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 (P_o) and thereby affects postnatal changes in maximum specific force (P_o) and thereby affects postnatal changes in maximum specific force (P_o) and thereby affects postnatal changes in maximum specific force (P_o) and thereby affects postnatal changes in maximum specific force (P_o). At each age, P_o was reduced and reduced in DNV compared with control (CTL) animals. By D-21 and D-28, relative expression of MHC2X and MHC2B was reduced. 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, P_o was reduced and V_o was slowed by DNV, compared with CTL. In CTL Dia_m, postnatal changes in P_o 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.

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

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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) CTL 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 polyneuronal innervation of the Dia_m (1, 41) and the emergence of MHC_{2x} and MHC_{2h} 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) intramuscularly. In both groups, 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

\[
\text{CSA} = \text{muscle weight (g) / } \left( \frac{1.056 \, \text{g/cm}^3 \cdot L_{o}}{\text{cm}} \right) \]

For measurements of \( V_o \), 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 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 digitalized 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

\[
\text{CSA} = \text{muscle weight (g) / } \left( \frac{1.056 \, \text{g/cm}^3 \cdot L_{o}}{\text{cm}} \right) \]

For measurements of \( V_o \), 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 (Cambridge Technology, model 300B) via a fine glass pipette. This connection provided a noncompliant attachment of the muscle segment to the force transducer and prevented tearing of the central tendon. The slack test was used to determine \( V_o \). (13). In this procedure, muscle length was rapidly shortened in a series of four to six steps, ranging from 5 to 15% of \( L_{o} \), while the muscle was maximally activated at 75 Hz. During the slack in muscle length (dL), force fell to zero, and the time required for force to redevelop (dt) was then used to calculate \( V_o \) (dL/dt; expressed as \( L_{o}/s \)). Because \( dt \) was prolonged at 15°C, this lower bath temperature improved the accuracy of measuring \( V_o \).

**MHC isoform composition**. Myosin was extracted from the muscle segments by scissors mincing in a high-salt solution (in mM): 135 Na, 5 K, 2 Ca, 1 Mg, 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 \( V_o \) using the “slack test”). In both cases, the muscle segments were suspended vertically in glass tissue chambers containing Rees-Simpson solution (pH 7.4) with the following composition (in mM): 135 Na, 5 K, 2 Ca, 1 Mg, 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 \( V_o \) using the “slack test”). In both cases, the muscle segments were suspended vertically in glass tissue chambers containing Rees-Simpson solution (pH 7.4) with the following composition (in mM): 135 Na, 5 K, 2 Ca, 1 Mg, 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 \( V_o \) using the “slack test”). In both cases, the muscle segments were suspended vertically in glass tissue chambers containing Rees-Simpson solution (pH 7.4) with the following composition (in mM): 135 Na, 5 K, 2 Ca, 1 Mg, 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 \( V_o \) using the “slack test”). In both cases, the muscle segments were suspended vertically in glass tissue chambers containing Rees-Simpson solution (pH 7.4) with the following composition (in mM): 135 Na, 5 K, 2 Ca, 1 Mg, 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 iso...
Table 1. Body weights and optimal Dia_m fiber lengths in CTL and DNV rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Body wt, g</th>
<th>L_m, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>29.6±1.3</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>DNV, 1 wk</td>
<td>32.7±1.7</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>D-21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>48.1±1.4</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>DNV, 2 wk</td>
<td>50.1±2.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>D-28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>64.4±3.6</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>DNV, 3 wk</td>
<td>70.7±2.7</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>317.8±15.1</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>DNV, 1 wk</td>
<td>339.3±7.6</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>DNV, 2 wk</td>
<td>342.5±6.2</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>DNV, 3 wk</td>
<td>345.9±3.2</td>
<td>2.0±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 6–7 for each group). Dia_m, diaphragm muscle; L_m, optimal fiber length; CTL, control; DNV, denervation; D-14, D-21, D-28, postnatal days 14, 21, and 28, respectively.

