Skeletal Muscle Injury and Repair in Marathon Runners After Competition

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Elevated serum creatine kinase MB isoenzyme (CK-MB) activity in marathon runners after competition may arise from injury to skeletal muscle, myocardium, or a combined tissue source. Normal radionuclide myocardial scintigraphy and the selective increase in skeletal muscle CK-MB reported in such runners strongly suggest a peripheral source. To understand this biochemical finding, the authors examined gastrocnemius muscles by electron microscopy from 40 male marathon runners at intervals after competition and from 12 male nonrunners. Muscle from runners showed post-race ultrastructural changes of focal fiber injury and repair: intra- and extracellular edema with endothelial injury; myofibrillar lysis, dilation and disruption of the T-tubule system, and focal mitochondrial degeneration without inflammatory infiltrate (1–3 days). The mitochondrial and myofibrillar damage showed progressive repair by 3–4 weeks. Late biopsies showed central nuclei and satellite cells characteristic of the regenerative response (8–12 weeks). Muscle from veteran runners showed intercellular collagen deposition suggestive of a fibrotic response to repetitive injury. Control tissue from nonrunners showed none of these findings. The sequential morphologic changes in runners suggest that the increase in skeletal muscle CK-MB is a marker of cellular regeneration. (Am J Pathol 1985, 118:331–339)

ASYMPTOMATIC marathon runners after competition may show marked elevations in serum of the so-called heart-specific or MB isoenzyme of creatine kinase (CK-MB)[1]. Peak values after the race and time-activity curves for CK-MB release are quantitatively comparable to abnormalities in patients undergoing acute myocardial infarction.[2] Elevated CK-MB in runners can arise either from injury to skeletal muscle through exertional rhabdomyolysis, from silent injury to myocardium, or from a combined tissue source. Normal radionuclide myocardial scintigraphy using Tc 99m pyrophosphate and thallium 201 argues against myocardial ischemia or injury in these runners,[1,2] as postulated by others.[3,4]

Biochemical analysis of trained skeletal muscle from marathon runners shows an increase in CK-MB concentration comparable to activity in serum after competition.[5] Elevation of CK-MB in skeletal muscle after strenuous training may be due to its selective presence in high oxidative or slow twitch fibers which predominate in endurance athletes, but CK-MB content and percent fiber type showed no significant correlation.[5]

An alternate explanation is that the increase in CK-MB is related to its higher concentrations in regenerating fibers, which recapitulate the isoenzyme profile of fetal skeletal muscle.[6,7] To test this hypothesis, we examined skeletal muscle from trained marathon runners at various intervals after competition using ultrastructural analysis by electron microscopy. Sequential changes of injury and repair would suggest that skeletal muscle CK-MB is a marker for tissue regeneration.

Materials and Methods

Subjects consisted of 40 male marathon runners who completed a marathon race of 26.2 miles (42.195 km) in a competitive effort and who volunteered to participate after informed consent. All runners were asymp-

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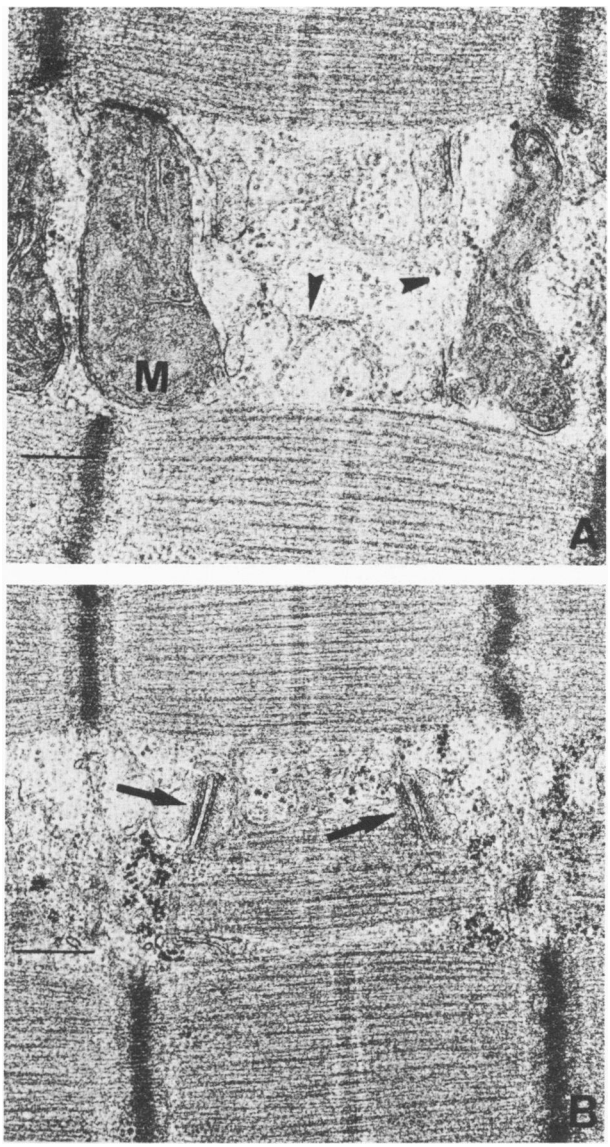

Figure 1—Control muscle biopsy specimens. **A**—Specimens with sarcomeric units separated by mitochondria (*M*) and sarcoplasmic reticulum (*arrowheads*). The mitochondria have a dense matrix and platelike cristae. There are numerous glycogen granules. (×30,000; bar = 0.3 μ) **B**—A view of the sarcomeric units with prominent Z bands and thick and thin filaments. The *arrows* indicate the junction of the transverse tubules and the sarcoplasmic reticulum. (×20,000; bar = 0.45 μ)

tomatic following competition except for a variable degree of leg stiffness. Sedentary age-matched males (10) and marathon runners out of training for a minimum of 6 months (2) served as control subjects (12).

