Isotonic contractile and fatigue properties of developing rat diaphragm muscle

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Isotonic contractile and fatigue properties of developing rat diaphragm muscle. J. Appl. Physiol. 84(4): 1260–1268, 1998.—Postnatal transitions in myosin heavy chain (MHC) isoform expression were found to be associated with changes in both isotropic and isotonic contractile properties of rat diaphragm muscle (Dia m). Expression of MHCneo predominated in neonatal Dia m fibers but was usually coexpressed with MHCslow or MHC2A isoforms. Expression of MHCneo disappeared by day 28. Expression of MHC2X and MHC2B emerged at day 14 and increased thereafter. Associated with these MHC transitions in the Dia m, maximum isometric tetanic force (P0), maximum shortening velocity, and maximum power output progressively increased during early postnatal development. Maximum power output of the Dia m occurred at 40% P0 at days 0 and 7 and at 30% P0 in older animals. Susceptibility to isometric and isotonic fatigue, defined as a decline in force and power output during repetitive activation, respectively, increased with maturation. Isometric endurance time, defined as the time for maximum power output to decline to zero, progressively decreased with maturation. In contrast, isotonic endurance time, defined as the time for force to decline to 30–40% P0, remained >300 s until after day 28. We speculate that with the postnatal transition to MHC2X and MHC2B expression energy requirements for contraction increase, especially during isotonic shortening, leading to a greater imbalance between energy supply and demand.

During early postnatal development, the Dia m becomes increasingly more susceptible to fatigue induced by repetitive isometric activation (24, 26, 35, 36). The increase in susceptibility to fatigue of the Dia m is also associated with an increase in the relative expression of MHC2X and MHC2B isoforms (36). Fibers expressing the MHC2X and MHC2B isoforms have higher ATP consumption rates (1, 29, 30, 32, 33) and lower oxidative capacities (27, 32, 36), compared with fibers expressing the MHCslow and MHC2A isoforms. Thus we hypothesized that the postnatal increase in susceptibility to fatigue of the Dia m was due, at least in part, to a greater imbalance between ATP consumption and aerobic capacity of fibers expressing the MHC2X and MHC2B isoforms (24, 36).

During shortening contractions, ATP consumption rate increases compared with that during isometric activation (6, 11). Thus the imbalance between energy supply and demand should be exaggerated during shortening contractions, and fatigue should be more rapid. Accordingly, Seow and Stephens (20) reported that in the mouse Dia m fatigue induced by repetitive shortening contractions was more pronounced than that induced by repetitive isometric activation. We hypothesized that, with the postnatal transition to MHC2X and MHC2B isoform expression in the Dia m, fatigue induced by repetitive shortening contractions would become progressively more pronounced. Accordingly, at different postnatal ages, we compared Dia m fatigue induced by repetitive isometric and isotonic contractions.

METHODS

Animal model and in vitro Dia m preparation. Experiments were performed on male Sprague-Dawley rats at postnatal days 0, 7, 14, 21, and 28 (D-0 to D-28, respectively) and on 84-day-old rats (adults) (n = 8 rats for each age group). Pregnant mothers were received at 14 days gestation, and after parturition the litter size was culled to eight pups. Pups from smaller litters were not studied to assure normal body growth. Body weights of the pups were measured daily. At D-21, the pups were weaned and, thereafter, they were housed two per cage. All experimental procedures were approved by the Institutional Animal Care and Use Committee at Mayo Clinic and were in strict accordance with the American Physiological Society animal care guidelines.

Rats were anesthetized by intramuscular injections of ketamine (60 mg/kg) and xylazine (2.5 mg/kg). The Dia m was then rapidly excised, and three muscle segments were dissected from the right midcostal region. One of the muscle segments was used to determine MHC isoform expression by electrophoretic analysis and immunohistochemistry. The other two muscle segments were used for in vitro measurement of isotonic and isometric fatigue properties (see below).
Electrophoretic determination of MHC isoform composition of the Diam. Muscle segments were stretched to 1.5 times resting excised muscle length [approximate optimal length (L_o) for muscle force generation in both adults and neonates (15)] and rapidly frozen in isopentane cooled to its melting point by liquid nitrogen. From one-half of the frozen muscle segment, myosin was extracted by scissor mining. The myosin extracts were centrifuged and supernatants recovered. After overnight storage to allow precipitation of myosin filaments, the solution was again centrifuged, and the pellet was dissolved in sample buffer, boiled, and then stored frozen. MHC isoforms were separated by SDS-PAGE. Specific MHC bands were identified by immunoblotting, as previously described (13, 27).

