Arch. Gerontol. Geriatr., 4 (1985) 101–115
Elsevier

AGG 00099

Skeletal muscle aging in the hind limb of the old male Wistar rat: inhibitory effect of hypophysectomy and food restriction

A.V. Everitt [1], C.D. Shorey [2] and Maria A. Ficarra [2]

[1] *Department of Physiology, F13, and* [2] *Department of Histology and Embryology, University of Sydney, N.S.W. 2006, Australia*

(Received 8 October 1984; revised version received 24 December 1984; accepted 28 December 1984)

Summary

By age 1 100 days (37 mth) hind leg paralysis was found in 50% of ad libitum fed (control) male Wistar rats, but only 10% of food restricted rats and no hypophysectomized rats of that age had this disease. Gastrocnemius muscle weight declined at a faster rate than whole body weight in old ad libitum fed rats but not in old hypophysectomized or food restricted rats.

Light microscopic and ultrastructural changes were studied in the muscles of the hind limbs of 11 control, 5 food-restricted and 5 hypophysectomized rats aged 805 to 1 307 days. Light microscopic changes in muscles involved progressive degeneration demonstrated by the accumulation of adipocytes and degenerative inclusion bodies. The main ultrastructural changes were associated with myofibrillar breakdown. In addition there was thickening of the basal lamina around blood capillaries.

However, muscle from hypophysectomized and food restricted rats of the same age range as controls possessed normal morphology with reduced thickening of the capillary basal lamina.

hind leg paralysis; muscle electron microscopy; light microscopic changes; gastrocnemius muscle weight

Introduction

The decline in muscle strength in old age is associated with a reduction in muscle mass and loss of muscle fibres (Grimby and Saltin, 1983). In the hind leg muscles of the old rat there is a fall in muscle weight (Tauchi et al., 1971; Yu et al., 1982), a loss of entire physiological muscle units (Caccia et al., 1979), a reduced number of muscle fibres (Gutmann et al., 1971; Tauchi et al., 1971), atrophy of muscle fibres (Van Steenis and Kroes, 1971; Fujisawa, 1974), increased variation in fibre diameter (Fujisawa, 1974), accumulation of lipofuscin granules and lipid droplets (Hanzlikova and Gutmann, 1975) and degenerative histological changes (Berg, 1956, 1967; Fujisawa, 1975; Hanzlikova and Gutmann, 1975). There is evidence that these age changes in the hind limb muscles of the old rat may be retarded by either food restriction (Berg and Simms, 1961; Yu et al., 1982) or by hypophysectomy (Everitt et al., 1980).

The main purpose of this study is to observe the long term effects of hypophysectomy and food restriction on the aging muscle in the hind limb of the rat.

Materials and Methods

Animals

Studies of hind leg paralysis, the weight of gastrocnemius muscle and the histology of hind leg muscles were made on 393 outbred male Wistar rats of the University of Sydney strain. Of these, 118 were hypophysectomized at age 60 to 70 days using the technique of Koyama (1962), and a further 85 were food-restricted beginning at age 60 to 70 days. Details of housing, feeding, etc., are described elsewhere (Everitt et al., 1980). Food-restricted rats consumed the same amount of food as hypophysectomized rats (7 g food/day), averaging 40% of that of the ad libitum fed controls.

Hind leg paralysis

Rats that dragged their hind legs were said to have hind leg paralysis. Those whose walking was impaired due to muscle weakness were considered to have an early form of this disease.

Wet weight of the gastrocnemius muscle

At autopsy the gastrocnemius muscle was dissected from the left hind leg and weighed in 20 old control (including 5 paralysed rats), 8 old hypophysectomized and 8 old food restricted rats aged 802 to 1 307 days. In order to correct for differences in body weight, the muscle weights were compared with those from 23 young ad libitum fed controls of similar body weight. An estimate of maximum muscle weight was made in 18 middle aged rats (8 controls, 5 hypophysectomized and 5 food restricted) of age 378 to 654 days.

Light microscopy

Morphological studies of hind leg muscles were performed on 11 controls aged 805 to 1 120 days, 5 hypophysectomized rats aged 1 015 to 1 113 days and 5 food restricted rats aged 902 to 1 307 days. Muscles examined were the gastrocnemius (lateral head), soleus and biceps femoris, due to their different fibre type populations (Ariano et al., 1973).

Muscles were fixed in glutaraldehyde/formaldehyde fixative in 0.1 M cacodylate buffer at pH 7.3 (Karnovsky, 1965) for 2 h at room temperature. Subsequent post fixation in 1% osmium tetroxide was carried out for 4 h at 4°C followed by dehydration through a graded series of water–ethanol mixtures and embedding in Spurr's epoxy resin (Spurr, 1969). Semithin sections were cut at a thickness of 1–2 μm and stained with toluidine blue at pH 9.2.

Electron microscopy

Tissues for electron microscopy were processed in a similar way to that used for light microscopy up to the point of embedding. However in some cases aldehyde

fixation was omitted and osmium tetroxide was used on its own as described by Palade (1952).

Staining of ultrathin sections was 4% aqueous uranyl acetate at 50–60°C for 15 min and lead citrate (Venable and Coggeshall, 1965) for 3 min. Examination of sections was performed using a Philips 201 electron microscope at 60 kV.

Statistics

Quantitative data are reported as mean ± standard error of the mean. Student's *t*-test was used for statistical comparisons between means.

Results

Hind leg paralysis

Figure 1 shows a control rat of age 1 003 days (33 mth) dragging its hind legs. In 20 yr of observations, this disease has not been seen in any hypophysectomized rat up to the age of 1 350 days (45 mth), the oldest animal so far studied. It occurs in small numbers of old food restricted rats. The maximum life duration recorded in this colony is 1 232 days (41 mth) for ad libitum fed controls, 1 353 days (45 mth) for hypophysectomized rats and 1 515 days (50 mth) for food-restricted rats.