Percutaneous needle biopsies of the lateral gastrocnemius muscle were performed by the technique of Berstrom as modified by Evans to maximize sample size.[8] Two or more runners were biopsied at time intervals after the race as follows: same day; 1, 2, 3, 5, 7, and 10 days, 2, 3, 4, 6, 8, 10, and 12 weeks. Three run-

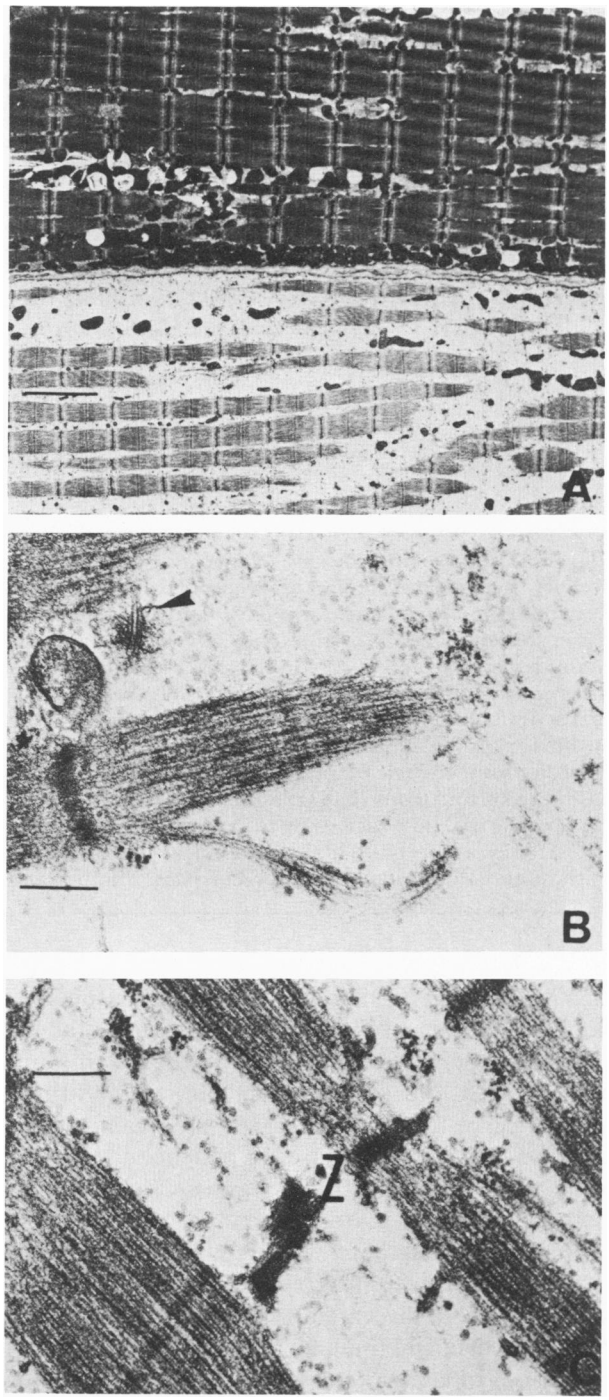

Figure 2—The acute changes of muscle fiber injury in biopsies obtained within 24–48 hours after competition. **A**—The selectivity of fiber injury is shown in this photograph. The upper fiber has morphologically normal myofibrils and a normal staining pattern. The bottom fiber has a "moth-eaten" appearance because of intracellular edema, myofibrillar lysis, and depletion of glycogen. (×8000; bar = 0.8 μ) **B**—Myofibrillar lysis with a disrupted and "frayed" sarcomeric unit. There is the remnant of the junction between the transverse tubule and sarcoplasmic reticulum (*arrowheads*). (×50,000; bar = 0.2 μ) **C**—An example of extreme myofibrillar lysis with only Z-band material (*Z*) remaining. The sarcoplasmic reticulum has also disappeared. (×50,000; bar = 0.2 μ)

