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Full Title: Muscle deficits with normal bone microarchitecture and geometry in young adults with well controlled childhood onset Crohn's disease

Short Title: Muscle-bone unit in Crohn's disease

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34

35 **Keywords (MeSH Terms)**

36 Crohn's disease; Magnetic resonance imaging; young adult; muscle; bone

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ABSTRACT

Muscle-bone deficits are common in children with Crohn's disease (CD); however, few studies have assessed long-term musculoskeletal outcomes in adults with childhood onset CD. This study assessed the prevalence of musculoskeletal deficits in a cohort of young adults with childhood onset CD in comparison with healthy age and sex matched controls.

This case-control study used 3-Tesla MRI and MRS to assess bone microarchitecture, cortical geometry and muscle area and adiposity at distal femur, and bone marrow adiposity of the lumbar spine, together with functional muscle tests and biochemical markers of the muscle-bone unit.

Twenty-seven adults with CD with median (range) age 23.2 years (18.0, 36.1) and 27 controls with median age 22.9 years (18.2, 37.0) were recruited. Trabecular microarchitecture and cortical geometry of the distal femur and bone marrow adiposity of the lumbar spine were not different between CD and controls ($p > 0.05$ for all). Muscle cross sectional area was lower ($p = 0.01$) and muscle fat fraction was higher ($p = 0.04$) at the distal femur in CD compared to controls. CD participants had significantly lower grip strength (-4.3 kg [95% CI: -6.8 , -1.8], $p = 0.001$) and lower limb muscle power relative to bodyweight (-5.0 W/kg [95% CI: -8.8 , -1.2], $p = 0.01$). CD activity scores were negatively associated with trabecular bone volume ($r = -0.40$, $p = 0.04$) and muscle area ($r = -0.41$, $p = 0.03$).

Young adults with well controlled CD managed with contemporary treatment strategies do not display abnormal bone microarchitecture or geometry at the distal femur but exhibit muscle deficits. The observed muscle deficits may predispose to musculoskeletal morbidity in future and interventions to improve muscle mass and function should be investigated.

Keywords: bone microarchitecture; muscle-bone; Crohn's disease; MRI

Introduction

In paediatric Crohn's disease (CD), the underlying inflammatory process, poor nutritional status and exposure to glucocorticoid (GC) disrupt regulatory pathways involved in muscle-bone development [1-4]. These factors can contribute to delayed puberty in CD resulting in poor bone mineral accrual and sub-optimal peak bone mass [5-7]. Impaired skeletal development may augment the deleterious effect of CD on bone, further increasing the future risk of osteoporosis and fracture associated with inflammatory bowel disease (IBD) in adults [8,9]. Despite this, few studies have directly assessed long-term musculoskeletal outcomes in adults with childhood onset CD, especially managed with contemporary treatment regimen.

Bone and muscle deficits have been observed in paediatric CD at diagnosis [10,11] and with follow-up after initiation of treatment [7,11]. Areal bone mineral density (BMD) assessed by dual energy x-ray absorptiometry (DXA) decreased following approximately 5 years follow up into late adolescence in young people with IBD [7] and childhood onset CD was associated with reduced lumbar spine bone area and mineral content in pre-menopausal women [12]. Indeed, in a recent population study, adults with childhood onset IBD had increased risk of hip fracture compared to those with adult onset disease [13]. These studies, however, represent previous treatment paradigms, with high proportions of systemic GC exposure and no use of GC sparing therapies like anti-tumour necrosis factor (TNF) therapies. Modern GC sparing therapies, such as anti-TNF therapy, are associated with improvement in markers of growth and bone formation in paediatric CD [14] and facilitate catch up growth [15]. Despite this, anti-TNF α was also associated with persistent musculoskeletal deficits after up to 12 months of therapy in paediatric CD, despite mild improvement [16,17]. It is however unclear whether muscle-bone deficits are present in young adults with childhood onset CD managed with contemporary therapies.

88

89 High resolution MRI offers an attractive method for assessing the muscle-bone unit at macro-
90 and micro-architectural level. Trabecular microarchitecture assessed by MRI was closely
91 associated with bone biopsy parameters in adults with chronic kidney disease [18], while the
92 addition of MR spectroscopy (MRS) allows concurrent quantification of bone marrow
93 adiposity (BMA), a recently emerged indicator of skeletal health [19,20]. Two recent studies
94 using high resolution peripheral quantitative computed tomography (HR-pQCT) identified
95 trabecular microarchitecture deficits in adults with IBD but did not distinguish between those
96 diagnosed in childhood from those diagnosed as adults [21,22]. To date, such a comprehensive
97 assessment of musculoskeletal health in young adults with childhood onset CD has not been
98 conducted. The aim of this study, therefore, was to assess musculoskeletal health in young
99 adults with childhood onset CD using novel methods of MRI in conjunction with MRS,
100 functional muscle testing and biochemical markers.

101

102 **Methods**

103 *Participants and Study Design*

104 Between June 2017 and January 2019, adults with childhood onset CD were recruited to take
105 part in this cross-sectional, case-control study. Healthy age- and sex-matched controls were
106 recruited via advertisement at one university hospital campus and from associates of CD
107 participants. One control participant was a third degree relative (first cousin) of a CD
108 participant. This individual had no symptoms or biomarker suggestive of CD and would have
109 little increased genetic risk for CD [23]. Inclusion criteria were aged 18-40 years (all
110 participants), and CD diagnosed at ≤ 17 years old and at least 12 months following diagnosis
111 (CD group). Exclusion criteria for all participants were contraindication to MRI, significant
112 comorbidity affecting muscle and/or bone, pregnancy, or use of medications known to affect

bone (anti-resorptive therapies like bisphosphonate, anti-receptor activator of nuclear factor kappa β and anabolic bone therapies like teriparatide and sclerostin antibody). Each participant with CD was age and sex matched to one healthy control (± 1 year).

Height was measured to the nearest mm and weight to the nearest 0.1 kg. Health and lifestyle data were collected; including current medications, use of nutritional supplements, fracture history, comorbidities, use of oral contraceptives and habitual physical activity (International physical activity questionnaire [IPAQ] long form)[24].

Age at diagnosis, history of CD related surgery and number of GC courses in the pediatric age (<18 years) were self-reported. Diagnosis, surgery and disease location according to Paris classification [25] were verified in electronic health records by a member of the study team. All participants were requested to return a stool sample for analysis of faecal calprotectin. Disease activity in CD was measured using the Crohn's Disease Activity Index (CDAI) [26]. Participants were classified as severe disease (CDAI score >451); moderate disease (221-450); mild disease (150-220) or clinical remission (0-149). This 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.

