NASA Technical Memorandum 103904

Muscle Ultrastructural Changes from Exhaustive Exercise Performed After Prolonged Restricted Activity and Retraining in Dogs

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ABSTRACT

The effect of exhaustive treadmill exercise on ultrastructural changes in the quadriceps femoris muscle was studied in 7 normal, healthy mongrel dogs (2-4 yr, 15-20 kg) before and after restricted activity (RA) (2 mo for five dogs, 5 mo for two dogs), and following a subsequent two-month treadmill exercise retraining period for the 5-mo group. Mean (±SD) time to exhaustion in the 2-mo group decreased from 177 ± 22 min before to 90 ± 32 min ($\Delta = 49\%$, $P < 0.05$) after RA. Retraining increased tolerance to 219 ± 73 min; 24% ($P < 0.05$) above the before-RA and 143% ($P < 0.05$) above the after-RA time. After-RA exhaustion time in the 5-mo group was 25 and 45 min. Before RA (2-mo group), pre-exercise muscle structure was normal and post-exercise there was only slight swelling of mitochondria. After RA (2-mo group) pre-exercise, numerous glycogen granules and lipid droplets appeared in the muscle fibers, mitochondria were smaller, and sarcoplasmic reticulum channels widened; post-exercise these changes were accentuated and some areas were devoid of glycogen, and there was fiber degeneration. After 5 mo RA pre-exercise there were more pronounced changes: mitochondria were very small and dense, there were many lipid droplets, myofibrils were often separated, and the fibers appeared edematous and degenerating; post-exercise the sarcoplasmic reticulum was swollen, no glycogen was present, and there was marked swelling and deformation of mitochondria. After retraining (2-mo group) both pre- and post-exercise there was still evidence of fiber degeneration. Thus, susceptibility of active skeletal muscle structures and subcellular elements, e.g., mitochondria, to the action of damaging factors occurring during exhaustive exercise is enhanced considerably by prolonged disuse. Retraining these muscles significantly increases their resistance to the damage caused by restricted activity and exhaustive exercise, but does not return them to their normal, ambulatory condition.

INTRODUCTION

Both physical exercise training and restriction of physical activity by limb immobilization and body confinement cause changes in the ultrastructure of skeletal muscles. Endurance training increases the size and number of mitochondria in exercise-trained skeletal muscle fibers (refs. 10 and 12) that contribute to enhanced ATP-producing capacity, one of the primary factors responsible for improvement of prolonged exercise tolerance (ref. 9). On the other hand, limb immobilization causes atrophy and degenerative changes in skeletal muscle fibers (refs. 1-3, 5, 6, 13, 17, 20, 22, and 24). In addition, a single bout of exhaustive exercise can produce changes in skeletal muscle ultrastructure similar to those following immobilization; e.g., swelling and disruption of mitochondria as well as muscle fiber degeneration (refs. 4, 7, 8, 10, 14-16, 19, and 23). Mitochondrial integrity may depend on the functional innervation of sympathetic and motor nerves as well as on humoral factors; e.g., thyroid hormones can influence training-induced changes in mitochondria (ref. 26). Mitochondrial disruption may contribute to the development of fatigue in both atrophied muscles and in those following prolonged exercise.

The purpose of the present study was to investigate the ultrastructure of the quadriceps femoris muscle in dogs at rest and after exhaustive exercise before and after restricted acitivity (RA), i.e., cage confinement, and after subsequent retraining. Contrary to some previous experimental designs,

where limbs of animals were immobilized but total body mobility was permitted, we have used total body confinement which permits some body movement but essentially no limb immobilization.

The authors thank Bridget Eller and Debra Fegan-Meyer for data analysis and manuscript preparation.

METHODS

This study was approved by the review committee of the Polish Academy of Sciences Department of Applied Physiology. The study was conducted on seven male, mongrel dogs (2 to 4 yr, 15 to 20 kg). They were housed in an indoor kennel and fed a nutritionally adequate diet that included cereals, beef broth with vegetables, and meat scraps.

Five dogs were confined in cages for two months (2-mo group), and two other dogs were confined for five months (5-mo group). The five 2-mo group dogs were retrained with daily, 60-min exercise sessions for two months after their period of confinement. The cages measured 40 cm wide, 80 cm high, and 110 cm in length which limited movement to standing, lying down, and slight forward and backward movement; they could not turn around. The animals were familiarized with treadmill running and all other experimental procedures, were very cooperative, and were eager to run on the treadmill.

The endurance exercise test was administered before confinement (both groups), the day after confinement (both groups), and the day following 60 days of non-confined retraining (2-mo group). Before each endurance test the dogs had been deprived of food for 18-24 hr but they had free access to water. The exercise test involved running to volitional fatigue on a motor-driven treadmill up a 12° slope at a speed of 1.4 to 1.8 m/sec. The exercise loads ranged from 2.85 to 3.26 W/kg body wt.

After RA the dogs were returned to their kennels. The daily 60-min retraining sessions were conducted 6 days/wk and involved running on the treadmill (12° slope) at 1.4-1.6 m/sec during the first month, and increased by 0.2 m/sec and then by 0.4 m/sec in the final weeks.

Immediately before and after each of the three endurance tests, samples of the quadriceps femoris muscle were obtained from the unrestrained dogs by incision under local anesthesia with lignocain (Polfa, Poland). The muscle samples were immediately fixed in 4% gluteraldehyde in phosphate buffer and postfixed in 1% OsO_4 in phosphate buffer. The tissue samples were dehydrated using increasing concentrations of ethyl alcohol and propylene oxide, and then embedded in Epon 812. Thin sections (600 Å), cut using glass knives on an LKB ultratome, were mounted on copper grids, stained in alcoholic uranyl acetate followed by lead citrate, and photographed with an electron microscope (JEM model 7A, Japan). The micrographs were observed and visual comparisons were made, but no morphometric analyses were performed.

