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Vitamin supplementation and resistance exercise-induced muscle hypertrophy: shifting the redox balance scale?

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Exercise training adaptations are the result of the cumulative changes in the cell consisting of at least transcription, translation, post-translational modifications, and overall cellular remodelling occurring in response to an exercise bout. While there are numerous stimuli for changes in transcription and translation, the transient increase in reactive oxygen species (ROS) appears to play a key role in exercise training adaptation (Powers *et al.* 2010). Thus, ingestion of antioxidant supplements, such as vitamins C and E, during exercise training could blunt exercise-induced ROS production and impair cellular responses to exercise training. As a result, investigators have examined the intracellular responses to exercise with and without antioxidants to determine the role of antioxidant supplementation on ROS-induced changes in protein signalling, gene transcription and protein translation. In general, antioxidant supplementation studies using both animal and human models have produced divergent results on overall exercise performance, protein signalling, gene expression and mitochondrial biogenesis. To date, most studies examine responses to aerobic exercise training interventions, and do not investigate resistance exercise training. In light of the lack of consensus, determining the interaction between antioxidant supplementation and exercise-induced ROS production and subsequent adaptations remains an essential quest for improving health outcomes related to exercise and redox state.

In the 15 December 2014 issue of *The Journal of Physiology*, Paulsen and colleagues sought to determine the impact of co-supplementation with vitamins C and E on the skeletal muscle adaptations to 10 weeks of resistance exercise training in healthy young individuals (Paulsen *et al.* 2014). The scientific community has not thoroughly investigated the impact of antioxidant supplementation on resistance exercise training adaptations. However, previous data suggest that antioxidant supplementation hampers aerobic exercise training-induced signalling associated with mitochondrial biogenesis and blunts beneficial exercise training adaptations (Gomez-Cabrera *et al.* 2008). The purpose of this paper is not to review the impact of vitamin C and E supplementation on aerobic exercise training adaptations, but rather to discuss and evaluate the lack of an effect of antioxidant supplementation on skeletal muscle growth, muscle strength, protein synthetic rates and ROS production following 10 weeks of resistance exercise with antioxidant supplementation. We will also discuss potential approaches for follow-up studies to further elucidate the impact of antioxidant supplementation on resistance exercise trainingst adaptations.

In the study by Paulsen and colleagues (2014), there was no effect of supplementation on changes in whole muscle cross sectional area (CSA) of the upper arm or thigh, myofibre CSA, or percentage myofibre distribution change in response to training in either group. Furthermore, antioxidant supplementation did not have an effect on training-induced changes in lower body one-repetition max (1RM), although there was a statistical trend for antioxidant supplementation to reduce upper body 1RM compared to placebo following exercise training. Thus, while antioxidant supplementation does not appear to significantly alter lean mass accretion or 1RM compared to placebo following resistance exercise training, antioxidant supplementation did attenuate skeletal muscle specific force of the thigh. We were intrigued that antioxidant supplementation resulted in a decreased specific strength, due to the lack of differences in the training-induced increases in lean mass and 1RM. However, this may be due to non-significant differences between groups for both maximal voluntary contraction and muscle CSA that became significant when combined. Overall, the impact of antioxidant supplementation on resistance exercise training adaptations seems to be minimal, although the acute exercise responses are slightly more divergent.

In response to the acute experiment, Paulsen and colleagues reported significantly elevated ubiquitin proteasome pathway (UPP) activity in the placebo compared to the antioxidant supplementation group. Specifically, total protein ubiquitination, as determined using immunoblot, was elevated in the placebo group, but not the supplementation group. Such an increase in proteolytic activity may explain the blunting effect of antioxidant supplementation on specific strength. The observed reduction in UPP activity in the supplementation group in addition to there being no significant difference in protein synthesis rates between groups could lead to a greater increase in lean mass accretion in the supplementation group compared to placebo. Unfortunately, it is impossible to extrapolate the protein synthesis and breakdown rates to overall muscle growth, as the protein synthesis measurements were short, and the total ubiquitination measurement is not an overall assessment of cellular proteolytic activity. Thus, we feel that the ubiquitination data are interesting, but post-training assessments would provide more insight into the impact of antioxidant supplementation on regulation of muscle growth and more specifically proteolytic activity.