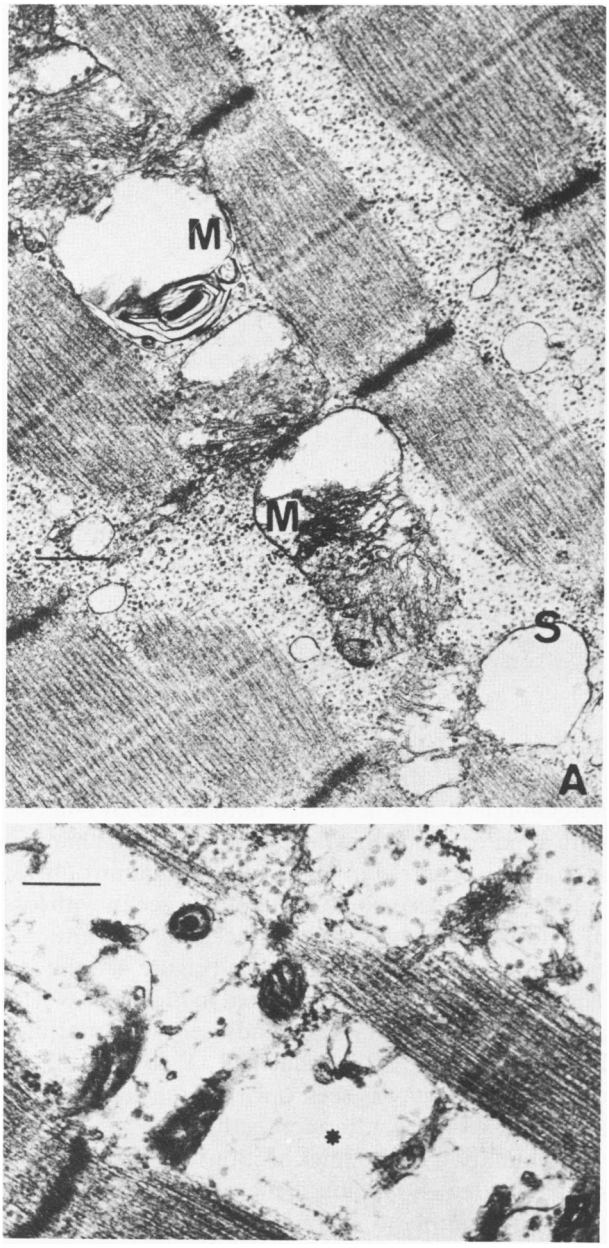

Figure 3—Biopsies at 48 hours after competition. **A**—The mitochondria (*M*) have lost both their cristae and matrixes with their formation of intramitochondrial myelin figures. The sarcoplasmic reticulum (*S*) is also dilated. (×20,000; bar = 0.45 μ) **B**—This fiber exhibits loss of both myofibrils and sarcoplasmic reticulum (*). (×20,000; bar = 0.45 μ)

ners underwent multiple biopsies contributing to this analysis, although the changes observed are based upon observations made in the group as a whole rather than on serial changes in the same individual.

A portion of each fragment was utilized for ultrastructural analysis. This fragment was immediately minced into 1-mm cubes and placed in half-strength Karnovsky's fixative. Tissue was stored in this fixative at 4 C from 3 days to 1 week. The tissue was then

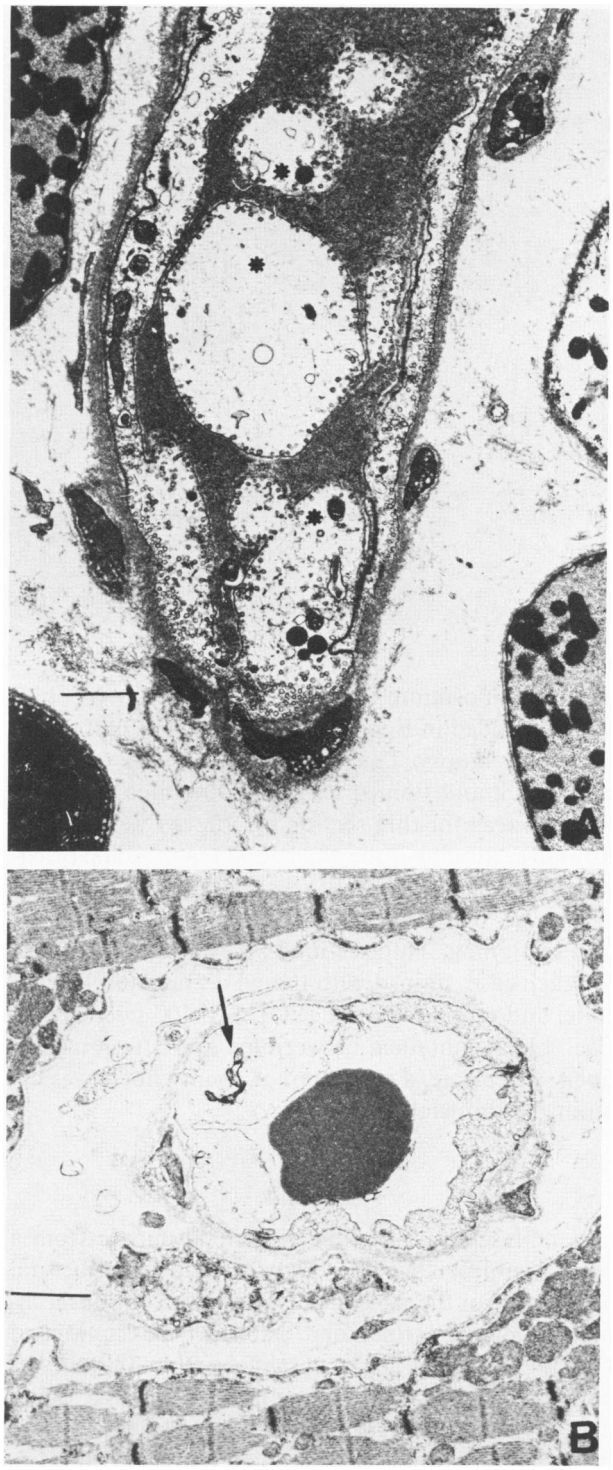

Figure 4—The interstitium of muscle fiber biopsy specimens 24–48 hours after competition. **A**—A capillary with severe endothelial damage, as indicated by the lack of staining of the endothelial cytoplasm (*). There is marked interstitial edema. (×5000; bar = 0.5 μ) **B**—An endothelial cell has lost its cytoplasmic content and contains an intracellular myelin figure (*arrow*). The adjacent cells are unaffected. (×8000; bar = 0.8 μ)