MRI Assessment of Muscle-Bone

MRI images of the distal femur were obtained using a 3-Tesla MRI scanner (Prisma, Siemens, Erlangen, Germany) with an 18-channel anterior array coil and 32-channel spine array. Femur length was measured using localiser scans as the distance between the superior aspect of the femoral head to the distal aspect of the medial femoral condyle. Trabecular and cortical images were then obtained at 15% and muscle images at 33% from the distal aspect of the right femur.

Trabecular images were obtained using a constructive interference in the steady state sequence, cortical images obtained using a T1-weighted turbo spin echo pulse sequence, and muscle images obtained using a six-point VIBE Dixon sequence. Pulse sequence parameters are available in Supplementary Table S1. Images were coded and analysed within MatLab software (MathWorks Inc., San Mateo, CA), using code developed in-house. Standardized analyses were performed in triplicate and the mean value used for subsequent analysis. Trabecular parameters were: apparent trabecular bone volume to total volume fraction (appBV/TV), apparent trabecular thickness (appTbTh [mm]), apparent trabecular number (appTbN [mm^{-1}]) and apparent trabecular separation (appTbSp [mm]). Cortical parameters were: cortical area (mm^2), cortical thickness (mm), endosteal circumference (mm) and periosteal circumference (mm). Muscle parameters were total cross-sectional area (mm^2), muscle fat fraction (%) and residual muscle area (muscle area after accounting for muscle fat fraction, calculated as described elsewhere [27] [mm^2]). Mean intra- and inter-observer CVs were both ~1% for trabecular parameters, 2% and 1% for cortical parameters, and 1.7% and 1% for muscle parameters, respectively.

MRS Assessment of Bone Marrow Adiposity

^1H -MRS was also performed using an 18-channel anterior array coil and 32-channel spine array. A point resolved spectroscopy pulse sequence with no water suppression was used to obtain spectra from a 20mm x 20mm x 20mm volume within a vertebral body at lumbar spine (L3) as described before [28]. The Java-based magnetic resonance user interface software package was used for analysis [29], which was performed following fitting of the spectrum in the time domain using a least-squares algorithm [30]. Area under the curve was then used to calculate BMA, expressed as percentage fat fraction [31].

Muscle Function

Maximal isometric grip strength was assessed by dynamometry (Takei 5401 Digital Hand Grip Dynamometer, Takei, Japan). Using the dominant hand, three attempts were made from which the highest value was used for analyses. Lower limb muscle function was assessed by jumping mechanography (Leonardo GRFP, Novotec Medical GmbH, Germany) [32]. Participants performed three standing two-legged jumps and the jump which achieved maximum height was used for analyses [33]. Mechanography data were only available in 37 participants (19 CD, 18 controls). Mechanography outcomes were absolute power (kilowatts [kW]), relative power (absolute power/body mass [W/kg]), absolute force (kilonewton [kN]), relative force (absolute force/weight; no units) and jump height (cm).

Biochemical Analyses

Participants arrived fasted and resting blood samples were drawn between 09:00-10:00h. Samples were centrifuged, aliquoted, and supernatant stored at -80°C until subsequent analyses. Blood samples were available for all participants with CD and in 28/29 healthy controls.

The following bone turnover markers were evaluated: Bone specific alkaline phosphatase (BSAP); C-terminal telopeptide of Type 1 collagen (CTX-1) and Sclerostin. All bone turnover markers were measured by ELISA (BSAP, CTX-1: Immunodiagnostic Systems, Boldon, UK; Intra-assay variance 10.8%, 2.3%, respectively. Sclerostin: TECO Medical AG, Sissach, Switzerland; Intra-assay variance 5%). Insulin like growth factor-1 (IGF-1) and its binding proteins (IGFBP) 2 and 3, and acid labile subunit (ALS) were measured by ELISA (Mediagnost, Reutlingen, Germany; Intra-assay variance 6.9%, <1%, <1%, 3.2%, respectively). Myostatin was measured by ELISA (Cusabio, Hubei Province, China; intra-assay variance 13.4%). Multiplex cytokine array analysis was performed using an eight-cytokine assay on a Luminex-

based Bio-Plex multi array system from which results for TNF α , IFN γ , interleukin (IL-) 6, and IL-8 were obtained (Bio-Rad, California, USA). 25-Hydroxyvitamin D (25-OH vitamin D) was measured by liquid chromatography–tandem mass spectroscopy with an intra-assay variation of <10%.

Sample Size Calculation

This study was powered to detect a 3.0% difference in MRI trabecular appBV/TV between CD and controls. Based on the variation in measurements from the first six healthy controls recruited, we required 19 participants in each group to detect 3.0% reduction in appBV/TV in CD compared with controls at $\alpha < 0.05$ with a power of 0.80.

Statistical Analysis

Data were assessed for normality using Shapiro-Wilks tests. Between group comparisons were made using t-tests for normally distributed continuous variables and Mann Whitney U tests for non-normally distributed data. Categorical variables were compared using Chi-squared tests. Differences between CD and controls were further compared following adjustment for co-variates using multiple regression. Mechanography outcomes were also compared using multiple regression analyses, adjusted for age and sex, as mechanography data were only available in unmatched participants (Mechanography cohort characteristics available in Supplementary Table 2). Univariate analyses with Spearman correlation were used to determine relationships between continuous variables in the CD cohort. Due to the importance of skeletal size in determining bone geometry, associations between cortical geometry (excluding cortical thickness) and other variables were adjusted for height. Continuous variables are presented as median (range) and categorical variables as frequency (percentage). Statistical analyses were performed using SPSS (Version 24, IBM Corp, USA).

Results

Clinical Characteristics

Twenty-seven adults (15 female) with CD with median age of 23.2 (18.0, 36.1) years, and 27 controls (15 female) with median age of 22.9 (18.2, 37.0) years were recruited (Table 1). Body mass index (BMI) was lower in participants with CD ($p = 0.03$). Both groups had similar levels of physical activity, history of fractures (all long bone fractures with history of trauma) and did not differ in other general characteristics (Table 1). No participant (CD and control) had a history of symptomatic vertebral fracture. None had previous or current use of bisphosphonates.

Median age at diagnosis of CD was 12 (6, 17) years. Using the Paris disease classification, ileocolonic (59%) and isolated colonic disease (33%) were most common. Median self-reported course(s) of GC in the pediatric age range (i.e. <18 years) was 1 (0, 8) (Table 1). Out of 27 participants with CD, 20 (74%) were in remission and seven (26%) had mildly active disease, according to CDAI scores at study visit. Most CD participants were using thiopurines (63%) or anti-TNF α (48%) therapy at time of study visit. Two thirds (18/27; 67%) were currently, or had previously been, on anti-TNF α therapy (Table 1). Faecal calprotectin was higher in CD (n, 13) compared with controls (n, 11) ($p = 0.05$), however in CD only 3/13 samples had a clinically relevant faecal calprotectin of >250 $\mu\text{g/g}$. Pro-inflammatory cytokines TNF α , IFN- γ , IL-8 and IGFBP-2 were higher in CD ($p < 0.05$ for all), however serum albumin was not different between CD and controls (Table 2).

