty of exercise; and general wellbeing was assessed using a five-point Likert scale (from 0 'very well' to 4 'terrible') as used in the validated and routinely applied Crohn's disease activity index. Participants were also asked to provide free-text comments regarding potential barriers during participation in jumping based exercise. The questionnaires can be accessed in full in Appendix 3.

6.3.3 Statistical Analysis

The primary aim of this survey was to gather data on the feasibility and acceptability of jumping based exercise in individuals with CD. Data were collected for descriptive purposes and no formal statistical testing was conducted. Data are presented as median (range) for continuous variables and frequency (percentage) for categorical variables. Data for the adolescent and adult survey are presented together unless otherwise stated.

6.4 Results

6.4.1 Cohort Characteristics

Forty-eight of 56 approached individuals completed the questionnaire (86% response rate). There were 22 adolescent (68% male) and 26 adult (50% male) respondents with an overall median age of 19 years (13, 40) and median age at diagnosis of 11 years (2, 34). Current medications were most commonly anti-TNFα therapy (69%, 33/48) and immunomodulators (67%, 32/48). One respondent was currently on GC therapy. Approximately one third (35%, 17/48) had a history of CD related surgery (

Table 6-1). Median recent disease control was rated as good (visual analogue scale: 79 [8, 100]) (Figure 6-1). General wellbeing was rated as ‘very well in 50% (24/48) or ‘slightly below average’ in 40% (19/48). Nearly half of respondents stated they were not concerned about their bone health (44%, 21/48), with 46% (22/48) and 10% (5/48) ‘slightly’ and ‘very’ concerned about bone health, respectively.

Table 6-1 Cohort characteristics stratified by age group.

	Adolescents (n = 22)	Adults (n = 26)
Male, n (%)	15 (68)	13 (50)
Age (years)	15 (13, 17)	24 (18, 40)
Age at diagnosis (years)	11 (5, 17)	12 (2, 34)
Disease duration (years)	4 (0, 9)	9.5 (1, 30)
Medications, n (%)		
Anti-TNFα	16 (73)	17 (65)
Immunomodulators	18 (82)	14 (54)
5-ASA	0 (0)	2 (8)
GC	1 (5)	0 (0)
Nutritional Supps.	2 (9)	3 (12)
Other	7 (32)	6 (23)
Wellbeing, n (%) [a]		
Very well	13 (59)	11 (44)
Slightly below average	8 (36)	11 (44)
Poor	0 (0)	3 (12)
Very poor	1 (5)	0 (0)
Terrible	0 (0)	0 (0)
Previous CD surgery, n(%)		
None	13 (59)	18 (69)
One	6 (27)	3 (12)
Two or more	3 (14)	5 (19)

Cohort characteristics of adolescents and adults with CD. Continuous data are displayed as median (range). [a] n = 47 (22 adolescents, 25 adults). Anti-TNFα – infliximab, adalimumab; 5-ASA – 5-aminosalicylates; immunomodulators – azathioprine, mercaptopurine, methotrexate; other – proton pump inhibitors (n = 7), antispasmodics (n = 2), thalidomide (n = 1), cholecalciferol (n = 1), hydrocortisone (n = 1), unknown (n = 1).

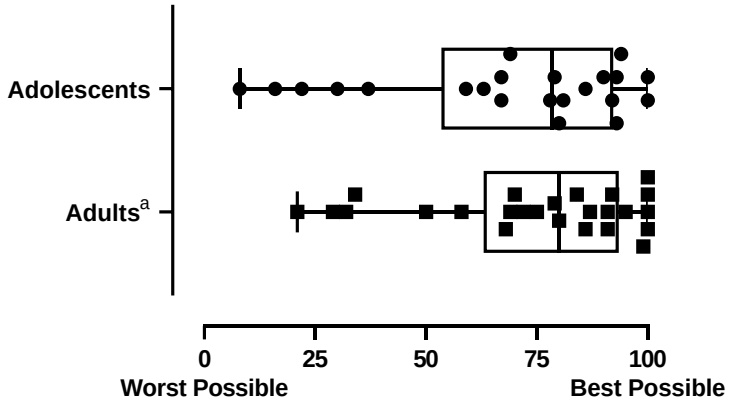

Figure 6-1 Assessment of recent disease control among adolescents and adults with CD. Box plot with individual data points illustrating answers to the question 'How would you rate the overall control of your Crohn's disease over the last 3 months?'. Answers were recorded using a visual analogue scale rated 0 (Worst possible) to 100 (Best possible). [a] n = 25.

6.4.2 PA & Exercise Habits

Seventy-five percent (36/48) of respondents reported regularly participating in at least one form of exercise and 40% participated in two or more. Team sports were most common among adolescents (41%, 9/22), whereas walking (42%, 11/26) and

resistance training (39%, 10/26) were most common in adults. Twelve respondents (25%, 12/48) stated they did not take part in any exercise at all (Figure 6-2). Fifteen percent reported walking as their only form of PA and if this was excluded the number of people who did not undertake any exercise rose to 40% (19/48).

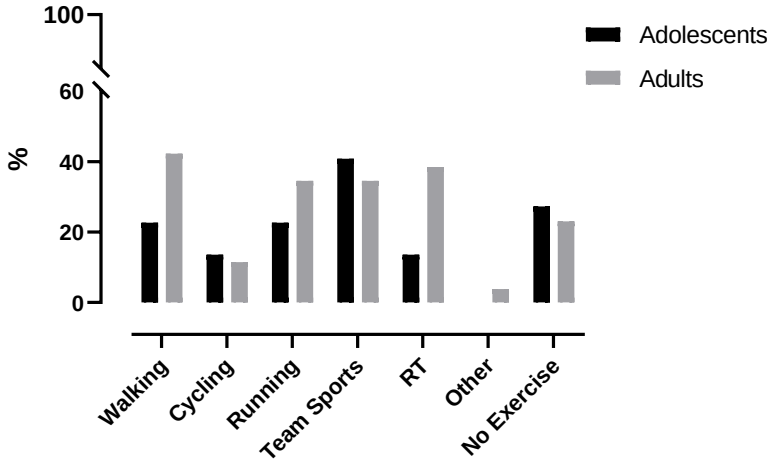

Figure 6-2 Current exercise habits of adolescents and adults with CD.
Bar chart displaying percentage responses to the question 'In a typical week, which of the following exercises do you do?'. In the adolescent survey, this question included the phrase 'excluding during school time' to ensure only leisure time activity was included. Black bars – adolescents; grey bars – adults. RT – resistance training; Other - yoga (n = 1).

6.4.3 Barriers to exercise

Fifty-six percent of respondents (27/48) stated that having CD made exercise participation more difficult (10/22 [46%] adolescents; 17/26 [65%] adults). The most common barriers to exercise participation in these individuals were fatigue (78%, 21/27), joint pain (56%, 15/27) and abdominal pain (52%, 14/27) (Figure 6-3).

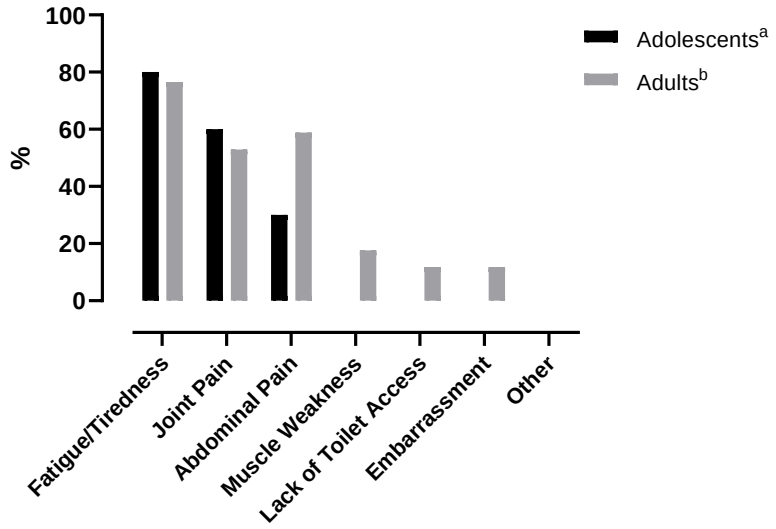

Figure 6-3 Barriers to exercise participation among adolescents and adults with CD.
Bar chart displaying percentage responses to the question 'In what way does your Crohn's disease make exercising more difficult?'. N = 27 ([a] n = 10, [b] = 17). Grey bars – adolescents, black bars – adults.

6.4.4 Effects of exercise on CD symptoms

Perceived benefits of exercise participation were improved mood (77%, 37/48), improved strength and fitness (56%, 27/48) and improved energy/fatigue levels (31%, 15/48) (Figure 6-4). Two adolescent respondents stated that exercise had no positive effects on their health or disease symptoms.

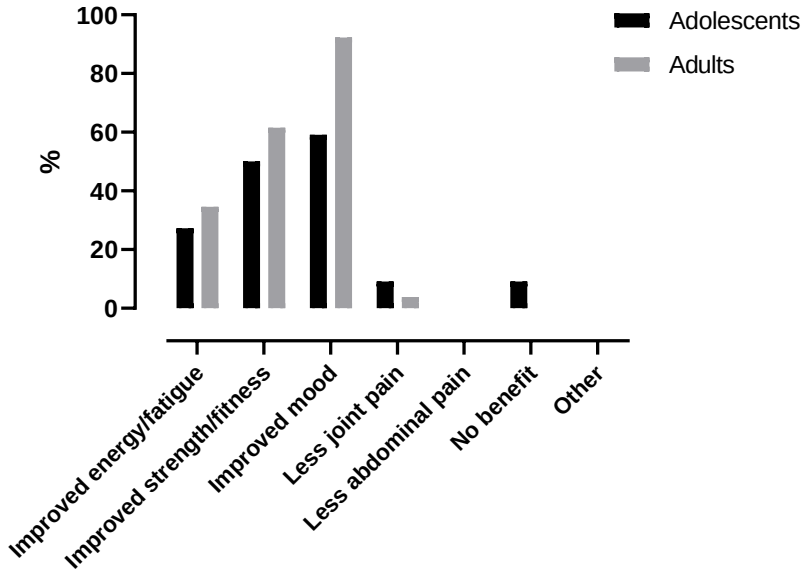

Figure 6-4 Perceived benefits of exercise in adolescents and adults with CD.
Bar chart displaying percentage responses to the question 'When you take part in exercise, does it make you feel better in any of the following ways?'. Grey bars – adolescents, black bars – adults.

Perceived negative effects of exercise were exacerbation of fatigue (44%, 21/48), increased joint pain (31%, 15/48) and feelings of weakness (15%, 7/48). Thirty-eight percent (18/48) stated that exercise had no negative effects on their health or disease symptoms (Figure 6-5).

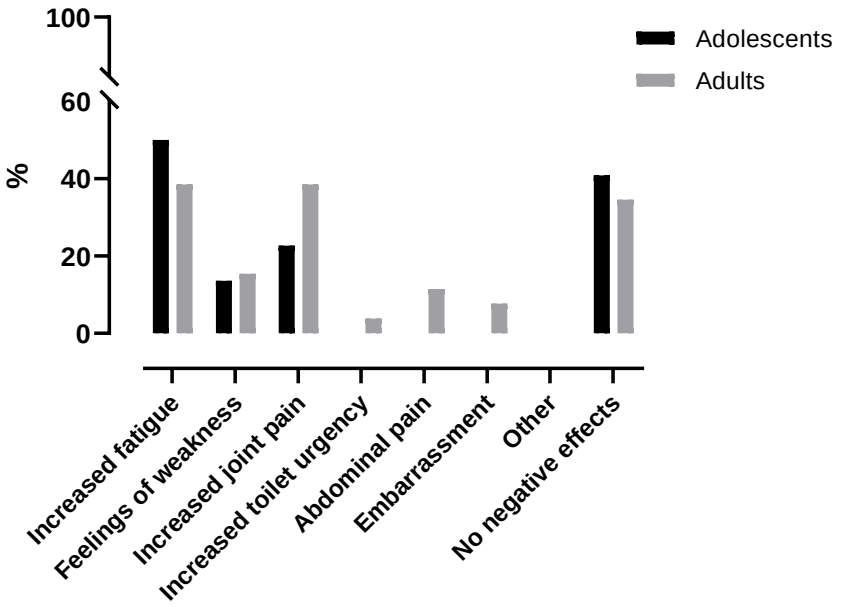

Figure 6-5 Perceived negative effects of exercise in adolescents and adults with CD.
Bar chart displaying percentage responses to the question 'When you take part in exercise, does it make you feel worse in any of the following ways?'. Grey bars – adolescents, black bars – adults.

6.4.5 Acceptability of exercise participation for improving skeletal health

Sixty-five percent (31/48) of participants stated they would be able to exercise for 30 minutes on three or more occasions per week (Figure 6-6). After viewing the video, 98% (47/48) believed that, for potential improvements in bone health, participating in similar types of exercise three times per week would be worthwhile. Perceived difficulty of jumping exercise was generally rated as low (median VAS: 2.5 [0, 87]) (Figure 6-7). Thirteen participants provided free text responses about potential barriers to participation in jumping exercise. These responses contained themes regarding concerns of fatigue (n = 5), joint pain (n = 5), abdominal pain (n = 3), physical inability (n = 2), and inability due to disease flares (n = 1). Five free text responses were given to a question asking for general comments regarding exercise, all of which contained positive responses (Table 6-2). Intentions towards participation in future research studies exploring the

utility of jumping exercise in CD were generally positive, with 83% saying they would take part in such research. Intentions to participate in exercise research among those who currently did no exercise was also high at 80%.

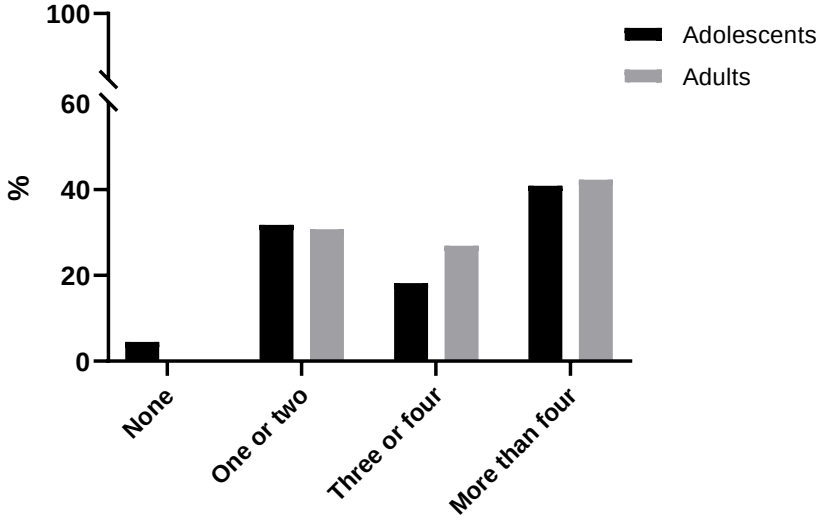

Figure 6-6 How often adolescents and adults with CD are willing to exercise per week.
Bar chart displaying percentage responses of adolescents and adults with CD to the question 'How many times per week do you think you would be able to set aside 30 minutes for exercise?' Grey bars – adolescents, black bars – adults.

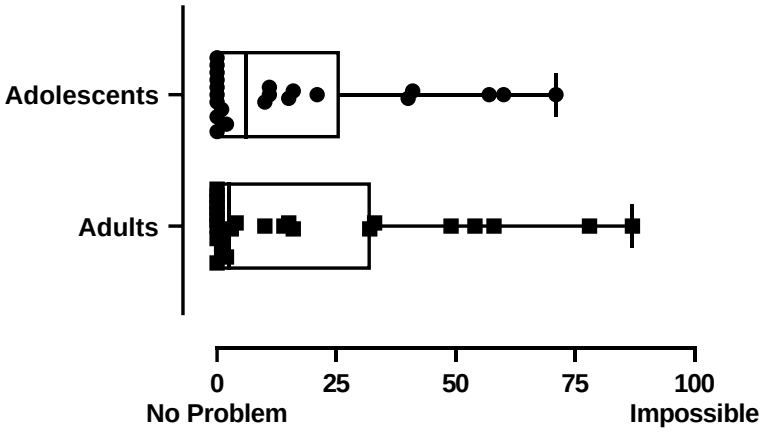

Figure 6-7 Perceived difficulty of participation in jumping based exercise.
Boxplot with individual data points illustrating answers to the question 'How easy do you think it would be to participate in exercise like this, three times per week?'. Answers were recorded using a visual analogue scale rated 0 (No problem) to 100 (Impossible).

Table 6-2 Qualitative responses to question regarding general comments about jumping exercise and research.

Question	Responses (n = 5)
Q20. Do you have any additional comments that you would like to make regarding exercise, or the research studies we are going to conduct?	*"I am very interested in this research and would like further info on this"* *"Think it would be beneficial"* *"The real struggle I have is sometimes getting the energy to get up and go to the gym to exercise. Once I'm over that hurdle, I enjoy being there and feel it helps me both mentally and physically"* *"After long periods of time (weeks with continued exercise) it seems to help"* *"I would be keen to participate"*

Free text responses (n = 5) to the question 'Do you have any additional comments that you would like to make regarding exercise, or the research studies we are going to conduct?'. All responses were provided by adult participants.

6.5 Discussion

This preliminary study investigated the feasibility and acceptability of jumping based exercise in adolescents and adults with CD. The primary results indicate a good level of acceptability of jumping based exercise. Attitudes were positive towards participation in jumping exercise and future research studies exploring its utility as a complementary therapy in CD. Perceived difficulty of exercise participation was low, although some potential barriers to jumping exercise were also identified.

Epidemiological data suggests high levels of habitual PA may be protective against the onset of developing CD [395]. However, whether the implementation of PA or exercise in those with established CD may improve disease status remains unclear from the limited published data (Chapter 5). Seventy five percent of respondents regularly participated in one or more forms of exercise, suggesting this to be an active cohort. Team sports (41%) and walking (42%) were most common among adolescents and adults, respectively. Nearly all participants reported some benefits of exercise participation, with perceived improvements in psychological wellbeing (77%), physical strength and fitness (56%), and fatigue (31%) being most common. This agrees with prior exercise research in CD that reported improved HRQoL from exercise interventions, including walking [377,378], running [376], and resistance training [375,383].

Musculoskeletal deficits remain a commonly reported complication in studies of CD, despite contemporary therapies (Chapters 2 & 3). In this survey, around half of the respondents stated they were not concerned about their bone health. This may be linked to a lack of routine bone monitoring, which is generally reserved for those exposed to chronic GC [44]. Despite this, most respondents reported a willingness to investigate the role of jumping based exercise for improving bone health through research. Even among those who reported no current exercise, 80% stated a willingness to participate in exercise research. Almost all respondents (47/48) also believed participation in jumping based exercise would be worthwhile for potential improvements to bone health. Finally, the generally low perceived difficulty of jumping exercise among respondents highlights the potential feasibility of the intervention, even for those who were currently undertaking no exercise at all. This demonstrates good preliminary acceptability of jumping

exercise among adolescents and adults with CD, regardless of current activity levels. Intentions to participate in hypothetical research may not translate into consent for a clinical trial. For this reason, pilot studies of jumping exercise are warranted prior to designing larger controlled trials, to test its feasibility in practice.

As mentioned previously, research assessing the effects of high impact exercise on musculoskeletal health in CD is currently lacking. One study of combined resistance and impact exercise in middle-aged CD reported improvements in bone and muscle parameters compared to non-exercising controls [375]. Jumping exercise provides high mechanical strains that may induce skeletal adaptation more effectively than previous low-impact interventions in CD [320,379]. Jumping protocols have been observed to improve bone health in several paediatric populations [317]. Additionally, plyometric exercise has been reported to induce comparable lower limb muscle hypertrophy to resistance training in previously untrained adults [162]. These data suggest jumping exercise to be an effective strategy for improving bone and muscle that warrants investigation in young CD populations.

Individuals with CD experience multiple barriers to exercise participation. Interventions that can be easily implemented at home should therefore be given increased consideration. Over half of the current cohort stated that their CD diagnosis makes exercising more difficult. These figures are comparable with a previous survey of children with IBD (45%) [393] but lower than previously reported in adults with CD (81%) [272]. Primary barriers to exercise among these participants were fatigue and abdominal or joint pain, again like other reports [272,309,393]. Fatigue is among the most common clinical problems in CD [308] and has been previously associated with reduced PA [274]. Only one study, however, has assessed the effects of exercise in CD patients with fatigue at baseline, reporting marked improvements [383]. In addition to fatigue, fear of incontinence and subsequent social avoidance are highly prevalent among those with IBD [396]. This concern adversely affects QoL [396] and has also been cited as a barrier to exercise participation [272]. Implementing accessible, home-based exercise, such as a simple jumping protocol, may provide a suitable intervention for those who are unable or do not wish to seek out specialist exercise facilities due to these concerns.

