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Effects of ageing and chronic dietary restriction on the morphology of fast and slow muscles of the rat

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INTRODUCTION

Study of the development and ageing of skeletal muscle is important for several reasons. Firstly, skeletal muscle constitutes the largest single tissue mass in the mammal, representing 25–45% of total body weight between birth and the mature adult (Goldspink, 1980) so that an understanding of the developmental changes taking place in this tissue will shed light on the growth of the whole body. Secondly, the considerable plasticity exhibited by muscle in response to environmental changes makes it an ideal tissue for the identification of the physiological factors that determine gene expression in terms of cell size, metabolism and contractile properties (Pette, 1980). Thirdly, many of the results of ageing manifest themselves as declining motor abilities, such as a loss of strength, speed, flexibility and to a lesser extent, endurance. Many of these changes are associated with altered structural and functional characteristics of skeletal muscle (Grimby & Saltin, 1983).

A useful model for studying the processes of ageing is that of dietary restriction in rodents. Chronic reduction of calorie intake in rats, particularly if imposed soon after weaning, is associated with an increased lifespan of between 36–66% (Merry & Holehan, 1985). Although the exact mechanisms underlying this dramatic extension of longevity remain obscure, controlled underfeeding is known to reduce the frequency of spontaneous tumours and postpone the onset of many age-related diseases, such as the cardiomyopathies and nephropathies. The effects of such dietary restriction from weaning to senescence have been examined in relation to protein turnover and developmental growth, both of the whole body (Lewis, Goldspink, Phillips & Merry, 1985) and of individual skeletal muscles (El Haj *et al.* 1986). However, little is currently known about the morphological changes in skeletal muscle brought about by such dietary manipulations. This study primarily investigates these changes from weaning to senescence in a fast- and a slow-twitch muscle of the rat. In addition, this investigation affords the opportunity to evaluate normal developmental changes throughout the lifespan of the rat from a range of structural and functional parameters in these two types of skeletal muscle.

MATERIALS AND METHODS

Male Sprague–Dawley rats (CFY strain) were supplied by the Wolfson Institute of Gerontology, the University of Hull, England. Animals were weaned at 21 days, randomly allocated to control (C) or dietary-restricted (DR) groups and individually caged. Control animals were fed *ad libitum* with a pelleted diet (formula 41 B, E. H. Bradshaw & Sons Ltd, Driffield, England). Dietary-restricted animals were pair-fed an identical diet, to 50% of the control group food intake. All animals were kept in conditions of constant temperature (21 °C) and humidity (50–70%) with a regimen of continuous light from 08.00 to 20.00 hours (B.S.T.) and with free access to water. In all, ten groups of 4–6 animals were killed at the following ages: 3, 7, 52, 91, 103, 121 and 149 weeks. Animal weights and the soleus and extensor digitorum longus (EDL) muscle weights were measured.

The soleus and EDL muscles of the hindlimb were selected for quantitative analysis, while a third muscle, the flexor digitorum profundus of the forearm, was subject to qualitative examination only.

Portions were taken from the mid-belly of all three muscles and frozen in isopentane cooled in liquid nitrogen. Transverse sections 10 μm thick were cut in a cryostat and serially stained using a variety of histochemical and histological procedures. Muscle fibre types were identified using the myosin ATPase reaction (Tunnell & Hart, 1977) as a determinant of slow oxidative (SO), fast oxidative glycolytic (FOG), fast glycolytic (FG) and intermediate (INTER) fibres (Peter *et al.* 1972; Kugelberg, 1976). Capillaries were visualised with the indoxyl-tetrazolium method for alkaline phosphate (Ziada, Hudlická, Tyler & Wright, 1984), and connective tissue with Picro Sirius Red/Acid Fuchsin (Constantine, 1969).

Sections stained for myosin ATPase activity were analysed for fibre type composition and total fibre number using direct counts from composite photomicrographs. Muscle fibre diameters were measured using a Leitz TAS Plus image analyser system (Leitz, Wetzlar, West Germany). The 'lesser fibre diameter' (Dubowitz & Brooke, 1973) of at least 150 fibres per fibre type was measured for each muscle and the means combined to give an overall mean fibre diameter.

Capillary analysis was carried out using the image analyser on sections stained for alkaline phosphatase (Ziada *et al.* 1984) at a magnification that ensured visualisation of capillaries with or without reaction product. For each section, 6–12 randomly chosen fields containing approximately 300 muscle fibres were analysed. Fields were rejected if the orientation of the section appeared to be oblique to the fibres' long axis. All capillary profiles in the field were counted, while vessels obviously larger than capillaries were not. Muscle fibre numbers were also counted for each field, including those fibres bisected by the perimeter of the frame on two sides only. Results were expressed as the capillary density (number of capillaries per mm^{-2}) and the capillary to fibre ratio.

