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Connective tissue changes and physical properties of developing and ageing skeletal muscle

M. A. ALNAQEEB, N. S. AL ZAID AND G. GOLDSPINK

Muscle Research Unit, University of Hull, U.K. Departments of Zoology and Physiology, Kuwait University and Department of Anatomy and Cellular Biology, Tufts University Schools of Medicine, Boston, Mass., 02111 *U.S.A.*

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INTRODUCTION

Senescence has been described as a "process of unfavourable progressive change" (Lansing, 1978). As far as the musculoskeletal system is concerned, this unfavourable change usually takes the form of loss of the ability to develop force and an increase in stiffness of joints, ligaments and muscles.

The decline of muscle strength is to some extent a reflection of a reduction in muscle mass, and, it is claimed, a reduction in the number of motor units (Campbell, McComas & Petito, 1973). However, it is clear that for some muscles or muscle groups, there is no close correlation between loss of muscle mass and loss of strength. McLennan, Hall, Timothy & Robinson (1980) showed a striking decline in grip strength in both men and women of 65 and over but only a marginal decline in forearm fat free mass. They suggest that this may be explained by a replacement of muscle tissue by fibrous tissue.

Connective tissue has been noted to increase in dystrophic muscle (Lowry, Hastings, Hull & Brown, 1942; Dreyfus, Schapira & Bourliere, 1954), denervated muscle (Tomanek & Lund, 1973) and immobilised muscle (Williams & Goldspink, 1981). In most cases such changes are associated with a reduction in muscle fibre size and/or loss of muscle fibres. There are conflicting reports concerning muscle connective tissue during development. Chiakulas & Pauly (1965) reported a decrease in the connective tissue content of abdominal muscles in both male and female human subjects up to 50 years of age. On the other hand, biochemical estimates of collagen in several muscles have shown that the decrease in connective tissue early in life is followed by a rise, continuing well into adult life (Schaub, 1963).

The situation for senile muscles is also unclear. Inokuchi, Ishikawa, Iwamoto & Kimura (1975), using histological techniques, claimed that the ratio of connective tissue to muscle tissue in the human rectus abdominis decreases in extreme age. However, this is in conflict with reports for a variety of ageing muscles using biochemical techniques in which connective tissue is said to increase (Schaub, 1963; Mohan & Radha, 1980). These discrepancies appear to be due, in part, to the different methods of measurement. In the light of the conflicting evidence, it was felt that further studies of muscle connective tissue content were desirable and, where possible, these should be related to the passive and active properties of the muscle.

MATERIALS AND METHODS

Male CFY Sprague–Dawley rats of different ages were purchased from the Wolfson Institute of Gerontology, the University of Hull. This strain shows a 50% loss of the initial population by 694 days and has a maximum life span of 1056 days. The mortality rate beyond 700 days continues at a high rate and only 10% of the original population survives past 900 days (Merry & Holehan, 1981). The mortality curve of this strain is typical for a population exhibiting senescence and, in shape, compares well with mortality curves for man (Comfort, 1979). The animals were reared under closely monitored conditions; temperature 21 + 1 °C, humidity 35–45% and a 12 hour photoperiod (light 8.00–20.00 hours). The litter size was standardised to eight and weaning was carried out at 21 days *post partum*. Food (formula 41B Bradshaw, Driffield) and water were available *ad libitum*.

Two hind limb muscles were selected for study; the soleus muscle which is predominantly made up of slow motor units and the extensor digitorum longus muscle which has a majority of fast contracting motor units. Both muscles have a reasonably fusiform structure and are small enough to permit rapid freezing of the tissue for histochemical analysis.

Animals were selected from the inbred colony at the ages of 21, 387 + 2 and 714 + 6 days and anaesthetised. The older obese animals were found to be highly sensitive to anaesthesia but a combination of low barbiturate levels and chloroform was found to be suitable. Sagatal (pentobarbitone sodium; May & Baker Ltd, Dagenham, England) was diluted in a mixture of 2 ml propylene glycol, 1 ml ethanol and 7 ml distilled water at a ratio of one part mixture to one part Sagatal stock solution (60 mg/ml). An initial dose of 20 mg/kg was administered intraperitoneally to senile animals, followed by small doses of 5 mg/kg of Sagatal until surgical anaesthesia was reached, not exceeding 50 mg/kg total dose. Young animals required a total dose of 60 mg/kg administered in a similar fashion. Maintenance of anaesthesia was assured by regular application of chloroform through a mask dispenser.

The active and passive properties were measured using the right hind limb of each animal; usually the muscles of the contralateral limb were used for histochemical and, in some cases, for biochemical analysis.

