Denervation alters myosin heavy chain expression and contractility of developing rat diaphragm muscle

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Sieck, Gary C. and Wen-Zhi Zhan. Denervation alters myosin heavy chain expression and contractility of developing rat diaphragm muscle. J Appl Physiol 89: 1106–1113, 2000.—We hypothesized that unilateral denervation (DNV) of the rat diaphragm muscle (Dia m) in neonates at postnatal day 7 (D-7) alters normal transitions of myosin heavy chain (MHC) isoform expression and thereby affects postnatal changes in maximum specific force (Po) and maximum unloaded shortening velocity (V o). The relative expression of different MHC isoforms was analyzed electrophoretically. With DNV at D-7, expression of MHC neo in the Dia m persisted, and emergence of MHC2X and MHC2B was delayed. By D-21 and D-28, relative expression of MHC2A and MHC2B was reduced in DNV compared with control (CTL) animals. Expression of MHC neo also reappeared in adult Dia m by 2–3 wk after DNV, and relative expression of MHC2B was reduced. At each age, Po was reduced and V o was slowed by DNV, compared with CTL. In CTL Dia m, postnatal changes in Po and V o were associated with an increase in fast MHC isoform expression. In DNV Dia m, no such association existed. We conclude that, in the Dia m, DNV induces alterations in both MHC isoform expression and contractile properties, which are not necessarily causally linked.

contractile properties; maturation; specific force; shortening velocity

SEVERAL MYOSIN HEAVY CHAIN (MHC) isoforms exist in skeletal muscle, which, in adults, correspond with the histochemical classification of different fiber types (22, 48, 52). During the first four postnatal weeks, there are dramatic transitions in MHC isoform expression in the rat diaphragm muscle (Dia m) (29, 30, 51, 59). At birth (postnatal day 0; D-0), the MHC neo isoform is predominantly expressed, but, by postnatal day 28 (D-28), the MHC neo isoform completely disappears. In contrast, MHC2X and MHC2B isoform expression appears in the rat Dia m only by the end of the second postnatal week.

The mechanism underlying postnatal transitions in MHC isoform expression is unknown. Transition in MHC isoform expression may be affected by changes in the pattern of innervation, circulating thyroid hormones, and/or genetic program (17, 25, 28, 51). Innervation of the rat Dia m is established by embryological day 18 (25), and a pattern of multiple motoneuron innervation of Dia m fibers persists until postnatal day 14 (D-14) (1, 41). Therefore, during the first two postnatal weeks, MHC isoform expression in the rat Dia m fibers can be influenced by more than one motoneuron. Although previous studies have suggested that innervation is not a requirement for the ultimate expression of adult fast MHC isoforms in hindlimb muscles (3, 8, 35, 43), this does not exclude an influence of innervation pattern on the normal postnatal transitions in MHC isoform expression. In the adult rat Dia m, removal of neural influence by denervation (DNV) results in the coexpression of slow and fast MHC isoforms within single fibers (7, 46, 62). In the present study, we hypothesize that DNV will disrupt the normal postnatal transitions in MHC isoform expression in the rat Dia m.

Several previous studies have demonstrated that the contractile properties of muscle fibers correspond with MHC isoform expression. For example, fibers expressing MHC slow or MHC neo isoforms have slower maximum unloaded shortening velocities (V o) compared with fibers expressing fast MHC isoforms (39, 40, 49, 50, 58). This explains, at least in part, the slower V o of the Dia m during early postnatal development, when there is predominant expression of MHC neo and MHC slow isoforms (29, 51, 58). The predominant expression of MHC neo and MHC slow isoforms may also explain the lower maximum specific force (force normalized for fiber cross-sectional area, P o) and greater fatigue resistance of the neonatal Dia m (20, 21, 29, 51, 58, 59, 64) in the adult rat Dia m, DNV leads to a dramatic slowing of V o and a marked reduction in P o, which are not directly proportional to changes in MHC isoform expression (37). Accordingly, in the present study, we hypothesize that unilateral DNV of the rat Dia m during early postnatal development also leads to a slowing of V o and a reduction in P o, and that these effects are independent of altered MHC isoform expression.

