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A Novel Stereological Method used to Quantify Muscle Damage Induced by Injurious Stretch-Shortening Cycles

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To date there has been little evidence quantifying the amount of degenerative myofibers induced by injurious stretch-shortening cycles (SSC's). PURPOSE: To use a novel stereological method to quantify skeletal muscle damage induced by injurious SSC's. Methods: Testing was performed on the dorsiflexor muscles of Sprague-Dawley rats *in vivo* (n = 24). Briefly, rats were anesthetized with isoflurane and randomly exposed to either 3 sets of 10 SSC's, 7 sets of 10 SSC's, 15 sets of 10 SSC's, or 15 isometric contractions of equal intensity. After exposure to an assigned protocol, animals were sacrificed at either 30 minutes or 48 hours, and changes in muscle morphometry were assessed at these time points. The tibialis anterior muscle tissue was excised and weighed, and the midbelly region was cut from the muscle and mounted on cork, immersed in OCT, frozen in isopentane cooled with liquid nitrogen, and stored at -80°C. Next, transverse sections (12 µm) were cut, mounted on precoated microscope slides, air dried, and stained using a routine procedure with Harris Hematoxin & Eosin. Tissue sections were evaluated on a Leica DMLB microscope. Stereology (121 point counting) was used to quantify the degree of myofiber damage in muscle from each group and was also used to measure the volume fraction, surface densities and average thickness of normal myofibrils, degenerative myofibers and the interstitial space. Results: There is an increase in volume of both non-cellular and cellular interstitium of animals exposed to 70 and 150 SSC's as compared to animals exposed to 30 SSC's and isometric controls ($p = 0.0098$). The volume of degenerative myofibers was greater in the 150 repetition group versus the 70 repetition group ($p = 0.016$), and both were greater than the 30 repetition and isometric control groups ($p = 0.004$). Conclusions: There is an increase in myofiber damage and edema with exposure to increasing number of SSC repetitions as assessed by the increase in volume of degenerative myofibers and cellular and non-cellular interstitium.

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