Skeletal myopathy in chronic magnesium depletion*) **)

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Zusammenfassung

21 Tage alte Sprague-Dawley-Ratten mit einem durchschnittlichen Körpergewicht von 56 g erhielten eine Mg-arme Diät (Teklad Test Diets: TD 76446) und deionisiertes, destilliertes Wasser, während die Kontrollen die gleiche Diät, aber 200 mg $MgCl_2$/100 ml Wasser bekamen. Nach 5 Wochen betrug das Körpergewicht der Mangeltiere 180 g (Kontrollen = 330 g), und das Plasma-Mg lag bei 0,28 mEq/l gegenüber 1,6 mEq/l bei den Kontrollen (Bestimmung mittels AAS). Die Skelettmuskelfasern wurden typisiert mittels Myofibrillen-ATP-ase (sowohl alkali- als auch säurestabile) und NADH-Diaphorase. Im langsam zuckenden M.soleus schien es, daß nur die Typ II-Fasern verändert waren (d. h. sie zeigten entweder zentralständige Kerne oder deutliche Faserdegeneration). Im schnellzuckenden M.tibialis anterior schien es, daß das Gebiet reich an Typ II oxidativen Fasern den höchsten Schweregrad der Myopathie aufwies. Wir schließen, daß Ratten bei chronischem Mg-Mangel eine degenerative Myopathie der Skelettmuskulatur entwickeln.

Summary

Twenty one day old weanling sprague-Dawley rats with an average weight of 56 gm were given magnesium (Mg) deficient diet (Teklad Test Diets: TD 76446) and deionized distilled water while the controls had the same diet and 200 mg $MgCl_2$ /100 ml of water. After 5 weeks, the average weight of Mg deficient rats was 180 g (control = 330 g) and their plasma magnesium, measured by atomic absorption photometry, was 0.28 mEq/l (control = 1.6 mEq/l). Skeletal muscle fiber types were identified by means of myofibrillar ATPase (both alkali and acid stable) and NADH diaphorase. In the slow-twitch soleus muscle it appeared that only type II fibers were affected (i.e. showed either central nucleation or marked fiber degeneration). In the fast-twitch anterior tibialis it appeared that the area which is rich in type II oxidative fibers showed the greatest degree of myopathy. We conclude that rats chronically depleted of magnesium develop degenerative skeletal myopathy.

Résumé

Des rats Sprague-Dawley sevrés, âgés de 21 j, d'un poids moyen de 56 g, ont reçu un régime déficitaire en Mg (régimes d'essai Teklad: TD 76446) et de l'eau distillée déionisée et 200 mg de $MgCl_2$/100 ml d'eau. Après 5 semaines, le poids moyen des rats déficitaires en Mg a été de 180 g (contrôle = 330 g) et leur Mg plasmatique, mesuré par spectrométrie d'absorption atomique a été de 0,28 meq/l (contrôle = 1,6 meq/l).

Les types de fibres ont été identifiés au moyen de l'ATP-ase myofibrillaire (stable à la fois envers les alcalis et les acides) et de la NADH-diaphorase. Dans le muscle soléaire à contractions lentes, il est apparu que seules les fibres de type II étaient affectées (c'est-à-dire qu'elles ont présenté ou bien une nucléation centrale ou bien une dégénérescence marquée des fibres). Dans le muscle tibial antérieur, il est apparu que l'aire, qui est riche en fibres oxydatives de type II, a présenté le degré le plus élevé de myopathie.

Nous concluons qu'une myopathie dégénérative des muscles du squelette de développe chez les rats soumis à une déplétion chronique du magnésium.

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Introduction

Dystrophic changes in skeletal muscles induced by magnesium deficiency were first reported by *Moore* et al in 1938 [5]. In a detailed sequential study, *Heggtveit* [2] showed that changes occurred as early as 7 days after magnesium deprivation. Focal lesions, which began as swelling with loss of striations, progressed to floccular necrosis of myofibers. Frequently, calcification developed in necrotic as well as in apparently healthy myofibers [2]. Despite these considerable morphological changes, the magnesium-deficient rats rarely show clinically obvious muscular disabilities, and it is not known if the mechanical properties of the muscles are affected.

