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Delving into disability in Crohn's disease: Dysregulation of molecular pathways may explain skeletal muscle loss in Crohn's disease☆

D.R. van Langenberg[a,*], P. Della Gatta[b], B. Hill[b], E. Zacharewicz[b], P.R. Gibson[a], A.P. Russell[b]

[a] *Department of Gastroenterology & Hepatology, Eastern Health Clinical School, Monash University, Box Hill, Victoria, Australia*
[b] *Centre of Physical Activity and Nutrition (C-PAN) Research, School of Exercise and Nutrition Science, Deakin University, Burwood, Victoria, Australia*

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Abstract

Background/aims: In Crohn's disease (CD), skeletal muscle mass and function are reduced compared to healthy controls, potentially resulting in disability. Mechanisms contributing to muscle impairment, and thus potential therapeutic targets, are poorly understood. This study aimed to measure and compare skeletal muscle size and molecular targets involved in skeletal muscle growth, in CD subjects and healthy controls.
Methods: CD (n = 27) and healthy (n = 22) subjects were recruited from the IBD outpatient clinic and via local advertisement respectively. Demographics and clinical data were collected via survey and interview. Quadriceps muscle cross-sectional area was measured using peripheral quantitative CT scanning. Levels of muscle hypertrophy and atrophy signalling targets using quantitative PCR and western blotting were measured in muscle biopsies.
Results: Muscle size was 14% lower ($p = 0.055$) and a 54% lower phosphorylated:total (p:t) Akt ratio was measured in the muscle samples ($p < 0.05$), indicating an attenuated muscle hypertrophy pathway in CD compared with controls. In those with CD, a lower p:t Akt ratio (<0.97) was associated with lower serum vitamin D3, lower physical activity indices (49 vs 64 mmol/L, 1.7 vs 2.2 × 10^6 accelerometer counts respectively, each $p < 0.05$) and a trend

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; pQCT, peripheral quantitative computed tomography.

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* Corresponding author at: Department of Gastroenterology & Hepatology, Eastern Health Clinical School, Monash University, Level 2, 5 Arnold Street, Box Hill, Victoria 3128, Australia. Tel.: +61 3 9094 9533; fax: +61 3 9899 9137.
E-mail address: Daniel.van-Langenberg@monash.edu (D.R. van Langenberg).

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towards lower serum ferritin levels (128 vs 322 mg/L, p = 0.07), compared with CD subjects with normal/high p:t Akt ratios.
Conclusion: The reduced muscle mass in CD may be explained, in part, by impaired activation of muscle protein synthesis pathways, notably the IGF1–Akt pathway. Normal vitamin D levels and regular exercise may be protective in CD against this trend, though confirmatory longitudinal studies are needed.

1. Introduction

Skeletal muscle is the primary organ for movement and metabolism and, therefore, plays a key role in maintaining human health. Conversely, loss of muscle mass and function associated with chronic disease or ageing is an important predictor of future disability.[1] In patients with Crohn's disease (CD), when compared to healthy controls, skeletal muscle mass and strength are reduced[2–4] and local muscle fatigue is increased.[5] Multiple factors including poor nutrition, physical inactivity, hormonal changes, prolonged corticosteroid therapy as well as direct effects of the underlying chronic disease,[4] would appear to negatively influence the molecular targets controlling skeletal muscle mass and function. However at present the molecular mechanisms involved in skeletal muscle dysfunction in CD are not well characterized.

Maintaining skeletal muscle mass is a tightly regulated process controlled by the fine balance between muscle hypertrophy and atrophy signalling pathways. IGF-1 activates several downstream signalling cascades including Akt/mTOR/$p70^{s6k}$/S6K, Akt/mTOR/4E-BP1 and Akt/GSK3β/eIF2B that are known to stimulate muscle hypertrophy.[6] Additionally, Akt activation also inhibits the upregulation of the muscle atrophy genes, MuRF1 and atrogin-1.[7,8] CD patients have a decrease in circulating IGF-1 levels and elevated TBARS, a marker of oxidative stress.[5] As IGF-1 and oxidative stress increases and decreases Akt signalling respectively,[9] these factors may a play role in the reduction in muscle mass in CD.[2] Pro-inflammatory factors, such as TNF-α and IL-6 are also increased in CD patients[10,11] and their elevation is associated with lower muscle mass and strength in elderly populations.[12,13] Whether or not their elevation is related to a reduction in muscle mass and Akt, $p70^{s6k}$ and GSK3β protein levels in CD are unknown.

The dysregulation of the Akt/mTOR/$p70^{s6k}$/S6K, Akt/mTOR/4E-BP1 and Akt/GSK3β/eIF2B and MuRF1/atrogin-1 pathways has been observed in atrophied skeletal muscle of patients with other chronic diseases such as amyotrophic lateral sclerosis and chronic obstructive pulmonary disease, as well as in the elderly.[14–16] Presently, the regulation of these pathways in skeletal muscle of subjects with CD has not been investigated, but is a vital step in understanding the mechanisms of muscle dysfunction as a potential major contributor to the increasingly recognized long term disability incurred in CD.[17–19]

Hence, the aim of the present study was to develop an understanding of the signalling factors that may negatively impact skeletal muscle size and function in CD patients. To achieve this, cross-sectional area of the quadriceps muscle and the levels of hypertrophy and atrophy signalling targets, including Akt, $p70^{S6k}$, 4E-BP1, GSK-3β, MuRF1 and atrogin-1 in skeletal muscle biopsies were measured. Correlations were made between these variables and previously measured levels of physical activity, circulating pro-inflammatory cytokines including TNFα, IL-6, IL-17 and IFN-γ, anabolic factors such as IGF-1 and testosterone, as well as other factors putatively important in muscle health, including vitamin D and magnesium.[20,21] All measurements were performed in patients with CD and healthy age and sex matched controls.

2. Materials & methods

2.1. Ethical approval

The study was approved by the Deakin University and Eastern Health research ethics committees (on 15/06/2009). For all investigations conducted, informed consent was obtained from all participants and the study was performed in concordance with the *Declaration of Helsinki* (2008 version).

2.2. Subject recruitment

Patients with Crohn's disease who had recently completed a survey in relation to a wider research programme were then consecutively, in order of survey completion date, invited to participate in the present cross-sectional study at their next visit, subject to informed consent. They were recruited from the Box Hill Hospital Inflammatory Bowel Disease Clinic and all had a confirmed diagnosis of CD according to standard criteria. Healthy volunteers were consecutively recruited via local advertisement in hospital and university publications, and the local newspaper in the same period. Exclusion criteria for the study included those aged less than 18 years or greater than 65 years, those with significant medical or psychiatric comorbidities likely to cause restrictions in functional performance and/or those who were pregnant. Also, healthy controls with first degree relatives with known IBD and those unable to give informed consent were excluded from the study. All subjects attended one study visit with an investigator prior the muscle testing in order to assess for these exclusion criteria and to ensure that they did not engage in regular high intensity exercise which may have otherwise biased the study results.

2.3. Calculation of muscle size

Muscle cross-sectional area (CSA) measurements (units of mm^2) were performed using peripheral quantitative CT (pQCT) scanning with a *Stratec XCT 3000* scanner (Stratec Medical, Pforzheim, Germany) at the level of the quadriceps muscle (at 25% of the bone's length measured from distal end of the lateral condyle of the femur). Scout views of the distal femur