Those muscle sections stained with Picro Sirius Red/Acid Fuchsin were used to analyse connective tissue content. This dye stains the connective tissue bright red which, under a green optical filter, appears black and contrasts well with the pale muscle fibres. Slides were examined with a Leitz microscope to which was attached a high resolution video camera. The video information was subsequently fed into a digitising interface (Computec Diplomat) connected to a peripheral slot of microcomputer (Apple II) which converted the video signal into a computer graphic image with two selectable grey levels. Using a control slide, the video image was adjusted by means of a threshold facility so that there was good correspondence

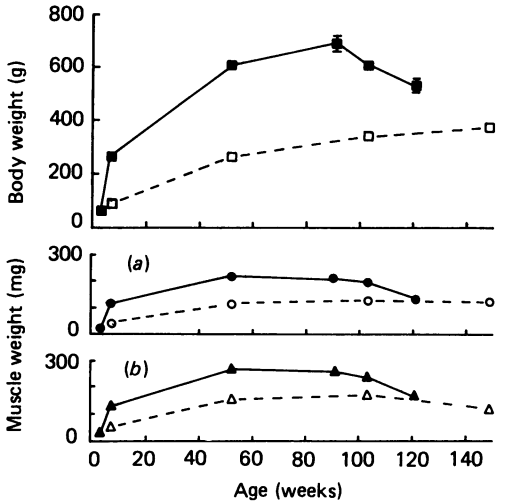

Fig. 1 (*a–b*). Whole body weights (top) and soleus (*a*) and EDL (*b*) muscle weights for control (closed symbols) and diet-restricted (open symbols) animals. Each point represents $\bar{x} \pm$ S.E.M. for a minimum of 5 animals. All values for diet-restricted groups are significantly different ($P < 0{\cdot}01$) from age-matched control values.

between the optical and video images. No further adjustments were made while taking readings of slides from all experimental and control tissues for a given age group. Muscle sections were scanned using a small area of known dimensions, and readings taken of the connective tissue content. Four experimental and four control muscles were scanned for each age group and ten readings taken from each muscle.

Data were subjected to standard statistical procedures (Sokal & Rohlf, 1981). Group means and standard errors were calculated. Both one- and two-way ANOVAs for independent samples were carried out to test for significant differences over time and between the different dietary regimes. Data in the form of percentages were transformed using the angular transformation into a variable meeting the assumptions of the analysis of variance. Muscle capillary data were also analysed using linear regression. F ratios were calculated to test for significant differences between regression slopes for control and experimental groups, and between the different muscles. For all statistical tests, differences between means were regarded as significant when a value of $P < 0{\cdot}05$ was obtained.

RESULTS

Body and muscle weights

Whole body growth and that of individual muscles followed broadly parallel courses throughout the lifespan of the rat (Fig. 1). Growth in the control animals was most rapid between 3 and 7 weeks, followed by a period of steady weight gain to 52 weeks. Between 52 and 91 weeks of age, body and muscle weights remained relatively stable, but fell rapidly thereafter in old age (Fig. 1). Dietary restriction suppressed growth, most noticeably at 7 weeks of age. This resulted in reduced body and muscle weights to approximately 50% of control values by one year (Fig. 1). In old age, dietary intervention prevented weight losses in the whole body and in the soleus muscle, and delayed the atrophic changes in the EDL.

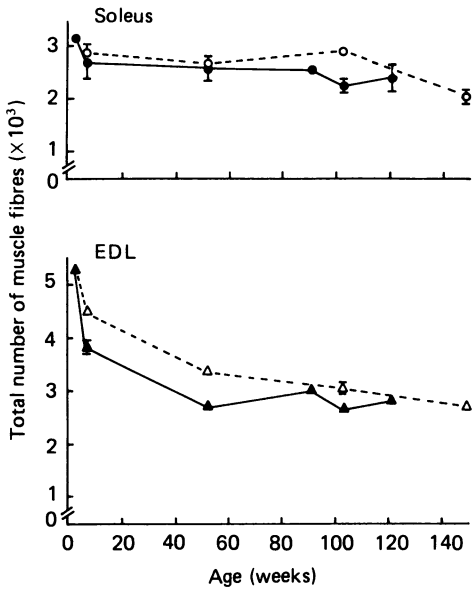

Fig. 2. Fibre numbers were counted directly from transverse sections of muscles from both control (closed symbols) and diet-restricted (open symbols) animals. Each value represents $\bar{x} \pm$ S.E.M. for a minium of 5 tissues.