The exercise endurance results were analyzed with Student's t-test for paired samples and the null hypothesis was rejected when $P < 0.05$.

RESULTS

Exercise Tolerance

Mean (±SD) time to exhaustion in the 2-mo group decreased from 177 ± 22 min before to 90 ± 32 min ($\Delta = 49\%$, $P < 0.05$) after RA. After two months of retraining tolerance increased to 219 ± 73 min: i.e., 24% ($P < 0.05$) above the pre-RA time and 143% ($P < 0.05$) above the post-RA time. The times to exhaustion in the two dogs in the 5-mo group after RA were 25 and 45 min.

Ultrastructure

Before 2-mo confinement: pre exercise– Morphology of the quadriceps muscle was normal. In the longitudinal section (fig. 1), the sarcomeres were separated by the Z-bands and the area between sarcomeres contained numerous glycogen granules and narrow horizontal and longitudinal channels of the triad system of the sarcoplasmic reticulum. In the transverse section (fig. 2), round mitochondria were evident having a delicate granular matrix with numerous regularly distributed cristae. The double walled nuclear membrane had narrow channels between its walls, and the karyoplasm had a uniform content of heterochromatin and euchromatin.

Before 2-mo confinement: post exercise– There was slight swelling of the mitochondria (fig. 3).

After 2-mo confinement: pre exercise– At the level of the I-band there were numerous glycogen granules and lipid droplets (fig. 4). The cristae configurations in the smaller round mitochondria were changed (fig. 5), and there were myelin-like bodies between myofibrils (fig. 6). The sarcoplasmic reticulum channels were widened in a majority of the fibers (figs. 4-6).

After 2-mo confinement: post exercise– In comparison with the micrographs taken before exercise, there was a slight swelling of the mitochondria and a more pronounced widening of the sarcoplasmic reticulum channels (fig. 7). Also, a lipofuchsin granule is present indicating cell degeneration. The nuclear intramembrane space was widened and heterochromatin material was translocated toward the nuclear membrane (fig. 7). Glycogen granules were prevalent in the fiber on the left, whereas the adjacent fiber was nearly void of glycogen (fig. 8). This fiber is clearly in a state of degeneration and, with the presence of a lipofuchsin granule, suggests a pathological condition.

After 5-mo confinement: pre exercise. The muscle ultrastructural changes were more pronounced when compared with the morphology pre-exercise after the two-month confinement. The mitochondria were smaller, round, and contained a very thick (electron dense) matrix; occasionally there were crystal-like inclusions within the mitochondria, and lipid droplets were evident (fig. 9). In the longitudinal section (fig. 10) the fibers appeared to be edematous and degenerating.

After 5-mo confinement: post exercise– The sarcoplasmic reticulum was swollen and there were essentially no glycogen granules present. There was marked swelling of many mitochondria; they had very irregular shapes, the cristae were short (often only fragments remained), and the matrix was pale (fig. 11).

After retraining 2-mo confinement: pre exercise– Between myofibrils occasionally there were light lipid droplets and a few small myloid bodies. There were increased subsarcolemma mitochondria, loss of myofibrils, and increased variability in myofibrillar diameters; these are responses of degenerating fibers (fig. 12).

After retraining 2-mo confinement: post exercise– The Golgi apparatus below the nucleus had widened channels (fig. 13), and there was an accumulation of myeloid bodies between fibers and around blood vessels (fig. 14) and under the sarcolemma (fig. 15).

DISCUSSION

Results from a prior study (ref. 21) and the present study demonstrated that two-month restricted activity of dogs dramatically reduces treadmill running endurance, which was accompanied by early development of excess hyperthermia during exercise (ref. 18).

In the present study after two months of restricted activity there were abnormalities of muscle ultrastructure. In the pre-exercise muscle, myelin-like bodies were found between myofibrils that perhaps were indicative of the onset of degeneration, and the mitochondria were somewhat smaller than those observed before confinement. This diminished size corresponds with a reduction in mitochondrial enzyme activities in humans after a few weeks of detraining (ref. 11). But a decrease in size and number of mitochondria may decrease their capacity just as disruption of their normal size. In the post-exercise muscle there was enlargement of the sarcoplasmic reticulum and of the nuclear intermembraneous space, there was no evidence of mitochondrial disruption disruption, but there were clear indications of muscle degeneration.

Compared with muscle ultrastructural changes observed in dogs confined for two months, far more extensive changes were found in the two dogs after five months of restricted activity. In pre-exercise muscle there were numerous degenerative changes within myofibrils, and post-exercise mitochondrial damage was clearly evident even though duration of exercise was much shorter (25 and 45 min) in this group than after confinement in the 2-mo group (90 ± 32 min). This significant mitochondrial disruption was found only post-exercise in the 5-mo group in spite of the fact that the muscle samples were taken from all seven dogs at a similar state of exhaustion at widely different endurance times. Thus the three additional months of confinement caused further deconditioning responses.

Similar mitochondrial changes following various types of exhaustive exercise have been described previously in skeletal muscles of rats (refs. 4, 7, 8, and 14) and in horses (ref. 19). The extent of mitochondrial changes after exercise in our 5-mo dogs suggests that the capacity of these

skeletal muscles to oxidize energy fuels may be severely impaired. If so, it could be a performance-limiting factor (ref. 9) as reflected in the shortest times to exhaustion. It has been suggested that the mitochondrial disruption visible after the sample fixation procedure does not accurately represent in-vivo conditions (refs. 8, 19, 25), but freeze fracture was not used to confirm this theory. Nevertheless, the changes in mitochondrial structure should be valid because the fixation procedure would have been the same throughout the study and the findings appear to be consistent.

