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REDUCED SKELETAL MUSCLE PROTEIN BALANCE IN PAEDIATRIC CROHN'S DISEASE.

SHORT Title: Muscle Physiology in paediatric Crohn's disease

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Abbreviations: ASMI: appendicular skeletal muscle index; a-v: arterio-venous**;** BCAA: branched chain amino acids; BMI: body mass index; CD: Crohn's disease; CDM: male CD; Con: controls; ConM: male controls; CRP: C-reactive protein; dom: dominant arm; EGTA: Ethyleneglycol-Bis-β-Aminoethylether Tetraacetate**;** FCP: faecal calprotectin; FFM: fat free mass; FM: fat mass; HBI: Harvey Bradshaw Index; IL-1β: Interleukin 1 beta**;** IL-6: Interleukin 6; IPAQ: international physical activity questionnaire; LBM: lean body mass; LM: lean mass; non-dom: non-dominant arm; REE: resting energy expenditure; RER: respiratory exchange ratio (volume CO_2 expired / volume of O_2 inspired); SDS: standard deviation score; TNFα: Tumor necrosis factor alpha; TBM: total body mass.

ABSTRACT

Background and Aims: An inability to respond to nutrition could be implicated in low muscle mass in Crohn's disease. We aim to determine skeletal muscle metabolic response to feeding in Crohn's disease and healthy volunteers.

Methods: Twenty asymptomatic Crohn's disease participants (15.6 ± 0.5 yrs; BMI 20.6 ± 0.9 kg/m^2); 9 with active disease (faecal calprotectin, 808 ± 225ug/g and C-reactive protein, 2.2 ± 1.2 mg/dl), 11 in deep remission (faecal calprotectin, 61 ± 12ug/g and C-reactive protein, 0.3 ± 0.2 mg/dl) and 9 matched healthy volunteers (16.0±0.6 yrs; BMI 20.7±0.6 kg/m^2) were recruited. Participants had a dual energy X-ray absorptiometry scan, handgrip dynamometer test, wore a pedometer and completed a food diary. Arterialised hand and venous forearm blood samples were collected concurrently and brachial artery blood flow measured at baseline and every 20mins for 2hrs after the ingestion of a standardised liquid meal. Net balance of branched chain amino acids and glucose were derived.

Results:

Controls had a positive mean BCAA balance. CD participants had an initial anabolic response to the meal, with increasing BCAA balance between t=0 & t=20, but returned to negative by t=60. This was associated with reduced FFM z-scores in CD but not with insulin resistance or disease activity. Exploratory analyses suggest that negative postprandial BCAA response seen in CD is predominant in males (p=0.049), with associated lower appendicular muscle mass (p=0.034), higher muscle fatigue (p=0.014) and reduced protein intake (p=0.026).

Conclusions: The inability to sustain a positive protein balance postprandially could provide an explanation for the reduced muscle mass seen in CD. Further mechanistic studies will be needed to confirm these findings.

Keywords: Crohn's disease; Inflammatory Bowel Disease; Nutrition; Sarcopenia.

INTRODUCTION

In the era of biological therapy and rising obesity in the general population [1] malnutrition is less prevalent in paediatric Crohn's disease (CD), though still an apparent complication in a substantial minority [2,3,4]. Many of these patients also have disproportionately lower skeletal muscle mass [5,6]. Reduced muscle mass is seen in active disease [7], persists in remission [8] and is negatively correlated with disease duration [9]. Low muscle mass has been linked to decreased muscle function [10,11,12], low bone mass and density [13,14], reduced physical activity [14], fatigue and impaired quality of life [15].

Skeletal muscle mass is determined by the balance between muscle protein synthesis and breakdown, rates of which determine protein turnover. Children in active disease a have high protein turnover [16]. However, whereas conventional medical treatments such as corticosteroids[17], elemental diets [17] and biological therapies [18] reduce fasting protein breakdown, they also reduce fasting protein synthesis, resulting in no change in net fasting protein balance when patients are in remission.

Protein synthesis is stimulated by protein nutrition, particularly the branched chain amino acids (BCAA). Twelve weeks of elemental diet induce partial normalisation of muscle cross-sectional area with no further change at 52 weeks even when patients are in remission [14]. Once the inflammatory burden is reduced, patients in remission may still suffer from low muscle mass [8], despite receiving apparently adequate protein nutrition [16,19].

This suggests that reduced muscle mass in active disease is driven by inflammation and a sub-optimal muscle protein synthetic response to protein feeding, termed anabolic resistance [20] that may persist in remission. Anabolic resistance is thought to be the main driver of muscle loss in critical illness, ageing and disuse [21].

We hypothesise that reduced muscle mass and function in paediatric CD patients could be associated with anabolic resistance. We aimed to measure skeletal muscle protein balance in a

cohort of paediatric CD patients and age- and BMI-matched controls in both the overnight-fasted and fed states.