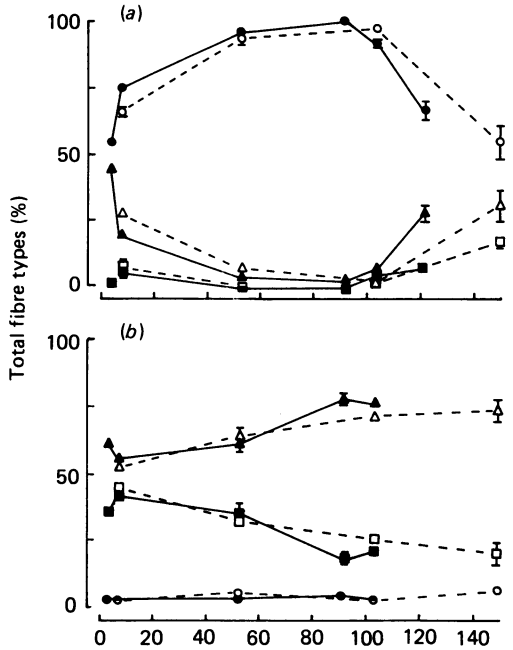

Fig. 3(*a–b*). Fibre type proportions were assessed directly from transverse sections of muscle stained histochemically for formalin preincubated myosin ATPase in both soleus (*a*) and EDL (*b*) muscles. Each point represents $\bar{x} \pm$ S.E.M. for a minumum of 5 muscles. Control: SO, ●—●; FOG, ▲—▲; INTER (Sol.) FG (EDL) ■—■. Diet-restricted: SO, ○----○; FOG, △----△; INTER (Sol.) FG (EDL) □---□.

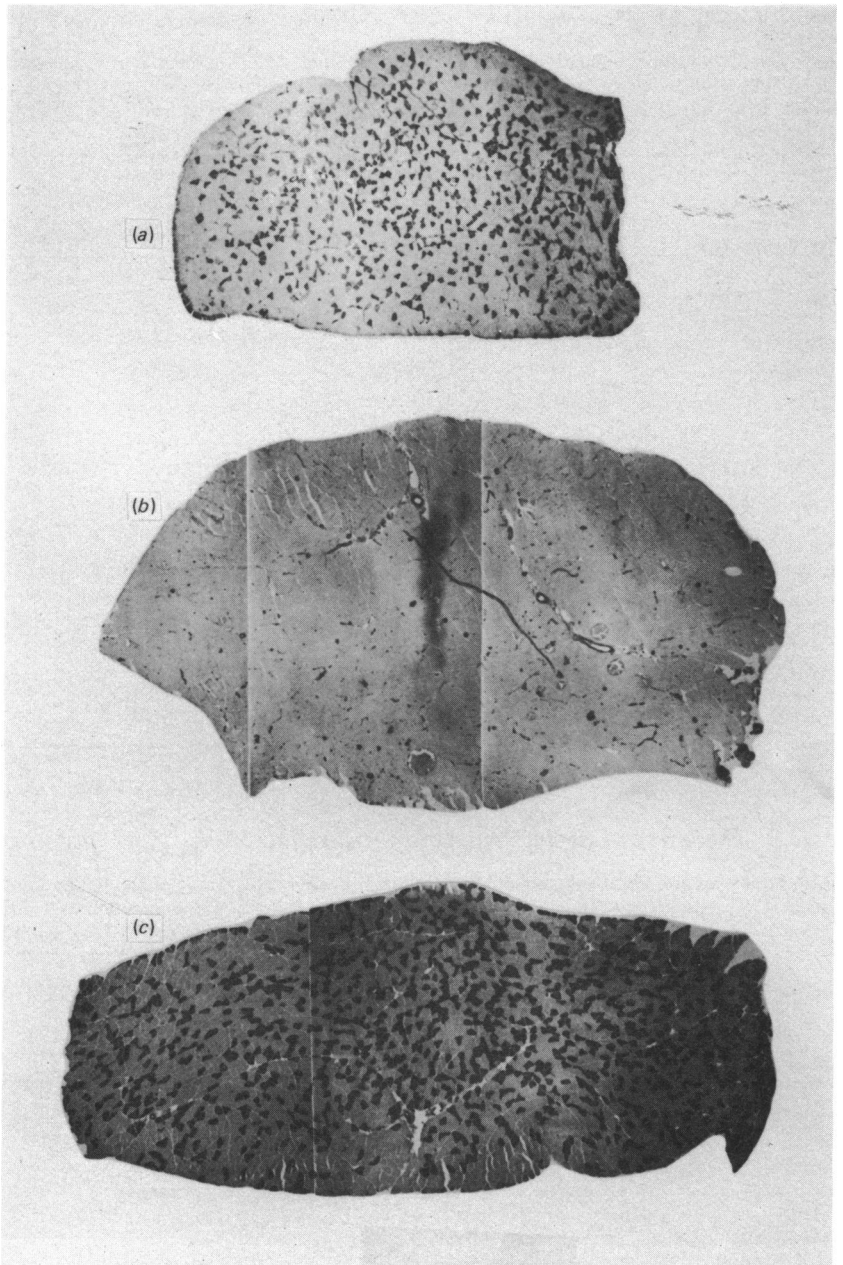

Fig. 4(*a–c*). The transformation in the soleus muscle from a mixed fibre type population at 7 weeks (*a*) to a totally slow fibre population at 91 weeks (*b*) and back to a mixed population at 121 weeks (*c*) is illustrated in these transverse sections of whole muscles. All sections were taken from control animals, and were stained for formalin preincubated ATPase.

Muscle fibre numbers and fibre types

Irrespective of diet, muscle fibre numbers in the soleus remained stable between 3 and 103 weeks of age (Fig. 2). By 149 weeks, however, the soleus of the food-restricted rats displayed a 30% reduction in fibre numbers ($P < 0{\cdot}01$). In contrast, the fibre complement of the EDL from normally fed rats fell dramatically by 50% between

Table 1. *The effect of ageing and dietary restriction on muscle capillary density*

(Muscle capillaries from control (C) and dietary restricted (DR) animals were counted from randomly chosen fields of known dimensions and expressed as capillaries per mm^{-2}. ($\bar{x} \pm$ S.E.M. for a minimum of 3 muscles per group). Brackets indicate the direction of statistical comparison (**$P < 0.01$).)

Age (weeks)	Soleus C		Soleus DR	E.D.L. C		E.D.L. DR
3	1339 ±24			1721 ±48		
7	666 ±47	〈**〉	903 ±36	677 ±55	〈**〉	1063 ±63
52	656 ±34		729 ±32	552 ±61		705 ±78
103	564 ±46		677 ±16	764 ±111		682 ±27
121	859 ±86			949 ±262		
149			660 ±88			898 ±128

Table 2. *The effect of ageing and dietary restriction on capillary/fibre ratio*

(Muscle capillaries and fibres were counted in randomly chosen fields from control (C) and dietary restricted (DR) muscles and expressed as capillaries per fibre ($\bar{x} \pm$ S.E.M. for a minimum of 3 muscles per group). Brackets indicate the direction of statistical comparison (*$P < 0.05$; **$P < 0.01$).)

Age (weeks)	Soleus C		Soleus DR	E.D.L. C		E.D.L. DR
3	1·26 ±0·03			0·89 ±0·02		
7	2·17 ±0·05	〈**〉	1·30 ±0·03	1·46 ±0·06	〈*〉	0·96 ±0·03
52	3·07 ±0·07	〈**〉	1·96 ±0·08	2·12 ±0·12	〈**〉	1·62 ±0·08
103	3·33 ±0·19	〈**〉	2·21 ±0·12	2·34 ±0·25	〈*〉	1·90 ±0·04
121	2·46 ±0·24			1·83 ±0·18		
149			2·49 ±0·08			1·90 ±0·13

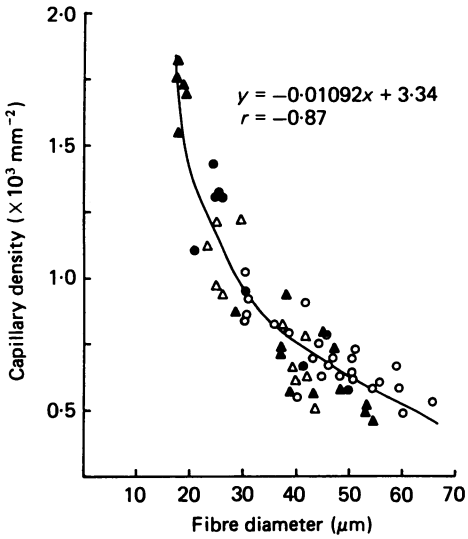

Fig. 5. The relationship between fibre diameter and capillary density. Linear regression analysis was carried out on transformed (log_{10}) data for both soleus (circles) and EDL (triangles) muscles from control (closed symbols) and diet-restricted (open symbols) animals. No significant differences were found between either muscle types or dietary regimes, resulting in the common slope of regression shown here.

weaning and one year of age. Although dietary restriction did not prevent this fibre loss over the animals' lifespan, it did effectively delay it (Fig. 2).

Developmental changes in the proportions of the various fibre types were observed in both hindlimb muscles (Fig. 3). These were most noticeable in the soleus, which nearly doubled its percentage of SO fibres with a corresponding fall in the proportion of FOG fibres between weaning and 91 weeks (Figs. 3, 4). This was then followed by a significant ($P < 0{\cdot}01$) reversal of this trend, so that in senescence the fibre type profile of the soleus resembled that of the 7 weeks old animals once again (Figs. 3, 4). Interestingly, the proportion of intermediate fibres, thought to be muscle cells undergoing transformation (Kugelberg, 1976), was highest at periods of greatest fibre type transition, i.e. in adolescence and old age (Fig. 3). The EDL displayed a more gradual, but significant ($P < 0{\cdot}01$) transformation of FG to FOG fibres from weaning to senescence (Fig. 3). Thus, both muscles underwent significant shifts in their fibre type 'profiles' over the lifespan of the rats. These changes appeared to be minimally affected by the imposition of dietary restriction (Fig. 3), except for possible delays in these transitions in the soleus in senescence (Fig. 3*a*).

Muscle capillarisation

When age- and dietary-matched tissues were compared, the EDL generally displayed higher values for capillary density than the soleus muscle (Table 1). There was a developmental fall in the capillary density in both muscles between 3 and 7 weeks. Food restriction delayed and extended this developmental fall in capillary density (Table 1). In contrast with capillary density, the capillary:fibre ratio showed the opposite developmental trend, i.e. a general increase with age (Table 2). At any given age ratios were consistently higher in the soleus than the EDL, and in control muscles compared with the experimental muscles from diet-restricted animals. These

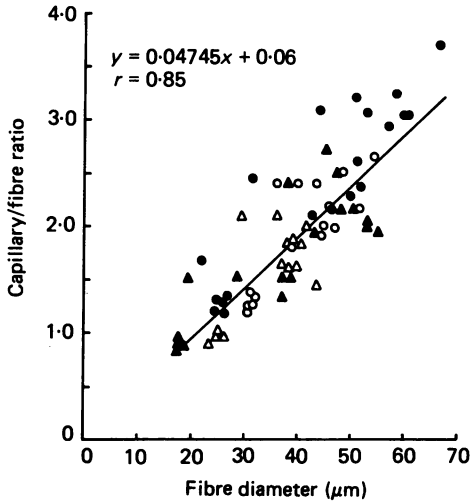

Fig. 6. The relationship between fibre diameter and capillary/fibre ratio. Linear regression analysis was carried out on data from both soleus (circles) and EDL (triangles) muscles from controlled (closed symbols) and diet-restricted (open symbols) animals. No significant differences were found between either muscle types or dietary regimes, resulting in the common slope shown here.

results may be misleading, however, as several authors have noted a dependence of muscle capillarity upon muscle fibre size (Plyley & Groom, 1975; Aquin, Sillau, Lechner & Banchero, 1980). When these two variables were plotted graphically, characteristic slopes of regression emerged for both capillary density and the capillary:fibre ratio (Figs. 5 and 6 respectively). In both cases, further analysis revealed no significant difference between slopes for the two muscle types or the two dietary regimes.

Connective tissue

Both the soleus and the EDL displayed large and significant increments in the ratio of endomysial connective tissue:muscle tissue during growth (Table 3). Measurements made on the muscles from diet-restricted animals indicated a generally slower rate of increase, so that by 103 weeks of age the connective tissue content was approximately one half that of the control muscles. At almost all ages, the slow-twitch soleus contained more connective tissue than the faster EDL, regardless of dietary status (Table 3). Although these results give a good indication of relative differences in connective tissue, no account is taken of variations in muscle fibre cross sectional area. It is evident that the amount of interstitial space per unit of muscle area is reduced as the individual fibres grow. Thus the differences in connective tissue content between young and older (larger) muscles, and similarly between age-matched, diet-restricted and control muscles, are likely to be underestimated by image analysis.

Other morphological changes

Qualitative examination of muscle sections from older rats revealed several features characteristic of denervation/reinnervation processes (Gutmann, 1977). These included fibre atrophy, compensatory fibre hypertrophy, irregular fibre shapes, fibre splitting and fibre type grouping (Fig 7*a*). Such features tended to occur earlier in control (103 weeks) than in diet restricted animals (149 weeks), and more frequently

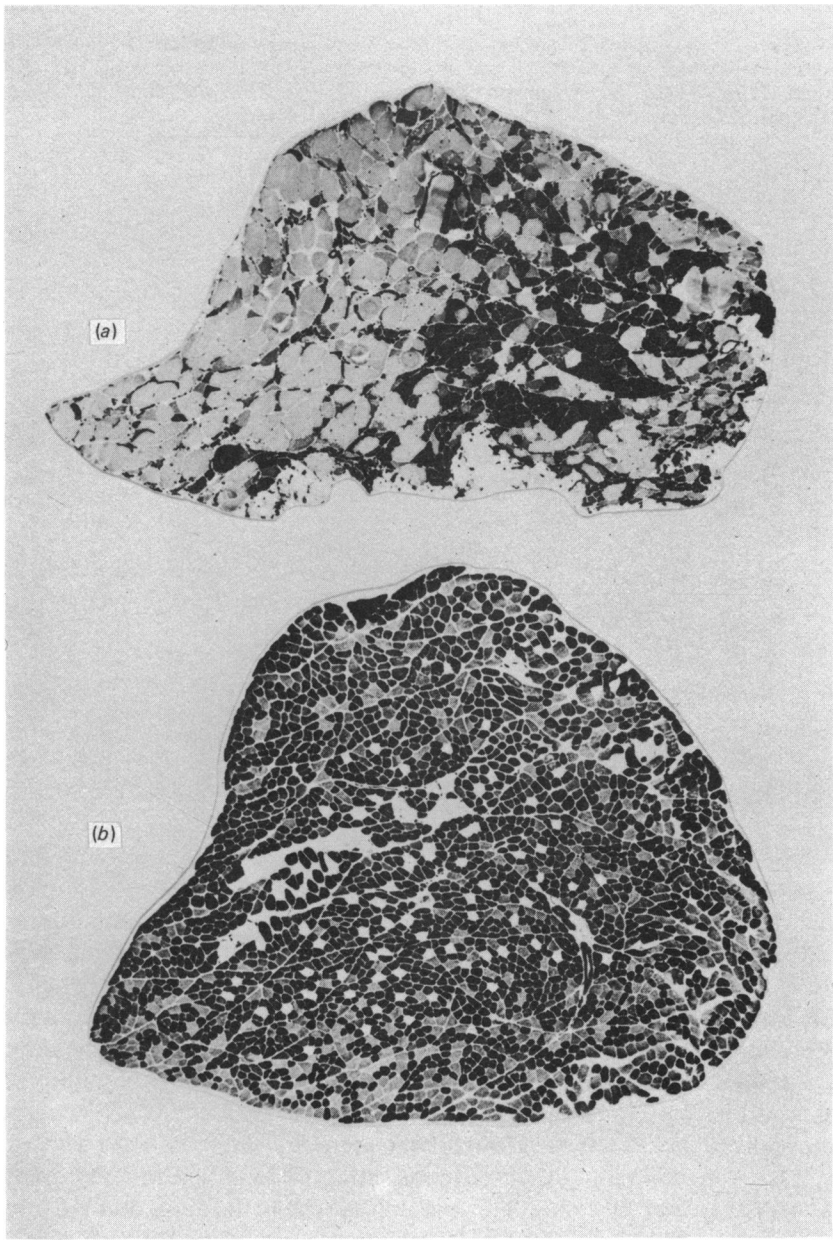

Fig. 7. (*a–b*). Transverse sections of whole soleus (*a*) and flexor digitorum profundus (*b*) muscles from the same animal (149 weeks, DR) illustrate the earlier age-related decline of the slower soleus. Formalin preincubated ATPase.

in the slow soleus than the faster muscle at a given age. This was most apparent when comparing the soleus and the flexor digitorum profundus muscle of the forelimb in rats 149 weeks old (Fig. 7). However, it should be stressed that the onset of age-related pathological features showed considerable animal to animal variation within a given cohort of rats.

Table 3. *The effects of ageing and dietary restriction on muscle connective tissue*

(Transverse sections of muscles from both control (C) and dietary restricted (DR) animals were histochemically stained for connective tissue and subjected to quantitative image analysis. Results are expressed as $\bar{x} \pm$ S.E.M. for a minimum of 4 muscles per group. Brackets indicate the direction of statistical comparison (**$P < 0.01$).)

	Endomysial connective tissue (counts per unit area)			
	Soleus		E.D.L.	
Age (weeks)	C	DR	C	DR
3	1068 ±61		1189 ±340	
7	4848 ±158	3715 ±155	3694 ±172	4009 ±145
52	7586 ±290	5114 ±235	5273 ±334	2792 ±333
103	12169 ±447	6752 ±375	7812 ±334	4423 ±333
149		6639 ±445		6194 ±386

DISCUSSION

Normal developmental growth of the whole animal and its musculature seems to rely upon a combination of hormonal, nutritional and mechanical factors (Pette, 1980). It is clear from the present study that the imposition of chronic dietary restriction at weaning had profound effects upon the subsequent growth of muscle in these experimental animals. These effects manifested themselves both as a reduction in the rate of growth and a prolongation of that growth into old age, compared with age-matched controls.

The most profound effects on growth were seen at 7 weeks of age, when the diet-restricted rats were still eating their reduced ration of food within a few hours of its presentation (El Haj *et al.* 1986). This piecemeal type of feeding, and the prolonged post-absorptive state associated with it, have been shown to have acute effects on skeletal muscle protein synthesis, with attendant reductions in the protein contents of tissues (El Haj *et al.* 1986). By 52 weeks of age these animals had fully adjusted to eating their reduced daily ration of food over a full 24 hour period, in a similar manner to the control animals. Therefore, the continued retardation of growth in the mature experimental rats may be regarded as resulting solely from the long-term chronic effects of dietary restriction, without the superimposed acute effects of piecemeal feeding. These chronic effects of dietary restriction on growth and ageing in the rat have been shown to be associated with a slowing of the normal developmental decline in the fractional rates of whole body protein synthesis and breakdown as observed in normally fed control animals (Lewis *et al.* 1985).

