AuPS/ASB Meeting - Adelaide 2010

Free communications: Systemic influences in skeletal muscle

Monday 29th November 2010 - Broughton Room - 09:30

Chair: Rod Snow

Effect of hypoxia on the dynamic response of leg blood flow during exercise

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Systemic hypoxia increases the muscle hyperaemic response during steady-state exercise. However, the effect of hypoxia on dynamic response characteristics of muscle blood flow is not known. To test this effect, eight subjects performed eight exercise trials while breathing a normoxic (F_IO_2 = 0.2094) or hypoxic (F_IO_2 = 0.105) gas mixture. Exercise consisted of five minutes of intermittent contractions of the left calf muscle (3s duty cycle) at a low intensity (20% MVC) during which leg blood flow (LBF) and mean arterial pressure (MAP) were measured between each contraction. Four sets of LBF responses were averaged for each subject under normoxia and hypoxia and fitted using a multiphasic exponential function. This enabled amplitudes and temporal parameters of a fast and slow growth phase, as well as a rapid decay phase, to be estimated. Hypoxia did not significantly affect MAP at rest but resulted in a 7% lower value by the end of exercise ($p < 0.05$). In contrast, hypoxia increased the change in LBF from the start to end of exercise by 13% ($p = 0.07$) and the amplitude of the rapid growth phase of the LBF response by 16% ($p < 0.001$). Hypoxia also increased the amplitude of the slow growth phase by 24% ($p = 0.08$) but it had no effect on the decay phase. These results suggest that the effect of hypoxia on exercise hyperaemia is targeted at rapid and slow phases of the response.

Citrulline supplementation does not prevent atrophy during limb-casting in mice

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Essential amino acids, particularly the branched-chain amino acids, have been shown to play a major role in the regulation of muscle protein synthesis and breakdown (Koopman, 2007). Thus, ingestion of specific amino acids (AAs) could be an effective therapeutic strategy to attenuate the muscle wasting and weakness common in many disease states and conditions. Although studies have indicated that supplementation with non-proteinogenic amino acids such as citrulline, can manipulate the anabolic response, their application for treating muscle wasting has received little attention. Interestingly, oral administration of citrulline to old malnourished rats enhanced muscle protein synthesis (Osowska *et al.*, 2006). Citrulline can be converted to arginine in the kidneys and thus plays an important role in protein homeostasis, controlling urea production and arginine availability. We hypothesized that citrulline administration increases muscle protein synthesis thereby preventing skeletal muscle wasting during limb-casting. Our aims were to establish the stimulating/protective properties of citrulline *in vitro* on muscle cell hypertrophy and atrophy, and to examine whether citrulline could attenuate the loss of muscle function during casting.

Atrophy was induced in cultured C2C12 myotubes by switching the medium to HBS, with or without the addition of 2.5 mM citrulline. After 6h of treatment, cells were fixed in 3.7% formaldehyde and reacted with myosin antibodies to determine myotube diameter, or prepared for western blot and RT-PCR analyses. Mice (n=24) were subjected to unilateral limb-casting. Mice were anaesthetised with an intraperitoneal (*i.p.*) injection of Ketamine/Xylazine (100 mg/kg Ketamine; 10 mg/kg Xylazine) so there was no response to tactile stimulation. The left hindlimb was wrapped in a special veterinary plaster with the foot positioned in plantar flexion to induce maximal atrophy of the gastrocnemius and other hindlimb muscles. Mice received citrulline (n=12, 1 g/kg/day) or alanine (n=12, control) during the 2 weeks of limb-casting. At the end of the treatment, mice were anaesthetised and *tibialis anterior* (TA) muscle function was assessed *in situ* (Murphy *et al.*, 2010). Mice were killed by cardiac excision while anaesthetised deeply. Muscles were analysed for changes in muscle fibre cross sectional area, fibre type distribution and oxidative capacity.

C2C12 myotubes incubated in HBS for 6h had a 40% reduction in myotube diameter. Incubation with citrulline partly prevented this wasting, with citrulline incubated myotubes being 18% bigger than the HBS or HBS-alanine treated myotubes ($p<0.05$). Incubation with citrulline did not enhance the phosphorylation status of p70-S6K1 or Akt. Two weeks of unilateral limb-casting resulted in 25% reductions ($p<0.05$) in quadriceps and TA muscle mass, and 33% and 15% reductions in peak and specific force, respectively, of TA muscles. These changes in muscle mass and function were associated with a specific atrophy of the type IIb/x fibres, without changes in the size of type IIa muscle fibres. Citrulline treatment during limb-casting did not attenuate muscle wasting.