Thus, it appears that the factors causing structural and mitochondrial disruption after restricted activity are not related as much to the state of muscular fatigue as to the pre-exercise "quality" of the muscle. That is, the susceptibility of actively trained skeletal muscle to exercise-induced disruption is increased markedly with increased restriction of activity, and retraining increases the resistance of muscle mitochondria and restores some of the damage produced by exhaustive exercise.

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Figure 1. Electron micrograph of normal quadriceps femoris muscle (longitudinal section) taken at rest before confinement (10,300X).

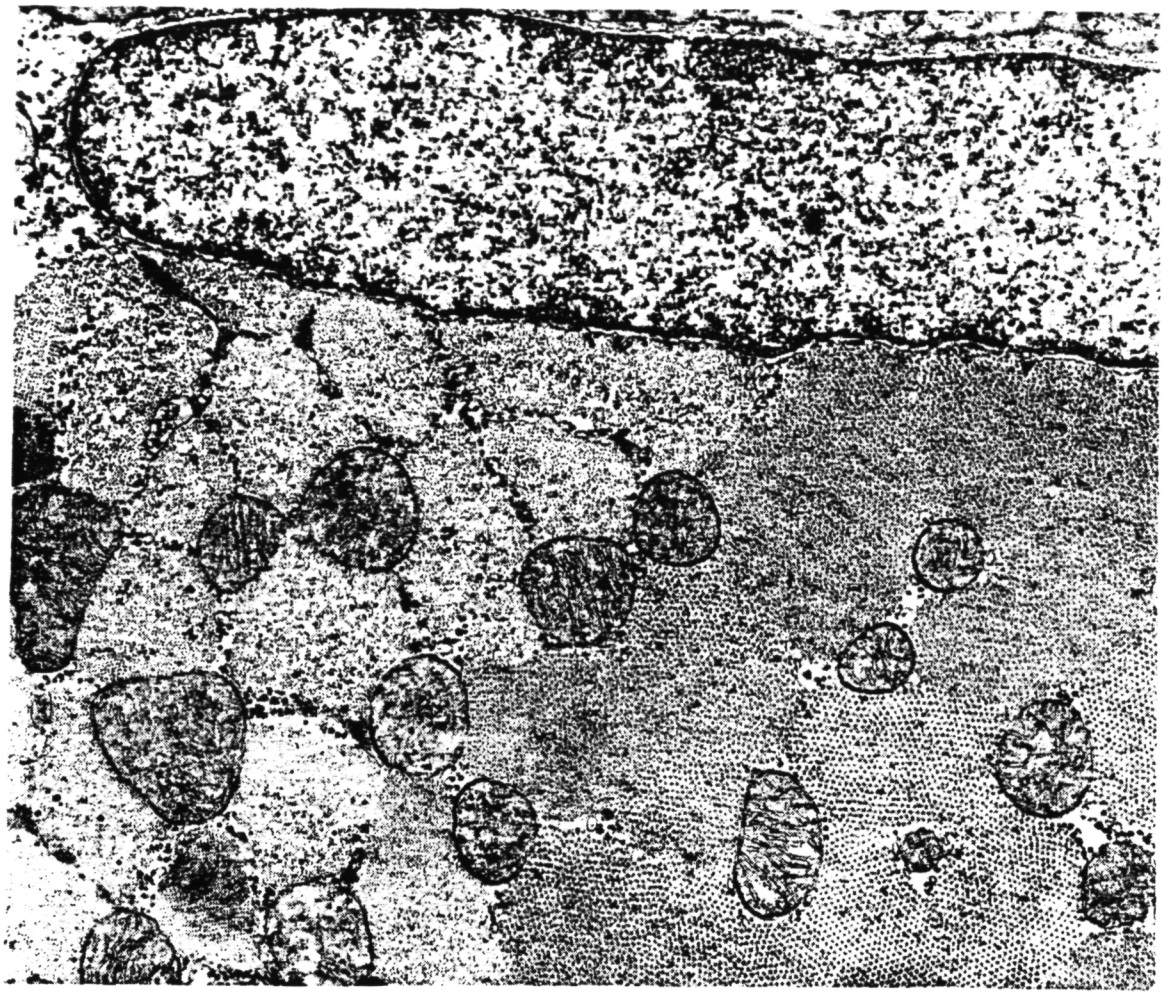

Figure 2. Electron micrograph of normal quadriceps femoris muscle (transverse section) taken at rest before confinement (10,300X).

