Clinical Nutrition xxx (2016) 1–7

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Supplementing essential amino acids with the nitric oxide precursor, L-arginine, enhances skeletal muscle perfusion without impacting anabolism in older men

W.K. Mitchell, B.E. Phillips, D.J. Wilkinson, J.P. Williams, D. Rankin, J. Lund, K. Smith, P.J. Atherton*

MRC-ARUK Centre of Excellence for Musculoskeletal Ageing Research, School of Medicine, University of Nottingham, Derby, DE22 3DT, UK

ARTICLE INFO

Article history:
Received 16 December 2015
Accepted 30 September 2016

Keywords:
Muscle
Ageing
Blood flow
Arginine
Metabolism
Nutrition

SUMMARY

Postprandial limb blood flow and skeletal muscle microvascular perfusion reduce with aging. Here we tested the impact of providing bolus essential amino acids (EAA) in the presence and absence of the nitric oxide precursor, L-Arginine (ARG), upon skeletal muscle blood flow and anabolism in older men. Healthy young (YOUNG: 19.7 ± 0.5 y, N = 8) and older men (OLD, 70 ± 0.8 y, N = 8) received 15 g EAA or (older only) 15 g EAA +3 g ARG (OLD-ARG, 69.2 ± 1.2 y, N = 8). We quantified responses in muscle protein synthesis (MPS; incorporation of ^{13}C phenylalanine into myofibrillar proteins), leg and muscle microvascular blood flow (Doppler/contrast enhanced ultrasound (CEUS)) and insulin/EAA in response to EEA ± ARG. Plasma EAA increased similarly across groups but argininemia was evident solely in OLD-ARG (~320 mmol, 65 min post feed); increases in plasma insulin (to ~13 IU ml^{-1}) were similar across groups. Increases in femoral flow were evident in YOUNG >2 h after feeding; these effects were blunted in OLD and OLD-ARG. Increases in muscle blood volume (MBV) occurred only in YOUNG and these effects were isolated to the early postprandial phase (+45% at ~45 min after feeding) coinciding with detectable arterio-venous differences in EAA reflecting net uptake by muscle. Increases in microvascular flow velocity (MFV) and tissue perfusion (MBV × MFV) occurred (~2 h) in YOUNG and OLD-ARG, but not OLD. Postprandial protein accretion was greater in YOUNG than OLD or OLD-ARG; the latter two groups being indistinguishable. Therefore, ARG rescues aspects of muscle perfusion in OLD without impacting anabolic blunting, perhaps due to the "rescue" being beyond the period of active EAA-uptake.

1. Introduction

Numerous factors have been implicated in the development of sarcopenia including an age-related compromise in the anabolic response to feeding [1]. Ingestion of essential amino acids (EAA) or high quality protein achieves a transient increase in muscle myofibrillar protein synthesis (MPS) above fasting rates and the magnitude of this appears reduced in older age, so called "anabolic resistance" [2]. The aetiology of anabolic resistance i.e. whether anabolic deficits reside in relation to impaired/altered AA digestion and absorption [3], tissue delivery and uptake [4], or intracellular signalling pathways involved in sensing and signalling to the translational machinery [5], remains poorly defined. It has been proposed that anabolic resistance may in part be due to an age-related compromise in vascular responsiveness to feeding with compromised EAA delivery to the capillary/myocyte interface [6,7]. In the context of this, recent methodological developments have facilitated less invasive assessment of skeletal muscle microvascular blood flow *in vivo*. The detection by ultrasound of injected gas-filled microbubbles, which remain within the vasculature, allows measurement of changes in microvascular blood volume (MBV) and microvascular flow velocity (MFV) [6,8]. Perfusion is the product of MBV and MFV. Increased MBV reflects capillary recruitment, facilitating delivery by increasing surface area for exchange and reducing capillary-to-myocyte distance. By comparison, increases in MFV will increase extraction only when there is a significant extraction ratio, that is, a concentration gradient *along*

Abbreviations: ARG, L-Arginine; AV, Arterio-venous; CEUS, Contrast Enhanced Ultrasound; EAA, Essential Amino Acids; eNOS, endothelial nitric oxide synthase; FSR, Fractional synthesis rate; LBF, Leg blood flow; MBV, Microvascular blood volume; MFV, Microvascular flow velocity; MPS, Myofibrillar protein synthesis.