MATERIALS AND METHODS

Study population

We aimed to recruit male and female, stable, asymptomatic CD outpatients (age 11-18 years). Relevant CD symptoms were measured through a Harvey Bradshaw index [22]. Patients were approached from Nottingham University Hospitals Trust and Birmingham Women's and Children's NHS Foundation Trust existing patient population. Disease activity was quantified through C-reactive protein (CRP), faecal calprotectin (FCP) and ileocolonoscopy or magnetic resonance imaging (MRI). Active disease was defined as CRP >5mg/dl or FCP >250μg/g or the presence of ulceration at ileocolonoscopy or MRI scan. In the absence of these findings patients were classed as in deep remission. Any CD-related medication apart from corticosteroids within 3 months prior to recruitment was permitted. Age-, gender- and BMI-matched healthy volunteers were recruited through advertisements at both institutions, through local press, and social media posts to parents. All potential participants were given comprehensive written and verbal explanations of the study before giving written informed consent (parental consent at ≤15yrs) and were free to withdraw at any time. Participants completed a general health questionnaire and underwent a short medical screening prior to participation. The study was approved by the Health Research Authority (15/WM/0285) on the 26th July 2016 with the study conforming to the recognised standards of the Declaration of Helsinki.

Outcome Measures

The primary outcome was forearm skeletal muscle protein net balance under fasted and fed conditions. Secondary outcomes were; forearm skeletal muscle glucose net balance; forearm

and whole body insulin sensitivity; resting and fed metabolic rate, daily physical activity; forearm muscle isometric strength and fatigability; appendicular lean mass (LM) and appendicular skeletal muscle index (ASMI); daily energy intake and dietary macronutrient composition; and markers of active disease (interleukin (IL)-1β, IL-6, TNFα, CRP and FCP). We investigated the effect of gender and disease activity on the primary outcome in an exploratory sub-analysis.

Differences between arterialised venous and venous concentrations (a-v difference) of BCAA, and glucose, multiplied by brachial artery blood flow and corrected for forearm lean mass were used to determine the net balance of these nutrients across the forearm under fasted and fed conditions. Positive values indicated net tissue uptake, whereas negative values indicated net release of nutrients. The following equation was used for a given nutrient (N):

Net balance of N (μmol/min/100g of forearm lean mass) = {Blood flow (ml/min) x ([N]arterialised – [N]venous (mmol/L or μmol/L)}/1000 (not for glucose)/ {lean mass (g) x 100}.

Lean mass (LM) was measured by Dual-energy X-ray absorptiometry (DEXA) (Luna Prodigy, GE Healthcare). The Matsuda index was used as an index of whole body insulin sensitivity [23,24], with a lower index indicating a higher level of insulin resistance. Muscle strength measurements were standardized for muscle size, as well as for height and age, using the method developed by Rauch et.al [25], to facilitate comparison. Appendicular LM (sum of lean mass in the limbs measured by DEXA) and ASMI (appendicular lean mass (kg) / height $(m)^2$) were calculated to give more precise indices of skeletal muscle mass than total lean mass alone.

Experimental protocol

Participants reported to the laboratory at 0800, following an overnight fast, having abstained from strenuous exercise for the previous 48 hours. On arrival, their body composition was

assessed by DEXA. Body mass, whole body, and regional body composition and body mass index were calculated. Subsequently, participants were asked to rest in a semi-supine position on a bed while a cannula was inserted in a retrograde fashion into a superficial vein on the dorsal surface of the dominant hand. This hand was kept in a hand-warming unit (air temperature 55°C) to arterialize the venous drainage of the hand [26]. A second cannula was placed in an antecubital vein in the non-dominant forearm. Both these cannulas were used for blood sampling. After baseline blood samples, and measurements of brachial artery blood flow (in the non-dominant arm), as measured by Doppler ultrasound (Toshiba Aplio 300), and resting energy expenditure by indirect calorimetry (Cosmed, Italy), all participants ingested a 220ml bottle of Ensure plus nutrition shake (t = 0). This meal provided 330kcal, consisting of 30% of energy as fat (11g), 53% of energy as carbohydrates (44g), and 17% of energy as protein (14g). This meal composition mirrors dietary recommendations and specifically the protein content equates to 32% of the daily protein requirements of the CD subjects (0.75g/kg bw).

Arterialized-venous (2 ml) and venous (2 ml) blood were obtained concurrently from the heated hand vein and antecubital vein along with brachial artery blood flow measurements at t = 0 and every 20 minutes thereafter for 2 hours, so that forearm muscle net balance of amino acids and glucose could be calculated in the fasted and fed states. At t = 100 a final indirect calorimetry was performed providing resting energy expenditure (REE) and respiratory exchange ratio (RER) to compare with fasting levels.

At the end of the 2-hour postprandial period, an assessment of forearm muscle function was undertaken. Participants performed 12 maximal static voluntary contractions using a dynamometer (MIE medical research Ltd. UK), with both dominant and non-dominant arms. The peak contraction was taken as maximal handgrip isometric strength (kg), usually achieved within the first 2-3 contractions. Level of fatigue was derived from the difference in

peak strength and strength measured at the end of 12 maximal contractions (mean of the last 3) [27]. Participants were familiarised with the protocol during screening and the same trained operator both gave instructions and took measurements from all participants [28]. SDS (standard deviation scores) were calculated for height dependent and weight dependent forearm strength as previously shown [25].

Blood metabolite, cytokine & hormone analysis

Blood glucose levels were measured using Yellow Springs Instrument Analyzer, YSI, 2300 STAT PLUS. Plasma separated from Ethyleneglycol-Bis-(β-Aminoethylether) Tetraacetate (EGTA) treated blood was analysed for BCAA concentrations by spectrophometric assay [29]. Serum separated from arterialised blood was analysed for insulin concentration with an enzyme-linked immunosorbent assay (ELISA) technique (DRG diagnostics, Germany). CRP was measured by ELISA at the Department of Clinical Chemistry, Queen's Medical Centre, Nottingham, as part of the initial screening process or in baseline blood samples. Inflammatory cytokines tumour necrosis factor (TNF)α; interleukin (IL)-6; IL-1β, and bioavailable testosterone, were measured in baseline (over-night fasted) arterialized plasma samples by colorimetric ELISA (R&D systems, Minneapolis, US) according to manufacturer's instructions.