METHODS

General procedures. Experiments were performed on 39 young and 31 adult male Sprague-Dawley rats. In the
younger animal groups, pregnant mothers were received at 14 days gestation, and at the time of birth, neonatal rats from each litter were randomly assigned to one of the six groups: 1) control (CTL) animals studied at D-14 (n = 6); 2) DNV animals studied at postnatal day 21 (D-21; n = 7); 3) CTL animals studied at D-28 (n = 7); 4) DNV animals studied at D-14 (n = 6); 5) DNV animals studied at D-21 (n = 7); and 6) DNV animals studied at D-28 (n = 6). Adult animals were divided into one of four groups: 1) Sham CTL animals (n = 8); 2) DNV animals studied after 1 wk (n = 7); 3) DNV animals studied after 2 wk (n = 8); and 4) DNV animals studied after 3 wk (n = 8). Animals were housed in separate cages under a 12:12-h light-dark cycle. Adult animals and the mothers were fed with Purina rat chow and provided with water ad libitum. Body weights were monitored daily in all groups. Surgical procedures were performed under aseptic conditions, and recovery of animals from surgery was carefully monitored. The Institutional Animal Care and Use Committee of the Mayo Clinic approved all procedures.

Phrenicotomy. In both young and adult groups, unilateral, rather than bilateral, DNV was performed to enhance survival of the animals and to match similar procedures performed in previous studies (23, 37, 61–63). In each of the younger DNV groups, the right phrenic nerve was transected in the neck at D-7. This age was selected because it precedes both the elimination of polynuronal innervation of the Dia_{m} (1, 41) and the emergence of MHC_{2a} and MHC_{2b} isoform expression (29–31, 51, 59).

In the D-7 rats, surgeries were performed using hypothermic anesthesia. The young rat was placed beneath a shallow layer of ice chips until the righting reflex was lost and spontaneous breathing ceased. The animal was then placed supine on a strip of aluminum foil, cushioned, and surrounded by ice chips. The surgical procedure was completed within 10 min, during which time the animal’s body temperature was maintained at 10°C. Adult animals were anesthetized using ketamine (60 mg/kg) and xylazine (2.5 mg/kg) that was administered intramuscularly. In both groups, a midline incision was made over the trachea, and the right phrenic nerve was sectioned in the neck at a point beneath the sternomastoid muscle. A portion of the distal end of the phrenic nerve was removed to prevent re-innervation of the Dia_{m} and to minimize neurotrophic effects emanating from the remaining nerve stump. The wound was closed with 6–0 silk sutures, and the surgical wounds were treated topically with Neosporin ointment (containing aerobosporin, neomycin, and bacitracin). After surgery, younger animals were gradually rewarmed under an infrared lamp. Using a pair of cotton-tipped applicators, the animal was vigorously but gently manipulated, with occasional pressing on the thoracic and abdominal walls, to promote ventilation. Within 4–8 min, the rat pups fully recovered, and they were then returned to their mother, who readily accepted them for care and feeding. A 95% success rate was achieved using this DNV procedure. Adult animals fully recovered within 1 h after surgery. In previous studies, our laboratory demonstrated that blood-gas levels were normal in the DNV animals, indicating ventilatory compensation for unilateral paralysis of the Dia_{m} (37).

