ISB 2013 BRAZIL
XXIV CONGRESS OF THE INTERNATIONAL SOCIETY OF BIOMECHANICS
XV BRAZILIAN CONGRESS OF BIOMECHANICS

KNOCKOUT OF BOTH NESPRIN 1 AND DESMIN RESULTS IN ABERRANT NUCLEAR MECHANICS AND FIBROSIS IN MURINE SKELETAL MUSCLE

[1]Mark A Chapman, [3]Jianlin Zhang, [3]Ju Chen, and [1,2]Richard L Lieber
Departments of [1]Bioengineering, [2]Orthopaedic Surgery, and [3]Medicine, University of California, San Diego, La Jolla, California, USA; email: rlieber@ucsd.edu, web: http://muscle.ucsd.edu

INTRODUCTION

Mechanical integration between the nucleus and the rest of the cell is critical for cellular strength, nuclear localization, and proper mechanotransduction [1,2,3]. Specifically, in skeletal muscle, faulty integration between the nucleus and the cytoskeletal network has been implicated in Emery-Dreifuss muscular dystrophy [3]. Two proteins found in skeletal muscle, nesprin and desmin, link the nucleus to the cytoskeletal network. By creating the nesprin-desmin double knockout mouse (DKO), we have eliminated these connections, which may enable increased understanding of the significance of nuclear anchoring. It is clear that loss of such connections can lead to fibrosis and dystrophy, but no mechanism has yet been proposed. Therefore, the purpose of this study was to measure nuclear mechanics, passive muscle mechanics, and collagen content of skeletal muscle in wild-type, as well as nesprin $1^{-/-}$, $desmin^{-/-}$, and DKO mice in order to characterize how skeletal muscle is affected by aberrant nuclear connectivity.

METHODS

Nuclear mechanical testing was performed on muscle fiber bundles from tibialis anterior (TA) muscles of 3-month-old mice. Samples were isolated from 3 animals per group (wild-type, nesprin $1^{-/-}$, $desmin^{-/-}$, and DKO) with 4 bundles per animal and 4 nuclei per bundle tested. Muscle bundles were carefully dissected, secured in a custom apparatus with 10-0 monofilament nylon suture, and placed onto a custom made stage of an inverted confocal microscope (LSM 510, Zeiss). Bundles were stretched using a micrometer until slack was removed. Four nuclei were imaged at slack length, and the slack sarcomere length was determined from the bright field image. Bundles were then stretched in sarcomere length increments of ~0.25 μm until achieving a sarcomere length of 4.0 μm. After each stretch, the same nuclei were imaged and the sarcomere length recorded. Changes in nuclear aspect ratio with increasing strain were quantified using ImageJ (NIH, Bethesda, MD). From these data, the deformability of each nucleus was determined from the slope of the aspect ratio versus sarcomere length plot.

Passive mechanical testing was performed on single fibers and bundles from TA muscles (n≥8 mice/genotype). Single muscle fibers as well as fiber bundles were dissected from the TA and secured in a custom chamber. On one side, the sample was secured to a force transducer (Aurora Scientific 405A; Aurora, ON, Canada) and the other side was secured to a titanium wire rigidly attached to a rotational bearing (Newport MT-RS; Irvine, CA). An incremental stress-strain protocol was then implemented. Briefly, 10% strain at 2000%/s was imposed on the samples, and samples were allowed to stress-relax for 3 minutes. Samples were stretched to failure, or until achieving a sarcomere length of 4 μm, whichever occurred first. From these data, tangent stiffness was calculated by fitting a quadratic equation to the stress-strain curve, and defining the slope at a sarcomere length of 3.2μm, the length as which passive tension develops in the TA.

Collagen content of TA muscles (n≥7 mice/genotype) was determined using a modified version of a previously published protocol [4]. Samples were placed in 6M HCl at 110°C for 24 hours for digestion. Following digestion, samples were evaporated to dryness, reacted with a chloramine-T solution, and incubated with a para-dimethylaminobenzaldehyde solution at 60°C. Absorbance readings at 550nm were measured using a spectrophotometer, and the amount of collagen in each sample was calculated by using the conversion factor of 7.46 OHP residues per collagen molecule.

RESULTS AND DISCUSSION

Nuclear mechanics: Nuclei of each genotype were deformed to different extents under our testing protocol (Fig. 1). The amount of nuclear deformation was quantified by determining the aspect ratio of the nuclei at each sarcomere length (Fig. 2). The slope of the aspect ratio versus sarcomere length line indicated the amount of deformation imposed upon the nucleus via the cytoskeletal network. The nuclei of the nesprin $1^{-/-}$ and DKO mice deformed significantly less compared to the wild-type and $desmin^{-/-}$ nuclei ($p<0.05$, via one-way ANOVA).

