# Blood Flow Restriction Promotes Mitochondrial Transfer and Reduces Muscle Damage in a Duchenne Muscular Dystrophy Mouse Model.

Hiroyori Fusagawa[1,2], Yusef Samimi[1,2], Ashley Fang[1,2], Nesa Milan[1,2], Alex Youn[1,2], Luke Sang[1,2], Ryan Sadjadi[1,2], Aboubacar Wague[1,2], Brian Feeley[1,2], Xuhui Liu[1,2],
[1] San Francisco Veterans Affairs Health Care System, San Francisco, California, USA, [2] Department of Orthopaedic Surgery, University of California, San Francisco, California, USA

Nothing to declare

**Introduction:**
Duchenne muscular dystrophy (DMD) is a severe muscle-wasting disease marked by mitochondrial dysfunction and progressive muscle degeneration due largely to the deficiency of dystrophin, a key protein essential for maintenance of muscle membrane integrity. Blood flow restriction (BFR) is a rehabilitation technique that involves temporarily reducing blood flow to a limb. It has been used increasingly in postoperative orthopedic patients to promote muscle regeneration and improve recovery. We previously discovered that the transient hypoxia induced by BFR promotes mitochondrial transfer from fibro/adipogenic progenitors (FAPs) to myocytes and reduces muscle degeneration in a rotator cuff injury mouse model (N Milan. ORS 2025 Annual Meeting Paper No.334). In this study, we aimed to investigate whether BFR can induce FAP mitochondria transfer and mitigate muscle degeneration in a DMD mouse model. We hypothesized that BFR promotes mitochondrial transfer from FAPs to muscle fibers, thereby ameliorating muscle damage in DMD.

**Methods:**
To track FAP mitochondria traffic in DMD muscle, we generated FAP mitochondria reporter mice in D2-mdx, a DMD background with severe muscle degeneration phenotype similar to DMD patients. C57 background Prrx1-Cre/MitoTag FAP-mitochondria reporter mice were crossed with D2-mdx mice for 6 generations. Because DMD is a X Chromosome-linked disease that only effects boys, only Male Prrx1-Cre/MitoTag/D2-mdx mice (total n = 15) were used in this study. Mice were randomly divided into BFR treatment and sham control groups. Mice in the BFR group (n = 3) underwent unilateral hindlimb BFR with a rubber band on the base of the right thigh for 10 minutes followed by a 10-min break for three cycles under general anesthesia of 1-2% isoflurane [1] twice a week for a total of six sessions over three weeks. Mice in the control (CTL) group (n = 4) underwent the same anesthesia procedure without BFR. Twenty-four hours after the final BFR session, mice were sacrificed, and the plantar flexor muscles were harvested for histologic analysis. Mice received Evans Blue Dye (EBD) intraperitoneal injection at 24 hours prior to sacrifice to evaluate the extent of muscle damage. Furthermore, to determine whether BFR promotes the transfer of mitochondria from FAPs to muscle fibers in DMD muscle, tibialis anterior muscles were harvested from both the non-BFR group (n = 4) and 24 hours after the single BFR group (n = 4). The distribution of MitoTag signaling was then compared between the groups. Unpaired t-test was used to analyze differences between groups, and the differences were considered statistically significant at $p < 0.05$ (*).

**Results:**
After a 3-week BFR intervention, EBD staining revealed that the area of damaged muscle fibers was significantly reduced in the BFR group compared to the CTL group (CTL: 8.8 ± 5.1 % vs BFR: 0.5 ± 0.5 %, $p$= 0.041) (**Figure 1**). Immunostaining showed that BFR induced robust FAP mitochondria transfer evidenced with existing of GFP+ FAP-derived mitochondria within the myofibers (**Figure 2**).