The frequency of hind leg paralysis is seen in Table I for control rats to rise from 7% at 800 days (26 mth) to 50% in rats aged 1 050 days (35 mth) or more. In food restricted rats aged 1 050 days or more the incidence was 9%, but in hypophysectomized rats it was zero.

Impairment of walking (Table I), which is a sign of early hind leg paralysis, is found in about 30% of controls at 800 days. However, it is not evident in

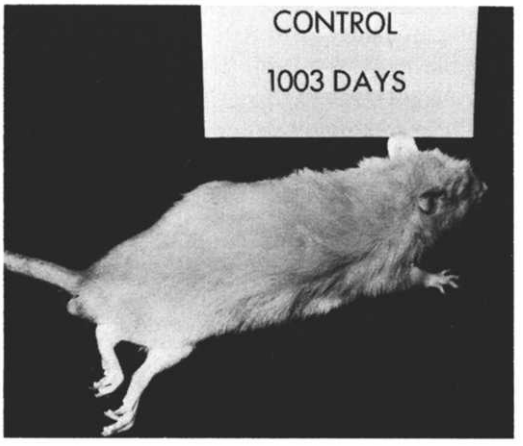


Fig. 1. A control male Wistar rat aged 1 003 days with paralysis of leg muscles. This condition forces the animal to drag its hind limbs while propelling itself along with its forelimbs. At age 1 000 days this disease is seen in 20% of ad libitum fed control rats (see Table I), but is rarely ever seen in food restricted rats and does not occur in hypophysectomized animals.

TABLE I

The number (n) of animals with impaired walking or hind leg paralysis in 129 control, 101 hypophysectomized and 68 food restricted male Wistar rats between ages 650 and 1 350 days.

Age (days)	Normal walking (n)	Impaired walking (n)	Hind leg paralysis (n)	Total (n)
Control rats				
650–749	22	3	0	25
750–849	18	9	2 (7%)	29
850–949	22	9	9 (23%)	40
950–1 049	1	14	4 (21%)	19
1 050–1 220	2	6	8 (50%)	16
Hypophysectomized rats				
650–949	67	0	0	67
950–1 049	14	1	0	15
1 050–1 350	18	1	0	19
Food restricted rats				
650–949	34	0	0	34
950–1 049	12	1	0	13
1 050–1 350	16	3	2 (9%)	21

hypophysectomized or food restricted rats until about 1 000 days (33 mth). At 1 000 days only about 5% of control rats walk normally, compared with 90 to 95% of hypophysectomized or food restricted rats.

Weight of gastrocnemius muscle

The gastrocnemius muscle, like most other organs, undergoes a growth and decline during the life span. The weight of the gastrocnemius muscle increased during growth from 0.88 g in the young ad libitum fed rat at 60 days (2 mth) to 2.89 g at 500 days (17 mth), and then decreased significantly ($P < 0.01$) in old age to 1.53 g at 1 000 days (33 mth) in animals still able to walk (Table II). In old rats that developed hind leg paralysis the mean weight was 0.87 g, but this was not significantly less than the weight (1.28 g) in old rats matched for body size and still able to walk.

In old ad libitum fed rats the percentage weight loss of the gastrocnemius muscle was twice as great as the loss in whole body weight (Table II). This difference was not seen in hypophysectomized or food restricted rats. In ad libitum fed rats between 500 and 1 000 days, muscle weight loss (2.89 to 1.53 g) was 47%, compared with only 22% loss in body weight (511 to 396 g). The corresponding losses in hypophysectomized rats were 20% muscle and 23% body weight and in food restricted rats 25% muscle and 19% body weight.

The relative weights of gastrocnemius muscle (Table II) to whole body weight (mg muscle/100 g body weight) declined significantly ($P < 0.01$) in ad libitum fed rats between middle age (5.65) and old age whether rats walked (3.72) or were paralysed (2.56). However, neither the old hypophysectomized (5.60) nor old food restricted (4.70) rats showed this accelerated weight loss from the gastrocnemius muscle.

TABLE II

Autopsy weights of gastrocnemius muscle in old male Wistar rats that were ad libitum fed (walking or with hind leg paralysis), hypophysectomized (hyp) or food restricted, compared with muscle weights in healthy young rats matched for body weight and in healthy middle aged rats.

Group	Mobility	No. of rats	Age range (days)	Body weight (g ± SE)	Gastrocnemius	
					(g ± SE)	(mg/100 g)
Middle aged ad lib	walking	8	405–615	511 ± 25	2.89 ± 0.12	5.65
Middle aged hyp ad lib	walking	5	379–646	208 ± 9	1.11 ± 0.11	5.40
Middle aged food restr.	walking	5	378–654	204 ± 5	1.04 ± 0.08	5.08
Old ad lib	walking	10	896–1041	396 ± 34	1.53 ± 0.09 [a]	3.86 [a]
Young ad lib	walking	10	55–225	396 ± 34	2.49 ± 0.24	6.28
Old ad lib	paralysed	5	891–1046	340 ± 42	0.87 ± 0.15 [a]	2.56 [a]
Old ad lib	walking	5	910–1040	344 ± 42	1.28 ± 0.15 [a]	3.72 [a]
Young ad lib	walking	5	55–175	345 ± 46	2.24 ± 0.37	6.49
Old hyp ad lib	walking	8	831–1298	159 ± 11	0.89 ± 0.070	5.60
Old food restricted	walking	8	802–1307	166 ± 11	0.78 ± 0.068	4.70
Young ad lib	walking	8	50–65	161 ± 11	0.88 ± 0.076	5.47

[a] Significantly less ($P < 0.01$) than young control, matched for body weight and fed ad libitum.