Quantitative histological analysis

Male animals aged 21, 84, 185, 508 and 758 days were killed by cervical dislocation or by an overdose of pentobarbital in the case of those animals that were used for the measurement of passive and active muscle properties. The extensor digitorum longus and the soleus muscles from both legs were dissected out. The muscles were cleaned of excess tissue, weighed and frozen in supercooled isopentane. Sections, 10 μm thick, were then cut in a cryostat and were used to examine the histology and histochemistry of the muscles. Most of these data will be reported elsewhere. For the purpose of this work, some sections were used for quantitative connective tissue estimation and some stained sections for muscle area measurement. The latter were projected using a Leitz projection microscope at a suitable magnification, and the outline of the muscles traced. Using a Planimeter (Allbrit Planimeter, U.K.) the total cross sectional area of each muscle was obtained and used in later calculations.

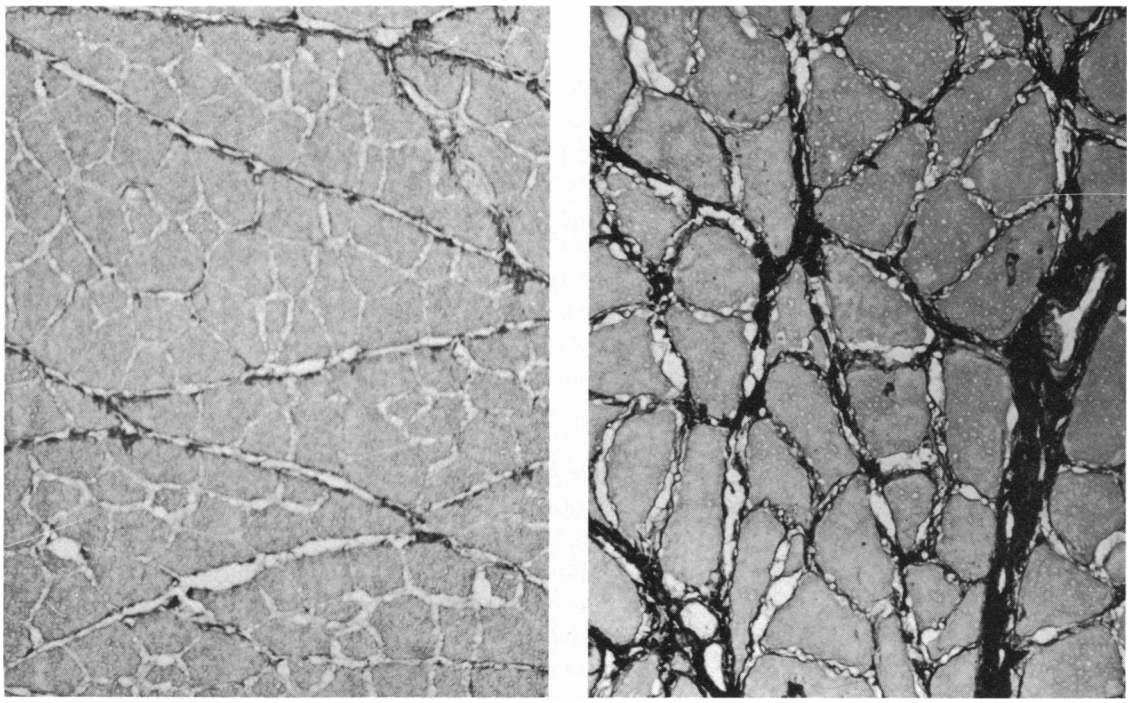

Fig. 1. Transverse sections taken from the rat soleus muscle from a young (left) and an old (right) rat after staining with Sirius red. × 160.

Determination of muscle connective tissue from sections

The amount of collagen was determined in sections taken from the muscle belly using a video image analyser after selective staining of the connective tissue with Sirius red.

The stain was prepared as a 1% solution of Sirius red F3BA in water. Picro-Sirius red, obtained as a solution of 10% stock solution in saturated aqueous picric acid, was kept saturated by adding picric acid crystals to the freshly prepared solution. The solution was allowed to stand for 24 hours before use. Frozen sections were rehydrated under running water for at least ten minutes. This step was necessary to allow the large molecules of Sirius red to penetrate connective tissue fibres (Sweat, Puchtler & Rosenthal, 1964) and, perhaps more important, it assisted the action of the poorly soluble picric acid in preventing the reaction of Sirius red with background tissue. The sections were then stained in saturated aqueous picric acid for 25 minutes followed by one minute in Picro-Sirius red. Sections were dehydrated in absolute alcohol using three changes each of one minute duration and were then cleared in xylene and mounted in DePex. The duration of staining time in Picro-Sirius red was adjusted so that a single time period would produce good staining of all age groups and muscles (Fig. 1). Furthermore, all slides were processed in one of three batches, each batch containing a mixture of ages and muscles.