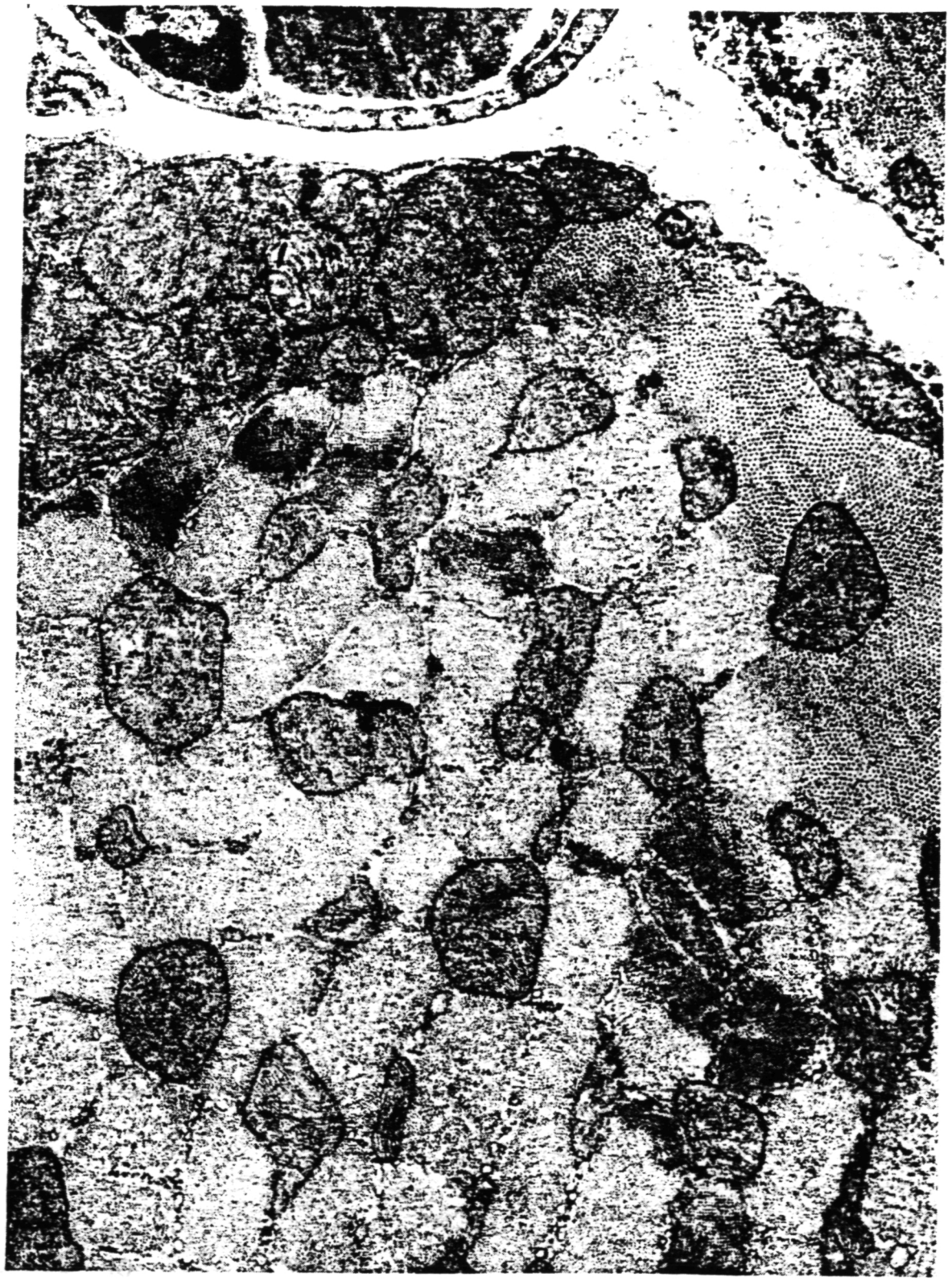

Figure 3. Electron micrograph of quadriceps femoris muscle (transverse section) taken after exhaustive exercise before confinement (10,300X).

Figure 4. Electron micrograph of quadriceps femoris muscle (longitudinal section) taken at rest after 2-month confinement (20,000X).

Figure 5. Electron micrograph of quadriceps femoris muscle (longitudinal section) taken at rest after 2-month confinement (20,000X).

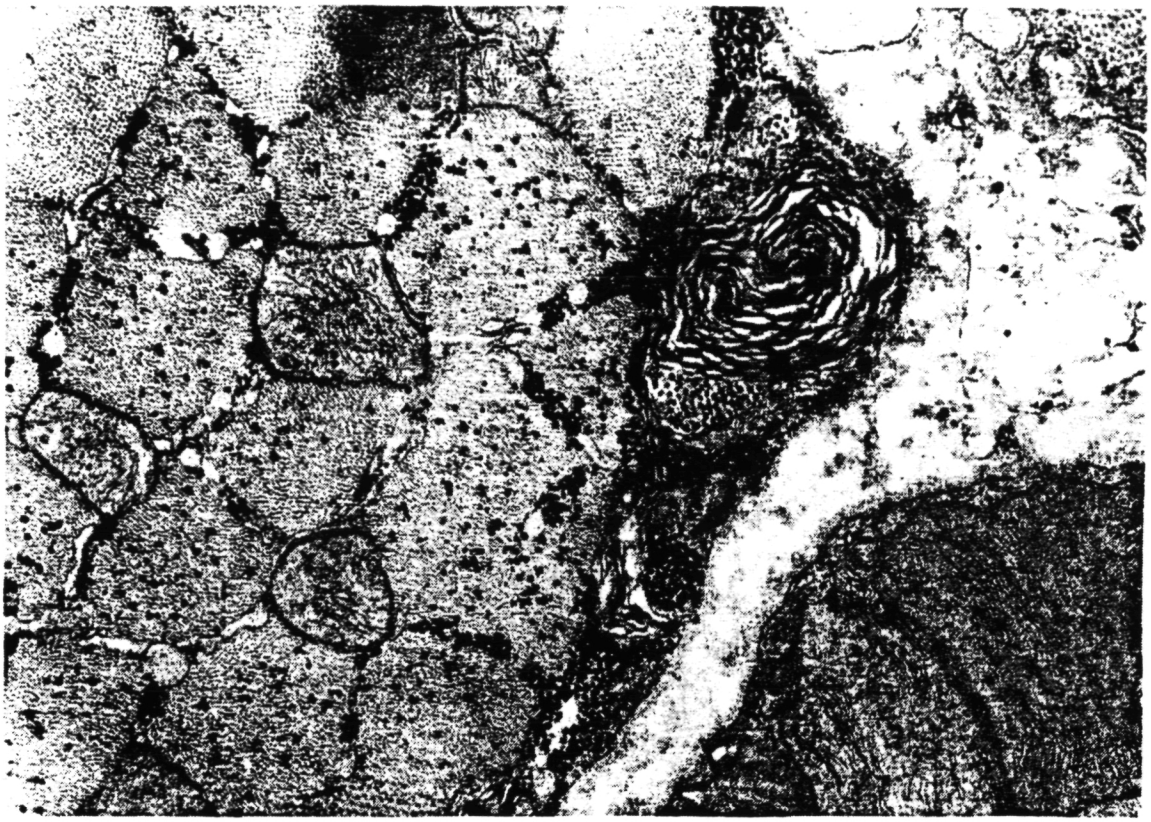

Figure 6. Electron micrograph of quadriceps femoris muscle (transverse section) taken at rest after 2-month confinement (20,000X).

