# The Functional Status of Dystrophic Muscles and Functional Recovery by Skeletal Muscles Following Myoblast Transfer

John A. Faulkner, Susan V. Brooks, Robert G. Dennis and Gordon S. Lynch

*Institute of Gerontology, University of Michigan, Ann Arbor, USA*

**Abstract**

The diversity of movement in mammals requires three different types of muscle contraction: isometric (fixed end), miometric (shortening), and pliometric (lengthening). Consequently, an adequate evaluation of the functional status of dystrophic muscles and of the recovery of dystrophic muscles after myoblast transfer requires measurements during each type of contraction. Although subject to some criticism as a model for Duchenne muscular dystrophy (DMD) in human beings, the lack of expression of dystrophin and the ongoing degeneration of fibres in striated muscles provide strong support for the *mdx* mouse as a suitable model. The diaphragm muscles of *mdx* mice show a progressive loss of mass and maximum and normalized force throughout the life span, whereas during adulthood the limb muscles *of mdx* mice appear to be larger but weaker than those of age-matched control mice. Muscle power *of mdx* mice is impaired compared with control mice to an even greater extent than force development, due to lower optimum velocities for power. The limb muscles *of mdx* compared with control mice appear to be more susceptible to injury during pliometric contractions, but muscles in young *mdx* mice show remarkable powers for recovery. Following transfer of myoblasts, survival and fusion of myoblasts is minimal and no evidence exists to support the conclusion that myoblast transfer into limb muscles provides any functional improvement in the severely impaired muscles of teen-age boys, or to the less impaired muscles *of mdx* mice.

Key words: mdx mice, limb muscles, diaphragm muscles, weakness, power, contraction-induced injury.

*Basic Appl. Myol. 7 (3&4): 257-264; 1997*

Movement is the essence of animal life and lack of movement is an essential element of the termination of life. The movements of all animals are totally dependent on the contractions of skeletal muscles. The diversity of movement is a function of complex interactions within and between skeletal muscles in the *structure-function relationships* of different types of muscle fibres, the architectural organization of muscle fibres, and the three types of contractions that muscle fibres perform. A muscle *contraction* is defined as the initiation of crossbridge cycling by increasing intracellular calcium concentration with an attempt of the muscle to shorten. Whether the muscle fibres remain at the same length, shorten, or are stretched depends on the interaction between the force developed by the muscle and the load on the muscle. An equal force and load or a fixed fibre length results in an *isometric* contraction. When the force is greater than the load, the muscle fibres shorten and a *miometric* contraction occurs. Finally, a load greater than the force developed stretches the fibres in a *pliometric* contraction. An adequate assessment of the functional capabilities of experimental and control skeletal muscles requires measurements of contractile properties during isometric, miometric and pliometric contractions.

In Duchenne muscular dystrophy (DMD) and in *mdx* mice, the dystrophic symptoms arise from a complete lack of dystrophin expression in skeletal muscles due to mutations in the dystrophin gene on the X-chromosome [7, 23]. In the skeletal muscles of patients with DMD, the absence of dystrophin, a large membrane-associated protein, and the resultant down-regulation of the dystrophin associated glycoprotein (DAP) complex within the sarcolemma [36] produces widespread and progressive degeneration, necrosis and loss of myofibres with a subsequent increase in connective and fatty tissue. Partial expression of truncated forms of dystrophin produce less severe necrosis for patients with Becker muscular dystrophy (BMD). The diaphragm muscle of the *mdx* mouse mimics the rapid deterioration of the skeletal muscles of young boys with DMD [14, 30, 41, 42, 47], whereas the limb muscles respond to the absence of dystrophin, at least early in life, with muscle hypertrophy accompanied by a decrease in specific force [14, 30]. The *mdx* mouse provides a valid model of DMD in terms of the complete lack of dystrophin

expression in skeletal muscles and comparable structural and functional impairments in the diaphragm muscle, but with some reservations due to the difference in the response of limb muscles.

The measurements of the *contractile properties* of dystrophic and control muscles as well as those of dystrophic muscles following myoblast transfer provide useful quantitative assessments of the functional capabilities only when valid measurements are made with the appropriate units of measurement and the appropriate procedures of normalization by cross-sectional area (CSA) for force and by muscle mass for power [18]. Data on age-matched and gender-matched controls of the same species are also prerequisites for valid comparisons. Unfortunately, the measurements of the capability of limb muscles *of mdx* mice to develop force are even more complex than for muscles from control mice and the evaluations have been compromised by a litany of problems. A large proportion of the data on dystrophic *mdx* mice compares mixed groups of males and females [14]; compares *mdx* mice with other than C57BL/10 controls [1, 11]; normalizes force data by muscle mass rather than by total muscle fibre cross-sectional area [1, 26, 39, 48]; or reports values for maximum isometric tetanic force ($P_o$, mN), specific $P_o$ ($kN/m^2$), or both, significantly below accepted values even for control mice [1, 39, 44. 48]. The result is highly variable sets of data that are almost impossible to interpret.