The Image Analysis System consisted of a Leitz Ortholux microscope fitted with a high resolution monochrome video camera (Link camera type 109A, Link Electronics). A Farnell stabilised power supply type B30/10 (Farnell Inst. Ltd, Wetherby, Yorks) was used to provide the power for the microscope lamp. The

optical image of the section under study was passed through a green filter (wavelength, 530 nm). The video image generated by the camera was further processed electronically to enhance the contrast. A variable threshold level was set to remove noise and grey gradation, so that the new image was a mixture of either black or white points with no greys. Sections from muscles of animals from all age groups were measured using the same threshold setting. The associated electronic circuitry of the analyser was capable of estimating the area of either the white or the black regions and of averaging many video raster scans to give greater accuracy. The scanned image was in the form of pulses which were recorded in digital form. The final processed image was displayed on a video monitor. In addition to being able to select the area on the section mechanically by moving the microscope stage, an electronic circuit allowed the blanking of the processed video image so that a small area of the total field of known dimensions could be studied. Once an area had been selected, a counter measured the number of black points in any one frame, or in an average of 20 frames.

Two types of counts were obtained for each muscle; the first at a final magnification of $\times 485$ and a second at a final lower magnification of $\times 127$. The former readings were obtained using blanked fields covering an area of 0·0144 mm^2. The fields were selected to contain mainly endomysial connective tissue. Between ten and twenty fields were counted and each count was an average of ten readings. Also, the number of whole muscle fibres enclosed within the field and the fibres touching two perpendicular sides of the blanking were counted in each field. At least five fields at the lower magnification, each an average of ten readings, were used to estimate perimysial connective tissue. The field area covered by each reading at this lower magnification was 0·6006 mm^2. The combination of low magnification and lower threshold settings produced estimates of the thicker perimysial connective tissue. Perimysium was sampled systematically by sampling fields from the deep to the superficial aspects of the muscle; the spacing between any two adjacent scans was wide enough to cover the whole muscle when the required number of fields had been measured. Results were expressed as counts per unit area (where the unit area was equivalent to the field covered at each magnification, i.e. 0·6006 mm^2 and 0·0144 mm^2). Endomysial connective tissue was also expressed as counts per fibre.

Biochemical estimation of connective tissue (collagen)

Male rats aged 21, 84, 188, 299 and 716 days were killed by a blow on the head followed by the dislocation of cervical vertebrae. The extensor digitorum longus and the soleus of the left limb were dissected out complete with both tendons intact and cut as close as possible to the bone. Excess superficial connective tissue and fat was removed. The belly of the muscle was carefully cut out with extra care being taken not to include any tendon, especially in the older animals where a thin sheath extends on both sides of the muscle from each tendon, overlapping in the middle. The belly and the remaining two segments were weighed and placed separately in vented PVC vials (No. 690 KSartedt) and were then quenched in liquid nitrogen.

All muscles were usually excised and frozen within one hour of death. The muscles were then freeze-dried for 12–18 hours at 13·33 Pa in a freeze dryer (Chem. Lab. Instruments Ltd, London). The muscles were weighed again to obtain dry weight and were stored in a dessicator to await further processing.

The freeze-dried muscles were dissolved in 5 ml of 6N-HCl and sealed in a 20 ml glass tube with a screw-on top lined with Teflon (Kimax, U.S.A.) and autoclaved

at 172 kPa for five hours. Hydroxyproline content of the hydrolysates was then determined by the method of Grant (1964). The analysis was carried out on a Technicon Autoanalyser II (Technico Inst. Co.).

Physical properties of muscles

A skin incision was made on the inner side of the right leg extending from the ankle to the knee. A similar incision through the fascia and parallel to the fibula was cut to expose the proximal tendon of the extensor digitorum longus. Excessive connective tissue interfering with the free movement of the extensor digitorum longus muscle was carefully parted and a thread was secured to the lower tendon. The blood and nerve supply to the muscle was kept intact. Stimulation electrodes of the cuff type were placed around the nerve supplying the extensor digitorum (see below). The animal was rigidly secured in the framework in the supine position. The thread was then tied to the transducer using surgical silk and the extensor digitorum longus and the transducer were carefully aligned. Stimulation was commenced after allowing the muscle to recover for ten minutes whilst being bathed in physiological solution. In the case of the soleus, the skin was further removed from the right limb and the Achilles tendon and muscle were exposed. The muscle was freed of connective tissue, taking care not to damage the nerve supply to the muscle. The animal was placed in the prone position and the distal tendon was secured to the force transducer. The transducer was manipulated to obtain the best position for the recording and the whole preparation was firmly clamped. Warm physiological solution (37·5 °C) was slowly dripped over the muscle for ten minutes and the muscle was then stimulated.