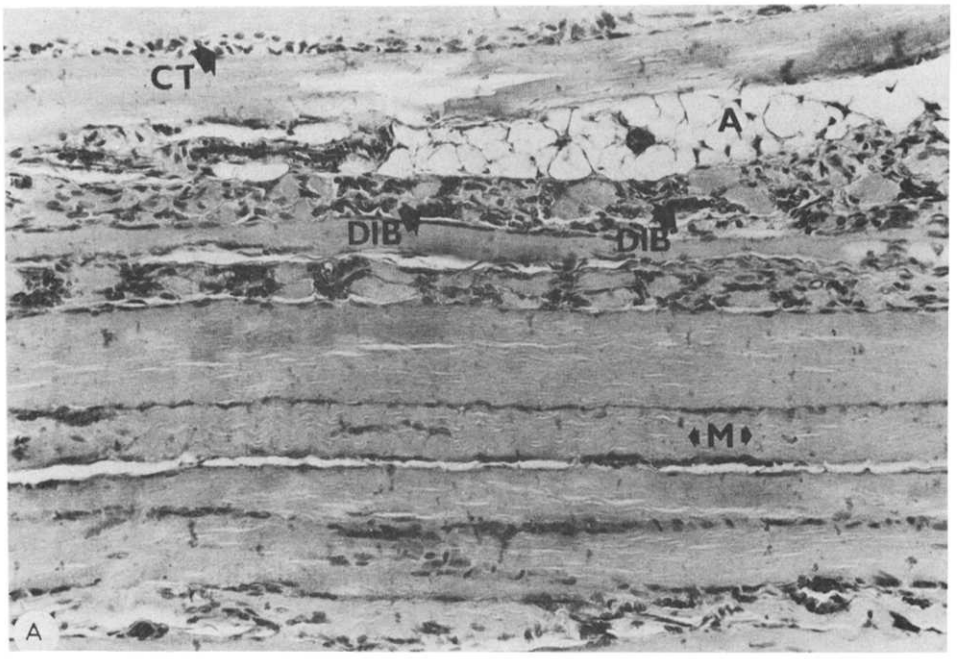
Light microscopy

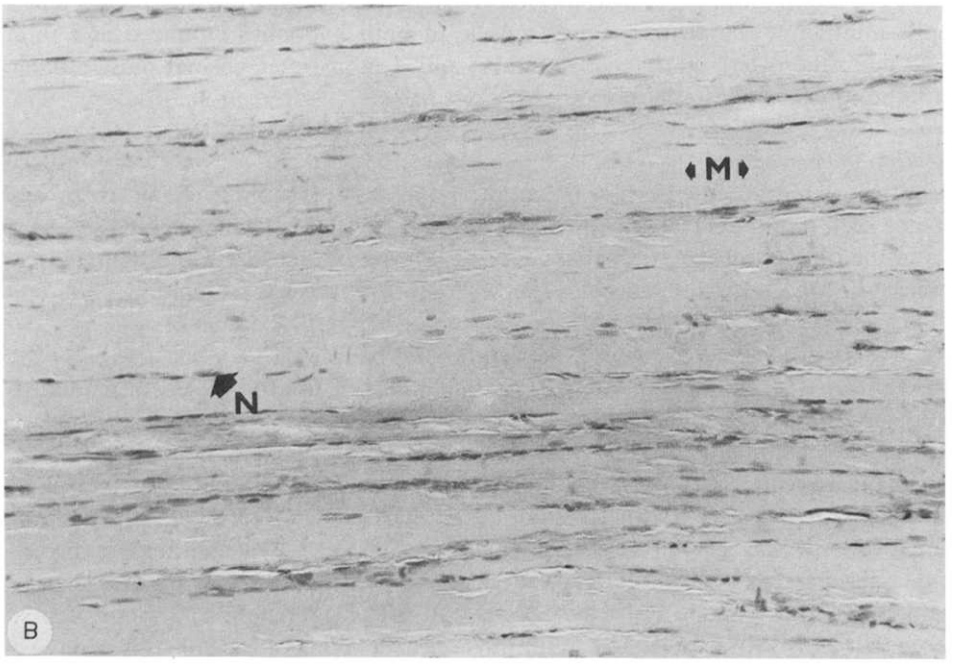
At the light microscopic level the most obvious aging features in 1 000-day-old rats were the presence of degenerative inclusion bodies and adipocytes (Fig. 2A). From overall observations the presence of connective tissue in the old control rats was more abundant than in hypophysectomized or food restricted rats. Figure 2B is representative of the gastrocnemius muscle in both hypophysectomized and food restricted rats in old age (800–1 300 days) and demonstrates normal muscle tissue with the conspicuous absence of degenerative inclusion bodies and lipid.

Electron microscopy

Ultrastructurally, myofibrillar breakdown (Fig. 3A) is the most characteristic age change seen in old control rats aged 800 to 1 100 days. This change is seen in all ad libitum fed intact rats so far examined, but is of rare occurrence in old hypophysectomized or food restricted rats (Fig. 3B), where the normal muscle banding pattern is usually seen.

Early changes were observed in the central and peripheral perinuclear area of the muscle fibres, in the form of varying accumulations of lipid vacuoles, degenerative inclusion bodies, autophagic vacuoles, dilated elements of sarcoplasmic reticulum and subsarcolemmal aggregates of membranous bodies, similar to myelin figures, and many vesicular outlines. Large lipid vacuoles with unusual vesicular inclusions were encountered near the fibres' periphery. The frequency of all these structures increased with age. They were accompanied by some degree of myofibrillar disruption, a degenerative change that markedly progressed with age to the point where the regular banding pattern was lost leaving thin diffuse Z-bands to support sparse degenerated myofibrils (Fig. 3A). With time only remnants of the Z-bands remained


CT
A
DIB
DIB
M
A


M
N
B

often in the form of dense rod-like structures irregularly distributed through the muscle fibre and frequently with small, short bundles of myofilaments attached, a few mitochondria, and vesicles of unknown origin. Eventually the breakdown process was complete and the muscle fibre was devoid of organelles and myofibrillar material, leaving a fine protein–polysaccharide ground cytoplasmic background. These degenerative changes were seen in all three muscle types, gastrocnemius, soleus and biceps femoris.