**Discussion:**
Appropriate animal models are critical for studying disease mechanisms and developing therapies. The C57BL/10ScSn-Dmdmdx/J (BL10-mdx) mouse is the most widely used model for DMD, but it displays only mild limb muscle phenotypes and minimal cardiac abnormalities compared to patients. These differences are largely due to genetic background, which strongly influences disease severity. The D2-mdx strain, created by backcrossing BL10-mdx mice onto the DBA/2J background, exhibits more severe and clinically relevant features, including marked muscle degeneration and extensive skeletal and cardiac fibrosis [2, 3]. To study in vivo mitochondrial transfer from FAPs in DMD, we generated a novel FAP-specific mitochondrial reporter model on the D2-mdx background. Our data show that this Prrx1-Cre/MitoTag/D2-mdx reporter mouse exhibits severe dystrophic pathology closely resembling that of DMD patients. Previously, we used the Prrx1-Cre/MitoTag mice as in the present study to create a rotator cuff injury mouse model and investigated the effects of BFR. In this present study, we demonstrate that BFR induces mitochondrial transfer from FAPs to myocytes in DMD muscle as well. Further studies are needed to elucidate the mechanisms by which mitochondrial transfer mitigates muscle degeneration in DMD. In addition, long-term BFR intervention studies will be necessary.

**Significance/Clinical Relevance:**
This study demonstrated the therapeutic potential of BFR—a clinically applied and safe intervention—in a severe model of DMD. These findings support the development of novel strategies aimed at preserving muscle health and ultimately improve the quality of life (QOL) of DMD patients

**Reference:** [1]. Zhang et al. J Orthop Res. 2021 Sep;39(9):1889-1897, [2].Putten et al. Faseb J. 2019;33(7):8110-8124, [3]. Swideerski and Lynch. Am J Physiol Cell Physiol. 2021;321(2):C409-412

**Acknowledgements:** We thank our funding sources from Department of Defense (DMDRP grant #MD230014) and Muscular Dystrophy Association (MDA, research grant #MDA 957140)

## Figure 1

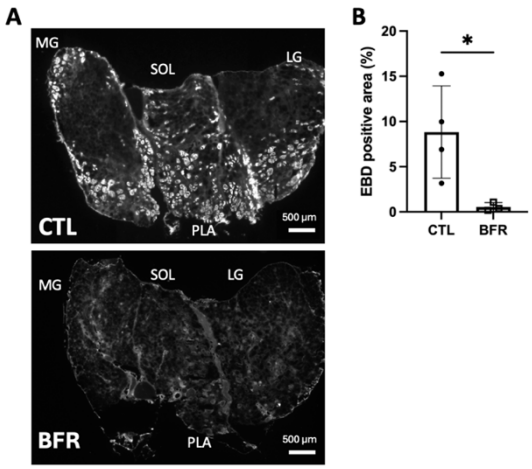


**Figure 1 Blood flow restriction (BFR) performed twice a week for three weeks reduced the number of muscle-damaged fibers. (A).** Representative images of Evans Blue Dye (EBD) for plantar flexor muscles from CTL group and BFR group. MG: medial gastrocnemius muscle, LG: lateral gastrocnemius muscle, SOL: soleus muscle, PLA: plantaris muscle **(B).** Average EBD positive area decreased in BFR group.*p<0.05 with unpaired t-test.

## Figure 2

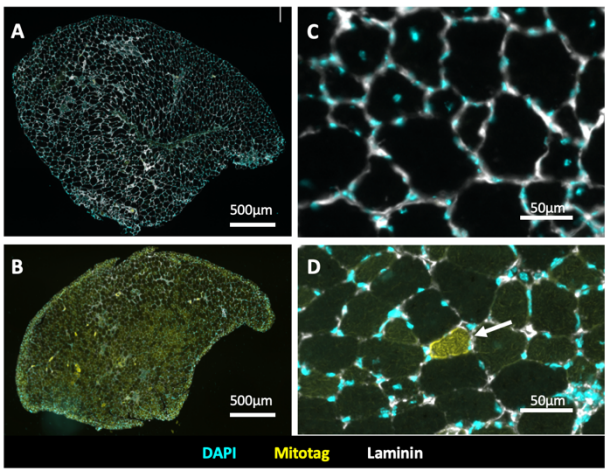


**Figure 2 Blood flow restriction (BFR) induced mitochondrial transfer from fibro/adipogenic progenitor cells (FAPs) to myocytes.** Representative Immunohistochemistry images of tibialis anterior muscles from non-BFR group (**A,C: enlarged view of A**) and 24 h after single BFR group (**B,D: enlarged view of B**). 24 h after single BFR session, mitochondria transferred from FAPs to myocytes are recognized with GFP reporter Mitotag signal (white arrow).