The capacity of *mdx* mouse diaphragm muscle to do oscillatory work

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1. *Mdx* mice were used as a model for Duchenne muscular dystrophy; both lack dystrophin. It was hypothesized that the *mdx* condition would have a marked effect on the ability of diaphragm muscle from *mdx* mice to do active net work and generate power. This hypothesis was tested using the work-loop technique.

2. Specific twitch force, specific tetanic force and maximum power were all significantly less in diaphragm strips from *mdx* mice than those from control mice.

3. In all preparations muscle length at which maximum power was achieved (L_w) was about 8% less than that at which maximum tetanic force was achieved (L_0), both in *mdx* and control muscle.

4. The isometric force–length curve for *mdx* muscle was steeper on both sides of the plateau. Similarly, the curve relating net work per cycle to muscle length was steeper for *mdx* muscle on both sides of the plateau.

5. Maximum power of *mdx* muscle was achieved at a lower strain than for control muscle; maximum power occurred at a strain of 10·2% for *mdx* and 14·7% for control. Further increases in strain caused a marked decrease of power production in *mdx* muscle, whereas they caused a smaller decrease in control muscle.

6. In summary, at muscle lengths longer than L_w and at high strains, performance of *mdx* muscle was compromised relative to that of control muscle. Work and power were compromised more than isometric force.

Mdx mice are commonly used as a model for Duchenne muscular dystrophy (DMD) because both are characterized by a complete absence of the expression of dystrophin in skeletal muscle, that is they are homologous. Dystrophin is a cytoskeletal protein located on the inner face of the sarcolemma associated with cytosolic filament integral membrane glycoproteins. Dystrophin has been proposed as a stabilizer of the sarcolemma during contraction (Petrof *et al.* 1993), but its exact function is unknown (Koenig *et al.* 1988). The absence of the protein dystrophin in skeletal limb muscle of *mdx* mice is associated with constant progressive muscle degeneration and necrosis. The degeneration of myofibres activates satellite cells and initiates a regenerative response. In limb muscles of *mdx* mice an equilibrium between degeneration and regeneration is achieved. In contrast, in diaphragm muscles of *mdx* mice, the regeneration does not compensate for the degeneration and a progressive decrease in muscle function occurs similarly to that observed in skeletal limb muscle of boys with DMD (Stedman *et al.* 1991; Dupont-Versteegden & McCarter, 1992; Petrof *et al.* 1993; Louboutin *et al.* 1993; Lefaucheur *et al.* 1995; Lynch *et al.* 1997).

One consequence of the dystrophic process in diaphragm muscles of *mdx* mice, as with most myopathies, is the increase in the amount of connective tissue (Stedman *et al.* 1991). The increase in collagen within the diaphragm muscle causes a decrease in muscle elasticity and a marked increase in passive resting tension (Marshall *et al.* 1989; Stedman *et al.* 1991). We hypothesized that the *mdx* condition would have a marked effect on the ability of diaphragm muscle from *mdx* mice to do active net work (sometimes called work during miometric contractions) and generate power. This hypothesis was tested using the work-loop technique developed by Josephson (1985).

The work-loop technique uses imposed length changes to simulate *in vivo* muscle action. *In vivo*, skeletal muscle must spend some time lengthening and some time shortening. During shortening the diaphragm muscle does positive work. During lengthening of the diaphragm, work is done on the muscle by structures to which it is attached. Thus, net work per cycle during cyclic contraction and relaxation depends on both the positive work during shortening and the negative work that the muscle absorbs during lengthening. Figure 1 shows traces of length and force as a

function of time and the work-loop generated by these traces. If we start at rest length and integrate over 100% of one length change cycle, then positive work occurs during shortening from 25 to 75% of the cycle, negative work occurs from 0 to 25% and from 75 to 100% of the cycle.

$$\text{Net work} = \text{positive work} - \text{negative work}$$
$$= \int_{25}^{75} F\mathrm{d}l - \left(\left[\int_{0}^{25} F\mathrm{d}l + \int_{75}^{100} F\mathrm{d}l\right]\right),$$

where F is force and $\mathrm{d}l$ is displacement or the imposed length change.

By definition, a muscle with high passive tension is one in which there is a large change in F for a standard passive length change (Woledge *et al.* 1985). Therefore, a muscle with high passive tension will have high values of negative work. Thus we would predict that because diaphragm muscles from *mdx* mice have high passive tension (Marshall *et al.* 1989; Stedman *et al.* 1991) they should have high values of negative work. One important functional consequence of the high value of negative work per cycle is a lower value of net work. Furthermore, because the relation between passive force and length tends to be exponential rather than linear (Woledge *et al.* 1985), we would expect that diaphragm muscle from *mdx* mice would have even higher values of negative work and thus even lower values of net work at long muscle lengths or at large muscle strains. The purpose of the present study was to test these expectations.

METHODS

Cage sedentary male C57BL/10 *mdx* and C57BL/10SNJ (control) SPF mice from Jackson Laboratories, age 9–12 weeks, were used in all of the experiments. All procedures were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 86-23). Average weight of *mdx* mice was 29·3 ± 0·6 g and of control mice was 30·6 ± 0·6 g. Mice were anaesthetized with sodium pentobarbitone (70 mg kg^{-1} I.P.) and the diaphragm with ribs attached was removed and placed in oxygenated saline. The mice were killed immediately with an overdose of sodium pentobarbitone. A single strip approximately 1 mm wide was dissected carefully from each diaphragm muscle. At the rib end all intercostal muscle was removed from the rib and the rib was pierced with a P-6 needle and silk suture (7-0) threaded through in order to tie this end to the fixed arm of an isometric force transducer (Harvard apparatus). The central tendon was tied as close to the muscle as possible with silk suture (6-0); this end was attached to the movable arm of an ergometer (Cambridge Technology). Throughout, *mdx* muscle means diaphragm strips from *mdx* mice and control muscle means diaphragm strips from control mice. In addition, a passive loop refers to a work-loop during which length changes were imposed on a passive (unstimulated) muscle and an active loop refers to a work-loop during which length changes were imposed on an active (electrically stimulated and contracting) muscle.

The mean muscle length of the diaphragm strips at maximum isometric tetanic force (L_0) was 8·39 ± 0·14 mm (*mdx* 8·22 ± 0·19, control 8·65 ± 0·19, mean ± S.E.M. for 12 *mdx* and 9 control mice, P_{diff} (the P value from one-way analysis of variance) = 0·158). The mean muscle length at maximum net work per cycle (L_w) was 7·78 ± 0·15 mm (*mdx* 7·64 ± 0·19, control 7·99 ± 0·25, P_{diff} = 0·269). The mean muscle wet weight was 5·10 ± 0·29 mg (*mdx* 5·80 ± 0·33, control 4·05 ± 0·29, P_{diff} = 0·002). These values for L_0 and L_w were not significantly different between diaphragm muscles from *mdx* and control mice, but the muscle preparations from *mdx* mice weighed significantly more.

The diaphragm strips were bathed in physiological saline solution of composition (mM): Na^+ 198; K^+ 3·8; Ca^{2+} 2·0; Mg^{2+} 1; Cl^- 159; SO_4^{2-} 1; HCO_3^- 24; temperature was 25 °C. All saline solutions contained substrate as pyruvate (20 mM) and glucose (10 mM) and antibiotic (streptomycin and penicillin-g). Tubocurarine (Sigma T-2379) was present at a concentration of 0·017 g l^{-1} to ensure that stimulation was via the depolarization of the muscle membrane. The high [Na^+] did not decrease force or work (authors' unpublished observations). The diaphragm strips were stimulated with platinum plate electrodes. Stimulus voltage (about 5 V) was adjusted to 1·2 × that required to produce maximal isometric twitch force. Before actual experimental trials, muscle length was adjusted approximately to L_w and the muscle was given one passive length change cycle (4 Hz cycle frequency, excursion amplitude = 0·05L_w) and one twitch contraction every 60 s for 10 min to allow the muscle to stabilize.

Effect of changes in length

Since previous data (Stedman *et al.* 1991) indicated that diaphragm strips from *mdx* are very sensitive to length changes, special precautions were taken to estimate optimum length. At each length isometric force was measured using a short tetanic stimulus; then after a 3 min wait period, a work trial was performed. The work trial consisted of three passive cycles and one active cycle (cycle frequency = 4 Hz, excursion amplitude = 0·05L_w, duty cycle = 0·25). The stimulus train durations for the isometric trial and the work trial were the same (62·5 ms). The stimulus train consisted of nine pulses at 96 Hz with a triplet at the start of the train to increase the rate of force development (Stevens, 1996). This triplet stimulus pattern increased the rate of force development in control mouse muscle (authors' unpublished observations), and it was presumed (but was not tested) that it would do the same in *mdx* diaphragm. The stimulus train is shown in Fig. 1. Isometric force with this stimulus was 87 ± 3·5% of isometric force with a longer train for both *mdx* and control muscle. Then muscle length was increased a small amount (0·2–0·5 mm) and force measured after another 3 min rest period. Muscle length at maximum net work (and power) under these conditions could be sharply defined and is called L_w. Except in trials in which the effect of long muscle lengths were studied, length was increased up to two increments in length beyond L_0 (i.e. 0·4 mm, or about 5% of L_0); where L_0 is the length at which maximum isometric tetanic force was developed.

To measure work and power, a sinusoidal signal was used to cycle muscle length about L_w (see Fig. 1) (Josephson, 1985). We use the term excursion amplitude to describe the amplitude of the imposed length change cycle (i.e. strain) and express its magnitude as a fraction of L_w. The standard excursion amplitude was 0·05 L_w, that is L_w ± 0·025 L_w, for all trials except when the effect of varying strain was being studied. An excursion amplitude of 0·05 L_w was chosen because preliminary experiments showed that the preparation could be stimulated for many trials using this strain with no decrement in force or power as long as there was a recovery period between trials. Fibre length was the same as muscle length so the actual strain on the fibres was the same. The cycle frequency, that is the frequency of the imposed length change, was 4 Hz except in trials in which the effect of varying cycle frequency was being studied.

Table 1. Force and power in control and *mdx* diaphragm

	Mdx	Control	Ratio	*P*
P_t (kN m^{-2})	40·6 ± 3·7	59·2 ± 3·1	0·69	0·001
P_o (kN m^{-2})	122·5 ± 10·5	207·4 ± 13·4	0·59	0·0001
P_t/P_o	0·33 ± 0·01	0·29 ± 0·01	1·14	0·01
Power at 4 Hz (W kg^{-1})	12·73 ± 0·87	21·25 ± 1·21	0·60	0·0001
Maximum power (W kg^{-1})	13·48 ± 1·42	30·78 ± 1·20	0·44	0·0001
Strain at max power (% of L_w)	10·47 ± 0·35	14·14 ± 0·43	0·74	0·0001
L_w/L_0	0·930 ± 0·012	0·928 ± 0·015	1·00	0·46
Time to dF/dt (ms)	19·4 ± 0·7	21·1 ± 0·7	0·92	0·11
Time to −dF/dt (ms)	44·5 ± 1·2	47·0 ± 1·5	0·95	0·20

Twitch force (P_t), tetanic force (P_o), power produced, strain at which maximum power is produced by diaphragm strips from *mdx* mice compared with those from control mice. Time to dF/dt is the time from the first stimulus pulse to the peak of dForce/dt and time to −dF/dt is time from the last stimulus pulse to the peak of −dF/dt; these parameters were used as estimates of rise time and relaxation time. Values are means ± S.E.M., $n = 12$ mice for *mdx*, $n = 9$ mice for control; P values in the last column are probabilities from one-way analysis of variance.

The key variables in the present study were: muscle length, strain, cycle frequency and duty cycle and these are illustrated in Fig. 1. Duty cycle is the fraction of the length change cycle that the muscle is stimulated. The phase of stimulation refers to the timing of the start of the stimulus train compared with the start of the imposed length change (Fig. 1). In the present study phase of stimulation is given as a percentage of the length change cycle; muscle shortening occurs from 25 to 75%. Phases of stimulation were optimized for maximum work and power for each individual preparation. Average phases were: 21·0 ± 0·3% at 1 Hz cycle frequency, 16·7 ± 0·6% at

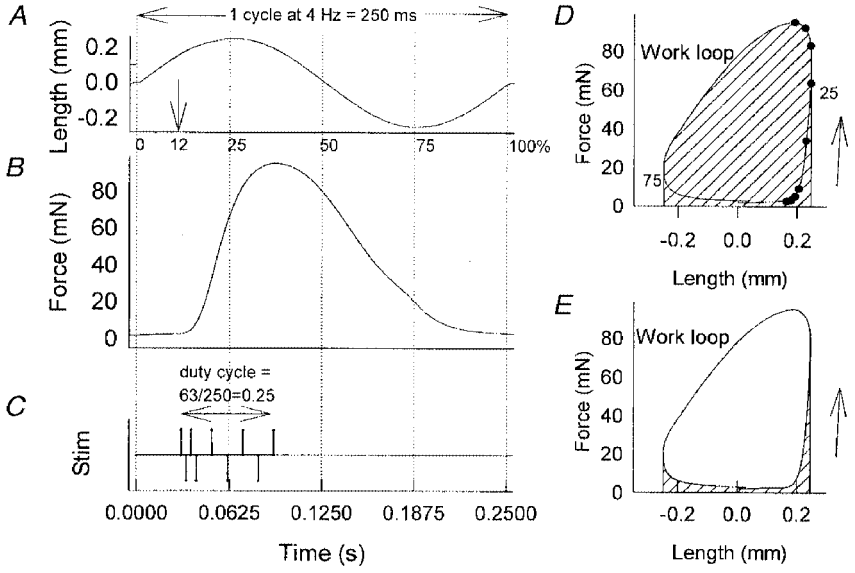

Figure 1. Traces to show conditions used to generate standard work-loops (4 Hz cycle frequency, 0·25 duty cycle), to define duty cycle and to define phase

A, the imposed length change cycle started at rest length, the muscle was stretched from 0 to 25% of cycle length, then shortened from 25 to 75% of cycle length (62·5 to 187·5 ms), and then stretched back to rest length. The vertical arrow indicates the onset of stimulation at a phase of 12% of cycle length. Excursion amplitude was 0·05 *L*. *B*, force developed by the stimulated muscle; most of the force developed during the shortening phase of the cycle. *C*, the stimulus train consisted of alternate positive and negative pulses at a base frequency of 96 Hz so the train duration of 7 pulses was 62·5 ms. The stimulus train had 2 additional pulses at the beginning of the pulse train to activate the muscle rapidly. Duty cycle (stimulus train duration as a fraction of cycle duration was 62·5/250 = 0·25. *D*, work-loop generated by the imposed length change cycle and force trace; arrow shows direction of the loop and symbols indicate the timing of the stimulus pulses; shortening is from 25 to 75 indicated on the loop; shaded area indicates positive work. *E*, same work-loop with negative work shaded; net work is the unshaded area and is equal to positive work minus negative work.

2 Hz, 13·2 ± 0·7 % at 4 Hz and 8·1 ± 0·7 % at 6 Hz. The rise time for contraction of the muscle was estimated from the time taken from the first stimulus pulse in the pulse train to the peak of the dF/dt (where F is force) trace during a standard working contraction (cycle frequency = 4 Hz, excursion amplitude = $0{\cdot}05L_w$, duty cycle = 0·25, phase = 12 %). The relaxation time was estimated as the time taken from the last stimulus pulse in the pulse train to the peak of $-dF/dt$ for the same contraction.

Displacement, the change in muscle length, was measured with the ergometer and force was measured with the isometric transducer; both were recorded with a Nicolet digital storage oscilloscope. Work is the product of force and displacement and was calculated by integration of the force–displacement curve. Positive work is the work done by the muscle during shortening and negative work is the work done on the muscle to lengthen it. Net work was calculated as the difference between positive and negative work.