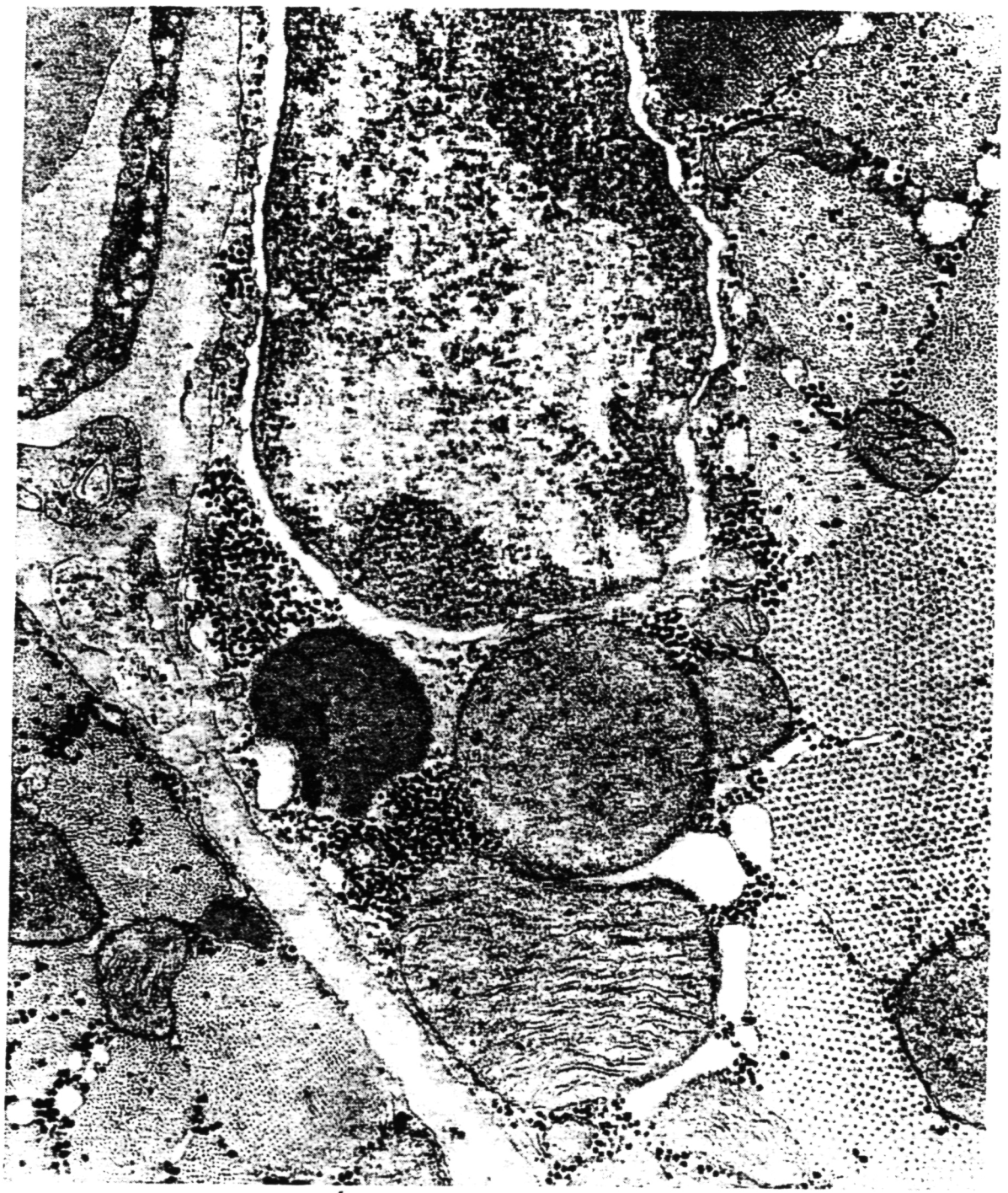

Figure 7. Electron micrograph of quadriceps femoris muscle (transverse section) taken after exercise after 2-month confinement (40,000X).

Figure 8. Electron micrograph of quadriceps femoris muscle (longitudinal section) taken after exercise after 2-month confinement (10,300X).