**Body Mass and Mass of Dystrophic Muscles**

Unlike boys with DMD, who have extreme muscle wasting and low body masses, the body masses of *mdx* mice show a normal growth curve and stabilize at values slightly higher (10%) than values for control mice [40, 44]. The low body masses (13 grams) reported for 2 year old *mdx* compared with control mice [40] appear to be due to the conventional housing of the animals since at 2 years of age specific pathogen free (SPF) *mdx* mice maintained in a barrier-protected facility at the University of Michigan Unit for Laboratory Animal Medicine have body masses 2.0-fold greater (unpublished data, University of Michigan). Only one study [9] reports that the mice were housed in SPF facilities. Despite the absence of dystrophin expression and a phase of extensive degeneration between ~ 21 and 28 days of age, the limb muscles *of mdx* mice appear to grow normally throughout the growth spurt with no evidence of impairment [39, 40] and perhaps even an accelerated rate of growth in the soleus muscle [9]. The mass of limb muscles in *mdx* mice stabilizes between 5 and 7 months of age [9, 40], suggesting an equilibrium has been established between continued degeneration and regeneration. For extensor digitorum longus (EDL) and soleus muscles in *mdx* mice, the muscle masses stabilize at values 20% to 30% greater than age-matched control mice [9, 14, 27, 39, 40], but values for the tibialis anterior muscle range from 20% [44] to 60% [40] to even 85% greater [46]. Apparently, the early muscle fibre degeneration in the muscles *of mdx* mice initiates a paradoxical hypertrophy.

Data are abundant on the growth and stabilization of the fibre length of control C57BL/6 mice, but only one study provides data on fibre lengths in *mdx* and C57BL/10 control mice and data are reported for only the soleus muscle [9]. Based on this limited data set, the growth in fibre length appears to lag in the soleus muscles of *mdx* mice which produces an extremely rapid increase in total muscle fibre CSA during the spurt in muscle growth [9]. Muscle fibre lengths in *mdx* mice stabilize at about 3 months of age, roughly 1 month later than those of the control mice, with values for fibre lengths not different between the two groups.

Unlike the limb muscles *of mdx* mice, and comparable with data on limb and diaphragm muscles of boys with DMD, the diaphragm muscles of *mdx* mice show a progressive degeneration, necrosis, and loss of fibres throughout their life span [14, 30, 41, 42, 47]. Data on diaphragm muscles are obtained on strips of variable width made at the discretion of the investigator. As a result of the variable masses, only comparisons of the percentage of CSA of the strip composed of viable fibres are valid [30]. The proportion of the total CSA of the diaphragm strip composed of viable muscle fibres decreases from ~70% in young (4 to 6 month old) mice to ~40% in old (24 month old) *mdx* mice [30, 42]. The lengths of fibres in the diaphragm muscles of *mdx* mice are -20% shorter throughout the life span [30].

**Maximum and Specific Force of Dystrophic Muscles**

Most of the data on the $P_o$ and specific $P_o$ of skeletal muscles *of mdx* mice have been measured *in vitro* on whole EDL and/or soleus muscles [1, 9, 14], and strips of diaphragm muscle fibres [14, 30, 41, 42, 47]. In contrast, Pastoret & Sebille [39] measured the isometric forces of soleus, EDL and plantaris muscles of C57BL/10 and *mdx* mice *in situ.* Unfortunately, the extensive series with eight samplings extending from 5 to 52 weeks of age presented by Pastoret & Sebille [39] report $P_o$s for the control muscles ~60% of the values for control C57BL/6 [4, 43] and C57BL/10 (unpublished data, University of Michigan) mice of comparable age and body mass. The data on the $P_o$ of limb muscles *of mdx* and C57BL/10 control mice are highly variable and many values for young (4 to 6 month) and adult (10 to 12 month) mice are less than 50% [1, 26, 39, 44, 48] of the accepted values of ~500 mN for EDL muscles and ~250 mN for soleus muscles measured *in vitro* [4, 43]. The low values and high variability for $P_o$ of muscles *of mdx* mice reported in different studies may be due to the high susceptibility of fibres in muscles *of mdx* mice to contraction-induced injury [3, 35, 41 ]. Despite the limitations in the data set, during the growth spurt from birth to 5 months of age, the rapid rise in $P_o$ displayed by control mice is equalled or surpassed by the limb muscles of the *mdx* mice [9, 13]. One factor may be the greater CSA due to the delayed growth in fibre length [9]. The $P_o$ then plateaus with values reported that are both above and below the values for age-matched control mice [1, 9, 14, 26, 39, 48]. Although inconclusive due to the low values

for Po for muscles of both *mdx* and control mice, the $P_o$ of the soleus muscles in *mdx* mice appears to decrease by 12 months of age. In contrast, the $P_o$ of EDL muscles in *mdx* mice does not change appreciably at this age. In old (24 month) *mdx* mice, Pastoret & Sebille [40] have reported more dramatic signs of degeneration in soleus than in EDL muscles.

Few investigations of *mdx* mice contain force data normalized correctly by total muscle fibre CSA. The two best data sets on the relationship between specific $P_o$ and age are marred by the inclusion of only one or two measurements at each age [9, 14]. The plots of specific $P_o$ with age (Figure 1A & 1B) indicate an impairment in the ability of the EDL and soleus muscles to generate force per unit area from the earliest age with the discrepancy increasing throughout the growth spurt. Although the specific $P_o$s of the muscles of control mice plateau by 4 months, the attainment of the plateau by muscles *of mdx* mice is more gradual and delayed at least 7 months. Compared with the specific $P_o$s of muscles from the control mice, those *of mdx* mice are ~20% lower. The paradoxical hypertrophy induced by fibre degeneration is successful in increasing the PO close to control values, but the degenerating and necrotic fibres reduce the percentage of the total muscle fibre CSA composed of viable myofibres and consequently the specific $P_o$.

