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IN MICE, THE MUSCLE WEAKNESS DUE TO AGE IS ABSENT DURING STRETCHING

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SUMMARY

1. The contractile force was compared in isolated soleus muscles from young (2·5–8 months old) and aged (28–31 months old) mice. Force was measured at 25 °C during isometric tetanic contractions during isovelocity stretching and shortening contractions.

2. The normalized isometric force was lower by 13·3% in muscles from aged mice. Muscles from young and aged mice produced $0{\cdot}951 \pm 0{\cdot}031$ N mg^{-1} ($n = 12$) and $0{\cdot}824 \pm 0{\cdot}048$ N mg^{-1} ($n = 9$) respectively. The relaxation time, from 90 to 10% of the tetanic force, of muscles from aged mice was $102{\cdot}1 \pm 3{\cdot}7$ ms ($n = 6$), which was longer than that for muscles from young mice, $84{\cdot}4 \pm 3{\cdot}8$ ms ($n = 6$) (means ± S.E.M.).

3. The force during shortening was also reduced in muscles from aged animals by the same proportion as the isometric force. Therefore the force during shortening relative to the isometric force was the same in muscles from young and aged mice.

4. During rapid stretching soleus muscles from aged mice produced a similar force to those from young mice. Therefore stretch can remove the weakness in muscles of aged mice.

5. These changes in muscles from aged mice are similar to those produced when inorganic phosphate (P_i) levels are raised, in skinned rabbit psoas fibres, or during fatigue or with low intracellular pH (pH_i), in frog muscle. It is possible therefore that the force loss due to ageing may be due to a higher P_i level or a lower pH_i.

INTRODUCTION

With old age muscle becomes weaker. This is not only due to muscle atrophy but also to a reduction in force per cross-sectional area. In a recent study, Brooks & Faulkner (1988) compared the contractile properties of soleus and extensor digitorum longus (EDL) muscles from young, adult and aged mice. They found that the force produced per cross-sectional area was reduced in muscles from aged mice during both isometric and shortening contractions. The force was reduced by a proportion (about 20%) which was independent of shortening velocity. This proportional decrease in force per cross-sectional area, independent of shortening velocity, has also been

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described when the inorganic phosphate (P_i) level is raised, in skinned rabbit muscle fibres (Elzinga, Stienen & Versteeg, 1989), and when the intracellular pH (pH_i) is lowered, in frog muscle (Curtin, 1991). Fatigued muscle produces a reduced force but the force during shortening is affected to a greater extent than the isometric force (de Haan, Jones & Sargeant, 1989). Hypertonicity also reduces isometric force production (for example, Howarth, 1958). However, under all these conditions of raised P_i levels, low pH_i, fatigue or hypertonicity, the force exerted during stretching is reduced by a much smaller proportion than the isometric force (Curtin & Edman, 1989; Elzinga *et al.* 1989; Månsson, 1989; Curtin, 1991). This can readily be explained by the hypothesis that these treatments lower force by altering the equilibrium between two attached cross-bridge states which produce differing amounts of force (Pate & Cooke, 1989). Stretching the muscle would be expected to shift all the cross-bridges into the high-force state, and thus force would be less dependent on the equilibrium constant.

We have tested whether stretching also removes the force loss due to ageing, to determine whether a similar explanation can be given for the weakness characteristic of muscles of old animals.

METHODS

Experiments were performed on mouse soleus muscles at 25 °C. Both left and right soleus muscles of young (2·5–8 months old) or aged (28–31 months old) mice (C57BL/10 strain or the tan coat mutation of the C57 black animal) were dissected after the mice had been killed by dislocation of the neck.

