Diaphragm contractile dysfunction in MyoD gene-inactivated mice

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Staib, Jessica L., Steven J. Swoap, and Scott K. Powers. Diaphragm contractile dysfunction in MyoD gene-inactivated mice. Am J Physiol Regul Integr Comp Physiol 283: R583–R590, 2002. First published May 23, 2002; 10.1152/ajpregu.00080.2002.—MyoD is one of four myogenic regulatory factors found exclusively in skeletal muscle. In an effort to better understand the role that MyoD plays in determining muscle contractile properties, we examined the effects of MyoD deletion on both diaphragmatic contractile properties and myosin heavy chain (MHC) phenotype. Regions of the costal diaphragm from wild-type and MyoD knockout [MyoD (−/−)] adult male BALB/c mice (n = 8/group) were removed, and in vitro diaphragmatic contractile properties were measured. Diaphragmatic contractile measurements revealed that MyoD (−/−) animals exhibited a significant (P < 0.05) downward shift in the force-frequency relationship, a decrement in maximal specific tension (P0 ′, −33%), a decline in maximal shortening velocity (Vmax, −37%), and concomitant decrease in peak power output (−47%). Determination of MHC isoforms in the diaphragm via gel electrophoresis revealed that MyoD elimination resulted in a fast-to-slow shift (P < 0.05) in the MHC phenotype toward MHC types IIA and IIX in MyoD (−/−) animals. These data indicate that MyoD deletion results in a decrease in diaphragmatic submaximal force generation and P0 ′ along with decrements in both Vmax and peak power output. Hence, MyoD plays an important role in determining diaphragmatic contractile properties.

The specific MHC isoform expressed within the skeletal muscle fibers contributes to the contractile characteristics of the fiber. For example, type I fibers generally have high levels of oxidative enzymes and greater endurance, whereas type IIB fibers have high levels of glycolytic enzymes and fatigue more rapidly (22). Single-fiber contractile studies also suggest that the intrinsic force generating properties of a fiber (i.e., specific tension) depend on MHC content (12). Furthermore, it is widely accepted that MHC isoforms correlate closely with the maximal shortening velocity (Vmax) of single muscle fibers (5, 14, 40, 45, 52, 53). Specifically, the presence of type IIB MHC is correlated with a faster shortening velocity, higher power output, and a higher curvature of the force-velocity relationship compared with the presence of type I MHC (5, 18, 40, 50, 52).

Four regulatory proteins found exclusively in skeletal muscle have been identified as important regulators of muscle-specific gene expression and include: MyoD, myogenin, Myf-5, and MRF4 (19, 22, 24, 53). The primary function of these myogenic regulatory factors (MRFs) appears to be involved with determination and development of muscle, including activation of muscle-specific genes. Some evidence suggests that MRFs may also play a more extended role in the maintenance of mature skeletal muscle fiber phenotypes (22, 24, 31, 33, 37, 42, 53, 56).

The continuous expression of MyoD mRNA beyond myoblast formation and differentiation in adult muscle suggests that it also functions to control gene expression in the adult. In this regard, MyoD mRNA is expressed primarily in fast muscle of adult rats and mice, and this expression changes with manipulation of muscle fiber type, indicating that MyoD may be involved in regulating fiber type-specific gene expression (24, 49, 56). Furthermore, both MyoD and myogenin have been implicated as regulators of fiber phenotype as MyoD and myogenin mRNA transcripts are preferentially located in fast and slow muscle fibers, respectively (53). Hence, expression of specific MRFs in adult muscle may contribute to the diversity of indi-
individual muscle phenotypes as well as to skeletal muscle plasticity (24, 56).