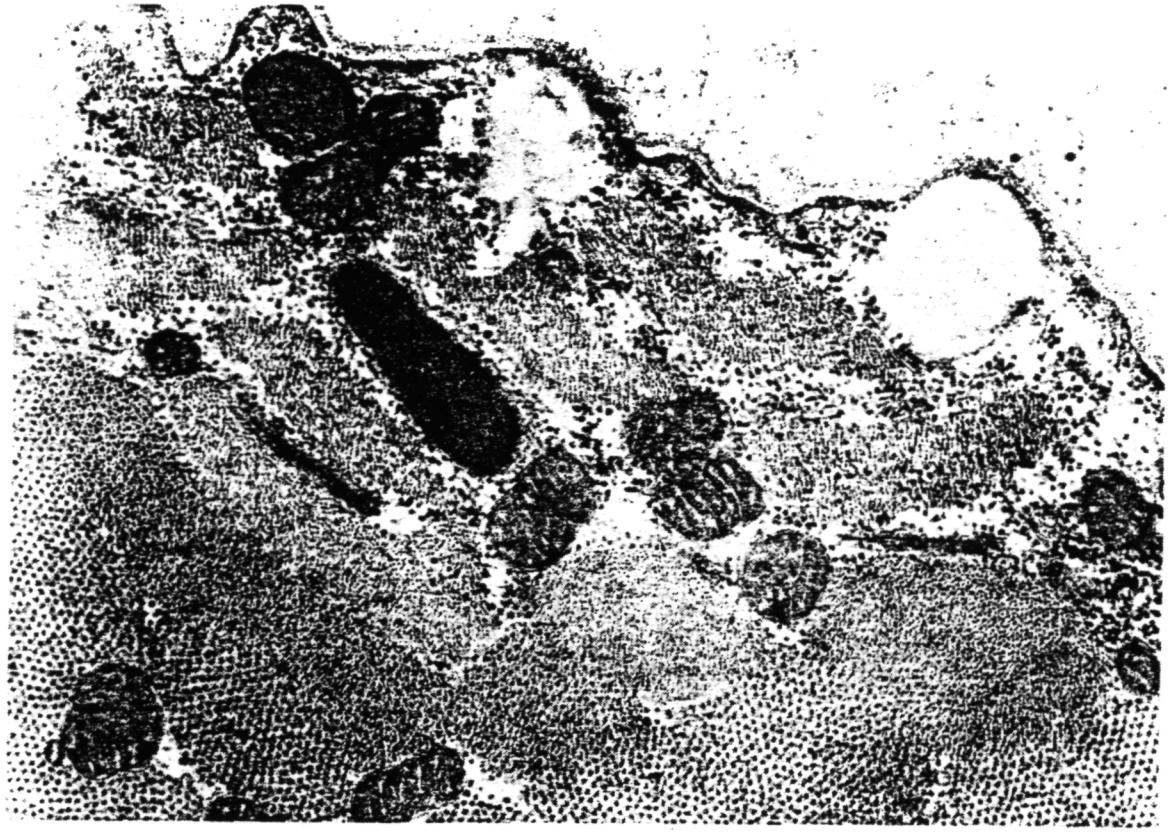

Figure 9. Electron micrograph of quadriceps femoris muscle (transverse section) taken at rest after 5-month confinement (30,000X).

Figure 10. Electron micrograph of quadriceps femoris muscle (longitudinal section) taken at rest after 5-month confinement (30,000X).

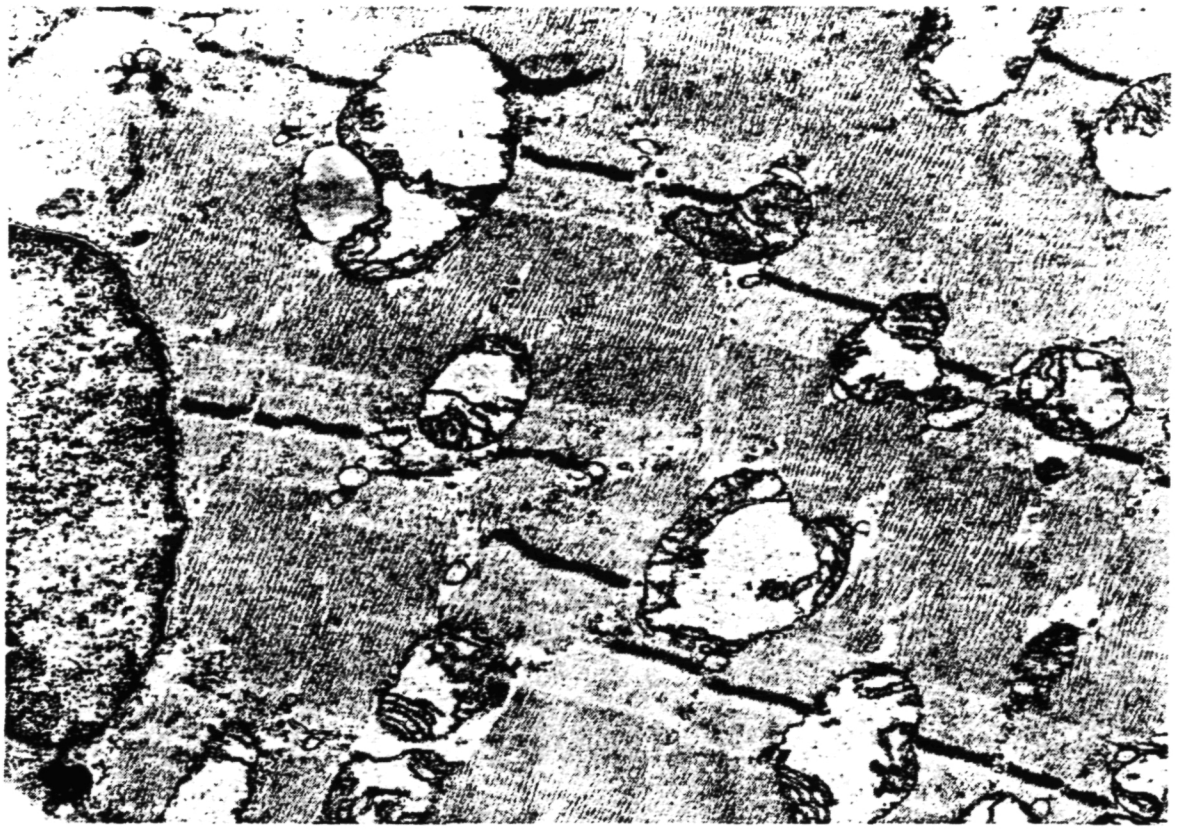

Figure 11. Electron micrograph of quadriceps femoris muscle (transverse section) taken after exercise after 5-month confinement (10,300X).

Figure 12. Electron micrograph of quadriceps femoris muscle (transverse section) taken at rest after 2 months retraining (10,300X).