The recording system was built around the Devices UF1 isometric force-displacement transducer amplifier 3559. Slow events, of the order of seconds, were recorded on a Devices multichannel thermal recorder type MX412 (Devices Ltd, Hertfordshire, England). Faster events were stored on a Telequipment storage oscilloscope DM53A with a type B differential amplifier. Permanent records were obtained using a Polaroid camera. Events were timed with a programmable Digitizer D100 (Devices Ltd) which also served as a trigger and synchronisation control. Stimulation was carried out with an SD9 Grass stimulator (Grass Instruments, Quincy, Massachusetts, U.S.A.) using teflon covered stainless steel wire which was attached to the nerve using a cuff made from polyethylene cannula tubing.

The temperature of the immediate environment was controlled with a thermostatically governed infrared lamp. The muscle was bathed continually with a physiological solution containing 137 mM-NaCl, 4 mM-KCl, 1 mM-$MgCl_2$, 1 mM-KH_2PO_4, 12 mM-$NaHCO_3$ and 2 mM-$CaCl_2$. This solution was gassed with a mixture of 95 % O_2 and 5 % CO_2. The temperature of the solution was regulated to maintain 37·5 + 0·5 °C by passing it through a thermostatically controlled water jacket. The transducer was coupled to the main framework through a multidirection rack and pinion and a micrometer. This arrangement allowed the correct alignment of the transducer and the muscle; it also permitted coarse and fine adjustment of muscle length and position.

The muscles were stimulated at a rate not exceeding two stimuli per minute. The muscle length was increased by turning the micrometer after each stimulation until optimum length was reached. This continued until the muscle length reached 105 % optimum length for the extensor digitorum longus and 110 % optimum length for the soleus. Stimulation pulses were between 3 and 5 volts and 0·5 milliseconds in dura-

tion. The isometric twitches were recorded on both the oscilloscope and pen recorder. The muscle was then readjusted back to its optimum length. Passive and active length–tension plots and isometric twitch parameters were then derived in the usual way. In order to compare the curve for the different age groups, it was necessary to express the length as a percentage of optimum length and the active tensions as a percentage of maximum twitch tension. The curves for both the passive and active tensions were compared using linear regression analysis (NAGF Library routine GO2CAF Mark 5). The passive tension curves were essentially exponential and were therefore subjected to logarithmic transformation prior to comparison by linear regression.

RESULTS

The results of the image analysis of connective tissue in sections are shown in Tables 1 and 2. In both the soleus and extensor digitorum longus muscles, the endomysial connective tissue expressed as counts per unit area showed a significant correlation with age as revealed by the analysis of variance. The amount of endomysial connective tissue per unit area increased initially up to 84 days of age. It then remained more or less constant until old age when it increased considerably.

When endomysial connective tissue was related to the number of fibres present in the field counted, similar trends appeared (Tables 1 and 2). That is to say, young muscle fibres had the least amount of endomysial connective tissue associated with them. However, by 84 days the amount of connective tissue per fibre had increased more than sevenfold, which indicated the rapidity with which connective tissue built up around fibres during early postnatal development. Further accumulation then occurred in ageing and senile animals at a relatively high rate, increasing by 54 % between 299 and 508 days and later by another 19 % (extensor digitorum) and 29 % (soleus) between 508 and 754 days.

It was interesting to note that the perimysial connective tissue in the extensor digitorum longus and the soleus remained more or less constant throughout life except in old age when again there was a very considerable increase, particularly in the extensor digitorum longus (68 %).

Biochemical estimates of collagen are shown in Tables 3 and 4. The total collagen content in whole muscles including both tendons increased continuously with age. Between 21 days and 84 days of age, there was approximately a thirteenfold increase in collagen of both muscles. This increase continued but at a much reduced rate thereafter. When collagen content was related to muscle weight, there was still a very significant increase in collagen per milligram of muscle between 21 and 84 days. After this, there was a trend towards (by 299 days) a lower collagen content per milligram of muscle until old age when a greater proportion of collagen was again recorded. Senile animals showed an increase in collagen concentration whether related to dry or wet muscle weight.

Collagen in the belly region only (excluding both tendons) of the extensor digitorum longus muscle (Table 3) increased early in life and then again in senility ($P < 0{\cdot}001$). In the soleus muscle (Table 4) there was a steady increase in collagen in the muscle belly throughout life, but with a more noticeable increase during senility (29 %).

Table 1. *Connective tissue content in sections of the developing and ageing extensor digitorum longus muscle*

Age (days)	Number of animals	Endomysium		Perimysium
		per unit area*	per fibre	per unit area†
21	4	14·31±2·71	0·44±0·10	49·16±4·22
84	5	32·95±5·83	3·87±0·69	44·82±7·91
185	5	31·58×4·74	4·48±0·79	46·98±4·51
299	5	30·76±3·50	4·70±0·46	44·28±6·86
508	5	44·48±4·32	7·22±0·78	59·19±4·59
758	5	55·02±6·66	8·62±1·18	99·78±11·67

All values are ±S.D.; * unit area = 0·0144 mm²; † unit area = 0·6006 mm².

Table 2. *Connective tissue content in sections of the developing and ageing soleus muscle*

Age (days)	Number of animals	Endomysium		Perimysium
		Per unit area*	Per fibre	Per unit area†
21	5	21·68±5·33	0·97±0·35	66·02±6·51
84	5	35·01±3·62	7·05±0·82	56·56±6·34
185	5	37·23±2·18	7·74±1·07	62·98±3·71
299	5	37·88±1·37	8·31±0·80	67·43±5·77
508	5	48·25±4·47	11·26±2·35	78·58±11·42
758	5	64·81±5·15	14·49±1·92	94·09±1·53

All values are ±S.D.; * unit area = 0·0144 mm²; † unit area = 0·6006 mm².

Table 3. *Collagen content of the developing and ageing extensor digitorum longus muscle*

Age (days)	Number of animals	Absolute collagen content mg	Collagen in whole muscle		Collagen in muscle (belly)	
			μg/mg dry muscle	μg/mg wet muscle	μg/mg dry muscle	μg/mg wet muscle
21	5	0·3966±0·05	68·96±8·45	17·50±1·70	20·54±2·37	4·89±0·87
86	5	5·0170±0·391	107·92±6·97	30·28±2·04	44·82±8·66	12·03±2·54
188	5	6·0618±0·294	102·07±7·21	28·29±2·09	58·99±1·72	15·73±0·76
299	5	6·0474±0·4784	87·51±5·22	24·40±1·41	42·12±6·81	11·04±1·77
716	5	6·7436±0·1697	104·41±7·75	28·45±2·31	61·39±5·23	18·33±5·81

All values are ±S.D.

Passive and active tension

The relationship between muscle length and passive tension was of an exponential nature in both muscles for all age groups investigated. Some examples of the passive tension curves are shown in Figure 2 and the logarithmic transformations and fitted regressions in Figure 3. Initial increases in muscle length around the *in vivo* resting length of the muscles produced small changes in passive tension. As the muscle was stretched, passive tension increased exponentially. The rate of this increase, however,

Table 4. *Collagen content of the developing and ageing soleus muscle*

Age (days)	Number of animals	Absolute collagen content mg	Collagen in whole muscle		Collagen in muscle (belly)	
			μg/mg dry muscle	μg/mg wet muscle	μg/mg dry muscle	μg/mg wet muscle
21	5	0·3786±0·154	47·68×8·29	18·18±7·08	36·16±10·83	8·80±2·33
84	5	5·0308±0·899	106·96×19·32	29·33±5·19	33·17±4·78	8·29±1·11
188	5	7·4218±1·694	125·27±26·11	34·13±8·41	44·97±13·43	10·99±3·50
299	4	7·5473±2·200	100·73±23·22	28·61±7·91	34·70±5·22	8·77±1·37
716	5	9·7524±1·338	132·86±22·34	39·74±5·92	49·96±11·25	12·50±2·40

All values are ±S.D.

varied with age. The young, 21 days old, extensor digitorum longus muscle showed a lower rate of passive tension development for every unit increase in length. The adult muscle developed passive tension at a higher rate when compared with the young muscle, and the senile extensor digitorum longus muscle showed the highest increase in tension per unit length. In contrast with the situation for the extensor digitorum longus, the elderly soleus muscle did not differ appreciably from the adult.

The length–active tension curves were similar to those obtained for other whole muscle preparations; therefore, sets of graphs have not been presented. In all cases the response (the ascending portion) was reasonably linear until a few length increments before optimum length was reached. Therefore, the relationship between length and active tension in the different age groups could be compared by regression analysis. These data (Table 5) showed that the adult muscles had steeper length–active tension curves than the young muscles and that senile muscles had even steeper curves than the adult muscles ($P < 0{\cdot}01$). That is to say, a smaller decrement of length (below optimum length) brought about a larger decrease in tension as the muscle became older. Again, the soleus muscle in the aged animal was the exception, since the length dependance of the active tension developed by this muscle did not change with age.

DISCUSSION

Collagen, the chief component in connective tissue, has an extremely low compliance which compares favourably with copper. This low compliance implies that small increases in the quantity of collagen in a muscle would increase the stiffness of the tissue considerably. Biochemical estimates of absolute collagen content in the two muscles investigated show progressive increases with age but relative collagen content (μg/mg muscle) presents a more complex picture. This is because the muscle fibres are also increasing in size, sometimes at a greater rate than the collagen content. It is for this reason that the collagen concentration actually decreases slightly in both muscles as the animals reach adulthood, although, even on a relative basis, collagen increases markedly in senility.

Estimates of collagen in the soleus and extensor digitorum longus muscles show, as expected, a considerably greater content when tendons are included as compared with estimates of the muscle belly alone. The tendons from the extensor digitorum longus contribute similar proportions of collagen to the whole muscle throughout the life of the animal. The same is true for the soleus except in very young muscles.