Limited nuclear deformation suggested that mechanical integration between the nucleus and the cytoskeleton was reduced in nesprin$^{-/-}$ and DKO mice. Although not significantly different from nesprin$^{-/-}$, the DKO trended toward a lower slope. This signifies that desmin plays only a minor role in nuclear anchorage. This is intuitive because the main role of desmin is not to secure the nucleus, but to mechanically stabilize and link adjacent myofibrils.

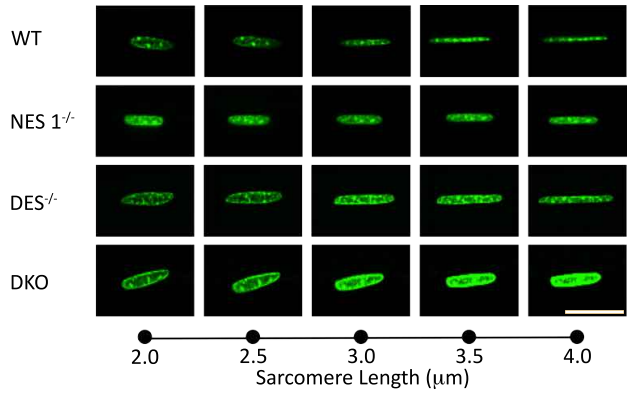

Figure 1: Nuclear deformation caused by controlled deformation of muscle bundles in the TA muscle. Wild-type and desmin$^{-/-}$ nuclei deformed, while this deformation was attenuated in the nesprin$^{-/-}$ and

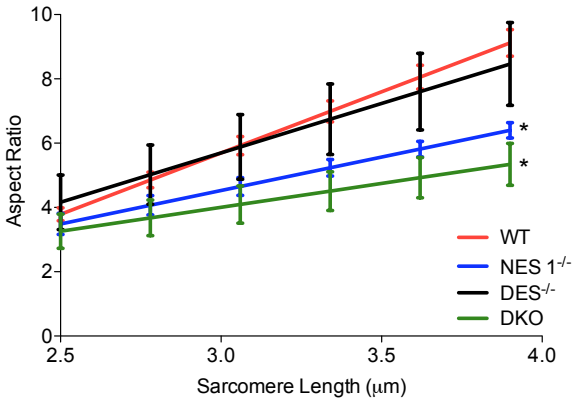

Figure 2: Nuclear aspect ratio versus sarcomere length. The trend line is an average of the trend lines from each experiment. Larger slope indicates larger nuclear deformation. Asterisk signifies slope is different from wild-type. *p<0.05.

Passive mechanics: Muscle fiber and fiber bundle tangent stiffness was calculated to determine whether passive stiffness was altered in the various genotypes. Single fiber testing, indicating cellular changes within muscles, revealed no genotypic differences in tangent stiffness (Fig. 3). However, bundle testing, indicating extracellular matrix changes, revealed a dramatic increase in tangent stiffness in the DKO group (Fig. 4).

These data suggest that there is a dramatic change in the extracellular matrix (ECM) in the DKO model. Increased stiffness could stem from deposition of additional ECM, reorganization or crosslinking of the existing matrix proteins, or a combination of the two. To investigate this further, collagen content, the main component of the ECM, was quantified.

Collagen content: TA collagen content was determined using a modified version of a previously published hydroxyproline assay protocol [4]. Collagen content was lowest in wild-type and nesprin$^{-/-}$ animals, with desmin$^{-/-}$ samples being significantly greater than wild-type, and DKO samples significantly greater than both wild-type and nesprin$^{-/-}$ samples (Fig. 5).

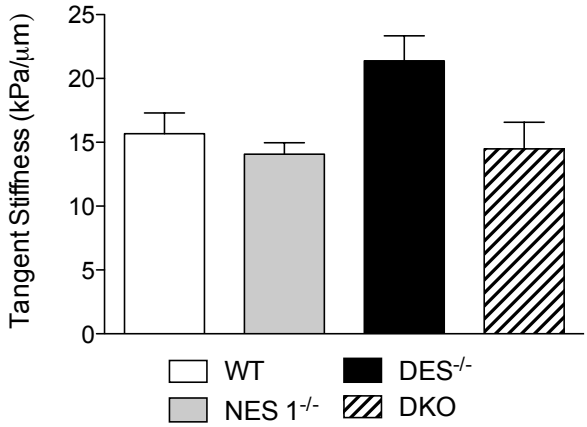

Figure 3: Tangent stiffness values for each genotype from passive mechanical tests of single muscle fibers. No significant differences were found from the one-way ANOVA. Data are presented as mean±SEM.