Atrophied muscle fibres preferentially displayed dilations of their sarcoplasmic reticulum, easily identifiable in their triad configurations with transverse system tubules. Mitochondrial atrophy coupled with their eventual disappearance from large areas of the muscle fibre, and myofibrillar splitting at numerous points along the banding pattern, were common features of the fast glycolytic type IIB muscle fibres in muscle. Fast glycolytic type IIB muscle fibres were differentiated from slow, type I fibres on the basis of mitochondrial content at the electron microscope level. Slow type I fibres have numerous large mitochondria (George and Scaria, 1958), whereas type IIB fibers have only few very small mitochondria.

A prominent feature of the microvasculature in the skeletal muscle of old rats was thickening of the basal lamina around capillaries (Fig. 4A) up to 1.2 μm, whereas normal widths in young adult rats average 0.1 μm. In old hypophysectomized rats (Fig. 4B) the basal lamina is usually about 0.1 μm and in old food restricted rats (Fig. 4C) somewhat thicker, although less than in old controls.

Discussion

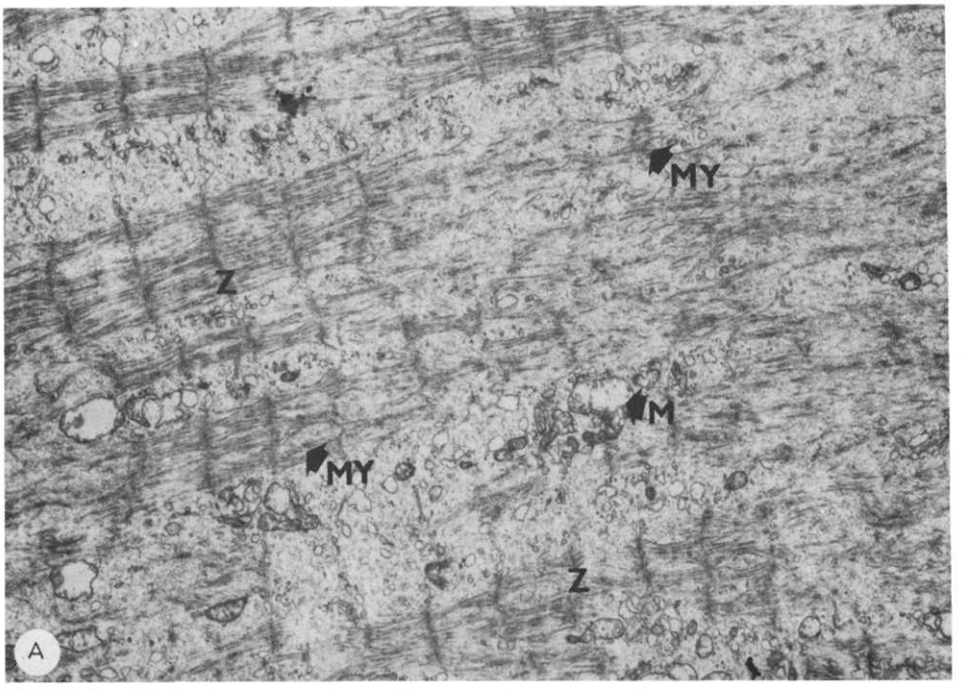
The major finding in this paper is that either hypophysectomy or food restriction is able to inhibit the development of gross and microscopic changes in the hind leg muscles of the old male Wistar rat.

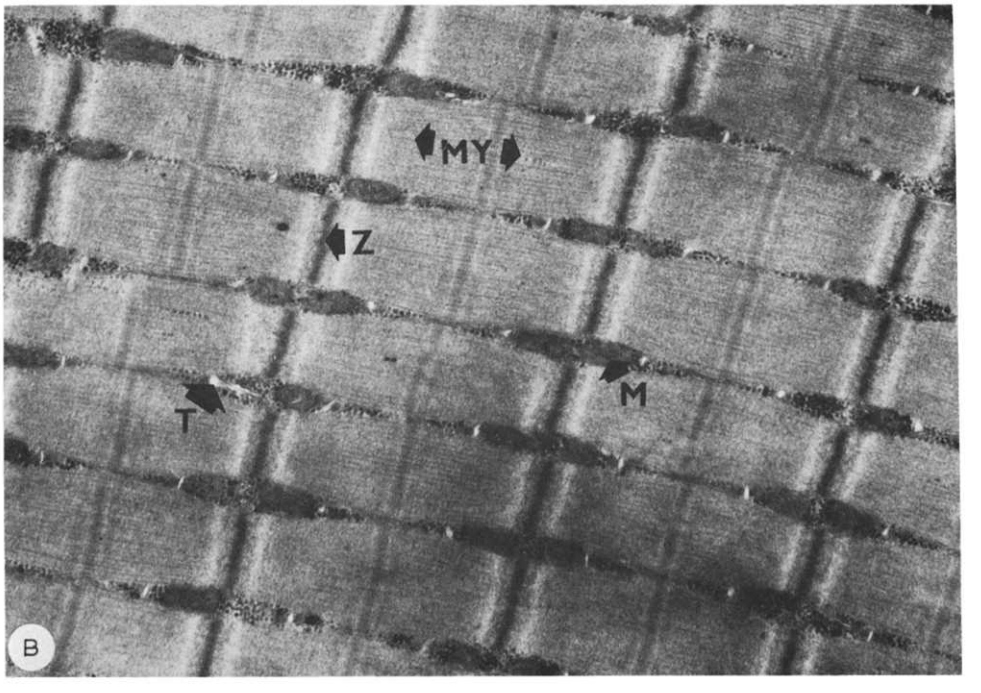
The principal gross changes are a decrease in muscle mass with a general wasting of the hindquarters eventually leading to hind leg paralysis (Berg, 1967; Van Steenis and Kroes, 1971). Microscopically, degenerative changes are seen both in the skeletal muscles of the hind limb and in the peripheral nerve supply (Van Steenis and Kroes, 1971). This paper describes the intrafibrillar changes that are found in the gastrocnemius, soleus and biceps femoris muscles of old rats approximately 1 000 days old. No attempt was made to quantify these morphological changes, since this will be the subject of a later paper.

