Accepted Manuscript

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PII: S0261-5614(16)31328-0

DOI: 10.1016/j.clnu.2016.11.012

Reference: YCLNU 2978

To appear in: *Clinical Nutrition*

Received Date: 28 July 2016

Revised Date: 12 October 2016

Accepted Date: 11 November 2016

Please cite this article as: Cuthbertson DJ, Babraj J, Leese G, Siervo M, Anabolic resistance does not explain sarcopenia in patients with type 2 diabetes mellitus, compared with healthy controls, despite reduced mTOR pathway activity, *Clinical Nutrition* (2016), doi: 10.1016/j.clnu.2016.11.012.

Anabolic resistance does not explain sarcopenia in patients with type 2 diabetes mellitus, compared with healthy controls, despite reduced mTOR pathway activity

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Conflicts of interest: None to declare

Funding: Medical Research Council and Arthritis UK

Word count: Abstract (250 words), Text (1500 words), References (12), Figures (2), OSM (1)

Journal: Clinical Nutrition (Short Communication)

Abstract

Background Ageing and type 2 diabetes mellitus (T2DM) are risk factors for skeletal muscle loss. We investigated whether anabolic resistance to feeding might underlie accelerated muscle loss in older people with T2DM and whether dysregulated mTOR signalling was implicated.

Subjects 8 obese men with T2DM, and 12 age-matched controls were studied (age 68±3 *vs*. 68±6y; BMI: 30±2 *vs.* 27±5 $kg \cdot m^{-2}$).

Methods Body composition was measured by dual-X-ray absorptiometry. Insulin and glucose were clamped at post-absorptive concentrations (13±2 *vs.* 9±3 $mU \cdot l^{-1}$; 7.4±1.9 *vs.* 4.6±0.4 $mmol \cdot l^{-1}$; T2DM *vs.* controls). Fractional synthetic rates (FSR) of myofibrillar and sarcoplasmic proteins were measured as the rate of incorporation of [^{13}C] leucine during a primed, constant infusion of [1-^{13}C] α-ketoisocaproic acid, 3 h after 10 or 20g of essential amino acids (EAA) were orally administered. Protein expression of total and phosphorylated mTOR signalling proteins was determined by Western blot analysis.

Results Despite a significantly lower appendicular lean mass index and a greater fat mass index in T2DM *vs*. controls, basal myofibrillar and sarcoplasmic and post-prandial myofibrillar FSR were similar. After 20g EAA, stimulation of sarcoplasmic FSR was slightly blunted in T2DM patients. Furthermore, feeding 20g EAA increased phosphorylation of mTOR, $p70^{S6k}$ and 4E-BP1 by 60-100% in controls with no response observed in T2DM.

Conclusions There was clear dissociation between changes in mTOR signalling *versus* changes in protein synthesis rates. However, the intact anabolic response of myofibrillar FSR to feeding in both groups suggests anabolic resistance may not explain accelerated muscle loss in T2DM.

Introduction

Sarcopenia is characterised by a progressive decline in skeletal muscle mass resulting in low muscle strength and impaired physical performance [1]. The risk for sarcopenia and physical disability is greater in older patients with type 2 diabetes mellitus (T2DM) [2]. The biological mechanisms associated with accelerated loss of muscle remain uncertain but alterations in skeletal muscle protein turnover are clearly implicated in such modifications in T2DM with evidence of impaired stimulation of protein synthesis related to ageing and insulin resistance [3, 4].

We determined whether an impaired anabolic response to feeding (with essential amino acids, (EAA)) was evident in skeletal muscle of older patients with T2D and whether diminished anabolic sensing and signalling through the PKB/mTOR pathway was implicated in any impairment.

Methods

Participant characteristics Twelve healthy older men and eight men with T2DM were recruited (age 68 ± 3 *vs*. 68 ± 6 y; BMI: 30 ± 2 *vs*. 27 ± 5 $kg \cdot m^{-2}$). All subjects gave informed written consent and the study was approved by the Tayside Regional Ethics Committee (Ref 224/01). Detailed information on inclusion and exclusion criteria is reported in the online supplemental material.