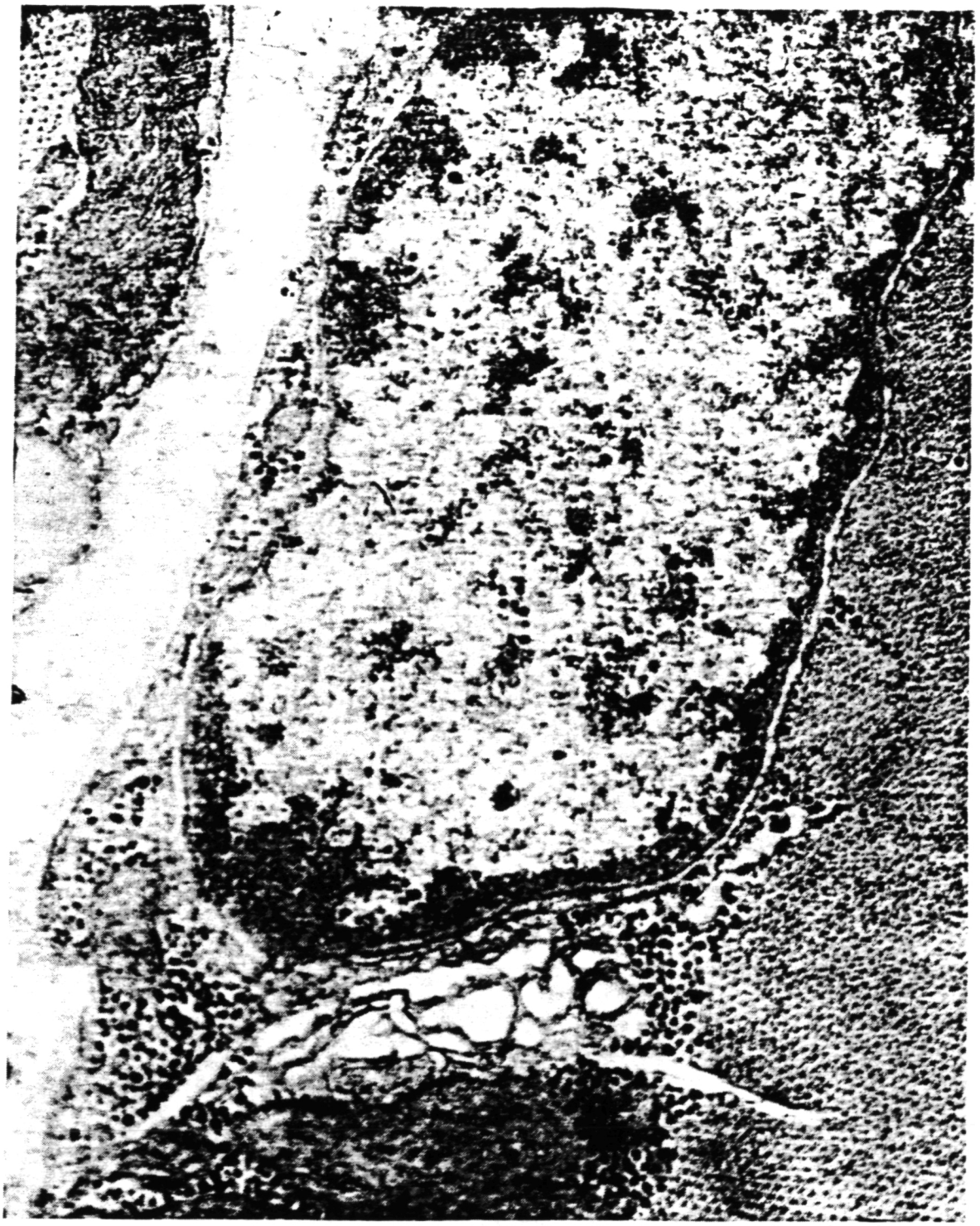

Figure 13. Electron micrograph of quadriceps femoris muscle (transverse section) taken after exercise after 2 months retraining (20,000X).

