Rapid communication

Ultrastructural evidence for loss of calcium homeostasis in exercised skeletal muscle

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Muscle injury has been documented following eccentric exercise bouts in skeletal muscle (Faulkner 1985, Duan et al. 1988, Lieber & Fridén 1988). Insights into the early events causing muscle injury have been obtained from mechanical studies (Lieber & Fridén 1993, Warren et al. 1993) as well as immunohistochemical studies of cellular and extracellular proteins (Lieber et al. 1996). From these studies has emerged the concept that a very early or even initial event associated with eccentric exercise is the loss in cellular calcium homeostasis. Indirect evidence for this phenomenon crosses experimental methods and exercise models including increased mitochondrial calcium content in rats subjected to downhill running (Duan et al. 1990), increased activity of the calcium activated neutral protease calpain after running in rats (Belcastro 1993), loss of cellular integrity with eccentric contraction of mouse muscle in vitro (Petrof et al. 1993), and selective loss of the intermediate filament protein desmin, after only 5 min of eccentric activation in rabbit tibialis anterior (TA) muscle (Lieber et al. 1996).

The most common ultrastructural change accompanying eccentric exercise is the disruption of the Z-disk structure with concomitant loss of myofibrillar register (Fridén et al. 1981). However, in this report, we present unique ultrastructural observations that may link the loss of calcium homeostasis to initial muscle injury.

Eighteen rabbits were anaesthetized with a subcutaneous injection of a ketamine-xyazine-acepromazine cocktail (50, 5 and 1 mg kg⁻¹ body mass, respectively) and maintained on halothane anaesthesia. The distal tibialis anterior tendon was secured to dual-mode servo-motor and aligned with the motor’s measuring and translation axis. The peroneal nerve was isolated for direct muscle activation and, under computer control, muscle length was adjusted to the length at which twitch tension was maximum. Cyclic linear length changes of 25% of the fibre length were imposed upon the TA muscle that were completed in 400 ms and repeated, along with muscle activation, every 2 s for 30 min for a total of 900 stretches. A small portion of the superficial TA was secured by nylon sutures to a wooden stick in situ and fixed overnight in a chilled 2.5% glutaraldehyde in isotonic phosphate buffer. During rinsing in buffer, the middle, mechanically undamaged portion of the biopsy, was transversely cut into slices about 1 mm thick. From

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Figure 1 High magnification electron micrograph showing crystalline structure within the SR. Original magnification × 80000.
Loss of calcium homeostasis

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Figure 2

Electron micrograph demonstrating loss of electron density in the region of Z-disk (arrows). Note also the Z-disk streaming and misalignment of the myofibrillar A- and I-band. Original magnification × 10000.

these slices, 8–10 tissue blocks were post-fixed for 2 h in 1% osmium tetroxide, dehydrated in graded alcohols and infiltrated with Epon. Blocks were orientated so that muscle fibres could be sectioned either longitudinally or transversely. Survey sections (1 μm) were stained with toluidine blue and a region selected for electron microscopy. Section thickness was kept as close to 60 nm as possible. Morphometry of the volume density of sarcoplasmic reticulum (SR) was made using standard point-counting procedure.

In addition to the commonly observed Z-disk streaming, we found two other features to be characteristic of eccentrically-exercised muscle: crystallized structures within the SR lumen in 14 of the biopsies (78%, Fig. 1) and the absence of electron density in the region normally occupied by the Z-disk (Fig. 2). The periodicity of the crystalline structure within the Z-disk was 42 nm and the volume density of SR was 3 ± 2% which was significantly greater than normal 0 ± 9% (P < 0.001). Loss of Z-disk material was accompanied by loss of myofibrillar band registry including A-band sliding to one end of the sarcomere.

While we had made many of these observations previously, their significance was not fully appreciated until the putative role of calcium activated proteolysis in muscle injury was made (Lieber et al. 1996). These observations are consistent with the proteolytic role of calpain since it is known that α-actinin, the main component of the Z-disk is a specific substrate for calpain, unlike actin or myosin (Belcastro 1993). Similarly, in the process of programmed cell death, formation of intracellular crystalline structures indicates the increased calcium concentration.

Taken together these ultrastructural observations support the role of disrupted calcium homeostasis in the early muscle injury process associated with eccentric contraction. Of course, further studies are required to detail both the source of the calcium (intracellular vs. extracellular), and the precise structures affected as well as their relative timing.

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