Height and weight were measured and body mass indices and body surface areas were determined. Dual energy photon X-ray absorptiometry (DEXA; HOLOGIC Discovery W, Bedford, MA) was used to determine whole-body and appendicular lean (ALM) and fat (FM) masses. Age, gender and body mass index (BMI)-specific reference curves were used to calculate the individual *percentile* for height-adjusted

ALM (appendicular lean mass index, ALMI) and FM (fat mass index, FMI) according to Prado *et al* [5].

Experimental protocol (including) Myofibrillar and sarcoplasmic protein FSR under basal, post-absorptive conditions (0-3 h) and after stimulation by EAA (3-6 h) were measured. In all cases a prior 0.5 h period (-0.5-0 h) ensured a plateau enrichment of the stable isotope. A protocol schematic and the composition of the oral EAAs that participants were fed is provided in the online supplemental material (Figure S1).

Analytical methods More detailed information on the analytical procedures are reported in the online supplemental material but methods are described briefly below.

Plasma glucose was measured with a YSI Stat2300 (Yellow Spring Instruments, Yellow Spring, OH) immediately after collection of each sample. Insulin was determined using an Abbott IMx analyzer (Abbott Inc Deerfield, IL, USA), based upon a microparticle enzyme immunoassay.

The labelling of leucine in plasma and free muscle water was determined by GC-MS after conversion to the *t*- BDMS derivative; the $^{13}C/^{12}C$ ratio of leucine was determined by selected-ion monitoring MS by standard methods as previously described. Plasma amino acids were quantified as their *t*-BDMS derivative after the addition of norleucine as internal standard.

Frozen muscle was processed in buffer containing inhibitors of phosphatase and protease activity (e.g. vanadate and microcystine) to maintain the phosphorylation state of the sarcoplasmic fraction signalling proteins. Samples (60–80 mg) were processed to separate myofibrillar and sarcoplasmic protein as described previously.

We estimated the relative concentrations and extent of phosphorylation using Western blotting in the basal state and after stimulation by EAA examining components of the

mammalian target of rapamycin (mTOR) signalling pathway, known to be important for regulation of mammalian MPS.

Statistical analysis Patient/subject anthropometric, body composition characteristics and biochemical concentrations were compared using an unpaired t-test. One-way ANOVA with multiple data sets were applied for the FSR measurements with Bonferroni post test procedures for comparison of group means. *P* was taken as being significant at 0.05 or less. Values are given as mean ± SD.

Results

Body composition The T2DM group had a significantly greater percentage body fat (33±2 *vs.* 24±5 %; p<0.001) compared to controls but the two groups had similar total (*P* = 0.84) and appendicular lean mass (*P* = 0.60) (Table S1, online supplemental material). However, with application of age, gender and BMI-specific models of body composition, significant differences between the two groups emerged using percentiles for FMI and ALMI. T2DM patients had higher FMI (49.8±26.4 *vs.* 16.0±14.9; *P* = 0.003) and lower ALMI (34.0±29.5 vs. 66.2±31.3; *P* = 0.04) percentiles compared to the controls, respectively (Figure 1A).

Biochemical characteristics Fasting plasma glucose and insulin concentration were maintained at the post-absorptive concentration throughout the duration of the study. There was a significant difference between the plateau glucose and plateau insulin concentrations in the T2DM patients and the elderly (both p<0.001) (Figure 2, online supplemental material).

Rates of protein synthesis Basal myofibrillar and sarcoplasmic FSR were similar between groups. Myofibrillar FSR was not significantly different after feeding either 10 or 20 g EAA between the controls and T2DM. The same pattern of anabolic

stimulation was observed in controls and T2DM in that 10 g EAA significantly stimulated myofibrillar MPS with no additional stimulation with 20 g beyond that seen with 10 g EAA (Figure 1B). Sarcoplasmic FSR was similar between the control and T2DM after 10 g EAA. After 20g EAA, there was reduced responsiveness of sarcoplasmic protein in T2DM compared with the control (Figure 1C).