MRI Trabecular Bone Microarchitecture and Cortical Bone Geometry

MRI assessment of trabecular microarchitecture of the distal femur indicated no differences between CD and controls (Table 3). For cortical bone, endosteal circumference ($p = 0.03$) and

periosteal circumference ($p = 0.03$) were lower in CD compared with controls. However, in multiple regression analyses (adjusted for sex and height), there were no differences between CD and controls for cortical parameters (Table 4).

Bone-muscle metabolism and GH axis

Median serum 25-OH Vitamin D was not different between groups. Nineteen percent (5/27) of CD and 8% (2/26) of controls had 25-OHD Vitamin D levels <25 nmol/l, respectively. Participants with CD had higher PTH than controls ($p = 0.01$), with nine samples (33%) above the upper limit of reference range (>7.5 pmol/l) (Table 2). Serum BSAP, CTX-1 and sclerostin were not different between groups. Similarly, serum myostatin, IGF-1, IGFBP-3 and ALS were not different between groups (Table 2).

MRI Muscle Area, Adiposity and Function

MRI muscle and fat imaging

Mean muscle cross sectional area was $7024 \text{ mm}^2 (\pm 1764)$ and $8540 \text{ mm}^2 (\pm 2297)$ in CD and controls, respectively ($p = 0.01$) (Figure 1A). Mean muscle fat fraction was 5.3% (± 2.0) and 4.1% (± 2.1) in CD and controls, respectively ($p = 0.04$) (Figure 1B). After accounting for muscle fat fraction, mean residual muscle area was nearly 20% lower in CD compared with controls ($6665 \text{ mm}^2 [\pm 1674]$ vs $8201 \text{ mm}^2 [\pm 2280]$, $p = 0.01$) (Figure 1C). Multiple regression analysis showed that participants with CD had 1124 mm^2 (95% CI: -1758, -489) lower residual muscle area than controls after adjustment for sex, height and weight (Model $R^2 = 0.74$, $p = 0.001$).

Grip strength

Median maximal isometric grip strength was 26.3 kg (17.0, 41.8) and 31.5 kg (22.1, 52.5) in CD and controls, respectively ($p = 0.04$). After adjustment for sex, height and weight, grip strength was 4.3 kg (95% CI: -6.8, -1.8) lower in CD compared to controls (Model $R^2 = 0.71$, $p = 0.001$).

Jumping mechanography

After adjustment for age and sex, participants with CD had 0.45 kW lower absolute power (95% CI: -0.80, -0.10; model $R^2 = 0.65$, $p = 0.01$), 0.21 kN lower absolute force (95% CI: -0.41, 0.00; model $R^2 = 0.28$, $p = 0.05$) and 5.1 cm lower jump height (95% CI: -8.6, -1.6; model $R^2 = 0.64$, $p = 0.01$). After adjustment for age and sex, participants with CD had 5.0 W/kg lower power relative to bodyweight (95% CI: -8.8, -1.2; model $R^2 = 0.6$, $p = 0.01$) but no difference in relative force (95% CI: -0.48, 0.03; model $R^2 = 0.1$, $p = 0.09$).

MRS Bone Marrow Adiposity

Mean L3 BMA was 32.0% (± 11.9) and 30.1% (± 11.2) in CD and controls, respectively ($p = 0.57$).

Bone and BMA associations with disease and muscle

In CD, appBV/TV was negatively associated with CDAI ($r = -0.40$, $p = 0.04$) and number of courses of GC under 18 years of age ($r = -0.42$, $p = 0.03$), but positively associated with BMI ($r = 0.53$, $p = 0.01$), residual muscle area ($r = 0.53$, $p = 0.01$), mechanography power ($r = 0.55$, $p = 0.02$) and grip strength ($r = 0.52$, $p = 0.01$). Similarly, appTbTh was negatively associated with CDAI ($r = -0.56$, $p = 0.003$) and number of CD related surgeries ($r = -0.58$, $p = 0.002$), but positively associated with residual muscle area ($r = 0.64$, $p < 0.001$) and grip strength ($r = 0.63$, $p = 0.001$). A positive association between residual muscle area and appBV/TV was also

observed in the control group ($r = 0.41$, $p = 0.04$). In height adjusted models, all cortical geometry parameters were positively associated with mechanography data; cortical area was positively associated with residual muscle area and periosteal circumference was positively associated with grip strength (Table 5). Cortical thickness was not associated with any other musculoskeletal outcomes and no cortical geometry parameters were associated with indices of disease. Additionally, BMA was not associated with other musculoskeletal or disease outcomes (data not shown).

Muscle associations with disease

Residual muscle area was negatively associated with CDAI ($r = -0.41$, $p = 0.03$) and number of CD related surgeries ($r = -0.44$, $p = 0.02$). Grip strength was negatively associated with number of CD related surgeries ($r = -0.48$, $p = 0.01$) but not with CDAI ($r = -0.33$, $p = 0.09$), and was positively associated with serum albumin ($r = 0.38$, $p = 0.05$). Jumping mechanography data were not associated with any disease related outcomes, and residual muscle area and grip strength were not associated with inflammatory cytokines (data not shown).

Muscle associations with GH axis and myostatin

Residual muscle area was not associated with IGF-1 ($r = 0.25$, $p = 0.22$), IGFBP-3 ($r = 0.09$, $p = 0.66$) or myostatin ($r = -0.13$, $p = 0.53$) in CD. Grip strength was positively associated with IGF-1 ($r = 0.41$, $p = 0.04$), but not associated with IGFBP-3 ($r = 0.24$, $p = 0.22$) or myostatin ($r = -0.24$, $p = 0.24$). Mechanography outcomes were not associated with IGF-1 or IGFBP-3, but mechanography absolute force and force relative to bodyweight were negatively associated with myostatin ($r = -0.59$, $p = 0.01$ and $r = -0.51$, $p = 0.02$, respectively).

DISCUSSION

This is the first study to evaluate the muscle-bone unit in young adults with childhood onset CD managed with contemporary therapy. Adults with childhood onset CD had lower thigh muscle mass and muscle function, despite mild disease activity and similar self-reported physical activity compared with controls. Muscle deficits were not associated with systemic abnormalities of the GH axis or myostatin. High-resolution MRI of the distal femur indicated no deficits of trabecular microarchitecture or cortical geometry in CD, and there were no differences in lumbar spine BMA. Trabecular microarchitecture and cortical geometry, however, were associated with muscle mass and function in CD.