Hind leg paralysis

Hypophysectomy in young rats was able to prevent the development in old age of

Fig. 2. (A) Longitudinal section from the gastrocnemius muscle of a control male rat aged 999 days. Fixed in glutaraldehyde/formaldehyde and resin embedded. Sections stained with toluidine blue at pH 9.2. Magnification×254. A, adipocytes; M, myofibre; CT, connective tissue; DIB, degenerative inclusion bodies. (B) Longitudinal section from the gastrocnemius muscle of a hypophysectomized male rat aged 1124 days. Muscle processed as in (A). Magnification×254. Note the absence of adipocytes and lipofuscin in this muscle. M, myofibre; N, nucleus.


MY
Z
M
MY
Z
A


MY
Z
M
T
B

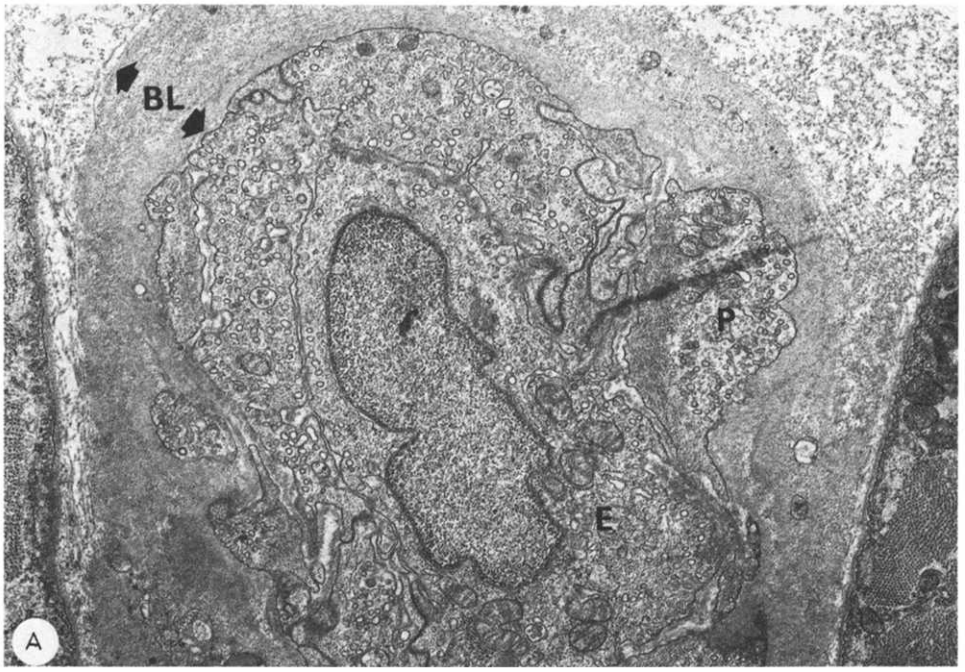


Fig. 4. (A) Transverse section through the gastrocnemius muscle of a control male Wistar rat aged 920 days. This collapsed blood capillary displays a highly thickened basal lamina from 500 to 1250 nm that contains within many vesicular outlines. Endothelial cell and pericyte cytoplasm appear quite normal. Magnification × 15,900. OsO_4 fixation. BL, basal lamina; E, endothelial cell; P, pericyte. (B) Transverse section through the soleus muscle of a male hypophysectomized Wistar rat aged 1050 days. This blood capillary shows no abnormal features. Endothelial cells and pericytes appear quite active, the former containing many pinocytotic vesicles, the latter displaying a Golgi body. Basal lamina (arrow) is of normal width, approximately 80 nm. Magnification × 15,000. E, endothelial cells; G, golgi body; P, pericyte; Pv, pinocytic vesicles; BL, basal lamina. (C) Electron micrograph from a longitudinal section through the gastrocnemius muscle of a food restricted male Wistar rat aged 1284 days. The transverse profile of the blood capillary demonstrates that only marginal thickening of the basal lamina (arrow) occurs when compared with aged control rats. At its thinnest (transverse) point it measures 360 nm in thickness. Magnification × 8,750. BL, basal lamina; E, endothelial cell.

Fig. 3. (A) Longitudinal section through the gastrocnemius muscle of a control male Wistar rat aged 1010 days. Myofibrillar breakdown is significant with only thin diffuse Z-bands (arrowed) remaining to support the sparse, degenerated myofibrils. The sarcoplasm contains few mitochondria, vesicles and fine filamentous remnants. Magnification × 14,700. OsO_4 fixation. Z, Z band; M, mitochondrion; MY, myofilaments. (B) Electron micrograph of a longitudinal section through the gastrocnemius muscle of a food restricted male Wistar rat aged 1284 days. There is no evidence of myofibrillar breakdown or structural abnormalities in mitochondria or t-tubules. Abnormal amounts of lipid were not detected. Similar normal electron micrographs were obtained from all old hypophysectomized rats. Magnification × 14,700. Fixed in Karnovsky's glutaraldehyde/formaldehyde fixative for 45 min and post-fixed in 1% OsO_4 for 2 h. Uranyl acetate and lead stained. MY, myofibril; Z, Z line; T, tubule; M, mitochondrion.