The Ringer solution contained (mM): NaCL, 115; KCl, 5; $MgCl_2$, 0·5; $CaCl_2$, 2·5; NaH_2PO_4, 1; $NaHCO_3$, 24; glucose, 11; curare, 15 mg l^{-1}; and was gassed continuously with 95% O_2, 5% CO_2. This gave a pH of 7·4. Aluminium T-shaped clips were folded around each tendon close to the muscle. A hole in each clip was used to attach it to the apparatus. Muscles were mounted between a fixed hook and a hook attached to the lever of a motor (Cambridge Technology Inc., USA., model no. 350). While one muscle was studied the second was kept, pinned out just above slack length, in a Petri dish containing gassed Ringer solution, at room temperature.

Muscles were stimulated directly with supramaximal pulses of 2 ms duration; the tetanic contractions were 0·5–1·2 s long. The force–frequency relationship is different at low frequencies of stimulation in soleus muscles from aged compared to young mice but no difference is seen at or above 50 Hz (Brooks & Faulkner, 1988). Hence muscles were stimulated at 50 Hz which gave an almost fused tetanus.

Both force and position were measured from the motor system and the output was displayed and stored on an oscilloscope (Nicolet 4094). Step and ramp movements were produced by a ramp generator signal fed to the motor.

The optimum length for force (L_0) was determined using isometric tetanic contractions. Each muscle then underwent a series of release and stretches at different velocities during tetanic contractions with 2–3 min between tetani. Corrections were made for the change in resting force which occurred due to a change in muscle length. Isometric tetanic contractions of sufficient duration to produce maximum force were recorded several times during each experiment at L_0. The final isometric force compared with the initial force did not decline by more than 16% in any experiment. The force rise time and relaxation time for isometric tetanic contractions were measured: force rise time was the time taken for force to rise from 10 to 90% of plateau force. Relaxation time was the time taken for force to fall from 90 to 10% of plateau force.

At the end of the experiment muscles were fixed at L_0 in 2% gluteraldehyde overnight. Small fibre bundles, with tendons at each end, were teased from the fixed muscle and used to estimated fibre length (L_{fibre}). Following removal of the tendons, muscles were dried and dry weight (W) was measured. These values were used to normalize force (F) as $F \times L_{fibre}/W$ in units of N m g^{-1}, and velocity was expressed as fibre lengths s^{-1}.

TABLE 1. Contractile properties of muscle in mice

	Plateau force (N)	W/L_{fibre} (mg mm^{-1})	$F \times L_{fibre}/W$ (N m g^{-1})	Relaxation time (10–90%) (ms)	Force rise time (10–90%) (ms)
This study					
Young	0·198 ± 0·007	0·211 ± 0·013	0·951 ± 0·031	84·4 ± 3·8	176·1 ± 12·9
(2·5–8 months)	$n = 11$	$n = 11$	$n = 12$	$n = 6$	$n = 6$
Aged	0·183 ± 0·013	0·220 ± 0·0007	0·824 ± 0·048	102·1 ± 3·7	180·0 ± 12·9
(28–31 months)	$n = 9$	$n = 9$	$n = 9$	$n = 6$	$n = 6$
Aged − young	−0·015 ± 0·015	0·009 ± 0·013	−0·127 ± 0·057	17·7 ± 5·3	3·9 ± 18·2
t	1·04	0·54	2·31	3·32	0·34
P	n.s.	n.s.	< 0·05	< 0·01	n.s.
Brooks & Faulkner (1988)					
Young			0·871 ± 0·019		
(2–3 and 9–10 months)			$n = 25$		
Aged			0·779 ± 0·043		
(26–27 months)			$n = 14$		
Aged − young			0·092		
t			1·95		
P			< 0·10		

Data (means ± S.E.M.) are given from young and aged mice, including: plateau force, dry weight/fibre length (W/L_{fibre}), normalized plateau force ($F \times L_{fibre}/W$), relaxation time and force rise time of isometric tetani. The t value is that from an unpaired Student's t test; the null hypothesis is that there is no difference between muscles from young and aged mice. The number of muscles is shown as n.