Importantly, some potential barriers to participation in jumping exercise were identified in the current study. Exercise was reported to negatively influence fatigue and joint pain in some participants These were highlighted as potential concerns in free text responses. As mentioned, chronic exercise training may be an effective strategy for improving fatigue in chronic disease, including CD [383]. Joint pain, which is a common complaint in people with CD [397], is adversely affected by physical inactivity in inflammatory disease and can potentially be alleviated by regular exercise [398]. Even carefully implemented high-impact exercise has been reported as safe in cohorts with established joint degeneration, without any exacerbation of pain [399]. In the only study including assessment of impact exercise in CD, there were no adverse events reported relating to increased joint pain [375]. While these barriers to exercise cannot be discounted, strategies to educate individuals with CD on the potential alleviation of fatigue and joint pain via exercise may address their concerns. Nonetheless, careful consideration and detailed reporting of these should be a feature of any future exercise trials.

The results of this study are limited to respondents from a single geographical area and may not be representative of the wider CD population. The aim of the study, however, was to assess the acceptability of exploring jumping exercise as a potential complementary therapy for CD patients in this region. For this purpose, the survey has provided important positive feedback. Three quarters of participants currently undertook at least one form of exercise per week and the results could be confounded by non-response bias, whereby those interested in exercise were more likely to participate and thus provide a more positive outlook than may be expected from the whole CD population. Most participants also reported good recent disease control and jumping exercise could be viewed less favourably by those with ongoing active disease.

In conclusion, adolescents and adults with CD displayed positive attitudes towards the exploration of jumping based exercise as a complementary therapy for potentially improving bone health in CD. The results of this questionnaire suggest that jumping based exercise is an acceptable intervention for people with CD and pilot studies assessing the feasibility of such interventions will follow.

7 Feasibility of jumping based exercise in children and adolescents with Crohn's disease

7.1 Abstract

Background. Jumping based exercise is an accessible intervention that may reduce the musculoskeletal burden associated with paediatric CD. Prior studies have not investigated jumping exercise in paediatric CD, although survey results suggest this to be an acceptable mode of exercise in young people with CD.

Aim. Assess the feasibility and safety of a short-term, jumping based exercise intervention in children with CD.

Methods. A pilot study of four-weeks, primarily home-based, jumping exercise. The intervention consisted of three sessions per week, with approximately 50 – 100 loading cycles per session. Primary outcome was feasibility and acceptability, measured through recruitment rate and adherence to protocol. Secondary outcomes were HRQoL, fatigue, and muscle function assessed by mechanography.

Results. Six children with CD and median age 13.1 (10.2, 14.9) years participated in this study. Median baseline wPCDAI was 8.75 (0, 15) and 5/6 were in clinical remission. Median adherence to the exercise intervention was 95.8% (66.7, 100), equating to 11/12 sessions. Recruitment rate was lower than expected at 20.7%. No clear effect was observed for exercise on HRQoL, fatigue, or muscle function. A total of 23 AEs were reported, although these were all minor in nature.

Conclusions. Adherence to short-term exercise appears feasible in quiescent paediatric CD. Acceptability may be lower than expected as a recruitment rate of only 20% was observed. Expansion of the study will provide further insight into the potential effects of jumping exercise on muscle-bone outcomes.

7.2 Introduction

Musculoskeletal deficits remain a prominent issue in paediatric and adult CD despite modern therapies, yet few interventions have addressed this issue. A twelve-month intervention of daily vibration therapy was ineffective for improving bone mass compared to sham therapy in paediatric CD patients with low trabecular vBMD at baseline [320]. This level of mechanical stimuli may have been insufficient to upregulate accelerated bone formation in an ambulatory cohort, and greater mechanical loads may be necessary, potentially through targeted exercise interventions. In middle-aged adults with CD, low impact core strengthening [379] and resistance plus impact exercise [375] were associated with small improvements in aBMD [375,379] and large gains in muscle function of up to 50% [375]. These data highlight how exercise may successfully improve muscle-bone outcomes in adults with CD, but no studies have been conducted assessing its utility for augmenting muscle-bone in paediatric cohorts.

Despite the known beneficial effects of PA and exercise in muscle-bone development, no studies have assessed the utility of exercise for improving musculoskeletal outcomes in paediatric CD. One observational study reported a positive association between accelerometer measured MVPA and BMD in paediatric IBD [273], while self-reported PA was positively associated with trabecular bone volume and muscle CSA in the prior MRI study of paediatric CD reported in Chapter 2. The current paucity of exercise research in paediatric CD means the feasibility and utility of different modes of exercise are currently unknown. Jumping exercise has been demonstrated as an accessible and effective strategy for improving bone mass in other paediatric populations [117-119,317,392] and has been reported to be as effective as resistance at inducing muscle hypertrophy in untrained individuals [162]. Feedback from patient survey in Chapter 6 suggests this to be an acceptable method of exercise among young people with CD, with >80% of respondents stating they would be interested in participating in jumping based exercise research.

This study therefore aimed to assess the feasibility and safety of participation in jumping based exercise as a potential method for improving musculoskeletal outcomes in paediatric CD.

7.3 Methods

7.3.1 Study population

This was a pilot feasibility study of jumping based exercise in children and adolescents with IBD. For this work, only children with CD were targeted for recruitment in line with the other studies of this thesis. Recruitment activity commenced in October 2019 and participants recruited up to October 2020 were included in this write up. Eligible CD participants were identified and approached by a member of their clinical IBD team and asked if they were happy to be contacted by the research team. Eligibility criteria for this study were a confirmed diagnosis of IBD at least 6 months prior to intervention, age 10 to 17 years (inclusive), stable medication >4 weeks prior to recruitment, ability to mobilise and perform the exercises independently, and mild, inactive, or moderately active disease at time of recruitment (according to HBI). Exclusion criteria were recent (<12 weeks) or planned surgery, contraindication to jumping exercise, any significant comorbidity, medications known to affect bone turnover (excluding GC), BMI > +3.5 SDS or a bodyweight >120kg, and pregnancy. All participants provided written informed consent or assent based on competency, and a parent or legal guardian of all participants also provided written informed consent. The study was approved by the South East Scotland Research Ethics Committee 01 (REC Ref: 19/SS/0084). Participants were provided with £40 of gift vouchers to compensate for their time and effort during the study. They were advised that gift vouchers were not linked to their adherence to the protocol to explicitly remove this as incentive for improved participation.

7.3.2 Study design & intervention

This was a single-arm pilot study. Children and adolescents with CD were recruited and were enrolled into a four-week, home-based intervention of jumping exercise.

This four-week intervention consisted of exercises designed to dynamically load the muscle-bone unit, as high mechanical loading and plyometric movements are known to promote improvements in bone mass and muscle function. All participants were enrolled on the same four-week exercise intervention of three jumping based sessions per week (12 total sessions) (Appendix 4). The intervention

required no equipment other than an adjustable step, which was provided at the baseline assessment. Exercise intensity was progressed over the intervention period to increase mechanical loading and introduce plyometric movements. Basic movements such as vertical jumps, jumping jacks, and drop landings were progressed to movements including one-legged hopping, drop jumps, and plyometric jumps. Each session included between 50 – 110 loading cycles and took approximately 15 to 20 minutes to complete. Participants were instructed to complete a five-minute warm up prior to each exercise session. Participants were asked to attend the first exercise session of each week in-person at the clinical research facility, QEUH. These sessions were used to introduce the new exercises for the respective week and ensure participants were able to perform these safely and with the correct technique. Any concerns or required modifications to the protocol could also be discussed at these sessions. The remaining two exercise sessions were to be completed at home and participants were asked to leave at least 24 hours between sessions. Baseline and follow up visits were arranged within 14 days of the start and end of the intervention, respectively.

7.3.3 Data collection

7.3.3.1 Baseline characteristics

Disease specific data including age at diagnosis, current medications, history of GI surgery, and history of GC exposure were self-reported. Participants' height was measured to the nearest 1mm using a wall mounted stadiometer, weight was measured to the nearest 0.1kg, and BMI (kg/m^2) was calculated. Height, weight, and BMI were converted into age- and sex-specific Z-scores [400]. Anthropometrics were measured again at follow up. Pubertal status was self-reported according to Tanner. Fracture history and menarche (females) were also self-reported. Participants were provided a wrist worn accelerometer (ActiGraph wGT3X-BT, ActiGraph Corp, FL, USA) to wear for seven days after their baseline assessment to measure habitual PA. Accelerometers were worn on the non-dominant wrist and sampled at a frequency of 30Hz. Previously published activity cut-offs for children were used to define time spent in light, moderate, and vigorous PA [401]. PA outcomes of interest were how much time was spent in each domain of PA and sedentary behaviour, and whether the participants were meeting the current guidelines of 60 mins of MVPA per day.

7.3.3.2 Primary outcomes

This was a pilot feasibility study and the primary outcomes assessed were rate of recruitment and adherence to the protocol. Recruitment rate was reported as a percentage of the total individuals approached who consented to take part in the study. Adherence to the protocol was self-reported by the participants. Each participant was provided an exercise diary to be completed after each session. This diary contained specific guidance on how to complete the prescribed exercises, in case participants were unable to attend any of the in-person study visits. The specific sets and repetitions prescribed for each session were also listed. Participants recorded the number of sets and repetitions they completed of each of the prescribed exercises during the session. If the session was missed for any reason, they were requested to provide a reason for this. Exercise safety was also recorded with participants asked to record if they experienced any pain either during or after exercise, and any trips or falls during the exercise. Participants were also asked to self-report any exacerbations of disease symptoms.

7.3.3.3 Secondary outcomes

7.3.3.3.1 Disease activity, HRQoL, and fatigue

To assess any potential effects of short-term jumping-based exercise several secondary outcomes were measured at baseline and follow up. Disease activity was assessed using the wPCDAI (CD). HRQoL was assessed using the paediatric IBD specific IMPACT III questionnaire [402]. IMPACT III is a 35-item, self-administered questionnaire regarding aspects of HRQoL including general wellbeing, emotional and social functioning, and disease symptoms over the previous two-week period. Questions are answered on a five-point Likert scale with total scores ranging from 35 (poor) to 175 (best). A cut off value of 143 points has been suggested for remission, and an improvement of ≥11 points reflects a clinically meaningful change [402].

Generic HRQoL was assessed using the PedsQL Generic Core Scale v4.0 [403] and fatigue was assessed using the PedsQL Multidimensional Fatigue scale [404]. These tools assess child and parent perspectives via self-administered and parent proxy questionnaires. The acute versions of these tools were used, which assess HRQoL

and fatigue over the previous seven-day period. Results are transformed onto a linear scale of 0 (worst) to 100 (best). Both tools have been previously validated in healthy children and paediatric IBD and found to negatively associate with disease activity [405,406].

7.3.3.3.2 Muscle function assessment

Assessment of the muscle unit was conducted using jumping mechanography at baseline and follow up, as described in Chapter 2. Briefly, the S2LJ and M1LH tests were used, providing data for dynamic muscle function (power [kW], power relative to bodyweight [W/kg]) and maximum applicable force to the muscle-bone unit (force [kN] and force as a multiple of bodyweight), respectively. Results were converted into and weight-adjusted, sex-specific Z-scores using previously published data [202].

7.3.3.3.3 Biochemical analyses

At baseline and follow up, non-fasted blood samples were drawn to evaluate potential effects of exercise on biomarkers of bone, muscle, and inflammation. Serum levels of 25-OHD, PTH, ESR, CRP were measured at the local NHS accredited clinical laboratory, following standard procedures.

7.3.3.4 Statistical Analyses

The primary outcomes of this feasibility study were descriptive statistics regarding recruitment rate and protocol adherence, which are reported as such. Due to the very small sample size and lack of follow up data available for two participants, no formal statistical testing was performed. All data are presented descriptively, and secondary outcome data reported at the individual participant level.

7.3.4 Impact of COVID-19 on this study

All non-essential clinical research activity in NHS GGC was suspended in March 2020 for a period of approximately six months because of the COVID-19 pandemic. This had a profound negative impact on recruitment and data collection for this study. Prior to the suspension of recruitment, three individuals who expressed an interest in participating in the study were in contact with the research team (LS).

These individuals were re-contacted when recruitment activity resumed but were no longer interested in participating. When study activity was suspended, two participants were currently on study. At this point, one participant was entering the final week of the intervention and the other was approximately halfway through the intervention. These two participants were unable to attend a follow up visit and therefore did not have follow up data available for secondary outcomes. They did, however, have data available for the primary outcome via self-completed exercise diaries. These diaries were collected in October 2020, after study activity was approved to resume. Recruitment activity resumed in late September 2020 and three further participants were recruited. Recruitment activity post-lockdown was impacted by much reduced clinical activity at RHC, including no regular IBD outpatient clinic, which moved to online. Those who were recruited post-lockdown attended only baseline and follow up visits and completed the four-week intervention at home, with weekly telephone or email contact. As the original ethical approval allowed for participants to complete the four-week intervention exclusively at home, this did not require an amendment to the protocol.

7.4 Results

7.4.1 Recruitment

Recruitment for this study occurred across approximately seven months within the period of October 2019 – October 2020. A targeted recruitment approach was taken involving pre-screening of potential participants with the paediatric IBD research nurse. A total of 41 children with CD were referred for potential inclusion in this study, of which 29 were approached to participate. Potential recruits were sent study information by post and followed up at outpatient clinic appointments where possible or contacted directly by the research nurse. Of the 29 children and adolescents who were approached, 17 refused and 12 expressed an initial interest in participating in the study. Reasons cited for refusal included general non-interest in research (n = 4), no interest in the proposed exercise (n = 2), concerns exercise exacerbates fatigue (n = 1), a recent injury (n = 1), and being too busy with school (n = 3). The other six refusals did not provide a specific reason. From the 12 interested in participating, a further six dropped out after initial interest due to concerns regarding the ongoing COVID-19 situation (n = 3), fatigue (n = 1),

a current disease flare (n = 1), and failure to reply (n = 1). Finally, six participants were recruited to the study, resulting in a successful recruitment rate of 20.7% of the patients approached.

7.4.2 Baseline cohort characteristics

Six children (4 male, 2 female) with CD consented to participate in this study. Median age at baseline was 13.1 (10.2, 14.9) years and median age at diagnosis was 8 (7, 11) years. Median wPCDAI score was 8.75 (0, 15) and 5/6 were in clinical remission, with one having mildly active disease. All six participants were currently treated with anti-TNF-α biologic therapy (5 infliximab, 1 adalimumab) and concurrent immunosuppression (4 azathioprine, 1 methotrexate, 1 6-mercaptopurine). Only one participant had a history of GI surgery and two had previously been treated with GC. Median self-reported Tanner stage of puberty was 2.5 (1, 5) and both girls had achieved menarche. None of the participants reported a history of fracture. Median self-efficacy for exercise at baseline was 68.5 (52, 80). All participants were meeting current PA guidelines with a median of 126 (73, 81) minutes of MVPA on average per day. Percentage of time spent in sedentary behaviour was 51.2% (47.3, 54.2), in light PA was 38.4% (35.1, 41.8), and in moderate PA was 11.5% (5.5, 14.5). Participation in vigorous PA was zero percent for all participants.

7.4.3 Exercise adherence

No participants formally withdrew from the study, although two did were unable to attend a follow up visit due to COVID-19 lockdowns. All six participants completed and returned the self-reported exercise diaries. Median self-reported adherence to the exercise intervention was 95.8% (66.7, 100), which equated to a median 11/12 sessions completed. Overall, 65 out of 72 total sessions were completed. In 64/65 completed sessions, participants self-reported completing 100% of the prescribed sets and repetitions of jumping-based exercises. Reasons provided for not completing exercise sessions included forgetting (n = 4), not feeling ‘up for it’ (n = 1), abdominal pain (n = 1), and a non-related injury (n = 1).

7.4.4 Exercise safety

There were no self-reported exacerbations of disease symptoms during the intervention period and clinical activity scores remained low at follow up in the four participants with available data (median wPCDAI 3.75 [0, 12.5]). A total of 23 AEs were reported during the intervention. Median self-reported AEs per participant was 2.5 (1, 10). Twenty-two of 23 AEs were related to the exercise intervention (sore knee from unrelated fall [n = 1]). All reported AEs were minor, and no SAEs were reported. Exercise related AEs were pain in the forefoot during hopping or plyometric type exercises (n = 10), non-specific knee pain (n = 4), ankle pain during hopping or plyometric exercises (n = 3), trip or fall during exercise (n = 2), back pain (n = 1), stitch (n = 1), knee 'gave way' the day after exercise (n = 1). One participant, who completed the intervention exclusively at home, reported 9/10 AEs relating to sore feet during hopping or plyometric exercises. This was not raised as a concern during weekly contact by the participant or their parent, so no adjustments to the protocol were made. None of the reported AEs were cited as a reason for subsequent non-participation in exercise.

7.4.5 HRQoL and fatigue

Individual participant data for HRQoL and fatigue are presented in Figure 7-1. Median IMPACT III score was 155 (136, 166) and 159 (140, 164) at baseline and follow up, respectively (Figure 7-1 A). Median change of IMPACT III score was -1.5 (-4, 9) (n = 4). Self-reported HRQoL scores were 77.2 (66.4, 96.7) and 83.9 (80, 94.2) at baseline and follow up, respectively (Figure 7-1 B). Parent proxy HRQoL scores were 80.2 (67.2, 94.2) and 74.3 (65.3, 97.5) at baseline and follow up, respectively (Figure 7-1 C). Median changes in the self-reported and parent proxy HRQoL scores were 3.7 (-4.8, 13.6) and -0.7 (-5.8, 4.1), respectively (n = 4). Median self-reported fatigue scores were 68.8 (59.7, 83.3) and 66.0 (45.8, 94.4) at baseline and follow up, respectively (Figure 7-1 D). Median parent proxy fatigue scores were 59.7 (51.4, 95.8) and 51.4 (38.9, 98.6) at baseline and follow up, respectively (Figure 7-1 E).

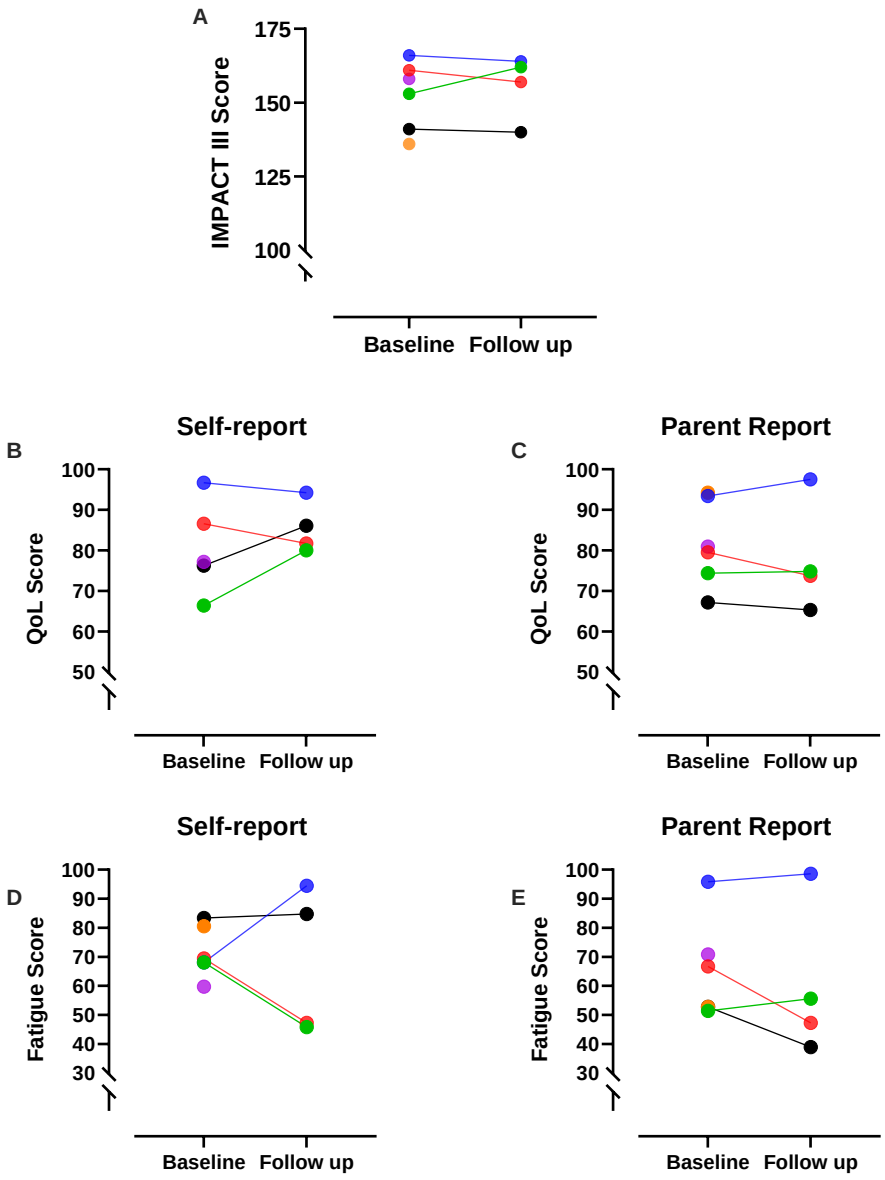

Figure 7-1 HRQoL and fatigue in paediatric CD.
Individual data for HRQoL and fatigue before and after four weeks of jumping based exercise. Each participant is represented by an individual colour. Participant 1 – black, 2 – purple, 3 – orange, 4 – red, 5 – blue, 6 – green. No follow up data were available for participants 2 & 3. Data presented are for disease specific HRQoL (IMPACT III) (A); self-reported and parent proxy generic HRQoL (PedsQL generic core scale) (B & C); self-reported and parent proxy generic fatigue (PedsQL Multidimensional fatigue scale) (D & E). For all assessment tools, higher scores are better.