Assessment of physical activity, habitual dietary intake & anthropometrics

Step counts measured using a pedometer (Omron, Kyoto, Japan) for 3-days in advance of the study visit and self-reported levels of physical activity, using short form International Physical Activity Questionnaire (IPAQ) [30] were used to assess habitual physical activity levels. Routine energy intake was measured using a 3-day paper-based food diary completed by participants in the days preceding their study visit (1 weekend day and 2 week days). Great care was taken in briefing participants, checking diaries during the study visit and the same operator analysed all the diaries using Nutritics software (Dublin, Ireland) in an effort to

minimise inaccuracy. Anthropometrics were collected by a single investigator using a standard protocol and z-scores were calculated for height and BMI (WHO Growth Reference data for 5-19 year olds) and for fat mass (FM) and fat free mass (FFM)[31].

Statistical analyses

The parametric or non-parametric nature of the data was determined with a Shapiro-Wilk test. Parametric data are presented as mean ± standard error of the mean (SEM) and non-parametric as median plus interquartile range (IQR). Parametric data has been examined using t-tests or where time is also a variable, two-way analysis of variance (experimental group x time). Where data were not normally distributed, these were analysed using a Mann-Whitney test. Area under the curve (AUC) has also been calculated to illustrate glucose net uptake in response to feeding. P value of <0.05 was considered significant. Data analysis was undertaken with Prism software V.7.0 (La Jolla, San Diego, US)

RESULTS

Subject characteristics

Twenty CD participants (15.6 ± 0.5 yrs; BMI 20.6 ± 0.9 kg/m^2) were recruited (**Table 1**). Mean number of years since diagnosis was 4.3 ± 0.6. All CD participants were asymptomatic with an HBI of < 4. Nine CD participants had evidence of disease activity with a mean FCP of 808 ± 225ug/g, mean CRP of 2.2 ± 1.2 mg/dl or evidence of ulceration at ileocolonoscopy or magnetic resonance enterography (MRE). The other eleven CD participants were in deep remission with mean FCP of 61 ± 12ug/g and CRP of 0.3 ± 0.2 mg/dl and absence of disease activity at ileocolonoscopy or MRE. Eleven participants were being treated with anti-TNF therapy (Adalimumab or Infliximab) while the rest were naïve to biological therapies. Five CD participants had a history of intestinal resection for stricturing (n=3) or penetrating (n=2) disease behaviour. See supplementary table for individual CD participant details. Nine

healthy control (Con) participants (16.0 ± 0.6 years; BMI 20.7 ± 0.6 kg/m^2) were recruited and matched to CD participants. Although there were no significant differences in anthropometrics between groups 10% of CD patients had a BMI z-score of <-2. It was not possible to cannulate two of the female CD participants, so for mechanistic studies in CD n=18. All endoscopic or imaging investigations were undertaken as part of the patients' standard care.

Muscle physiology: protein and glucose metabolism

Arterialized plasma BCAA (BCAA) peaked at t = 40 in response to feeding (time p<0.0001) (**Figure 1A**). Con but not CD were in positive forearm muscle BCAA balance (**Figure 1B**), although both groups mounted an initial response to feeding (t = 0 to t = 20) (**Figure 1C**). Glucose a-v difference across the forearm increased in response to feeding, with this and glucose net uptake not different between CD and Con suggesting no skeletal muscle insulin resistance (**Figures 2A & C**).

Body composition and muscle function

There was a significant difference between Con and CD FFM z-scores (0.10 and -0.84 respectively, p=0.015) but only trends towards a difference in LBM, appendicular LM and ASMI (**Table 2**). All other body composition measures were comparable between Con and CD. No differences were found in muscle strength or fatigue although there was a trend for CD to fatigue more than Con in the dominant arm.

Whole body physiology: insulin sensitivity and energy expenditure

Arterialized blood glucose and serum insulin levels increased in both groups post-feeding (t = 20) and peaked at t = 40, (time p<0.0001), (**Figures 3A & B**). Glucose concentrations then dropped back, stabilizing above fasting levels by t = 60, whereas insulin continued to decline until t = 120. CD response to feeding in terms of these parameters and Matsuda index was not different to Con and therefore no whole-body insulin resistance was detected (**Figures 3A**

- C). REE and RER increased post-feeding (t = 0 vs. t = 20-120) in CD and Con (all $p<0.05$) with no differences between groups (**Table 3**).

Cytokine analyses

No differences in TNFα, IL-1β and IL-6 were observed between CD and Con groups although there was a trend for higher TNFα in CD (**Table 1**).

Testosterone levels

Testosterone levels were significantly higher in CD (median 9.0 ng/ml, IQR: 3.1-16.6) when compared to Con (1.8ng/ml, 1.4-8.2, p=0.031) (**Table 1**).

Physical activity and diet

Neither activity levels nor total energy intake differed between groups (**Table 3**).

Gender and disease activity sub-analyses

Male CD participants (CDM) n=11, were in overall negative BCAA balance, in contrast to the positive balance of male control participants (ConM) n=5, (p=0.049). CDM also had lower levels of arterialised BCAA than ConM at baseline and post feeding (condition p=0.027). CDM had a lower height for age when compared to ConM (z-scores -0.2 and 0.5 respectively, p=0.021) and appendicular LM was 24% lower than in ConM (p=0.034). CDM fatigued significantly more than ConM in the dominant arm (p=0.014). Protein intake was lower in CDM (p=0.026) with 75 ± 5g/kg body mass/day reported in CDM and 105 ± 15g/kg/day reported in ConM.

There was a difference in skeletal muscle insulin sensitivity in active (n=9) versus deep remission (n=11). In active disease forearm glucose net uptake was more than 2-fold greater than deep remission (p=0.036) implying skeletal muscle insulin resistance in CD in deep remission. Serum insulin also peaked sooner in active disease, at 20 min, versus 40 mins in deep remission (interaction p=0.0014). Active disease also had higher serum IL-6 levels than deep remission (median: 206 pg/ml, IQR: 83-390 vs. 41 pg/ml, 0-135, respectively; p=0.025).

There was however no difference in anabolic response between those in active disease and deep remission.

DISCUSSION

We hypothesised that the reduced muscle mass in CD could be driven through anabolic resistance of skeletal muscle to a mixed oral meal that may persist even when in clinical remission. Con but not CD were in a positive net protein balance and this was associated with reduced FFM z-scores. However, CD participants were able to mount an initial positive BCAA balance response to feeding and so may not be completely anabolically resistant as hypothesised. Protein balance, and the ability to increase muscle protein mass, is also determined by muscle protein breakdown. The overall neutral net balance observed in the current study may suggest that muscle protein synthesis and/or breakdown are affected. Male CD participants had an overall negative protein balance, with associated lower appendicular LM, higher levels of muscle fatigue and reduced habitual dietary protein intake. Moreover, CD patients with active disease had similar net muscle protein balance, but a higher skeletal muscle glucose uptake, compared to those in deep remission. These exploratory findings may imply that anabolic resistance plays a role in the aetiology of reduced LM in males irrespective of disease status. These findings will need to be validated in a larger cohort. Adolescent CD patients may therefore require more protein per meal compared to age-matched controls in order to maintain a positive protein balance and muscle mass. This is the first study to have investigated skeletal muscle protein balance in paediatric CD.

Findings of reduced muscle mass in paediatric CD in remission are congruent with the majority of the literature [5,6,7,8]. Reduced muscle function has been previously found in paediatric CD patients with mild disease and in clinical remission [11].

CD participants in the current study still consumed more protein than recommended and their consumption was above National Diet and Nutrition Survey (NDNS) 2014-16 [32] average values. CD: 79g and NDNS: 67g (NDNS aged 11-18 and weighted to match our gender split). However, a higher protein recommendation may be appropriate as a linear relationship between amino acid availability and protein balance holds true above current recommended intake levels because amino acids suppress muscle protein breakdown as well as stimulate protein synthesis[33]. An overall increase in protein intake, rather than specific amino acid supplements, has recently been suggested to be most effective in both stimulating muscle protein synthesis and suppressing muscle protein breakdown [34]. Greater anabolic response to higher doses of protein in healthy adults has been shown to be largely due to the suppression of protein breakdown with only a small increase in protein synthesis [35]. Despite experimental studies indicating leucine is key in stimulating the mTOR pathway [36] chronic supplementation has not consistently produced positive results [34]. It has now been demonstrated that stimulation of muscle protein synthesis by protein ingestion is not solely due to leucine [37]. A study in CD adults showed that protein supplementation equivalent to a 25% increase in protein intake for 16 weeks improved LM; though no detail of protein turn-over was available[38]. Our observation of a lower net protein balance in the face of adequate amino acid availability would further support the notion of an impaired anabolic response in CD.

Testosterone levels were raised in CD. This contrasts with previous studies that have reported both reduced androgens and delayed maturation in CD [39,40], though it is likely that these studies reported on total rather than bioavailable testosterone but did not specify.

Insulin inhibits protein breakdown and has a permissive effect in stimulating protein synthesis via the Akt/mTOR signalling pathway [41,42]. Whole body insulin resistance has been found in CD patients with active disease [43] with TNFα being shown to reduce insulin sensitivity in humans [44]. In the present study skeletal muscle insulin sensitivity is similar between CD and

Con as has been previously shown [45]. However skeletal muscle glucose uptake was higher in active disease vs. deep remission. Although plasma TNFα levels were similar between these groups, plasma IL-6 was significantly higher in patients with active disease. Chronically elevated levels of IL-6 have been implicated in insulin resistance and reduced skeletal muscle mass[46]. Conversely acute administration of IL-6 has been found to increase glucose disposal [47]. It remains unclear why skeletal muscle insulin sensitivity may be higher in active disease vs. deep remission.

Higher basal metabolic rate per FFM ratio and diet-induced thermogenesis have regularly been reported in CD, which could explain lower body mass[48]. However, in accordance with the current study, several other studies in both paediatric CD[49] and adults[50,51] have found no relationship between the two.

The association of reduced protein balance with reduced FFM z-scores in CD is a possible explanation as to why patients fail to gain muscle mass even when in remission. CD may not be able to re-build muscle mass because they cannot maintain a positive protein balance in response to feeding. In a study of young adult males with inflammatory bowel disease, a fifth of participants had significantly reduced muscle mass raising the possibility that these LM deficits persist into adulthood [52]. A longitudinal study would be needed to investigate this.