The impairment in the capacity of limb muscles in *mdx* mice to develop specific $P_o$ early in life, suggests that the regeneration of degenerating muscle fibres impairs transiently the development of force, but not of muscle mass [39]. Embryonic and neonatal muscle fibres are known to have low capacities for the generation of force and power [45] and the continued presence of embryonic and neonatal isoforms of myosin in regenerating muscle fibres in *mdx* mice may contribute to the functional impairments [13]. Throughout the adulthood *of mdx* mice, the hypertrophied state of EDL and soleus muscles with no concomitant increase in absolute $P_o$ reflects an increase in non-contractile tissue, probably due to the presence of degenerating and necrotic fibres. The presence of this non-contractile tissue results in lower values for specific $P_o$ for both EDL and soleus muscles (Figure 1A & 1B).

The variation of the mass and CSA of the diaphragm muscle strips at the discretion of the investigator make the $P_o$ equally variable [30], consequently only comparisons of the specific $P_o$s are valid. As with the limb muscles of *mdx* mice, the diaphragm muscle shows early signs of rapid and significant gains in specific $P_o$ suggestive that the total diaphragmatic mass is increasing, but the gains plateau at 7 weeks of age (Figure 1C). From 7 weeks on, in contrast to the limb muscles of the *mdx* mice which show gains out to 4 to 6 months and then plateau, the specific $P_o$ of the diaphragm muscle, show progressive loss in specific $P_o$ with age (Figure 1C). As a result of continual fibre necrosis, by 12 months of age, the specific $P_o$ ($kN/m^2$) of the diaphragm muscles *of mdx* mice is 60% of the value for the control diaphragm and by 24 months of age, the specific $P_o$ is 48% [30].

The functional properties of skeletal muscles of boys with DMD have been studied with voluntary tests of strength development [15, 28, 29, 34]. Compared with normal control values which demonstrate significant gains in strength during growth and development, the boys with DMD demonstrate no gain in strength and consequently a dramatic loss of strength relative to normal values (Figure 2A). By 10 years of age, the quadriceps [14, 25] and biceps [25] strength of boys with DMD are ten to fifteen percent of the values for normal boys of the same age [4, 34].

### The Power of Dystrophic Muscles

Much less data are available on the capability of muscles *of mdx* mice to develop power [10, 30] than on their ability to develop force. At 3 months of age, the deficit in normalized power, is not different from the deficit in specific $P_o$ for diaphragm muscles from *mdx* mice with each ~ 60% of the control value. The lack of a difference indicates that at this early age no difference has developed between diaphragm muscles in *mdx* and control mice in the optimum velocity for the development of power. By 4 to 6 months of age, the specific $P_o$ of diaphragm muscles *of mdx* mice is still decreased to 60% of control values, but the normalized power has decreased to 46%. For old *mdx* mice at 24 months of age, the specific $P_o$ has decreased to 48% and the normalized power to 31 % of the aged-matched control mice [30]. The optimum velocity for power typically occurs at ~30% of a muscles maximum velocity of shortening, and the maximum velocity of shortening is a function of the proportion of fast and slow fibres present in the muscle [5]. Therefore, the lack of a difference in the deficit in force and power of diaphragm muscles in 3 month old *mdx* compared with control mice suggests that the loss of fibres resulting from dystrophic fibre necrosis is not specific to one fibre type, whereas in the *mdx* mice older than 4 months the fast fibres appear to be more susceptibile to injury and necrosis. Compared with slow fibres, fast fibres are injured more easily and more severely during pliometric contractions in control animals [31]. Consequently, a selective injury and loss of fast fibre types and a significant shift to a greater proportion of slow myosin isoforms may not be unexpected. On this basis, the greater impairment in the soleus than the EDL muscles in older *mdx* mice is incongruous, but this incongruity may reflect more frequent recruitment [22] and progressive injury to fibres in the soleus muscles [31] being balanced out by less frequent recruitment [22] and less injury of the fast fibres in the EDL muscles despite a greater susceptibility to injury [31].

### Contraction-Induced Injury

The complex phenomenon of contraction-induced injury is experienced by actively contracting muscles of control animals as well as by those in *mdx* mice, particularly when the muscles are stretched during contractions [2, 19]. Contraction-induced injury is initiated by a mechanical event,