7.4.6 Muscle-bone unit

Weight-adjusted Z-scores for dynamic muscle power and maximum force applicable are presented in Figure 7-2. Median weight-adjusted Z-scores for absolute muscle power were -0.4 (-1.73, 0.5) and -0.57 (-1.47, 0.68) at baseline and follow up, respectively (Figure 7-2 A). Weight-adjusted Z-scores for relative muscle power were -0.31 (-1.68, -0.1) and -0.36 (-1.56, -0.01) at baseline and follow up, respectively (Figure 7-2 B). Median weight-adjusted Z-scores for

absolute force were -1.87 (-2.86, 0.39) and -0.77 (-2.62, 0.33) at baseline and follow up, respectively. (Figure 7-2 C). Median weight-adjusted Z-scores for force relative to bodyweight during hopping were -2.15 (-2.51, -0.42) and -1.06 (-2.03, -0.45) at baseline and follow up, respectively (Figure 7-2 D).

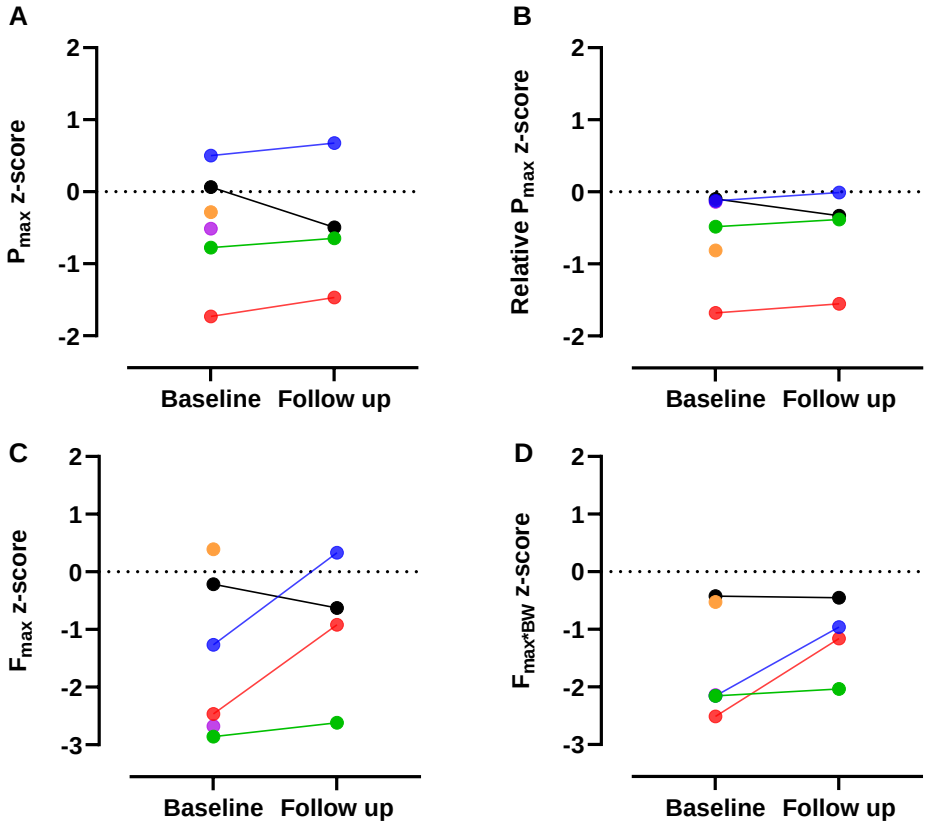

Figure 7-2 Weight-adjusted Z-scores for jumping mechanography in paediatric CD.
Individual data for weight-adjusted, sex-specific Z-scores for jumping mechanography before and after four weeks of jumping based exercise. Participant 1 – black, 2 – purple, 3 – orange, 4 – red, 5 – blue, 6 – green. No follow up data were available for participants 2 & 3. Data presented are for absolute (A) and relative (B) muscle power during countermovement jumping, and absolute (C) and relative (D) force generated during one legged hopping.

7.5 Discussion

This study provides preliminary data on the feasibility and safety of jumping based exercise in children and adolescents with CD. Despite the very small sample size, high adherence to the intervention suggests that jumping based exercise is a feasible mode of exercise, at least in children with well controlled CD. Recruitment activity was disrupted by the COVID-19 pandemic and it is unclear how much this contributed to the low recruitment rate of 20%. Jumping based exercise was safe, as no exacerbations of disease symptoms were reported, although several minor AEs were reported; suggesting greater supervision or a slower progression of exercise intensity may be required.

Exercise may be an effective strategy for improving bone and muscle mass in children with CD. Particularly, jumping based exercise interventions have been shown to increase bone mass and strength in various paediatric populations [117-119,317]. Despite this, the feasibility of impact exercise in paediatric CD had not been explored to date. In this study, six children with CD completed a jumping based exercise intervention with high levels of adherence. Median adherence was >95% and only one participant reported an adherence below 80%. This participant was on study at the time of COVID-19 national lockdown, which may have contributed to their non-adherence, as they had completed 5/6 of sessions until that point but only completed three of the remaining six. Adherence to short term exercise therefore appears feasible, albeit in a very small sample. This agrees with the data from Chapter 6 stating that most children with CD would find jumping based exercise easy to participate in. However, whether short-term adherence translates into long-term participation in jumping based exercise requires future investigation. One study reported good adherence in the first four weeks (median 72.5%) of a six-month exercise intervention aimed at improving muscle-bone outcomes, including jumping exercise, in juvenile arthritis [407]. Adherence rapidly dropped off after this period and six-month median adherence was only 47%. At follow up, no improvements in DXA, HR-pQCT or mechanography assessed Z-scores for muscle-bone outcomes were reported [407].

Jumping exercise was not associated with any worsening of subjective disease symptoms or disease activity scores, suggesting it to be safe for children with mild CD. This concurs with all previous exercise research in CD, in which no exercise

related exacerbations of disease have been reported (Chapter 5), although primarily in adults. Despite this, several minor AEs were reported that were specific to the type of exercise being employed. Particularly, several AEs occurred during plyometric type movements, such as pain of the forefeet and joints. However, although several AEs were reported, these did not negatively impact exercise adherence. Additionally, none of the participants reported AEs during weekly contact and no adjustments to the protocol were required, suggesting these were likely transient and mild in nature. Nevertheless, a slower progression to plyometric movements and increased supervision and teaching of exercise techniques may be warranted.

This cohort, despite meeting the criteria of no more than two hours of leisure time weight bearing exercise per week, contained participants who did routinely exercise. According to baseline accelerometer data, all participants were meeting the guidelines of >60 minutes of MVPA per day. This suggests potential selection bias within the cohort of children who have interests in exercise being more likely to take part. Two participants indicated they did not participate in any leisure time activity which was reflected in their lower habitual MVPA compared to the others. Despite this, these participants still met the recommended guidelines of >60 minutes of MVPA on average per day. The recruitment of children not currently meeting PA guidelines would provide more information on whether jumping exercise is feasible in an inactive paediatric CD cohort, although being able to successfully screen inactive children based on self-reported PA is difficult.

Exercise has been shown to have a moderate positive influence on HRQoL in adults with CD [376-378,383], but no effects were observed in two paediatric studies to date [384,386]. The influence of exercise on HRQoL in CD may be mediated by its effects on fatigue, physical function, or body composition. Here, descriptive data suggests short-term jumping exercise did not have any effect on disease specific HRQoL scores. Self-report of disease specific and generic HRQoL scores were generally high at baseline and remained so at follow up, suggesting a potential ceiling effect. Discrepant results were found between participants for fatigue, although these data are hindered by the very small sample size.

Baseline jumping mechanography produced weight-adjusted Z-scores that were generally below reference values, particularly for muscle assessment during one

legged hopping, suggesting abnormal function of the muscle-bone unit. At follow up, improvements in Z-scores for absolute and relative force were observed in two participants, which could be linked to neuromuscular adaptations associated with short-term jump training [408,409]. A descriptive upward trend in all mechanography Z-scores was observed for 3/4 participants although the relevance of these changes is unclear. One participant had lower muscle power Z-scores at follow up compared to baseline, despite reporting full adherence to exercise. This may have been linked to underlying iron deficiency that was subsequently identified shortly after study cessation and treated clinically. While this study was not powered to detect changes in mechanography outcomes, these initial data suggest short-term improvements are possible. Jumping mechanography may, therefore, be a sensitive marker for evaluating adaptations in muscle function after short-term exercise even in children who currently meet PA guidelines.

This study was primarily limited by its very small sample size. The 20% recruitment rate is in stark contrast to the data collected in Chapter 6, which suggested >80% of CD patients would be interested in participating in jumping based exercise. Even among the survey respondents who did not currently participate in any exercise, 80% expressed an interest in research participation. This perhaps suggests that assessing feasibility of exercise interventions in this group of patients via a survey may provide quite inaccurate information on possible recruitment rate. However, the negative influence of COVID-19 on study recruitment cannot be disregarded and COVID-19 concerns were understandably cited by several potential recruits as a reason for non-participation. The study would have benefited from subjective feedback regarding the acceptability of jumping based exercise to guide the development of a more definitive trial in this population of patients. Despite these limitations, some useful preliminary data has been generated and the expansion of the study in future will provide a deeper understanding of the feasibility of jumping exercise in paediatric CD.

In conclusion, this study provides preliminary data on the feasibility of jumping based exercise in children with well controlled CD. Adherence to short-term exercise appears feasible in this population. However, the acceptability of exercise participation may be lower than previously stated as reflected by a low recruitment rate. Improving the sample size will provide further data regarding

exercise feasibility and the potential influence on markers of the muscle-bone unit in CD.

8 General discussion and future directions

8.1 General discussion

Crohn's disease is associated with multifactorial insult to the muscle-bone unit. Paediatric CD is associated with reduced bone and muscle mass and strength that, if unresolved, may predispose to increased risk of adverse musculoskeletal outcomes as adults. Despite this, few studies have performed a comprehensive evaluation of the muscle-bone unit in children and adults with CD using high-resolution imaging. As such, there is a dearth of information regarding skeletal microstructure in young populations of CD, particularly in contemporary cohorts. Such detailed evaluations of bone and muscle health in young CD cohorts may identify specific deficits that can be targeted in future interventions.

Exercise is an effective strategy for improving bone and muscle health across the lifespan, yet its utility in CD has not been well established. This thesis aimed to characterise the muscle-bone unit in children and young adults with childhood onset CD. Furthermore, the associations between IBD and adverse musculoskeletal outcomes in middle aged and older adults were investigated in a large population-based cohort study. Finally, the potential role for jumping based exercise in CD was explored via an online survey and short-term feasibility intervention of

8.1.1 The muscle-bone unit in children & adults with CD (Chapters 2, 3, & 4)

The first half of this thesis was dedicated to investigating the muscle-bone unit and adverse musculoskeletal outcomes in paediatric (Chapter 2), young adults (Chapter 3), and middle-aged and older adults with CD (Chapter 4). In each of these studies, CD was associated with musculoskeletal deficits, although with some discrepancy. High-resolution MRI revealed deficits in trabecular microarchitecture in paediatric CD but not young adults with paediatric onset CD, when compared to respective healthy age and sex matched control cohorts. Estimated BMD of the calcaneus, a trabecular rich region, was also low in the older CD cohort compared to controls, indicative of trabecular bone deficits. Discordant trabecular results between paediatric and young adult cohorts opens questions regarding the natural history of trabecular development in paediatric CD. Early

trabecular loss, or impaired development, in paediatric CD may be alleviated by effective long-term disease control with contemporary therapies, which have been found to improve biochemical markers of bone and muscle development [49,217,339]. Short term prospective studies (12 - 24 months follow up) have reported only modest improvements in muscle-bone deficits [49,217,225]; however, longer term contemporary disease management may facilitate continued normalisation of previously incurred skeletal deficits into young adulthood. Disease severity and prior GC exposure were both negatively associated with trabecular bone volume in these studies, while a proxy of disease severity (i.e. current immunosuppressant use) also negatively associated with eBMD in the older cohort. These factors are therefore again highlighted as primary precipitators of poor bone health in CD across different age groups, even in those with generally mild disease at time of study. The implications of skeletal deficits were highlighted in the middle-aged CD cohort as low eBMD was associated with likelihood of any fracture in this group.

Disparity in trabecular bone between paediatric and young adult cohorts could also be moderated by the influence of PA and mechanical loading on recovering disease or GC mediated bone loss. The importance of PA for trabecular bone development has been demonstrated in children with impaired mobility, who display marked trabecular deficits [115]. In paediatric CD, PA was positively associated with trabecular bone volume. Those with combined low PA and trabecular bone volume also tended to have a history of severe disease. This may have reduced tolerance or motivation for PA, subsequently inhibiting the trabecular bone development. In young adults, no association between PA and trabecular bone was discovered, but all PA scores reported were indicative of being physically active. Of course, the two studies presented here are independent, cross-sectional CD cohorts, and longitudinal assessment of trabecular bone in a large cohort of paediatric CD into young adulthood would be required to test these hypotheses.

Deficits in skeletal muscle were consistent across all the CD cohorts. Paediatric and young adults with CD had low muscle function compared to controls and reference data, respectively. Muscle mass was also low in the young adult cohort but not in the paediatric cohort, likely due to the large heterogeneity of muscle mass in children matched by age only. Jumping mechanography identified muscle

function deficits in paediatric CD that were not evident using grip strength dynamometry. Therefore, mechanography may be a more sensitive and useful tool for muscle function assessment in younger populations. The ability to dynamically assess several components of the muscle-bone unit using typical paediatric movement patterns makes mechanography easy to implement and preferable over the limited data available via isometric grip dynamometry. In both cohorts, after size adjustment, muscle function remained low. This suggests some intrinsic cause of reduced muscle function in CD that requires future investigation. Accurately defining muscle function deficits and their associated mechanisms in young CD cohorts may facilitate the development of targeted interventions, ultimately reducing risk of future adverse musculoskeletal events. Such events, including muscle weakness indicative of sarcopenia and falls, were significantly more likely in middle aged and older adults with CD compared to non-IBD peers. These issues were present despite representing a mild disease cohort, again highlighting the musculoskeletal burden of CD even in those with mild disease. The increased risk of adverse musculoskeletal outcomes in the older CD cohort has extended implications for younger CD populations. Childhood onset CD is generally accepted as a more severe disease phenotype than adult-onset disease. Childhood onset CD may therefore be associated with higher risk of poor long term musculoskeletal outcomes compared to adult-onset disease, as has been demonstrated for risk of fractures [324]. Together, these data emphasise the importance of addressing muscle-bone deficits early in the disease course in young people with CD.

The studies highlight the sensitivity of high-resolution MRI for identifying trabecular differences between cases and controls. Conversely, cortical geometry results were somewhat surprising. No deficits in cortical geometry between CD and control groups were observed, which is feasible considering no evidence of growth disturbance in either CD cohort. However, previous data consistently indicate cortical deficits in paediatric [49,217,276,277] and adult CD [282,283] using pQCT or HR-pQCT. This discrepancy could indicate the limitations of MRI for cortical assessment in CD. The hypointense signal of cortical bone and surrounding connective tissue, and corticalisation of peripheral trabeculae at the endocortical boundary of the metaphysis, make clear definition of the periosteal and endosteal circumferences difficult. Resultantly, subtle differences in cortical geometry may not have been identified, making these analyses less useful in mild disease

cohorts. The calculation of cortical thickness was also based on assumption of concentric cortical bone, which did not represent the asymmetrical geometry observed in most participants. Similarly, lumbar spine BMA was heterogeneous among paediatric and young adult CD and does not appear to be a useful outcome in these groups, although its association with patients with greater degree of inflammation deserves future investigation.

8.1.2 The potential role for exercise in improving muscle-bone in CD (Chapters 5, 6, & 7)

The second half of this thesis investigated the evidence and potential utility for exercise as an intervention to improve bone and muscle in children and adults with CD. Systematic review revealed sparse evidence for exercise in IBD, with only one prior study designed to address this issue (Chapter 5). In paediatric IBD, the evidence base for utility of exercise was almost non-existent, despite its potential to improve muscle-bone development. Themes among the limited evidence base included a mild positive effect of exercise on HRQoL and improvements in markers of physical function. Importantly, few adverse events and no exacerbations of disease related to exercise had been reported, although generally the reporting of these was poor. One method of exercise not studied in children or adults with CD was jumping based exercise, which may be an effective and accessible intervention for improving muscle mass and function and bone health.

The feasibility of jumping based exercise was surveyed in local adolescents and adults with CD, with positive feedback (Chapter 6). Attitudes towards exercise were positive and almost all respondents agreed that participation in jumping exercise would be worthwhile for potential musculoskeletal improvements. In survey, intentions to participate in jumping based exercise research were high, with >80% saying they would participate. However, in practice, the recruitment rate in paediatric CD patients for a short-term jumping-based exercise intervention was much lower at 20% (Chapter 7). This suggests a selection bias among survey respondents, although 80% of those who reported current participation in no exercise also stated an interest in research participation. The discrepancy between signalled interest and actual uptake may represent difference in attitudes between respondents versus those approached for the intervention study. Nonetheless, this brings into question the relative usefulness

of small patient surveys for assessing feasibility of future interventions. The direct implementation of feasibility interventions with close monitoring of recruitment uptake and reasons for non-enrolment may be more useful for gathering information about the relative feasibility and acceptability data. Recruitment activity for the intervention is likely to have been negatively impacted by the Covid-19 pandemic and the lower-than-expected recruitment rate was reflective of this. Several prospective participants cited the pandemic as a reason for not enrolling and recruitment would likely have been more successful under different circumstances. In the small cohort who did participate, jumping based exercise was a feasible intervention, highlighted by the high overall adherence. Minor AEs were common and linked to more high-impact exercises, suggesting more supervision or slower progression of exercise intensity may be preferable. This provides useful preliminary data on jumping exercise in paediatric CD which should be expanded on in future as a potential intervention for improving the functional muscle-bone unit.

8.2 Conclusion

In conclusion, the data presented in this thesis has provided novel insights into the macro- and microarchitecture of the muscle-bone unit in contemporary cohorts of children and young adults with CD, using high-resolution MRI. Despite advances in therapies, mild CD remains associated with adverse musculoskeletal outcomes, including low trabecular bone volume in paediatrics and low muscle mass in young adults. Poor muscle function was characteristic in these populations and present at increased rates and linked to an increased risk of falls in early old adults with CD. In all, these data further highlight associations between CD and poor muscle-bone outcomes and underline the need for effective strategies to combat this. This research will help inform the development of future prospective and interventional studies aiming to characterise the natural development of muscle and bone in young CD populations, risk of adverse musculoskeletal outcomes in older adults with CD, and investigation the role of exercise in alleviating musculoskeletal and other complications of disease.

8.3 Future directions

The data presented can provide a framework upon which several future avenues of research can be based. The primary areas of research that can be expanded upon from this work include further development of high-resolution MRI assessment of bone; investigation of the underlying mechanisms for muscle dysfunction in CD; prospective assessment of musculoskeletal outcomes in young and older populations with CD; and further exploration of the feasibility and utility of exercise for the management of muscle-bone deficits and other secondary complications of CD.

This work and previously published studies have highlighted MRI as a promising tool for holistically assessing the muscle-bone unit at both the macro and microstructural level. If MRI is to advance towards a clinically viable method of assessing skeletal health, there is a need to develop standardised methods of assessment and to explore the utility of commercially available MRI pulse sequences. Currently, no accepted standard for MRI assessment of bone exists, meaning studies - including those reported here - have implemented custom pulse sequences at independently selected ROIs. Here, data are reported from 15% distal femur; an area representative of the distal metaphysis that should be further developed as a standardised ROI. This ROI provides clear delineation of the trabecular microarchitecture and sufficient cortical geometry to make a combined assessment of these compartments at the same level. Other studies have utilised the growth plate as a reference point, which adds subjectivity to analyses and cannot be applied in adults. Furthermore, although uncommon in CD, the distal femur is a common site of major osteoporotic fracture and therefore a clinically relevant site for skeletal evaluation in populations with severe skeletal phenotypes. Further developmental work is required using high field (≥3 Tesla) and ultra-high field (≥7 Tesla) MRI scanners to optimise pulse sequences for assessment of skeletal micro and macrostructure and to identify the parameters that may be the most useful prognostic indicators of skeletal fragility. Investigations of the repeatability of longitudinal MRI scanning of bone is also required as there is currently no data on the variability between scans taken at different timepoints, which currently limits the certainty of any observed longitudinal.