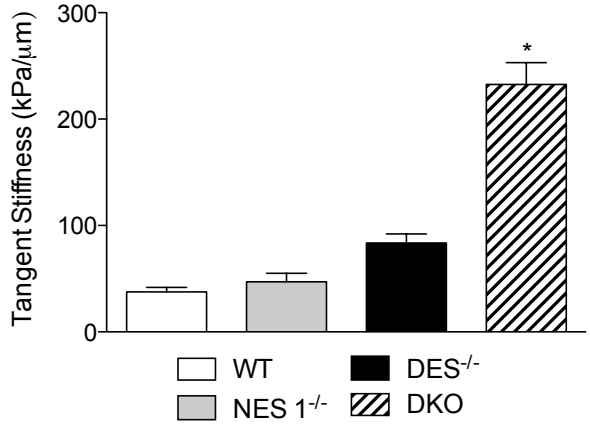

Figure 4: Tangent stiffness values for each genotype from passive mechanical tests of muscle bundles. The tangent stiffness for DKO samples was found to be significantly greater than the other genotypes. *p<0.05. Data are presented as mean±SEM.

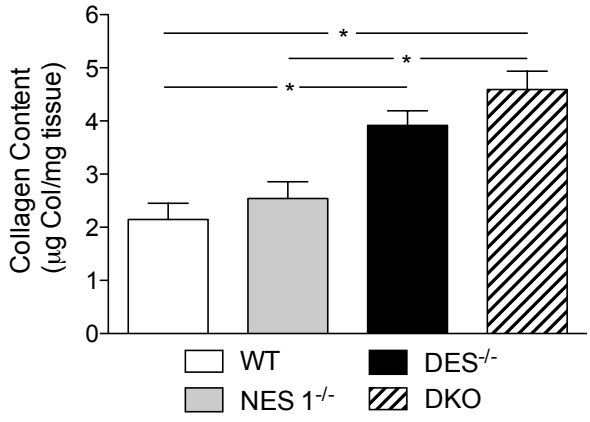

Figure 5: Collagen content from hydroxyproline assay. DKO samples were significantly more collagenous compared to wild-type and nesprin$^{-/-}$ samples, while desmin$^{-/-}$ samples were significantly greater than wild-type samples as determined by a one-way ANOVA. *p<0.05. Data are presented as mean±SEM.

While collagen content correlates with passive mechanical data (Figs. 4&5), it does not scale linearly with bundle passive mechanical data. When plotted against each other and fit with an exponential, the tangent stiffness versus collagen content graph has an R^2 value of 0.344. This weak correlation suggests that there is another factor that explains the dramatic increase in passive mechanics with a small increase in collagen. Since collagen did not become much more abundant in the DKO, a possible explanation is that collagen organization, not collagen quantity, was altered. Crosslinks within the collagen molecule, which have been implicated in both cardiovascular and pulmonary fibrosis, could be causing this increase in the tangent stiffness [5,6].

CONCLUSIONS

Collectively, these data demonstrate that the nuclei of the nesprin-desmin double knockout mice have dramatically reduced connectivity to the cytoskeletal network in skeletal muscle. This lack of connectivity coincides with an increase in the tangent stiffness of muscle bundles in the DKO, signifying a fibrotic response in the tissue. These data are corroborated, although not completely, by the collagen data. An increase in collagen crosslinking could also be attributing to the dramatic increase in bundle tangent stiffness of the DKO, but further studies would have to be conducted to verify this.

ACKNOWLEDGEMENTS

This work has been supported by a grant from the NIH (R01AR059334), M.A.C. thanks the NSF Graduate Research Fellowship Program and an NIH T32 training grant for support.

REFERENCES

1. Brosig M, et al., *Int J Biochem Cell Biol*. **42**: 1717-28, 2010.
2. Houben F, et al., *Biochim Biophys Acta*. **1773**: 675-86, 2007.
3. Zhang J, et al., *Hum Mol Genet*. **19**: 329-41, 2010.
4. Edwards CA, and O'Brien WD Jr, *Clin Chim Acta*. **104**: 161-7, 1980.
5. Last JA, et al., *Am Rev Respir Dis*. **141**:307-13, 1990.
6. Lopez B, et al. *Hypertension*. **60**:677-83, 2012.