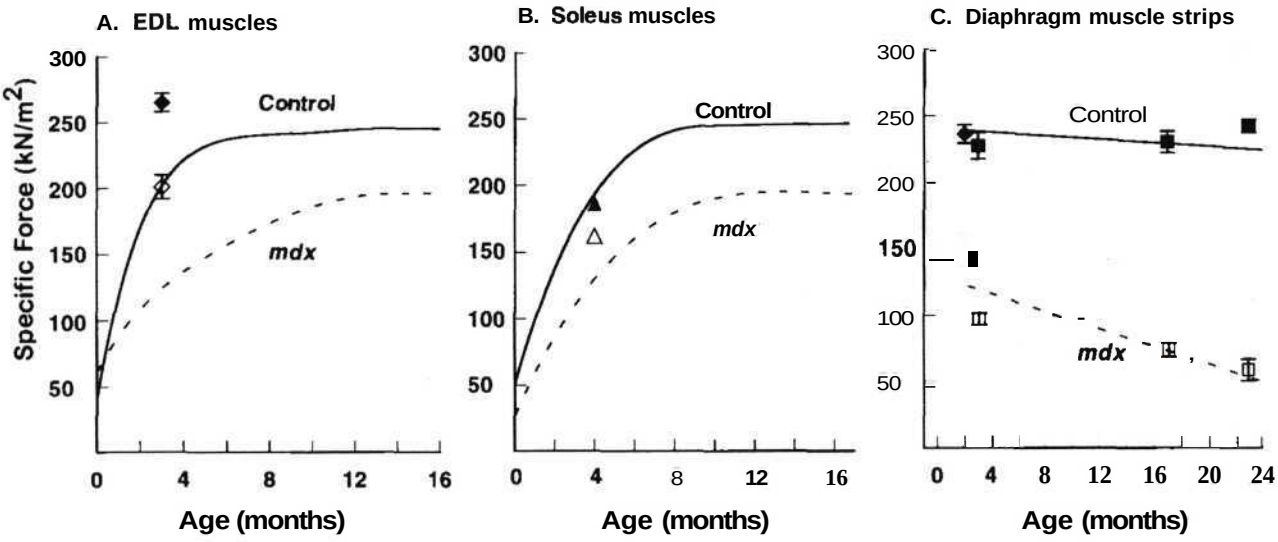


*Figure 1. Maximum isometric specific forces (kN/m)² of A. extensor digitorum longus (EDL) and* B. *soleus muscles, and* C. *small bundles of diaphragm muscle fibres of control and mdx mice, at sampling points between 14 days and 24 months of age. Solid and dashed lines represent data adapted from the study of Dupont-Versteegden and McCarter [14]. Filled and open symbols represent mean data from control and mdx mice, respectively, at specific ages, and values given are means ± 1 S.E.M. Diamonds* (4, ◇) *are data reproduced from Cox et al. [10]; triangles* (A, Δ) *from Coulton et al. [9]; and squares* (□, ▪ *from Lynch et al. [30] with permission.*

which in the absence of fatigue is measured most effectively by a force deficit [19]. The severity of contraction-induced injury of muscles *in situ* or *in vivo* is a function of the initial length of the fibres [25], the average force during the stretch, and the magnitude of the stretch [6]. The mechanical injury can produce a cascade of events that lead to a more severe injury ~3 days later. For human beings, the secondary injury is termed *delayed onset muscle soreness* [2].

Surprisingly, three studies found no difference in the susceptibility of muscles from *mdx* and control mice to contraction-induced injury [32, 33, 46]. The studies of McArdle et al. consisted of 900 isometric contractions during a 30 minute protocol *in vitro* with no differences observed between muscles *of mdx* and control mice in the accumulation of calcium [33] or in the release of muscle enzymes [32]. With a 20 minute protocol consisting of 270 pliometric contractions administered *in vivo*, Sacco et al. [46] reported no differences between muscles *of mdx* and control mice for the force deficit during 12 days of recovery. Each of these protocols was particularly arduous and may simply have gone beyond the ability to discriminate differences. Less demanding protocols of 5 to 6 pliometric contractions *in vitro* produced a force deficit of 57% for diaphragm muscle strips from *mdx* mice and 29% for those from control mice [41] and 38% and 13% for EDL muscles from *mdx* and control mice, respectively [35]. In contrast, after 6 pliometric contractions of soleus muscles *in vitro*, muscles from both *mdx* and control mice had force deficits of ~ 10% [35]. Studies of contraction-injured skeletal muscles conducted *in vitro* through protocols of repeated contractions are especially difficult to interpret since both isometric and pliometric contractions of *in vitro* muscles result in injury to muscle fibres and significant loss of muscle enzymes [27, 32, 33]. In contrast, a protocol of 225 contractions *in situ* with stretches through 20% of muscle fibre length produced a ~50% force deficit to EDL muscles of control mice 3 days later but produced no deficit when the 225 contractions were isometric, miometric, or the stretches were to passive muscles [19].

To resolve some of the problems in interpretation due to both the type of preparation and the arduous nature of the protocol, Brooks [3] compared the susceptibility to contraction-induced injury of EDL muscles of *mdx* and C57BL/10 mice *in situ* with a 10 pliometric contraction protocol with one contraction every 10 s. One hour after the protocol, the muscles from *mdx* mice demonstrated a force deficit of 40% compared with 20% for muscles of control mice. Despite the two-fold greater initial force deficit, the muscles from *mdx* mice recovered completely within 72 hours, whereas the muscles of the control mice showed no recovery. Brooks [3] attributed the greater force deficit and the more rapid recovery for the muscles in the *mdx* mice to the manifestation of a type of injury unique to dystrophic muscle. The most defensible hypothesis is that, in addition to some myofibrillar disruption, fibres in the muscles of *mdx* mice experienced disruption of the sarcolemma [8]. A more rapid recovery for muscle fibres in *mdx* mice might also arise from an up-regulation of the regenerative response that is credited for the hypertrophy of muscles after 28 days of age and for the maintenance of absolute $P_o$ throughout the adult life-span [1,9, 14,26,39, 48].