The purpose of the present study of magnesium-depleted rats was to reevaluate our earlier findings and to correlate contractile parameters with the structural alterations of slow and fast twitch muscles.

Materials and methods

Male Sprague-Dawley rats, (Canadian Breeding Farm and Laboratories Ltd, St. Constant, P. Q.), weighing around 100 g, were given a magnesium deficient diet which was obtained from Teklad Test Diets (Madison, Wisconsin). According to the supplier, the magnesium content of the diet (TD 76446) was less than 10 ppm. The animals drank distilled water **ad libitum.** The control animals were either pair-fed or fed **ad libitum** with

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the same diet but having $MgCl_2$ (200 mg/100 ml) in their drinking water.

For contractile measurements, the rats were anesthetized with sodium pentobarbitone (Nembutal). Both hind limbs were shaved and the fast-twitch extensor digitorum longus muscle (EDL) was exposed in one limb by reflecting the anterior tibialis (AT). The branches of the lateral popliteal nerve supplying EDL were identified and the remaining branches cut with care being taken not to damage the blood supply to the muscle. A light stainless steel rod was tied to the tendon of insertion and this subsequently served to attach the muscle to a force transducer (Statham UC-2 with load cell attachment) for measurement of muscle contractions. The tendon of origin was exposed so that it could be held firmly in a clamp to maintain the muscle under isometric conditions. In the other hind limb the slow-twitch soleus muscle was similarly prepared. In this case no denervation of adjacent muscles was necessary because a sufficient length of soleus nerve could be exposed to permit stimulation of the soleus muscle alone.

Once the muscles were prepared the rat was placed on a bed of *Sylgard* resin in a plexiglass bath with its entire hind quarters immersed in oxygenated *Krebs-Ringer* solution. The muscle was attached to the transducer, the upper-tendon clamped and stimulating stainless steel wire electrodes were placed on the nerve. Stimuli (0.05 msec duration) were applied to the nerve from a *Grass* SD9 stimulator. Muscle length was adjusted to yield maximum twitch tension and stimulus intensity was adjusted to 1.5 x which produced a maximal response. For tetanic contractions stimuli were applied at 100/sec for soleus and 180/sec for EDL. Bath temperature ranged from 28° to 30° C. Contractile responses were filmed from a Tektronix 502A oscilloscope. Measurements of contractile force as well as of the time to reach peak twitch tension and the time to relax to half of that tension (½RT) were made from negatives by means of a film reader.

Blood was taken from the aorta of anesthetized animals by a heparinized syringe. Plasma magnesium was measured by atomic absorption photometry. For histochemical studies on muscles, segments of slow-twitch soleus and fast-twitch AT and EDL were frozen by immersion for 10 seconds in isopentane cooled to liquid nitrogen temperature. Frozen sections were cut in a cryostat at 10 μ thickness and stained for myofibrillar ATPase at pH 10.4 and 4.35 [1] and succinic dehydrogenase [6].

For light microscopy, soleus and anterior tibial muscles (representing fast-twitch) were fixed in 10 % neutral buffered formalin. Sections from paraffin-embedded tissues were stained by hematoxylin and eosin, phosphotungstic acid hematoxylin and *Masson*'s trichrome methods. The heart and kidneys were also processed and examined for characteristic lesions of magnesium depletion.

For electron microscopy, tiny pieces of muscle were fixed by immersion into one-half strength *Karnovsky*'s fixative for 2—4 hours at 4° C, washed with 0.1 m sodium cacodylate buffer and then post-fixed in 1 % osmium tetroxide. After dehydration by graded ethyl alcohol and propylene oxide, tissues were embedded in epon-araldite resin. Thin sections were stained with uranyl acetate and lead citrate.

Results

In our preliminary experiments, magnesium deficiency was induced in male weanling rats soon after they were brought to our laboratory, weighing between 40 g and 60 g. We found that 10—20 per cent of the deficient animals failed to survive the entire experimental period of 4—5 weeks. To improve survival, we left the weanling rats under our laboratory conditions with Purina rat diet and tap water for several days until they reached the weight of 90—110 g before inducing magnesium depletion. The characteristic clinicopathological changes of magnesium depletion, however, were not different in the weanling from those in the slightly older rats. Results on the latter group only are described in this report.