On the basis of correlations between myogenic factors and MHC expression, it has been suggested that the myogenin:MyoD ratio may regulate fiber phenotype (24). Nonetheless, this issue remains controversial. Although some investigations suggest that a connection between the myogenin:MyoD ratio and muscle phenotype exists (22–24), other studies report a limited association between muscle phenotype and these myogenic factors (11, 13, 27, 35, 36, 44). For example, it has been shown that MyoD and myogenin may be directly involved in controlling fiber type-specific gene expression in response to external signals such as hypothyroidism, chronic low-level frequency stimulation, cross-reinnervation, denervation, and hindlimb suspension (9, 11, 15, 35, 60). Although these interventions promote a slow-to-fast myosin transition, no changes in myogenin expression occurred. Collectively, these results question the notion that MyoD and myogenin exclusively control the myosin phenotype of skeletal muscle.

Although MyoD has been identified as an important transcription factor in skeletal muscle and the absence of MyoD impairs expression of type IIB MHC, the impact of MyoD deletion on whole muscle contractile properties is unknown (23, 24, 31, 41, 53, 56). Therefore, this investigation determined the effects MyoD deletion on in vitro contractile performance in the mouse diaphragm. On the basis of observations provided by Seward et al. (49), we anticipated that MyoD deletion would result in a fast-to-slow shift in diaphragmatic MHC phenotype. Therefore, compared with diaphragms from wild-type (WT) animals, we hypothesized that MyoD deletion would alter diaphragmatic contractile properties resulting in a reduction in both maximal specific force production (P0) and Vmax.

**METHODS**

**Animals and Experimental Design**

To test our hypotheses, these experiments examined the in vitro diaphragmatic contractile properties of both WT (BALB/c) and MyoD knockout [MyoD (−/−)] adult (9 mo old) mice. The WT control animals were true WTs generated by separate breeders and were distinguished from mice. The WT control animals were true WTs generated by separate breeders and were distinguished from mice. The WT control animals were true WTs generated by separate breeders and were distinguished from mice.

**In Vitro Measurement of Costal Diaphragm Contractile Function**

Diaphragmatic strip preparation. Anesthesia was induced by an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt). After a surgical plane of anesthesia was reached, the diaphragm was quickly excised with the ribs and central tendon attached and placed in a dissecting chamber bath containing Krebs-Hensleit solution aerated with 95% O2-5% CO2 gas. Two adjacent strips of muscle (dimensions −8 × 4 mm) from the ventral costal diaphragm were cut parallel with the connective tissue fibers retaining a portion of rib and central tendon on each strip to enable the attachment of a clamp. After the two strips were cut, remaining costal diaphragm tissue was carefully trimmed of fat and connective tissue, rinsed free of blood, blotted dry, and then rapidly frozen in liquid nitrogen and stored at −80°C for subsequent biochemical analyses. Specifically, the left posterior costal diaphragm was retained for measurement of citrate synthase (CS) activity and the remainder of the costal diaphragm for MHC analysis.

The two muscle strips designated for contractile measurements were individually suspended vertically in two separate organ baths between two lightweight Plexiglas clamps connected to a force transducer in a jacketed tissue bath (Radnoti, Monrovia, CA) containing Krebs-Hensleit solution. The jacketed tissue bath pH was maintained at 7.4 ± 0.05, the osmolality of the bath was ~290 mosmol/kgH2O, and temperature was maintained at 37 ± 0.5°C for both isometric and isotonic measurements.

Isometric and isotonic contractile properties of each muscle strip were measured simultaneously using the two individual organ baths. Because our experiments required the measurement of a large number of contractile properties, two muscle strips were studied to reduce the metabolic load placed on each muscle. Specifically, one muscle strip was used to measure muscle isometric contractile properties (e.g., force-frequency relationship and muscle fatigue properties), whereas the second muscle strip was used to measure muscle isotonic contractile properties (e.g., muscle-shortening velocities). Isometric muscle force production was monitored using an isometric force transducer (Grass, model FT03, Quincy, MA), whereas isotonic muscle force production was monitored with a combined force and position transducer capable of monitoring both force and shortening velocity (Aurora Instruments, model 300B, Ontario, Canada). Each transducer output was amplified and differentiated by operational amplifiers subjected to analog-to-digital conversion for analysis using a computer-based data-acquisition system (LabVIEW 6i, National Instruments, Houston, TX). The force transducers were calibrated before and after each experiment using calibration weights.

**Determination of optimal length-tension relationship.** After a 15-min thermoequilibration period in the bath, each strip was field stimulated (modified Grass Instruments S48 stimulator) along its entire length using platinum wire electrodes with a 500-ms train of supramaximal (~120 V) monophasic pulses delivered at 300 Hz. In vitro contractile measurements began with determination of the muscle’s optimal length (L0) for isometric tetanic tension development. The muscle was adjusted to its L0, at which maximal tetanic tension was obtained by systematically adjusting the length of the muscle with a micrometer while evoking single-twitch, isometric contractions. Both isometric and isotonic contractile properties were measured at L0.

**Peak twitch tension, time-to-peak tension, and half relaxation time.** Peak isometric twitch tension (P0) was determined from a series of single pulses (2-ms duration). A computer-based algorithm was used to determine time-to-peak tension (TPT). One-half relaxation time (1/2 RT) was analyzed by the temporal pattern of force decline after a maximal isometric twitch.

**Determination of the force-frequency relationship.** Maximal isometric tetanic force was measured at 10, 20, 40, 80, 100,
150, 200, 250, and 300 Hz with a supramaximal stimulus train of 250-ms duration. At the completion of all contractile measurements, L0 was measured using calipers while the strips remained suspended between the two Plexiglas clamps.

Peak tetanic tension. Peak isometric tetanic contractions were produced with a supramaximal stimulus train of 250-ms duration (300 Hz). Maximal isometric tetanic tension (P0) was determined from a series of two contractions with a 2-min recovery between measurements to prevent muscle fatigue.

Determination of the force-velocity relationship. The force-velocity relationship was determined by measurement of the force and velocity of muscle shortening at 12 different isometric loads (150-ms train at 150 Hz) over the range of force and velocity of muscle shortening at 37°C. Force-velocity data were fit to the Hill equation with a least-squares technique (20). Vmax was determined by solving for velocity when force equals zero (10). To assess muscle function after the force-velocity protocol, maximal tetanic P0 before the force-velocity data collection. Peak power was determined by finding the product of force and velocity.

Rate of fatigue and recovery. Muscle fatigue was defined as the rate of decline in muscle force production. The rate of diaphragmatic fatigue development was determined at 37°C by monitoring the decrease in isometric force production over a 30-min contractile protocol. The costal diaphragm strip was stimulated by unfused tetanic contractions using a stimulus train of 30 Hz every 2 s with a train duration of 250 ms. The ratio of the period of muscle contraction to rest (duty cycle) was 12.5%. Tolerance to fatigue was assessed by the percentage of initial force maintained at the end of the 30-min protocol. Recovery from the fatigue protocol was determined by the measurement of three maximal tetanic contractions at 1, 5, and 10 min postfatigue. These times were chosen because our preliminary experiments revealed that the healthy adult mouse diaphragm regains ~80% of initial force-generating capacity within 10 min following this type of fatigue protocol (unpublished data).

Measurement of costal diaphragm cross-sectional area. After the measurements of contractile properties, the strips were removed, blotted dry, and weighed. The total muscle cross-sectional area (CSA) of the in vitro preparation was calculated by the algorithm CSA (cm^2) = [wet mass (g)/fiber length (cm) × 1.056 (g/cm^2)], where wet mass was the weight of the diaphragm strip, 1.056 g/cm^2 was the density of the muscle, and fiber length was expressed in centimeters measured at L0 (26).

Biochemical Analysis

Tissue homogenization for enzyme assay. Each muscle sample from the costal region of the diaphragm (~10 mg) was added to 1 ml of cold 100 mM phosphate buffer (pH = 7.4) in a 3-ml glass homogenization tube. The homogenization process consisted of eight passes of the glass pestle through the homogenate using a low-speed (~50 revolution/min) motorized homogenizer (Eberbach ConTorque, Ann Arbor, MI). At the completion of homogenization, additional cold 100 mM phosphate buffer was added to further dilute the sample (1:101 wt/vol). Homogenates were then centrifuged (3°C; 700 g for 10 min) to remove the insoluble protein from the homogenate. The supernatant was removed and immediately assayed to determine CS activity and protein concentration.

Analysis of CS activity. CS activity was analyzed as a marker of muscle-oxidative capacity using the technique described by Srere (51). Briefly, CS activity was measured indirectly via the reaction of CoASH with DTNB spectrophotometrically detected by a colorimetric change at 412 nm. Each sample was assayed at 25°C in triplicate, and specific activities were normalized to protein concentrations.

Analysis of protein concentration. Protein concentrations in the muscle homogenates were determined using the technique described by Bradford (6).

MHC analysis. Myofibrillar protein was isolated from diaphragmatic samples using techniques described by Baldwin et al. (1). Separation of MHC isofroms was performed with the SDS-PAGE technique using a modified procedure described by Talmadge and Roy (54). A 1- to 2-µg sample of myofibrillar protein diluted in sample buffer was loaded into a 0.75-mm thick minigel (8% SDS-PAGE separating; 4% SDS-PAGE stacking) and electrophoresed using the Bio-Rad mini-PROTEAN II cell apparatus for ~20 h at 4°C (Bio-Rad Laboratories, Hercules, CA). In each gel, duplicate myofibrillar samples from the diaphragm were run along with lanes containing molecular weight standards as well as control samples of soleus and plantaris muscles. Gels were stained with Rapid Coomassie Blue and subsequently analyzed (Research Products, Mt. Prospect, IL). The relative concentrations of myosin isofroms were determined by scanning the gels with a gel Doc Chemi Doc 2000 Gel Documentation System (Bio-Rad Laboratories). Each MHC band from the video image was then digitized and analyzed twice for optical density with video-analysis software (Quantity One, BioRad Laboratories).

Statistical Analysis

The experiment was designed to test the hypothesis that loss of MyoD in skeletal muscle would alter muscle contractile properties compared with skeletal muscle with MyoD present. Comparisons between experimental groups (WT vs. MyoD [−/−]) for each dependent variable (P0, P0, TPT, 1/2 RT, V0, peak power, CS, and MHC) were subjected to a Student’s t-test. Force-frequency curves between groups were statistically analyzed using a Bonferroni’s corrected t-test. Repeated-measures ANOVA was implemented to compare fatigue (2 × 8) and force recovery (2 × 3) indexes (group × time). Significance was established a priori at *P < 0.05.*

RESULTS

Morphometric Characteristics

Animal body weights did not differ between the two experimental groups [WT = 28.25 ± 4.06 g; MyoD (−/−) = 30.64 ± 2.62 g; *P > 0.05*]. Furthermore, the CSA of the costal diaphragm strips used for contractile property measurements did not differ between groups (*P > 0.05*).

In Vitro Costal Diaphragm Contractile Measurements

Twitch force development and maximal specific tension. The impact of MyoD deletion on diaphragmatic twitch characteristics and maximal tetanic forces in the MyoD (−/−) and WT animals is depicted in Table 1. Compared with WT, MyoD (−/−) animals exhibited a 33% reduction in P0 and a 44% decrement in P0 forces (*P < 0.05*). Although MyoD (−/−) animals displayed slower 1/2 RT, no differences were observed in TPT compared with their WT counterparts. Although the
Diaphragm contractile characteristics in wild-type (WT) and MyoD knockout (MyoD (-/-)) animals. A: diaphragm force-frequency characteristics expressed as a function of specific force production. B: diaphragm force-frequency characteristics expressed as a percent of maximal specific tension (% P0). Values are means ± SE; n = 8/group. *P < 0.05.

Fig. 1. Isometric diaphragm contractile characteristics in wild-type (WT) and MyoD knockout (MyoD (-/-)) animals. A: diaphragm force-frequency characteristics expressed as a function of specific force production. B: diaphragm force-frequency characteristics expressed as a percent of maximal specific tension (% P0). Values are means ± SE; n = 8/group. *P < 0.05.

**Table 1. Diaphragm isometric contractile characteristics**

<table>
<thead>
<tr>
<th></th>
<th>P0, N/cm²</th>
<th>P0, N/cm²</th>
<th>1/2 RT, ms</th>
<th>TPT, ms</th>
<th>Pt/Po, %</th>
</tr>
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<tbody>
<tr>
<td>WT</td>
<td>23.39 ± 2.82</td>
<td>5.20 ± 0.49</td>
<td>16.42 ± 0.87</td>
<td>16.96 ± 0.43</td>
<td>22.04 ± 1.43</td>
</tr>
<tr>
<td>MyoD (-/-)</td>
<td>15.75 ± 3.36*</td>
<td>2.92 ± 0.39*</td>
<td>12.71 ± 1.44*</td>
<td>17.81 ± 0.61</td>
<td>18.27 ± 1.77</td>
</tr>
</tbody>
</table>

Values are means ± SE. P0, maximal specific tension; P0, maximal twitch force; 1/2 RT, twitch half relaxation time; TPT, time-to-peak twitch force, WT, wild type; MyoD (-/-), MyoD knockout. *P < 0.05 WT vs. MyoD (-/-).

P/P0 ratio tended to be lower in MyoD (-/-) animals, these differences were not significant.

**Force-frequency characteristics.** Figure 1 illustrates the relationship between diaphragm force production and muscle stimulation frequency. Compared with WT, MyoD (-/-) animals exhibited a marked reduction (P < 0.05) in diaphragm tension during in vitro stimulation as illustrated by the downward shift in the force-frequency curve (Fig. 1A). Indeed, specific force production was markedly diminished in MyoD (-/-) animals compared with WT at all stimulation frequencies ranging from 10 to 300 Hz. Note, however, when expressed as percent P0, no differences existed between groups (P > 0.05; Fig. 1B).

**Force-velocity characteristics and peak power output.** To assess the effect of MyoD deletion on isotonic contractile performance, in vitro force-velocity measurements were performed. Diaphragms from the MyoD (-/-) animals displayed a significant downward shift in the force-velocity relationship compared with WT (Fig. 2). Furthermore, as illustrated in Fig. 3A, diaphragmatic Vmax was 37% (P < 0.05) higher in WT compared with MyoD (-/-) animals. Similarly, gene deletion had deleterious effects (~47%; P < 0.05) on the diaphragm’s peak power output (Fig. 3B).

**Fatigue.** Costal diaphragm endurance was evaluated during a 30-min fatigue protocol. Depicted in Fig. 4, no differences existed in the rate of diaphragmatic fatigue development between groups during the fatigue protocol, and no differences existed in the rate of recovery from fatigue (P > 0.05).

**Biochemical Characteristics**

**MHC profile.** Portions of the costal diaphragm were used for quantification of MHC profiles. Results, summarized in Fig. 5, indicate that MyoD deletion resulted in a significant (P < 0.05) shift from MHC type IIB toward both MHC type IIA (from 30% of the total pool to 42%) and MHC type IIX (33–45%). No differences existed in the percent of type I MHC isoforms between MyoD (-/-) and WT animals.

**Diaphragmatic oxidative capacity.** CS activity was measured as a marker of diaphragmatic oxidative capacity in the costal diaphragm of each group. No significant (P > 0.05) differences in CS activity existed between groups [WT = 84.22 ± 2.50 μmol·g⁻¹·min⁻¹; MyoD (-/-) = 91.41 ± 3.68 μmol·g⁻¹·min⁻¹; P > 0.05].
DISCUSSION

Overview of Principle Findings

These are the first experiments to examine the effect of MyoD deletion on diaphragmatic contractile function. Our results clearly demonstrate that MyoD is essential for normal diaphragmatic contractile function in adult rodents. Specifically, compared with WT controls, MyoD knockout results in dramatic decreases in diaphragmatic $P_o$, $V_{\text{max}}$, and maximal power output. Furthermore, MHC analysis revealed that MyoD deletion results in a shift in diaphragmatic MHC phenotype from MHC type IIB toward the slower types IIA and IIX MHC phenotypes. Interestingly, MyoD deletion did not influence the rate of diaphragmatic fatigue or CS activity. Collectively, these findings support the hypothesis that MyoD knockout results in a decrease in submaximal and maximal specific tension, $V_{\text{max}}$, and peak power output in the diaphragm. A brief discussion of these experimental results follows.

MyoD Deletion Impairs Diaphragmatic Maximal Isometric Force Production

Although our results clearly indicate that MyoD deletion results in a large reduction in diaphragmatic specific $P_o$, our data do not reveal the mechanism(s) responsible for this observation. Theoretically, decrements in MyoD ($\rightarrow$) diaphragmatic $P_o$ could be due to 1) compromised cytoskeletal proteins associated with the sarcomere, 2) decreased myofibrillar protein concentration, 3) impaired intracellular Ca$^{2+}$ handling and excitation-contraction coupling, 4) reduced force production per individual cross bridge, or 5) a combination of these factors. On the basis of previous reports, it appears that MyoD knockout could negatively impact muscle-specific $P_o$ by altering at least three of these factors.

First, MyoD ($\rightarrow$) skeletal muscle has been shown to have altered sarcomere scaffolding (7, 8, 28, 29). Healthy skeletal muscle contains a complex cytoskeletal system that is essential to normal contractile function. A key cytoskeletal protein that is impaired or missing in MyoD ($\rightarrow$) animals is desmin (7, 8, 28, 29). Desmin is a muscle-specific intermediate filament and is the primary structural protein contained within the extrasarcomeric cytoskeleton theorized to serve in the transmission of mechanical forces longitudinally, lat-
erally, and to propagate mechanochemical signals throughout the myofibers (12, 25, 34, 55). Therefore, any abnormality in desmin production within muscle fibers could result in impaired function of the sarcomere (57).

Several lines of evidence indicate that deletion of MyoD results in impaired expression of desmin in skeletal muscle. First, Sabourin et al. (43) failed to detect desmin in cultures of MyoD-deleted satellite cells. However, using more precise detection methods, Yablokova-Reuveni et al. (61) later found that desmin is expressed in muscle of MyoD-deficient mice but at a lower level than WT counterparts. Moreover, work by White et al. (59) revealed that although MyoD (−/−) myoblasts are able to synthesize desmin, the expressed desmin does not appear to be appropriately organized within the muscle. As such, given that distinct morphological abnormalities have been observed in adult skeletal muscle from MyoD (−/−) mice, it seems possible that MyoD knockout could impair muscle contractile properties via the reduced expression of desmin (28–30).

A second factor, in addition to the thick filaments, the Ca2+-regulated thin filaments may also contribute to alterations in muscle force production. Troponin and tropomyosin constitute the Ca2+-sensitive switch that regulates the contraction of striated muscle fibers (48). Although myosin generates tension during muscle contraction, actin and Ca2+-regulatory proteins regulate tension generation. Incidentally, it has been shown that MyoD (−/−) mice differ from WT littermates in the response of fast muscle fibers to Ca2+ activation that parallels differences in troponin T isoform expression (32). Thus the basis of altered Ca2+ regulation of contraction in MyoD (−/−) fibers could arise from a disruption in the normal expression of contractile and regulatory protein isoforms in these fibers. Consequently, altered troponin T expression provides a theoretical basis for the disruption in Ca2+ sensitivity during contraction observed in MyoD (−/−) fibers.

MyoD Deletion Reduced Diaphragmatic Vmax

Another important finding in the current investigation is that diaphragmatic Vmax was 37% lower in MyoD (−/−) animals compared with WT. Skeletal muscle-shortening velocity is directly proportional to the rate of cross-bridge cycling and, theoretically, cross-bridge cycling rates are altered by changing attachment rate constants, dissociation constants, or both (16, 17). Studies investigating skeletal muscle Vmax reveal that cross-bridge cycling rates could be influenced by a variety of factors including MHC composition/myosin head binding states, myosin light chain isoforms, and myosin binding protein C (3).

Regarding MHC content, many studies reported a strong correlation between Vmax, maximum actin-activated myosin ATPase activity, and MHC isoform composition (2, 4, 52). Because there is a robust correlation between MyoD and MHC IIB gene expression patterns as well as evidence from in vitro and cell culture experiments suggesting that MyoD activates the MHC IIB gene, we hypothesized that MyoD knockout would lead to a decrease in diaphragmatic Vmax (58). Our data clearly support this hypothesis. However, it is unclear if the decrease in diaphragmatic Vmax observed in the MyoD (−/−) animals was due entirely to the fast-to-slow shift in MHC phenotype. Indeed, it seems possible that a portion of the reduced diaphragmatic Vmax in the MyoD (−/−) animals was due to altered expression of other myofibrillar proteins that also exist in a number of different isoforms (e.g., myosin light chain isoforms, myosin binding protein C, etc.) (3, 40, 47, 52). To date, it is unknown if MyoD deletion alters the expression of myofibrillar proteins involved in the regulation of muscle Vmax; this is an interesting area for future research.

MyoD Deletion Does Not Alter Diaphragmatic Fatigue Properties

Paradoxically, the change in MHC phenotype from fast to slow was not accompanied by a concomitant change in either fatigue tolerance or CS activity (Figs. 4 and 5). This observation is somewhat surprising given that metabolic changes usually accompany myofibrillar changes. The dysregulation between myofibrillar phenotype and metabolic phenotype in these MyoD (−/−) mice adds to a growing body of evidence that indicates myofibrillar phenotype conversion is not always mirrored by a proportional shift in oxidative enzymes during transition toward a slower fiber type (11, 13, 27, 35, 36, 44). Although overall oxidative capacities are generally higher in MHC type I and IIA fibers compared with type IIB, individual oxidative capacities differ across a single MHC fiber population. For example, Powers et al. (39) reported that succinate dehydrogenase activity varies widely within MHC types I, IIA, and IIB fiber types in the rat costal diaphragm. The range of oxidative capacities across the MHC fiber types supports the notion that MHC shifts do not always parallel changes in fiber-oxidative enzyme activities. In fact, it is likely that the oxidative capacity of the diaphragms from both of our experimental groups is determined by the biochemical adaptation to functional demands placed on the muscle and is not simply determined by intrinsic myogenic factors alone (21).

The lack of change in the metabolic phenotype was also unexpected given that the ratio of myogenin:MyoD has been shown to be important in regulating metabolic phenotype in some experimental models (24). Others showed that overexpression of myogenin can lead to an increase in oxidative capacity, and therefore, likely induce fatigue resistance in skeletal muscle of mice (22–24). Consequently, we would have expected the oxidative capacity of these MyoD (−/−) diaphragms, where the ratio of MyoD:myogenin is zero, to increase dramatically. However, both CS activity and measurements of fatigue tolerance suggest no alteration in the metabolic profile of these muscles (Fig. 4). Instead, it is more likely that a delicate balance between repression and activation of the MRFs prevails.

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and an equilibrium is maintained through a highly regulated integrative network of factors rather than a single, direct control (13).

Conclusions

Our results suggest that MyoD is required for the normal function of adult skeletal muscle, and mice missing this gene display distinct muscle phenotypes with impaired contractile characteristics. In the MyoD (−/−) mouse diaphragm, it seems likely that several factors could contribute to the impaired diaphragmatic $P_s$, $V_{\text{max}}$, and peak power output. Specifically, deletion of MyoD expression could result in reduced expression of desmin as well as the elimination of the MHC IIB phenotype and the expression of MyoD. Differences in $P_s$, $V_{\text{max}}$, and peak power output. Additionally, studies are required to elucidate the specific downstream proteins regulated by MyoD and the role that these proteins play in the regulation of muscle contractile function.

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