RESULTS

Both left and right soleus muscles were used, twelve muscles from young mice and nine muscles from aged mice. The results obtained from isometric tetanic contractions are summarized in Table 1. The plateau force is less in the muscles from

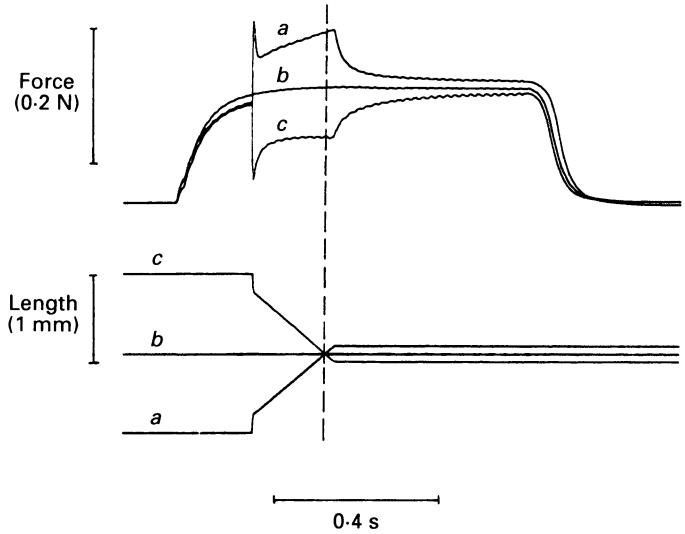

Fig. 1. Records of force during tetani and the corresponding length changes. Contractions are either with stretching (*a*), isometric (*b*) or with shortening (*c*). The isometric contraction (*b*) was at optimum muscle length for force. The dashed line shows when force was measured. The muscle was from a young mouse, stimulated at 50 Hz. Ramp length changes were at a velocity of 0·5 fibre lengths s^{-1}; L_{fibre} is 7·7 mm, W/L_{fibre} = 0·18 mg mm^{-1}.

aged mice. This is not due to change in size of the muscles, as estimated by dry weight per unit fibre length, which was the same in both groups of muscles. The reduction in normalized force ($F \times L_{fibre}/W$) was 13·3%. The relaxation time significantly increased with ageing, but there was no significant change in the force rise time.

The protocol used for obtaining force–velocity data is illustrated in Fig. 1, which shows superimposed records of force and length. Records (*b*) are from an isometric contraction at optimum length. For shortening (*c*) or lengthening (*a*) contractions a length step of 0·2 mm and an isovelocity ramp of 0·8 mm were applied after 0·2 s of isometric stimulation. Before a shortening contraction the muscle was just above optimum length, whereas before a lengthening contraction the muscle was below optimum length. The dashed line in Fig. 1 shows the point at which force was measured from all three records, which was when the length is at the optimum for isometric force. The normalized force (N m g^{-1}) during shortening and during stretching is shown in Fig. 2. Like the isometric force, the mean force during shortening was reduced in muscles from aged mice as compared to that from young mice. These differences were significant ($P < 0·1$) for most of the shortening velocities and for slow stretches, whereas at high velocities of stretching there was no

significant difference in the force produced. However, the errors are such that proportional difference in force during stretching as large as that in isometric contraction cannot be excluded statistically.

A clearer picture can be obtained by plotting the isovelocity force relative to the isometric force for the same muscle, as shown in Fig. 3. The force during shortening

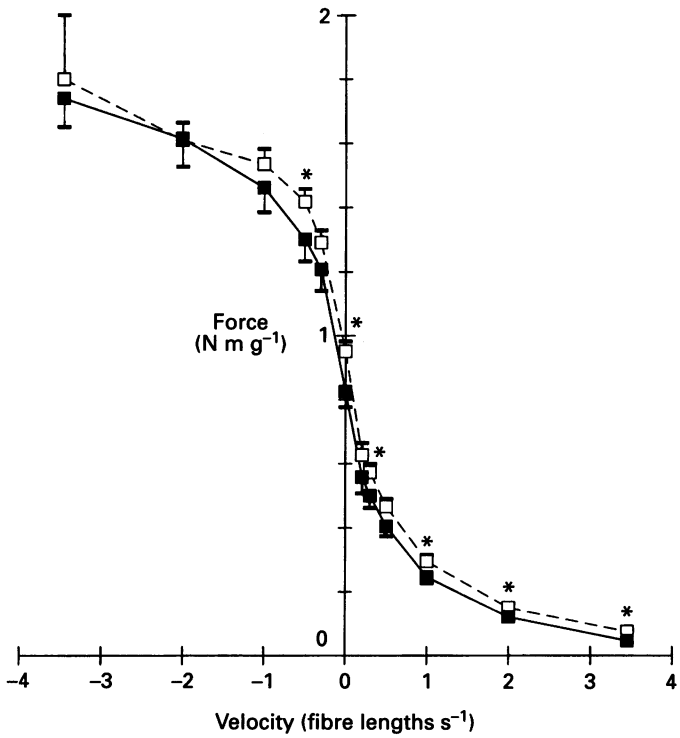

Fig. 2. Mean force–velocity relationship during shortening and stretching (negative values) from muscles of young (□) and aged (■) mice. The results are means ± S.E.M. (error bars are not included when they fall within the symbol). The asterisks indicate a significant difference between the means of the two groups in a one-tailed unpaired *t* test ($P = 0{\cdot}10$). The numbers of observations were as follows (from left to right): young, 2, 8, 11, 11, 12, 12, 9, 12, 12, 12, 10, 2; aged, 6, 8, 9, 9, 9, 9, 7, 9, 9, 9, 9, 2.

relative to isometric contraction is almost identical for muscles from young and aged mice at all velocities; that is, the proportion of the force lost due to ageing is similar during shortening and when isometric. In contrast, during stretching the relative force was clearly greater in muscles from aged mice. An unpaired *t* test showed that this increase in force was significant at all velocities of stretch ($P < 0{\cdot}005$ for each velocity except for $-3{\cdot}45$ fibre lengths s^{-1}; $P < 0{\cdot}10$). The three fastest velocities of stretch are all in the region of the force–velocity curve for which force is almost independent of velocity. We therefore combined all these results (from the three fastest velocities) to find the best estimate of the extent to which force during rapid stretching is increased, relative to isometric force, in the muscles from aged mice; the

result is 12·7 ± 2·1 %. Thus the reduction in isometric force due to ageing appears to be very similar to the enhancement of the relative force during rapid stretching.

The kinetics of the force change due to stretching or shortening appear to be unchanged by ageing. This conclusion was reached by measuring the change in force

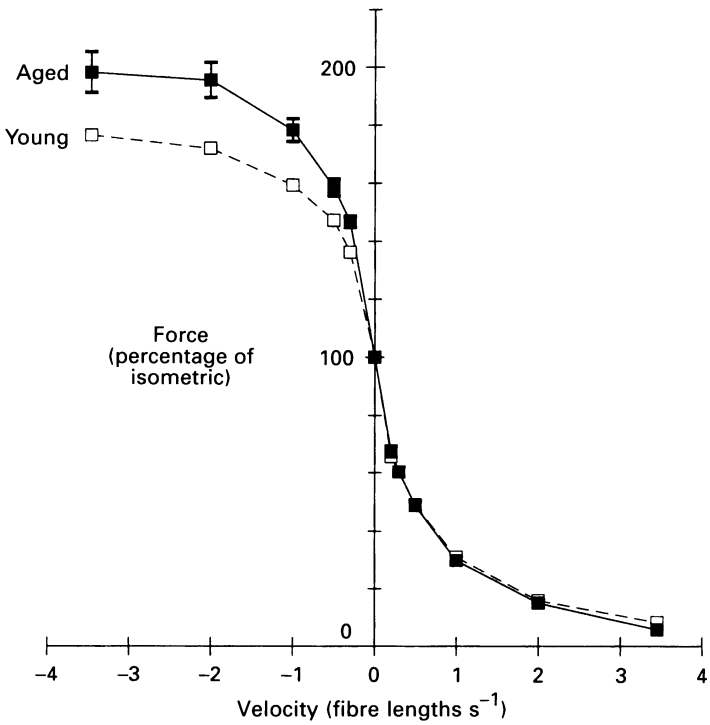

Fig. 3. Relative force–velocity relationship for muscles from young (□) and aged (■) mice. The force during isovelocity movement is expressed as a percentage of the isometric force. The results are means ± S.E.M. (error bars are not included when they fall within the symbol). The numbers of observations were the same as in Fig. 2.

during the releases and stretches at approximately 2 fibre lengths s^{-1} at 20, 40, 60 and 80 % of the length change, and comparing it with that at 90 %. These ratios were compared in muscles of young and aged mice by subtracting the ratio for young from the ratio for old. The results for stretching (means ± S.E.M., $n = 12$) were −5·5 ± 5·6, −2·6 ± 3·6, 1·2 ± 4·2, −4·2 ± 4·2 %. For shortening the figures were 2·7 ± 2·9, 2·5 ± 1·4, 0·6 ± 1·1 and 1·9 ± 0·7 %. Thus no significant difference in the time course of force response to length change was apparent.

DISCUSSION

Recent experiments using mouse and human skeletal muscle have shown that muscle weakness associated with old age is not only due to atrophy but that there is also a reduction in force per cross-sectional area (Brooks & Faulkner, 1988; Bruce, Newton & Woledge, 1989). The mouse muscles that Brooks and Faulkner studied were soleus and EDL, and both produced less force per cross-sectional area when

from an aged mouse. The results of Brooks and Faulkner for soleus muscles are shown in Table 1, converted to the units that we used in this study. We find a significant force reduction with soleus muscles from aged mice of 13%. A similar decrease (11%) was found by Brooks and Faulkner in soleus muscles. They also studied the force produced by soleus and EDL muscles in aged mice during shortening and found it to be reduced compared to that in young mice by the same proportion as the isometric force. This is also what we found in soleus muscle during shortening. In addition we found that during stretching the force is reduced much less and if the stretch is sufficiently rapid there is no reduction in the force exerted. A similar phenomenon may occur in human muscle as a recent study showed that the weakness in elderly subjects was less for eccentric contractions than for concentric contractions (Vandervoort, Kramer & Wharram, 1990).

There have been two possible explanations put forward for the force reduction per cross-sectional area associated with ageing in muscles studied *in vitro*. These possibilities are (1) a failure of activation or (2) the reduced amount of myofibrillar proteins, either because of the replacement of muscle cells by other material (e.g. connective tissue) or because of the intracellular accumulation of lipofuscin, which is known to occur in some muscles (Jennekens, Tomlinson & Walton, 1971). In both cases the force loss is due to a reduction in the number of active myosin sites. Our results suggest that this cannot be correct because it predicts that the force deficit during stretching would be of the same proportion as that during isometric and shortening contractions. In fact the force in muscle from aged mice, when it is rapidly stretched, seems to be the same as that in muscle from young mice, in contrast to the deficit during isometric and shortening contractions. The simple explanation of this observation is that aged mice have as many myosin sites as young mice. We found no difference in the isometric force rise time nor in the time course of the stretch or release response in muscles from young and aged mice. This suggests that the level of activation is comparable in muscles from young and aged mice under all the conditions of our experiments.

Stretching an active muscle causes it to produce extra force, above the isometric level (Katz, 1939). This extra force could be due to (1) parallel elasticity, such as that from connective tissue, (2) an increased number of attached cross-bridges, or (3) greater force per cross-bridge. The extra force does not come from parallel elasticity because the experiments have been designed to eliminate this by subtracting the resting force. Neither is it likely to come from an increase in the number of attached cross-bridges, because if we can extrapolate from experiments on frog muscle fibres, there is only a slight increase in the stiffness of muscle during stretch (Lombardi & Piazzesi, 1990). It seems therefore that the main effect of stretching on muscle force is to increase the force exerted by each cross-bridge. Lombardi and Piazzesi consider that this is due to moving more of the cross-bridges into a high force state. If we identify this state with the high force state suggested by Pate & Cooke (1989), which differs from the low-force state in not having P_i bound to the active site, then we have an explanation of why raised P_i levels lower force during isometric contraction, but not during stretching as reported by Elzinga *et al.* (1989). In the former case the P_i shifts the equilibrium of the cross-bridges towards the low force, P_i-bound state, but during sufficiently rapid stretching all the cross-bridges are forced into the

low-force state irrespective of the P_i concentration. If pH_i and hypertonicity also altered the equilibrium between these states a similar explanation would account for the results of Curtin (1991) and Månsson (1989). Therefore it seems likely that the cause of the force loss in ageing is that the behaviour of the myosin molecule changes, favouring the low-force state. Perhaps this is because the muscles from old mice are more acid and/or have a higher P_i than those from young mice, a point that now should be investigated. Activation or excitation–contraction coupling involving calcium release and uptake, and binding to troponin, etc. may change with age in some way. Thus the possibility that ageing causes a reduction in activation which can be reversed by stretching cannot be ruled out. It is not likely that myofibrils of ageing mice are in a hypertonic environment. However, hypertonicity may reduce the myofibrillar lattice spacing, and it may be this lattice that is altered in aged animals. Perhaps there is a change in the environment that is increasing the net charge on the proteins. The lattice spacing should therefore be measured in muscles from young and aged mice to see if this is contributing to the force reduction in aged animals.

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REFERENCES

BROOKS, S. V. & FAULKNER, J. A. (1988). Contractile properties of skeletal muscles from young, adult and aged mice. *Journal of Physiology* **404**, 71–82.

BRUCE, S. A., NEWTON, D. & WOLEDGE, R. C. (1989). Effect of age on voluntary force and cross-sectional area of human adductor pollicis muscle. *Quarterly Journal of Experimental Physiology* **74**, 359–362.

CURTIN, N. A. (1991). Force during stretch and shortening of frog sartorius muscle: effects of intracellular acidification due to increased carbon dioxide. *Journal of Muscle Research and Cell Motility* (in the Press).

CURTIN, N. A. & EDMAN, K. A. P. (1989). Effects of fatigue and reduced intracellular pH on segment dynamics in 'isometric' relaxation of frog muscle fibres. *Journal of Physiology* **413**, 159–174.

DE HAAN, A., JONES, D. A. & SARGEANT, A. J. (1989). Changes in velocity of shortening, power output and relaxation rate during fatigue of rat medial gastrocnemius muscle. *Pflügers Archiv* **413**, 422–428.

ELZINGA, G., STIENEN, G. J. M. & VERSTEEG, P. G. A. (1989). Effect of inorganic phosphate on length responses to changes in load in skinned rabbit psoas muscle. *Journal of Physiology* **415**, 132*P*.

HOWARTH, J. V. (1958). The behaviour of frog muscle in hypertonic solutions. *Journal of Physiology* **144**, 167–175.

JENNEKENS, F. G. I., TOMLINSON, B. E. & WALTON, J. N. (1971). Histochemical aspects of five limb muscles in old age. *Journal of Neurological Sciences* **14**, 259–276.

KATZ, B. K. (1939). The relationship between force and speed in muscular contraction. *Journal of Physiology* **96**, 45–64.

LOMBARDI, V. & PIAZZESI, G. (1990). The contractile response during steady lengthening of stimulated frog muscle fibres. *Journal of Physiology* **431**, 141–171.

MÅNSSON, A. (1989). Changes in force and stiffness during stretch of skeletal muscle fibres, effects of hypertonicity. *Biophysics Journal* **56**, 429–433.

PATE, E. & COOKE, R. (1989). A model of crossbridge action: the effects of ATP, ADP and P_i. *Journal of Muscle Research and Cell Motility* **10**, 181–196.

VANDERVOORT, A. A., KRAMER, J. F. & WHARRAM, E. R. (1990). Eccentric knee strength of elderly females. *Journal of Gerontology* **45**, B125–128.