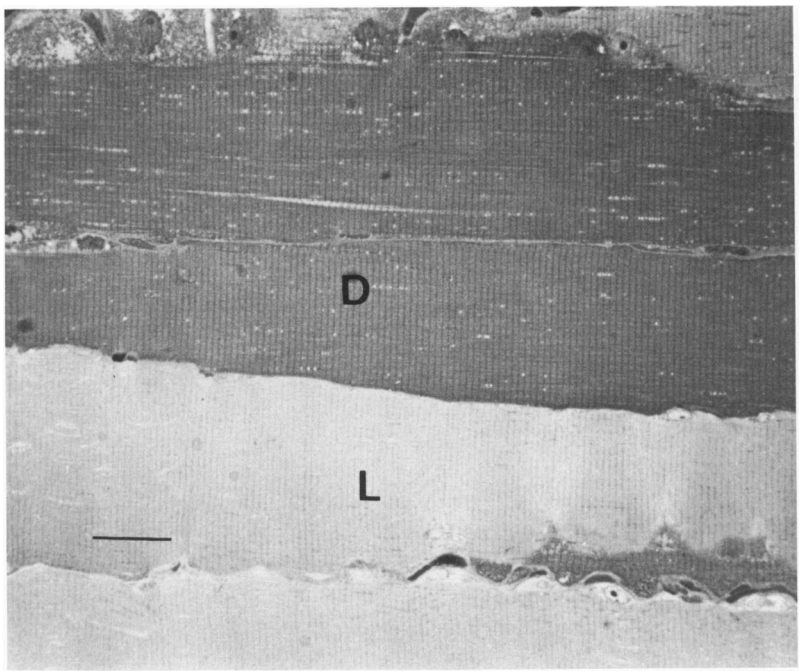

Figure 5—A 1-μ-thick Epon section stained with toluidine blue from a marathon runner 24 hours after competition. The fibers reveal little damage but exhibit a variegated light (*L*) and dark (*D*) staining pattern. (×400; bar = 30 μ)

postfixed in osmium tetroxide, dehydrated in alcohol, and embedded in Epon. Eight blocks were embedded from each biopsy. Thick sections (1 μ) were cut from each block and stained with toluidine blue for selection of areas for thin sectioning. Blocks were chosen for thin sectioning if they had good longitudinal orientation of muscle fibers. Blocks that appeared to have lesions (ie, by light microscopy) were also chosen for thin sectioning. Thin sections were cut on two blocks for each case, stained with uranyl acetate and lead citrate, and examined on a JEOL EM-100 microscope. Each block contained between ten and fifteen muscle fibers. Therefore, a minimum of twenty fibers was examined from each sample.

Results

A representative section of skeletal muscle from a control subject is shown in Figure 1. The ultrastructural appearance of the skeletal muscle obtained by our technique is identical to standard published descriptions of skeletal muscle. Biopsies obtained within 48 hours of competition are shown in Figures 2, 3, and 4. Figure 2A demonstrates the focality of the muscle injury. Some fibers have a normal dark staining pattern with residual intracellular lipid and glycogen. Other fibers have a "lighter" staining because of depletion of both glycogen and lipid with intracellular edema. As seen in Figures 2B and C, there is both profound myofibrillar lysis and disappearance of the sarcoplasmic reticulum. This loss of sarcoplasmic reticulum is best appreciated in Figure 3B. Mitochondrial damage (Figure 3A) is manifested by dissolution of cristae and loss of mitochondrial matrix. These mitochondrial changes were apparently independent of myofibrillar changes, in that they occurred in fibers with normal myofibrils.

Damage was also noted in endothelial cells, with loss of cytoplasmic matrix and intracellular constituents creating a "clear-cell" appearance (Figure 4A and B). The focality of this change is noted in Figure 4B, where a damaged endothelial cell is adjacent to a morphologically normal endothelial cell. Evidence of endothelial damage was seen only in biopsies obtained at 24 and 48 hours after competition.

Examination of the thick sections of these biopsies revealed extremely variable damage from runner to runner. Some samples displayed only "light" and "dark" fibers with no obvious myofibrillar damage (Figure 5). Other samples revealed only mild focal areas of myofibrillar loss (Figure 6A). In extreme cases, 25% of the fibers exhibited areas of myofibrillar loss (Figure 6B and C). The myofibrillar loss was extremely patchy. In no case was it greater than 10% of the fiber length.

Biopsies obtained 1 week after competition showed beginning resolution of the acute injury. Most fibers have reconstituted their glycogen, as seen in Figure 7. Some fibers, however, now appear as empty myotubes containing only scattered mitochondria (Figure 7B). Also noted in these biopsies are both "satellite" cells and interstitial cells resembling fibroblasts (Figure 7A).