In vitro measurement of contractile properties. The in vitro preparations for determining isometric and isotonic contractile properties of the Dia_{m} have been previously described (29, 37, 64). Briefly, at D-14, D-21, and D-28, rat pups were reanesthetized using furane inhalation, whereas adult animals were anesthetized with ketamine (60 mg/kg) and xylazine (2.5 mg/kg). The Dia_{m} was rapidly excised, and two muscle segments (3- to 4-mm width) were dissected from the right midcostal region, one for isometric measurements and the other for isotonic measurements. The isometric and isotonic experiments were performed simultaneously using two different systems. In both cases, the muscle segments were suspended vertically in glass tissue chambers containing Rrees-Simpson solution (pH 7.4) with the following composition (in mM): 135 Na^{+}, 5 K^{+}, 2 Ca^{2+}, 1 Mg^{2+}, 120 Cl^{−}, 25 HCO_{3}^{−}, and 0.012 d-tubocurarine. The solution was aerated with 95% O_{2}-5% CO_{2} and maintained at either 26°C (isometric) or 15°C (isotonic). The cooler temperature was used in the isotonic studies to improve the accuracy of measurements of the time required for force to redevelop after a step change in muscle length (see below for methods of measuring V_e using the “slack test”). In both cases, the muscle segments were stimulated directly with current pulses (0.5-ms pulse duration) via platinum plate electrodes placed on either side of the muscle. A computer controlled the stimulus pattern. Stimulus intensity was raised until a maximal twitch response was obtained and was then set at 125% of this maximal stimulus intensity (supramaximal stimulation, ~230 mA). During single-pulse stimulation, muscle fiber length was adjusted until a maximal twitch force was obtained. This optimal length (L_{o}) was measured using digital calipers.

For isotonic force measurements, the costal margin origin of muscle fibers was clamped to a steel rod that was fixed to a micromanipulator. The central tendon of the Dia_{m} segment was glued to a nylon mesh that was then attached to a calibrated force transducer (Grass FT 03). All force responses were displayed on a storage oscilloscope ( Nicolet 410), recorded on a chart recorder (Gould TA2000), and digitized at a 1-kHz sampling rate using Lab View software. Subsequently, force was normalized for the cross-sectional area (CSA) of the muscle segment, which was estimated using the following formula

\[ CSA = \text{muscle weight} \times \text{[muscle specific density (1.056 g/cm}^3\text{)]} \times \text{[L}_{o}\text{]} \times \text{[cm]} \]

For measurements of V_e, the costal margin origin of fibers was fixed in series with a micromanipulator for length adjustments in establishing L_{o}. The central tendon of the muscle segment was glued to a small piece of aluminum foil that was then attached to a force transducer (Grass FT 03). All force responses were displayed on a storage oscilloscope (Nicolet 410), recorded on a chart recorder (Gould TA2000), and digitized at a 1-kHz sampling rate using Lab View software. Subsequently, force was normalized for the cross-sectional area (CSA) of the muscle segment, which was estimated using the following formula

\[ CSA = \text{muscle weight} \times \text{[muscle specific density (1.056 g/cm}^3\text{)]} \times \text{[L}_{o}\text{]} \times \text{[cm]} \]

\[ MHC\text{ isoform composition. Myosin was extracted from the muscle segments by scissors mincing in a high-salt solution (in mM: 300 NaCl, 100 Na_{2}HPO_{4}, 50 Na_{2}HPO_{4}, 1 Na_{2}P_{2}O_{7}, 10 EDTA, pH 6.5) at 4°C for 40 min (4). Extracts were centrifuged, and the supernatants were recovered. Ten microliters of supernatant were diluted (1:10) in a low-salt buffer consisting of 1 mM EDTA and 0.1% 2-mercaptoethanol (vol/vol) and stored overnight at 4°C to allow precipitation of myosin filaments. The filament solution was subsequently centrifuged to form a pellet, which was then dissolved in myosin sample buffer (0.5 M CaCl_{2}, 10mM Na_{2}HPO_{4}), followed by dilution (1:200) in SDS samples buffer [62.5 mM}
Different MHC isoforms were separated by SDS-PAGE gel electrophoresis. Gel preparation was based on a modification of the procedure by Sugiura (54). A 3.5% acrylamide concentration (pH 8.8) was used in the stacking gel, and the resolving gel (8 × 10 cm in size, 0.75-mm thick, Hoefer SE250) consisted of a gradient of 5–8% acrylamide (pH 8.8) with 25% (vol/vol) glycerol. All samples were run at a constant current of 20 mA/gel until the tracking dye reached the bottom of the gel (~1.75 h). After completion of the gel run, the gels were removed from the plates and silver stained according to the procedure of Oakley et al. (38). The relative expression of different MHC isoforms was then quantified by densitometry.