Signaling protein analysis In T2DM the concentration of $p70^{S6k}$ was reduced to 0.76±0.06 of control (P<0.01) but mTOR and 4E-BP1 were similar. Feeding 20 g EAA increased phosphorylation of mTOR, $p70^{S6k}$ and 4E-BP1 by 60-100 % without any effect in T2DM (P<0.05) (Figure 2).

Discussion

We have shown that in obese older patients with T2DM and good glycaemic control, rates of myofibrillar or sarcoplasmic muscle protein synthesis were similar to those in age-matched, non-diabetic individuals. Only the stimulation of sarcoplasmic protein synthesis, in response to the highest dose (20 g) of EAA, was blunted in the patients with T2DM. Despite the similarity in the anabolic response of MPS to feeding, there was diminished phosphorylation of the proteins involved in amino acid sensing/signalling pathways, namely mTOR, $p70^{S6k}$ and 4E-BP1 in patients with T2DM compared with controls.

In examining the effects of feeding in T2DM, several pathophysiological considerations beyond that of ageing must be accounted for, particularly the degree of insulin resistance. Recently there have been conflicting results regarding the magnitude of the anabolic response to feeding in non-diabetic individuals in the presence of acute lipid-induced insulin resistance. Stephens *et al*., demonstrated anabolic resistance to a bolus of oral amino acids when muscle lipid was increased

during a lipid infusion in young men [6]. In contrast, others have found that elevated circulating free fatty acid concentrations do not impair the anabolic response to feeding [7].

However, it has been reported that T2DM hyperaminoacidaemia stimulates rates of whole body or skeletal muscle protein synthesis to a similar extent to those observed in lean and weight-matched control subjects [8]. In the current study, the influence of insulin resistance has been removed by maintaining insulin concentrations at their respective post-absorptive concentrations to study the isolated effect of hyperaminoacidaemia.

In patients with T2DM, we observed a diminution of the activation of mTOR, $p70^{S6k}$ and 4E-BP1, despite apparently similar rates of myofibrillar protein synthesis to those in the healthy older people and attenuated rates of sarcoplasmic protein synthesis only in response to the highest dose of EAA. We have previously demonstrated that in older people, only basal, post-absorptive insulin concentrations accompanying ingestion of EAA is required to significantly activate the mTOR signalling pathway and so we might expect similar activation of this pathway in both groups [4]. Paradoxically, stimulation of MPS in diabetic rats has been demonstrated to occur in the absence of increases in the phosphorylation of components of the mTOR pathway (4E-BP1 and $p70^{S6k}$) suggesting there may be alternate, insulin-independent, signal transduction pathways that mediate changes in MPS. However we have also noted a lack of a coherent response between the activation of signalling proteins regulating translation initiation and directly measured rates of protein synthesis in human muscle in previous studies [9, 10].

These findings suggest that the mechanism underlying accelerated muscle loss in T2DM does not involve anabolic resistance to feeding. Similarly, evidence of

anabolic resistance to exercise in T2DM is lacking considering the findings from exercise interventions in obese T2DM in which the magnitude of accretion of muscle mass is similar to that observed in healthy controls [12]. Taking these data together, it might be speculated that the more pronounced muscle loss in T2DM perhaps reflects greater levels of physical inactivity in T2DM compared to age-matched healthy controls; individuals with T2DM have a demonstrably more sedentary lifestyle than age-matched healthy controls [11]. In the absence of measures of the participants' habitual physical activity, the mechanism underlying the reduced lean body mass in those with type 2 diabetes *versus* healthy controls can only be speculated in the current study but is likely to involve reduced physical activity in those with type 2 diabetes.

In conclusion, we demonstrate that despite evidence of aberrant mTOR signalling responses to feeding in obese patients with T2DM, compared to overweight non-diabetic controls, the anabolic response to feeding is similar in T2DM and controls. Thus, the results suggest that anabolic resistance to feeding may not be responsible for the accelerated muscle loss observed in T2DM with mechanisms linked to reduced physical activity potentially contributory.