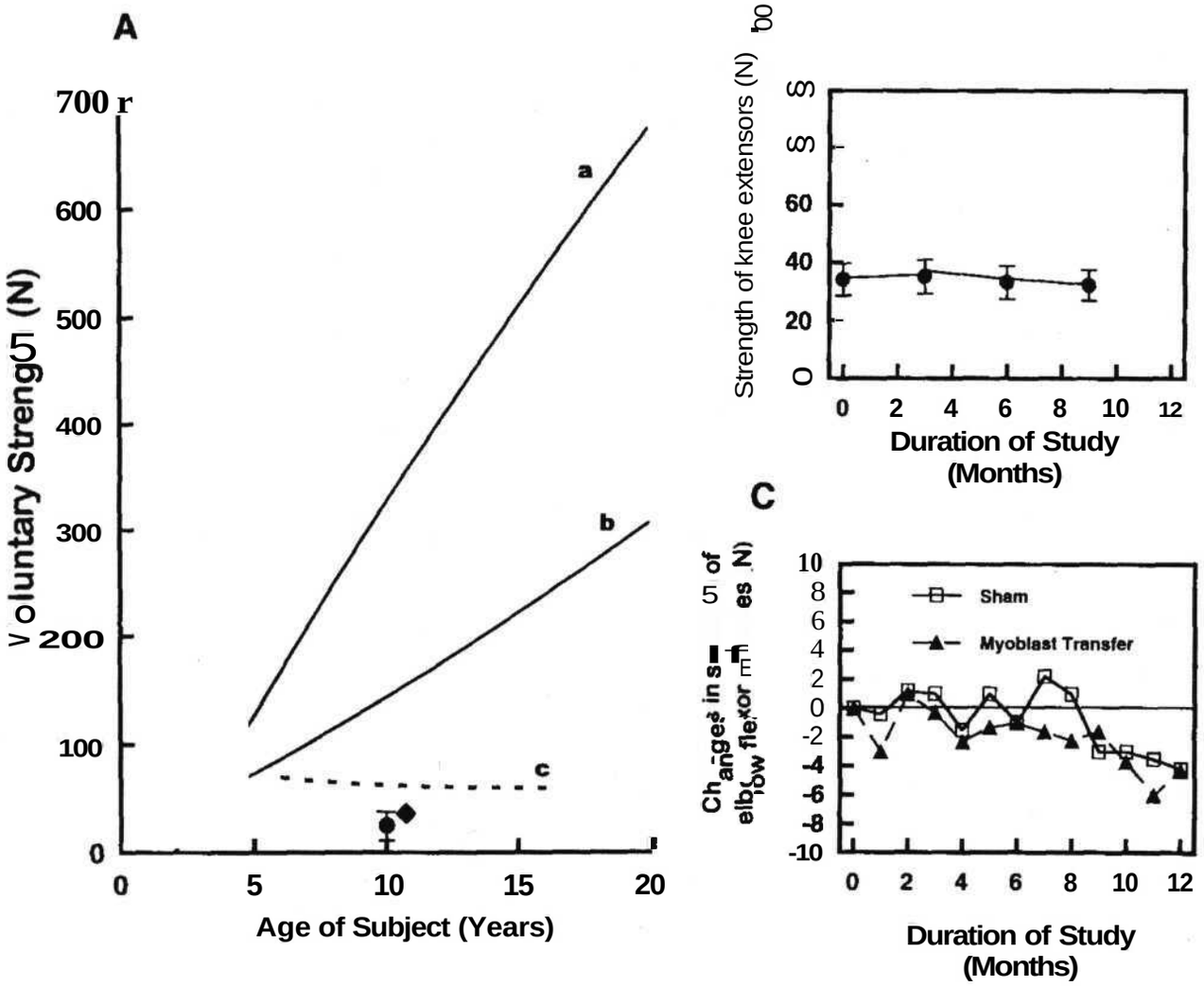


*Figure 2. Data are presented for maximum voluntary strength of knee extensor and elbow flexor muscles of control subjects, patients with Duchenne muscular dystrophy (DMD), DMD patients following therapeutic myoblast transfer. Panel* A. *Voluntary strength (in newtons, N) is plotted against the age, in years, of the subjects. Solid lines* a *and* b *represent the strengths of knee extensor and elbow flexor muscles, respectively, for control subjects, and dashed line* c *shows data for knee extensor muscle strength for DMD patients. Knee extensor data are adapted from Edwards [15], and elbow flexor strength data are adapted from Parker et al. [3 7J. Also shown in panel A by the filled diamond (◆) is the elbow flexor muscle strength of a single DMD patient prior to myoblast transfer therapy (from Karpati et al. [28]), and the mean knee extensor muscle strength following myoblast transfer therapy (●) for subjects in Law et al. [29] from all time points shown in panel B. Panel* B. *The mean knee extensor muscle strength is shown for subjects from Law et al. [29] for the 9 months following myoblast transfer treatment. Panel C. Changes in elbow flexor muscle strength (in newtons. N) are shown for 5- to 10-year-old DMD patients (▲) that underwent myoblast transfer therapy or a sham operation (O) for the 12 months following treatment (adapted from Mendell et al. [34]). Taken together the data demonstrate the lack of success of this treatment as a means of improving muscle function in DMD patients.*

**Single Skinned Fibre Segments**

From any muscle and from any species including human beings, small bundles of muscle fibres may be obtained either by open or needle biopsies. Following either a chemical permeabilization or mechanical skinning procedure, single fibre segments may be mounted between a force transducer and the lever arm of a servomotor suspended in a *relaxing* solution with low calcium concentration. The single fibres are activated by exposing the fibre segment to solutions with higher calcium concentrations. The disruption of the sarcolemma and the activation by exogenous calcium permits investigation of the viability of the myofibrillar proteins. An early report suggested that single permeabilized fibre segments from the muscles of boys with DMD had values of $P_o$ ~50% lower than those

of fibres from control muscles as well as abnormal calcium regulation [50]. More recent studies range from showing values of $P_o$ for single permeabilized fibres from muscles of DMD patients and normal controls that are not different [24] to concluding that single muscle fibres from DMD patients generate maximum forces that are less than 20% of control values [20]. Horowits and his associates [24] concluded that "the absence of dystrophin, leads to clinical weakness by causing the breakdown of muscle fibres that were once capable of generating normal force, while the surviving fibres exhibit normal contractility." This statement overstates the normalcy of the surviving fibres which are in large part actually regenerated, rather than surviving, fibres. Many of these fibres display abnormalities in both structure and function [21] and in an intact muscle maintain a greater susceptibility to contraction-induced injury [3, 35, 41].

The most reasonable explanation for the disparate findings in the studies of single fibres from muscle of DMD patients is that the 30-80 fibres studied in each case do not constitute a representative sample, not surprising in light of the many tens of thousands of fibres which make up the human muscles studied. In the main, significant differences in contractile properties were not observed for single permeabilized fibres from muscles of *mdx* mice compared with those from age-matched control C57BL/10 mice [49]. Generally, the functional data on single permeabilized fibres are consistent with the early biochemical reports that the myosin, actomyosin, troponin and tropomyosin show no definitive abnormalities, but secondary effects mediated through the absence of dystrophin, muscle fibre degeneration, regeneration, and repair impact on the contractility of many individual fibres in dystrophic muscle [20, 49].

**Isometric Contractile Properties After Myoblast Transfer**

Myoblast transfer into a skeletal muscle has the potential to impair, maintain, or enhance specific structural and functional properties of the skeletal muscles [28]. Consequently, following myoblast transfer, or any other experimental intervention involving skeletal muscle, the most important assessments are the degree of impairment in the structure-function relationships of the muscle and the degree to which the *treated* muscle has regained control values for structure and function and for the relationships between the two. The determination of the structure-function relationships is of vital importance in the evaluation of myoblast transfer because the structure-function relationships are disrupted by the dystrophic condition and frequently, an intervention may produce differential magnitudes of recovery in structural and functional variables.

The successful transfer of myoblasts into the limb muscles of *mdx* mice can be evaluated morphologically through the presence or absence of dystrophin expression, percentage of damaged fibres, and percentage of fibres with central nuclei [38]. In contrast to the efficacy of the *mdx* mouse model for morphological assessments, the lack of large differences in the $P_o$ and specific $P_o$ of limb muscles from adult *mdx* compared with control C57BL/10Sn mice makes a functional evaluation of myoblast transfer equivocal. For the evaluation of models of transgenic *mdx* mice, the diaphragm muscle has provided an effective measure of the degree of restoration [10, 30]. With the expression of the full length dystrophin gene [10] or selected truncated minigenes [30] full recovery of both specific $P_o$ and normalized power has been demonstrated as well as a sustained expression of dystrophin throughout the life-span of the mouse [30]. The diaphragm muscle is not convenient for myoblast transfer. Myoblasts injected into "untreated" limb muscles of *mdx* and control host mice with [17] and without [17, 48] preinjury to the host muscles show a "rapid and massive" die off shortly after injection [17] with large and permanent decreases in muscle mass and $P_o$ [48]. The short-comings of myoblast transfer therapies have led to the development of alternative strategies. A "sliced" muscle graft has shown "excellent" long term survival and movement of male donor cells in the muscles of female *mdx* host mice [16] and injection of a recombinant adenovirus which contained a dystrophin minigene into limb muscles of *mdx* mice provided protection from the fibre damage and force deficit associated with a protocol of pliometric contractions [12].

In a number of clinical trials [28, 29, 34], the efficacy of myoblast transfer for the treatment of DMD has been evaluated with measurements of both the expression of dystrophin and the development of force. In each trial, although many millions of myoblasts were injected into a single muscle, within several months fewer than 1 % of the fibres in the injected muscles expressed dystrophin. The poor outcomes demonstrated by morphological assessments are consistent with no improvement in the ability of the patients with DMD to develop force [28, 29, 34]. No differences were observed in the strength of myoblast-treated muscles compared with untreated sham-injected muscles (Figure 2B & 2C). A consistent conclusion from all the clinical trials was that a clearer understanding is required of the factors that govern the survival, fusion, and expression of donor myoblasts in the muscles of dystrophic patients.

**Acknowledgment**

Richard T. Hinkle and Gabriele Wienert provided valuable assistance in the preparation of the manuscript. Supported by NIA through AG06157 and the Nathan Shock Center for the Basic Biology of Aging AG13283 to JAF. SVB, RGD, and GSL received fellowship support from the Brookdale Foundation, a Whitaker Opportunity Award, and the National Health & Medical Research Council (Australia), respectively.

**Address correspondence to:**

John A. Faulkner, Institute of Gerontology, 300 North Ingalls Building, University of Michigan, Ann Arbor, MI,

48109-2007, USA, tel. 313 764 4378, fax 313 936 2116, Email JAFAULK@UMICH.EDU.