Muscle mass deficits are a typical characteristic of pediatric CD. Longitudinal studies suggest muscle mass deficits persist even with medical therapy following diagnosis [34] and anti-TNF α therapy [16,17]. Our results suggest deficits in muscle mass and function persist into adulthood. We report significantly reduced muscle mass of the thigh, lower grip strength and jumping mechanography outcomes in participants with CD. Mechanography data have not been reported in adults with CD, but were lower [10] or unaffected [35] in pediatric CD compared to reference data. Our data corroborate previous reports of low grip strength in adults with CD [36,37], and the observed deficit is equivalent to that associated with increased risk of multiple morbidities in healthy older adults [38], highlighting its likely clinical relevance. Furthermore, low muscle mass may have therapeutic implications in CD, recently being associated with early failure of anti-TNF α therapy [39,40] and the need for surgical intervention [41]. To our knowledge, our study is the first to investigate muscle fat fraction in CD compared with healthy controls, demonstrating higher muscle fat fraction in CD. In adults with CD undergoing surgical resection, muscle fat was associated with length of stay and short-term re-admission

[42]. The clinical significance of muscle fat in CD (independent of muscle mass) requires further investigation.

Despite mild disease activity, associations between disease and muscle outcomes were evident. The precise mechanism of muscle deficits in CD remains unclear, with some suggesting impaired muscle protein synthesis [43,44], potentially linked to reduced systemic IGF-1 [44]. Our data did not identify abnormalities in systemic GH axis; however, grip strength was positively associated with serum IGF-1 in CD. Low systemic IGF-1 has been previously associated with reduced muscle mass in adults with rheumatoid arthritis [45]. Changes in systemic IGF-1 also mirrored changes in muscle mass following initiation of anti-TNF α therapy in pediatric CD [46]. Myostatin, a protein of the transforming growth factor beta family and potent inhibitor of muscle growth [47], was negatively associated with mechanography outcomes in CD. Myostatin and IGF-1 were both associated with muscle mass in adults with chronic kidney disease on haemodialysis [48]. Pro-inflammatory cytokines were elevated in CD compared with controls, indicating sub-clinical inflammation that may contribute to muscle deficits, as observed in healthy older adults [49]. Additionally, the regenerative capacity of skeletal muscle may be compromised in CD as excessive TNF α can induce inflammation activated p38 signalling leading to epigenetic alterations in muscle satellite cells that may inhibit myogenesis [50,51]. While faecal calprotectin was also higher in CD, this was not to a level of clinical relevance, and the small number of samples (n, 13) precluded assessment of relationships with other outcomes. Longitudinal studies of more severe CD may clarify the role and relative contribution of the GH axis, myostatin and cytokines on muscle outcomes.

Studies using peripheral quantitative computed tomography (pQCT) in pediatric CD identified low trabecular volumetric BMD and cortical area, which were associated with muscle mass

and function deficits [10,11,34]. Histomorphometry in newly diagnosed children with IBD revealed thin cortical bone with maintained trabecular microarchitecture, although trabecular turnover activity was low [52]. Two recent HR-pQCT studies identified low trabecular bone volume and trabecular thickness at the distal tibia in adults with IBD (60% CD) [22] and low trabecular number and increased trabecular separation at the tibia and radius in adolescents and young adults with IBD (74% CD) [21]. Greater exposure to GC in these cohorts may have contributed to trabecular erosion, concordant with the negative associations between GC exposure and trabecular bone volume observed in our results. Exclusive enteral nutrition is the primary management strategy for active CD at diagnosis and during subsequent relapse during paediatric care in our centre [53], common in most centres in the United Kingdom and some European countries. In addition, HR-pQCT provides greater spatial resolution versus MRI (< 100µm vs 200µm, respectively) allowing for detection of more subtle differences in trabecular bone.

Deficits in cortical area in pediatric CD have been shown to improve with follow-up [34] and with anti-TNFα therapy [16], although complete normalization was not observed. Greater improvement in linear growth was associated with greater increase in cortical area [16,34], highlighting the importance of growth and puberty on improvements in cortical bone size. We hypothesize that, through improved control of underlying mechanisms, contemporary CD therapies ameliorate disease associated growth dysfunction thus leading to ongoing improvements in cortical bone geometry, potentially explaining the lack of cortical deficits in our adults with childhood onset CD.

There is growing interest for the role of BMA on health outcomes, including osteoporosis. Adipocytes and osteoblasts originate from mesenchymal stem cells and regulatory factors

including cytokines and GC influence differentiation into either bone or fat forming cells [54]. Our observation of no difference in BMA between CD and controls agrees with a previous study of adults with IBD in remission [55]. BMA has been reported to be increased at diagnosis of Cushing's disease [56,57], however it normalizes following treatment of the GC excess [57]. Further studies in those with active CD are required to determine the effects of chronic inflammation on BMA.

Despite no obvious trabecular or cortical bone abnormalities in adults with childhood onset CD, our results show associations between muscle area and function with trabecular bone volume and thickness and cortical area. Associations between trabecular microarchitecture and muscle have been previously reported in a large cohort of healthy adults aged 20 to 97 years, although we did not observe such a relationship in our relatively small sample size of healthy young adults [58]. The long term impact of low muscle mass on bone remodelling in young adults with childhood onset CD requires attention, as this has been reported as a predictor of osteopenia in CD [59]. As MRI does not assess BMD, we cannot rule out a potential mineralization defect in our CD cohort, as has been widely reported elsewhere [21,22,59].

This study is limited by a relatively small sample size and the cross-sectional nature of the data precludes definitive comment on relationship between CD associated factors (like disease and treatment factors) and muscle-bone outcomes. Additionally, we are unable to comment on the musculoskeletal health of CD participants prior to onset of disease or study visit. No data was collected regarding current dietary intake which is likely to be an important contributor to musculoskeletal health in CD. However, this study is not powered to investigate the influence of nutrition on musculoskeletal outcomes in CD. Prospective or interventional studies investigating the influence of different dietary factors on bone and muscle health in CD are

411 currently lacking and should be addressed in future. Despite these limitations, this study
412 provides important insight into the muscle and bone health of young adults with childhood
413 onset CD and suggests future strategies should be devised to directly target improvements in
414 muscle mass and function.
415

Conclusion

In conclusion, this study has for the first time characterised the muscle-bone unit of young adults with well controlled childhood onset CD, identifying deficits in muscle mass and function, but no apparent abnormalities in MRI derived measures of trabecular bone microarchitecture and cortical geometry. Low muscle mass in this population may augment the deleterious effects of disease on muscle-bone in the longer term, and strategies to improve muscle mass and function warrant future investigation.