In one set of experiments to study the effect of varying strain, strain was increased in increments of $0{\cdot}03L_w$; cycle frequency was kept at 4 Hz, and stimulus duty cycle was kept at 0·25. Usually, measurements were made up to a strain of $0{\cdot}21L_w$; in a few trials strain was increased further. Control trials with strain of $0{\cdot}05L_w$ were carried out before the series and after strains of 0·12, 0·15, 0·18 and $0{\cdot}21L_w$. There was no work deficit in these control trials so muscle damage at these strains was negligible. During all experiments, trials were made at 3 min intervals to allow for metabolic recovery. For each preparation the strain for maximum power was interpolated by fitting a quadratic to the relation. We report the average of these interpolated values in the results.

In another set of experiments, cycle frequency and duty cycle were varied. In this case cycle frequencies were done in the following order: 4, 6, 2 and 1 Hz because preliminary trials showed that low frequency trials were more likely to result in a force and work deficit. The cause of the greater force deficit at low frequency is

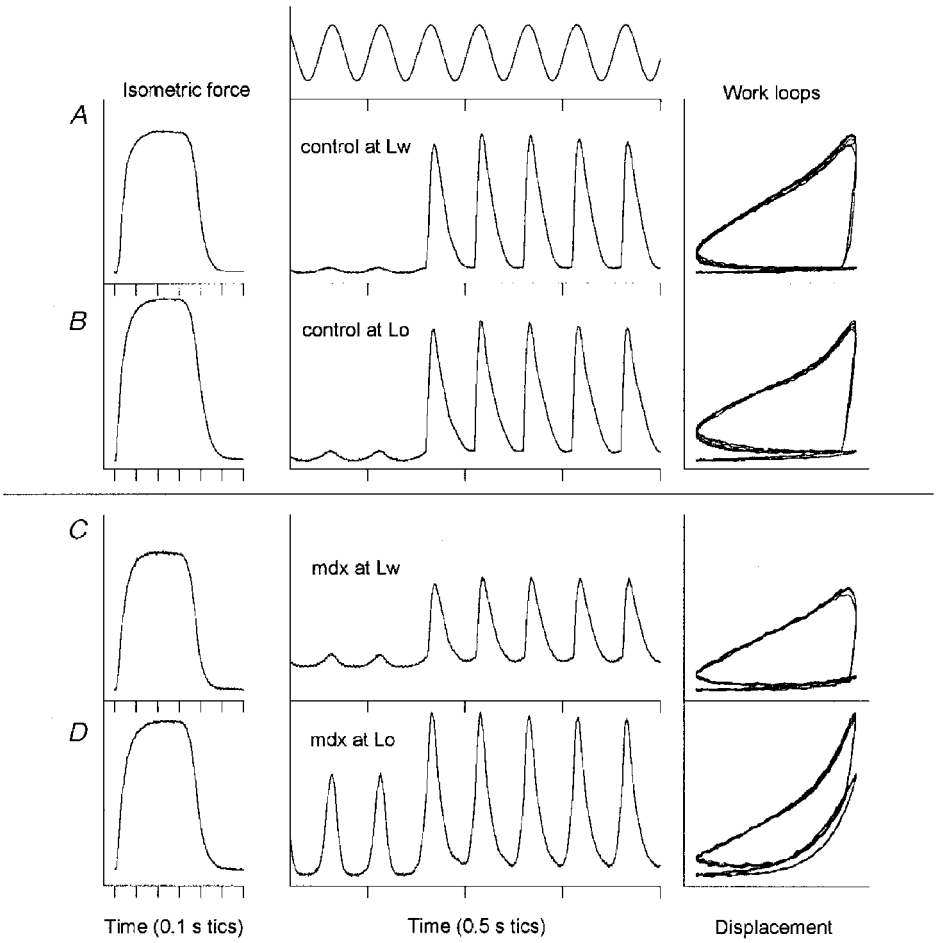

Figure 2

Exemplary traces to show the effect of a change in length on isometric force, rest force during the imposed cyclic length change of passive muscle, active force during the imposed cyclic length change of contracting muscle and work-loops in diaphragm strips from control mice (*A* and *B*) and from *mdx* mice (*C* and *D*). Traces are shown for each muscle at two muscle lengths: one near L_w (the length at which maximum power was achieved; *A* and *C*) and the other at L_0 (the length at which maximum developed isometric force was achieved; *B* and *D*). L_0 was always longer than L_w. For the work-loops the small loops at the bottom are for 3 consecutive loops from passive muscle (i.e. no electrical stimulation) and are clockwise. The larger loops are for 5 consecutive work-loops from contracting muscle and are counter-clockwise (imposed length change cycle = 4 Hz, excursion amplitude = $0{\cdot}05\,L_w$). The very top centre panel shows the imposed length change cycle.