The present data show that, regardless of diet, the large increments in skeletal musculature associated with maturation were due to cell hypertrophy and not hyperplasia. Indeed, while the fibre complement of the soleus muscle remained relatively stable between 3 and 53 weeks of age, the number of fibres in the EDL control muscle declined by approximately 50%. Fibre loss during growth in the rat EDL has been reported previously (Layman, Hegarty & Swan, 1980). However, these results are at variance with the generally held opinion that muscle fibre numbers remain constant from soon after birth (Goldspink, G., 1980; Gollnick, Timson, Moore & Riedy, 1981). Many of the discrepancies between studies can be explained by differences in the methodologies and species employed. For example, the age and strain of rat, method of fibre counting (direct or indirect) and architecture of the muscle chosen for study may all affect the results obtained. Despite the careful methods employed in this study and the magnitude of the reported changes during early post-weaning growth, it remains questionable whether there is a true developmental loss of fibres from the EDL for two reasons. Firstly, there was no microscopic evidence of the processes of fibre degeneration or fusion which would be necessary to explain a true disappearance of cells (Layman *et al.* 1980). Secondly, Gollnick *et al.* (1981), employing a direct method of counting whole, isolated fibres from the EDL muscle of female Sprague–Dawley rats, showed that fibre counts at 13–15 weeks of age were approximately 5200, i.e. comparable with the counts for the EDL muscles of the newly weaned male animals in the present study. Compared with the simple geometry of the soleus muscle, the arrangement of fibres in the rat EDL is relatively complex. It is possible that the total fibre complement may not be included in a single transverse section through the mid-belly of this muscle, particularly as the muscle enlarges during growth (Gollnick *et al.* 1981). Thus it seems likely that the substantial loss of fibres reported here for the growing EDL may be more apparent than real.

The loss of fibres in the ageing soleus has been noted previously (Larsson & Edström, 1986). Gutmann (1977) stated that senescent muscle fibre loss is an end product of the progressive deterioration of neuromuscular contact leading to a random loss of fibres. This would appear to be the case, as those solei undergoing the greatest fibre losses also tend to demonstrate many of the pathological features associated with denervation and reinnervation (see Fig. 7).

The dramatic shift towards a slower fibre type profile in the soleus muscle of the growing rat is in agreement with previous findings (Kugelberg, 1976; Cacchia, Harris & Johnson, 1979). Since the increase in the proportion of SO fibres occurred within a relatively stable total fibre population, it can be concluded that a true conversion of fast fibres to slow occurred, rather than a selective depletion of the fast fibre population. It is well established that the specialisation of fibre types is determined primarily by the innervation supplying individual motor units (Pette & Vrbová, 1985). It follows that interconversions of muscle fibre types may involve some sort of modification to their neural input. Although this could conceivably have involved processes of denervation and reinnervation in the rat solei, there were no signs of the morphological features associated with these processes. A more likely cause was an increase in the total daily contractile activity of the FOG fibres of the soleus, as the relative postural load increased in the growing rats. Chronic increases in the postural load of the soleus by removal of its synergists (Guth & Yellin, 1971) or by enhancing gravitational forces (Martin & Romond, 1975) result in longer contraction times and a relative increase in slow fibres of the rat soleus. On the other hand, a reduction in the activity of this muscle by joint immobilisation (Booth & Kelso, 1973) or

suspension (Templeton *et al.* 1984) results in a shortening of contraction time and a relative increase in the proportion of fast fibres of the soleus, while the faster muscles of the hindlimb are not affected to any great extent (Elder, 1983). These observations suggest that slow motor units may be uniquely dependent upon contractile activity for the development and maintenance of their properties. As slow motor units are more efficient at maintaining sustained contractions (Crow & Kushmerick, 1982), the developmental change to a slower soleus probably conveys a greater metabolic economy in this muscle as its habitual workload increases. This may also apply to the EDL, which showed evidence of fibre type conversion (from FG to FOG) between one and two years of age ($P < 0{\cdot}05$) when total fibre numbers were stable. Similar changes consequent upon enhanced contractile activity of fast muscles have been recorded (Watt, Kelly, Goldspink & Goldspink, 1982).

Increased proportions of fast fibres have previously been noted in the solei of senile rats (Cacchia *et al.* 1979). These fibre type changes are confirmed in the present study. In the light of the preceding discussion, it seems plausible that this fibre type reconversion was at least partly due to a reduced postural load, as the ageing rats became increasingly lighter and more sedentary. Evidence relating contractile activity to fibre type expression in old age is provided by Stebbins, Schultz, Smith & Smith (1985), who found significantly more slow-twitch fibres in the soleus muscles of exercised senile rats than sedentary controls.