The main limitation of this study is the relatively small sample size that will not allow detailed sub-analyses by disease activity and gender. Moreover, it is cross-sectional and acts as a precursor to a larger prospective study. Furthermore, although reduced serum Vitamin D has been implicated in reduced LM [53] values were unavailable in this cohort.

A key strength was our use of the a-v balance technique giving a holistic view of the anabolic response and high temporal resolution. The discovery that CD can respond to anabolic stimuli, albeit to a lesser extent than healthy controls, has important implications for treatment.

In conclusion, we have shown that reduced FFM z-scores in CD patients are associated with a reduced protein balance in response to feeding, thus providing a possible explanation for the persistence of low muscle mass in children regardless of disease activity. Moreover, male CD participants were in overall negative protein balance with associated lower appendicular LM, height for age, fatigue in the dominant arm and protein intake. This is an interesting preliminary observation that requires further elucidation. A large prospective interventional study is warranted to confirm the effects of disease activity and gender, protein intake and exercise, with the aim of restoring muscle mass to healthy levels in children and young adult CD patients.

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Statement of Authorship: Dr Gordon W. Moran is the guarantor of the article. Gordon W. Moran and Francis B. Stephens designed the research. Amanda Davies, Aline Nixon, Rafeeq Muhammed, Sian Kirkham conducted the study and acquired the data. Kostas Tsintzas, Francis B. Stephens and Gordon W. Moran provided study supervision. Amanda Davies, Kostas Tsintzas, Francis B. Stephens and Gordon W Moran analyzed and interpreted the data. Amanda Davies and Gordon W. Moran drafted the article. All authors critically revised the article for important intellectual content and approved the final version of the article, including the authorship list.

Conflicts of interest: Dr Moran has received: educational support from Abbvie, Janssen, NAPP, Takeda Pharmaceuticals, Merck Sharp & Dohme Ltd, Ferring and Dr Falk. He has received speaker honoraria from Merck Sharp & Dohme Ltd, Abbvie, Janssen, Ferring and Takeda Pharmaceuticals. He attended advisory boards for Abbvie, Takeda Pharmaceuticals, Janssen, Medtronic, Phebra Pharmaceuticals, Servertus Associates Ltd and Dr Falk.

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552

FIGURE LEGENDS

Figure 1. Indicators of protein metabolism in CD vs. Con: arterialized plasma BCAA concentrations (A), mean BCAA net balance across the forearm standardized for forearm lean mass (B) and BCAA net balance across the forearm, over time, standardized for forearm lean mass (C).

Values are means ± SEM. Significant differences ($p<0.05$) & trends ($p<0.1$) are marked. BCAA=branched chain amino acids, CD=Crohn's disease, Con=Control. SEM=standard error of the mean.

Figure 2. Skeletal muscle insulin sensitivity in CD vs. Con: glucose arterio-venous difference across the forearm (A), brachial artery blood flow (B) and AUC glucose net uptake across the forearm standardized for forearm lean mass (C).

Values are means ± SEM. Significant differences ($p<0.05$) & trends ($p<0.1$) are marked. AUC=area under the curve, CD=Crohn's disease, Con=Control, SEM=standard error of the mean.

Figure 3. Indicators of whole body insulin sensitivity in CD vs. Con: arterialized blood glucose concentrations (A), serum insulin (B) and Matsuda index (C).

Values are means ± SEM. Significant time effects are marked. CD=Crohn's disease, Con=Control.

Table 1. Subject characteristics of Control and Crohn's disease patients

	Con	**CD**	**p value**
	n=9	n=20[w] / n=18[v]	
Age (yrs)[w]	16.0 ± 0.6	15.6 ± 0.5	n.s.
Height (m)[w]	1.71 ± 0.05	1.66 ± 0.02	n.s.
Height-for-age (z-scores)[w]	0.5 ± 0.4	0.1 ± 0.2	n.s.
Weight (kg)[w]	60.7 ± 3.7	56.8 ± 2.7	n.s.
BMI (kg/m^2)[w]	20.7 ± 0.6	20.6 ± 0.9	n.s.
BMI-for-age (z-scores)[w]	0.03 ± 0.27	-0.2 ± 0.3	n.s.
Years since diagnosis[w]	n/a	4.2 ± 0.6	n/a
HBI[w]	n/a	1 ± 0	n/a
FCP (µg/g)[w]	n/a	132 ± 41	n/a
CRP (mg/dl)[w]	<5 ± 0	2.6 ± 0.6	n.s.
IL-1β (pg/ml)[v]	169 (37.5-517)	454 (30.5-1835)	n.s.
IL-6 (pg/ml)[v]	101 (50-335)	83 (28-287)	n.s.
TNFα (pg/ml)[v]	37 (0-111)	212 (0-661)	p=0.078
Testosterone (ng/ml)[v]	1.8 (1.4-8.2)	9.0 (3.1-16.6)	p=0.031

All values are means ± SEM except for non-parametric test results (cytokines & testosterone) where values are median and interquartile range and z-scores which were calculated for height-for-age and BMI-for-age using WHO standards. Statistically significant differences ($p<0.05$) and trends ($p<0.1$) are listed and n.s. = no significant differences between groups. CD=Crohn's disease, Con=Control.