Within 1 month after competition, most of the myofibrillar damage has been resolved. Only small foci

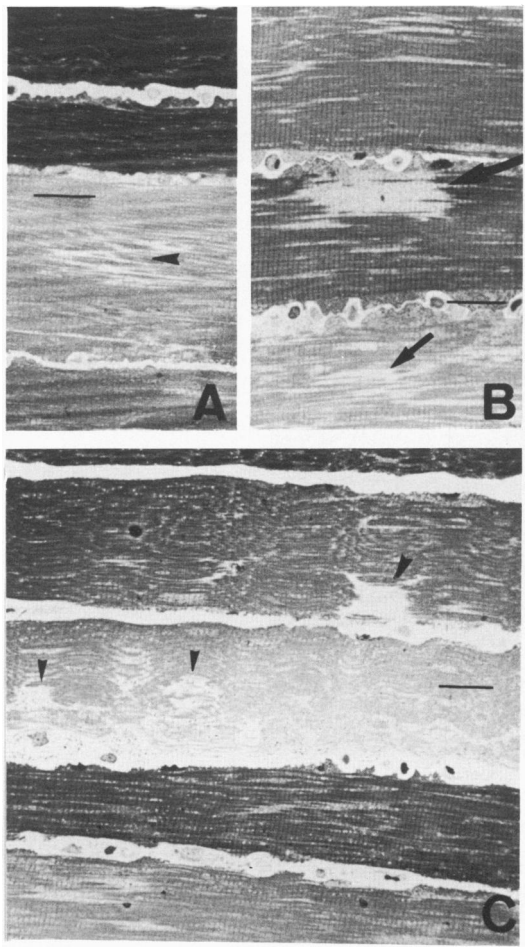

Figure 6—One-micron-thick epon sections of muscle biopsy specimens obtained 24–48 hours after competition. **A**—A biopsy specimen with light and dark fibers exhibiting only minimal sarcomeric damage (*arrowhead*). (×400); bar = 30 μ) **B**—A biopsy specimen with extensive focal damage in one fiber (*large arrow*) and more diffuse damage in another fiber (*small arrow*). (×400; bar = 30 μ) **C**—A biopsy specimen with extensive damage in several foci in two fibers (*arrowheads*). (×400; bar = 30 μ)

of damaged myofibrils remain. One no longer finds empty myotubes. Fibers contain abundant glycogen. Mitochondria are numerous and exhibit marked variation in size and shape. These mitochondrial changes may be a manifestation of earlier mitochondrial injury. Prominent in these biopsies are central nuclei (Figure 8), a morphologic change that correlates with regenerative activity.

Biopsies obtained 8 and 10 weeks after competition demonstrated the resolution of fiber injury and changes of regeneration and repair. Satellite cells (Figure 9), the presumed precursor of skeletal muscle cells, are prominent in these biopsies. These cells can be recognized because of their characteristic location immediately outside the basal lamina of the muscle cells. Many of the satellite cells appeared to be metabolically "active," with