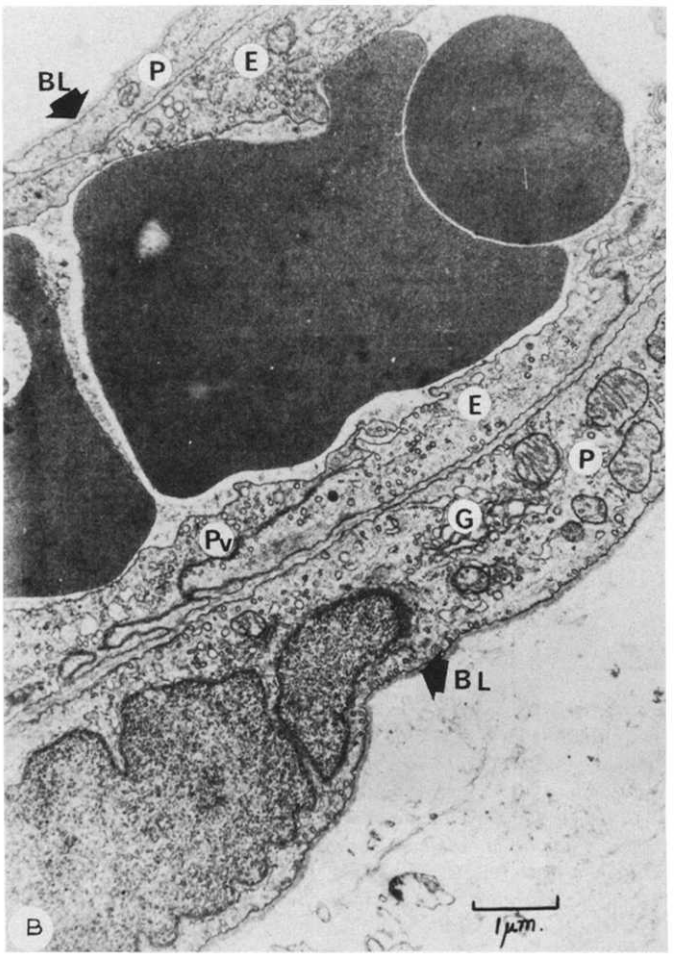


Fig. 4 (continued).

the paralysis of the hind legs, which is seen in 50% of ad libitum fed intact male rats aged 1 100 days (Table II). Similarly, long term food restriction beginning at 60 days was able to inhibit the development of this disease, which was seen in about 10% of old underfed rats. These observations confirm the earlier reports of the inhibitory actions of food restriction (Berg and Simms, 1961) and hypophysectomy (Everitt et al., 1980) on the development of hind leg paralysis in the old rat. Whether the hind leg paralysis is due to age related lesions in the peripheral nerves (Van Steenis and Kroes, 1971; Burek et al., 1976) is not clear. Berg et al. (1962) found that caloric restriction was able to retard the onset of hind leg muscle lesions, but did not affect the incidence of radiculoneuropathy in the cauda equina and other spinal roots.

Gastrocnemius muscle weight loss

In the old male Wistar rat between ages 500 and 1 000 days (17 and 33 mth) the

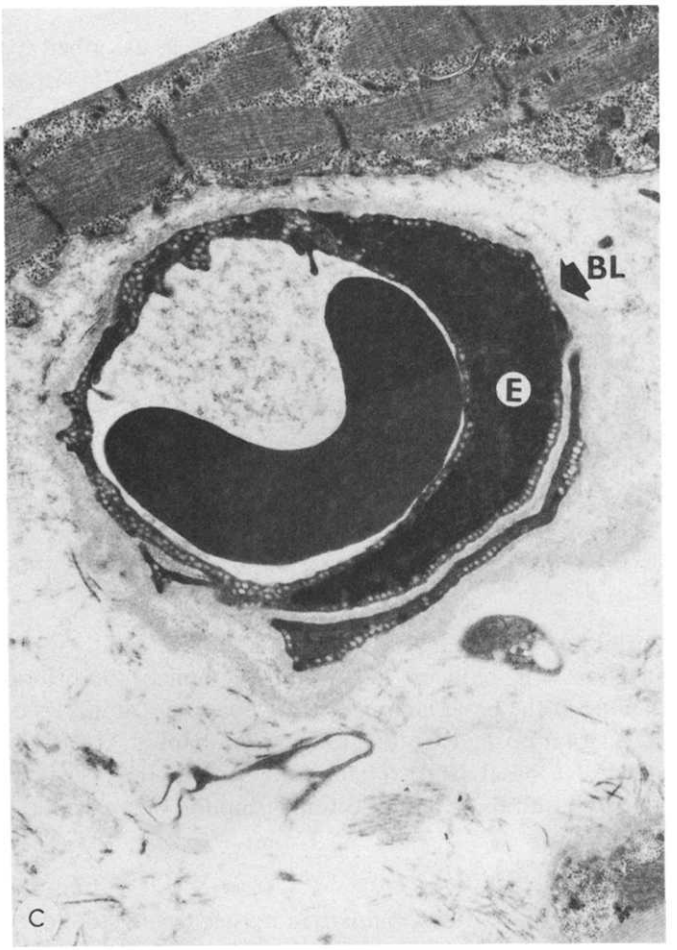


Fig. 4 (continued).

loss of weight from the gastrocnemius muscle occurred twice as fast as from the whole body (Table II). Similarly, Tauchi et al. (1971) observed a faster weight loss from the tibialis anterior muscle than the whole body between ages 12 and 24 mth in the male Wistar rat.

The age-related loss of weight from the gastrocnemius muscle occurred at a similar rate as whole body weight loss in rats that were hypophysectomized or food restricted from early life (Table II). The inhibition by food restriction has been reported by Yu et al. (1982) who noted that the weight of the gastrocnemius muscle of ad libitum fed rats began to decline after age 18 mth (540 days), while in food restricted rats the decline was delayed until 30 mth (900 days). The delay in weight loss could account for the difference observed between food restricted and ad libitum fed rats in the present study.

Degenerative changes

The myofibrillar breakdown and fibre atrophy in skeletal muscle described by earlier workers (Berg, 1967; Fujisawa, 1974; Hanzlikova and Gutmann, 1975; Anver and Cohen, 1979) were observed in old rats fed ad libitum throughout life (Figs. 2A and 3A). In the present study these changes were of rare occurrence in old hypophysectomized or food restricted rats, whose skeletal muscles usually displayed the normal banding pattern (Figs. 2B and 3B) seen in the young rat. This is consistent with the histopathological studies of Berg and Simms (1961) who showed that at age 900 days degenerative skeletal muscle lesions occurred in 30% of food restricted rats compared with 74% of ad libitum fed rats.

The degenerative changes were seen in all three muscles examined (gastrocnemius, soleus and biceps femoris). However, in the absence of quantitation it was not possible to estimate the percentage of affected fibres in different muscle types. There is some evidence for selective aging in certain muscle types. Gutmann (1977) has observed a more rapid decline in the number of muscle fibres in the slow soleus muscle compared with the fast extensor digitorum longus; there was a preferential atrophy of type II muscle fibers in the soleus.

Capillary basal lamina

Old ad libitum fed rats exhibited thickening of the basal lamina of capillaries in skeletal muscle. This thickening was reduced in hypophysectomized and food restricted rats. Similar thickening of the basal lamina of the glomerular capillaries of rat kidney has been reported (Johnson and Cutler, 1980; Wyndham et al., 1983). Our studies show that glomerular basal lamina thickening is inhibited by food restriction (Wyndham et al., 1983) and by hypophysectomy (unpubl. obs.).

Role of nerve supply and other factors

Whether the degenerative changes in muscle originate in muscle or are secondary to changes in the nerve supply is controversial. It is widely believed that 'events in the muscles are only secondary to the fate of motor neurons in the central nervous system' (Grimby and Saltin, 1983). On the basis of extensive physiological and morphological studies Gutmann and Hanzlikova (1972) have proposed functional denervation to explain the senile changes in rat skeletal muscle. This is supported by the work of Fujisawa (1976) whose morphological studies suggest denervation of motor end-plates in old age. However, as mentioned earlier, the work of Berg et al. (1962) casts doubt on the neurotrophic hypothesis, since food restriction retarded the onset of muscle lesions but did not affect the incidence of spinal nerve lesions.

Other factors may lead to muscle atrophy and degeneration such as lack of exercise, inadequate nutrition and hormonal deficiencies (Gutmann, 1977). Disuse or the immobilization of a limb (Cooper, 1972) produces degenerative changes in muscle fibres which are very similar to those seen in normal aging. Disuse atrophy may contribute to muscle aging changes in old ad libitum fed control rats which are more sluggish in their movements than young controls and are less active than food restricted and hypophysectomized rats of similar age.

In the old rat the accumulation of lipofuscin granules or degenerative inclusion bodies may act as a diffusion barrier around muscle capillaries restricting nutrient supply and so accelerate muscle fibre atrophy and degeneration.

It might be thought that age-related increases in body fat content in ad libitum fed control rats could contribute to the decrease in gastrocnemius muscle weight: body weight ratio from 5.65 mg/100 g at 500 days to 3.72 mg/100 g at 950 days (Table II). This is not supported by the work of Bertrand et al. (1980) which indicates that the maximum fat content of the ad libitum fed male Fischer rat is found at 70% of the life span at about 20 mth (600 days) and that the loss of fat with age usually occurs more rapidly than the loss of body mass. Lesser et al. (1973) also find a fall in the percentage of body fat in the old male Sprague–Dawley rat. These studies suggest that both fat depots and gastrocnemius muscle are losing weight at a faster rate than the whole body.

Mechanism of anti-aging actions of food restriction and hypophysectomy

Food restriction and hypophysectomy have very similar actions since both inhibit the aging of collagen fibres in tail tendon, delay the onset of various diseases of old age (renal disease, hind leg paralysis, tumours) and prolong life (Everitt et al., 1980; Everitt et al., 1983). The mechanisms are not clear, but there is considerable interaction. Chronic food restriction causes a fall in the secretion of pituitary hormones (Campbell et al., 1977) while hypophysectomy reduces food intake (Everitt et al., 1980). Apart from the direct aging effects of dietary and hormonal factors, nutrients can alter the secretion of pituitary and other hormones which have aging actions (Everitt et al., 1983). Although the mechanisms of the anti-aging effects of food restriction and hypophysectomy are controversial, the inhibitory actions of these procedures on aging hind leg muscle are clear cut.

Acknowledgements

These studies were supported in part by grants from the Consolidated Medical Research Fund of the University of Sydney and the University Research Grant. Cortisone acetate was generously supplied by Roussel Pharmaceuticals, Sydney. The authors are especially grateful to Frank Jones, who performed the hypophysectomies.

References

Anver, M.R. and Cohen, B.J. (1979): Lesions associated with aging. In: The Laboratory Rat, pp. 377–399. Editors: H.J. Baker, J.R. Lindsey and S.H. Weisbroth. Academic Press, New York.