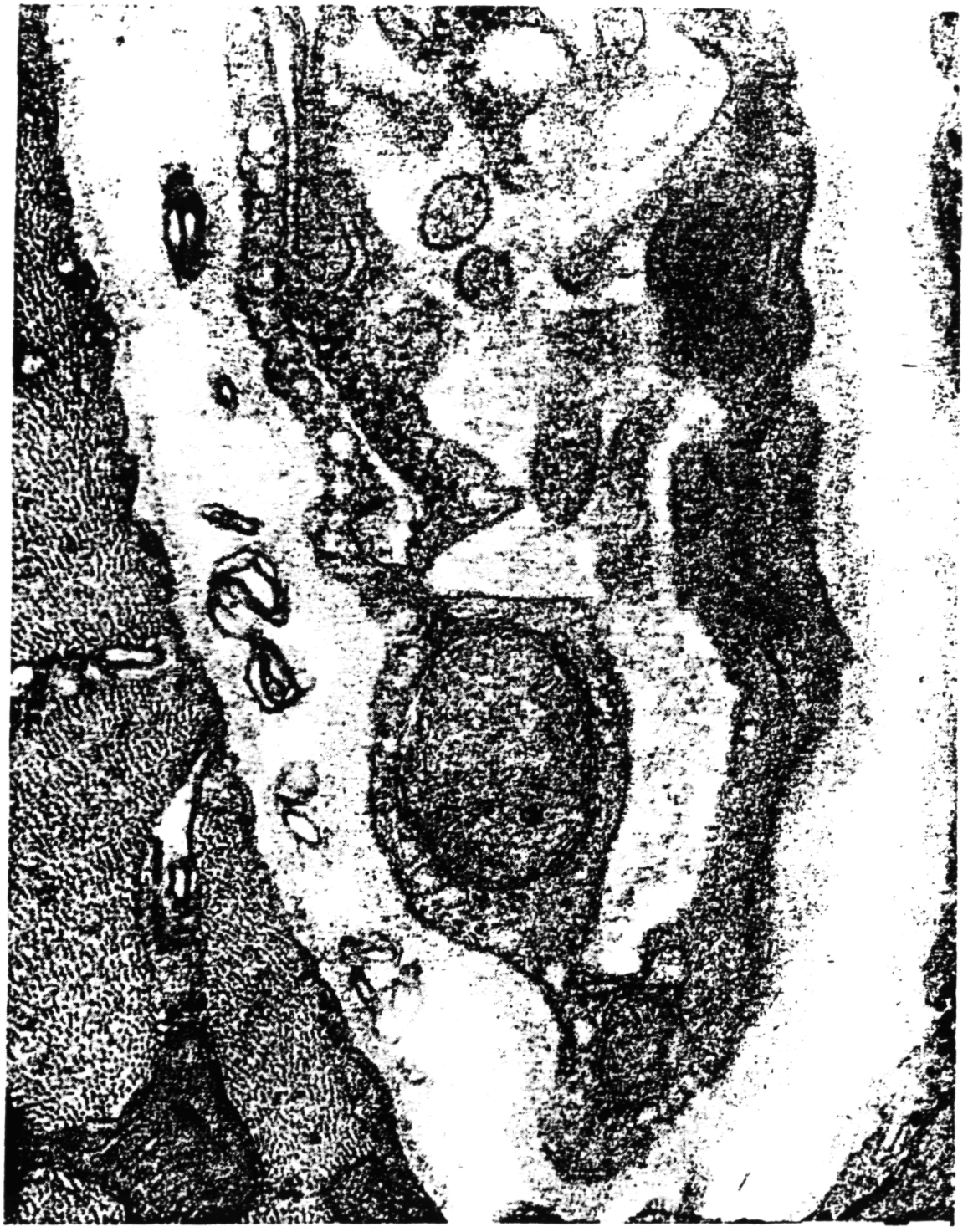

Figure 14. Electron micrograph of quadriceps femoris muscle (transverse section) taken after exercise after 2 months retraining (20,000X).

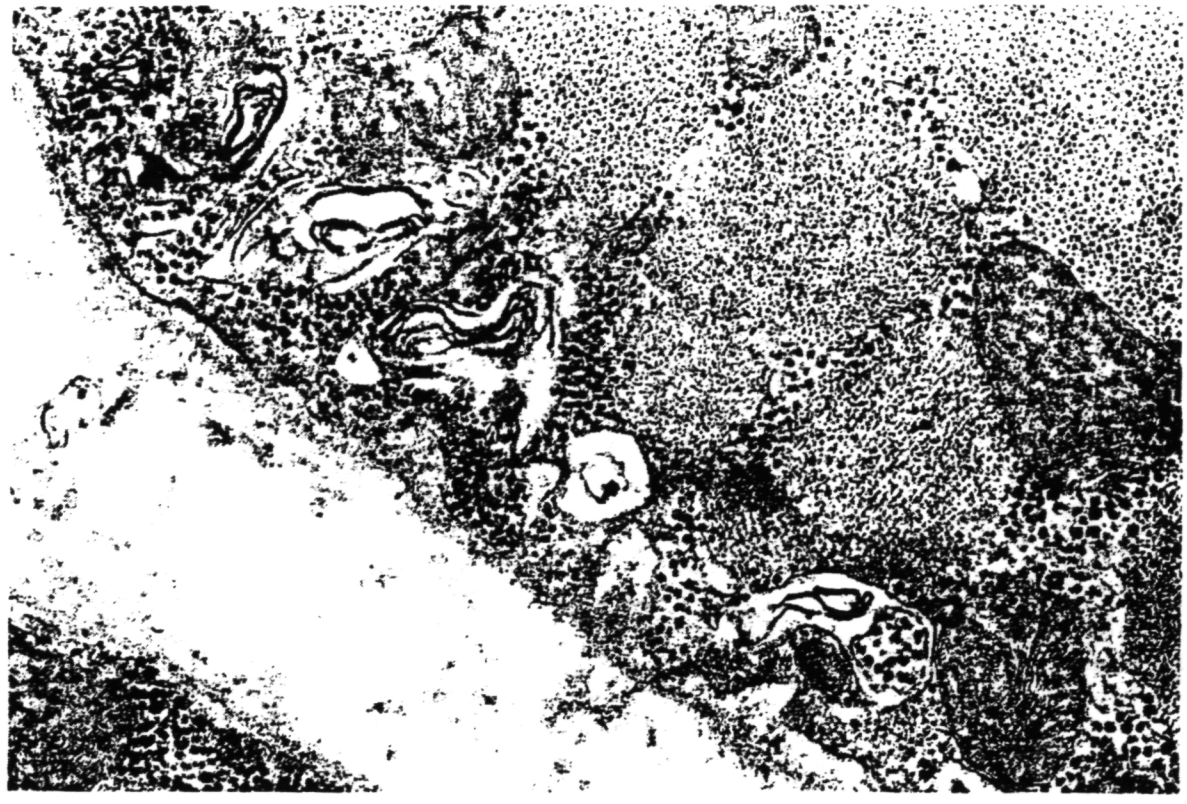

Figure 15. Electron micrograph of quadriceps femoris muscle (transverse section) taken after exercise after 2 months retraining (30,000X).

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1991	Technical Memorandum

4. TITLE AND SUBTITLE

Muscle Ultrastructural Changes from Exhaustive Exercise Performed After Prolonged Restricted Activity and Retraining in Dogs

5. FUNDING NUMBERS

199-18-12-07

6. AUTHOR(S)

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8. PERFORMING ORGANIZATION REPORT NUMBER

A-92018

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

National Aeronautics and Space Administration
Washington, DC 20546-0001

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

NASA TM-103904

11. SUPPLEMENTARY NOTES

Point of Contact: John Greenleaf, Ames Research Center, MS 239-11, Moffett Field, CA 94035-1000; (415) 604-6604 or FTS 464-6604

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Unclassified — Unlimited
Subject Category 52

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 words)***

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14. SUBJECT TERMS

Mitochondria, Skeletal muscle, Electron microscopy, Treadmill exercise

15. NUMBER OF PAGES

23

16. PRICE CODE

A02

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified		