Properties of single motor units in medial gastrocnemius muscles of adult and old rats

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1. The purpose of this study was to determine the role of motor unit remodelling in the deficit that develops in the maximum isometric tetanic force (F_0) of whole medial gastrocnemius (MGN) muscles in old compared with adult rats. The F_0 values and morphological data were determined for MGN muscles and eighty-two single motor units in muscles of adult (10–12 months) and sixty-two units in those of old (24–26 months) F344 rats. During an unfused tetanus, fast and slow (S) motor units were identified by the presence and absence of sag, respectively. Fast-fatigable (FF) and fast-fatigue-resistant (FR) units were classified by fatigue indices less than or greater than 0·50, respectively.

2. For old rats, whole MGN muscle F_0 was 29% less than the value of 11·2 N measured for adult rats. The deficit in whole muscle F_0 of old rats resulted from equivalent decreases in the number of motor units, 16% smaller than the adult value of ninety-seven, and in the mean motor unit F_0 value, 14% less than the adult value of 117 mN.

3. With ageing, little motor unit remodelling occurred in FR units, whereas the S and FF motor units demonstrated dramatic, but opposing, changes. For S units, the number of units remained constant, but the number of fibres per motor unit increased 3-fold from 57 to 165. In contrast, the number of FF units decreased by 34% and the number of fibres per motor unit of the remaining units decreased to 86% of the adult value of 333. The age-related remodelling of motor units appeared to involve denervation of fast muscle fibres with reinnervation of denervated fibres by axonal sprouting from slow fibres.

Old age is associated with a progressive loss of muscle mass and muscle strength (Grimby & Saltin, 1983). The decline in muscle mass is caused by atrophy of single muscle fibres, a reduction in the number of fibres, or most probably a combination of the two (Faulkner, Brooks & Zerba, 1995). Denervation causes both the atrophy of muscle fibres and ultimately the loss of muscle fibres (Schmalbruch, Al-Amood & Lewis, 1991). Decreases in the number of α-motoneurons in the motor nucleus (Hashizume, Kanda & Burke, 1988) and in the number of motor units in skeletal muscles have been reported for old compared with young animals (Einsiedel & Luff, 1992), including humans (Doherty, Vandervoort, Taylor & Brown, 1993). In addition, with ageing, cross-sections of skeletal muscles show a dramatic decrease in the type II/type I fibre ratio (Caccia, Harris & Johnson, 1979; Larsson, 1983; Kanda & Hashizume, 1989).

The changes in the number and in the contractile properties of motor units with age have been investigated in several different skeletal muscles in both rats and humans. In the rat, the properties of fast motor units have been investigated in tibialis anterior (TBA) muscles, those of slow motor units in soleus muscles (Edström & Larsson, 1987) and those of fast and slow motor units in the medial gastrocnemius (MGN) muscle (Kanda & Hashizume, 1989; Einsiedel & Luff, 1992). Remodelling of motor units in a muscle with a heterogeneous population of fast and slow motor units (Kanda & Hashizume, 1989; Einsiedel & Luff, 1992) may be quite different from remodelling in a muscle with a homogeneous population of motor units (Edström & Larsson, 1987). In extensor digitorum longus (EDL) muscles of rats, in which 97% of the muscle fibres are fast (Edström & Larsson, 1987), the absence of slow motoneurons may affect the magnitude of reinnervation of denervated fibres in old age. Most of the skeletal muscles in humans contain almost equal proportions of type II (fast) and type I (slow) fibres (Larsson, 1983). Consequently, to reflect the changes that occur with age in the skeletal muscles of humans, changes with age in structure and function of fast and slow type motor units need to be studied in the same muscle.

In the rat, the MGN muscle is one of the few muscles in which significant proportions of both fast and slow motor units are present. Furthermore, the MGN muscle in the rat

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displays a significant decrease in maximum isometric tetanic force (F_0) of ~30% with age (Einsiedel & Luff, 1992). Despite the advantages offered by this muscle, only a few investigations of motor unit properties have utilized the MGN muscle of the rat (Kanda & Hashizume, 1989, 1992; Einsiedel & Luff, 1992). Kanda & Hashizume (1989) used the glycogen-depletion method to investigate the morphology and contractile properties of motor units in the MGN muscle of adult and old rats. Although meticulously detailed regarding the characteristics of motor units, the lack of a measurement of the whole muscle maximum force in the Kanda & Hashizume (1989) study precluded assessments of the relative contribution of the mean motor unit F_0 and the number of motor units to the deficit in whole muscle F_0 in old rats. Einsiedel & Luff (1992) measured the F_0 of the whole MGN muscle, but the changes at the motor unit level were not compared with changes at the whole muscle level and the number of fibres in different types of motor units, the innervation ratios, were not determined.

Controversy exists as to the effects of ageing on the F_0 of slow and fast motor units in the MGN muscles of rats. Kanda & Hashizume (1989) reported a 2-fold increase in the F_0 of slow motor units with ageing, whereas Einsiedel & Luff (1992) observed a small 7% increase. Each study reported a 30–40% decrease in the F_0 of fast-fatiguable units with ageing, but, for the F_0 of the fast-fatigue-resistant units, Kanda & Hashizume (1989) noted a 36% decrease in contrast to the 34% increase cited by Einsiedel & Luff (1992). Most importantly, although Kanda & Hashizume (1989) showed a dramatic increase in the innervation ratios of slow motor units with ageing, the effect of ageing on the innervation ratios of fast motor units was not reported. The purpose of our study was to compare the changes in the F_0 of fast-fatiguable (FF), fast-intermediate (FI), fast-fatigue-resistant (FR) and slow (S) type motor units with the change in the F_0 of the MGN muscles of adult and old rats. For MGN muscles of old compared with adult Sprague–Dawley rats, Einsiedel & Luff (1992) reported a 30% deficit in F_0 and both Kanda & Hashizume (1989) and Einsiedel & Luff (1992) demonstrated changes in the F_0 values of different types of motor units in the MGN muscles with ageing. Based on these observations, we proposed that the deficit in the F_0 of MGN muscles in old compared with adult rats must result from a combination of: (1) a decrease in the total number of motor units, and (2) a decrease in the mean F_0 of the remaining motor units. We tested the hypothesis that the underlying mechanism responsible for the deficits in number and force of motor units is a complex remodelling of motor units, with no change in the number of S units and an increase in the mean F_0 of S units and a decrease in the number and F_0 values of motor units for each of the two types of fast units (FF and FR).

METHODS

Experiments were performed on ten adult (10–12 months) and eight old (24–26 months) F344 rats. The rats were obtained from the specific pathogen free colony of the National Institute of Aging (Bethesda, MD, USA). Prior to experimentation, the rats were housed in a pathogen free–barrier protected animal room in the Unit for Laboratory Animal Medicine at the University of Michigan.

Operative procedures

All experiments were performed in accordance with the Guide for Care and Use of Laboratory Animals (United States Public Health Service, Publication No. 85-23). The rats were anaesthetized by intraperitoneal injection of pentobarbitone sodium (65 mg kg^{-1} for adult rats and 40 mg kg^{-1} for old rats). Supplementary doses (10–15 mg kg^{-1} for adult and 5–10 kg mg^{-1} for old rats) were administered to maintain a depth of anaesthesia that prevented responses to tactile stimuli. An incision was made on the medial part of the lower leg to expose the MGN muscle. The MGN muscle was dissected free of surrounding tissue and separated from the lateral gastrocnemius muscle by careful dissection through the interdigitating muscle fibres. Care was taken to maintain the blood and nerve supply to the muscle intact. The tibial nerve was exposed and dissected free of surrounding tissue. All the branches of the tibial nerve except the one to the MGN muscle were severed. A 1-0 suture was tied firmly to the distal end of the MGN muscle and a loop was formed. The loop was placed on a stainless steel hook that was attached to a force transducer (BG-1000; Kulite Semiconductor Products, Leonia, NJ, USA). The output of the force transducer was amplified and sampled by a microcomputer and simultaneously displayed on a digital storage oscilloscope (Gould Inc., Valley View, OH, USA). A skin pouch was formed around the muscle to hold mineral oil. A heating lamp combined with a thermocouple operating on a feedback loop maintained the temperature of the muscle at 37 °C.

A lumbosacral laminectomy was performed to expose the ventral roots in the lumbar and sacral regions of the spinal cord. The spinal cord was transected at the cephalic end below the sacral region and the dorsal roots were separated to expose the ventral roots. The ventral roots were bathed in warm mineral oil and the temperature was maintained at 37 °C using a heating lamp. A pair of platinum electrodes were sutured to the muscle fascia with a distance between them of 15 mm for recording electrical activity on the surface of the muscle. The common electrode was placed on the muscle tendon junction. The electrical signals were preamplified, filtered and amplified again by a commercial electromyograph (model M; TECA Corporation, Pleasantville, NY, USA), and simultaneously displayed on an oscilloscope. The body temperature of the rat was maintained at 37 °C by a separate water-bath placed underneath the animal.

Contractile properties of the whole muscle

An 8 mm long tubular bipolar nerve cuff with stainless steel wire electrodes was inserted under the tibial nerve for stimulation of the MGN muscle. The nerve was lifted gently using a special hook made of glass to avoid any trauma. Square voltage pulses, 0·2 ms in duration, were used to stimulate the nerve with the distal electrode as the cathode. The length of the muscle was adjusted to the optimal length (L_0) for the development of maximum twitch force. The L_0 of the muscle for the development of force during a maximum isometric tetanic contraction was not different from that obtained during a twitch contraction (Brooks & Faulkner, 1988).

During non-potentiated twitches, time-to-peak tension (TPT) was measured. To measure the tetanic force, trains of stimuli at one frequency were delivered for a period of 300 ms. During repeated contractions, the stimulation frequency was increased gradually from 10 Hz with step increments until the force reached a plateau. The force plateau was defined as the maximum isometric tetanic force (F_0). A period of 2 min was allowed between contractions to allow the muscle to recover. During each tetanic contraction, the maximum rate of force development (dF/dt) was also measured. At the end of the experiment, the L_0 of the MGN muscle was measured.

Contractile properties of single motor units

After the measurement of whole muscle contractile properties, the ventral roots innervating the MGN muscle, namely L4, L5 and L6, were identified. The isolated ventral roots were dissected repeatedly with fine watch makers forceps until a filament containing only one functioning axon remained (Kanda & Hashizume, 1992). The criteria for a single motor axon were that, with the intensity of stimulation increased gradually, there was: (1) an all-or-none mechanical twitch, and (2) an all-or-none action potential, as measured on the surface of the muscle. Following the identification of a single motor unit, contractile properties of the motor unit were measured as described for the whole muscle.

Classification of motor units

Motor units were classified based on the presence or absence of sag and an index of fatigability (Burke, Levine, Tsairis & Zajac, 1973). Sag was defined as the gradual decrease in force during a partially fused tetanic contraction with a duration of 200–400 ms. The frequency of stimulation (in Hz) for assessing sag was determined as the inverse of 1·25 times the motor unit TPT (in s). The stimulation frequency for assessing sag varied from 35 to 80 Hz for fast units and from 25 to 40 Hz for slow units. All motor units that exhibited the property of sag were classified as fast motor units and the remainder were classified as slow. The fast motor units were classified additionally on the basis of a fatigue index (Burke, *et al.* 1973).

The resistance to fatigue was measured by a stimulation protocol with trains of 325 ms in duration, at a frequency of 40 Hz, and repeated every 1 s for 2 min (Burke *et al.* 1973; Kanda & Hashizume, 1989, 1992). The isometric force for fast motor units actually increased during the initial seconds, reached a plateau and then decreased. The fatigue index was defined as the ratio of the force measured at the end of the 2 min period to the force at the plateau. The isometric force of slow motor units declined slightly, or did not change significantly, during the fatigue test. Consequently, the fatigue index of slow units was defined as the ratio of the force measured at the end of the 2 min period to the force measured during the first tetanic contraction. Those units with fatigue indices of less than 0·5 were classified as FF, units with fatigue indices between 0·5 and 0·75 as FI, and units with fatigue indices above 0·75 as FR. The criteria for classifying motor units were similar to the criteria proposed in several other studies (Burke *et al.* 1973; Chamberlein & Lewis, 1989; Kanda & Hashizume, 1989, 1992).

Morphological and histochemical assessments

After the measurement of contractile properties, both the experimental and contralateral muscles were removed from each rat. The anaesthetized rat was then killed by an intravenous injection of 1 ml kg^{-1} of 1 M potassium chloride into an exposed femoral vein. Death was ensured by the induction of a pneumothorax. The tendons were trimmed and the muscles were blotted and weighed. The contralateral muscles were fixed at L_0 in a Petri dish using metal pins. The fascia covering the muscles were cut open using fine scissors and the fibre length (L_f) was measured under a dissection microscope. A small portion of the muscle was removed for determination of the dry mass/wet mass ratio. The experimental muscles were frozen at −70 °C using dry ice and isopentane. In a cryostat, serial sections 14 μm thick were cut from the medial, proximal and distal one-thirds of the muscle length. Sections were stained for myofibrillar ATPase activity at pH 9·4 and 4·3 (Brooke & Kaiser, 1970). Muscle fibres were classified as type I, type IIa or type IIb based upon ATPase staining. Type I fibres appeared light at pH 9·4 and dark at pH 4·3 (Brooke & Kaiser, 1970). Fibres which were dark at pH 9·4 were classified as type II. The type II fibres were further classified as IIa if they appeared light at pH 4·3, or IIb if they were dark at pH 4·3. If the classification based on pH 4·3 was not conclusive, serial sections stained for succinate dehydrogenase activity were used to classify type IIa and IIb fibres (Brooke & Kaiser, 1970). Following staining for succinate dehydrogenase activity, type IIa fibres appeared dark purple, whereas type IIb fibres appeared light purple.

In each muscle, from all three sections (proximal, medial and distal), no fewer than 2000 fibres were randomly chosen by placing an imaginary grid on the section and sampling fibres from every other square on the grid. The method ensured that the sample represented the whole cross-section of the muscle. The fibre type was then classified, and the cross-sectional areas (CSAs) were determined using an image analysing system (Bioquant Imaging System, Nashville, TN, USA). Since the muscle sections were cut at right angles to the long axis of the muscle, the single fibre CSA was corrected for the angle of pennation of the MGN muscle. The correction was achieved by multiplying the calculated CSA by the cosine of the 21 deg angle of pennation. The mean single fibre CSA of all fibre types in the muscle was obtained by weighting the value for each fibre type by the percentage present. The percentage of the total fibre CSA occupied by each fibre type was also determined. The total fibre CSA of each muscle was calculated from the formula: muscle mass (in kg) divided by the product of L_f (in m) and density (in kg m^{-3}). The density of skeletal muscle was taken as 1060 kg m^{-3} (Brooks & Faulkner, 1988). The specific F_0 (in kN m^{-2}) of the whole muscle was determined by dividing F_0 (in kN) by the total fibre CSA (in m^2).

Number and proportion of motor units

For each muscle, the mean F_0 for all of the motor units measured was calculated. The F_0 for each muscle was divided by the mean F_0 for all of the motor units to estimate the number of motor units in each muscle. The estimates for MGN muscles of adult and old rats were then averaged separately to obtain the mean number of motor units for the adult and old age groups. For each muscle, the proportion of different types of motor units was calculated based on the number of motor units sampled for that type and the total number of motor units sampled. The proportion of motor units of each type calculated for each muscle was averaged for the adult and old age groups. For each muscle, based on the proportion of each motor unit type and estimated number of motor units for the whole muscle, the total number of motor units of each type was calculated. For each type of motor unit in each muscle, the total force developed by was obtained by multiplying the mean F_0 by

Table 1. Structural and functional data for whole MGN muscles and for overall properties of motor units for adult and old rats

	Adult	Old
Sample size	10	8
Mass (mg)	732 ± 28	619 ± 15*
Dry/wet mass ratio	0·28 ± 0·01	0·29 ± 0·02
Fibre length (mm)	12·7 ± 0·1	12·7 ± 0·1
CSA (mm^2)	54·1 ± 1·6	45·9 ± 1·4*
TPT (ms)	18·8 ± 1·0	22·9 ± 1·1
F_0 (N)	11·2 ± 0·4	8·0 ± 0·3*
Specific F_0 ($kN\ m^{-2}$)	207 ± 4	175 ± 7*
Motor unit F_0 (mN)	117 ± 7	101 ± 3**
Number of motor units	97 ± 4	81 ± 3**
Number of fibres in MGN †	19437 ± 620	17764 ± 690

Values are given as means ± 1 S.E.M. * Value for old rats significantly different from value for adult rats with two-tailed test; $P < 0·05$. ** Value for old rats significantly different from value for adult rats with one-tailed test; $P < 0·05$. See text for explanation. † Total number of fibres calculated from the functional method (see Methods). CSA, cross-sectional area; TPT, time-to-peak force; F_0, maximum isometric tetanic force.

the number of motor units. The percentage contribution of each motor unit type to the whole muscle F_0 was calculated by dividing the whole muscle F_0 by the total force obtained for each motor unit type. If the number of motor units sampled in a muscle was less than four, the data from that muscle were removed from the calculation.

Innervation ratio

The innervation ratios of the different types of motor units were determined indirectly. Burke & Tsairis (1973) used a similar rationale, but a slightly different method, to estimate indirectly the innervation ratios of motor units in the MGN muscles of cats. Our indirect estimate was based on two assumptions regarding the MGN muscle of rats: that (1) muscle fibres classified by contractile properties are not different from those classified by histochemical techniques, and (2) slow and fast fibres develop the same specific F_0. The first assumption, that classifications based on contractile properties (Burke *et al.* 1973) and those based on histochemical demonstration of myofibrillar ATPase activity (Brooke & Kaiser, 1970) are not different, is supported by data on the MGN muscles of cats (Burke *et al.* 1973) and rats (Einsiedel & Luff, 1992). Thus, in the MGN muscles of cats and rats, fibres in FF motor units demonstrate the myofibrillar ATPase activities of type II b fibres, fibres in FR motor units that of type II a fibres and fibres in S motor units that of type I fibres.

A second assumption made for the indirect estimate of single fibre F_0 values was that no difference existed in the development of specific F_0 by fast and slow fibres, motor units or muscles. Several studies have found no difference in the value of specific F_0 for fast and slow single permeabilized fibres (Lucas, Ruff & Binder, 1987; Macpherson, 1995) and whole muscles (Brooks & Faulkner, 1988). In contrast, the specific F_0 values reported for slow motor units have been invariably lower than those for fast motor units and the values have been highly variable (Burke & Tsairis, 1973; Bodine, Roy, Eldred & Edgerton, 1987; Chamberlein & Lewis, 1989; Kanda & Hashizume, 1989, 1992). Difficulty in identifying glycogen-depleted fibres in slow motor units has been noted repeatedly (Burke & Tsairis, 1973; Chamberlein & Lewis, 1989; Kanda & Hashizume, 1989, 1992). Despite significant effort, the explanation for the low estimates of specific F_0 values for slow motor units has not been resolved. We conclude that the measurements of the specific F_0 of single fibres and whole muscles are more valid than those of the motor units and that, in animals of the same age, slow and fast single fibres, and consequently motor units, develop specific F_0 values not different from one another.

The F_0 generated by a single fibre of each muscle fibre type was calculated from its mean single fibre CSA and the whole muscle specific F_0. For MGN muscles of either adult or old rats, the specific F_0 appropriate for a particular MGN muscle was used to calculate the F_0 for each type of single fibre in that muscle. The innervation ratio of each type of motor unit in each muscle was obtained by dividing the F_0 of a given motor unit by the mean single fibre F_0 of the appropriate fibre type.

Total number of fibres per MGN muscle

To validate the indirect estimates of the innervation ratios of the motor units, we proposed to use two methods for determining the number of fibres in the MGN muscle. The ~18000 fibres estimated for the MGN muscles of rats (Kanda & Hashizume, 1989) make a direct count of the number of fibres following nitric acid digestion unreasonable. Consequently, the number of fibres in each MGN muscle was estimated by an exclusively 'structural' method and by a predominantly 'functional' method. The structural method consisted of dividing the total fibre CSA by the mean single fibre CSA of each type of fibre and then summing the values to obtain the total number of fibres in the whole muscle. The functional method involved obtaining the number of fibres for each type of motor unit from the product of the innervation ratio and the number of motor units. The values for each type of motor unit were then summed to obtain the total number of fibres in each muscle. The functional method is not completely devoid of structural measurements, but, despite this limitation, the comparison provides some verification of the assumptions underlying the estimation of the innervation ratios of motor units in adult and old rats.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare means of whole muscle variables and of different motor unit types between the two age groups. When ANOVA indicated a statistical significance between motor unit types, Student's t tests were used

Table 2. Morphological and functional data on single fibres in MGN muscles and estimates of the number of fibres per motor unit and per muscle for adult and old rats

	Adult	Old
Sample size	10	8
Single fibre CSA (μm^2)		
Type I	1869 ± 55	1839 ± 103
Type IIa	2521 ± 94	2593 ± 67
Type IIb	3176 ± 84	2925 ± 64*
Mean	2834 ± 74	2559 ± 51*
Total fibre CSA (%)		
Type I	4·3 ± 0·2	14·7 ± 0·7*
Type IIa	36·3 ± 0·7	43·7 ± 0·6*
Type IIb	59·4 ± 0·7	41·6 ± 0·8*
Single fibre F_0 (μN)†		
Type I	386 ± 11	323 ± 22*
Type IIa	522 ± 23	454 ± 20*
Type IIb	657 ± 21	513 ± 23*
Number of fibres‡		
Type I	1233 ± 46	3676 ± 94*
Type IIa	7849 ± 295	7751 ± 198
Type IIb	10166 ± 382	6527 ± 167*
Total	19249 ± 492	17954 ± 295*

Values are given as means ± 1 S.E.M. * Value for old rats significantly different from value for adult rats with two-tailed test; $P < 0·05$. † Single fibre F_0 was calculated by the product of single fibre CSA and specific F_0 (values of specific F_0 are given in Table 1). ‡ The number of fibres was calculated by the structural method (see Methods). CSA, cross-sectional area; F_0, maximum isometric tetanic force.

to compare the means further. Generally a two-tailed test was used except in the testing of specific hypotheses, when a one-tailed t test was appropriate. The level of significance was set *a priori* at 0·05.

RESULTS

Despite the use of specific pathogen free rats in this study, some of the diseases normally acquired by old F344 rats were observed. Three of the old rats were diagnosed with interstitial cell tumour of the testis, nephrosclerosis and pituitary adenoma. The functional and morphological properties of the MGN muscles of these rats were not different when compared with other rats in the old age group. Consequently, the data from all the rats were pooled. All the rats were in good condition judged by their movement, body mass and food intake.

The body mass values of the rats from the adult and old age groups were not different, but the mass of MGN muscles in old compared with adult rats was 15% smaller (Table 1). Due to the smaller muscle mass and the lack of any change in L_f, the total fibre CSA of muscles of old rats was also 15% smaller. The TPT values of the twitch response for muscles in adult and old rats were not different (Table 1). Compared with the value of F_0 for muscles in adult rats, the value for muscles in old rats was 71% (Table 1). When the F_0 was normalized by the total fibre CSA, the specific F_0 (in kN m^{-2}) for MGN muscles in old rats was 85% of the value for adult rats. The F_0 was obtained at a stimulation frequency of 120 Hz for muscles in both adult and old rats. The frequency–force curves, normalized by the F_0 for adult and old muscles, were not different. For example, the stimulation frequency which produced 50% of F_0 was 35 Hz for adult and 32 Hz for old rats.

Compared with the single fibre CSA in MGN muscles of adult rats, the values for type I and type IIa fibres in muscles of old rats were not different, whereas the values for type IIb fibres and for the total of all the fibres in muscles of old rats were 8 and 10% smaller, respectively (Table 2). When the total CSA occupied by a specific fibre type was expressed as a percentage of the total fibre CSA of the whole MGN muscle, compared with the values for muscles of adult rats, the values for muscles of old rats were 80% for type IIa fibres, 70% for type IIb fibres, and 342% for type I fibres (Table 2).

For motor units in MGN muscles of adult and old rats, force traces showed the often reported differences among the three fibre types (Burke *et al.* 1973), but no differences between the same type of motor unit in muscles of old compared with adult rats. The TPT values of specific motor unit types in muscles of old rats were not different from those of comparable motor units in muscles of adult rats (Table 3). In MGN muscle of two old rats less than four motor units were sampled. Consequently, these two muscles were removed from the calculations of the motor unit properties. For each of the other sixteen MGN muscles,

Table 3. Structural and functional data on the types of motor units in MGN muscles of adult and old rats

	FF	FI	FR	S
Sample size				
Adult	27	—	36	19
Old	18	3	26	15
TPT (ms)				
Adult	15·1 ± 0·5	—	14·8 ± 0·4	28·7 ± 0·5†
Old	15·3 ± 0·7	14·0 ± 0·6	15·5 ± 0·6	25·2 ± 0·7*†
F_0 (mN)				
Adult	206 ± 11†	—	94 ± 4†	19 ± 2†
Old	149 ± 6*†	125 ± 6	96 ± 3†	50 ± 3*†
Fatigue index				
Adult	0·19 ± 0·02†	—	0·87 ± 0·01†	0·95 ± 0·01†
Old	0·19 ± 0·02†	0·60 ± 0·01	0·89 ± 0·01	0·90 ± 0·01*
Proportion of motor units				
Adult	0·34 ± 0·01†	—	0·44 ± 0·03†	0·22 ± 0·02†
Old	0·26 ± 0·01*†	0·05 ± 0·03	0·42 ± 0·01†	0·27 ± 0·01*†
Number of motor units				
Adult	32 ± 2†	—	42 ± 3†	23 ± 3†
Old	21 ± 1*	—	38 ± 2*†	22 ± 2
Innervation ratio				
Adult	333 ± 17†	—	185 ± 7†	57 ± 4†
Old	286 ± 9*†	—	205 ± 7†	165 ± 13*†
Number of fibres				
Adult	10358 ± 731†	—	7796 ± 505†	1283 ± 206†
Old	6205 ± 289*†	—	7879 ± 292†	3680 ± 467*†

Values are given as means ± S.E.M. * Value significantly different from the value for adult rats of the same type; $P < 0·05$. † Value significantly different from the value for the other two types of motor units within the same age group; $P < 0·05$. Note: FI motor units are not included in the statistical analysis. FF, fast-fatigable motor unit; FI, fast-fatigue-intermediate motor unit; FR, fast-fatigue-resistant motor unit; S, slow motor unit.

complete data were obtained for between five and fourteen motor units. The properties of eighty-two motor units were determined in muscles of adult rats and sixty-two units in those of old rats. FI motor units were observed only in muscles of old rats. Even in muscles of old rats, the FI units constituted less than 5% of the total number of motor units sampled.

For MGN muscles of adult and old rats, the frequency distribution of F_0 values for each of the three types of motor units and for all motor units are shown in Fig. 1. Since FI motor units were not observed in adult rats and only three FI motor units were observed in old rats, the distribution of FI motor units is not shown. While the distribution of FR motor units changed little in old age (Fig. 1*B*), the FF and S motor units showed marked changes (Fig. 1*A* and *C*). Compared with the frequency distribution of F_0 values for FF and S units in muscles of adult rats, the same distributions of F_0 values for FF and S units in muscles of old rats were shifted to the left and right, respectively (Fig. 1*A* and *C*).

For FR motor units in MGN muscles of adult and old rats, the innervation ratios were not significantly different (Table 3). In contrast, compared with the innervation ratios of FF and S motor units in adult rats, the innervation ratios for comparable units in old rats were 86 and 289%, respectively (Table 3). The F_0 of FF and S motor units in muscles of old rats were 72 and 263% of the values for adult rats, respectively, whereas the F_0 of the FR units did not change significantly (Table 3).

Compared with the numbers of FF and FR motor units in MGN muscles of adult rats, the numbers in MGN muscles of old rats were 66 and 81%, respectively (Table 3), whereas the number of S units in muscles of adult and old rats was not different. Furthermore, although the contribution of FR motor unit F_0 to whole muscle F_0 did not change with ageing (Fig. 2), for the FF and S units in old rats, the contribution of motor unit F_0 to whole muscle F_0 decreased to 67% and increased to 349% of their respective values for adult rats (Fig. 2). As a consequence of the overall remodelling of motor units in MGN muscles of

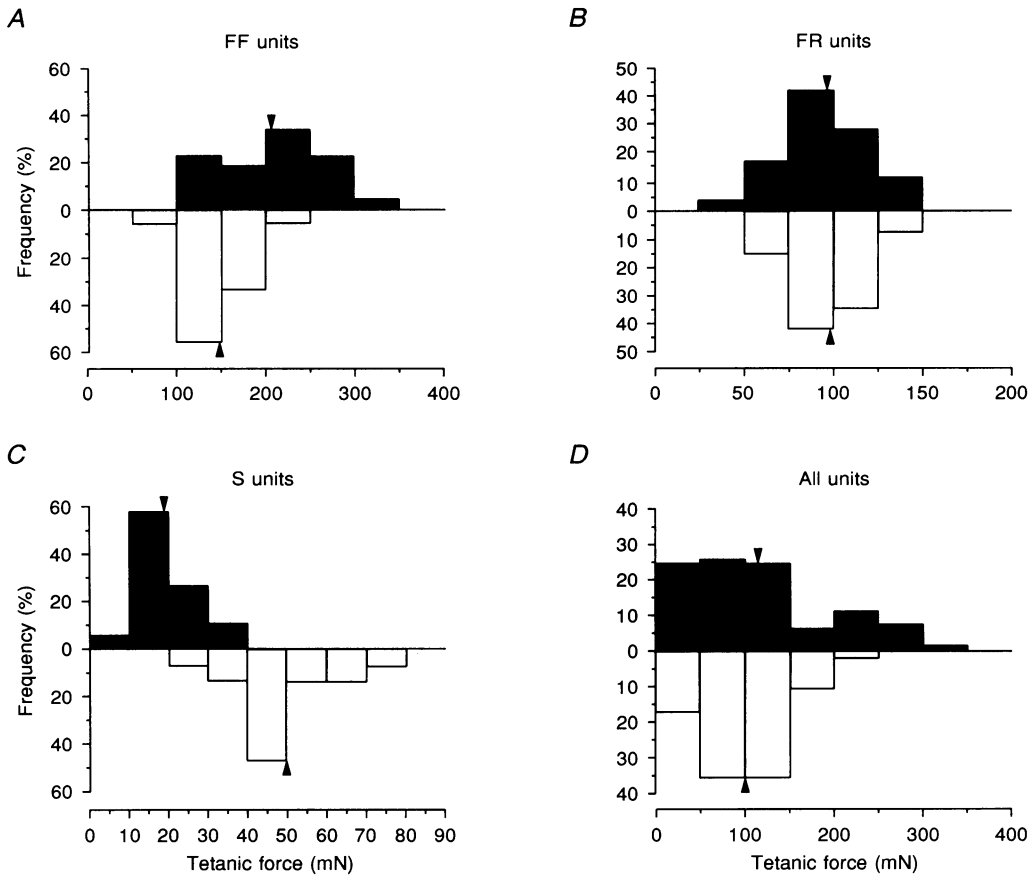

Figure 1. Frequency distribution of the maximum isometric tetanic force developed by each of the three types of motor units and all the motor units in MGN muscles of adult and old rats

■, adult rats; □, old rats. The arrows indicate the mean value of the distribution. The four frequency distributions represent fast-fatigable motor units (FF, *A*), fast-fatigue-resistant motor units (FR, *B*), slow motor units (S, *C*), and all motor units (*D*).

old rats, the number of motor units decreased to 84% and the mean F_0 for the total population of motor units to 86% of the values for adult rats (Table 1).

Based on the structural method, the estimate of the total number of fibres in the MGN muscles of old rats was 93% of the total number of 19249 obtained for adult rats (Table 2). The functional method, the product of the innervation ratio and the number of motor units, provided an estimate of 19437 for the total number of fibres in the MGN muscle of adult rats with an estimate for old rats of 91% of this value (Table 1). The excellent agreement between these estimates strongly supports the assumptions made in the indirect calculations of the innervation ratios.

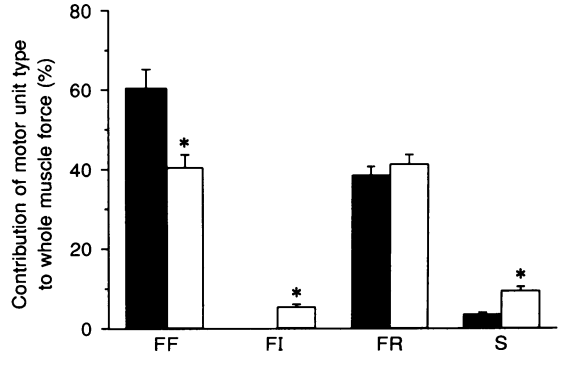

Figure 2. Percentage contribution of each type of motor unit to whole muscle force in adult and old rats

■, adult rats; □, old rats. The fast-intermediate (FI) motor units are only shown for old rats because no FI units were sampled in muscles of adult rats. * Significant difference from the value for motor units in muscles of adult rats ($P < 0{\cdot}05$). FF, fast-fatigable motor units; FR, fast-fatigue-resistant motor units; S, slow motor units. Error bars are S.E.M.

DISCUSSION

Our observations of dramatic changes in the F_0 values and innervation ratios of FF, FR and S motor units in MGN muscles of old compared with adult Fisher 344 rats confirm the reports of Kanda & Hashizume (1989) and Einsiedel & Luff (1992) that significant remodelling of motor units occurs with ageing. The lack of any difference between old and adult rats in the number of slow motor units in the MGN muscle has not been reported previously. Despite the absence of corroborative evidence, no change in the number of slow units with ageing is consistent with the hypothesis of a selective loss of fast motoneurons and of denervation of fast fibres in the remaining motor units (Kanda & Hashizume, 1989). For the MGN muscles of old compared with adult rats, the increase of 2·6-fold in the F_0 value for the slow units is slightly greater than the 2·1-fold increase observed by Kanda & Hashizume (1989) and considerably greater than the 7% increase reported by Einsiedel & Luff (1992). Of particular note are our indirect estimates of innervation ratios for slow units in MGN muscles of adult and old rats of 57 ± 4 and 165 ± 13, respectively, which are in excellent agreement with the values of 58 ± 3 and 154 ± 16 obtained by direct counts of glycogen-depleted fibres (Kanda & Hashizume, 1989).

Each study of motor units in MGN muscles of rats (Kanda & Hashizume, 1989; Einsiedel & Luff, 1992; and the present study) has reported a 30–40% decrease in the F_0 of the FF units. With ageing, the decrease in the F_0 of the FF units results from decreases in the single fibre CSA, innervation ratio and specific F_0. In contrast to the decreases in the FF units in MGN muscles of old rats, if the three FI units are included with the FR units, we found no difference between FR units in MGN muscles of adult and old rats for the F_0 or the innervation ratio and only a small 10% decrease in the number of FR units. In previous investigations of MGN muscles, Kanda & Hashizume (1989) reported a 36% decrease in the F_0 of the FR units, whereas Einsiedel & Luff (1992) cited a 34% increase. The data on F_0 of motor units in the Einsiedel & Luff (1992) study are difficult to interpret since 20–30% of the distribution of motor units in MGN muscles of adult and old rats were classified as FI units compared with 0–4% in our study and that of Kanda & Hashizume (1989). Einsiedel & Luff (1992) cited an even greater increase of 60% in the F_0 for FI units than the 34% increase they reported for FR units. Whether the increased proportion of FI units observed by Einsiedel & Luff is peculiar to Sprague–Dawley rats or represents a difference in classification criteria is not clear.

In the present study, the estimates based on single fibre and total fibre CSAs of 19250 fibres in the MGN muscles of adult ($n = 10$) and 17950 fibres in those of old ($n = 8$) rats are extremely close to the estimates of 18252 fibres in MGN muscles of three adult rats and 17515 fibres in those of two old rats (Kanda & Hashizume, 1989) estimated by a similar method. The overall effect of the remodelling of the three types of motor units is a large loss of 3600 muscle fibres from the FF motor unit pool and a minuscule loss of 100 muscle fibres from the FR unit pool. The increase of 2440 fibres in the S motor unit pool modulates the loss of the fast fibres significantly. As a consequence, the MGN muscle loses a total of 1300 fibres. In all species investigated, the number of muscle fibres in muscles decreases in old age. For old rats, our estimates of a 7–9% decrease in the number of fibres in MGN muscles are in reasonable agreement with direct measurements of EDL muscles (Daw, Starnes & White, 1988) and with a previous estimate of 5% obtained for MGN muscles (Kanda & Hashizume, 1989).

The large loss of muscle fibres from the FF motor unit pool is consistent with the hypothesis that, with ageing, fast fibres, and specifically FF (type II b) fibres, are particularly susceptible to denervation (Kanda & Hashizume, 1989). The hypothesis of a loss of fast type II b fibres is also supported by the substantial decrease in the type II b/type I fibre ratio in needle and open biopsy studies of muscles of humans (Larsson, 1983). The motoneurons of fast motor units differ from slow units in their larger membrane surface (Cullheim, Fleshman, Glenn & Burke, 1987), high metabolic enzyme activity (Campa & Engel, 1971), and the greater complexity of synaptic morphology (Burke *et al.* 1973). With advanced age, a declining synthesis of proteins and a decreasing ability to break down complex proteins (Gafni, 1990) as well as the increasing generation of partially reduced oxygen species (Sohal & Allen, 1990) may affect large fast motoneurons more than small slow motoneurons. Despite the attractiveness of the hypothesis, there is no direct evidence to indicate that during the course of ageing fast fibres are more susceptible to denervation than slow fibres.

The degeneration of nerve terminals at the motor end-plate may constitute an on-going process throughout life (Barker & Ip, 1966). Reinnervation by collateral and ultraterminal sprouting appears to be highly successful in young animals, but increasingly less so in older animals (Barker & Ip, 1966; Brown, Holland & Hopkins, 1981). Following experimentally induced partial denervation of muscles, nerve sprouting is less successful in muscles of old compared with adult animals (Rosenheimer, 1990). Furthermore, cross-reinnervation (Foehring, Sypert & Munson, 1986) and self-reinnervation (Desypres & Parry, 1990) studies have demonstrated slow motor nerves to be much more successful than fast motor nerves in re-innervating the denervated fibres. The influence of the type of motor nerve in determining the functional, histochemical and biochemical characteristics of muscle fibres is well established (Sreter, Luff & Gergeley, 1975). Consequently, the innervation of fast muscle fibres by slow motoneurons would transform the characteristics of the fibres to those of slow type I fibres. Despite the 3-fold increase in the innnervation ratio of slow units with age,

we observed no change in either the morphology of slow type I fibres or in their time-dependent contractile properties. Consequently, for MGN muscles of old compared with adult rats, the 3-fold increase in the number of slow type I muscle fibres and the 30–40% loss in the number of fast type IIb muscle fibres is consistent with the concept of denervation of the fast type IIb fibres, reinnervation by axonal sprouting from slow type I fibres with subsequent conversion of the fibre type. The process minimizes the effect of denervation, but does not eliminate it.

Substantial decreases in the number of motor units have been reported for muscles of elderly compared with adult humans (Doherty *et al.* 1993). Similarly, for old compared with adult rats, a smaller number of motor units have been observed in plantaris (Pettigrew & Gardiner, 1987), TBA and soleus (Edström & Larsson, 1987) and MGN (Kanda & Hashizume, 1989; Einsiedel & Luff, 1992) muscles. The number of motor units estimated for the MGN muscle of adult and old rats in our study is in good agreement with the data from other studies (Hashizume *et al.* 1988; Kanda & Hashizume, 1989; Einsiedel & Luff, 1992). Hashizume *et al.* (1988) attributed the smaller number of motor units in muscles of old compared with adult animals to the loss of α-motoneurons in the motor nuclei, but whether the initiating event is actually neurogenic or myogenic is unclear. The universal nature of the loss of motor units with ageing suggests that the phenomenon is an inevitable concomitant of ageing (Brooks & Faulkner, 1994).

The decline in muscle mass in old age has been observed in all mammalian species, including mice (Brooks & Faulkner, 1988), rats (Daw *et al.* 1988; Kanda & Hashizume, 1989), and humans (Grimby & Saltin, 1983). We report a 15% smaller muscle mass for the MGN muscles in old F344 rats compared with adult rats, whereas Kanda & Hashizume (1989) noted a 24% decrease. The larger decrease noted by Kanda & Hashizume could be due to their use of 28-month-old compared with our 25-month-old rats. The smaller muscle mass observed in old compared with adult rats is due to a combination of a decrease in the number of fibres (Daw *et al.* 1988), and a decrease in the single fibre CSA (Klitgaard, Brunet, Maton, Lamaziere, Lesty & Monad, 1989). Both mechanisms, a 10% decrease in single fibre CSA and a 7% decrease in the number of fibres, contribute to the 15% decrease in the mass of MGN muscles in old rats.

The deficit of ~30% observed for the F_0 of MGN muscles of old F344 rats agrees well with the deficit in F_0 of MGN muscles of Sprague–Dawley rats (Einsiedel & Luff, 1992). Similar decreases have been reported for F_0 of EDL and soleus muscles in mice (Brooks & Faulkner, 1988). The ~30% decrease in the F_0 of the MGN muscle of old compared with adult rats results partly from the 15% decrease in the total fibre CSA and partly from the 15% decrease in specific F_0. A decrease in the specific F_0 has been observed in the muscles of old mice, rats and humans (Brooks & Faulkner, 1994). The value of 207 kN m^{-2} for the specific F_0 of MGN muscles in adult rats is slightly lower than the value for most hindlimb muscles of rats (Eddinger, Moss & Cassens, 1985), or mice (Brooks & Faulkner, 1988). The lower value for specific F_0 results from the large angle of pennation for MGN muscle (Brooks & Faulkner, 1988; Ashton-Miller, He, Kadhiresan, McCubbrey & Faulkner, 1992) and the large aponeurosis (Holewijn, Plantinga, Woittiez & Huijing, 1984). After correcting for the 21 deg angle of pennation (Spector, Gardiner, Zernicke, Roy & Edgerton, 1980), and for the mass of the aponeurosis, the specific F_0 increases to a value of 233 kN m^{-2}, a value similar to that obtained for most hindlimb muscles of rodents (Brooks & Faulkner, 1988). The corrections do not affect the magnitude of the deficit in specific F_0 due to ageing (Brooks & Faulkner, 1988). Despite considerable effort, the cause of the deficit in specific F_0 that occurs with ageing is unknown (Faulkner *et al.* 1995).

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