**References**

[1] Anderson JE, Bressler BH, Ovalle WK: Functional regeneration in the hindlimb skeletal muscle of the *mdx* mouse. *J Muscle Res Cell Motil* 1988; 9: 499-515.

[2] Armstrong RB: Mechanism of exercise-induced delayed onset muscular soreness: a brief review. *Med Sci Sports Exercise* 1984; 16: 529-538.

[3] Brooks SV: Rapid recovery following contraction-induced injury to in situ skeletal muscles in *mdx* mice. *J Muscl Res Cell Motil* 1997 (In Press).

[4] Brooks SV, Faulkner JA: Contractile properties of skeletal muscles from young, adult and aged mice. *J Physiol (Lond)* 1988; 404: 71-82.

[5] Brooks SV, Faulkner JA: Forces and powers of slow and fast skeletal muscles in mice during repeated contractions. *J Physiol (Lond)* 1991; 436: 701-710.

[6] Brooks SV, Zerba E, Faulkner JA: Injury to muscle fibres after single stretches of passive and maximally stimulated muscles in mice. *J Physiol (Lond)* 1995; 488: 459-469.

[7] Bulfield G, Siller WG, Wight PAL., Moore KJ: X chromosome-linked muscular dystrophy *(mdx)* in the mouse. *Proc Nat Acad Sci* 1984; 81: 1189-1192.

[8] Clarke MSF., Khakee R, McNeil PL: Loss of cytoplasmic basic fibroblast growth factor from physiologically wounded myofibers of normal and dystrophic muscle. *J Cell Sci* 1993; 106: 121-133.

[9] Coulton GR, Curtin NA, Morgan JE, Partridge TA: The *mdx* mouse skeletal muscle myopathy: II. contractile properties. *Neuropathol Appl Neurobiol* 1988; 14: 299-314.

[10] Cox GA, Cole NM, Matsumura K, Phelps SF, Hauschka SD, Campbell KP, Faulkner JA, Chamberlain JS: Ovefexpression of dystrophin in transgenie *mdx* mice eliminates dystrophic symptoms without toxicity. *Nature* 1993; 364: 725-729.

[11] Dangain J, Vrbová G: Muscle development in *mdx* mutant mice. *Muscle Nerve* 1984; 7: 700-704.

[12] Deconinck N, Ragot T, Marechal G, Perricaudet M, Gillis JM: Functional protection of dystrophic mouse *(mdx)* muscles after adenovirus-mediated transfer of a dystrophin minigene. *Proc Natl Acad Sci USA* 1996; 93: 3570-3574.

[13] DiMario JX, Uzman A, Strohman RC: Fibre regeneration is not persistent in dystrophic *(mdx)* mouse skeletal muscle. *Develop Biol* 1991; 148: 314-321.

[14] Dupont-Versteegden EE, McCarter RJ: Differential expression of muscular dystrophy in diaphragm versus hindlimb muscles of *mdx* mice. *Muscle & Nerve* 1992; 15: 1105-1110.

[15] Edwards RHT: Studies of muscular performance in normal and dystrophic subjects. *Brit Med Bulletin* 1980; 36: 159-164.

[16] Fan Y, Beilharz Mw, Grounds MD: A potential alternative strategy for myoblast transfer therapy: The use of sliced muscle grafts. *Cell Transplantation* 1996; 5: 421-429.

[17] Fan Y, Maley M, Beilharz M, Grounds M: Rapid death of injected myoblasts in myoblast transfer therapy. *Muscle Nerve* 1996; 19: 853-860.

[18] Faulkner JA, Brooks SV, Dennis RG: Methods for the measurement of the recovery of function following whole muscle transfer, myoblast transfer and gene therapy. *Meth Tissue Eng* 1997 (In Press).

[19] Faulkner JA, Brooks SV, Opiteck JA: Injury to skeletal muscle fibers during contractions: conditions of occurrence and prevention. *Phys Ther* 1993; 73: 911-921.

[20] Fink RHA, Williams DA, Stephenson DG. Physiological properties of skinned fibres from normal and dystrophic (Duchenne) human muscle activated by Ca and $Sr^{2+}$. *J Physiol (Lond)* 1990; 420: 337-353.

[21] Head SI, Williams DA, Stephenson DG: Abnormalities in structure and function of limb skeletal muscle fibres of dystrophic *mdx* mice. *Proc R Soc Lond* 1992; 248: 163-169.

[22] Henneman E, Olsen CB: Relations between structure and function in the design of skeletal muscles. *J Neurophsyiol* 1965; 28: 581-598.

[23] Hoffman EP, Brown Jr. RH, Kunkel LM: Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987; 51: 919-928.

[24] Horowits R, Dalakas MC, Podolsky RJ: Single skinned muscle fibers in Duchenne muscular dystrophy generate normal force. *Ann Neurol* \ 990; 27: 637-641.

[25] Hunter KD, Faulker J: Pliometric contraction-induced injury of mouse skeletal muscle: effect of initial length. *J Appl Physiol* 1997; 82: 278-283.

[26] Irintcherv A, Zweyer M, Wernig A: Impaired function and structural recovery after muscle injury in dystrophic *mdx* mice. *Neuromusc Disorders* 1997 (In Press).

[27] Jones DA, Jackson MJ, Edwards RHT. Release of intracellular enzymes from an isolated mammalian skeletal muscle preparation. *Clin Sci* 1983; 65: 193-201.

[28] Karpati G, Ajdukovic D, Arnold D, Gledhill RB, Guttmann R, Holland P, Koch PA, Shoubridge E, Spence D, Vanasse M, Watters GV, Abrahamowicz M, Duff C, Worton RG: Myoblast transfer in

Duchenne muscular dystrophy. *Ann Neurol* 1993; 34: 8-17.

[29] Law PK, Goodwin TG, Fang Q, Deering MB, Duggirala V, Larkin C, Florendo JA, Kirby DS, Li HJ, Cornett J, Li LM, Shirzad A, Quinley T, Yoo TJ, Holcomb R: Cell transplantation as an experimental treatment for duchenne muscular dystrophy. *Cell Transplantation* 1993; 2: 485-505.

[30] Lynch GS, Rafael JA, Hinkle RT, Cole NM, Chamberlain JS, Faulkner JA: Contractile properties of diaphragm muscle segments from old *mdx* and old transgenic *mdx* mice. *Am J Physiol (Cell Physiol)* 1997 (In Press).

[31] Macpherson PCD, Schork AM, Faulkner JA: Contraction-induced injury to single fiber segments from fast and slow muscles of rats by single stretches. *Am J Physiol* 1996; 271: C1438-C1446.

[32] McArdle A, Edwards RHT, Jackson MJ: Effects of contractile activity on muscle damage in the dystrophin-deficient *mdx* mouse. *Clin Sci* 1991; 80: 367-371.

[33] McArdle A, Edwards RHT, Jackson MJ: Accumulation of calcium by normal and dystrophin-deficient mouse muscle during contractile activity *in vitro. Clin Sci* 1992; 82: 455-459.

[34] Mendell JR, Kissel JT, Amato AA, King W, Signore L, Prior TW, Sahenk Z, Benson S, McAndrew PE, Rice R, Nagaraja H, Stephens R, Lantry L. Morris GE, Burghes AHM: Myoblast transfer in the treatment of Duchenne's muscular dystrophy. *New Engl J Med* 1995; 333: 832-838.

[35] Moens P, Baatsen PHWW, Maréchal G: Increased susceptibility of EDL muscles from *mdx* mice to damage induced by contractions with stretch. *J Muscle Res Cell Motil* 1993; 14: 446-451.

[36] Ohlendieck K, Campbell KP: Dystrophin-associated proteins are greatly reduced in skeletal muscle from *mdx* mice. *J Cell Biol* 1991; 115 :1685-1694.

[37] Parker DF, Round JM, Sacco P, Jones DA: A cross-sectional survey of upper and lower limb strength in boys and girls during childhood and adolescence. *Ann Human Biol* 1990; 3: 199-211.

[38] Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM: Conversion of *mdx* myofibres from dystrophin-negative to -positive by injection of normal myoblasts. *Nature* 1989; 337: 176-179.

[39] Pastoret C, Sebille A: Time course study of the isometric contractile properties *of mdx* mouse striated muscles. *J Muscl Res Cell Motil* 1993; 14: 423-431.

[40] Pastoret C, Sebille A: *mdx* mice show progressive weakness and muscle deterioration with age. *J Neurol Sci* 1995; 129: 97-105.

[41] Petrof BJ, Shrager JB, Stedman HH, Kelly AM, Sweeney HL: Dystrophin protects the sarcolemma from stresses developed during muscle contraction. *Proc NatAcad Sci* 1993; 90: 3710-3714.

[42] Petrof BJ, Stedman HH, Shrager JB, Eby J, Sweeney HL, Kelly AM: Adaptation in myosin heavy chain expression and contractile function in dystrophic mouse diaphragm. *Am J Physiol* 1993; 265: C834-841.

[43] Phillips SK, Bruce SA, Woledge RC: In mice, the muscle weakness due to age is absent during stretching. *J Physiol (Lond)* 1991; 437: 63-70.

[44] Quinlan JG, Johnson SR, Mckee M, Lyden SP Twitch and tetanus in *mdx* mouse muscle. *Muscle Nerve* 1992; 15: 837-842.

[45] Reiser PJ, Moss RL, Giulian GG, Greaser ML: Shortening velocity and myosin heavy chains of developing rabbit muscle fibers. *J Biol Chem* 1985; 260: 14403-14405.

[46] Sacco P, Jones DA, Dick JRT, Vrbová G: Contractile properties and susceptibility to exercise-induced damage of normal and *mdx* mouse tibialis anterior muscle. *Clin Sci* 1992; 82: 227-236.

[47] Stedman HH, Sweeney HL, Shrager JB, Maguire HC, Panettieri RA, Petrof B, Narusawa M, Leferovich JM, Sladky JT, Kelly AM: The *mdx* mouse diaphragm reproduces the degenerative changes of Duchenne muscular dystrophy. *Nature* 1991; 352: 536-539.

[48] Wernig A, Irintchev A, Lange G: Functional effects of myoblast implantation into histoincompatible mice with or without immunosuppression. *J Physiol (Lond)* 1995; 484.2: 493-504.

[49] Williams DA, Head SI, Lynch GS, Stephenson DG: Contractile properties of skinned muscle fibres from young and adult normal and dystrophic (*mdx*) mice. *J Physiol (Lond)* 1993; 460: 51-67.

[50] Wood DS, Sorenson MM, Eastwood AB, Charash WE, Reuben JP: Duchenne dystrophy: abnormal generation of tension and Ca++ regulation in single skinned fibers. *Neurol* 1978; 28: 447-457.