Statistics. All data are reported as means ± SE. A two-way ANOVA was used to evaluate changes in contractile properties and MHC isoform expression, with age and experimental condition (CTL vs. DNV) used as grouping variables. When appropriate, an unpaired t-test was used as a post hoc analysis to compare CTL and DNV groups. A multiple stepwise linear regression was used to determine the contribution of each MHC isoform to the correlation between postnatal and MHC isoform transitions and changes in P_o and V_o. Statistical significance of group differences and regressions were tested at P < 0.05.

Table 2. Effect of DNV on MHC isoform composition of the rat Dia_m during early postnatal development

<table>
<thead>
<tr>
<th>Group</th>
<th>MHC_mna</th>
<th>MHC_slow</th>
<th>MHC_2A</th>
<th>MHC_2X</th>
<th>MHC_2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>24.4 ± 0.4</td>
<td>27.3 ± 0.4</td>
<td>35.2 ± 0.6</td>
<td>11.7 ± 0.7</td>
<td>1.4 ± 0.7</td>
</tr>
<tr>
<td>DNV, 1 wk</td>
<td>37.4 ± 2.5*</td>
<td>16.6 ± 2.5*</td>
<td>46.0 ± 2.4*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>D-21</td>
<td>6.3 ± 0.3</td>
<td>27.0 ± 1.7</td>
<td>37.7 ± 2.4</td>
<td>21.2 ± 2.2</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>DNV, 2 wk</td>
<td>20.2 ± 1.2*</td>
<td>21.2 ± 1.0*</td>
<td>29.8 ± 1.1*</td>
<td>24.1 ± 1.0</td>
<td>4.7 ± 0.9*</td>
</tr>
<tr>
<td>D-28</td>
<td>0</td>
<td>25.7 ± 1.9</td>
<td>35.3 ± 1.0</td>
<td>31.3 ± 0.9</td>
<td>7.7 ± 1.4</td>
</tr>
<tr>
<td>DNV, 3 wk</td>
<td>8.1 ± 0.7*</td>
<td>26.8 ± 0.9</td>
<td>27.4 ± 1.2*</td>
<td>34.8 ± 2.0</td>
<td>2.9 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6–7 for each group). MHC, myosin heavy chain. *Significant difference (P < 0.05) from CTL values at corresponding age.

RESULTS

During the first four postnatal weeks, rats displayed rapid weight gain, which was not significantly affected by DNV (Table 1). During early postnatal development, there was also a progressive increase in L_o of the Dia_m, which was not significantly affected by DNV (Table 1).

DNV-induced alterations in MHC isoform composition of the Dia_m. Postnatal transitions in MHC isoform composition of the Dia_m were altered in the DNV rats. During the first 3 wk after DNV, the relative expression of the MHC_mna isoform in the Dia_m was significantly higher in the DNV rats compared with CTL animals of the corresponding age (P < 0.05; Table 2). By D-28, in the CTL Dia_m, expression of the MHC_mna isoform was completely absent, whereas expression of the MHC_mna isoform persisted in the DNV animals. However, like CTL animals, expression of the MHC_mna isoform in the DNV Dia_m gradually decreased with postnatal development (P < 0.05).

After 1 and 2 wk of DNV in the younger animals (i.e., D-14 and D-21), the relative expression of the MHC_slow isoform was lower in the DNV Dia_m compared with the corresponding age-matched CTL (P < 0.05; Table 2). In the D-28 rats, the relative expression of the MHC_slow isoform was comparable between CTL and DNV Dia_m.

The relative expression of the MHC_2A isoform was higher in the DNV Dia_m compared with CTL at D-14 (P < 0.05; Table 2). However, by D-21 and D-28, the relative expression of the MHC_2A isoform was lower in the DNV animals compared with the corresponding age-matched CTL (P < 0.05).

Expression of the MHC_2X isoform appeared only by D-14 in the CTL Dia_m. In contrast, after 1 wk of DNV, there was no expression of the MHC_2X isoform in the D-14 DNV Dia_m (Table 2). Expression of the MHC_2X isoform in the DNV Dia_m was delayed until D-21. In D-21 and D-28 animals, the relative expression of the MHC_2X isoform did not differ between DNV and CTL animals.

Similar to the expression of the MHC_2X isoform, emergence of the MHC_2B isoform did not occur until D-14 in the CTL Dia_m. Expression of the MHC_2B isoform was completely absent in the DNV Dia_m at D-14.
and three weeks after DNV, expression of the MHC neo isoform was significantly lower in the DNV Diam compared with corresponding age-matched CTL (P < 0.05; Table 2).

The relative MHC isoform composition of the adult Diam was also altered by DNV (P < 0.05; Table 3). Two and three weeks after DNV, expression of the MHCneo isoform reappeared in the DNV Diam. The relative expression of the MHCslow isoform in the Diam increased 1 and 2 wk after DNV compared with CTL animals (P < 0.05; Table 3). By the third week after DNV, the relative expression of the MHCslow isoform was comparable between DNV and CTL Diam. The relative expression of the MHC2X isoform decreased in the DNV Diam after 1 wk compared with CTL (P < 0.05; Table 3). By the third week after DNV, the relative expression of adult fast MHC (MHC2A, MHC2X and MHC2B) isoforms were comparable between DNV and CTL Diam.

DISCUSSION

The results of the present study support our hypotheses that unilateral DNV 1) alters normal postnatal transitions in MHC isoform expression and 2) causes marked changes in contractile properties of the developing Diam. Although the pattern of DNV-induced alterations in MHC isoform expression were generally consistent with a reduction in Po and a slowing of Vso, there does not appear to be a strong causal relationship between these changes. Unilateral DNV at D-7 did delay the expression of MHC2X and MHC2B isoforms and prolonged the expression of MHCneo, but these alterations were disproportionate to the dramatic slowing of Diam Po, and the relative composition of fast MHC isoforms (r² = 0.06, P > 0.05; Fig. 4A).

In adult animals, DNV was also associated with a slowing of Diam Vso, which did not depend on the duration of DNV (P < 0.05; Fig. 3B). This DNV-related slowing of Diam Vso was not correlated with any change in MHC isoform composition of the muscle (r² = 0.06, P > 0.05; Fig. 4B).

Table 3. Effect of DNV on MHC isoform composition of the adult rat Diam

<table>
<thead>
<tr>
<th>Group</th>
<th>MHCneo</th>
<th>MHCslow</th>
<th>MHC2A</th>
<th>MHC2X</th>
<th>MHC2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTL</td>
<td>0</td>
<td>27.0 ± 0.7</td>
<td>28.6 ± 1.5</td>
<td>32.5 ± 1.3</td>
<td>11.9 ± 2.5</td>
</tr>
<tr>
<td>DNV, 1 wk</td>
<td>0</td>
<td>35.2 ± 1.8*</td>
<td>35.1 ± 1.8*</td>
<td>23.7 ± 0.7*</td>
<td>6.0 ± 2.3*</td>
</tr>
<tr>
<td>DNV, 2 wk</td>
<td>0.6 ± 0.5*</td>
<td>33.7 ± 1.3*</td>
<td>36.9 ± 1.9*</td>
<td>24.8 ± 1.8*</td>
<td>4.0 ± 1.0*</td>
</tr>
<tr>
<td>DNV, 3 wk</td>
<td>7.9 ± 1.9*</td>
<td>25.5 ± 2.0</td>
<td>31.8 ± 1.5</td>
<td>30.5 ± 2.1</td>
<td>4.3 ± 0.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 7–8 for each group). *Significant difference (P < 0.05) from CTL values.
changes in Dia m contractile properties induced by DNV. Thus, although alterations in MHC isoform expression may have contributed in part to the Dia m contractile changes induced by DNV, other factors must be involved.

Postnatal transitions in MHC isoform expression. Previous studies have suggested that innervation is required for the repression of MHCneo gene expression (16, 18, 44). This may account for the reexpression of MHCneo that occurs after DNV in adult skeletal muscle (46). However, other studies have reported that the transition from MHCneo to adult fast MHC isoform expression does not require intact innervation (3, 8, 35, 43). The results of the present study clearly indicate that intact innervation is not absolutely required for the postnatal transition in MHC isoform expression in the rat Dia m. However, DNV at D-7 did prolong the expression of MHCneo in the rat Dia m, which lends support to a possible suppression of MHCneo gene expression by factors emanating from phrenic motoneurons. It is possible that postnatal MHC isoform transitions ultimately depend on preprogrammed fiber phenotype (44) and that changes in the pattern of innervation (e.g., polyneuronal to single motoneuron innervation) or removal of neural influence only modulate the timing of this eventual transition.

Influence of DNV on muscle contractile properties. During early postnatal development of the rat Dia m, there is a progressive increase in P o and V o that reaches adult values by D-28 (29, 51, 58, 64). The results of the present study clearly demonstrate that unilateral DNV causes a marked reduction in Dia m P o and a slowing of V o, even in the youngest animals (D-14). Furthermore, the subsequent developmental increase in both P o and V o was completely blunted. These observations extend and confirm previous results in the adult Dia m, in which a dramatic reduction in P o and slowing of V o was observed after unilateral DNV (33, 37, 61, 63).

In the adult Dia m, we found that 2 wk of unilateral Dia m paralysis induced by tetrodotoxin blockade of phrenic nerve action potential propagation also caused changes in P o and V o that were comparable to DNV (37, 63). In contrast, 2 wk of unilateral Dia m paralysis induced by spinal cord hemisection at C 2 resulted in little, if any, change in P o and V o (37). It has been suggested that Dia m adaptations after unilateral DNV result from passive mechanical strain imposed by continued inspiratory-related contractions of the intact contralateral side (27, 53, 60). However, the passive mechanical effects induced by paralysis of the right side of the Dia m after C 2 spinal cord hemisection are entirely comparable to those induced by unilateral DNV and tetrodotoxin nerve blockade. Yet, the morphometric and contractile adaptations of the Dia m were quite different across these models. Furthermore, in another study in the rabbit, our laboratory found that the sternal region of the paralyzed Dia m passively shortened while the midcostal region was passively

![Fig. 2. Correlation between changes in Dia m P o and the relative expression of adult fast myosin heavy chain (MHC) isoforms from D-14 to D-28 (A) and in adults (B). ○, △, ●, ▲, values from CTL animals; ●*, ▲, ▲, values from DNV animals.](image)

![Fig. 3. Effects of DNV on maximum unloaded shortening velocity (V o) of the DM from D-14 to D-28 (A) and in adults (B). Open bars, values (means ± SE; n = 6–8 for each group) from CTL animals; solid bars, values from animals exposed to unilateral DNV for 1–3 wk.](image)
stretched by the continued inspiratory-related activation of the contralateral side. Despite these differences in passive strain between the two Dia_m regions after transection of the contralateral side, the changes of the Dia_m result from the removal of a nerve extract. It is more likely that the DNV-induced contractile changes of the Dia_m result from the removal of a neurotrophic influence. It has been reported that injection of nerve extracts can attenuate DNV-induced atrophy of the rat extensor digitorum longus muscle (9). Similarly, it has been shown that ciliary neurotrophic factor can attenuate DNV-induced atrophy of the same muscle (26). Therefore, it appears that phrenic motoneurons may express a neurotrophic factor that maintains muscle fiber morphometry and possibly contractile properties.

**Relationship between muscle contractile properties and MHC isoform expression.** In single muscle fibers, a number of studies have demonstrated a relationship between MHC isoform expression and fiber contractile properties (2, 12, 20, 21, 39, 40, 45, 49, 50, 55). Based on the results of these studies, it is well accepted that muscle fibers expressing fast MHC isoforms generate greater $V_o$ than fibers expressing MHC_{slow} or MHC_{neo} isoforms. The relationship between MHC isoform expression and $P_o$ of single muscle fibers is more controversial. Some studies have reported no difference in $P_o$ across fibers expressing MHC_{slow} and fast MHC isoforms (19, 36, 57), whereas other studies have reported that fibers expressing fast MHC isoforms generate greater $P_o$ than fibers expressing MHC_{slow} (2, 12, 20, 21, 49, 50).

In mixed skeletal muscles, it has been shown that contractile properties correlate with the relative composition of MHC isoforms (5, 15, 29, 42, 58, 59). In the present study, we found that postnatal changes in $P_o$ and $V_o$ in the CTL Dia_m were correlated with the relative proportion of fast MHC isoforms comprising the muscle. These results are consistent with previous observations in the rat Dia_m (29, 51, 58, 59, 64). The postnatal changes in Dia_m, $P_o$, and $V_o$ were blunted by DNV; yet transitions in MHC isoform expression in the Dia_m still occurred, albeit at a different rate compared with the normal postnatal transitions observed in CTL animals. Thus the DNV-induced changes in Dia_m contractile properties were not correlated with changes in MHC isoform composition.

In several previous studies, it has been reported that experimentally induced changes in muscle contractile properties are consistent with changes in MHC isoform composition, suggesting a cause and effect relationship. For example, after 2 wk of hindlimb suspension in the rat, $V_o$ of the soleus muscle becomes faster, which is consistent with an increase in the relative expression of fast MHC isoforms (15). Similarly, after spinal cord transection at T12-T13, $V_o$ of the cat soleus and medial gastrocnemius muscles becomes faster, consistent with an increase in the relative expression of fast MHC isoforms (42). Conversely, in response to hypothyroidism, there is a slowing of $V_o$ of the rat plantaris muscle and a decrease in the relative expression of fast MHC isoforms (5). In the rat Dia_m, our laboratory also found a dramatic slowing of $V_o$ in response to hypothyroidism but very little change in the relative expression of fast MHC isoforms (24). In the developing rat Dia_m, our laboratory found that hypothyroidism caused a reduction in $P_o$ and a slowing of $V_o$ which was consistent with, but directly proportional to, a small decrease in the relative expression of fast MHC isoforms (51). Thus, although alterations in MHC isoform composition may be consistent with contractile changes, the proportionality of these changes may be completely different. This raises important questions as to the actual cause and effect relationship between the concurrent changes in MHC isoform expression and contractile properties.

There are several alternative mechanisms by which DNV might have affected Dia_m contractile properties. Specific force is dependent on the number of cross bridges in parallel, the recruitment of cross bridges in response to elevated intracellular calcium concentration, and cross-bridge cycling kinetics (10, 56). DNV has been shown to influence protein synthesis (6, 32, 34) and fiber cross-sectional area (23, 37, 60–63). Thus an effect on the number of cross bridges in parallel...
ties. It is also possible that DNV influenced (e.g., MHC protein content per half sarcomere) is a possibility. It is also possible that DNV influenced cross-bridge cycling kinetics. Future studies are needed to elucidate the underlying mechanisms for the DNV-induced alterations in Dia_m contractile properties.

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