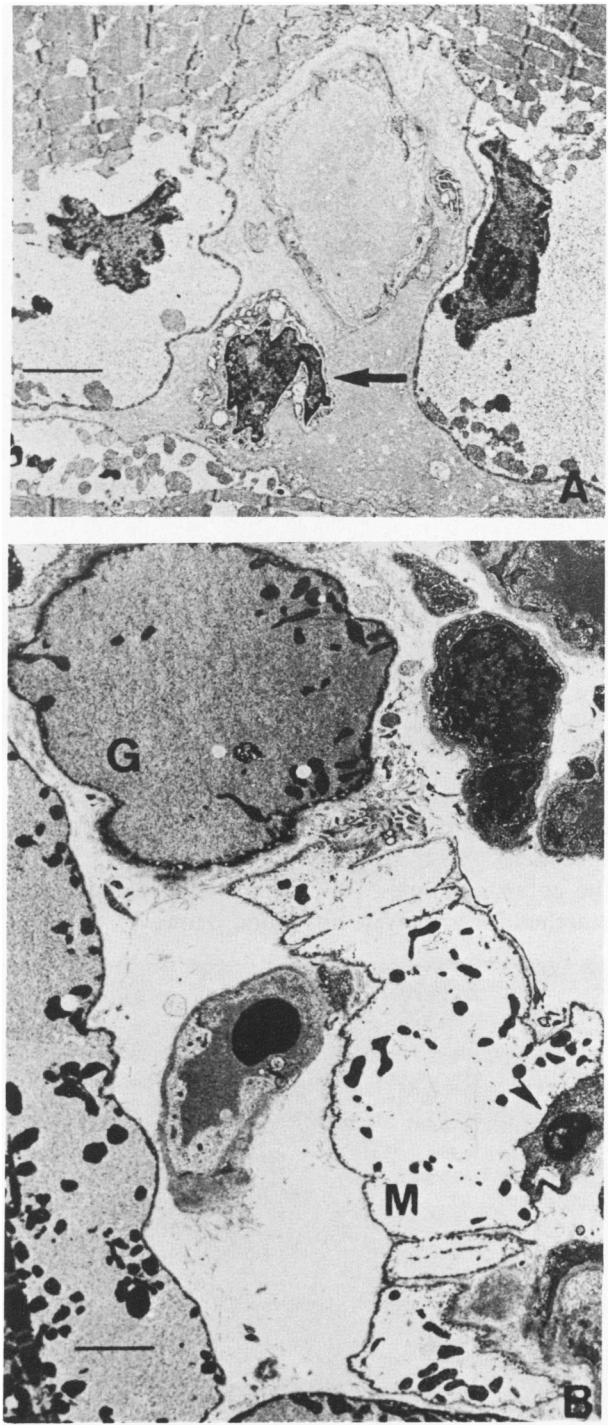

Figure 7—Muscle biopsy specimens 1 week after competition. **A**—Muscle fibers have reconstituted their glycogen. There is still residual interstitial edema. Mesenchymal cells (*arrow*) are seen within the interstitium (×8000; bar = 0.8 μ) **B**—The end result of severe myofibrillar lysis is an empty myotube (*M*). Adjacent to the myotube is a satellite cell (*arrow*). Other fibers have abundant glycogen (*G*). (×2000; bar = 0.12 μ)

numerous polysomes and the development of endoplasmic reticulum (Figure 9).

The muscle fibers themselves also exhibit morphologic evidence of regenerative activity. Rough endoplas-

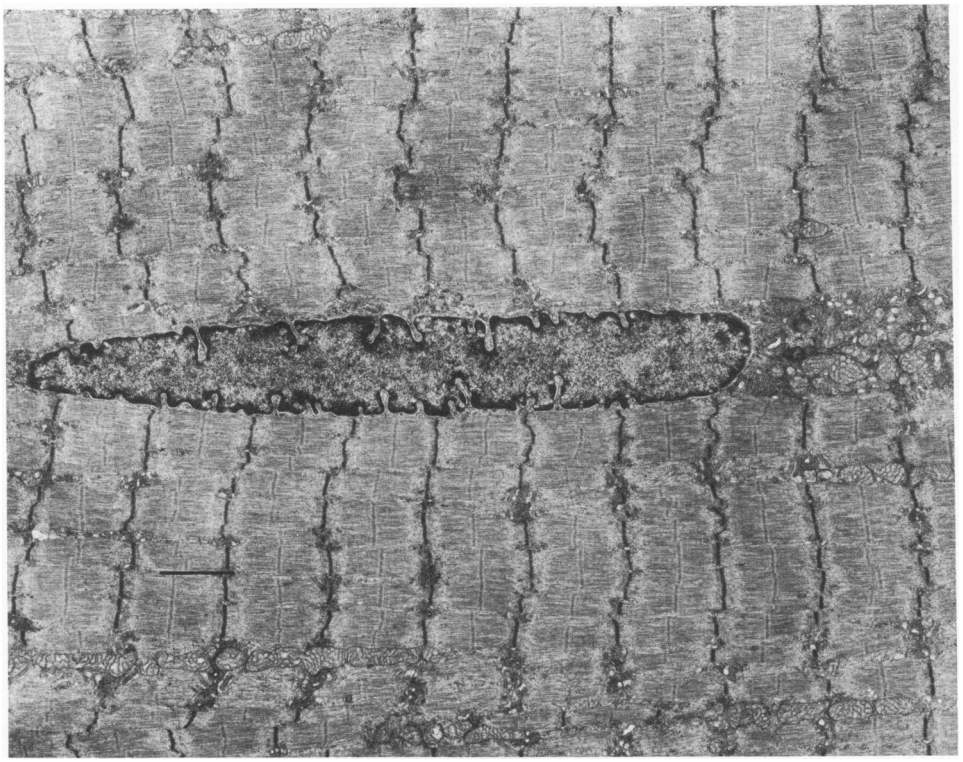

Figure 8—A myofiber with a central nucleus and a normal complement of myofibrils. (×5000; bar = 0.8 μ)

mic reticulum (Figure 10B) and Golgi complexes (Figure 10A) are particularly prominent in these biopsy specimens. Such structures were inconspicuous in the control samples. Although microfilaments were searched for, they were not found. However, the presence of intact myofibrils lacking the "gaps" and interruptions seen in earlier samples suggest that synthesis of myofilaments has occurred. Abnormalities of mitochondrial size and shape persisted.