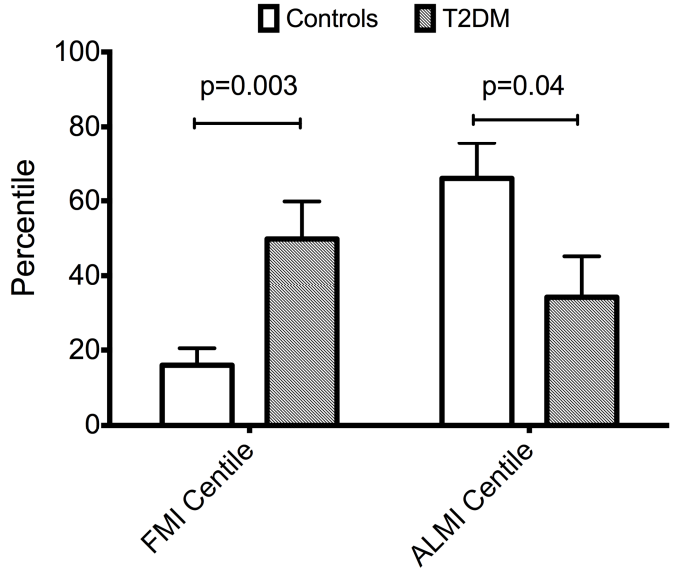
Figure legends

Figure 1 Patients with T2DM, despite greater body weight, higher fat mass and a higher percentile of fat mass index (FMI) had more pronounced sarcopenia with a significantly lower percentile appendicular lean mass index (ALMI) than the healthy controls (Fig 1A). Myofibrillar (Fig 1B) and sarcoplasmic (Fig 1C) fractional synthesis rates (FSR) in the post-absorptive state and in response to 10 g and 20 g EAA, in the healthy elderly (open bars) (n=12) and in patients with T2D (cross-hatched bars) (n=8). Values are mean ± SD; *P <0.001 compared to fasted; $^{\dagger}P$ < 0.01 compared to 10 g; $^{\dagger\dagger}P$ < 0.01 compared to old.

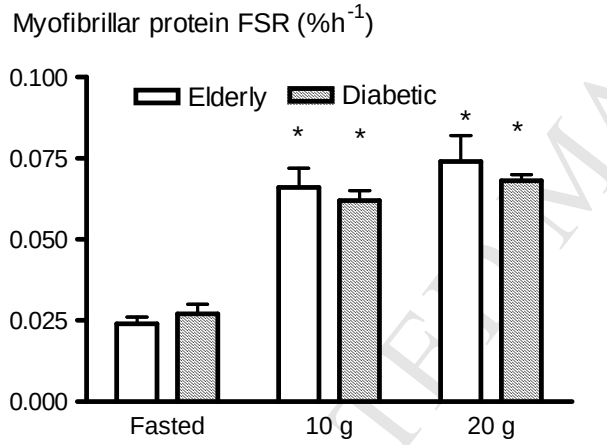
Figure 2 Western blot analysis of phosho-specific mTOR, $p70^{S6k}$ and 4E-BP1 in fasting and fed conditions in healthy elderly and T2D in response to 20g EAA. Values are mean ± SD; *P <0.05.

