Microcirculation in a mouse model of Duchenne muscular dystrophy: another blow to the vascular hypothesis?

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DUCHEEN MUSCULAR DYSTROPHY (DMD) is a devastating disease caused by a mutation in the gene coding dystrophin. As noted by Bagher et al. in their study published in this issue of the Journal of Applied Physiology (1) and as noted by others (5, 9, 10, 12), the affected protein, dystrophin, is part of a transmembrane complex that not only provides mechanical stability to the sarcolemma, but also localizes neuronal nitric oxide synthase (nNOS), an important regulator of muscle blood flow during exercise, to the cell membrane.

Given the importance of the cardiovascular system in supplying the needs of normal muscle, early searches for the underlying pathogenesis of muscular dystrophy focused on possible defects in the vasculature supplying the muscle. This led to the vascular hypothesis, which holds that muscular dystrophy arises from areas of anoxia and ischemia in the muscle that develop because of defects in the microcirculation. Early studies to evaluate the vascular hypothesis employed two main approaches: morphological approaches and evaluation of changes brought about by occlusion of the vasculature, e.g., injection of microbeads to plug downstream microvessels, both of which failed to provide strong support for the vasculature as a primary cause of dystrophic changes in the muscle (4). In addition, early studies utilizing tracer clearance (2) showed no obvious differences in flow response to exercise and occlusion in DMD and other muscular dystrophies, and no abnormal signs of ischemia were found in the muscle of mice or patients with muscular dystrophy, arguing against a primary vascular cause for the disease.

Until recently, there have been surprisingly few studies employing direct evaluations of the in situ microcirculation in muscular dystrophy models, despite its importance as a potential contributor to the pathology of the disease. Searches for differences in microvascular network morphology and degenerative changes in the microcirculation have been largely unsuccessful. While one study (6) suggested that vessel density is decreased in gracilis muscle of older mdx mice, studies in younger mice (3, 11) found that arteriole length density, capillary red blood cell velocity, and the density, length, diameter, and surface area of the capillaries are either increased in dystrophic mice relative to controls or similar under conditions of maximum vasodilation. Thus, while the issue is not completely settled, existing findings suggest that, contrary to the vascular hypothesis, dystrophic muscle has an unchanged or increased capillary density, surface area, and blood flow, most likely secondary to angiogenic signals arising from muscle fiber breakdown (8, 11).

Studies of functional responses of arterioles and microvessels in muscular dystrophy are also surprisingly limited, although recent reports indicate that vascular relaxation in response to elevated flow and shear stress (6, 7) are impaired in arteries of mdx mice. Consistent with the results of the study by Bagher et al. (1), vessel responses to vasoconstrictor and vasodilator stimuli were unaltered in mdx mice (6, 7), suggesting that selective defects in mechanical transduction mechanisms may be present and aggravate organ damage in muscular dystrophy.

A corollary to the vascular hypothesis of circulatory-dependent ischemia as a pathogenic mechanism in muscular dystrophy is that functional sympatholysis, i.e., the ability of local mechanisms to override sympathetic vasoconstriction during exercise, may be impaired in this disease (12). This defect would not only limit exercise tolerance but could also contribute to ischemia and possibly to muscle degeneration in muscular dystrophy patients. Interest in the role of impaired functional sympatholysis in contributing to muscle damage and impaired exercise tolerance in muscular dystrophy is heightened by the observation that the dystrophin mutation in mdx mice is accompanied by reduced expression of nNOS (a potentially important mediator of functional sympatholysis) in skeletal muscle and a failure to localize nNOS to the sarcolemma (10, 12). Recent studies by Lai and coworkers (5) have shown that targeting of nNOS to the sarcolemma depends on spectrin-like repeats R16 and R17 in the rod domain of the dystrophin molecule, and that transfection with a minigene containing the R16/R17 repeats improves perfusion, contractile force generation, muscle histopathology, sarcolemmal integrity, and exercise performance in mdx mice.

The possible involvement of impaired functional sympatholysis in muscular dystrophy is supported by studies reporting that functional sympatholysis is defective in the mdx mouse model of DMD (12). Importantly, sympatholysis is also blunted in nNOS−/− mice (12), suggesting a specific role of nNOS in modulating sympatholysis in exercising muscle. Other studies (9) have suggested the possible involvement of reduced nNOS in defective functional sympatholysis in children with DMD, but not in muscular disorders that are not associated with defective nNOS.

In the present study (1), Bagher et al. compare multiple highly informative characteristics of the microcirculation in the cremaster muscle of mdx mice vs. C57BL/10 wild-type controls with careful attention to experimental quality control and matching of microvascular network locations. These characteristics included arteriolar responses to the endothelium-dependent vasodilator acetylcholine (ACh), the α-adrenergic agonist norepinephrine, elevated PO2, perivascular nerve stimulation (PNS); and also spontaneous resting tone. An especially important component of the present study is the direct evaluation of conducted dilation and constriction in response to ACh and K+ (respectively), which provide direct insight into the integrity of electromechanical coupling mechanisms in the microcirculation. Contrary to predictions of the vascular hy-
In myenteric neurons in the colon (13), the impaired response to PNS in the present study may be due to a neuropathy in the adrenergic nerve endings. If this is the case, the mdx mouse model of DMD may provide more information regarding adrenergic neurotransmission in DMD rather than microvascular alterations per se. Regardless of the underlying mechanism(s), the findings of the present study imply that impaired functional sympatholysis may have a lesser role in the pathophysiology of DMD than originally believed.

In view of the careful investigation of microvascular parameters in the present model of DMD and the lack of compelling evidence for a microvascular basis for the pathogenesis of DMD, where do we go from here? One of the major and enduring questions regarding microvascular studies of muscular dystrophy is the appropriateness of the model in predicting the final phenotype in humans (4). While the specific mutation in the mdx mouse model suggests that it is well suited to evaluating mechanisms of pathogenesis of muscular dystrophy, the exact link between the dystrophin mutation and the final phenotype in humans is still an open question.

Another important question is whether the muscle is a source of physiological changes in muscular dystrophy, rather than a target. For example, recent studies suggest that angiogenic factors are released in response to inflammatory and degenerative changes in the muscle, which appear to stimulate neo-vascularization in dystrophic muscle and increase blood flow (11). Relevant to this issue, Saito et al. (8) reported that serum levels of VEGF are elevated in patients with DMD and Becker muscular dystrophy, suggesting that VEGF may reflect hypoxic or ischemic conditions in muscle tissue and may be related to the progression of the disease in muscular dystrophy patients. Taken together, those findings suggest that the microcirculation responds in a compensatory fashion, rather than contributing to muscle wasting and degeneration in DMD. In this respect, an area of investigation that may be especially important is development of methods that optimize the vasculature of muscular dystrophy patients as a delivery route for therapeutic approaches such as gene therapy.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES