Citrulline prevents fasting-induced muscle cell atrophy *in vitro*

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Essential amino acids, particularly leucine, have been shown to play a major role in the regulation of muscle protein metabolism (Koopman, 2011). 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. Citrulline can be converted to arginine in the kidneys and thus plays an important role in protein homeostasis, controlling urea production and arginine availability (Osowska *et al.*, 2006). However, little is known about the potential direct effects of citrulline on skeletal muscle. We hypothesized that citrulline can directly affect muscle protein metabolism in an arginine-independent manner. Our aims were to establish the stimulating/protective properties of citrulline *in vitro* on muscle cell development and atrophy.

Myotube formation was assessed by culturing C2C12 myoblasts in differentiation media supplemented for up to 5 days with either 2.5 mM alanine (isonitrogenous control), citrulline, arginine or leucine (positive control). Thereafter, 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. Atrophy was induced in cultured C2C12 myotubes by removing serum from the medium, with or without the addition of 2.5 mM alanine, citrulline, arginine or leucine. After 48 h of treatment, cells were fixed and stained as described above and myotube diameter and total myotube area was determined.

Incubation with citrulline enhanced C2C12 myotube formation by ~50% compared with administration of alanine. This improved muscle cell differentiation was associated with increased mRNA expression of calcineurin A. Serum withdrawal (SF) resulted in a 25% reduction in myotube diameter. Interestingly, incubation with citrulline completely prevented this wasting whereas additional supplementation with alanine, arginine, or leucine did not protect myotubes from wasting. Incubation with citrulline did not alter the p70-S6K1 or Akt phosphorylation suggesting that citrulline is not converted to arginine in skeletal muscle.

Citrulline administration reduces muscle wasting *in vitro*, but does not exert its effect *via* the classical amino acid-induced increase in Akt/mTOR signalling. Our preliminary data suggest that citrulline exerts direct effects on skeletal muscle cells, potentially through calcineurin signalling.

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