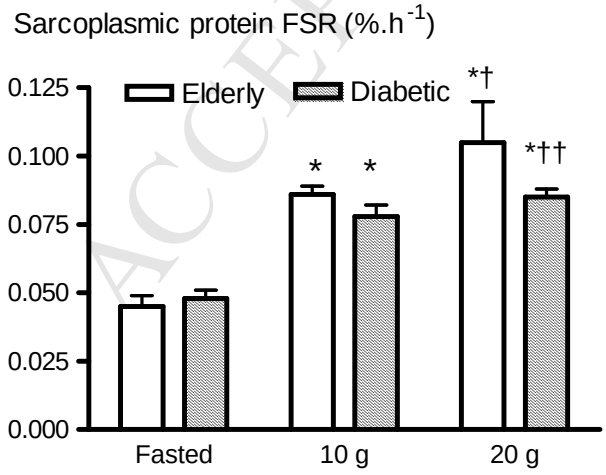
Figure 1

A



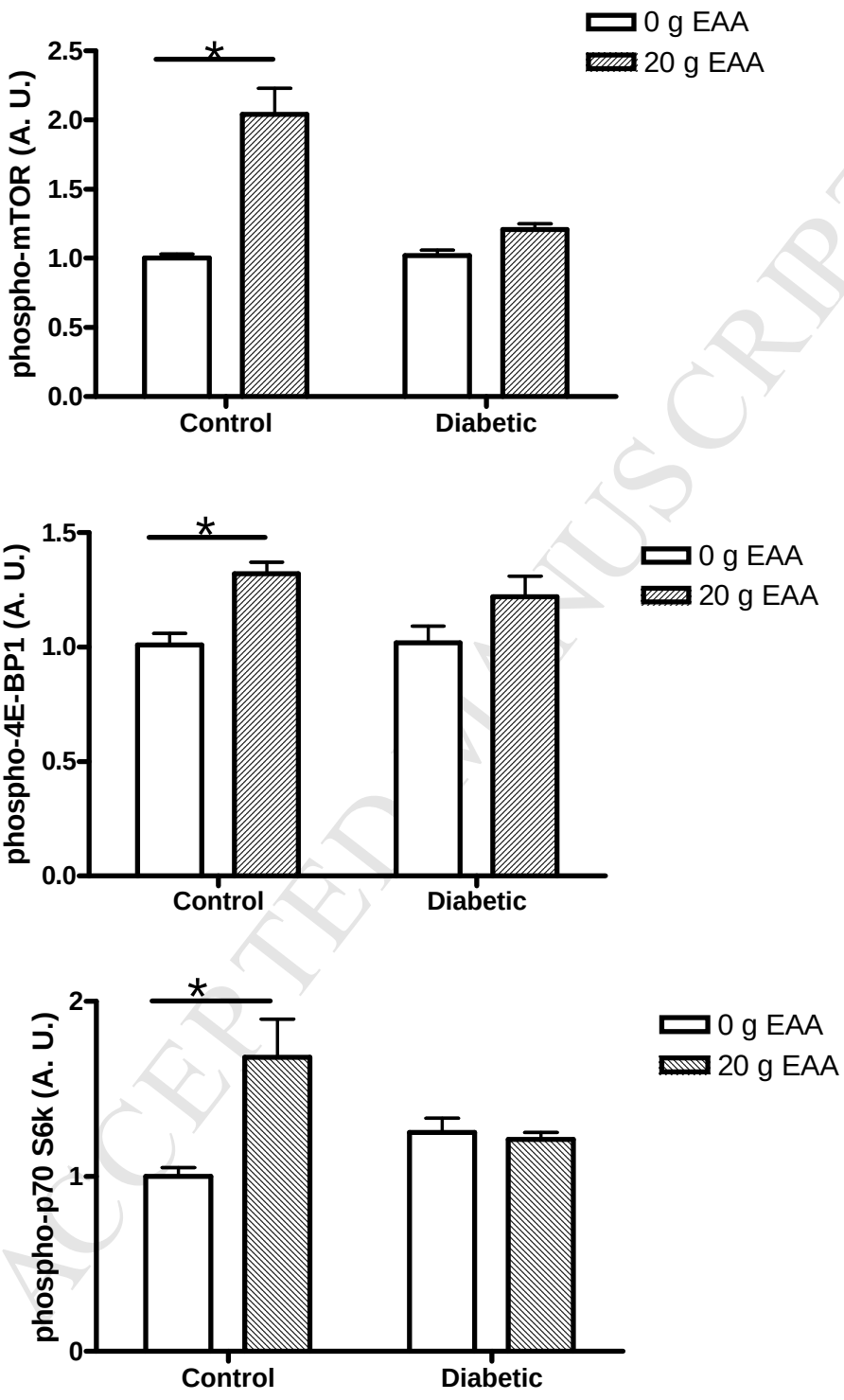
B





C

Figure 2



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