Changes in oxidative stress decrease the rate of protein synthesis in cultured C2C12 myotubes

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Skeletal muscle is a major component of body mass, and not only controls voluntary movements but also serves as a major source of body heat. A loss of muscle mass, also described as muscle wasting or atrophy, can be detrimental to overall health. An imbalance between protein synthesis and degradation has the potential to contribute to muscle wasting. Oxidative stress is considered to play a role in muscle wasting, but how oxidative stress affects protein turnover is unclear. We have hypothesized that increased in oxidative stress will decrease the rate of protein synthesis leading to a net loss of total protein.

Catalase (3000units/ml) and glucose oxidase (10munits/ml) were used to change the level of oxidative stress for 48h in day 7 multinucleated C2C12 myotubes. Catalase decreased the level of oxidative stress by removing hydrogen peroxide (H_2O_2), whereas glucose oxidase increased the level of oxidative stress by generating H_2O_2. To measure protein synthesis, myotubes were treated with radioactive labeled leucine for 24h. Radioactive incorporation was then be measured with a liquid scintillation counter.

As hypothesized, catalase caused a net increase in total protein and glucose oxidase caused a net loss of total protein from myotubes. As expected, glucose oxidase significantly reduced the rate of protein synthesis from 15.7 to 11.9μmol/dish/24h of leucine incorporation (n=3, $P<0.05$). Unexpectedly, catalase caused a significant decrease in the rate of protein synthesis from 13.2 to 9.8μmol/dish/24h of leucine incorporation (n=6, $P<0.05$) instead of the expected increase in protein synthesis.

As the decrease in protein synthesis was not consistent with the observed increase in net protein accumulation in the catalase treated myotubes, we hypothesized that catalase also caused a decrease in the level of protein degradation. To measure protein degradation in myotubes, day 6 myotubes were pre-treated with radioactive labeled leucine for 24h so that labeled leucine would be incorporated into protein. Day 7 myotubes were then treated (catalase or untreated), and radioactive release into the medium was measured. As hypothesized, catalase caused a significant decline in the level of protein degradation by 7.3% (n=4, $P<0.05$). Therefore, the increase in net total protein accumulation in the catalase treated model can be attributed largely to a decrease in the rate of protein degradation.

In summary, these data show that oxidative stress can affect the rate of both protein synthesis and protein degradation.