The rapid post-weaning growth of skeletal muscles resulted in substantial changes in the capillary bed, with a clear inverse relationship between muscle size and capillary density for both muscles. Despite this rapid reduction in capillary density during growth, adequate cellular diffusion is maintained by several compensatory mechanisms. The cross sectional profiles of the fibres may elongate to reduce diffusion distances to the cell core, concentrations of respiratory pigments may rise to aid oxygen transportation within the cell (Aquin *et al.* 1980) and, perhaps of greatest significance, new capillaries may form to increase the capillary:fibre ratio. Such angiogenesis may be initiated chemically by the intracellular accumulation of metabolites associated with hypoxia in the enlarging and increasingly active muscle fibres, or result from mechanical factors related to intracapillary blood flow (Hudlická, 1984). It is apparent from the present data that the large differences in muscle capillarity between diet-restricted and control groups of the same age were not significant when related to average fibre size. This indicates that prolonged food deprivation had no direct effect on muscle capillarity. In a similar manner, the marked differences in the indices of capillarity between the fast EDL and the slow soleus muscles disappeared when these indices were related to muscle fibre size. This is somewhat surprising in view of the reported correlation between muscle oxidative capacity and capillarity (Hoppeler, Hudlická & Uhlmann, 1983). A likely explanation for the similar patterns of vascularity for fast and slow muscles at a given average fibre size lies in the high proportion of FOG fibres in the rat EDL. These fibres, which constitute over half the cross sectional area of the EDL, are known to be highly oxidative and abundantly supplied with capillaries (Gray & Renkin, 1978). The results of this study, therefore, confirm that both fibre size and fibre oxidative capacity are important determinants of muscle capillarity in the growing rat.

Significant developmental increases in the ratio of endomysial connective tissue to muscle tissue were noted for both the soleus and the EDL, irrespective of diet. The greatest increments were seen in younger muscles, and were presumably a structural adaptation to ever-increasing contractile forces (Williams & Goldspink, 1981). The postulated relationship between contractile activity and structural adaptations in the

muscle's connective tissue framework may also explain the observed differences between tissues from age-matched control and diet-restricted animals, and between the EDL muscle and the more active soleus. The latter fibre type differences have been noted previously (Kovanen, Suominen & Heikkinen, 1980). Paradoxically, the continued accumulation of connective tissue in both muscles in old age may be due, at least partly, to reduced activity in the ageing muscles (Williams & Goldspink, 1984) and partly to an increased resistance of the ageing collagen molecules to protein breakdown (Mohan & Radha, 1980). Collagen metabolism may also be stimulated by muscle degeneration and regeneration (Myllyla *et al.* 1986), processes which are associated with old age (Gutmann, 1977). Although the relationship between the arrangement of connective tissue and the physiological properties of muscle is not yet clearly defined, one consequence of the accumulation of connective tissue is an increased passive resistance, or stiffness, of the the muscle as it gets older (Mohan & Radha, 1980). This may be augmented by a decreasing compliance of connective tissue with age, as the cross-links between collagen strands multiply. Such changes probably contribute to the overall decline in the efficiency of motor function with ageing (Gutmann, 1977).

In both the soleus and EDL control muscles, old age was associated with some degree of muscle wasting, due to collective fibre atrophy rather than a decrease in fibre numbers. Such senile fibre atrophy is common to both human (Essén-Gustavsson & Borges, 1986) and animal (Larsson & Edström, 1986) muscles, and may be primarily due to neuronal disturbances at the motor end-plate (Gutmann, 1977). The obvious delaying of this atrophy in the skeletal muscles of the diet-restricted rats, coupled with the relative scarcity of age-related pathological features, suggests that these muscles were maintained in a physiologically younger condition than those of the *ad libitum*-fed control rats. A similar phenomenon has been described for heart muscle (Goldspink, Lewis & Merry, 1986).

Finally, it has been reported that the neurogenic changes associated with ageing may affect distal muscles more than proximal ones (Grimby & Saltin, 1983) and postural muscles more than phasic ones (Larsson & Edström, 1986). These observations seem to be exemplified by the almost total absence of age-related pathological features in the fast flexor digitorum profundus muscle of the forearm, even at 149 weeks of age.

SUMMARY

The soleus and extensor digitorum longus muscles of the hindlimb and the flexor digitorum profundus muscle of the forelimb were studied in *ad libitum*-fed control and age-matched diet-restricted male rats at various ages from weaning to senescence. Growth of individual muscles was accomplished by fibre hypertrophy and not hyperplasia. Between weaning and one year, fibre numbers remained constant in the soleus but fell by 50% in the extensor digitorum longus. Both muscles displayed increasingly oxidative fibre type profiles with advancing age, irrespective of dietary status. This was particularly noticeable in the soleus, which transformed its fibre population from one containing 35% fast fibres at weaning to one with no fast fibres at 91 weeks. In senility, however, the fibre type population again displayed 25% fast fibres.

The capillary:fibre ratio and the capillary density were correlated with muscle fibre size in both hindlimb muscles. Although capillarity increased with age, expected differences between fast and slow muscles were probably minimised by the high proportion of FOG fibres in the extensor digitorum longus. Both hindlimb muscles

displayed significant increases in the ratio of connective:muscle tissue with increasing age. The soleus invariably contained more connective tissue than the extensor digitorum longus. Dietary restriction reduced the rate of increase, so that the connective tissue content was approximately one half that found in control muscles at one year. Various pathological features associated with old age were delayed considerably in the muscles of the diet-restricted rats. It is concluded that chronic dietary restriction imposed directly after weaning has a dramatic effect on the normal growth and ageing of skeletal muscle.

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