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HETEROGENEITY OF SARCOMERE LENGTHS IN PASSIVE LIVE AND FORMALIN FIXED MOUSE EDL MUSCLE MEASURED WITH 2 PHOTON MICROSCOPY

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INTRODUCTION

Sarcomere length (SL) is a key parameter underlying muscle function. Within a muscle, the heterogeneity of SL and its operating range affects global measures such as the muscle length-tension relationship and may provide insight into muscle stability and function. Measuring SL in muscle is difficult and often requires some surgical dissection of fibers and/or chemical fixation of muscle fibers [2,3]. Formalin fixation can produce shortening in muscle hence it may alter measurements of SL heterogeneity and operating range. Here, we take advantage of new 2 photon (2P) imaging techniques [1,4] to measure SL in passive whole live muscles. We then compare these results to fixed muscle, asking whether fixation alters mean SL and increases SL heterogeneity.

METHODS

We studied mouse extensor digitorum longus (EDL) muscles (n=6, CD-1 aged 45-60 days). The mice were anaesthetized with urethane. In the left leg, we carefully freed the EDL from surrounding tissue, tied the tendons with silk suture, and bathed the muscle in oxygenated Ringer's solution. While alive, we stretched the passive muscle to varying muscle-tendon lengths and imaged it with 2P microscopy. Next, we held the muscle at a predetermined length and fixed it in formalin (10% neutral buffered formalin). We later reimaged it using 2P. Since we felt uneven strain in the cut tendon may cause SL artifacts, we also examined the contralateral EDL using the more common procedure of whole limb fixation. We removed the right hindleg, retracted the skin, and pinned the leg to a board at joint angles designed to approximate the EDL length in the left leg. It was then bathed in formalin. After one day or longer we dissected the EDL free and imaged it. We studied three different muscle lengths: slack; moderate; and long, using 3 different conditions: live; fixed-isolated; and fixed-limb.

The 2P imaging technique we used is similar to that described by Llewellyn et al. [1] except the muscle was viewed from the surface and not penetrated with a microendoscope. We imaged surface fibers up to a depth of 200 µ using second harmonic generation (SHG) imaging (Bio-Rad 2100 MPD, tunable titanium-sapphire laser wavelength at 880 nm, Zeiss 25x water-dipping objective, filter and photo multiplier tube at λ 370-450 nm).

RESULTS

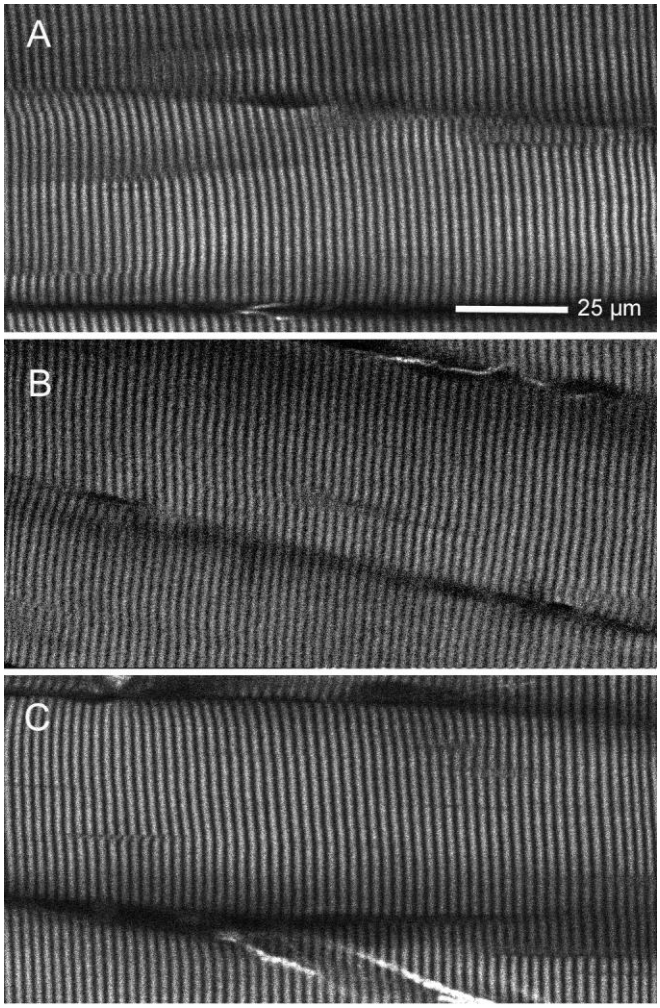

Figure 1: Slack length A) Live, in this image mean SL=2.50µm, sdev= 0.048; B) Fixed-isolated, mean SL=2.45µm, sdev=0.082. C) Fixed-limb, mean SL=2.55µm, sdev=0.088.

Fig. 1 shows images from a representative mouse EDL sampled at slack length. The dark vertical bands show the z

lines. The muscles fibers are oriented horizontally. Z bands were clearly visible in live and fixed tissue to a depth of 200 µm, except under areas of thick collagen from the aponeuroses.