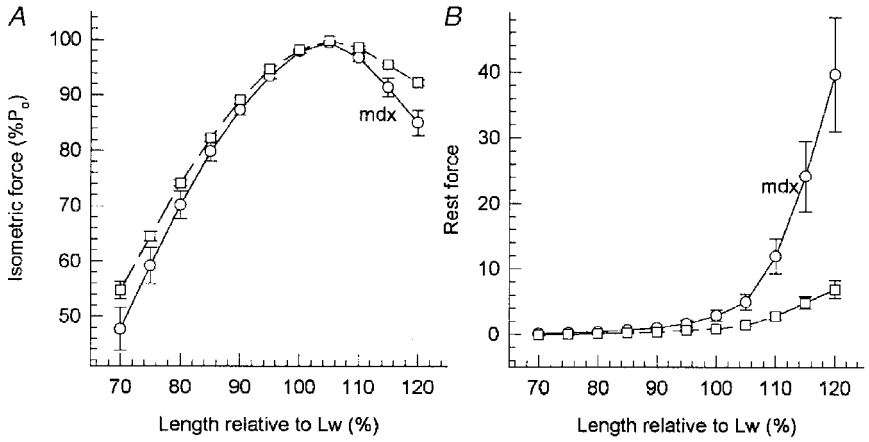

Figure 3

The effect of muscle length (normalized to L_w, the length at which work and power were maximum) on maximum developed isometric force (*A*) and on rest force (*B*) using diaphragm muscle strips from 8 *mdx* (○ and continuous lines) and from 6 control mice (□ and dashed lines). L_w is 100 on the *X*-axis. Values are means ± 1 S.E.M.; some S.E.M.S are smaller than the symbols. Scales are different for developed and rest force, but all force data are expressed as a percentage of P_o, maximum isometric tetanic force normalized for cross-sectional area.

probably due to the fact that stimulus trains were longer. Maximum power occurred at longer duty cycles at lower frequencies; for example duty cycle was 0·45 at 1 Hz cycle frequency whereas it was 0·3 at 6 Hz in control muscle. Stimulus train duration at a duty cycle of 0·45 at 1 Hz cycle frequency was 450 ms, whereas at a duty cycle of 0·3 at 6 Hz cycle frequency stimulus train duration was only 50 ms. During all experiments, trials were made at 3 min intervals to allow for metabolic recovery.

RESULTS

Specific twitch force (P_t), specific tetanic force (P_o), power under the standard conditions (4 Hz cycle frequency, 0·25 stimulus duty cycle) and maximum power were all significantly less in diaphragm strips from *mdx* mice than in diaphragm strips from control mice (Table 1). Twitch force/tetanus force ratio was significantly greater in diaphragm strips from *mdx* mice than from control mice. In all preparations the muscle length at which maximum power was achieved (L_w) was less than that at which maximum tetanic force was achieved (L_0). However, the ratio of L_w/L_0 was the same in diaphragm strips from *mdx* mice as from control mice. The times for contraction (dF/dt) and for relaxation ($-dF/dt$) were not significantly different between diaphragm strips from *mdx* mice compared with those from control mice. For most of the comparisons shown in the figures, work and power data have been normalized to that produced under standard conditions (4 Hz cycle frequency, 0·25 stimulus duty cycle) in order to facilitate comparisons of diaphragm strips from *mdx* mice with those from control mice.

Effect of varying muscle length

Exemplary traces for *mdx* and control muscle are shown in Fig. 2 at two different lengths, L_w and L_0. The force traces were similar at the two lengths, but the work-loops were quite different. The work-loop of *mdx* muscle at L_w was similar to that of control muscle, but the work-loop at L_0 (a slightly longer length) showed a marked increase in passive work and a decrease in net work. In Fig. 3, both tetanic and rest force data were normalized to maximum developed isometric force in the force–length curve. Length data were normalized to L_w, the length at which maximum work was achieved under standard conditions (4 Hz cycle frequency, 0·25 stimulus duty cycle). L_0 was 1·08 L_w for *mdx* muscle and 1·07 L_w for control muscle in Figs 3, 4 and 5. The isometric force–length curve for *mdx* muscle was steeper on both sides of the plateau. An increase in muscle length

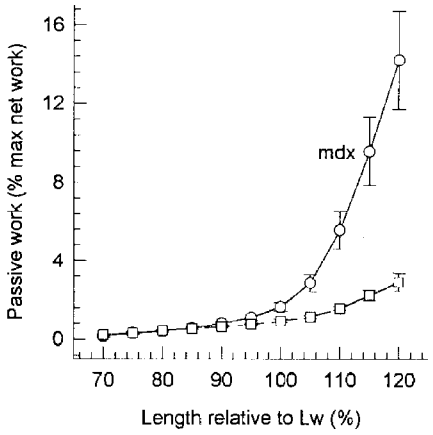

Figure 4

The effect of muscle length (normalized to L_w, the length at which net work is maximum) on work done during passive work-loops by diaphragm strips from 8 *mdx* mice (○ and continous lines) and from 6 control mice (□ and dashed lines). L_w is 100 on the *X*-axis. All work data are expressed as a percentage of maximum net work per cycle. Values are means ± 1 S.E.M.; some S.E.M.S are smaller than the symbols.

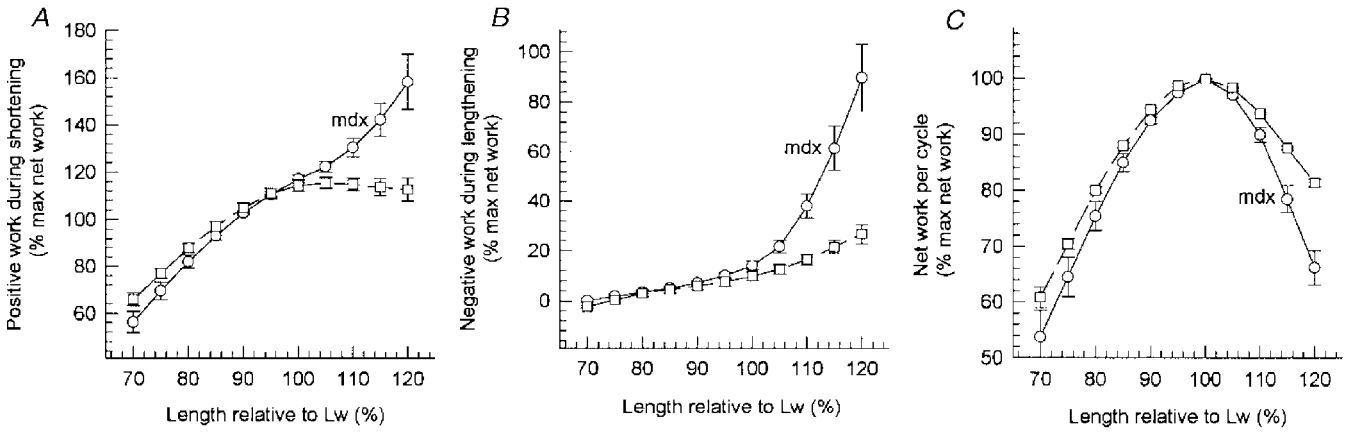

Figure 5

The effect of muscle length (normalized to L_w, the length at which work is maximum) on positive work (*A*), negative work (*B*) and net work per cycle (*C*) during active work-loops by diaphragm strips from 8 *mdx* mice and from 6 control mice. Other features are the same as in Fig. 4.

beyond L_w caused a marked increase in rest force of *mdx* muscles whereas the changes for control muscles were very small.

In the passive work–length curve (Fig. 4; exemplary work-loops are shown in Fig. 2), passive work data were normalized to maximum net work per cycle and length data were normalized to L_w. An increase in muscle length beyond L_w caused a marked increase in the amount of work done in a passive work-loop in *mdx* muscle, whereas the changes in control muscle were very small. Passive work is work done on the muscle; it is negative work.

In the active work-length curve (Fig. 5; exemplary work-loops are shown in Fig. 2), positive, negative and net work data were normalized to maximum net work per cycle and length data were normalized to L_w. An increase in muscle length beyond L_w caused a marked increase in negative work per cycle and positive work per cycle for *mdx* muscles, whereas the changes in control muscle were very small. The curve relating net work per cycle to muscle length was steeper for *mdx* muscle than for control muscle on both sides of the plateau. Thus the effect of length on negative work was larger than its effect on positive work and, as a result, net work per cycle was less at long lengths.

Effect of varying strain

As expected, maximum power production by diaphragm muscle strips from *mdx* mice was less than that of control mice (Fig. 6, Table 1). More interesting from the point of

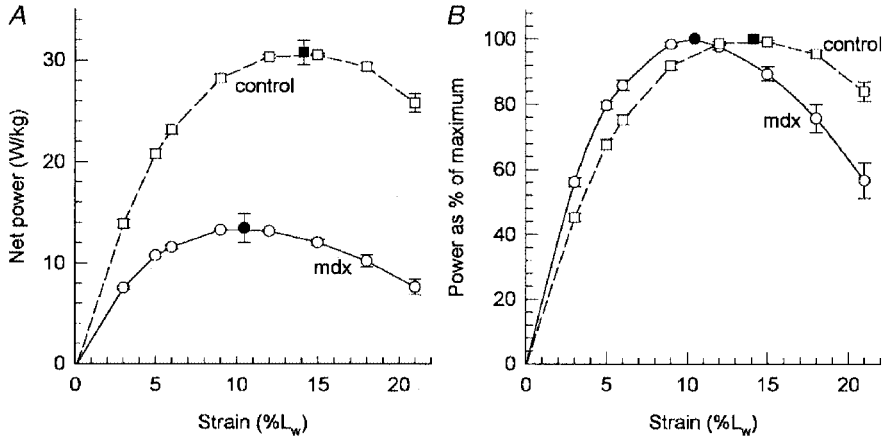

Figure 6

The effect of varying strain on power production by diaphragm strips from 10 *mdx* mice (○ and continuous lines) and from 7 control mice (□ and dashed lines). *A* shows absolute power values (W (kg muscle)$^{-1}$); weight specific peak power of muscle from *mdx* mice was about 44% that from control mice. In *B*, the values have been normalized to maximum power production for each preparation. The curve for muscle from *mdx* mice was steeper and peaked at a lower strain (10·5) compared with that from control mice (14·1). Cycle frequency was 4 Hz; stimulus duty cycle as a fraction of cycle duration was 0·25. Values are means ± 1 S.E.M.; many S.E.M.s are smaller than the symbols. Filled symbols show the interpolated values for peak power.

view of the present study was the observation that maximum power of *mdx* muscle was achieved at a lower strain than for control muscle (maximum at a strain of 10·2% for *mdx* mice and 14·7% for control mice). Moreover, further increases in strain caused a marked decrease in power production for *mdx* muscle, whereas they caused a smaller decrease for control muscle. Exemplary work-loops at a series of strains from 0·05 to 0·21 L_w are shown in Fig. 7. The larger loop occurred during an active contraction when the muscle was electrically stimulated and the smaller loop below the active loop occurred during a passive cycle when the muscle was not stimulated. Larger strains caused a marked increase in passive work and a marked decrease in the ability to do net work in *mdx* muscle compared with control muscle.

