DECREASED PHOSPHORYLATIVE CAPACITY AND RESPIRATORY RATE OF RABBIT SKELETAL MUSCLE MITOCHONDRIA IN VITAMIN E DYSTROPHY

J. J. A. HEFFRON,* A. C. CHAN, G. A. GRONERT and P. V. J. HEGARTY

Department of Anesthesiology, Mayo Clinic and Medical School, Rochester, Minnesota 55901, and Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108, U.S.A.

(Received 16 January 1978)

Abstract—1. Ultrastructural and biochemical changes occurring in mitochondria of skeletal muscle of vitamin E deficient rabbits and the extent of repair of the mitochondria after oral administration of the vitamin to the deficient rabbits have been studied.

2. Muscle of the dystrophic rabbits showed structural changes typical of the myopathic state, and these were reversed in the rehabilitated rabbits.

3. State 3 respiratory rate of the dystrophic mitochondria was severely reduced when measured in the presence or absence of albumin in the assay medium.

4. The ADP/O ratio of the dystrophic mitochondria utilizing glutamate malate was reduced by one-third when albumin was absent from the assay medium but was similar to the ratio of the control and rehabilitated mitochondria when albumin was present.

5. This result suggested that skeletal muscle mitochondria of dystrophic rabbits had a decreased ADP/O ratio in vitro.

INTRODUCTION

The lesions of vitamin E dystrophy in skeletal muscle of the rabbit are characterized in the early stages by mitochondrial swelling, fragmentation of the cristae and accumulation of dense granules within the mitochondria (van Vleet et al., 1967; 1968). Later changes, such as myofibrillar disruption, accumulation of lipid droplets in the sarcoplasm and intra-mitochondrial calcification, are considered to be secondary in nature (Mason, 1973). The lesions are generally regarded as being myogenic in origin. Biochemical studies of vitamin E deficient rabbit muscle indicate that increased oxygen uptake by skeletal muscle represents a primary response to the vitamin deficiency whereas other biochemical changes are secondary to this or some other metabolic disturbance (Mason, 1973; Victor, 1934). Increased oxygen uptake usually precedes the appearance of the histological lesions in rabbits and can be restored to normal within a few hours of intravenous administration of DL-α-tocopherol (Hummel & Melville, 1951). These studies suggest that vitamin E deficiency leads in some manner to increased mitochondrial oxygen consumption. Although this may be due in part to the increased energy demands of accelerated protein synthesis, the ultrastructural observations indicate that the major proportion of the increased oxygen consumption may be due to a loss of respiratory control or to uncoupling of oxidative phosphorylation or a combination of both of these factors.

In the present study we have attempted to correlate the ultrastructural damage and biochemical changes occurring in the mitochondria of skeletal muscle of vitamin E deficient rabbits. The extent of repair of the mitochondria after oral administration of vitamin E to the dystrophic animals has not been studied. Therefore, the correlation of biochemical and structural properties of the mitochondria in the rehabilitated muscle has not been determined.

MATERIALS AND METHODS

Animals

Four male New Zealand white rabbits (Oryctolagus cuniculus domesticus), body weight 1.3 kg, were fed a diet deficient in vitamin E for 4 weeks. The composition of the diet and the rationale for inducing acute dystrophy in a 4 week experimental period are explained elsewhere (Ghan & Hegarty, 1977). At the end of the 4 week period on the vitamin E deficient diet, two of the rabbits were given an oral dose of 50 mg DL-α-tocopherol acetate and then fed a diet containing vitamin E for about 4 weeks after which time the animals' body weight had returned to that of control animals. Rabbits were then killed by injection of potassium chloride into the heart, and the gastrocnemius muscles were excised immediately and placed in ice-cold 0.15 M NaCl. Muscles were obtained from 3 control animals in the same way.

Electron microscopy

Muscles samples from the control, dystrophic and rehabilitated animals were processed for electron microscopy as previously described (Hegarty et al., 1973). Briefly, the
samples were fixed in a medium containing 2%, glutaraldehyde and 2%, para-formaldehyde in 0.1 M cacodylate buffer pH 7.4 for 24 hr and post fixed in 1%, buffered OsO4. Sections (150 nm thick) were cut on an ultramicrotome and stained with uranyl acetate and finally lead citrate.