Muscle function deficits are a consistent problem across the lifespan in CD, even in cohorts of mild or well controlled disease managed with contemporary therapies. Historically, GC exposure and inflammation have been implicated in CD muscle dysfunction as these are known catabolic factors. However, particularly in young populations, contemporary management increasingly involves the use of anti-cytokine antibodies for induction and maintenance therapy, and very limited GC exposure. In these cohorts, therefore, it is unclear whether subclinical inflammatory activity contributes to skeletal dysfunction or whether other unknown factors are involved. Detailed assessments of skeletal muscle in a range of CD populations including young and old, and treated and non-treated, may provide insight into potential mechanisms of muscle dysfunction that could become therapeutic targets in future.

The current studies revealed various degree of musculoskeletal abnormality in separate cohorts of CD. What remains unknown, is the natural history of musculoskeletal development in children diagnosed with CD. Some studies suggest a potentially protracted period of skeletal growth in children with well controlled CD, potentially facilitating the improvement of any previously incurred musculoskeletal deficits. To adequately assess this, a large prospective study of muscle-bone assessment in children diagnosed with CD into young adulthood would be required. The use of MRI as a repeat assessment tool could be applied to such a study due to its lack of ionising radiation and ability to assess multiple components of muscle-bone in a single series of scans. This would provide insight into the longitudinal changes of bone and muscle in children with CD and how these relate to clinical and environmental factors.

The future projected increases in CD prevalence, particularly older patients, underlines the necessity to understand the risk of adverse musculoskeletal outcomes and their effects on daily living in this population. The use of population databases such as the clinical practice research datalink would provide access to prospectively study a non-selected cohort of CD patients with comprehensive primary care and linked data, including prescriptions and hospital episode statistics. Such studies may be able to provide perspective into clinical risk factors of CD that are associated with poor bone and muscle in older adults and how these relate to activities of daily living.

The preliminary data on exercise feasibility reported here should be built upon in future studies. Particularly, the effects of jumping and resistance exercise should be explored as a method for augmenting muscle-bone development and function in young CD populations. The potential added benefit of PA or exercise for improving bone and muscle mass could be assessed in observational or intervention studies. For example, a prospective cohort study of paediatric CD enrolled at the time of anti-TNF-α induction and followed up for 12 to 24 months with periodic assessment of habitual PA by accelerometry. This format of study has been successfully implemented previously when assessing the independent effects of treatment on muscle-bone outcomes in CD, and the addition of habitual PA monitoring could be easily implemented. A more ambitious study may involve a randomised controlled trial of resistance and impact exercise versus standard care in young people with CD at the point of transitioning to maintenance anti-TNF therapy. Not only would this provide data on the effects of exercise for improving muscle-bone but could also provide insight into the potential interactions between exercise and pharmacotherapy. The current lack of data regarding the role of exercise in the management of CD means this should be an active area of investigation not only for improving muscle-bone outcomes, but also other secondary complications of disease.

Appendices

Appendix 1: Roles and responsibilities within each study/chapter.

Chapter 2: Paediatric MRI study.

- Study design (**LS** & Dr Jarod Wong)
- Submission for ethical and R&D approval (**LS**)
- Recruitment of CD group (**LS** & Paediatric IBD Team at RHC, Glasgow)
- Recruitment of healthy controls (Dr Huda Elsharkasi [for separate MRI study])
- Study visits and data collection (**LS**)
- Blood samples (Research nurses at CRF)
- MRI Scans (Research radiographers)
- MATLAB code for MRI analyses (Dr Blair Johnston, Dr Christie McComb & Dr John Foster, NHSGGC Medical Physics)
- All MRI & MRS analyses (**LS**)
- Data curation and statistical analyses (**LS**)
- Write up (**LS**)

Chapter 3: Young adult MRI study.

- Study design (Jarod Wong)
- Ethical & R&D approvals (Jarod Wong)
- Recruitment (Jan – Sept 2017 = research nurses; Oct 2017 onwards = **LS**)
- Study visits and data collection (**LS** and Research nurses at CRF)
- Blood samples (Research nurses at CRF)
- Biochemical analyses of blood samples (LS and Martin McMillan)
- MRI Scans (Research radiographers)
- MATLAB code for MRI analyses (Dr Blair Johnston, Dr Christie McComb & Dr John Foster, NHSGGC Medical Physics)
- All MRI & MRS analyses (**LS**)
- Data curation and statistical analyses (**LS**)
- Write up (**LS**)

Chapter 4: Associations between IBD and musculoskeletal outcomes.

- UK Biobank study design, approvals, & data collection (UK Biobank)
- Design and analysis conception (**LS**)
- Data curation and cleaning (**LS**)
- Statistical analyses (**LS**)
- Write up (**LS**)

Chapter 5: Systematic review of exercise in IBD.

- Study conception and design (**LS**)
- Search strategy development (**LS**)
- Literature searches (**LS**)
- Study assessment and selection for review (**LS**)
- Study quality assessment (**LS** [primary], Jarod Wong [secondary, for quality control])
- Write up (**LS**)

Chapter 6: Feasibility questionnaire.

- Study conception and design (**LS**, Jarod Wong & Stuart Gray)
- Questionnaire development (**LS**)
- Questionnaire content validity check (Paediatric & Adult Gastroenterologists)
- Questionnaire readability (paediatric & adult laypersons)
- Data collection (**LS**)
- Data curation and analyses (**LS**)
- Write up (**LS)**

Chapter 7: Feasibility of jumping based exercise in paediatric CD

- Study conception and design (**LS,** Jarod Wong & Stuart Gray)
- Ethical & R&D approvals (**LS**)
- Recruitment (**LS** & Paediatric IBD Research nurse)
- Study visits & data collection (**LS**)
- Data curation and analyses (**LS**)
- Write up (**LS**)

Appendix 2: Systematic Review Search Strategy (Chapter 5)

EMBASE (1996 to 2021 Week 14)

1. exp inflammatory bowel disease/
2. exp Crohn disease/
3. exp ulcerative colitis/
4. (inflammatory bowel disease or ibd or ulcerative colitis or crohn* disease).tw.
5. 1 or 2 or 3 or 4
6. exp exercise/
7. exp kinesiotherapy/
8. exp fitness/
9. exp exercise test/
10. exp physical activity/
11. exp sport/
12. (exercis* or physical activit*).tw.
13. 6 or 7 or 8 or 9 or 10 or 11 or 12
14. clinical trial/
15. exp controlled clinical trial/
16. exp case control study/
17. exp pilot study/
18. "review"/
19. intervention.tw.
20. (randomi* control* trial or RCT).tw.
21. 14 or 15 or 16 or 17 or 18 or 19 or 20
22. 5 and 13 and 21

Results – 605

MEDLINE (1946 to April Week 2 2021)

1. exp Inflammatory Bowel Diseases/
2. exp Crohn Disease/
3. exp Colitis, Ulcerative/
4. (inflammatory bowel disease* or ibd or ulcerative colitis or crohn* disease).tw.
5. 1 or 2 or 3 or 4
6. exp EXERCISE/
7. exp Exercise Therapy/
8. exp Exercise Movement Techniques/
9. exp Physical Fitness/
10. exp Exercise Test/
11. exp SPORTS/
12. (exercis* or physical activit*).tw.
13. 6 or 7 or 8 or 9 or 10 or 11 or 12
14. exp Clinical Trial/
15. exp Controlled Clinical Trial/
16. exp Pilot Projects/
17. exp Case-Control Studies/
18. exp "REVIEW"/
19. intervention.tw.
20. (randomi* control* trial or RCT).tw.
21. exp Prospective Studies/
22. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21
23. 5 and 13 and 22

Results – 233

COCHRANE Database (Issue 3 of 12, March 2021)

#1 MeSH descriptor: [Inflammatory Bowel Diseases] explode all trees
#2 MeSH descriptor: [Crohn Disease] explode all trees
#3 MeSH descriptor: [Colitis, Ulcerative] explode all trees
#4 (inflammatory bowel disease* or IBD or crohn* disease or ulcerative colitis):ti,ab
#5 #1 or #2 or #3 or #4
#6 MeSH descriptor: [Exercise] explode all trees
#7 MeSH descriptor: [Exercise Movement Techniques] explode all trees
#8 MeSH descriptor: [Exercise Test] explode all trees
#9 MeSH descriptor: [Exercise Therapy] explode all trees
#10 MeSH descriptor: [Physical Fitness] explode all trees
#11 MeSH descriptor: [Sports] explode all trees
#12 (exercis* or physical activit*):ti,ab
#13 #6 or #7 or #8 or #9 or #10 or #11 or #12
#14 #5 and #13

Results – 270

CINAHL (Searched 18/04/2021)

1. (MH "Inflammatory Bowel Diseases+")
2. (MH "Crohn Disease")
3. (MH "Colitis, Ulcerative")
4. TI(inflammatory bowel disease* or ibd or ulcerative colitis or crohn* disease) OR AB(inflammatory bowel disease* or ibd or ulcerative colitis or crohn* disease)
5. S1 OR S2 OR S3 OR S4
6. (MH "Exercise+")
7. (MH "Therapeutic Exercise+")
8. (MH "Exercise Test+")
9. (MH "Physical Fitness+")
10. (MH "Exercise Physiology+")
11. (MH "Sports+")
12. (MH "Physical Activity")
13. TI exercis* OR AB exercis*
14. TI physical activit* OR AB physical activit*
15. S6 OR S7 OR S8 OR S9 OR S10 OR S11 OR S12 OR S13 OR S14
16. S5 AND S15

Results – 253

Appendix 3: Questionnaires for feasibility of high-impact exercise (Chapter 6)

Survey on Exercise and Crohn's Disease
(Young people with Crohn's [13 – 17 years])

People with Crohn's disease are at risk of poor bone and muscle health. We know that exercise is good for bone and muscle health, but we don't know if exercise has the same benefits for people with Crohn's disease.

We hope to design some research studies investigating the effects exercise in people with Crohn's disease. Before we do this, we would like to know the opinions of people with Crohn's towards taking part in this type of exercise. These opinions will be very helpful when designing our research.

Q1. Do you have Crohn's disease?