Table 2. Body composition and muscle function

	Con	**CD**	**p value**
	n=9	n=20[w] / n=18[v]	
Body composition[w]			
FM (z-scores)	0.08 ± 0.37	0.90 ± 0.40	n.s.
FFM (z-scores)	0.10 ± 0.44	-0.84 ± 0.15	p=0.015
LBM (kg)	46.0 ± 4.5	39.3 ± 1.5	p=0.084
Appendicular LM (kg)	22.9 ± 2.6	18.7 ± 0.8	p=0.057
ASMI (kg/m^2)	7.6 ± 0.4	6.8 ± 0.2	p=0.052
Muscle function[v]			
Fatigue (%) dom	18 ± 3	26 ± 2	p=0.061
Fatigue (%) non-dom	21 ± 4	23 ± 2	n.s.
Strength dom (kg/kg forearm LM)	23.8 ± 1.3	25.6 ± 1.5	n.s.
Strength non-dom (kg/kg forearm LM)	23.9 ± 1.1	24.3 ± 1.4	n.s.
Age dependent FS SDS dom	-1.41 ± 0.35	-1.50 ± 0.22	n.s.
Age dependent FS SDS non-dom	-1.62 ± 0.31	-1.90 ± 0.21	n.s.
Height dependent FS SDS dom	-1.13 ± 0.35	-0.93 ± 0.34	n.s.
Height dependent FS SDS non-dom	-1.36 ± 0.33	-1.37 ± 0.36	n.s.

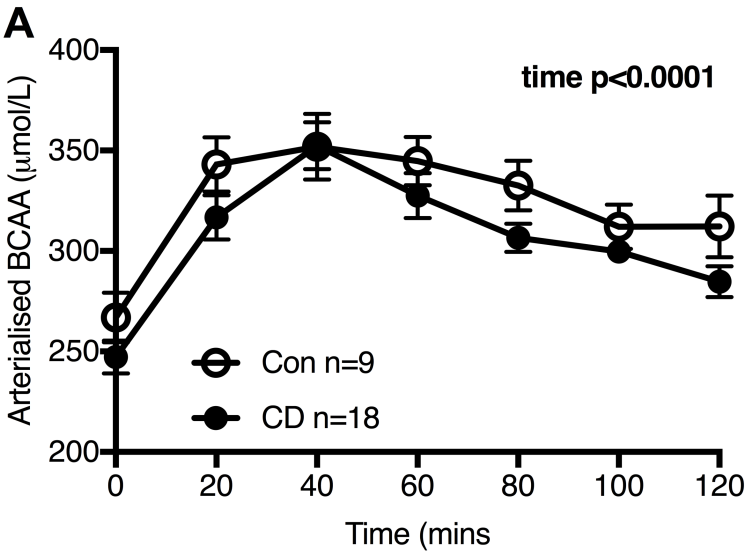
All values are means ± SEM except standard deviation scores (SDS) calculated for height and age dependent strength (see methods) and z-scores calculated for FM & FMM using age and sex appropriate reference data (see methods). Statistically significant differences ($p<0.05$) and trends ($p<0.1$) are listed and n.s. = no significant differences between groups.

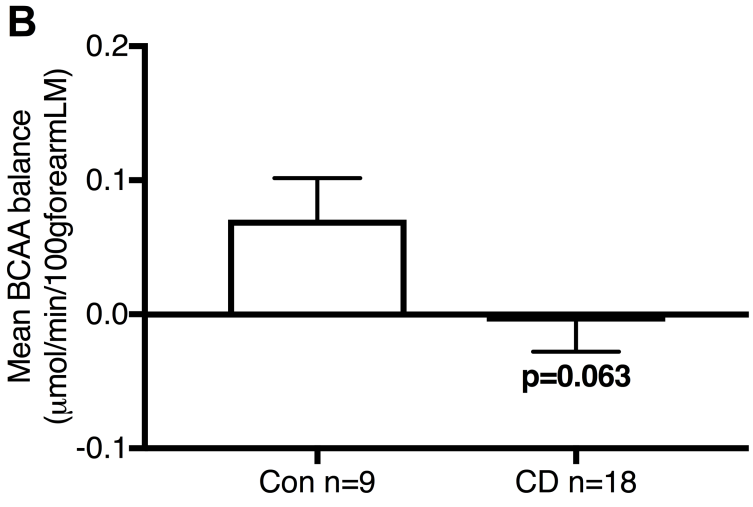
CD=Crohn's disease, Con=Control, FS=forearm strength, dom=dominant arm, non-dom=non dominant arm.

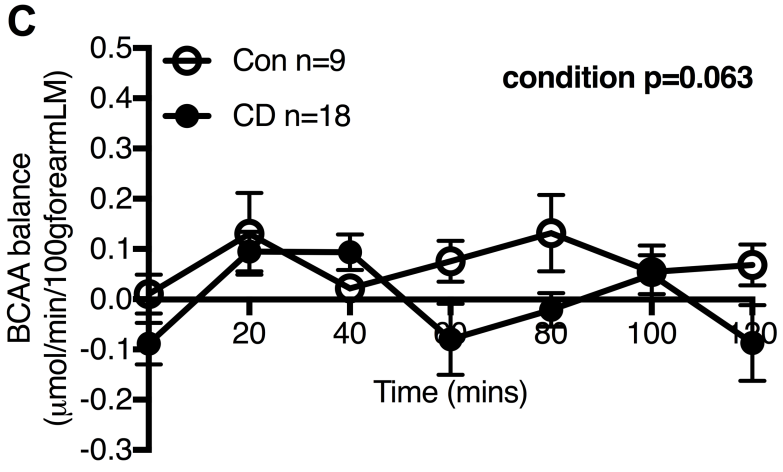
Table 3. Energy intake & energy expenditure