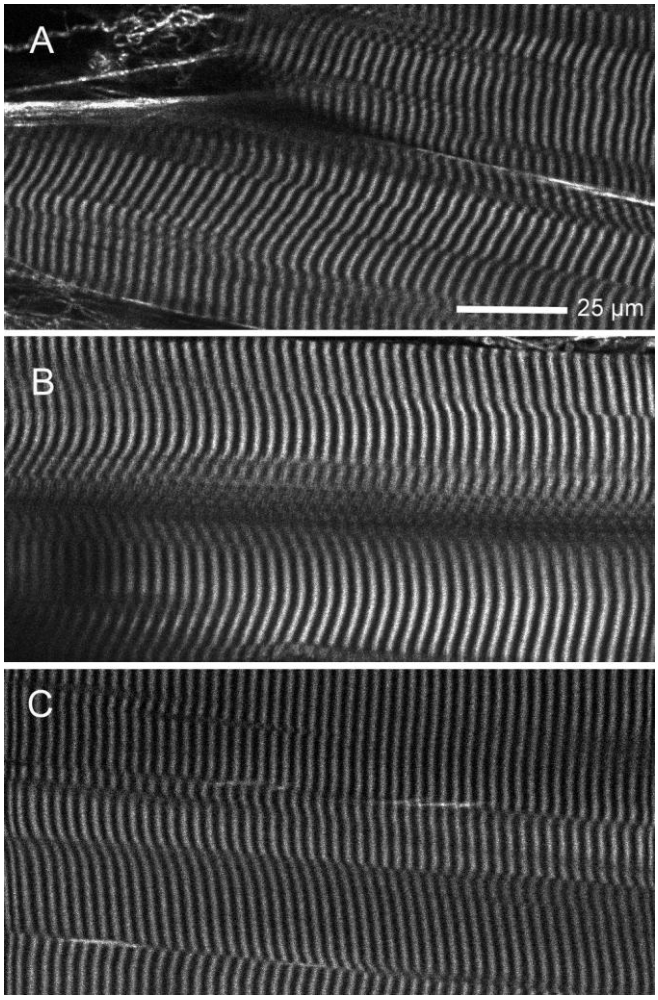

Figure 2: Moderate SL or physiological maximum length A) Live, in this image, mean SL=3.17µ sdev=0.152; B) Fixed isolated, mean SL=3.34µ sdev=0.064; C) Fixed limb, mean SL=2.99 µ sdev=0.052.

Live passive muscle did not shorten to SL less than 2.5 µm. At this length all muscles showed uniformity across the muscle fibers. When fixed, provided the muscle was held at slack length or longer, SL heterogeneity appeared the same to that observed before fixation. In isolated-fixed muscle, if the ends were not secured, formalin caused the muscles to shorten unpredictably, sometimes to less than 1.8 µm. In fixed-limb samples, SL below 2.5 µm were not observed in spite of pining the leg at what should produce shorter SL.

Figure 2 shows images from a representative mouse measured at moderate SL (3.1 µm). This SL corresponded to the longest physiological length we measured in the EDL. It was obtained with knee fully extended and the foot fully flexed. Shear is visible in the live (A), fixed-isolated (B), and fixed-limb (C). Shear was apparent in all muscles studied at medium and longer SL. Stretching the muscles farther (3.8 µm) increased the observed shear but did not seem to damage the muscle or alter the heterogeneity between live and fixed-isolated muscle.

We tested the hypothesis that formalin fixation altered mean sarcomere length by comparing the live and fixed-isolated samples from all areas of the muscle. The mean SL was computed for each mouse and used to normalize the data. Data from all lengths were pooled. A t-test did not show a significant difference in mean SL between the live and fixed-isolated samples ($P>0.05$). We tested the hypothesis that formalin increases SL heterogeneity using the local variations in SL for all three muscle treatments. Small regions, 10 to 40 sarcomeres in series, were normalized by the mean SL for this region. Data from all muscle lengths and mice were pooled for each muscle treatment. A Kolmogorov-Smirnov test showed no significant difference in SL heterogeneity between live and fixed-isolated or between live and fixed-limb.

Quantifying SL heterogeneity is complicated by shear (see Figure 2). The live and fixed muscle look qualitatively similar but, so far, we have not attempted to quantify shear.

CONCLUSIONS

1) 2P imaging of whole live passive muscle provided visible sarcomeres at depths up to 200µm in mouse EDL. SL can be measured without chemical fixation or any mechanical disruption of the muscle.

2) Whole muscles fixed in formalin could be also be imaged with 2P eliminating fascicle dissection as a confounding factor in SL measures.

3) At muscle lengths longer than slack length, fixation in formalin did not change mean SL or increase SL heterogeneity.

4) At short muscle lengths fixation in formalin produced variable responses. In some samples sarcomere lengths decreased, in others they remained at slack length (2.5 µ).

5) In mouse EDL the longest physiological sarcomere length was 3.1 µm. We could not determine the shortest because of variable shortening for slack muscles.

ACKNOWLEDGEMENTS

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REFERENCES

1. Llewellyn ME, et.al. Nature 454(7205): 784-788, 2008.
2. Loeb GE, and Gans C. Electromyography for experimentalists. Univ. of Chicago Press, 1986.
3. Ward SR, et al. J Biomech 42: 193-196, 2009.
4. Zipfel et al., Nature Biotech. 21: 1369-1377, 2003.