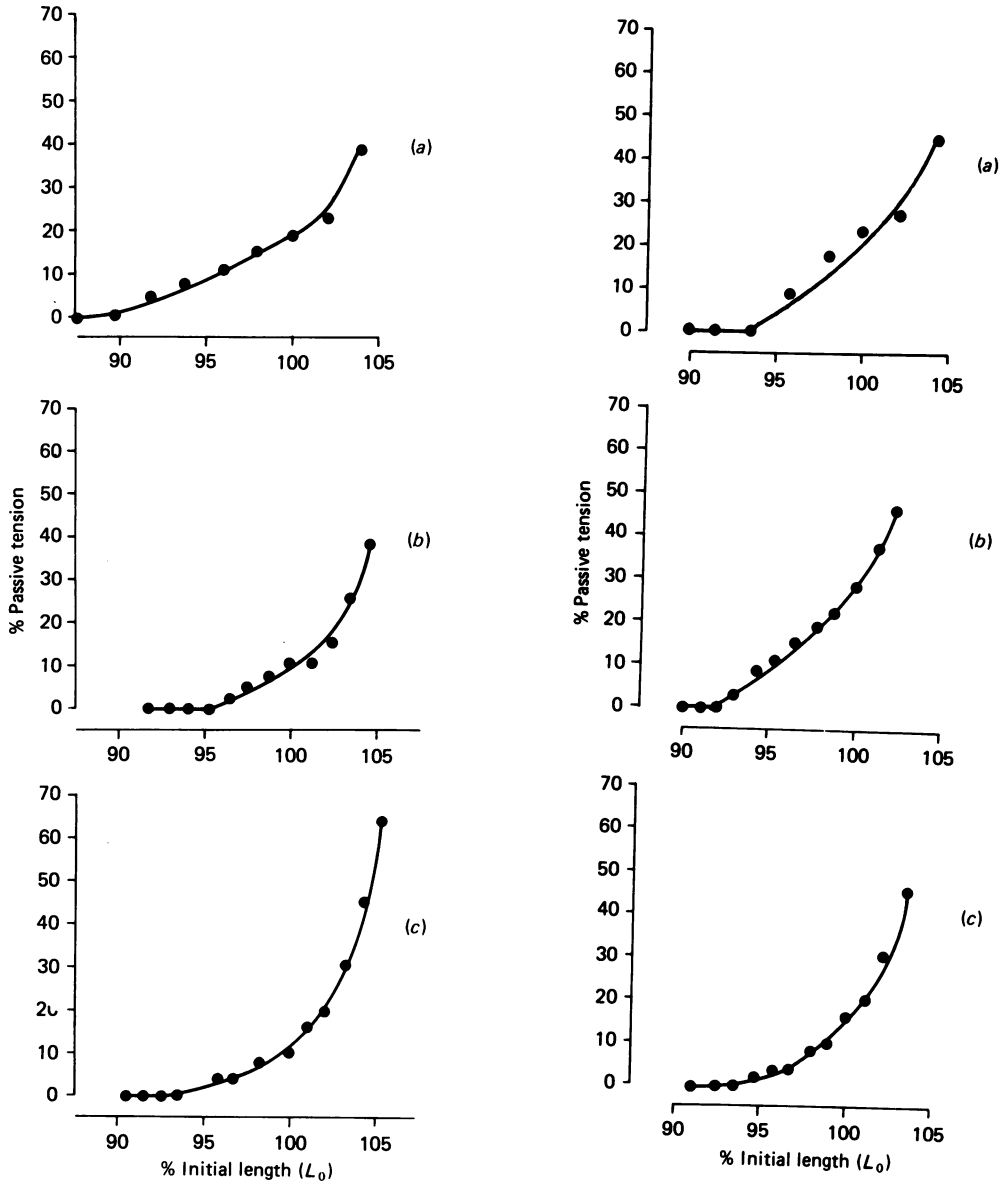

Fig. 2 (*a–c*). Examples of length–passive tension graphs for the extensor digitorum longus (left) and soleus (right) muscles at (*a*) 21 days), (*b*) 387 days and (*c*) 714 days. Tension is expressed as a percentage of maximum twitch tension produced at optimum length.