Immunohistochemical determination of MHC isoform expression in single Diam fibers. From the other one-half of the frozen muscle segment, five serial transverse sections were cut at 10-µm thickness and reacted with mouse primary antibodies against different MHC isoforms. In some cases, only a single antibody was used, e.g., anti-MHC_C (13, 27) [Schiaffino et al., BF3-35 (19), IgG]. However, in most cases, pairs of mouse IgG or IgM primary antibodies were used, e.g., anti-MHC_C (Novacorta, IgG), anti-MHC_B (Blau A4.A4.78, IgG) or Blau N1551 (IgM), anti-MHC_X (Schiaffino et al., BF3-19, IgM) and anti-MHC_H (Novacorta, IgG). Primary antibodies were diluted in PBS containing 0.5% bovine serum albumin (5 mg/ml) and applied to the muscle section for ~2 h at room temperature. Slides were then washed in PBS and reacted with Cy3- or Cy5-conjugated secondary antibodies (goat anti-mouse IgG or goat anti-mouse IgM) for 45 min at room temperature. The use of antibody pairs allowed for double labeling of MHC isoform expression in the same section with minimal cross-reactivity. This was confirmed by adding the opposite secondary antibody to a section incubated with only IgG or IgM primary antibody. Sections incubated with only the secondary antibodies served as controls for nonspecific reactivity of all primary antibodies. With the use of these methods, coexpression of different MHC isoforms could be determined within single fibers, with the exception of MHC_B, which coexpression was determined using digital calipers. Thereafter, the muscle segment was normalized for the CSA of the muscle, which was estimated by using the following formula: CSA = muscle weight (g)/[muscle specific density (1.056 g/cm^3)·L_o (cm)].

In one muscle segment, isotonic shortening velocities were measured at different loads ranging from 3 to 100% of P_o. At each load clamp level, muscle segments were stimulated at 75 Hz for 600 ms. The duration of stimulation was limited by the range of movement of the lever arm (~5 mm), especially at lower load clamp levels. For each isotonic load, the velocity of shortening was measured over a 30-ms period beginning 10 ms after the initiation of muscle shortening. The force-velocity measurements were least-squares fitted to a hyperbolic curve by using the Hill equation, and maximum velocity (V_{max}), expressed as muscle length (ML) per second, was determined by extrapolation (7). Power output of the Diam at each load was calculated as the product of isotonic load and shortening velocity (expressed as W·m^-2).

Isometric and isotonic fatigue properties. Isometric and isotonic fatigue were evaluated simultaneously in two separate muscle segments obtained from each Diam. In one muscle segment, isometric fatigue resistance was assessed during repetitive 40-Hz stimulation in 330-ms-duration trains repeated each second for a 5-min period. An isometric fatigue index was calculated as the ratio of force generated after 2 min of stimulation to the initial force.

In the second muscle segment, isotonic fatigue was assessed during repetitive 40-Hz stimulation in trains of 330-ms duration repeated each second. The load on the muscle during shortening corresponded to that at which maximum power output was observed (30–40% P_o). Fatigue was assessed as changes in shortening velocity and power output. Isometric endurance time was defined as the time required for power output to decline to 0 (i.e., the time when the muscle lost its ability to shorten). For comparison, isometric endurance time was defined as the time required for force to decline to the same %P_o as that used in the isotonic fatigue test.

Force and length signals were acquired and stimulation protocols were controlled by computer via a data-acquisition card (AT-M1016L-9, National Instruments) using LabView (National Instruments) software.

Statistical analysis. A one-way analysis of variance (age as the grouping variable) was used to evaluate maturational changes in P_o, V_{max}, maximum power output, maximum work performance, and isometric and isotonic endurance times. A two-way analysis of variance for repeated measures (age and time as grouping variables) was used to evaluate isometric and isotonic fatigue. When appropriate, post hoc analysis (unpaired Student’s t-test with Bonferroni correction) was also performed. In all cases, statistical significance was established at the 0.05 level. All data are represented as means ± SE.
Table 1. Transitions in MHC isoform composition of the rat diaphragm muscle during early postnatal development as determined by SDS-PAGE

<table>
<thead>
<tr>
<th>Age, days</th>
<th>MHC Isoform</th>
<th>MHC Isoform</th>
<th>MHC Isoform</th>
<th>MHC Isoform</th>
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<tbody>
<tr>
<td></td>
<td>MHCneo</td>
<td>MHCslow</td>
<td>MHC2A</td>
<td>MHC2X</td>
</tr>
<tr>
<td>D-0</td>
<td>66.3 ± 1.6*</td>
<td>11.8 ± 0.8*</td>
<td>21.9 ± 1.8*</td>
<td>0*</td>
</tr>
<tr>
<td>D-7</td>
<td>50.1 ± 1.4*</td>
<td>14.1 ± 1.1*</td>
<td>35.8 ± 1.8*</td>
<td>0*</td>
</tr>
<tr>
<td>D-14</td>
<td>26.5 ± 0.4*</td>
<td>24.8 ± 1.6*</td>
<td>36.3 ± 2.0*</td>
<td>12.4 ± 1.3*</td>
</tr>
<tr>
<td>D-21</td>
<td>8.7 ± 0.8*</td>
<td>23.2 ± 1.9*</td>
<td>39.7 ± 2.1*</td>
<td>23.7 ± 1.4*</td>
</tr>
<tr>
<td>D-28</td>
<td>0</td>
<td>28.6 ± 2.0*</td>
<td>34.6 ± 1.1*</td>
<td>32.4 ± 1.1*</td>
</tr>
<tr>
<td>Adult</td>
<td>0</td>
<td>25.7 ± 1.2*</td>
<td>30.4 ± 1.4*</td>
<td>29.7 ± 1.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference (P<0.05) from adult values.

RESULTS

Postnatal changes in body weight. During early postnatal development, body weights of the male rats progressively increased from 7.4 ± 0.3 g at D-0 to 307.4 ± 3.6 g in adults (P<0.05). During the first 3 postnatal wk, body weights increased at a rate of ~10 g/wk, whereas the growth rate increased to ~30 g/wk from D-21 to D-84 (adults).

MHC isoform composition of the Diam. Based on relative densitometric analysis of the SDS-PAGE, the relative contribution to total cross-sectional area of diaphragm muscle fibers expressing different MHC isoforms during early postnatal development is shown in Table 1. At D-0 and D-7, only MHCneo, MHCslow, and MHC2A were expressed in the Diam, with the MHCslow predominating at both ages. The MHC2X isoform appeared by D-14, and the MHC2B isoform by D-21. The MHCneo isoform was no longer expressed in the Diam by D-28. The relative expression of the MHCslow isoform increased after D-0 and reached adult values by D-14. The relative expression of the MHC2A isoform also increased after D-0, reaching highest levels between D-14 to D-28, before declining slightly in the adult. After D-14, the relative expression of the MHC2X isoform increased, reaching adult levels by D-28. The relative expression of the MHC2B isoform also increased after appearing at D-21.

Immunohistochemical determination of MHC isoform expression in Diam fibers. Immunohistochemical analysis was used to provide qualitative information regarding the distribution of MHC isoform expression within single Diam fibers at different postnatal ages (Table 2). However, with coexpression of MHC isoforms, immunohistochemistry could not determine the relative amounts of each MHC isoform expressed. In addition, coexpression of the MHC2X isoform could not be unambiguously determined based on immunohistochemistry.

At birth, ~92% of all Diam fibers expressed the MHCneo isoform, either alone or in combination with the MHCslow, and/or MHC2A isoforms (Fig. 1). Given the relative MHC isoform composition of the Diam, at D-0, determined based on SDS-PAGE analysis (Table 1), it is likely that expression of the MHCneo isoform predominated in most of these fibers.

The general pattern of coexpression of the MHCneo isoform with MHCslow and MHC2A isoforms was also found in the D-7, D-14, and D-21 Diam (Fig. 1; Table 2). However, based on SDS-PAGE data, the MHCneo isoform comprised far less of the total MHC isoform expression in the Diam at these ages (Table 2). There-

Table 2. Proportion, cross-sectional area, and relative contribution to total cross-sectional area of diaphragm muscle fibers expressing different MHC isoforms during early postnatal development

<table>
<thead>
<tr>
<th>Age, days</th>
<th>MHC Isoform Immunoreactivity</th>
<th>Proportion of fiber, %</th>
<th>Fiber cross-sectional area, μm²</th>
<th>Relative contribution to total area, %</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>MHCneo and MHCslow</td>
<td></td>
<td></td>
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<tr>
<td>D-0</td>
<td>17.3 ± 2.8</td>
<td>2.0 ± 1.2</td>
<td>72.6 ± 3.0</td>
<td>248 ± 16*</td>
</tr>
<tr>
<td>D-7</td>
<td>11.3 ± 1.1</td>
<td>71.1 ± 2.1</td>
<td>83.3 ± 0.9</td>
<td>211 ± 14*</td>
</tr>
<tr>
<td>D-14</td>
<td>19.5 ± 1.4</td>
<td>71.3 ± 1.7</td>
<td>9.2 ± 0.3</td>
<td>150 ± 11</td>
</tr>
<tr>
<td>D-21</td>
<td>4.1 ± 1.1</td>
<td>15.4 ± 1.3</td>
<td>25.9 ± 0.8*</td>
<td>201 ± 19</td>
</tr>
<tr>
<td>D-28</td>
<td>3.2 ± 0.5</td>
<td>31.0 ± 0.5*</td>
<td>50.7 ± 2.6*</td>
<td>210 ± 19</td>
</tr>
<tr>
<td>Adult</td>
<td>3.4 ± 1.9</td>
<td>0.5 ± 0.1</td>
<td>29.9 ± 1.4</td>
<td>578 ± 16</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>MHCslow and MHC2A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-0</td>
<td>114 ± 14</td>
<td>248 ± 16*</td>
<td>331 ± 19*</td>
<td>581 ± 2.7</td>
</tr>
<tr>
<td>D-7</td>
<td>177 ± 12</td>
<td>307 ± 28*</td>
<td>571 ± 29*</td>
<td>77.9 ± 3.1</td>
</tr>
<tr>
<td>D-14</td>
<td>274 ± 26</td>
<td>829 ± 54*</td>
<td>809 ± 59*</td>
<td>116.3 ± 11</td>
</tr>
<tr>
<td>D-21</td>
<td>388 ± 21*</td>
<td>1.67 ± 80</td>
<td>2.388 ± 177</td>
<td>201 ± 19</td>
</tr>
<tr>
<td>D-28</td>
<td>829 ± 54*</td>
<td>1.67 ± 80</td>
<td>2.388 ± 177</td>
<td>212 ± 18</td>
</tr>
<tr>
<td>Adult</td>
<td>1.67 ± 80</td>
<td>2.388 ± 177</td>
<td>2.388 ± 177</td>
<td>581 ± 2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference (P<0.05) from adult values.
fore, it is likely that the amount of MHCneo isoform coexpressed with Dia_m fibers progressively decreases with age. It should also be noted that based on SDS-PAGE the MHC2X isoform comprised ~12% of the total MHC isoform expression in the D-14 Dia_m and ~24% in the D-21 Dia_m (Table 1). At these ages, there were no fibers that singularly expressed the MHC2X isoform. Coexpression of the MHC2X isoform could not be detected by immunohistochemistry at these ages. Therefore, an unknown proportion of Dia_m fibers coexpressed the MHC2X isoform at D-14 and D-21.

By D-28, the MHCneo isoform was not expressed in the rat Dia_m (Fig. 1; Tables 1 and 2). Generally, the adult pattern of MHC isoform expression was observed by D-28. However, compared with the adult, a greater proportion of Dia_m fibers expressed the MHC2A isoform at D-28, fewer fibers expressed the MHC2X isoform, and no fibers singularly expressed the MHC2B isoform (Table 2; P < 0.05). These immunohistochemical results corresponded with the SDS-PAGE analysis, where the relative MHC isoform composition of the D-28 Dia_m was found to be comparable to that of the adult, with the exception of greater MHC2A expression and lower MHC2B expression at D-28 (P < 0.05; Table 1).

CSA of Dia_m fibers. The increase in body weight during early postnatal development was accompanied by a dramatic increase in the CSA of Dia_m fibers (Table 2). Fiber CSAs in the D-0 Dia_m were relatively uniform, although those fibers coexpressing the MHCneo and MHC2A isoforms were smaller than other fibers (P < 0.05; Table 2). In the D-0 Dia_m, fibers expressing the MHCneo isoform, either alone or in combination with other MHC isoforms, contributed ~85% to total Dia_m mass (Table 2).

The CSAs of Dia_m fibers at D-7 and D-14 continued to be relatively uniform compared with the ones in the adult (Table 2). Fibers coexpressing the MHCneo isoform were smaller than fibers expressing the MHCslow isoform alone (P < 0.05; Table 2). In the D-7 and D-14 Dia_m, fibers expressing the MHCneo isoform, either alone or in combination with other MHC isoforms, continued to provide a major contribution to total Dia_m mass (Table 2).

By D-28, Dia_m fibers expressing the MHC2X and MHC2B isoforms were significantly larger than other MHC phenotypes (P < 0.05; Table 2). However, this difference in CSA between fibers expressing the MHC2X and MHC2B isoforms and other MHC phenotypes was not as pronounced as that observed in the adult Dia_m (P < 0.05; Table 2). As a result, the relative contribution of fibers expressing the MHC2X and MHC2B isoforms in the D-28 Dia_m was only ~24% compared with ~58% in the adult (P < 0.05; Table 2).

Contractile properties. The L_o of Dia_m fibers increased threefold from D-0 to adulthood (P < 0.05; Table 3). Both the P_t and P_o of the Dia_m increased significantly with postnatal maturation (P < 0.05; Table 3). The increase in P_t and P_o was proportionate, such that the
P0/Po remained relatively constant across postnatal maturation.

At each postnatal age, the force-velocity relationship of the Dia_m was hyperbolic, but with postnatal maturation there was a significant upward shift in the force-velocity relationship at lower load clamp levels (P < 0.05; Fig. 2). Accordingly, Vmax increased more than fourfold from D-0 to adulthood (P < 0.05; Fig. 2).

With the age-related increase in both tetanic force and shortening velocity, the power generated at each load level also increased significantly (P < 0.05; Figs. 4, 5, and 6; Table 4). The two fatigue protocols were directly compared in older animals (Figs. 4, 5, and 6; Table 4). This isotonic endurance time became progressively shorter from D-14 to adulthood (P < 0.05; Fig. 6B). Fatigue during repetitive isotonic activation was also evidenced by a progressive decline in shortening velocity (Fig. 5B) and maximum power output (Fig. 5C) at each postnatal age (P < 0.05). This rate and extent of decline in shortening velocity and maximum power output varied with age, being greatest in the adult Dia_m and least at D-0 (P < 0.05; Fig. 5, B and C).

The Dia_m appeared to be more susceptible to fatigue during repetitive isotonic contractions compared with repetitive isometric activation, especially in older animals (Figs. 4, 5, and 6; Table 4). The two fatigue protocols were directly compared in older animals (D-14 and older) by determining the time required for force to decline to comparable levels (~30% of P0) during isotonic vs. isometric endurance times. In adults, the time required for force to decline to comparable levels (30% of P0) was significantly longer during isotonic activation (118 ± 5 s) compared with the isometric endurance time (82 ± 4 s; P < 0.05). At D-14, isotonic endurance time was longer than 300 s, compared with...
150 ± 4 s for isotonic endurance time (P < 0.05). Similarly, at D-28, isotonic endurance time was longer than isotonic endurance time (>300 vs. 109 ± 8 s, respectively; P < 0.05). At D-0 and D-7, the Diam continued to generate at least 40% of P0 for >5 min during both isometric and isotonic fatigue protocols. Thus a comparison of endurance times was not possible at these ages.

For older animals (D-14 and older), it was also possible to compare the amount of residual force generated during the isometric fatigue protocol at a time corresponding to the isotonic endurance time. For example, after 82 s in adults (corresponding to the isotonic endurance time), the Diam was still able to generate 44 ± 2% of P0 during the isometric fatigue protocol. Similarly, Diam at D-14, D-21, and D-28 was still able to generate ~60% of P0 during repetitive isometric activation, when the muscle failed to shorten during repetitive isotonic contraction (Table 4).

**DISCUSSION**

The present study examined the association between postnatal transitions in MHC isoform expression and changes in isometric and isotonic contractile and fatigue properties of the rat Diam. The disappearance of $\text{MHC}_{\text{iso}}$ isoform expression and the emergence of $\text{MHC}_{\text{2X}}$ and $\text{MHC}_{\text{2B}}$ isoform expression were associated with a maturational increase in $P_0$, $V_{\text{max}}$, and maximum power output of the Diam. However, with this postnatal increase in contractility, the Diam became more susceptible to fatigue, especially during shortening contractions.

In the present study, MHC isoform expression was determined by using both SDS-PAGE and immunohistochemistry. Based on densitometric analysis, the relative composition of different MHC isoforms in the Diam could be determined at each postnatal age. However, the distribution of MHC isoform expression within single fibers could not be evaluated by whole muscle fibers.
the progressive increase in Diam Po with development is studies (9, 15, 24, 31, 35). As we previously reported, the present study is consistent with several previous possible to detect coexpression of the MHC 2X isoform. 

SDS-PAGE. In a previous study in the adult Dia_m, we used SDS-PAGE to determine MHC isoform expression in single fibers (32). This method was particularly useful in assessing the relative composition of different MHC isoforms in those fibers coexpressing MHC isoforms. However, because of the small size and fragility of fibers in the developing Dia_m, it was not possible to reliably dissect single fibers and utilize SDS-PAGE analysis in the present study. Instead, we utilized immunohistochemistry to identify MHC isoform expression within single fibers. This method is limited by the fact that the extent of MHC isoform coexpression cannot be quantified. Furthermore, since no specific antibody for the MHC 2X isoform exists, it was not possible to detect coexpression of the MHC 2X isoform. Thus, whereas SDS-PAGE analysis revealed that the MHC 2X isoform was present in the Dia_m at D-14, the expression of this isoform could not be detected by immunohistochemistry until D-28, when it was singularly expressed in some fibers.

The increase in Dia_m Po with maturation observed in the present study is consistent with several previous studies (9, 15, 24, 31, 35). As we previously reported, the progressive increase in Dia_m Po with development is inversely correlated with the expression of the MHC neo isoform and positively correlated with the emergence of MHC 2X and MHC 2B isoform expression (9). In the neonatal Dia_m, Po is only one-half that of the adult. The lower specific force of the neonatal Dia_m may reflect, at least in part, the higher relative contribution of interstitial space to total muscle area in the neonate (15). However, it is clear that other factors must also contribute to the lower Po of the neonatal Dia_m. For example, it is possible that Dia_m fibers during early postnatal development have a lower myofibrillar volume density than do adult fibers (9, 14, 24, 31, 35). It is also possible that neonatal Dia_m fibers differ in the force per cross bridge or in cross-bridge cycling kinetics.

Several previous studies have demonstrated a relationship between MHC isoform expression and the V_o or V_max of single muscle fibers (2, 4, 16–18, 34). Generally, studies in adult animals have demonstrated that muscle fibers expressing fast MHC isoforms have faster V_o or V_max than fibers expressing the MHC slow isoform. In the adult rat Dia_m, Eddinger and Moss (4) reported that the V_o of fibers expressing fast MHC isoforms was ~3.5 times faster than that of fibers expressing the MHC slow isoform. In developing rat soleus and psoas muscle fibers, Reiser and colleagues (16, 17) reported that the V_o of fibers expressing developmental MHC isoforms was slower than that of fibers expressing fast MHC isoforms but faster than that of fibers expressing the MHC slow isoform.

In previous studies in the rat Dia_m (9, 31), we found that postnatal transitions in MHC isoform composition strongly correlated with an increase in V_o, as determined by using the slack method (5). During the first 3 postnatal wk, the increase in V_o of the Dia_m inversely correlated with the decrease in the relative contribution of the MHC neo isoform. After D-14, the increase in V_o positively correlated with the progressive increase in MHC 2X and MHC 2B isoform expression. In these previous studies, the slack test was used to determine V_o, whereas in the present study V_max was estimated by extrapolation of the force-velocity relationship to zero load. Moreover, measurements were obtained at 26°C rather than 15°C as in our previous studies (9, 31). In muscles consisting of different fiber types, such as the Dia_m, it has been suggested that V_max more accurately reflects the relative composition of different MHC isoforms while V_o may reflect primarily the fastest fibers (3). Despite the differences in technique, the increase in V_max that we observed during early postnatal development of the Dia_m in the present study was qualitatively similar to that previously observed. However, if a Q10 of ~2 is assumed for V_o measurements, the corrected V_o for the Dia_m at 26°C would be considerably faster than the V_max observed in the present study at each postnatal age. For example, at D-0, the V_max at 26°C observed in the present study was 1.2 ML/s, whereas the temperature-corrected V_o of the Dia_m at D-0 was 2.0 ML/s. In the adult Dia_m this discrepancy between V_o and V_max would be even more pronounced; a V_max of 5.1 ML/s in the present study vs. a temperature-corrected V_o of 12.6 ML/s. The greater discrepancy between V_max in the adult most likely reflects the contribution of fibers expressing the MHC 2X and MHC 2B isoforms.

As previously observed, susceptibility of the Dia_m to fatigue induced by repetitive isometric activation increases during early postnatal development (14, 15, 24, 31).
This age-related increase in the susceptibility of the Diaₘ fibers to fatigue cannot be explained by maturational changes in fiber oxidative capacity, since succinate dehydrogenase activity and capillary density of Diaₘ muscle fibers actually increase during the early phase of postnatal development when fatigue resistance is declining (22–24, 36). In the adult Diaₘ, the capacity for oxidative phosphorylation in fibers expressing the MHCslow and MHC₂A isoforms remains relatively high, whereas the oxidative capacity of fibers expressing the MHC₂X and MHC₂B isoforms is substantially lower (32). These fiber type differences in oxidative capacity in the adult do correspond with the fatigue resistance of the motor units that they comprise (27).

Differences in ATP consumption rate can also contribute to the susceptibility of different muscle fiber types to fatigue. The MHC is the site of hydrolysis of ATP during cross-bridge cycling, i.e., actomyosin ATPase. Recent studies have clearly demonstrated an association between MHC isoform expression, actomyosin ATPase activity, and vmax, in adult muscle fibers (1, 29, 30, 33). Using a quantitative histochemical technique to measure actomyosin ATPase activity of muscle fibers in the adult rat Diaₘ, we also found that fibers expressing the MHCslow isoform have the lowest actomyosin ATPase activity, followed in rank order by fibers expressing MHC₂A, MHC₂X, and MHC₂B isoforms (32). Furthermore, in a recent study, we found that the actomyosin ATPase activity of fibers coexpressing the MHCneo isoform with either MHCslow or MHC₂A was lower than that of fibers expressing adult fast MHC isoforms (28). These fiber type differences in actomyosin ATPase activities support the hypothesis that postnatal changes in energy demands of cross-bridge cycling may account, at least in part, for maturational changes in fatigue resistance (24, 36). The slower cross-bridge cycling rate and lower ATP consumption of fibers expressing the MHCslow isoform, together with a higher oxidative capacity, may lead to a better balance between energy supply and utilization and thus a lower susceptibility to fatigue. In contrast, the faster cross-bridge cycling rate and higher ATP consumption of fibers expressing the MHC₂X and MHC₂B isoforms, together with their lower oxidative capacity, may lead to an imbalance between the energy supply and utilization and thus a greater susceptibility to fatigue.

The observation that the Diaₘ fibers are more susceptible to fatigue during repetitive isotonic shortening contractions compared with repetitive isometric contractions is consistent with the previous study of Seow and Stephens (20) in the mouse Diaₘ. Because with shortening contractions ATP consumption rate increases (6, 11), these results also support the hypothesis that fatigue results, at least in part, from an imbalance between energy supply and demand. The fact that the difference between isotonic and isometric fatigue becomes increasingly more pronounced during postnatal development is consistent with the higher ATP consumption rates and lower oxidative capacity of fiber expressing the MHC₂X and MHC₂B isoforms, which emerge only after D-14 in the rat Diaₘ.

Because of more compliant lung and chest wall mechanics, it has been proposed that recruitment of a greater fraction of Diaₘ fibers is required to sustain normal ventilation in the neonate (24). Accordingly, polyneuronal innervation of rat Diaₘ fibers until D-14 facilitates the more complete recruitment of fibers during ventilatory behaviors (24). In contrast, in the adult Diaₘ, it is likely that recruitment of more fatigue-resistant motor units consisting of type IIX and IIB fibers is not required to accomplish normal ventilatory behaviors (21, 25). With maturation and the differentiation of fibers expressing MHC₂X and MHC₂B isoforms, the functional reserve capacity of the diaphragm increases (i.e., a greater difference between maximum force vs. force generation during ventilation) (21). However, because of the increased susceptibility of type IIX and IIB fibers to fatigue, it is unlikely that these fibers are recruited during normal ventilation. Thus the normal postnatal transitions in MHC isoform expression in the Diaₘ most likely have little impact on sustaining ventilation. Instead, the transition to MHC₂X and MHC₂B isoform expression allows for a greater diversity of Diaₘ motor behaviors, especially those requiring shorter duration activation with greater power output.

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