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REFERENCES

1. MacRae VE, Wong SC, Farquharson C, Ahmed SF. Cytokine actions in growth disorders associated with pediatric chronic inflammatory diseases (Review). *Int J Mol Med* 2006; 18 (6):1011-1018.
2. Sederquist B, Fernandez-Vojvodich P, Zaman F, Savendahl L. Recent research on the growth plate: Impact of inflammatory cytokines on longitudinal bone growth. *J Mol Endocrinol* 2014; 53 (1):T35-44.
3. Gerasimidis K, McGrogan P, Edwards CA. The aetiology and impact of malnutrition in paediatric inflammatory bowel disease. *J Hum Nutr Diet* 2011; 24 (4):313-326.
4. Von Scheven E, Corbin KJ, Stefano S, Cimaz R. Glucocorticoid-associated osteoporosis in chronic inflammatory diseases: Epidemiology, mechanisms, diagnosis, and treatment. *Curr Osteoporos Rep* 2014; 12 (3):289-299.
5. Mason A, Malik S, Russell RK, Bishop J, McGrogan P, Ahmed SF. Impact of inflammatory bowel disease on pubertal growth. *Horm Res Paediatr* 2011; 76 (5):293-299.
6. DeBoer MD, Denson LA. Delays in puberty, growth, and accrual of bone mineral density in pediatric Crohn's disease: despite temporal changes in disease severity, the need for monitoring remains. *J Pediatr* 2013; 163 (1):17-22.
7. Laakso S, Valta H, Verkasalo M, Toiviainen-Salo S, Makitie O. Compromised peak bone mass in patients with inflammatory bowel disease--a prospective study. *J Pediatr* 2014; 164 (6):1436-1443 e1431.
8. Szafors P, Che H, Barnette T, Morel J, Gaujoux-Viala C, Combe B, et al. Risk of fracture and low bone mineral density in adults with inflammatory bowel diseases. A systematic literature review with meta-analysis. *Osteoporosis Int* 2018; 29 (11):2389-2397.
9. Hakimian S, Kheder J, Arum S, Cave DR, Hyatt B. Re-evaluating osteoporosis and fracture risk in Crohn's disease patients in the era of TNF-alpha inhibitors. *Scand J Gastroentero* 2018; 53 (2):168-172.
10. Ward LM, Ma J, Rauch F, Benchimol EI, Hay J, Leonard MB, et al. Musculoskeletal health in newly diagnosed children with Crohn's disease. *Osteoporos Int* 2017; 28 (11):3169-3177.
11. Dubner SE, Shults J, Baldassano RN, Zemel BS, Thayu M, Burnham JM, et al. Longitudinal Assessment of Bone Density and Structure in an Incident Cohort of Children With Crohn's Disease. *Gastroenterology* 2009; 136 (1):123-130.
12. Mauro M, Armstrong D. Juvenile onset of Crohn's disease: a risk factor for reduced lumbar bone mass in premenopausal women. *Bone* 2007; 40 (5):1290-1293.
13. Ludvigsson JF, Mahl M, Sachs MC, Bjork J, Michaelsson K, Ekblom A, et al. Fracture Risk in Patients With Inflammatory Bowel Disease: A Nationwide Population-Based Cohort Study From 1964 to 2014. *Am J Gastroenterol* 2019; 114 (2):291-304.
14. Thayu M, Leonard MB, Hyams JS, Crandall WV, Kugathasan S, Otley AR, et al. Improvement in Biomarkers of Bone Formation During Infliximab Therapy in Pediatric Crohn's Disease: Results of the REACH Study. *Clin Gastroenterol H* 2008; 6 (12):1378-1384.
15. Malik S, Wong SC, Bishop J, Hassan K, McGrogan P, Ahmed SF, et al. Improvement in Growth of Children With Crohn Disease Following Anti-TNF-alpha Therapy Can Be Independent of Pubertal Progress and Glucocorticoid Reduction. *J Pediatr Gastr Nutr* 2011; 52 (1):31-37.
16. Griffin LM, Thayu M, Baldassano RN, DeBoer MD, Zemel BS, Denburg MR, et al. Improvements in Bone Density and Structure during Anti-TNF-alpha Therapy in Pediatric Crohn's Disease. *J Clin Endocr Metab* 2015; 100 (7):2630-2639.
17. Altowati MMA, Shepherd S, McMillan M, McGrogan P, Russell R, Ahmed SF, et al. Persistence of Muscle-bone Deficits Following Anti-tumour Necrosis Factor Therapy in Adolescents With Crohn Disease. *J Pediatr Gastr Nutr* 2018; 67 (6):738-744.
18. Sharma AK, Toussaint ND, Elder GJ, Masterson R, Holt SG, Robertson PL, et al. Magnetic resonance imaging based assessment of bone microstructure as a non-invasive alternative to histomorphometry in patients with chronic kidney disease. *Bone* 2018; 114:14-21.
19. Veldhuis-Vlug AG, Rosen CJ. Clinical implications of bone marrow adiposity. *J Intern Med* 2018; 283 (2):121-139.

20. McComb C, Harpur A, Yacoubian C, Leddy C, Anderson G, Shepherd S, et al. MRI-based abnormalities in young adults at risk of adverse bone health due to childhood-onset metabolic & endocrine conditions. *Clin Endocrinol* 2014; 80 (6):811-817.
21. Pepe J, Zawadynski S, Herrmann FR, Juillerat P, Michetti P, Ferrari-Lacraz S, et al. Structural Basis of Bone Fragility in Young Subjects with Inflammatory Bowel Disease: A High-resolution pQCT Study of the SWISS IBD Cohort (SIBDC). *Inflamm Bowel Dis* 2017; 23 (8):410-417.
22. Haschka J, Hirschmann S, Kleyer A, Englbrecht M, Faustini F, Simon D, et al. High-resolution Quantitative Computed Tomography Demonstrates Structural Defects in Cortical and Trabecular Bone in IBD Patients. *J Crohns Colitis* 2016; 10 (5):532-540.
23. Russell RK, Satsangi J. IBD: a family affair. *Best Pract Res Clin Gastroenterol* 2004; 18 (3):525-539.
24. Craig CL, Marshall AL, Sjoström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; 35 (8):1381-1395.
25. Levine A, Griffiths A, Markowitz J, Wilson DC, Turner D, Russell RK, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; 17 (6):1314-1321.
26. Best WR, Beckett JM, Singleton JW, Kern F, Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology* 1976; 70 (3):439-444.
27. Morrow JM, Sinclair CD, Fischmann A, Machado PM, Reilly MM, Yousry TA, et al. MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. *Lancet Neurol* 2016; 15 (1):65-77.
28. Chen SC, Shepherd S, McMillan M, McNeilly J, Foster J, Wong SC, et al. Skeletal Fragility and Its Clinical Determinants in Children With Type 1 Diabetes. *J Clin Endocrinol Metab* 2019; 104 (8):3585-3594.
29. Naressi A, Couturier C, Devos JM, Janssen M, Mangeat C, de Beer R, et al. Java-based graphical user interface for the MRUI quantitation package. *Magn Reson Mater Phys* 2001; 12 (2-3):141-152.
30. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. *J Magn Reson* 1997; 129 (1):35-43.
31. Schellinger D, Lin CS, Lim J, Hatipoglu HG, Pezzullo JC, Singer AJ. Bone marrow fat and bone mineral density on proton MR spectroscopy and dual-energy X-ray absorptiometry: their ratio as a new indicator of bone weakening. *AJR Am J Roentgenol* 2004; 183 (6):1761-1765.
32. Veilleux LN, Rauch F. Reproducibility of jumping mechanography in healthy children and adults. *J Musculoskelet Neuronal Interact* 2010; 10 (4):256-266.
33. Elmantaser M, McMillan M, Smith K, Khanna S, Chantler D, Panarelli M, et al. A comparison of the effect of two types of vibration exercise on the endocrine and musculoskeletal system. *J Musculoskelet Neuronal Interact* 2012; 12 (3):144-154.
34. Tsampalieros A, Lam CKL, Spencer JC, Thayu M, Shults J, Zemel BS, et al. Long-Term Inflammation and Glucocorticoid Therapy Impair Skeletal Modeling During Growth in Childhood Crohn Disease. *J Clin Endocr Metab* 2013; 98 (8):3438-3445.
35. Maratova K, Hradsky O, Matyskova J, Copova I, Soucek O, Sumnik Z, et al. Musculoskeletal system in children and adolescents with inflammatory bowel disease: normal muscle force, decreased trabecular bone mineral density and low prevalence of vertebral fractures. *Eur J Pediatr* 2017; 176 (10):1355-1363.
36. Wiroth JB, Filippi J, Schneider SM, Al-Jaouni R, Horvais N, Gavarry O, et al. Muscle performance in patients with Crohn's disease in clinical remission. *Inflamm Bowel Dis* 2005; 11 (3):296-303.
37. Lu ZL, Wang TR, Qiao YQ, Zheng Q, Sun Y, Lu JT, et al. Handgrip Strength Index Predicts Nutritional Status as a Complement to Body Mass Index in Crohn's Disease. *Journal of Crohn's and Colitis* 2016; 10 (12):1395-1400.
38. Celis-Morales CA, Welsh P, Lyall DM, Steell L, Petermann F, Anderson J, et al. Associations of grip strength with cardiovascular, respiratory, and cancer outcomes and all cause mortality: prospective cohort study of half a million UK Biobank participants. *BMJ* 2018; 361:k1651.