Estimations of tendon collagen are not considered to be very accurate because of the difficulty of dissecting the whole tendon from the bone; in any event, a more important determinant of muscle stiffness is the muscle belly connective tissue. The biochemical analyses indicate that the concentration of connective tissue within the muscle belly increases in the initial stages and then again in senility. The quantitative image analysis of sections following selective staining of the connective tissue (Fig. 5) indicates that this increase in muscle belly collagen is relatable to increases in both the endomysium and the perimysium. The data described show that concentration of

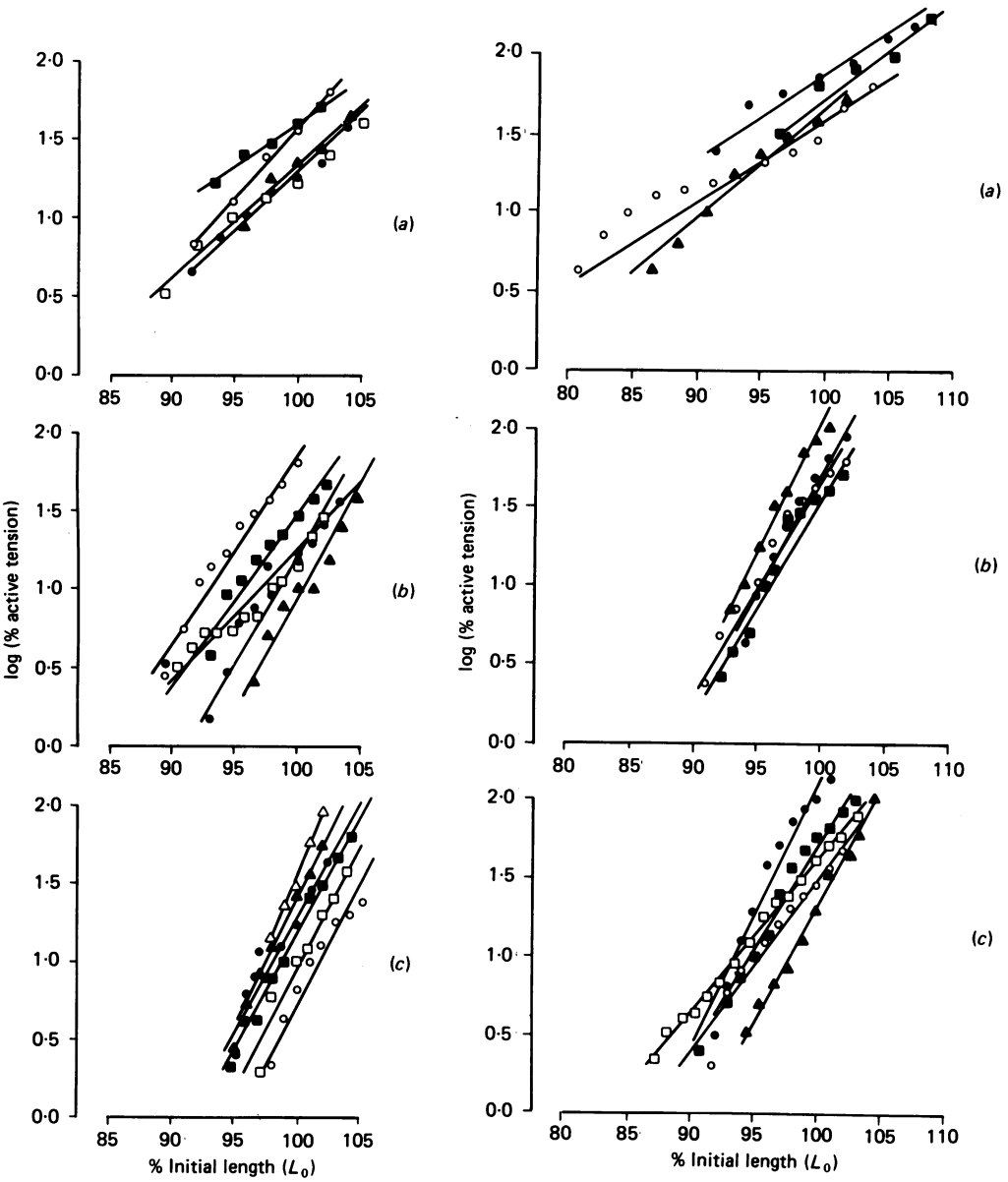

Fig. 3 (*a–c*). Length–passive tension graphs for the extensor digitorum longus (left) and soleus (right) muscles at (*a*) 21 days, (*b*) 387 days and (*c*) 714 days. The steepness of the log transform indicates the stiffness of the muscle.

endomysial connective tissue increases early in life and again in senility. However, this is not true for perimysial connective tissue; this remains constant throughout life except in old age when there is a very significant increase, particularly in the extensor digitorum longus muscle.