Most of the clinical signs which have been described in magnesium-deficient rats in previous reports [2, 4, 7] appeared in our animals within the first week. Those were characterized by erythema of ears, paws and tail, and focal loss of hair on the face as well as on the body. Convulsive seizures, however, were rarely seen. The average daily food intake of magnesium deficient rats was 10—13 g. After 4—5 weeks of magnesium depletion, the weight gain was 60—65 g, as opposed to 140 g in pair-fed controls and 250 g in controls which had access to food **ad libitum.**

Plasma magnesium of depleted animals measured 4.2 ppm while that in the control was 16.4 ppm on the average.

The most obvious change in the mechanical properties of the muscle of magnesium deficient rats was a marked reduction of tension, both in a single twitch and a tetanus (Table 1). This was true in the case of both fast-twitch EDL and slow-twitch soleus muscles. As is clearly shown in Table 1, however, this reduction of force development could be accounted for by the accompanying loss of muscle mass. Thus, although the absolute twitch tension of EDL from magnesium deficient rats was only 56 % of that produced by their control counterparts, when twitch tension was expressed per gram of muscle weight, there was no difference between control and experimental muscles. Essentially the same was true in soleus. Although there was a small decrease in normalized tension (to 82.5 % of control values) there was a much greater reduction in absolute tension (to 62 %).

The data on tetanic tensions, also shown in Table 1, show a slightly different pattern. In the case of both muscles there was a greater reduction of absolute tetanic tension than was seen for twitch tension, and normalized tetanic tension was only seen to fall in the experimental animals. This may reflect an abnormality of the calcium release system of magnesium deficient muscles which is manifested to a greater extent during a tetanus.

Both EDL and soleus contracted more slowly in the magnesium depleted animals than in control. Table 1 indicates that the time to peak tension was increased by 14 % in EDL and 22.5 % in soleus, while ½RT was prolonged by 41 % in EDL and 50.5 % in soleus.

Histologically, there was some variability in the size of myofibers. Individual fibers showed segmental lesions which varied from swelling and loss of striations to floccular necrosis. Dense cellular infiltration in the area of fiber lysis consisted of mononuclear cells, some of which were phagocytic (Fig. 1). Fiber diameter was substantially reduced and some fibers exhibited central nucleation in both EDL and soleus (Fig. 2). However, histochemically the muscles retained a rather normal mosaic arrangement of fiber types with no preferential involvement of one type.

The ultrastructure of both the soleus and anterior tibial muscles showed a spectrum of changes which were most remarkable in their involvement of myofilaments. Patchy dissolution of myofilaments imparted a rarefied appearance to some fibers (Fig. 3), but, at the other extreme, a whole segment underwent complete necrosis (Fig. 4). A frequent finding in affected myofibers was dedifferentiation with loss of sarcomeric arrangement and replacement by randomly disposed aggregates of myofilaments with distorted Z bands

Tab. 1: Contractile properties of Mg-deficient rat muscles.

	E.D.L.		SOLEUS	
	Control	Mg-Def	Control	Mg-Def
Twitch Tension (g)	32.5 ± 4.5 (9)	18.2 ± 1.8 (13)	20.2 ± 1.9 (11)	12.5 ± 1.0 (14)
(g/g wet wt)	266.4 ± 40.8 (7)	262.9 ±23.6 (10)	171.8 ±10.1 (8)	141.2 ±17.7 (8)
Tetanus Tension (g)	137.2 ± 15.0 (9)	53.8 ± 6.3 (10)	124.1 ±11.0 (10)	59.1 ± 3.9 (11)
(g/g wet wt)	1113.1 ±137.3 (7)	721.2 ±40.8 (7)	1080.7 ±49.2 (8)	614.4 ±44.0 (6)
Twitch/Tetanus	0.23± 0.01 (9)	0.34± 0.03 (10)	0.16± 0.01 (10)	0.21± 0.02 (11)
Time to Peak Tension	29.9 ± 1.0 (8)	34.1 ± 2.0 (13)	70.9 ± 3.6 (11)	86.7 ± 6.4 (14)
½ Relaxation Time	27.2 ± 1.7 (8)	38.4 ± 4.0 (13)	73.5 ± 5.9 (11)	110.7 ± 8.9 (14)

Values are mean ± S.E.M. Number of muscles in parentheses.