Evidence of prior cell injury and loss is found within

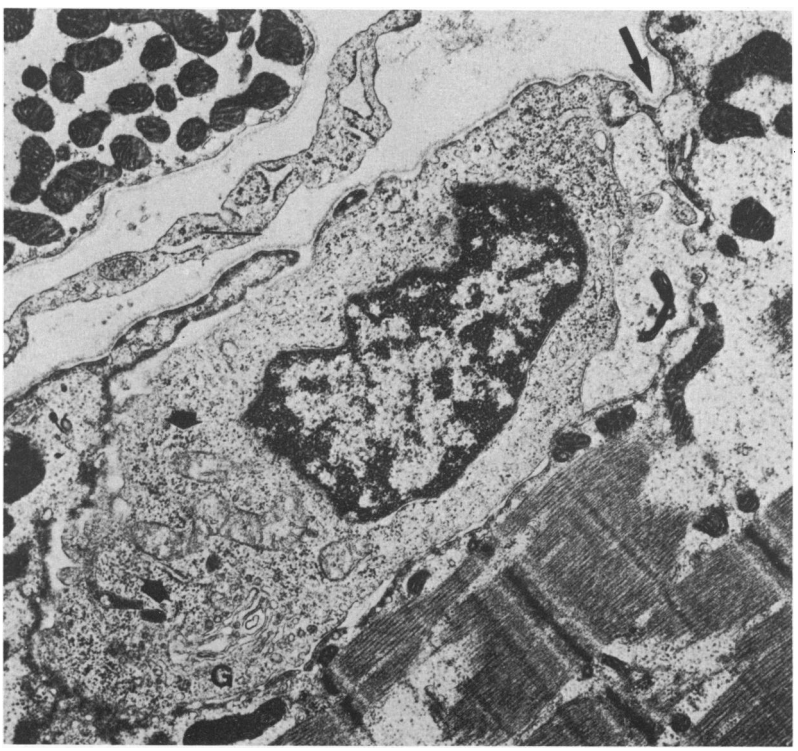

Figure 9—A satellite cell. The continuity with the basal lamina of the muscle fiber is evident (*arrow*). The satellite cell has a prominent Golgi region (*G*) and a rough endoplasmic reticulum (*arrows*). (×10,000; bar = 0.8 μ)

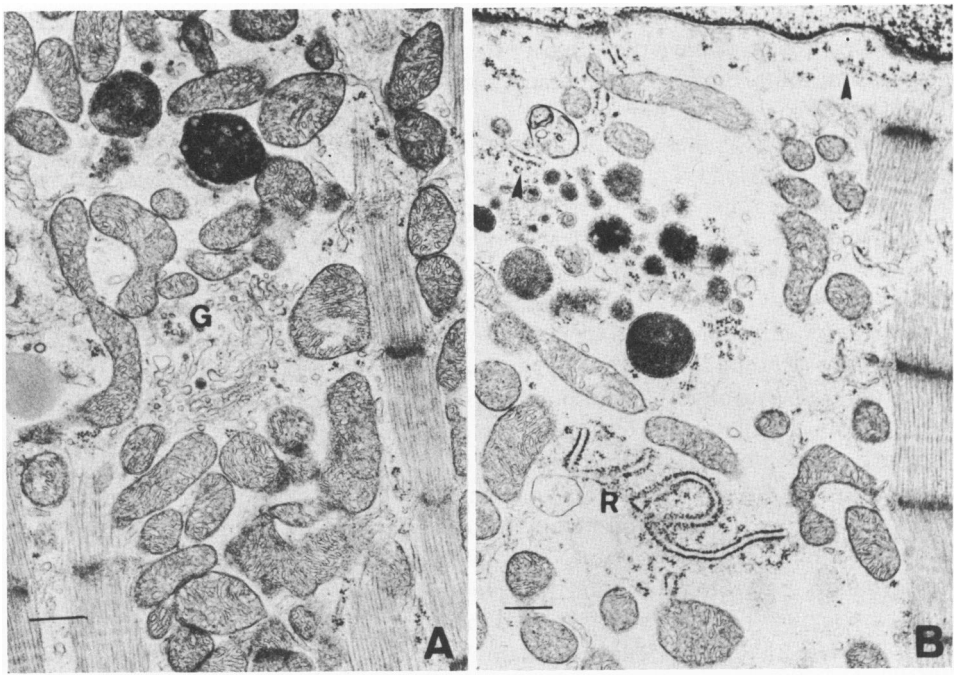

Figure 10—Muscle biopsy specimens 10 weeks after competition. **A**—A fiber with a prominent Golgi region (*G*). (×10,000; bar = 0.6 μ) **B**—A fiber with rough endoplasmic reticulum (*R*) and polysomes (*arrowheads*). (×10,000; bar = 0.6 μ)

the interstitium (Figure 11). There are variable amounts of interstitial collagen deposition. Capillaries also display the stigmata of previous injury. Lipofuchsin is particularly prominent in the endothelial cells of veteran competitors, indicating previous endothelial damage. The capillary basal lamina is thickened and laminated, suggesting repeated capillary injury. Collagen deposition is particularly prominent perivascularly.

None of the biopsy specimens revealed evidence of cellular inflammation. The dominant process observed was the development and resolution of myofibrillar lysis.

Discussion

Gastrocnemius muscle biopsies from marathon runners after competition reveal sequential ultrastructural changes of cellular injury and repair. The consistent finding in these biopsies was injury to sarcoplasmic units. The severity of these changes varied from individual to individual, but all runners exhibited some degree of myofibrillar lysis. The observed differences in severity are not surprising, since the runners varied in age, conditioning, and running speed. The observed damage was focal and confined to individual sarcomeric units. In the most severe case, less than 10% of the sarcomeric units appeared damaged. These myofibrillar alterations occurred in fibers depleted of glycogen and lipid. Our findings are suggestive of cell injury, but not frank necrosis. The absence of inflammatory cells, in particular, indicates that acute necrosis has not occurred.