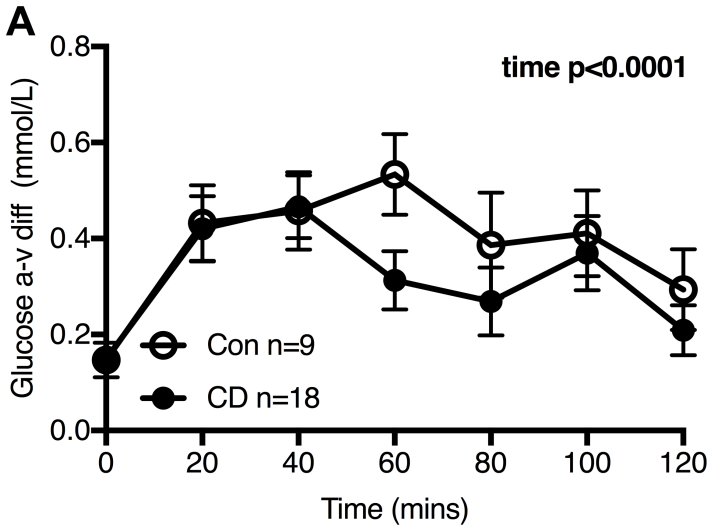
	Con	**CD**	**p value**
	n=9	n=20[w] / n=18[v]	
Energy intake[w]			
Energy intake (kJ/day)	9124 ± 1081	8333 ± 512	n.s.
Protein intake (kJ/day)	1531 ± 173	1350 ± 93	n.s.
Protein intake (g/day)	90 ± 10	79 ± 5	n.s.
Recommended protein intake (g/day)	46 ± 3	43 ± 2	n.s.
Indirect calorimetry[v]			
REE - fasted (kJ/hr/kgLBM)	6.1 ± 0.3	5.9 ± 0.4	n.s.
REE - post-feeding (kJ/hr/kgLBM)	6.5 ± 0.3[*]	6.0 ± 0.4[*]	n.s.
RER – fasted	0.81 ± 0.02	0.76 ± 0.05	n.s.
RER - post-feeding	0.87 ± 0.02[*]	0.80 ± 0.05[*]	n.s.
Physical Activity[w]			
Pedometer (no of steps/day)	8056 ± 849	7831 ± 725	n.s.
IPAQ (total MET-mins/week)	6196 ± 2158	4443 ± 994	n.s.

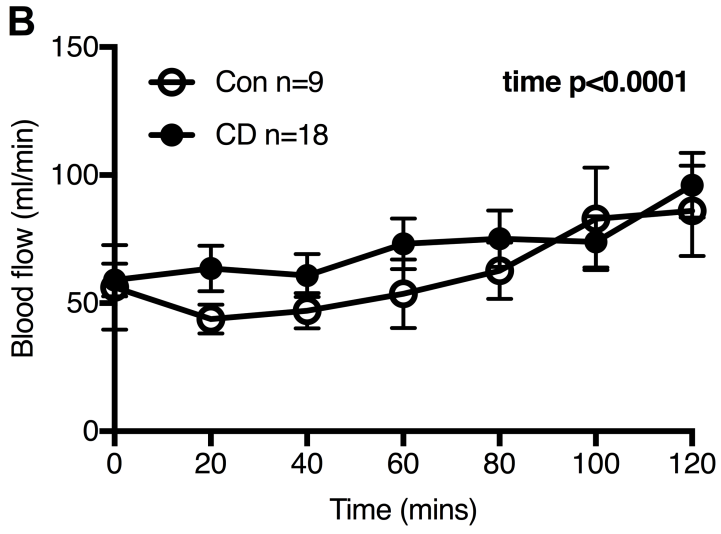
All values are means ± SEM. Statistically significant differences between fed and fasted states in both groups are marked *=$p<0.05$. n.s. = no significant differences between groups. CD=Crohn's disease, Con=Control.

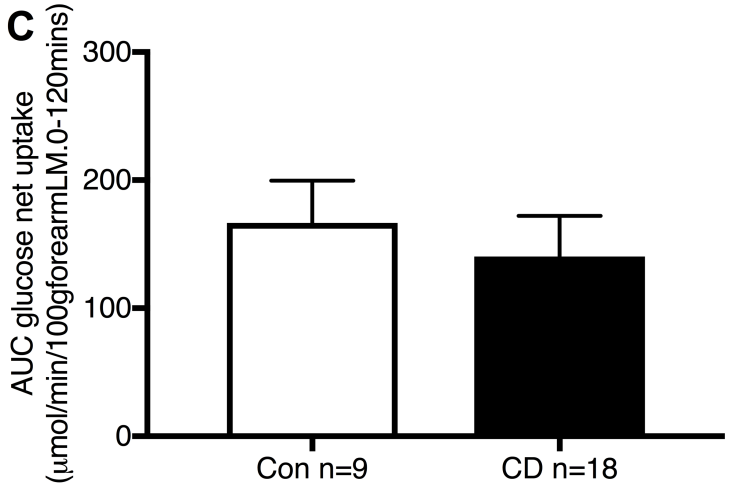

A
Arterialised BCAA (μmol/L)
400
350
300
250
200
time p<0.0001
Con n=9
CD n=18
0
20
40
60
80
100
120
Time (mins