Mitochondria

Mitochondria were isolated from the gastrocnemius muscle of the dystrophic, rehabilitated and control animals by a modification of the method of Dow (1967). Muscles were minced finely with a scissors and homogenized in 15 vols of ice-cold medium containing 0.25 M sucrose, 10 mM EDTA, 50,000 i.u. sodium heparin, and 50 mM Tris HCl pH 7.4 at 0 °C. Homogenization was carried out with a Poltron PT-20 homogenizer while the vessel containing the homogenate was cooled in ice during homogenization. The muscle was homogenized for 10 sec at speed 5 and this was repeated once after a 3 min interval at 0 °C. Cell debris and myofibrils were removed by centrifuging the homogenate at 750 g for 10 min at 0 °C. The supernatant was re-centrifuged at 750 g for 5 min. The resulting supernatant was centrifuged at 15,000 g for 20 min to sediment the mitochondria. The light fluffy layer was removed with a glass rod and the mitochondrial pellet was resuspended in 10 ml of homogenizing medium, and re-centrifuged at 15,000 g for 20 min. After decanting the supernatant and removing adhering drops of sucrose solution, the mitochondrial pellet was resuspended in 0.4 ml 0.25 M sucrose g of original tissue. Mitochondrial respiratory rates and ADP/O ratios were determined at 37 °C with a Gilson "Oxygraph" equipped with a Clark electrode. The assay medium for oxidative phosphorylation had the following composition: 15 mM KCl, 30 mM potassium phosphate buffer pH 7.4, 25 mM Tris HCl pH 7.4, 45 mM sucrose, 10 mM mannitol, 5 mM MgCl2, 7 mM EDTA, 20 mM glucose. Respiratory rates and ADP/O ratios of the mitochondria were determined in the presence and absence of 0.2%, crystalline bovine serum albumin (BSA) after adding 0.15 μmole samples of ADP to the reaction medium. The ADP stock solution was 15 mM as determined by a coupled enzymatic method (Jaworck et al., 1974) and was stored at −76 °C. Mitochondrial protein was determined with the Folin phenol reagent (Lowry et al., 1951). Respiratory rates were expressed as ng atoms O2/mg protein min at 37 °C.

RESULTS

Examples of mitochondria from normal (Fig. 1a) and dystrophic (Fig. 1b) muscle are similar to those described by other workers (van Vloten et al., 1967; 1968). The major characteristics of mitochondria of the dystrophic muscles are swelling, loss of matrix, and fragmentation of cristae. Muscle from the rehabilitated animals showed a normal fine structure (Fig. 1c). However, certain isolated areas showed incomplete recovery of the mitochondria with respect to the cristae and matrix density (Fig. 1d). Structural changes observed in the mitochondria would predict both changes in respiratory rates and respiratory control in the dystrophic animals, and that these changes should be almost completely reversed upon rehabilitation of the animals.

The rates of state 3 and state 4 respiration, respiratory control indices (RCI) and ADP/O ratios of mitochondria of normal, dystrophic and rehabilitated rabbit gastrocnemius muscles, using glutamate-malate as substrate, are shown in Table 1. In the mitochondria from the dystrophic animals, the state 3 rate, determined in the presence of albumin, was 31% less than the control rate, while in the absence of albumin, the rate was reduced by 54%, compared with the control. There was no difference between the state 4 rates of the dystrophic or control mitochondria when assayed in the presence or absence of albumin. Similarly, the RCI values of the dystrophic mitochondria were 40% and 51%, less than those of the controls assayed in the presence and absence of albumin, respectively. In the control mitochondria, the ADP/O ratio was not changed in the absence of albumin; however, the ADP/O ratio of the dystrophic mitochondria was 2.79 in the presence of albumin, and this was reduced to 2.06 when albumin was omitted from the assay medium. The RCI and ADP/O ratio of the mitochondria of the rehabilitated animals were similar to those of the controls both in the presence and absence of albumin (Table 1). In contrast to the mitochondria of the dystrophic animals, the mitochondria from the rehabilitated animals showed increased state 3 respiratory rates compared with the control values both in the presence and absence of albumin.

The same parameters of mitochondrial function were determined with succinate as substrate and in the presence of albumin (Table 2). The state 3 respiratory rates of the normal, dystrophic and rehabilitated mitochondria were similar with both succinate and glutamate-malate as substrates. However, succinate oxidation showed much looser coupling than glutamate-malate oxidation in the mitochondria, as indicated by the lower RCI values. The RCI of the dystrophic mitochondria with succinate as substrate was reduced to a similar extent as with glutamate-malate when compared with the control value. The RCI returned to the control value in the rehabilitated mitochondria. In contrast with glutamate-malate as a

| Table 1. Mitochondrial functions in normal, dystrophic and rehabilitated rabbit gastrocnemius muscle with glutamate-malate as substrate |
|----------------|----------------|----------------|----------------|
|                | State 3 (ng atoms O2/mg min at 37 °C) | State 4 | RCI | ADP/O |
| Normal: (n = 3) | + BSA 148.9 | 24.8 | 6.11 | 2.82 |
|                | − BSA 131.6 | 31.0 | 4.32 | 2.81 |
| Dystrophic: (n = 2) | + BSA 103.4 | 28.5 | 3.66 | 2.79 |
|                | − BSA 60.7 | 28.2 | 2.11 | 2.46 |
| Rehaptinated: (n = 2) | + BSA 191.0 | 50.5 | 7.06 | 2.91 |
|                | − BSA 143.1 | 36.0 | 4.28 | 2.84 |

The substrate was 8.3 mM glutamate malate. RCI = Respiratory control index i.e. State 3 rate/State 4 rate. n = number of animals. Three replicate determinations were made on each mitochondrial preparation. Values are means.
Fig. 1. (a) Gastrocnemius muscle from control rabbit fed a vitamin E supplemented diet showing intact fine structure of mitochondria × 42,600; (b) Dystrophic gastrocnemius muscle from rabbit fed a vitamin E deficient diet. Note the loss of matrix and fragmentation of cristae. × 32,500; (c) Rehabilitated gastrocnemius muscle after vitamin E supplementation, showing mitochondria with intact fine structure. × 15,000; (d) Rehabilitated gastrocnemius muscle after vitamin E supplementation. Localized areas still reveal abnormal mitochondria similar to Fig. 1b. × 12,000.
substrate, the dystrophic mitochondria showed a reduced ADP/O ratio with succinate in the presence of albumin. However, the ratio returned to the control value in the rehabilitated mitochondria.

**DISCUSSION**

The present results indicate that the ultrastructural changes which take place in the skeletal muscle of vitamin E dystrophic rabbits can be reversed completely by returning the vitamin to the diet. The muscle of the dystrophic animals exhibited the classical myelin figures, infolding and waviness of the sarcolemma, severe myofibrillar disruption and myofilament disintegration, Z-line streaming, a swelling and loss of mitochondrial cristae. Except for the presence of occasional electron-translucent mitochondria, the muscle of the rehabilitated rabbits displayed a normal fine structure.

The biochemical studies of the mitochondria show that the maximal respiratory rate of the dystrophic mitochondria was severely reduced irrespective of whether or not albumin was present in the medium. This finding was seen with both NAD- and FAD-dependent substrates. The most interesting result was the reduction in the ADP/O ratio of the dystrophic mitochondria when assayed in the absence of albumin. Normal ADP/O ratios were observed in the mitochondria of the rehabilitated rabbits independent of the presence of albumin in the assay medium. Furthermore, the respiratory rates of these mitochondria were greater than the rates of the control mitochondria. Therefore, it is concluded that vitamin E deficiency has probably two actions on mitochondrial function: (a) loss of dehydrogenase and electron-transporting activities; (b) a degree of uncoupling of phosphorylation of ADP from electron transport. The loss of cristae in the mitochondria of the deficient rabbits supports the view that the reduced respiratory rates are a consequence of the reduced density of the cristae in the mitochondria. As has been suggested by Schwarz (1972) for liver mitochondria from vitamin E deficient animals, this might be related to loss of an intrinsic component of the respiratory chain such as cytochrome c the least firmly bound cytochrome.

The fact that the presence of albumin in the assay medium masked the uncoupling of phosphorylation of ADP from electron transport would explain why this has not been observed previously. It should be emphasized that the reduction in ADP/O ratio was additional to lower RC1 values, which together indicate a severe loss of the structural integrity of the mitochondrial membrane and probably considerable alteration of enzyme conformation. This loss of membrane integrity was readily reversed by vitamin E administration to the dystrophic animals. Previous studies by Dow (1967) have shown that the respiratory control of normal rat muscle mitochondria was much improved by albumin, and this was confirmed in the present study of rabbit muscle mitochondria. Albumin appeared to increase the respiratory control of the normal and dystrophic mitochondria equally while it apparently reversed the decrease in ADP/O ratio of the dystrophic mitochondria. This suggested that skeletal muscle mitochondria of the dystrophic rabbits had a decreased ADP/O ratio in vivo.

**REFERENCES**


---

**Table 2. Mitochondrial functions in normal, dystrophic and rehabilitated rabbit gastrocnemius muscle with succinate as substrate**

<table>
<thead>
<tr>
<th>State 4 (ADP/O ratio)</th>
<th>State 3 (RC1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal: (n = 3)</td>
<td></td>
</tr>
<tr>
<td>Dystrophic: (n = 2)</td>
<td></td>
</tr>
<tr>
<td>Rehabilitated: (n = 2)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>State 4</th>
<th>State 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal: 152.8</td>
<td>43.1</td>
</tr>
<tr>
<td>Dystrophic: 103.5</td>
<td>55.3</td>
</tr>
<tr>
<td>Rehabilitated: 190.1</td>
<td>66.4</td>
</tr>
</tbody>
</table>

The substrate was 8.3 mM succinate. BSA was present in the assay medium. Other footnotes as in Table 1.