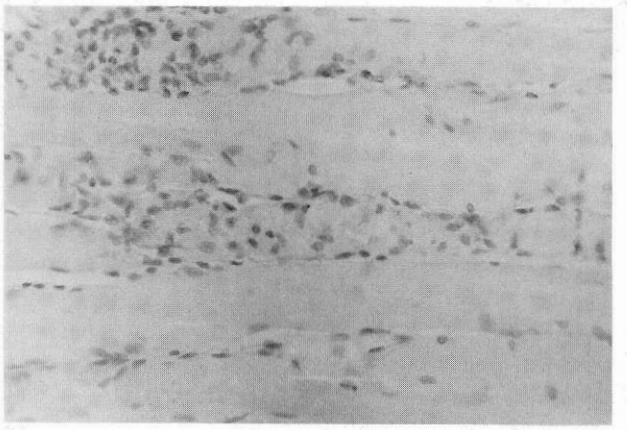

Fig. 1: Floccular necrosis of myofibers. Hematoxylin and eosin (HE).

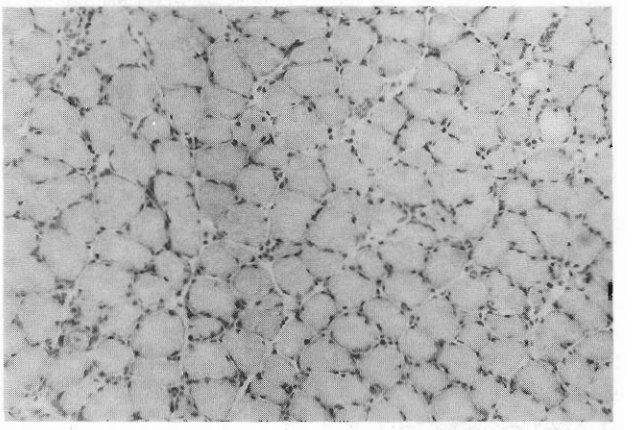

Fig. 2: Marked reduction in fiber diameters. HE.

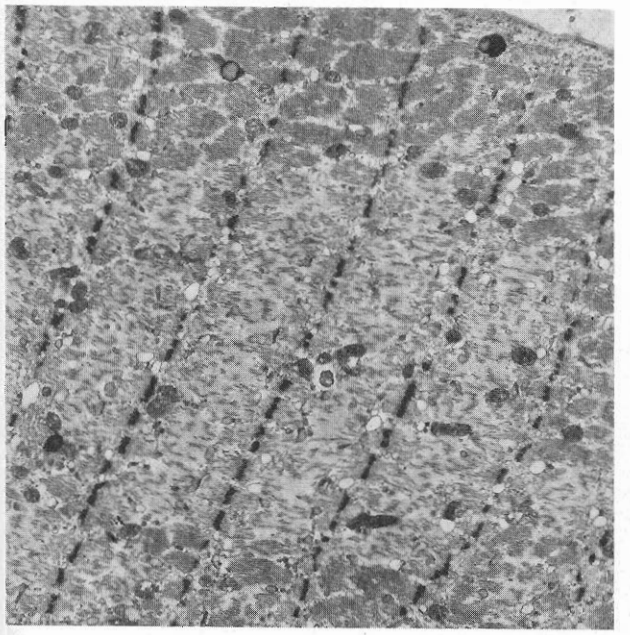

Fig. 3: Partial loss of myofilaments. Uranyl acetate (UA) and lead citrate (LC). x 10,800.