Ariano, M.A., Armstrong, R.B. and Edgerton, V.R. (1973): Hindlimb muscle fibre populations of five mammals. J. Histochem. Cytochem., 21, 51–55.

Berg, B.N. (1956): Muscular dystrophy in aging rats. J. Gerontol., 11, 134–139.

Berg, B.N. (1967): Longevity studies in rats. II. Pathology of ageing rats. In: Pathology of Laboratory Rats and Mice, pp. 749–786. Editors: E. Cotchin and F.J.C. Roe. Blackwell Scientific Publications, Oxford.

Berg, B.N. and Simms, H.S. (1961): Nutrition and longevity in the rat. III. Food restriction beyond 800 days. J. Nutr., 74, 23–32.

Berg, B.N., Wolf, A. and Simms, H.S. (1962): Nutrition and longevity in the rat. IV. Food restriction and the radiculoneuropathy of aging rats. J. Nutr., 77, 439–442.

Bertrand, H.A., Lynd, F.T., Masoro, E.J. and Yu, B.P. (1980): Changes in adipose mass and cellularity throughout the adult life of rats fed ad libitum or a life-prolonging restricted diet. J. Gerontol., 35, 827–835.

Burek, J.D., Van der Koge, A.J. and Hollander, C.F. (1976): Degenerative myelopathy in three strains of aging rats. Vet. Pathol., 13, 321–331.

Caccia, M.R., Harris, J.B. and Johnson, M.A. (1979): Morphology and physiology of skeletal muscle in aging rodents. Muscle Nerve, 2, 202–212.

Campbell, G.A., Kurcz, N., Marshall, S. and Meites, J. (1977): Effects of starvation in rats on serum levels of FSH, LH, thyrotropin, growth hormone and prolactin: response to LH-releasing hormone and thyrotropin releasing hormone. Endocrinology, 100, 580–587.

Cooper, R.R. (1972): Alterations during immobilization and regeneration of skeletal muscle in rats. J. Bone Joint Surg., 54A, 919–953.

Everitt, A.V., Seedsman, N.J. and Jones, F. (1980): The effects of hypophysectomy and continuous food restriction, begun at ages 70 and 400 days, on collagen aging, proteinuria, incidence of pathology and longevity in the male rat. Mech. Aging Dev., 12, 161–172.

Everitt, A.V., Wyndham, J.R. and Barnard, D.L. (1983): The anti-aging action of hypophysectomy in hypothalamic obese rats: effects on collagen aging, age-associated proteinuria development and renal histopathology. Mech. Aging Dev., 22, 233–251.

Fujisawa, K. (1974): Some observations of the skeletal musculature of aged rats. Part 1. Histological aspects. J. Neurol. Sci., 22, 353–366.

Fujisawa, K. (1975): Some observations of the skeletal musculature of aged rats. Part 2. Fine morphology of diseased muscle fibres. J. Neurol. Sci., 24, 447–469.

Fujisawa, K. (1976): Some observations on the skeletal musculature of aged rats. III. Abnormalities of terminal axons found in motor end plates. Exp. Gerontol., 11, 43–47.

George, J.C. and Scaria, K.S. (1958): A histochemical study of dehydrogenase activity in the pectoralis major muscle of the pigeon and certain other vertebrate skeletal muscles. Q. J. Microscopic Sci., 99, 469–473.

Grimby, G. and Salten, B. (1983): The ageing muscle. Clin. Physiol., 3, 209–218.

Gutmann, E. (1977): Muscle. In: Handbook of the Biology of Aging, pp. 445–469. Editors: C.E. Finch and L. Hayflick. Van Nostrand Reinhold Company, New York.

Gutmann, E. and Hanzlíková, V. (1972): Basic mechanisms of aging in the neuromuscular system. Mech. Ageing Dev., 1, 327–349.

Gutmann, E., Hanzlíková, V. and Vyskocil, F. (1971): Age changes in cross-striated muscle of the rat. J. Physiol., 219, 331–343.

Hanzlíková, V. and Gutmann, E. (1975): Ultrastructural changes in senile muscle. Adv. Exp. Med. Biol., 53, 421–429.

Johnson, J.E. Jr. and Cutler, R.G. (1980): Effect of hypophysectomy on age-related changes in the rat kidney glomerulus: observations by scanning and transmission electron microscope. Mech. Ageing Dev., 13, 63–74.

Karnovsky, J.J. (1965): A formaldehyde–glutaraldehyde fixation of high osmolarity for use in electron microscopy. J. Cell Biol., 27, 137–138.

Koyama, R. (1962): A simple method of hypophysectomy in rats. Endocrinol. Jpn., 9, 321–323.

Lesser, G.T., Deutsch, S. and Markofsky, J. (1973): Aging in the rat: longitudinal and cross-sectional studies of body composition. Am. J. Physiol., 225, 1472–1478.

Palade, G.E. (1952): A study of fixation in electron microscopy. J. Exp. Med., 95, 285–298.

Spurr, A.R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26, 31–43.

Tauchi, H., Yoshioka, T. and Kobayashi, H. (1971): Age change of skeletal muscles of rats. Gerontologia, 17, 219–227.

Van Steenis, G. and Kroes, R. (1971): Changes in the nervous system and musculature of old rats. Vet. Pathol., 8, 320–332.

Venable, J.H. and Coggeshall, R. (1965): A simplified lead citrate stain for use in electron microscopy. J. Cell Biol., 25, 407–408.

Wyndham, J.R., Everitt, A.V. and Everitt, S.F. (1983): Effects of isolation and food restriction begun at 50 days on the development of age-associated renal disease in the male Wistar rat. Arch. Gerontol. Geriatr., 2, 317–332.

Yu, B.P., Masoro, E.J., Murata, I., Bertrand, H.A. and Lynd, F.T. (1982): Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. J. Gerontol., 37, 130–141.