Effect of varying cycle frequency

The effect of cycle frequency was similar in both *mdx* and control muscle strips even though the absolute power levels were greater in the control strips (Fig. 8*C*). An increase in cycle frequency from 1 to 2 Hz resulted in an increase in power, as did an increase from 2 to 4 Hz (Fig. 8*A* and *B*), but the differences in power between 4 and 6 Hz were not significant. Also, as expected, maximum power was achieved at lower stimulus duty cycles as cycle frequency

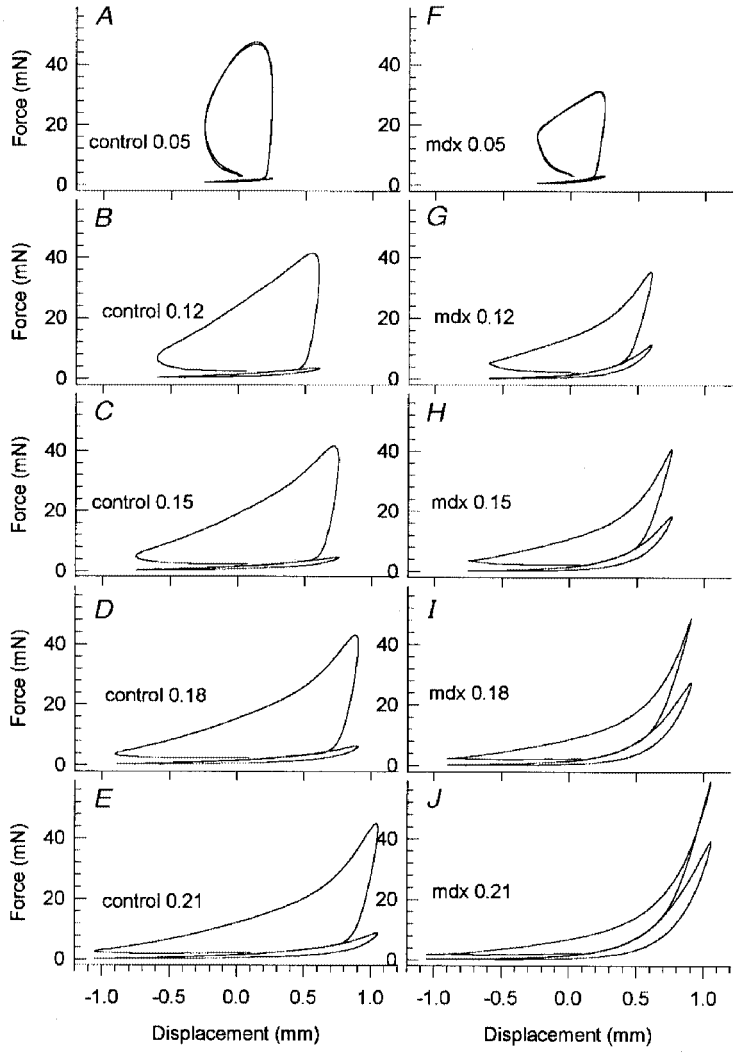

Figure 7

Exemplary work-loops at a series of different strains for diaphragm strips from a control mouse (*A*–*E*) and from an *mdx* mouse (*F*–*J*). In each case the work-loops are for a cycle from passive muscle followed by a cycle from active muscle. The passive work-loop was from a muscle passively stretched and not activated; it is the small loop at the bottom of the trace. The active loop is from the same muscle when electrically stimulated to contract during shortening so that it did positive net work per cycle. The top panels are for two loops under standard conditions with a strain of 0·05 L_w done before and after the other trials to show that the changes were reversible and that no damage was incurred at these strains. The muscle from the control mouse achieved maximum power (25·4 W kg^{-1}) at a phase of 14·5% whereas the muscle from the *mdx* mouse achieved maximum power (10·4 W kg^{-1}) at a phase of 10·4%. Both strips were about the same mass: control, 4·0 mg; *mdx*, 3·8 mg. The numbers in each panel give the strain.

was increased from 1 to 6 Hz. The stimulus duty cycle is the fraction stimulus train duration/total cycle duration.

In summary, at muscle lengths longer than L_w and at high strains, performance of *mdx* muscle was compromised relative to that of control muscle. Work and power were more compromised than isometric force.

DISCUSSION

The lower isometric force developed by diaphragm muscle strips from *mdx* mice compared with control mice observed in the present study is in agreement with previous reports. Petrof *et al.* (1993) reported that in young *mdx* mice, specific P_o of diaphragm strips is reduced to 50% of control values even when corrected for the portion of the diaphragm strip composed of damaged fibres. Lynch *et al.* (1997) reported that maximum isometric tension is reduced to 60% of control values in young *mdx* mice (4–6 months) and to 48% of control values in old *mdx* mice (24 months). The values for power reported by Lynch *et al.* (1997) are different from the values in this study because they did not use the work-loop method. Their measurements did not take into account the negative work required to return the muscle to its original length; they only reported positive work.

The main results of the present study are related to changes in the passive properties of diaphragm muscle. As explained in the Introduction, these changes in passive properties cause an increase in negative work and thus a decrease in net work per cycle. Our results show that these changes in passive properties narrow the range of lengths and strains over which *mdx* diaphragm muscle can do net positive work. Stedman *et al.* (1991) reported a reduction in elasticity related to a marked increase in collagen density. They showed one passive work-loop with a strain of ±7% and reported that the shape of this passive work-loop was independent of cycle frequency over the range 2–40 Hz. The fact that it is independent of cycle frequency is consistent with the results of Syme (1990) who showed that viscous work is an order of magnitude less than that of elastic work during passive work-loops of rodent diaphragm muscle; that is, passive properties are determined largely by elastic properties. Our results extend the observations of Stedman *et al.* (1991) to show that the marked increase in passive stiffness compromises the range of strains and lengths over which the *mdx* diaphragm can do useful work, that is, can do positive net work. The steeper curves for diaphragm muscle from *mdx* mice in Figs 3 and 5 can be accounted for in part by shorter average length of *mdx* fibres (Stedman *et al.* 1991).