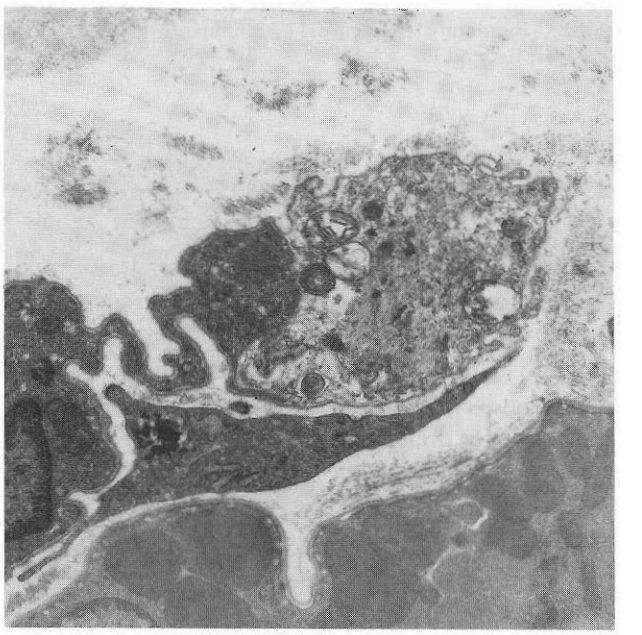

Fig. 4: Segmental necrosis of a myofiber. UA and LC. x 18,000.

(Fig. 5), or filamentous packing of cytoplasm without discernible Z bands (Fig. 6). In the latter, reduction in fiber diameter was suggested by a retraction from the basal lamina which showed numerous foldings. Occasionally, an area of widespread necrosis was entirely occupied by highly convoluted remnants of basal lamina. Cytoplasmic vacuolation and phagolysosomes were seen in several affected myofibers. In many myofibers without myofilamentous involvement, mitochondria were swollen and vacuolated.

Discussion

It is evident from our study that regardless of the slow or fast twitch nature of the muscles, they were equally affected by chronic magnesium depletion induced in rats by dietary means. Lesions involving individual myofibers were essentially segmental. Our earlier report on the myopathy of magnesium depleted rats showed calcification of dystrophic muscles along with other changes [2]. This finding had also been de-

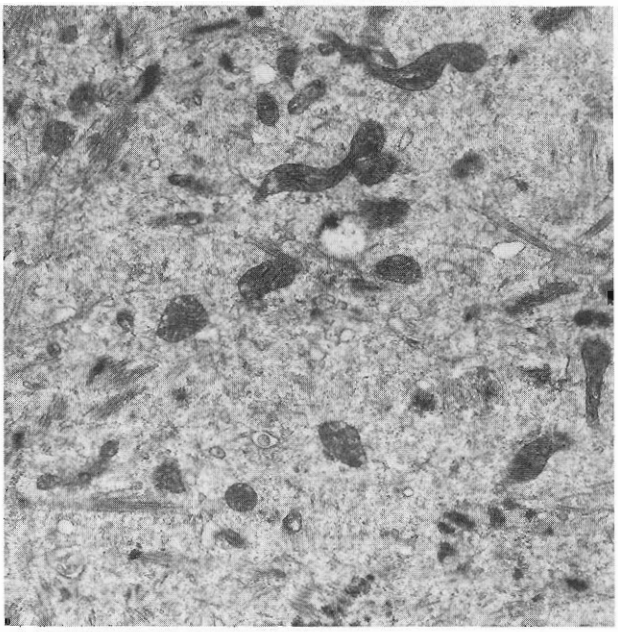

Fig. 5: Randomly aggregated myofilaments with distorted Z bands. UA and LC. x 16,000.

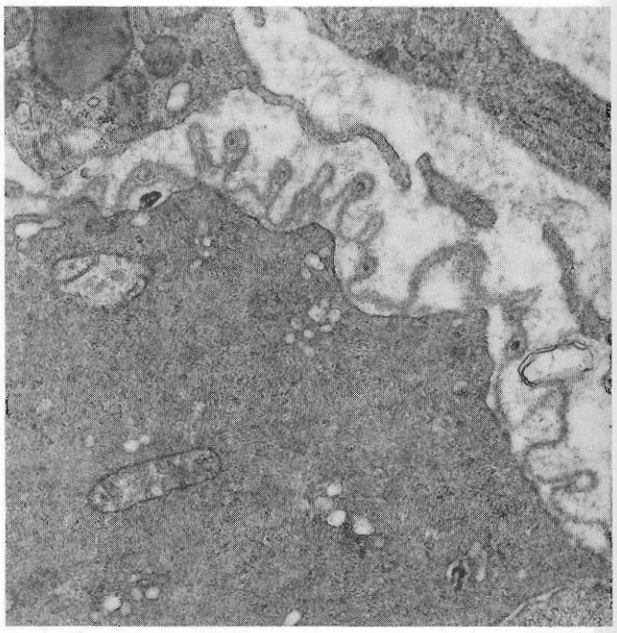

Fig. 6: Atrophying dedifferentiated myofiber retracting from its basal lamina. UA and LC. x 25,500.