The injury described involves profound focal damage in individual fibers as indicated by the empty myotubes and interstitial collagen in biopsies obtained days to weeks after the acute event, suggesting fiber degener-

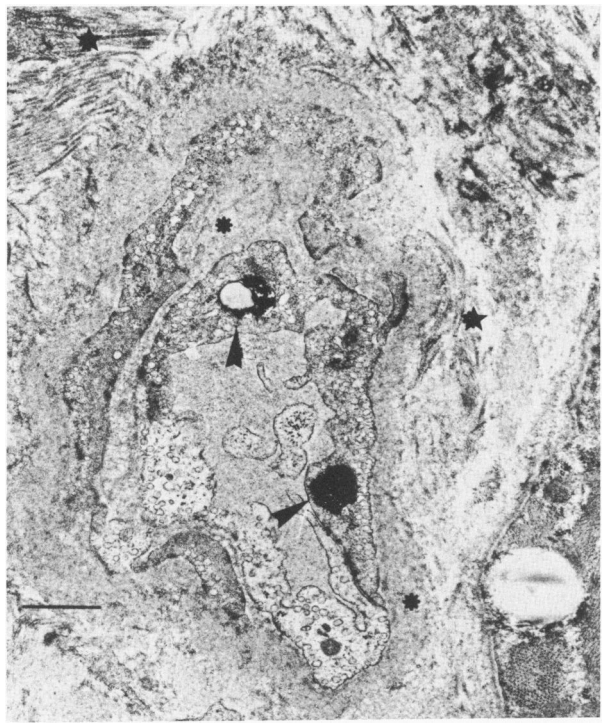

Figure 11—A biopsy 10 weeks after competition. The endothelial cell contains lipofuscin granules (*arrowheads*) suggestive of previous injury. The capillary basal lamina (*) is thickened and multilayered, also suggesting previous capillary damage. There is abundant perivascular collagen (*stars*). (×8000; bar = 0.8 μ)

ation and dropout. We should emphasize, however, that most fibers appear to have the capacity to regenerate sarcoplasmic units. Biopsies from veteran runners have abundant glycogen and morphologically abnormal mitochondria. These skeletal muscle biopsies resemble changes described in endomyocardial biopsies under conditions of hypertrophic stress.[9] Hypertrophic changes in cardiac muscle include degenerative findings without inflammation similar to the skeletal muscle pattern seen in these runners. Both instances may represent a type of fiber injury and degeneration without frank necrosis leading to fiber dropout, collagen deposition, and hypertrophy of remaining fibers.

Our findings differ from those of other observers, who have noted interstitial hemorrhage and an inflammatory cell infiltrate in similar biopsy specimens.[10] We noted areas of focal interstitial hemorrhage in many specimens, including most controls. We think that this hemorrhage is related to the trauma of the biopsy technique, a common artifact in biopsy specimens of all types. We were unable to document inflammatory cell infiltration even after reexamination of many thick sections specifically for this finding.

The focal nature of the muscle fiber damage may provide an anatomic basis for understanding enzyme release or leakage from muscle cells during injury. The segmental involvement of the sarcoplasmic reticulum and the transverse tubular system may produce transient membrane permeability. We are suggesting that enzyme efflux may take place from a reversibly injured portion of the cell and may not imply irreversible cell injury or cell death. This focal damage may result specifically from the metabolic stress of a continued demand for work output in the face of intracellular substrate depletion, similar to the pathophysiology of injury in muscle phosphorylase deficiency. The findings of glycogen depletion and repletion immediately after the race and during recovery correlate with restoration of mitochondrial architecture and repair of sarcomeric damage. The reversibility of the injury with restoration of sarcomeric integrity and glycogen repletion is shown in Figure 8.

Biopsy specimens taken 10–12 weeks after competition show central nuclei, an increased content of endoplasmic reticulum, and prominent Golgi areas. These are all morphologic correlates of the regenerative process.[11] Satellite cells are contained within the bilayer of the sarcoplasmic reticulum, as noted in Figure 9, and may be recruited in the process of cellular repair. The morphologic observations herein may provide an anatomic explanation for the increase in CK-MB content of endurance-trained skeletal muscle. One hypothesis is that CK-MB, as reported for lactic dehydrogenase isoenzymes, may be selectively concentrated in high oxidative or slow twitch fibers.[12]

An alternate explanation is that CK-MB is a marker for the regenerative tissue response to injury, which explains the increase in skeletal muscle CK-MB in selected disease states as well as in runners.[5,13,14] The balanced process of injury and repair involved in intensive endurance training induces a higher tissue level of CK-MB as an index of this ongoing regenerative process. Peaks in serum CK-MB in asymptomatic runners after competition represent acute isoenzyme release or exertional rhabdomyolysis from skeletal muscle altered during training. Biochemical alterations in skeletal muscle and serum of runners are similar to changes in chronic myopathies and dystrophies, in which CK-MB also increases as a marker of attempted tissue repair.[14] Veteran runners have foci of interstitial fibrosis but have preservation of basic architecture, in contrast to the progressive fibrosis and atrophy seen in genetically compromised or dystrophic tissue.

These findings leave several questions unanswered. We are unable to identify in this study the specific fiber types susceptible to injury as classified by myosin ATPase stains. Immunohistochemical techniques at the ultrastructural level would be required to resolve this question. In addition, studies in skeletal muscle after endurance training of other types would be of interest, because patterns of injury and repair may be most prominent after marathon running because of metabolic substrate depletion. Studies of veteran athletes would serve to elucidate concerns about long-term tissue changes. Finally, skeletal muscle in marathon runners may provide a convenient model for further studies in skeletal muscle injury and repair.

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