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

R. Koopman, D. Ham, B.G. Gleeson and G.S. Lynch, Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia.

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

Osowska S, Duchemann T, Walrand S, Paillard A, Boirie Y, Cynober L & Moinard C. (2006). *American Journal of Physiology. Endocrinology and Metabolism* **291:** E582-586.

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