

## **Citrulline protects muscle cells from wasting in an mTOR independent manner *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 diseases and conditions. Studies have indicated that the non-proteinogenic amino acid citrulline, through its conversion to arginine, can manipulate skeletal muscle protein metabolism *in vivo*. Additionally, we have recently observed a protective effect of citrulline on skeletal muscle cells *in vitro* (Ham, 2013) independent of arginine availability. However, the mechanisms responsible for this observed effect are still unclear. Our aims were to compare the effects of citrulline and arginine supplementation on: 1) the phosphorylation status of the mTOR protein synthetic pathway and; 2) protein synthetic rate in an *in vitro* model of muscle wasting. We hypothesized that both citrulline and arginine would affect protein synthesis but the activation pattern of the mTOR pathway would differ between the two amino acids.

Confluent C2C12 myoblasts were cultured in differentiation media for 5 days to form mature myotubes. Atrophy was induced by incubating myotubes in HEPES buffered saline (HBS) for 6 h. Media were supplemented with 2.5 mM citrulline, arginine, or leucine. After 6 h cells were fixed in 3.7% formaldehyde and reacted with myosin antibodies to determine myotube diameter. Protein synthesis and mTOR phosphorylation status were measured in cells incubated in HBS for 1 h. Protein synthetic rate was measured by incubating myotubes with puromycin for exactly 30 min immediately before the collection of cells.

Incubation in HBS for 6 h resulted in a 35% reduction in myotube diameter. Both citrulline and arginine significantly attenuated the reduction in myotube diameter and increased protein synthetic rate by ~70% compared to alanine treated controls ( $P < 0.05$ ). Both arginine and citrulline increased mTOR phosphorylation ( $P < 0.05$ ). Interestingly, only arginine increased S6 phosphorylation ( $P < 0.05$ ), while only citrulline increased 4EBP1 phosphorylation ( $P < 0.05$ ). Incubation with the mTOR inhibitor, rapamycin, prevented the protective effect of arginine, but not citrulline, on myotube diameter.

Both citrulline and arginine increase mTOR phosphorylation and protein synthetic rate in an *in vitro* model of starvation. Importantly, mTOR phosphorylation is critical for the protective effects of arginine, but not citrulline, on skeletal muscle. Therefore, citrulline may attenuate muscle wasting *in vitro* by directly activating proteins downstream of mTOR, such as 4EBP1.

Koopman R. (2011) *Proceedings of the Nutrition Society* **70**, 104-13.

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