* Corresponding author.
E-mail address: Philip.atherton@nottingham.ac.uk (P.J. Atherton).

http://dx.doi.org/10.1016/j.clnu.2016.09.031
0261-5614/

the exchange vessel [9]. As blood flow is regulated at multiple anatomical levels increases in MBV can occur with little or no change in limb blood flow due to matched decreases in MFV [10]. Thus separate resolution of these facets is vital. In healthy younger people, ingestion of a mixed meal [7], EAA feed [6] or insulin/glucose infusion [8] results in capillary recruitment and hyperaemia within muscles which would serve to facilitate nutrient and insulin delivery to myocytes [9]. These vascular responses are impaired in older people [6,11]. In a recent study we showed that resistance exercise training was able to enhance microvascular responses to the hyperaemic stimulus of nutrition (low dose EAA, ~8 g) in skeletal muscles of older men without enhancing protein accretion [12].

L-Arginine is widely considered to have beneficial effects on vascular health. There is evidence it rejuvenates endothelial function [13] and facilitates glucose disposal [14]. L-Arginine is the principle substrate of the endothelial Nitric Oxide Synthase (eNOS). Enzyme kinetics of eNOS predict that physiological L-arginine concentrations are adequate to ensure its availability should not be rate limiting in NO production [15]. Despite this, *in vivo* studies suggest that L-arginine supplementation promotes NO production and enhances exercise tolerance [16]. This has been termed the "arginine paradox" [17]. Few studies have directly investigated the influence of supplemental arginine or its associated amino acids (ornithine/citrulline) on muscle protein metabolism [18]. There have been conflicting results from previous literature with suggestions of either increased MPS [19,20] or no effect [21,22] with the supplemental argininemia, when provided either alone [20] or in combination with a low dose of EAA [21,22]. Only one study assessed the impact of argininemia in the context of muscle protein anabolism in older humans [22]. It reported that supplementation of low dose EAA (15 g Whey; ~7 g EAA) with 10 g L-citrulline, the precursor for L-arginine synthesis, failed to enhance microvascular blood flow or MPS, despite enhancing plasma argininemia. Therefore, albeit using distinct strategies to modulate microvascular blood flow, neither this study nor a previous one from our lab [23] reported favourable effects of enhancing anabolism under conditions of low EAA availability. In the present study we investigate the effect of L-arginine supplementation on the separate components (MBV, MFV) and time-course of microvascular physiology in relation to muscle protein anabolism (MPS) under conditions of greater EAA availability (15 g EAA; equivalent to ~30 g Whey), in older aged individuals provided 15 g EAA oral in the absence/presence of L-arginine. In order to investigate any "restoration" we included a younger group fed 15 g EAA.

2. Subjects and methods

2.1. Study design

Studies were undertaken in line with the Declaration of Helsinki and with ethical approval from University of Nottingham Medical School Ethics Committee (United Kingdom) and were preregistered (clinicaltrials.gov registration no. NTC01735539).