39. Ding NS, Malietzis G, Lung PFC, Penez L, Yip WM, Gabe S, et al. The body composition profile is associated with response to anti-TNF therapy in Crohn's disease and may offer an alternative dosing paradigm. *Aliment Pharmacol Ther* 2017; 46 (9):883-891.
40. Holt DQ, Varma P, Strauss BJG, Rajadurai AS, Moore GT. Low muscle mass at initiation of anti-TNF therapy for inflammatory bowel disease is associated with early treatment failure: a retrospective analysis. *Eur J Clin Nutr* 2017; 71 (6):773-777.
41. Bamba S, Sasaki M, Takaoka A, Takahashi K, Imaeda H, Nishida A, et al. Sarcopenia is a predictive factor for intestinal resection in admitted patients with Crohn's disease. *Plos One* 2017; 12 (6):e0180036.
42. O'Brien S, Kavanagh RG, Carey BW, Maher MM, O'Connor OJ, Andrews EJ. The impact of sarcopenia and myosteatosis on postoperative outcomes in patients with inflammatory bowel disease. *Eur Radiol Exp* 2018; 2 (1):37.
43. Davies A, Nixon A, Muhammed R, Tsintzas K, Kirkham S, Stephens FB, et al. Reduced skeletal muscle protein balance in paediatric Crohn's disease. *Clin Nutr* 2019.
44. van Langenberg DR, Della Gatta P, Hill B, Zacharewicz E, Gibson PR, Russell AP. Delving into disability in Crohn's disease: dysregulation of molecular pathways may explain skeletal muscle loss in Crohn's disease. *J Crohns Colitis* 2014; 8 (7):626-634.
45. Baker JF, Von Feldt JM, Mostoufi-Moab S, Kim W, Taratuta E, Leonard MB. Insulin-like Growth Factor 1 and Adiponectin and Associations with Muscle Deficits, Disease Characteristics, and Treatments in Rheumatoid Arthritis. *J Rheumatol* 2015; 42 (11):2038-2045.
46. DeBoer MD, Lee AM, Herbert K, Long J, Thayu M, Griffin LM, et al. Increases in IGF-1 After Anti-TNF-alpha Therapy Are Associated With Bone and Muscle Accrual in Pediatric Crohn Disease. *J Clin Endocrinol Metab* 2018; 103 (3):936-945.
47. Elliott B, Renshaw D, Getting S, Mackenzie R. The central role of myostatin in skeletal muscle and whole body homeostasis. *Acta Physiol (Oxf)* 2012; 205 (3):324-340.
48. Delanaye P, Bataille S, Quinonez K, Buckinx F, Warling X, Krzesinski JM, et al. Myostatin and Insulin-Like Growth Factor 1 Are Biomarkers of Muscle Strength, Muscle Mass, and Mortality in Patients on Hemodialysis. *J Ren Nutr* 2019.
49. Visser M, Pahor M, Taaffe DR, Goodpaster BH, Simonsick EM, Newman AB, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. *J Gerontol A Biol Sci Med Sci* 2002; 57 (5):M326-332.
50. Palacios D, Mozzetta C, Consalvi S, Caretti G, Saccone V, Proserpio V, et al. TNF/p38alpha/polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. *Cell Stem Cell* 2010; 7 (4):455-469.
51. Chen SE, Jin B, Li YP. TNF-alpha regulates myogenesis and muscle regeneration by activating p38 MAPK. *Am J Physiol Cell Physiol* 2007; 292 (5):C1660-1671.
52. Ward LM, Rauch F, Matzinger MA, Benchimol EI, Boland M, Mack DR. Iliac bone histomorphometry in children with newly diagnosed inflammatory bowel disease. *Osteoporos Int* 2010; 21 (2):331-337.
53. Cameron FL, Gerasimidis K, Papangelou A, Missiou D, Garrick V, Cardigan T, et al. Clinical progress in the two years following a course of exclusive enteral nutrition in 109 paediatric patients with Crohn's disease. *Aliment Pharmacol Ther* 2013; 37 (6):622-629.
54. Sebo ZL, Rendina-Ruedy E, Ables GP, Lindskog DM, Rodeheffer MS, Fazeli PK, et al. Bone Marrow Adiposity: Basic and Clinical Implications. *Endocr Rev* 2019; 40 (5):1187-1206.
55. Bastos CM, Araujo IM, Nogueira-Barbosa MH, Salmon CEG, de Paula FJA, Troncon LEA. Reduced bone mass and preserved marrow adipose tissue in patients with inflammatory bowel diseases in long-term remission. *Osteoporosis Int* 2017; 28 (7):2167-2176.
56. Ferrau F, Giovinazzo S, Messina E, Tessitore A, Vinci S, Mazziotti G, et al. High bone marrow fat in patients with Cushing's syndrome and vertebral fractures. *Endocrine* 2019.
57. Maurice F, Dutour A, Vincentelli C, Abdesselam I, Bernard M, Dufour H, et al. Active cushing syndrome patients have increased ectopic fat deposition and bone marrow fat content compared to cured patients and healthy subjects: a pilot 1H-MRS study. *Eur J Endocrinol* 2018; 179 (5):307-317.