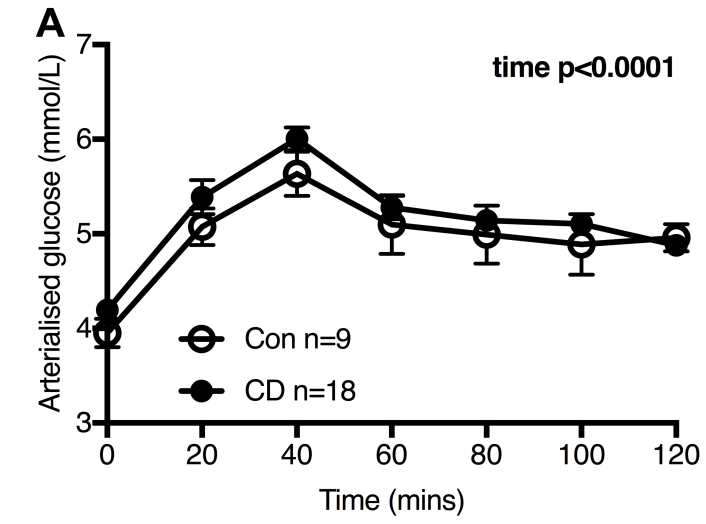

B
Mean BCAA balance
(μmol/min/100gforearmLM)
0.2
0.1
0.0
-0.1
p=0.063
Con n=9
CD n=18

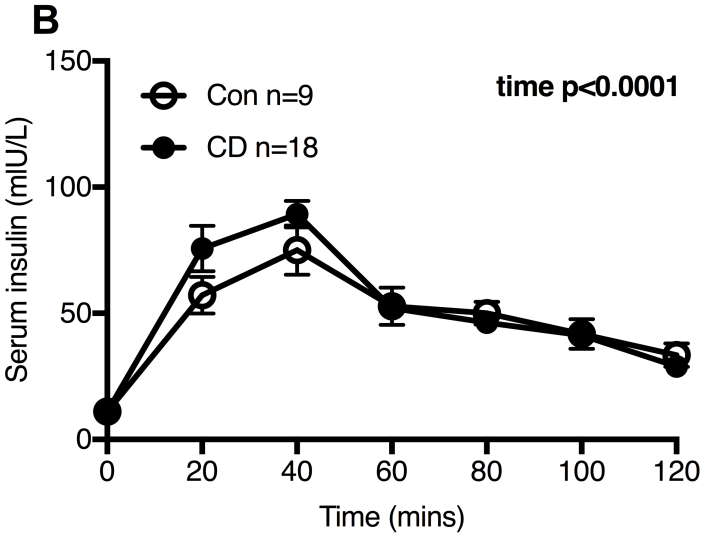

C
BCAA balance
(μmol/min/100gforearmLM)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Con n=9
CD n=18
condition p=0.063
20
40
100
Time (mins)

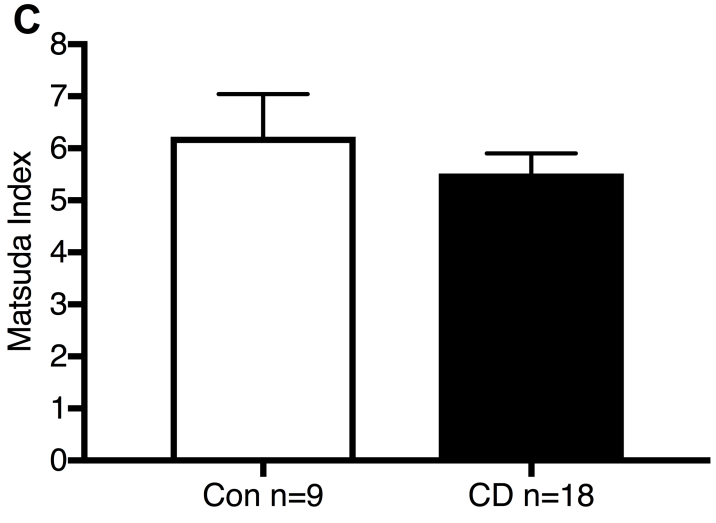

A
time p<0.0001
Glucose a-v diff (mmol/L)
0.8
0.6
0.4
0.2
0.0
Con n=9
CD n=18
0
20
40
60
80
100
120
Time (mins)


B
150
100
50
0
Con n=9
CD n=18
time p<0.0001
Blood flow (ml/min)
0
20
40
60
80
100
120
Time (mins)


C
AUC glucose net uptake
(µmol/min/100gforearmLM.0-120mins)
300
200
100
0
Con n=9
CD n=18


A
Arterialised glucose (mmol/L)
7
6
5
4
3
time p<0.0001
Con n=9
CD n=18
0
20
40
60
80
100
120
Time (mins)


B
Serum insulin (mIU/L)
150
100
50
0
Con n=9
CD n=18
time p<0.0001
0
20
40
60
80
100
120
Time (mins)


C
Matsuda Index
8
7
6
5
4
3
2
1
0
Con n=9
CD n=18

Highlights

- A positive skeletal muscle protein balance is only seen in healthy controls
- Neutral protein balance and reduced FFM z-scores in CD
- No other differences in body composition or muscle physiology between CD & control
- Negative protein balance, lower muscle mass and function in male CD