Citrulline protects muscle cells from cachectic stimuli and preserves protein metabolism *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 atrophy and protein metabolism.

Confluent C2C12 myoblasts were cultured in differentiation media for 5 days to form mature myotubes. Atrophy was induced by incubating myotubes in 1) serum free media for 48 h; 2) 25 μM hydrogen peroxide for 24 h to induce oxidative stress; or 3) 1 $\mu g \cdot ml^{-1}$ lipopolysaccharide to induce an inflammatory response. Media were supplemented with 2.5 mM citrulline, arginine, or leucine. After the indicated time, cells were fixed in 3.7% formaldehyde and reacted with myosin antibodies to determine myotube diameter or prepared for western blot analyses. Protein synthesis was measured by incubating treated myotubes with puromycin for exactly 30 min immediately before the collection of cells. To determine the acute effect of citrulline on protein synthesis, myotubes were incubated in serum free media for 5 h and then in HEPES-buffered saline (HBS) for 1 h before a 30 min stimulation with either citrulline, arginine or leucine in HBS.

Serum withdrawal, hydrogen peroxide and lipopolysaccharide all resulted in a 15% reduction in myotube diameter. Interestingly, incubation with citrulline completely prevented this wasting, whereas supplementation with equimolar amounts of arginine or leucine did not protect myotubes from wasting. Leucine, but not citrulline acutely stimulated protein synthesis, however, after 48 h serum withdrawal, incubation with citrulline completely prevented the 25% reduction in protein synthesis while neither arginine or leucine supplementation prevented this reduction.

Citrulline administration maintains normal protein metabolism and protects muscle cells from various cachectic stimuli *in vitro* but does not exert its effect *via* conversion to arginine or *via* the classical amino acid-induced increase in protein synthesis.

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