Our results show that at large strains, the diaphragm muscle from *mdx* mice experienced much greater stress compared with muscle from control mice. For example, as strain was increased from 12 to 21% there was a 40% decrease in power generated by diaphragm strips from *mdx* mice (Fig. 6) and, at the same time there was a 50% increase in stress (Fig. 7). In contrast, in diaphragm strips from control mice there was a much smaller decrease in power generated, 16%, with no change in stress.

In addition to the decrease in isometric force, a slowing of twitch contraction kinetics and increased endurance in skeletal limb muscle from old *mdx* mice or *mdx* mice forced to undergo treadmill exercise also has been reported. These changes usually are attributed to the marked increase in the proportion of type I fibres and the marked decrease in type II fibres so that the whole skeletal muscle gradually changes to one with properties similar to those of slow twitch muscle (e.g. Stedman *et al.* 1991; Hudecki *et al.* 1993; Petrof *et al.* 1993; Pastoret & Sebille, 1993, 1995*a*,*b*; Irintchev *et al.* 1997; Lynch *et al.* 1997). The results of the present study show that there were minimal changes in rate of force development in diaphragm muscle in young mice (Table 1). These results show that from a functional point of view, that

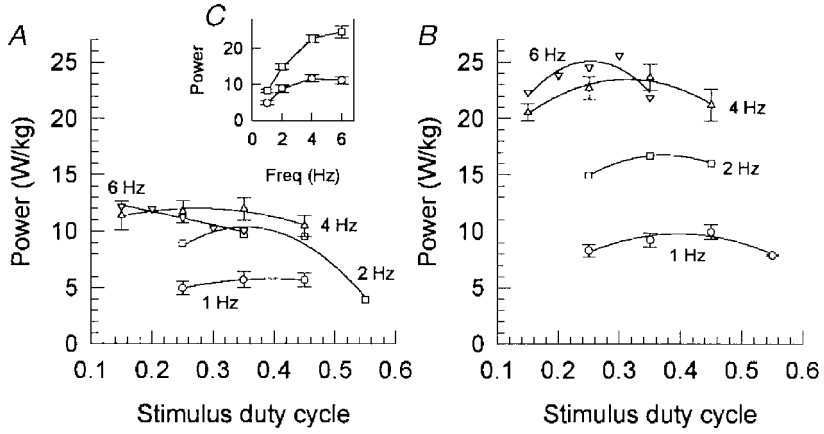

Figure 8

Power production by diaphragm muscle strips from *mdx* mice (*A*) was less than that from control mice (*B*) at all cycle frequencies. ○, 1 Hz cycle frequency; □, 2 Hz; △, 4 Hz; and ▽, 6 Hz. Values are means with S.E.M. shown for 1 and 4 Hz. Lines in *A* and *B* are 2nd order least square regression fits to the mean values. *C* shows the effect of cycle frequency on power at a duty cycle of 0·25. □, control mice; ○, *mdx* mice.

is from the point of view of doing active positive net work, the manifestation of the *mdx* condition on the passive properties of muscle seem to exert more effect than the slowing of twitch contraction. That is, differences between *mdx* muscle and control muscle were more marked with changes in muscle length and with muscle strain than with changes in cycle frequency. A change in contraction kinetics should be manifested by a large effect seen with changes in cycle frequency; we did not see a large effect.

In the present study muscles were not exposed to lengthening contractions to induce contraction-induced injury. However, it has been shown that the most important factor determining muscle damage during contraction-induced injury (as estimated by force deficit) is the amount of negative work done on the muscle during lengthening (Hunter & Faulkner, 1997). The results in the present study show that the amount of negative work in diaphragm strips from *mdx* mice increased dramatically with an increase in muscle length compared with muscle from control mice (Fig. 5). Thus any circumstances that would cause the diaphragm to oscillate and do active work around a longer mean length or at high strains, may be more likely to result in muscle damage in diaphragm from *mdx* mice than from control mice. This idea is not new and is not our idea. As *mdx* dystrophic muscle appears histologically similar to normal muscle damaged by lengthening contractions, Edwards *et al.* (1984) suggested that similar mechanisms may be involved. Furthermore, Stedman *et al.* (1991) hypothesized that because of the changes in collagen content and decrease in elasticity, *mdx* muscle would be more susceptible to contraction-induced injury than normal muscle.

Some studies show that muscle from *mdx* mice is more susceptible to contraction-induced injury than muscle from control mice (eg. Head *et al.* 1992; Moens *et al.* 1993). *In vivo*, Brussee *et al.* (1997) showed that a downhill running protocol produces a slight increase in the percentage of degenerating muscle fibres in normal mice, whereas it produces a marked increase in some *mdx* muscles. Surprisingly, diaphragm is the muscle most altered by downhill running; it is damaged more than the limb muscles actually involved in the downhill running. During normal respiration the diaphragm is still contracting while being stretched by the elastic recoil of the thorax during the last half of expiration (Gillis, 1996). Thus the *mdx* mouse diaphragm is continuously subjected to eccentric contractions with every breath even during eupnoea. We speculate that the damage to the diaphragm during downhill running may have been related to the increase in excursion amplitude of the diaphragm associated with the increased ventilation during running. *In vivo*, muscle action in *mdx* animals probably is restricted in that large strains at normal muscle lengths or even small strains at longer muscle lengths will result in relatively high stress levels and probably will cause contraction-induced injury.

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