scribed by other investigators [4]. In the present study, however, calcific deposits were consistently absent in both the soleus and anterior tibial muscles although the other dystrophic changes were similar to the previous study. We feel that this was most likely due to the considerably reduced phosphate content of the present diet compared to the earlier diet [3].

Recent studies have indicated that pathophysiological changes in skeletal muscles of magnesium-depleted rats are 'membrane-oriented' [7, 8]. This was evidenced by decreased membrane potential as measured in the soleus muscle and decreased Mg-ATPase activity. Ultrastructurally, both sarcoplasmic reticulum and mitochondria were affected, characterized by total disorganization of the former and swelling with vacuolation of the latter [8]. The disorganization of the sarcoplasmic reticulum may offer an explanation for the changes we have observed in the mechanical properties of muscle from magnesium deficient rats. If calcium uptake is impaired, as has been reported [7], contractions might be expected to be prolonged, particularly the relaxation phase. This is precisely what we observed; half relaxation time was prolonged between 40 and 50 %. Since the sarcoplasmic reticulum is also an essential component of the excitation-contraction coupling mechanism, any derangement of this system could lead to a reduction in coupling of the action potential to the contractile process. It would be reasonable to anticipate that this might lead to a greater proportional reduction of tetanic tension than of twitch tension if, for instance, successive action potentials could liberate less calcium than normal. This would explain our reduced tetanic tensions and rather high values of twitch: tetanus ratio. It is also possible that excitation itself was inadequate during tetanic stimulation of magnesium depleted muscles. It will be necessary to record action potentials during such stimulation to determine the importance of this.

We conclude, then, that chronic magnesium depletion causes irreversible cell damage in skeletal muscle in association with slower muscular contraction and prolongation of the relaxation phase. For adequate functional interpretation however, one must consider that rats deprived of magnesium have a significant reduction in growth as well as in muscular activities.

References

[1] *Guth, L., Samaha, F. J.:* Qualitative differences between actomysin ATPase of slow and fast mammalian muscles. Esp. Neurol. **25** (1969) 138—152.

[2] *Heggtveit, H. A.:* Myopathy in experimental magnesium deficiency. Ann. N. Y. Acad. Sci. **162** (1969) 758—765.

[3] *Heggtveit, H. A., Herman, Y., Mishra, R. K.:* Cardiac necrosis and calcification in experimental magnesium deficiency. A light and electron microscopic study. Am. J. Pathol. **45** (1964) 757—782.

[4] *Ko, K. W., Fellers, F. X., Craig, J. M.:* Observations on magnesium deficiency in the rat. Lab. Invest. **11** (1962) 294—305.

[5] *Moore, L. A., Hallman, E. T., Sholl, L. B.:* Cardiovascular and other lesions in calves fed diets low in magnesium. Arch. Path. **26** (1938) 820—838.

[6] *Nachlas, M. M., Tsou, K. C., Desouza, E., Cheng, C. S., Seligman, A. M.:* Cytochemical demonstration of succinate dehydrogenase by the use of new p-nitrophenyl substituted ditetrazole. J. Histochem. Cytochem. **5** (1957) 420—436.

[7] *Robeson, B. L., Maddox, T. L., Martin, W. G.:* Muscle changes in rats fed magnesium and calcium deficient diets. J. Nutr. **109** (1979) 1383—1389.

[8] *Robeson, B. L., Martin, W. G., Friedman, M. H.:* A biochemical and ultrastructural study of skeletal muscle from rats fed a magnesium-deficient diet. J. Nutr. **110** (1980) 2078—2084.

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