Duchenne muscular dystrophy is the most common and devastating genetic muscle disorder. It is caused by mutations in the gene that encodes DMD/dystrophin, a large (427 kDa) protein that links the actin cytoskeleton to a complex of proteins on the sarcolemma. DMD/dystrophin was originally proposed to be a structural protein that protects the sarcolemma from damage during high-force muscle contractions. However, direct experimental evidence supporting this theory is lacking. Rather, in recent years it has emerged that DMD/dystrophin serves as an important scaffold for localizing numerous signaling proteins at the sarcolemma, and its loss causes widespread perturbations in many essential signaling and homeostatic pathways that control normal muscle function. Currently, there are no effective, long-term treatments for Duchenne muscular dystrophy. Gene-based strategies to induce expression of truncated forms of DMD/dystrophin are currently in development and some have undergone clinical trials. However, it remains unclear how effective these strategies will be in terms of correcting the numerous signaling defects that occur in dystrophic muscle. Therefore, testing drugs that target specific pathways affected by the loss of DMD/dystrophin remains an important strategy for identifying potential treatments for Duchenne muscular dystrophy.

Several skeletal muscle diseases are characterized by dysregulation of autophagy. Excessive autophagy is associated with muscle atrophy while reduced autophagic flux is evident in several inherited muscle diseases. Recently, a number of studies have shown that the autophagy process is impaired in muscles from both dmd/mdx mice and Duchenne muscular dystrophy patients, as shown by reduced expression of the autophagy marker MAP1LC3B/LC3B (microtubule associated protein 1 light chain 3 beta) and increased accumulation of the protein SQSTM1/p62, indicative of damaged protein aggregate accumulation due to impaired autophagic flux. Recent evidence showed that CYBB/NOX2 (cytochrome b-245, beta polypeptide) plays a key role in the inhibition of autophagy in dmd/mdx mouse muscle. We and others have demonstrated that increased production of reactive oxygen species (ROS) by CYBB/NOX2 mediates many deleterious pathways in dystrophic muscles. CYBB/NOX2 is upregulated and its activity is very sensitive to mechanical stretch in muscles of dmd/mdx mice compared to normal, WT mice. The increased ROS production by CYBB/NOX2 triggers Ca^{2+} entry through stretch-activated ion channels. Together, the ROS and Ca^{2+} increase membrane permeability via lipid peroxidation, and induce muscle damage and reduced force production. CYBB/NOX2-derived ROS also activates SRC kinase, which, in turn, stimulates the AKT-MTOR pathway, leading to autophagy inhibition (Fig. 1). Therefore, drugs that inhibit CYBB/NOX2 should enhance autophagy and improve health and function in dystrophic muscles.

In our recent study, we aimed to inhibit CYBB/NOX2 and oxidative stress in dmd/mdx muscle by using simvastatin, a medication used by millions of people worldwide to lower oxidative stress in dmd/mdx muscle by using simvastatin, a medication used by millions of people worldwide to lower...
circulating LDL cholesterol. Several studies have previously shown that statins reduce CYBB/NOX2 expression and activity, which explains many of their reported antioxidant benefits. We showed that CYBB/NOX2 expression is reduced in dmd/mdx hindlimb muscle and this correlates with significantly improved muscle force production. We also found that simvastatin treatment in old dmd/mdx mice decreases oxidative stress (H2O2 levels) in the diaphragm, the most severely affected muscle in dmd/mdx mice. This is accompanied by increased force production and 50% less fibrosis in diaphragm muscles from simvastatin-treated dmd/mdx mice. Simvastatin significantly increases autophagy in many tissues, including skeletal muscle. Therefore, we measured the expression levels of LC3A and LC3B in the diaphragm of dmd/mdx mice, to determine whether simvastatin increases LC3B, indicative of increased autophagy induction. We found that the levels of LC3A are unaffected by simvastatin, but the levels of LC3B significantly increase by 40% in simvastatin-treated dmd/mdx mice. These data suggest that simvastatin enhances autophagy in severely dystrophic muscle. We are now using other established assays to further investigate the effects of simvastatin on autophagic flux in dmd/mdx muscles.

Our data indicate that the reduction in CYBB/NOX2 expression and oxidative stress is likely a key mechanism for the enhanced autophagy induction provided by simvastatin in dmd/mdx muscle (Fig. 1). However, additional pleiotropic effects of simvastatin may also contribute to these improvements. In addition to blocking cholesterol production, statins also inhibit the isoprenylation of many small GTPases, which is an essential post-translational modification for membrane localization of these proteins. One of these proteins, RAC1, is significantly upregulated in dystrophic muscle and is a key activator of CYBB/NOX2 in dmd/mdx mice. Therefore, by blocking the isoprenylation of RAC1, simvastatin may also reduce the activation of CYBB/NOX2, in addition to lowering its expression. Recent evidence has shown that RAC1 also inhibits autophagy via activation of MTOR, a process that is blocked by simvastatin treatment. Therefore, RAC1 can activate both CYBB/NOX2 and MTOR, effectively amplifying this pathway that leads to inhibition of autophagy (Fig. 1).

While treatments that enhance autophagy provide substantial benefits for dystrophic muscle, further research is now required to determine the cellular mechanisms by which increased autophagy reduces muscle damage and fibrosis while improving muscle function in dystrophic muscles. Given the major role of CYBB/NOX2 in reducing autophagy and stimulating pathways that produce muscle damage, identifying simvastatin as a drug that effectively targets CYBB/NOX2 in dystrophic muscle, and improves muscle health and function, suggests that it has great potential as a novel treatment approach for Duchenne muscular dystrophy.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

**Funding**

This research was supported by the National Institutes of Health (National Institute of Neurological Disorders and Stroke) award 1R21NS088691 to Nicholas Whitehead and Stanley Froehner.