The passive mechanical characteristics of developing and ageing skeletal muscles change appreciably in the two muscles investigated. The stiffness (compliance) of the extensor digitorum longus muscle increases progressively with age; in other words, higher passive tensions occur for smaller increases in length as the animal

Table 5. *The correspondence of isometric tension to length change in the muscles of the rat at different ages expressed as a regression coefficient of graphs of length–isometric twitch tension*

	Extensor digitorum longus		Soleus	
Age (days)	Regression coefficient	Probability	Regression coefficient	Probability
21	5·83		7·59	
	5·54		4·57	
	7·90		4·79	
	5·18			
	5·18		5·65 ± 1·69	
	5·93 ± 1·13			
387	9·85		7·16	Not significant
	6·76		6·26	
	6·62	< 0·05	6·30	
	8·12		5·62	
	10·64			
			6·34 ± 0·63	
	8·40 ± 1·80			
714	14·20		8·13	
	13·88		5·33	
	10·30	< 0·01	5·35	Not significant
	12·17		5·20	
	10·19		5·06	
	12·15 ± 1·90		5·81 ± 1·30	

ages. This is particularly true in the senile extensor digitorum longus. In contrast, the soleus muscle exhibits a change in stiffness only early in life; thereafter, it remains unchanged.

The data for the stiffness of the muscles and those for connective tissue content are in accord at the ages studied, with the exception of the soleus in the senile animal. With this exception, the passive mechanical behaviour of the muscle appears to be related directly to collagen concentration. In the extensor digitorum longus muscle, the average slope of the length–passive tension curve is 0·066 while the collagen content is 68·96 μg/mg: The soleus muscle, with a lower collagen content of 47·68 μg/mg, has a shallower slope (0·052). As collagen increases to 87·51 μg/mg during maturation of the extensor digitorum longus muscle, the trend continues into old age; a slope of 0·161 is associated with an increased collagen concentration of 104·41 μg/mg. The reason why the soleus muscle in the senile animal is an exception is not known: its stiffness is about the same as in the adult animal although it has an increased total collagen content. This may be due to the different rate of accumulation of the connective tissue components within the belly region of the muscle, particularly the lower rate of increase in perimysial content. Alternatively, it may be related to the degree of crosslinking and configuration of the collagen molecule. Crosslinking has been suggested in senile collagen (Verzar, 1963) and has been shown to alter the biochemical and mechanical properties of the protein. Crosslinking appears to increase the resistance of collagen to degradative enzymes (Mohan & Radha, 1980) and urea degradation (Harrison & Archer, 1978), and to increase its tensile strength and decrease its elasticity (Verzar, 1963). The reason why the soleus muscle in the aged animal shows no increase in stiffness (remaining supple) may be related to activity.

Electromyographic studies have shown that this muscle is active during both standing and walking (Goslow, Reinking & Stuart, 1973) and the fact that it does not age in the same way as the extensor digitorum longus muscle may be related to its high level of activity even in the senile animal.

The connective tissue within a muscle contributes to either the series elastic component or the parallel elastic component. The present study indicates that an increase in either component produces a reduction in the compliance of the muscle. However, an increase in connective tissue can also be expected to hamper the contraction or the relaxation processes as it may act as an extensible or even a compressible spring, depending on its length–tension properties. It is interesting, therefore, to see how accumulation of connective tissue affects the length–active tension curve. In the case of the soleus, the relationship between the relative force developed and the relative initial length is unchanged with age. The extensor digitorum longus, however, shows proportionally greater decrements of tension for decrements of length as the muscle ages. This implies that the optimum sarcomere length is reached with less length extension in the aged than in the younger extensor digitorum longus muscle. This is probably due to the more extensive development of the series and parallel elastic component with age; in turn, this leads to a better correspondence of fibre length changes with sarcomere length changes. Because of the poor development of the connective tissue in the young muscle, it is conceivable that the tendons and sarcolemma can be stretched without the sarcomeres being pulled out to the same extent. A decrease in sarcomere number in old age due to disuse, for example, reduced excursion of the limbs, would also have the same effect of increasing the steepness of the length–tension relationship. It is clear that further investigation of this point is required to establish whether sarcomere number does change in senility. Indeed, more extensive work on the ageing of the musculoskeletal system is required. It is difficult to know, for instance, whether the deterioration in muscle function reported here is due to the ageing process *per se* or whether it is a reflection of the change in activity pattern in senility. A combined study of exercise and ageing may provide a way of distinguishing between these two possibilities.

SUMMARY

Selective staining of the connective tissue and image analysis showed that in the extensor digitorum longus and soleus muscles of the rat there was an increase in the thickness of the endomysium in both early growth and senility. The perimysium thickness was more or less constant throughout life except in senility when the concentration of this component also increased. The stiffness (length–passive tension) of these muscles was found to increase throughout life. Log transforms of the length–passive tension plots had particularly steep slopes in the senile extensor digitorum longus muscle. Except in the senile soleus muscle, the increase in stiffness was closely correlated with the increase in endomysium and perimysium and with total muscle collagen (as measured biochemically) with age. The relationship between the initial length and the active tension in the extensor digitorum longus muscle changed with age. The older muscles showed a greater decline in tension for each decrement of length resulting from the increased development of the connective tissue.

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