Despite reporting significant differences in protein ubiquitination between the placebo and supplementation groups, Paulsen and colleagues did not find a change in skeletal muscle protein synthesis rates, which is in accordance with their overall finding of similar increases in lean mass for placebo and supplementation groups (Paulsen *et al.* 2014). Interestingly, $p70^{S6K}$ phosphorylation was significantly greater in the placebo group as compared to the supplementation group, although exercise increased phosphorylation in both groups. One potential reason for the disconnect between the signalling response and the protein synthetic rates may be the short duration of their tracer experiments. Specifically, the investigators measured protein synthesis for 1 h at baseline and for 1 h following the resistance exercise bout. While this transient approach could provide insight into an impact of antioxidant supplementation on acute changes in fractional synthesis rate (FSR), a longer isotope exposure time could potentially strengthen the findings.

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Recently, our laboratory developed a mathematical model to determine the relative contribution of individual protein synthetic rates and protein contents to overall cellular synthesis rate with different label duration (Miller *et al.* 2015). Because this model indicated that shorter experiments are biased towards assessment of rapidly synthesized proteins, we believe utilizing deuterium oxide (D_2O) for a week or more in the study by Paulsen and colleagues could have revealed a significant impact of antioxidant supplementation on skeletal muscle protein synthesis during resistance exercise training. Furthermore, D_2O supplementation allows for longer labelling periods without restricting participants to a laboratory setting, capturing free-living assessments of protein synthesis. Scalzo and colleagues present an important example of the benefit of this methodology, in which D_2O administration during sprint interval training revealed a sex-specific difference in protein synthesis for the first time (Scalzo *et al.* 2014). Thus, we believe that utilizing D_2O for a week or more in the current study may have provided more insight into the impact of antioxidant supplementation on skeletal muscle protein synthesis during resistance exercise training.

While increased duration of isotope exposure may have provided more insight into the impact of antioxidant supplementation on protein synthetic responses to resistance exercise training, examining subcellular fraction (e.g. mitochondrial and myofibrillar)-specific protein synthesis rates with the current approach may have revealed an impact of antioxidant supplementation. Specifically, Paulsen and colleagues assessed FSR in a whole cell lysate, while antioxidants have been reported to reduce mitochondrial biogenesis. We postulate that if Paulsen and colleagues had fractionated the skeletal muscle prior to assessing protein synthesis, they might have found differences in mitochondrial protein synthesis rates, and potentially the myofibrillar fraction as well. Furthermore, increased mitochondrial content may elevate intracellular ROS levels, impacting the overall rate of cellular protein synthesis, and potentially upregulating the UPP.

Unfortunately, Paulsen and colleagues did not investigate antioxidant enzyme content or capacity following antioxidant supplementation and resistance exercise training. Contraction leads to increased ROS levels within the skeletal muscle, which are essential for some of the exercise-induced adaptations, including upregulation of antioxidant enzymes to reduce the overall oxidative stress on the cell. One such antioxidant enzyme, the transcription factor Nrf2, is an important sensor of cellular oxidative stress and its activation leads to upregulation of numerous antioxidant enzymes. Including an assessment of Nrf2 activation in the study by Paulsen and colleagues might have elucidated differences in oxidative stress responses between the placebo and antioxidant supplementation groups. Furthermore, enzymatic activity and expression levels of common antioxidant enzymes such as superoxide dismutase, glutathione reductase and catalase might have added to the findings of the present study. Measurements of Nrf2 activation and common antioxidant enzyme expression and activity assay data could provide some insight into the impact of exogenous antioxidant supplementation on the endogenous antioxidant response. Specifically with respect to the study by Paulsen and colleagues, these measurements could provide some mechanistic insight into the antioxidant supplementation-induced reduction in strength gains.

Overall, Paulsen and colleagues provide intriguing insight into the potential role of ROS in resistance exercise training adaptations. Further, the investigators provide data for the development of a wide-range of potential follow-up investigations. Some examples include more direct assessments of the changes in ROS production within an exercising muscle with or without supplementation, longer-term measurements of protein synthesis using the D_2O technique, and thorough measurements of proteolytic markers (such as markers of autophagic flux and the three major proteolytic pathways) to attempt to determine changes in protein turnover. With a more thorough understanding of the impact of antioxidant supplementation on exercise training adaptations, health professionals can devise better dietary and exercise training programmes and incorporate them in exercise prescriptions to maintain or improve the health of the elderly and diseased populations.

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Additional information

Competing interests

The authors declare no competing interests.

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