Although citrulline administration reduced muscle wasting *in vitro*, it was unable to counteract muscle wasting *in vivo*. Citrulline does not exert its effect on skeletal muscle *via* the classical amino acid-induced increase in Akt/mTOR signalling.

Koopman R. (2007). *International Journal of Sports Nutrition and Exercise Metabolism* **17:** S47-S57.

Murphy KT, Koopman R, Naim T, Leger B, Trieu J, Ibebunjo C & Lynch GS. (2010). *FASEB Journal* doi:10.1096/fj.10-159608

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Inhibition of the renin-angiotensin system enhances whole body and skeletal muscle function in healthy and tumour-bearing mice

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Cancer cachexia describes the progressive skeletal muscle wasting and weakness in many cancer patients. Cancer cachexia impairs mobility, causes severe fatigue, and accounts for >20% of cancer-related deaths. The mechanisms underlying cancer cachexia are multifactorial and current treatments have proved ineffective as they have only targeted one of these mechanisms (Murphy & Lynch, 2009). Renin-angiotensin system (RAS) inhibition has typically been used in the treatment of hypertension, but recent evidence indicates that stimulation of RAS may contribute to skeletal muscle breakdown by a myriad of mechanisms, including inducing inflammation, causing insulin resistance, inducing skeletal muscle apoptosis, reducing protein synthesis and enhancing protein degradation. RAS inhibition may therefore preserve or enhance skeletal muscle strength and function and consequently, represents a potential therapeutic strategy for counteracting the skeletal muscle wasting and weakness associated with conditions such as cancer cachexia. We tested two hypotheses: i) that life-long RAS inhibition would enhance whole body and skeletal muscle function in healthy mice; and ii) that acute RAS inhibition would enhance whole body and skeletal muscle function in a commonly used murine model of cancer cachexia.

All experiments were approved by the Animal Experimental Ethics Committee of The University of Melbourne and conducted in accordance with the current codes of practice of the National Health and Medical Research Council (Australia). Animals were anaesthetised with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) prior to assessment of muscle contractile properties and were later killed as a consequence of cardiac excision while anaesthetised deeply.

In study 1, 12 week old wild-type control mice (n=13) and those lacking the angiotensin type 1A receptor ($AT_{1A}^{-/-}$, n=15) were tested for whole body strength (grip strength) and function (rotarod), glucose sensitivity during a glucose tolerance test and maximum tetanic force production and fatiguability *in situ* of the *tibialis anterior* (TA) muscle (Murphy *et al.*, 2010). Compared with controls, $AT_{1A}^{-/-}$ mice exhibited a 17% higher grip strength (p<0.01) and a 49% prolonged latency-to-fall during a rotarod test (p<0.01). Glucose sensitivity was improved by 23-26% in $AT_{1A}^{-/-}$ mice (p<0.01). Maximum *in situ* forces (normalised to CSA) of TA muscles was higher by 25% in $AT_{1A}^{-/-}$ mice (p<0.05), but the force decline during fatiguing intermittent stimulation was not different between groups.

In study 2, 15 week old CD2F1 (Balb/c × DBA) mice bearing Colon-26 (C-26) tumour cells were treated for 14 days with the ACE inhibitor, Perindopril (4 mg/kg/day, n=4-7) *via* the drinking water. Control mice were given water alone (n=4-6). Perindopril prevented the decline in body mass in C-26 tumour-bearing mice (p<0.01), enhanced grip strength by 29% (p<0.05) and prolonged the latency-to-fall during a rotarod test by 56% (p<0.05). Glucose sensitivity was improved with Perindopril (p<0.01). Perindopril had no effect on maximum force of TA muscles *in situ* or of diaphragm muscle strips *in vitro*, but attenuated the decline in force during fatiguing intermittent stimulation in both TA muscles and diaphragm muscle strips (p<0.01).

RAS inhibition enhanced whole body and skeletal muscle function, and improved glucose sensitivity in healthy mice and in mice bearing C-26 tumours. These findings highlight the therapeutic potential of RAS inhibition for cancer cachexia and other diseases associated with skeletal muscle wasting and weakness.

Murphy KT & Lynch GS. (2009) *Expert Opinion on Emerging Drugs* **:** 619-632.

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