Full Length Research Paper

Ultrastructure of extensor digitorum longus (EDL) muscle fibers of alloxan-diabetic rats

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The diabetic effects of alloxan (type I diabetes mellitus) were investigated in 40 Wistar albino rats (18 controls and 22 diabetics). Alloxan in sterile physiological saline was injected into the animals intravenously. The ultrastructure of type IIa, type IIb and type I fibers in extensor digitorum longus (EDL) muscle of normal and alloxan diabetic rats was studied. In diabetic animals, the mitochondria of type IIa and type I fibers showed a loss of cristae. There was also an increased number of lipid droplets. The shape of the nuclei was highly irregular in these fibers. The type IIb fibers showed some disorientation of the T-tubuler system. It is concluded that alloxan-diabetes has differential effect on the fine structure of the three fiber types ın rat skeletal muscle.

Key words: Diabetes mellitus, skeletal muscle, alloxan, ultrastructure.

INTRODUCTION

Mammalian skeletal muscle fibers have been classified in many different ways, such as: types I, IIa, and IIb fibers, based on histochemical studies (Stein and Padykula, 1962); white, red and intermediate fibers based on ultra-structural and cytohemical characteristics (Gauthier, 1969); and fast -twitch -glycolytic (FG), fast -twitch-oxidative –glycolytic (FOG), and slow-twitch-oxidative (SO) fibers respectively, based on their contractile pro-perties and metabolic profiles (Peter et al*.,* 1972).

Slow contracting, oxidative muscle cells are charac-terized by unique structural proteins. Slow isotype myosin heavy chains are involved in slow and sustained movements, whereas fast muscle cells are characterized by their fast isotype myosin heavy chains and by rapid response to stimulus (Francis-West et al., 2003).

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglisemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes include long-term da-mage, disfunction and failure of various organs (WHO, 1999). This disease may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss.

Many contractile, morphological and biochemical mea-sures of muscle function are changed in experimantel diabetes. Thus, there have been reports of contraction prolongation in slow muscles and reduced tetanic tension in fast muscles (Grossie, 1982; Paulus and Grossie, 1983; Cotter et al., 1989). Histochemical and ultrastruc-tural changes include a general differentiation of fast twitch fibers (Bestetti et al., 1981), and reduction in stain-ing for mitochondrial enzymes (Balogh et al., 1988; Cebesoy et al., 2000). At the biochemical level, there is depressed protein synthesis and enhanced proteolysis (Pain and Garlick, 1974), falls in amino acid and glucose transport (Manchester, 1970), reduced Na^+-K^+-ATPase activity and pump density (Moore et al., 1983; Kjeldsen et al., 1987); changes in sarcoplasmic reticulum Ca^{+2}-ATPase; (Ganguly et al., 1983; Eibschutz et al., 1984) and reductions in intracellular ATP concentration (Moore et al., 1983). Many of these contractile, morphological and biochemical changes are prevented or reserved by insulin.

The purpose of the present investigation was to study the ultrastructural alterations of skeletal muscle fibers in chronic alloxan-diabetic rats.

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MATERIALS AND METHODS

Forty Wistar albino rats were obtained from the Animal Laboratories
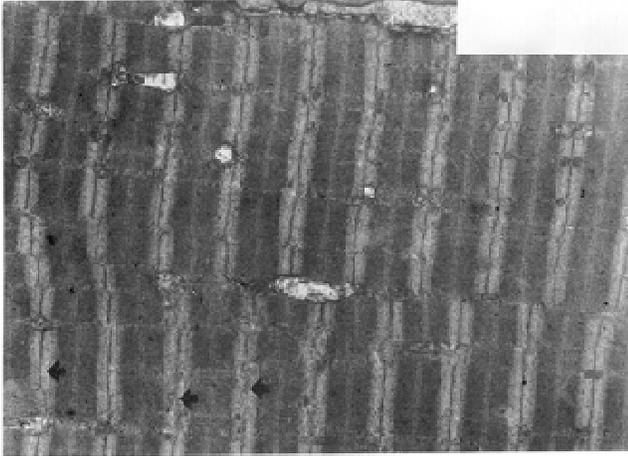
Figure 1. Longitudinal section of type I fiber from control animal (x 7200).
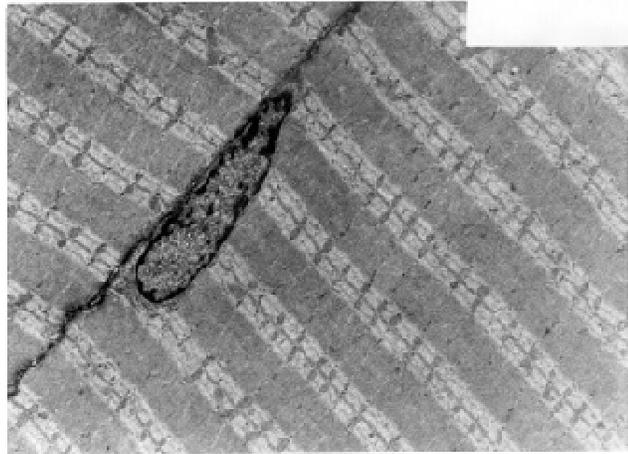
Figure 2. Longitudinal section of type II fiber from control animal (x 7200).
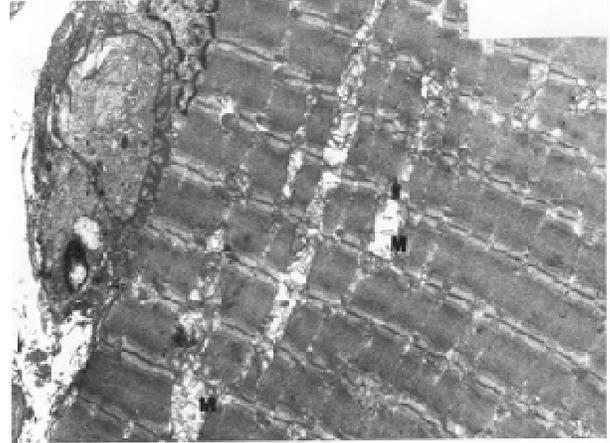
Figure 3. Loss of cristae in mitochondria (M) and irregular shape of the nuclei in type I fiber from diabetic animal (x 7200).
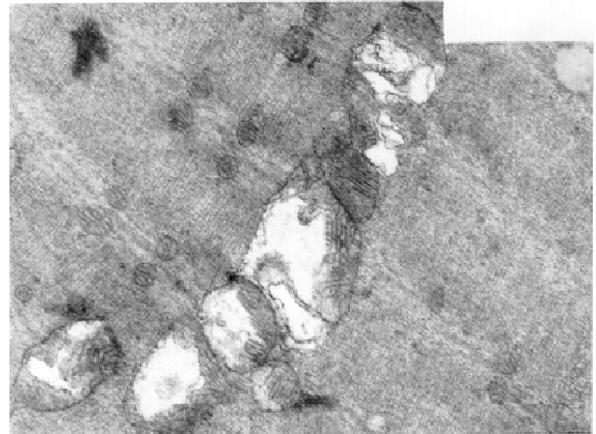
Figure 4. Loss of cristae in mitochondria and deformation of sarcoplasmic reticulum in type I fiber from diabetic animal (x 28500).

of Ankara University. Alloxan with 55 mg/kg dissolved in sterile physiooological saline was intravenously injected into 22 rats ranging in weight from 160-230 g (Reuterving et al., 1987).

Eighteen rats ranging in weight from 160-230 g were used as a control group. After 24 h administration of alloxan, glucose levels in urine were determined by Diastix test strips (Bayer, Germany). The alloxan-injected animals were kept under controlled conditions for 30 days. After 30 days, blood glucose concentrations were measured as > 400 mg dl^{-1}, determined with Glucostix strips (Bayer, Germany) from the cut tip of the tail of the alloxan-injected rats.

The animals were then decapitated under ether anaesthesia. Extensor Digitorum Longus (EDL) muscles were dissected free, cleaned of excess fascia, and blotted dry. Muscles were fixed in 2,5% phosphate-buffered glutaraldehyde, pH 7,2 and kept cold for 2 h. After rinsing several times in cold phosphate buffer, the tissues were postfixed in 1% osmium tetroxide solution for 2 h. Fixed tissues were dehydrated in a series of graded ethanol, placed into propylene oxide and embedded in araldite (Hayat, 1981). Ultrathin sections were stained with uranyl acetate and lead citrate and examined with transmission electron microscopy (TEM) (JOEL 100 CX 2) at 80 kv.

RESULTS

The different muscle fiber types were identified under the electron microscope on the basis of the frequency and location of their mitochondria, the development of the sarcotubular system, and the thickness of the Z lines.

In control animals, type I fiber has the highest mitochondrial content and the widest Z line (Figure 1), whereas type IIb fiber has the lowest mitochondrial content and the narrowest Z line (Figure 2). Type IIa fiber is intermediate in both characteristics. In type I fibers of the diabetic animals, the mitochondria contained fewer cristae and more granules than those in the control animals. A much greater number of lipid droplets of different sizes was observed. The shape of the nuclei was highly irregular in these fibers (Figures 3 and 4).

In the type IIa fibers from the diabetic animals, the mito-
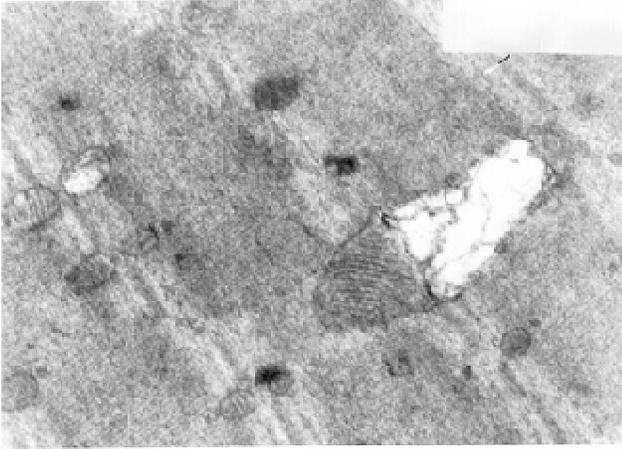
Figure 5. Melting of mitochondrial cristae in type IIa fiber of diabetic rat (x 28500).
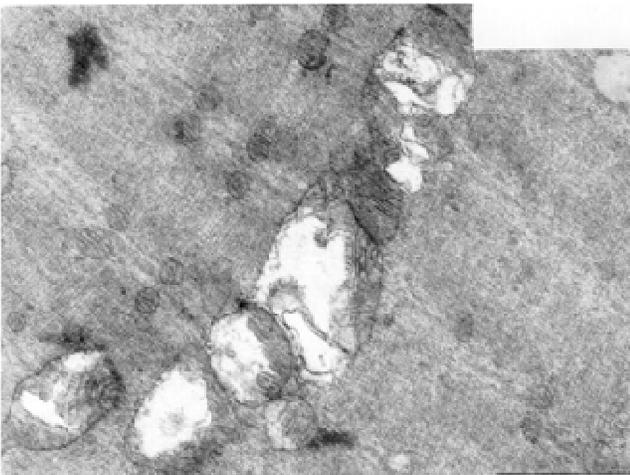
Figure 6. Loss of cristae in mitochondria in type IIa fiber of diabetic rat (x 21000).
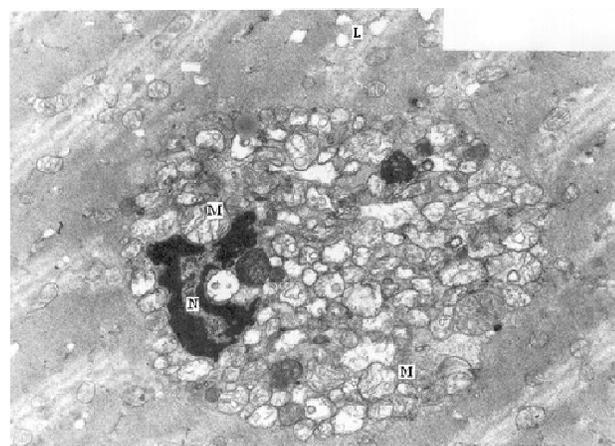
Figure 7. Nucleus (N) of type IIa fiber was surrounded by numerous mitochondria (M) and few lipid droblets (L). The nucleus had irregular shape (x 8700).
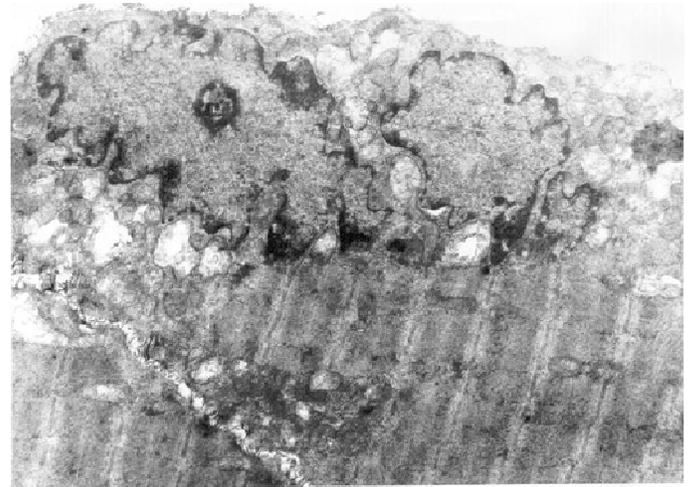
Figure 8. Irregular shape of the nuclei in type II a fiber and loss of chromatin material (x 8700).
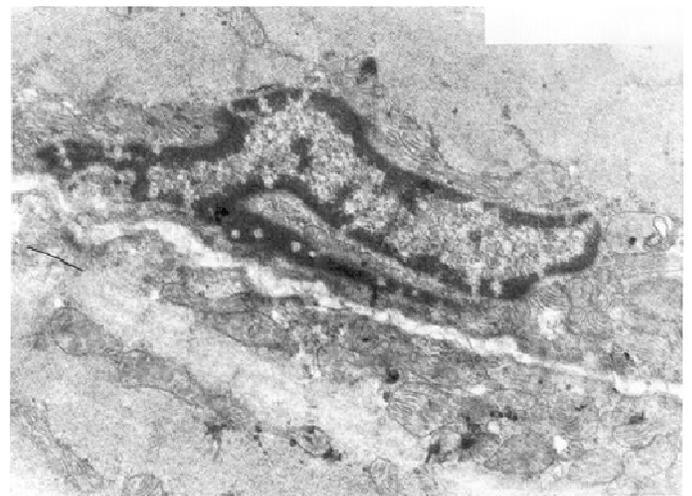
Figure 9. Irregular shape of the nuclei in type IIa fiber (x 15000).

chondrial cristae were less distinct and fewer in number than the controls (Figures 5 and 6). There were considerably more lipid droplets, usually in juxtaposition to the mitochondria or bulging out from underneath the sarcolemma (Figure 7). Irregular shape of the nuclei was seen in these fibers (Figures 8 and 9). The separation of a muscle nucleus with its adjacent sarcoplasm from a satellite cell was noticed in this fiber type of the diabetic rat (Figure 10).

The mitochondria in type IIb fibers of the diabetic rats seemed to be reduced in number; there were no apparent morphological changes. The extensive sarcotubular system was still well developed, but some disorientation of the T tubules and sarcomeres was observed (Figure 11). Relatively more lipid droplets were seen in these fibers than in the normal (Figure 12).
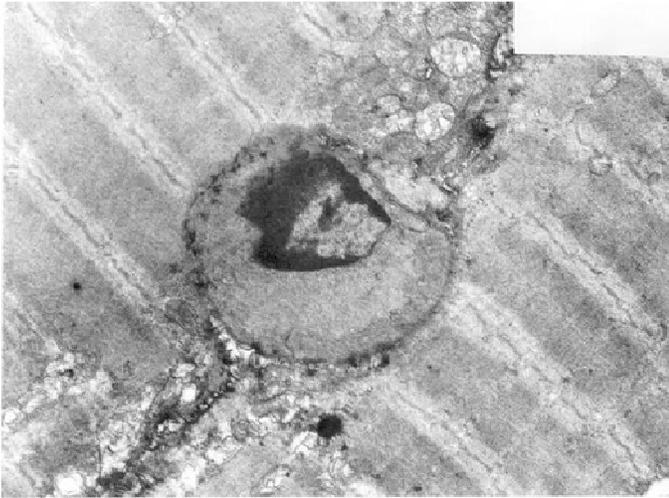
Figure 10. Formation of satellite cell in type IIa fiber of diabetic animal (x 10800).
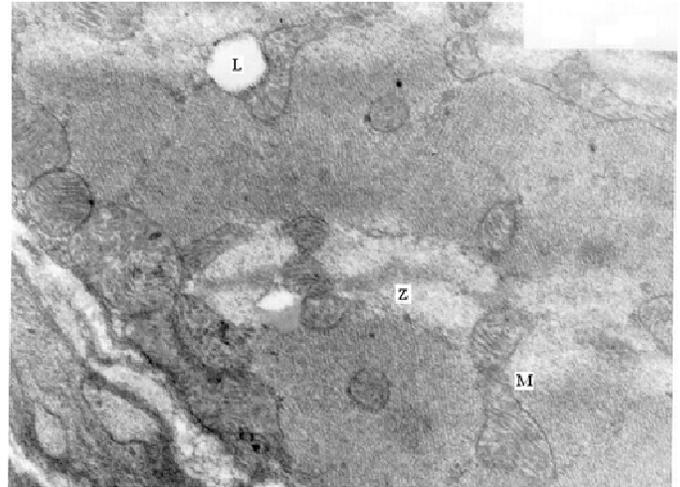
Figure 12. Disorientation of Z lines (Z) and the mitochondria seemed to be reduced in number (M), showing increased number of lipid droplets (L) (x 21000).
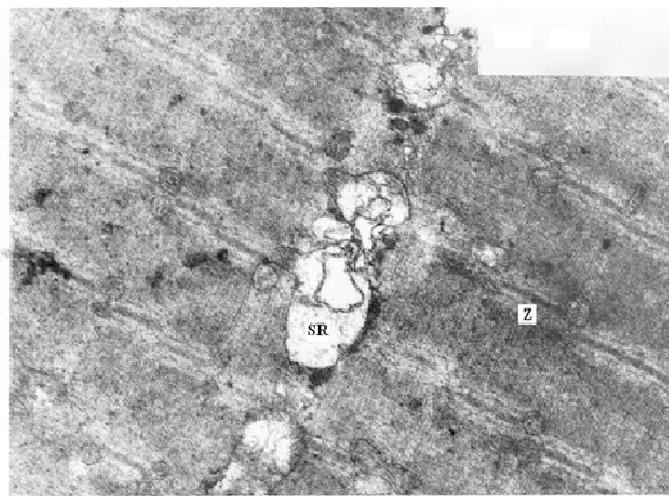
Figure 11. Deformation of sarcoplasmic reticulum (SR) and disorientation of Z lines (Z) in type IIa fiber of diabetic animal (x 15000).

DISCUSSION

Slow and fast twitch muscles were differentially affected by diabetes. In the former, contractile speed was further slowed and oxidative capacity and fatigue resistance were impaired. Fast muscles showed preferential fast - twitch -glycolytic (FG) fiber atrophy and reduced strength performance. Some of these deficits have been previously reported (Amstrong et al., 1975; Cotter et al., 1989; Paulus and Grossie, 1983; Baldwin et al., 1972). The reported findings indicate that fast-twitch skeletal muscle fibers are more dependent upon insulin for maintenance of their normal metabolic and morphological characteristics than slow-twitch fibers. In normal muscle slow-twitch-oxidative (SO) fibers posses a high capillary density (Cameron et al., 1990; Mai et al., 1970) and capacity for fatty acid (Baldwin et al.,1972) and ketone body oxidation (Kark et al., 1971). These factors suggest that SO fibers might adapt to the diabetic condition with minimal adverse metabolic effects. However, the fast-witch-oxidative-glycolytic (FOG) fibers have similar capabilities. In fact, FOG fibers in rat quadriceps muscle have a higher carnitine palmityl transferase activity and greater capacity for palmitate oxidation (Baldwin et al.,1972) than SO fibers, indicating the FOG fibers are better able to metabolize fatty acid.

The structural changes observed in muscle fibers of diabetic animals can be related to abnormal metabolism resulting from insulin deficiency. Previous histochemical and biochemical investigations in streptozotocin- diabetic rats suggest a greater loss in oxidative potential on the fast-twitch fiber population than in slow-twitch fibers (Amstrong et al., 1975; Cebesoy et al., 2000). Since the oxidative enzymes are located on the mitochondrial cristae, the altered mitochondrial morphology in FOG and SO fibers and the decreased number of mitochondria in FG fibers of diabetic animals seem to reflect this reduced oxidative potential.

The segregation of parts of FOG and SO fibers to form satellite cell in diabetic animals is interesting. Mono-nucleated satellite cells in normal adult muscles cover only 1-2% of the total nuclear population (Bischoff, 1974; Ontel, 1974). Their increase in FOG and SO fibers of diabetic animals is comparable to that occuring in denervated rat muscles (Ontel, 1974).

It is concluded that alloxan-diabetes has differential effects on the fine structure of the three fiber types in rat skeletal muscle.

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