- 588 58. LeBrasseur NK, Achenbach SJ, Melton LJ, Amin S, Khosla S. Skeletal muscle mass is associated with
589 bone geometry and microstructure and serum insulin-like growth factor binding protein-2 levels in
590 adult women and men. *J Bone Miner Res* 2012; 27 (10):2159-2169.
- 591 59. Bryant RV, Ooi S, Schultz CG, Goess C, Grafton R, Hughes J, et al. Low muscle mass and sarcopenia:
592 common and predictive of osteopenia in inflammatory bowel disease. *Aliment Pharm Ther* 2015; 41
593 (9):895-906.

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Figure Legends

Figure 1: MRI imaging of muscle and muscle fat fraction at 33% distal femur in CD and control

A: Muscle cross sectional area

B: Muscle fat fraction (%)

C: Residual muscle area

Individual dot plots for MRI-based measures of muscle area and adiposity at 33% distal femur.

Between group differences assessed using t-tests.

* $p < 0.05$

CD: Crohn's disease; mm: millimetres

611 **Table 1. Demographic, lifestyle and clinical characteristics in CD and controls**

	Crohn's Disease (n = 27)	Controls (n = 27)	p-value
<i>Demographics & Lifestyle</i>			
Sex (f/m), n	15/12	15/12	-
Age (years)	23.2 (18.0, 36.1)	22.9 (18.2, 37.0)	-
Height (cm), mean (SD)	169.1 (9.4)	171.8 (10.7)	0.33 ^a
Sitting Height (cm), mean (SD)	87.5 (4.6)	89.4 (4.4)	0.12 ^a
Leg Length (cm), mean (SD)	81.6 (6.9)	82.4 (7.9)	0.71 ^a
Weight (kg)	61.5 (38.2, 104.1)	69.4 (49.8, 93.3)	0.08 ^b
BMI (kg/m ²)	21.1 (16.1, 34.4)	23.5 (18.2, 28.2)	0.03 ^{b*}
BMI Category, n (%)			
Underweight	3 (11.1)	1 (3.7)	0.32
Normal weight	21 (77.8)	21 (77.8)	1.0
Overweight	1 (3.7)	5 (18.5)	0.1
Obese	2 (7.4)	0 (0)	0.16
Physical Activity (MET-mins/week)	2953 (585, 10392)	3699 (603, 12798)	0.99 ^b
Calcium/Vitamin D Supplements, n (%)	7 (25.9)	5 (18.5)	0.47
Oral Contraceptives, n (%)	3 (20.0)	5 (33.3)	0.41
<i>Clinical Characteristics</i>			
Age at menarche (years), mean (SD)	13.3 (1.2)	12.3 (1.4)	0.07 ^a
Previous Fracture, n (%)	12 (44.4)	9 (33.3)	0.4
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 (%)			
Anti-TNF α	13 (48.1)	-	
Glucocorticoids	1 (3.7)	-	
Thiopurines	17 (63.0)	-	

5-ASA	3 (11.1)	
Methotrexate	2 (7.4)	-
Vedolizumab	2 (7.4)	-
Previous CD Surgery, n (%)	10 (37.0)	-
Steroid use (<18 years)	1 (0, 8)	-
Biologic use, 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-TNF α – infliximab/adalimumab; CD – Crohn’s disease; steroid use – number of self-reported courses of GC; biologic use – previous or current anti-TNF α therapy.

Continuous variables displayed as median (range) unless otherwise stated.

^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

* p<0.05

Table 1. Clinical biochemistry, biochemical markers of muscle-bone metabolism, and pro-inflammatory cytokines

<i>Clinical biochemistry</i>	Crohn's Disease (n = 27)	Controls (n = 26)	p-value
Albumin (g/l), mean (SD)	40.6 (3.9)	42.2 (2.6)	0.1 ^a
Faecal Calprotectin (µg/g) [†]	81 (30, 1800)	30 (30, 217)	0.05 ^{b*}
ESR (mm/hour)	5 (1, 90)	2 (2, 8)	0.07 ^b
CRP (mg/l)	1 (0, 63)	0 (0, 9)	0.003 ^{b*}
25-OHD (nmol/l)	51 (14, 142)	45 (16, 101)	0.96 ^b
PTH (pmol/l), mean (SD)	7.0 (2.8)	5.3 (1.7)	0.01 ^{a*}
Alkaline Phosphatase (U/l)	70 (38, 124)	63 (23, 121)	0.12 ^b
<i>Markers of bone turnover</i> [‡]			
BSAP (µg/l)	13.9 (8.4, 33.8)	15.0 (1.9, 30.1)	0.85 ^b
CTX-1 (ng/ml)	0.38 (0.12, 1.43)	0.6 (0.15, 0.9)	0.92 ^b
Sclerostin (ng/ml), mean (SD)	0.4 (0.13)	0.47 (0.14)	0.06 ^a
<i>Markers of GH/IGF-1 axis</i> [‡]			
IGF-1 (ng/ml), mean (SD)	310 (90)	349 (81)	0.11 ^a
IGFBP2 (ng/ml)	377 (107, 858)	334 (111, 634)	0.03 ^{b*}
IGFBP3 (ng/ml), mean (SD)	4438 (663)	4448 (802)	0.96 ^a
ALS (ng/ml)	10802 (5484, 16348)	11770 (7566, 21974)	0.52 ^b
<i>Pro-inflammatory cytokines</i> [‡]			
TNFα (pg/ml)	10.53 (3.51, 31.59)	5.67 (2.27, 18.42)	0.01 ^{b*}
IFN-γ (pg/ml)	2.48 (1.56, 6.56)	1.56 (0.36, 3.4)	<0.001 ^{b*}
IL-6 (pg/ml) [§]	1.08 (0.36, 8.92)	1.2 (0.4, 5.72)	0.95 ^b
IL-8 (pg/ml)	11.40 (3.40, 122.72)	6.66 (3.40, 15.6)	0.01 ^{b*}
<i>Myokines</i> [‡]			
Myostatin (ng/ml)	2.77 (0.14, 21.95)	3.37 (0.09, 19.98)	0.33 ^b

25-OHD – 25hydroxyvitamin 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.

Results displayed as median (range) unless otherwise stated

^a t-test; ^b Mann Whitney U test

[†] n = 24 (13 CD, 11 controls), [‡] n = 52 (27 CD, 25 controls), [§] n = 14 (9 CD, 5 controls); [|] n = 37 (27 CD, 10 controls)

* p < 0.05

Table 3. MRI-based measures of trabecular bone microarchitecture and cortical geometry of the distal femur.

	Crohn's Disease (n = 27)	Controls (n = 27)	p- value
<i>Trabecular Microarchitecture</i> [†]			
AppBV/TV, mean (SD)	0.552 (0.034)	0.555 (0.018)	0.7 ^a
AppTbTh (mm), median (range)	0.297 (0.238, 0.368)	0.292 (0.265, 0.356)	0.51 ^b
AppTbN (/mm), mean (SD)	1.868 (0.148)	1.898 (0.106)	0.41 ^a
AppTbSp (mm), mean (SD)	0.241 (0.026)	0.235 (0.017)	0.34 ^a
<i>Cortical Geometry</i>			
Cortical Area (mm ²), mean (SD)	278.7 (45.4)	290.4 (41.3)	0.32 ^a
Cortical Thickness (mm), mean (SD)	2.6 (0.38)	2.53 (0.31)	0.47 ^a
Endosteal Circumference (mm), median (range)	97.4 (78.1, 141.4)	106.5 (78.4, 130.4)	0.03 ^{b*}
Periosteal Circumference (mm), median (range)	114.7 (94.6, 154.8)	124.2 (95.2, 145.4)	0.03 ^{b*}

App – apparent; BV/TV – bone volume/total volume fraction; TbTh – trabecular thickness; mm – millimetres; TbN – trabecular number; TbSp – trabecular separation

^a t-test; ^b Mann Whitney U test

[†] n = 52 (26 CD, 26 Controls)

* p < 0.05

Table 4. Multiple regression analysis of cortical bone geometry at 15% distal femur

	B (95% CI)	Std. β	Model R^2	P-value
<i>Cortical Geometry</i>				
Cortical Area (mm ²)	-6.0 (-22.1, 10.1)	-.07	0.58	0.38
Cortical Thickness (mm)	0.05 (-0.13, 0.23)	.068	0.14	0.61
Endosteal Circumference (mm)	-3.75 (-9.47, 1.96)	-.138	0.47	0.19
Periosteal Circumference (mm)	-3.23 (-8.3, 1.84)	-.124	0.55	0.21

Std – Standardized; mm - millimetres

Models are adjusted for sex and height.

Controls used as reference group.

Table 5. Multiple regression analyses for associations between cortical geometry and muscle outcomes in Crohn's disease.

	B (95% CI)	Std. β	Model R^2	p-value
<i>Residual Muscle Area (mm²)</i>				
Cortical Area (mm ²)	0.009 (0.11, 1.68)	.330	0.64	0.03*
Endosteal Circumference (mm)	0.002 (-0.001, 0.005)	.253	0.54	0.12
Periosteal Circumference (mm)	0.002 (0.000, 0.005)	.276	0.65	0.06
<i>Grip Strength (kg)</i>				
Cortical Area (mm ²)	2.03 (-0.54, 4.60)	.245	0.6	0.12
Endosteal Circumference (mm)	0.77 (-0.09, 1.61)	.291	0.56	0.08
Periosteal Circumference (mm)	0.75 (0.03, 1.47)	.297	0.66	0.04*
<i>Mechanography Power (kW)</i>				
Cortical Area (mm ²)	22.31 (3.97, 40.64)	.431	0.8	0.02*
Endosteal Circumference (mm)	11.50 (2.58, 20.41)	.704	0.51	0.02*
Periosteal Circumference (mm)	10.69 (3.43, 17.95)	.692	0.64	0.01*
<i>Mechanography Force (kN)</i>				
Cortical Area (mm ²)	37.36 (2.43, 72.28)	.317	0.78	0.04*
Endosteal Circumference (mm)	23.36 (7.81, 38.91)	.628	0.56	0.01*
Periosteal Circumference (mm)	21.29 (8.63, 33.94)	.604	0.68	0.003*

mm – millimetres; Std – standard; Std. β – standardized beta coefficient; kg – kilograms; kW – kilowatts; kN - kilonewtons

All models are adjusted for height.

* $p < 0.05$

Figure 1: MRI imaging of muscle and muscle fat fraction at 33% distal femur in CD and controls

Supplementary Material

Supplementary Table S1: MRI & MRS pulse sequence parameters for assessment of trabecular microarchitecture, cortical geometry, muscle fat fraction and bone marrow adiposity.

	<i>Trabecular</i>	<i>Cortical</i>	<i>Muscle Fat</i>	<i>Bone Marrow</i>
	<i>Microarchitecture</i>	<i>Geometry</i>	<i>Fraction</i>	<i>Adiposity</i>
Sequence Type	CISS	T1-weighted TSE	6-point VIBE DIXON	PRESS
Resolution (mm)	0.2 x 0.2 x 0.4	0.4 x 0.4 x 2	1.1 x 1.1 x 2	20 x 20 x 20
TE (ms)	5.68	11	1.54, 2.97, 4.40, 5.83, 7.26, 8.69	30
TR (ms)	12.62	650	10.7	2000
Flip Angle (°)	50	150	4	90
No. of Averages	4	1	6	80
FoV (mm²)	100 x 100	140 x 140	204 x 204	-
Matrix	448 x 448	320 x 240	192 x 192	-
Number of Slices	20	20	20	-
Bandwidth (Hz.pixel⁻¹)	228	240	1090	1200
Scan Time (mins:secs)	9:24	1:05	3:00	2:50

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

Supplementary Table S2. Cohort characteristics of participants with available mechanography data, comparing CD and controls.

	Crohn's Disease (n = 19)	Controls (n = 18)	p-value
<i>Demographics & Lifestyle</i>			
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 (p<0.05)

Trabecular bone analysis

A manual region of interest (ROI) was drawn around the endocortical boundary by a single observer, from which a central region equating to 50% of trabecular bone was selected for analyses (Supplementary Image 1A below). Images were binarized into bone and marrow phases prior to quantitative assessment, done by segmentation using a fuzzy c-means algorithm – allowing for allocation of individual pixels to either ‘bone’ or ‘marrow’ clusters.

Cortical bone analysis

User-defined boundaries were manually drawn around the endosteal and periosteal circumferences by a single observer. These were then smoothed using a Savitzky-Golay filter and superimposed into the original image, thus defining the region of cortical bone (Supplementary Image 1B below).

Muscle analysis

Ten ROIs were manually segmented around the muscle groups of the upper leg (Supplementary Image 1C below) by a single observer.

Inter-observer CoVs were obtained by a second observer independently analysing a subset of 30 (15 CD, 15 Controls) trabecular, cortical and muscle images, respectively.

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Figure 1. Representative images of MRI analyses. Left - trabecular analysis, blue region indicates central 50% of trabecular compartment; Middle - Cortical analysis, green line indicates endosteal circumference and blue line is periosteal circumference; Right – Muscle analysis, ten manually drawn regions of interest around the muscles at 33% distal femur

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