Healthy young (N = 8, 18–28 y; YOUNG) and older (N = 16, 65–75 y) men were recruited (by invitation letter posted to addresses close to the trial centre). Recruits were studied having fasted overnight and were requested not to undertake heavy exercise for 48 h before to the study. At 08.00 on the study day, an 18-g venous cannula was inserted into the dorsal aspect of the participant's left hand for delivery of a primed (0.3 mg kg^{-1}), constant infusion (0.6 mg kg^{-1} h^{-1}) of L-[ring-$^{13}C_6$]-phenylalanine tracer (Isotec, Sigma Aldrich, Poole, Dorset, UK). As per Fig. 1, blood samples and muscle biopsies were collected during the acute study period. Arterialised venous blood was collected by a retrograde 16-g intravenous cannula placed on the dorsal aspect of the right hand, the hand being heated to 55 °C. A conchotome technique was used to biopsy *m. vastus lateralis*, with prior infiltration of 5 ml of 1% lignocaine. Iced PBS was used to wash the muscle which was then frozen in liquid nitrogen and kept at −80 °C prior to analysis.

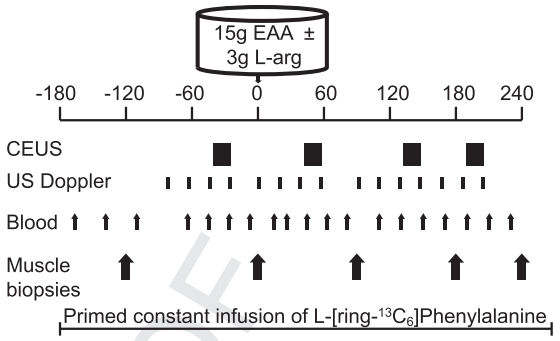

Fig. 1. Study protocol with contrast enhanced ultrasound (CEUS), ultrasound Doppler measurements, blood samples and muscle biopsies taken as indicated. Subjects received a 15 g essential amino acid (EAA) feed with or without 3 g L-Arginine supplementation.

Biopsies at 1 h and 3 h post-commencement of tracer (prior to EAA feeding) allowed measurement of postabsorptive MPS. Subjects were then provided 15 g essential amino acids (EAA; Histidine 1.21 g, Isoleucine 1.73 g, Leucine 3.59 g, Lysine 3.07 g, Methionine 0.95 g, Phenylalanine 0.91, Tryptophan 1.13 g, Threonine 0.48 g and Valine 1.86 g, all Sigma Aldrich) in aqueous solution (250 ml). Older men were allocated, by alternate study order, to receive 15 g EAA with (OLD-ARG) or without (OLD) 3 g L-arginine supplementation. Subjects were blinded to arginine administration. Biopsies, at 90, 180 and 240 min after feeding permitted measurement of MPS across the intervening periods. Table 1 summarises subject characteristics.

2.2. Measurement of plasma AA and insulin concentrations

Plasma insulin concentrations were measured using a high-sensitivity human insulin enzyme-linked immunosorbent assay (DRG Instruments GmbH, Marburg, Germany). Total insulin responses to feeding within each subject was calculated using the area under the insulin concentration/time curve above baseline (baseline equal to mean insulin concentrations measured before feeding and 155 and 195 min after feeding). To measure plasma amino acid (AA) concentration equal volumes of arterialised plasma and 10% sulfosalicylic acid were mixed and cooled to 4 °C for 30 min before centrifugation (8000*g*); the supernatant was passed through a 0.22 μm filter before AA analysis (Biochrom 30, Biochrom, Cambridge, Cambridgeshire, UK). The plasma concentration of all 20 AA was measured (with comparison to a standard AA mix and a norleucine internal standard). Older men fed 15 g EAA also underwent AA analysis of corresponding femoral venous blood samples to permit measurement of EAA uptake within the leg.

2.3. Measurement of muscle microvascular blood volume and leg blood flow

Contrast enhanced ultrasound (CEUS) permitted measurement of changes in microvascular blood volume (MBV) and microvascular flow velocity (MFV) with feeding and calculation of perfusion (which is proportional to MBV × MFV). As previously described in detail, an iU22 ultrasound scanner (Phillips Healthcare, Reigate, Surry, UK) was used to detect Sonovue™ microbubbles (Bracco, Milan, Italy), which were infused via an antecubital fossa vein [6].