Yes ☐
No ☐ (if you answered 'no', please do not complete this survey)

Q2. How old are you? (e.g. 14 years, 7 months)

_____ years _____ months

Q3. At what age were you diagnosed with Crohn's disease? (e.g. 11 years, 6 months)

_____ years _____ months

Q4. Are you:

Male ☐
Female ☐

Q5. What medications are you currently taking for your Crohn's disease? (*Tick all that apply. If you are taking a medication that is not listed, write it in the box below*.**)**

Prednisolone ☐
Mercaptopurine/Azathioprine ☐
Methotrexate ☐
Infliximab ☐

Adalimumab ☐
Mesalasine (e.g. Pentasa, Salofalk) ☐
Nutritional Supplements ☐
Other (please specify) ☐

_____

Q6. How many surgeries have you had for your Crohn's disease?

None ☐
One ☐
Two ☐
More than two ☐

Q7. How would you rate your general wellbeing?

Very well ☐
Slightly below average ☐
Poor ☐
Very poor ☐
Terrible ☐

Q8. How would you rate the overall control of your Crohn's disease in the past 3 months?

0 (Worst Possible) **100 (Best Possible)**

Q9. How concerned are you about your bone health?

Not concerned ☐
Slightly concerned ☐
Very concerned ☐

Q10. We will now ask some questions about your exercise habits, and how exercising makes you feel. Please answer as honestly as possible.

Not including during school time, in a typical week which of the following exercises do you do? *(Tick all that apply. If you do a type of exercise that is not listed, please write it below.)*

Walking/Hiking for leisure	☐
Jogging/Running	☐
Cycling	☐
Team sports (i.e. football, hockey, rugby etc.)	☐
Resistance training (i.e. lifting weights)	☐
I don't do any exercise	☐
Other *(explain below)*	☐

_____

Q11. Does your Crohn's disease make it harder for you to exercise?

Yes	☐	*if yes, go to Q12*
No	☐	*if no, go to Q13*

Q12. If you answered 'Yes' to Q11, is it harder for you to exercise because of any the following reasons? (*Tick all that apply. If you think of a reason that is not listed, please write it below.)*

Joint pain	☐
Muscle weakness	☐
Tiredness/fatigue	☐
Embarrassment	☐
Lack of access to toilet	☐
Painful stomach/abdomen	☐
Other *(explain below)*	☐

_____

Q13. When you take part in exercise, does it make you feel <u>BETTER</u> in any of the following ways? *(Tick all that apply. If you think of something not listed, please write it below.)*

I have more energy	☐
I feel stronger/fitter	☐
I feel less tired/fatigued	☐
I have less joint pain	☐
I have less abdominal pain	☐
It improves my mood	☐
Exercise doesn't make me feel any better	☐
Other *(explain below)*	☐

_____

Q14. When you take part in exercise, does it make you feel <u>WORSE</u> in any of the following ways? (*Tick all that apply. If you think of something that is not listed, please write it below.)*

I feel more tired	☐
I feel weak	☐
I have more joint pain	☐
I have increased need for the toilet	☐
I have more stomach/abdominal pain	☐
It makes me embarrassed	☐
Exercise doesn't make me feel any worse	☐
Other *(explain below)*	☐

_____

Q15. We are investigating whether exercise may be a useful treatment for people with Crohn's disease. We are going to carry out come research studies to see if certain types of exercise benefit people with Crohn's.

The following questions will ask your opinions about this type of exercise, and your thoughts about taking part in future research studies.

Not including during school time, how many times per week do you think you would be able to set aside 30 minutes for exercise?

None	☐
1 or 2 times	☐
3 or 4 times	☐
More than times	☐

Q16. Please follow the link below to watch a short video demonstrating the type of exercise we are investigating. Please watch the video then answer the following questions.

[VIDEO LINK HERE]

How easy do you think it would be to participate in exercise like this, three times per week?

0 (No Problem) **100 (Impossible)**

Q17. Do you think the opportunity of improving your health is worth doing this type of exercise three times per week?

Yes ☐
No ☐

Q18. Would you be interested in taking part in a research study looking at the effects of this type of exercise in people with Crohn's disease?

Yes ☐
No ☐

Q19. Can you think of any problems you might face if you were trying to participate in this type of exercise? *(Please explain below. Be as detailed as possible.)*

_____
___
_____
___

Q20. Do you have any additional comments that you would like to make regarding exercise, or the research studies we are going to conduct?

_____
___
_____
___
_____
_____

THANK YOU VERY MUCH FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.

Survey on Exercise and Crohn's Disease
(Adults with Crohn's [18 years+])

People with Crohn's disease are at risk of poor bone and muscle health. We know that exercise is good for bone and muscle health, but we don't know if exercise has the same benefits for people with Crohn's disease.

We hope to design some research studies investigating the effects exercise in people with Crohn's disease. Before we do this, we would like to know the opinions of people with Crohn's towards taking part in this type of exercise. These opinions will be very helpful when designing our research.

Q1. Do you have Crohn's disease?

Yes ☐
No ☐ (if you answered 'no', please do not complete this survey)

Q2. How old are you? (e.g. 29 years)

_____

Q3. At what age were you diagnosed with Crohn's disease? (e.g. 14 years)

_____

Q4. Are you:

Male ☐
Female ☐
Transgender ☐
Gender Neutral ☐

Q5. What medications are you currently taking for your Crohn's disease? (*Tick all that apply. If you are taking a medication that is not listed, write it in the box below*.**)**

Prednisolone ☐
Mercaptopurine/Azathioprine ☐
Methotrexate ☐
Infliximab ☐

Adalimumab ☐
Mesalasine (e.g. Pentasa, Salofalk) ☐
Nutritional Supplements ☐
Other (please specify) ☐

_____

Q6. How many surgeries have you had for your Crohn's disease?

None ☐
One ☐
Two ☐
More than two ☐

Q7. How would you rate your general wellbeing?

Very well ☐
Slightly below average ☐
Poor ☐
Very poor ☐
Terrible ☐

Q8. How would you rate the overall control of your Crohn's disease in the past 3 months?

0 (Worst Possible) **100 (Best Possible)**

Q9. How concerned are you about your bone health?

Not concerned ☐
Slightly concerned ☐
Very concerned ☐

Q10. We will now ask some questions about your exercise habits, and how exercising makes you feel. Please answer as honestly as possible.

In a typical week, which of the following exercises do you do? *(Tick all that apply. If you do a type of exercise that is not listed, please write it below.)*

Walking/Hiking for leisure ☐

Jogging/Running	☐
Cycling	☐
Team sports (i.e. football, hockey, rugby etc.)	☐
Resistance training (i.e. lifting weights)	☐
I don't do any exercise	☐
Other *(explain below)*	☐

_____

Q11. Does your Crohn's disease make it harder for you to exercise?

Yes	☐	*if yes, go to Q12*
No	☐	*if no, go to Q13*

Q12. If you answered 'Yes' to Q11, is it harder for you to exercise because of any the following reasons? (*Tick all that apply. If you think of a reason that is not listed, please write it below.)*

Joint pain	☐
Muscle weakness	☐
Tiredness/fatigue	☐
Embarrassment	☐
Lack of access to toilet	☐
Painful stomach/abdomen	☐
Other *(explain below)*	☐

_____

Q13. When you take part in exercise, does it make you feel <u>BETTER</u> in any of the following ways? *(Tick all that apply. If you think of something not listed, please write it below.)*

I have more energy	☐
I feel stronger/fitter	☐
I feel less tired/fatigued	☐
I have less joint pain	☐
I have less abdominal pain	☐
It improves my mood	☐
Exercise doesn't make me feel any better	☐
Other *(explain below)*	☐

_____

Q14. When you take part in exercise, does it make you feel <u>WORSE</u> in any of the following ways? (*Tick all that apply. If you think of something that is not listed, please write it below.)*

I feel more tired	☐
I feel weak	☐
I have more joint pain	☐
I have increased need for the toilet	☐
I have more stomach/abdominal pain	☐
It makes me embarrassed	☐
Exercise doesn't make me feel any worse	☐
Other *(explain below)*	☐

_____

Q15. We are investigating whether exercise may be a useful treatment for people with Crohn's disease. We are going to carry out come research studies to see if certain types of exercise benefit people with Crohn's.

The following questions will ask your opinions about this type of exercise, and your thoughts about taking part in future research studies.

How many times per week do you think you would be able to set aside 30 minutes for exercise?

None	☐
1 or 2 times	☐
3 or 4 times	☐
More than times	☐

Q16. Please follow the link below to watch a short video demonstrating the type of exercise we are investigating. Please watch the video then answer the following questions.

[VIDEO LINK HERE]

How easy do you think it would be to participate in exercise like this, three times per week?

0 (No Problem) **100 (Impossible)**

Q17. Do you think the opportunity of improving your health is worth doing this type of exercise three times per week?

Yes ☐
No ☐

Q18. Would you be interested in taking part in a research study looking at the effects of this type of exercise in people with Crohn's disease?

Yes ☐
No ☐

Q19. Can you think of any problems you might face if you were trying to participate in this type of exercise? *(Please explain below. Be as detailed as possible.)*

_____
___
_____
___

Q20. Do you have any additional comments that you would like to make regarding exercise, or the research studies we are going to conduct?

_____
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THANK YOU VERY MUCH FOR TAKING THE TIME TO COMPLETE THIS QUESTIONNAIRE.

Session				Sets x Reps (per exercise)
Week One				
1	Vertical Jumps	Drop Landings (40cm)	Jumping jacks	3 x 6
2	Drop Landings (40cm)	Jumping Jacks	Vertical Jumps	3 x 8
3	Drop Landings (40cm)	Vertical Jumps	Jumping Jacks	3 x 10
Week Two				
4	Tuck Jumps	Drop Landings (50cm)	Two-legged hops	3 x 6
5	Drop Landings (50cm)	Tuck Jumps	Two-legged hops	3 x 8
6	Two-legged hops	Drop Landings (50cm)	Tuck Jumps	3 x 10
Week Three				
7	Box Jumps (30cm)	Drop Jumps (40cm)	One-legged hops	3 x 6 (3 x 4 per leg for hops)
8	Drop Jumps (40cm)	One-legged hops	Box Jumps (30cm)	3 x 8 (3 x 6 per leg for hops)
9	One-legged hops	Box Jumps (30cm)	Drop Jumps (40cm)	3 x 10 (3 x 8 per leg for hops)
Week Four				
10	Box Jumps (40cm)	Plyometric Jumps	Drop Jumps (50cm)	3 x 6
11	Drop Jumps (50cm)	Box Jumps (40cm)	Plyometric Jumps	3 x 8
12	Plyometric Jumps	Drop Jumps (50cm)	Box Jumps (40cm)	3 x 10

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