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_m 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_neo 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_neo isoform was completely absent, whereas expression of the MHC_neo isoform persisted in the DNV animals. However, like CTL animals, expression of the MHC_neo 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.

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>MHCIsoform</th>
<th>%total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHC_neo</td>
<td>MHC_slow</td>
</tr>
<tr>
<td>D-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>24.4±0.4</td>
<td>27.3±0.4</td>
</tr>
<tr>
<td>DNV, 1 wk</td>
<td>37.4±2.5*</td>
<td>16.6±2.5*</td>
</tr>
<tr>
<td>D-21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>6.3±0.3</td>
<td>27.0±1.7</td>
</tr>
<tr>
<td>DNV, 2 wk</td>
<td>20.2±1.2*</td>
<td>21.2±1.0*</td>
</tr>
<tr>
<td>D-28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTL</td>
<td>0</td>
<td>25.7±1.9</td>
</tr>
<tr>
<td>DNV, 3 wk</td>
<td>8.1±0.7*</td>
<td>26.8±0.9</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.
Table 3. Effect of DNV on MHC isoform composition of the adult rat Dia m

<table>
<thead>
<tr>
<th>Group</th>
<th>MHC neo</th>
<th>MHC slow</th>
<th>MHC 2A</th>
<th>MHC 2X</th>
<th>MHC 2B</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.

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 Dia m. Although the pattern of DNV-induced alterations in MHC isoform expression were generally consistent with a reduction in P o and a slowing of V o, there does not appear to be a strong causal relationship between these changes. Unilateral DNV at D-7 did delay the expression of MHC 2X and MHC 2B isoforms and prolonged the expression of MHC neo, but these alterations were disproportionate to the dramatic changes in P o and V o, which did not depend on the duration of DNV (P < 0.05; Fig. 3B). This DNV-related slowing of Dia m V o was not correlated with any change in MHC isoform composition of the muscle (r² = 0.06, P > 0.05; Fig. 4B).

DISCUSSION

The relative expression of the MHC2B isoform was significantly lower in the DNV Dia m compared with corresponding age-matched CTL (P < 0.05; Table 2).

The relative MHC isoform composition of the adult Dia m was also altered by DNV (P < 0.05; Table 3). Two and three weeks after DNV, expression of the MHC neo reappeared in the DNV Dia m. The relative expression of the MHC slow isoform in the Dia m 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 MHC slow isoform was comparable between DNV and CTL Dia m. The relative expression of the MHC 2X isoform decreased in the DNV Dia m after 1 and 2 wk compared with CTL (P < 0.05; Table 3). By the third week after DNV, the relative expression of the MHC 2X isoform was comparable between DNV and CTL Dia m. In adult animals, DNV was also associated with a slowing of Dia m V o, which did not depend on the duration of DNV (P < 0.05; Fig. 3B). This DNV-related slowing of Dia m V o was not correlated with any change in MHC isoform composition of the muscle (r² = 0.06, P > 0.05; Fig. 4B).

DNV-induced alterations in Dia m P o and V o. DNV significantly reduced the P o of the developing Dia m at each age (P < 0.05; Fig. 1A). From D-14 to D-28 in CTL animals, P o increased by ∼28% (P < 0.05; Fig. 1A), whereas in the DNV group, P o remained constant at about one-half that of the CTL muscle. In CTL animals, the increase in P o from D-14 to D-28 correlated with an increase in the relative expression of adult fast MHC (MHC 2A, MHC 2X and MHC 2B) isoforms (r² = 0.28; P < 0.05; Fig. 2A). In contrast, in the DNV animals, there was no correlation between the increase in adult fast MHC isoform expression and P o (r² = 0.09, P > 0.05; Fig. 2A).

In the adult Dia m, DNV also resulted in a decrease in P o, which did not depend on the duration of DNV (P < 0.05; Fig. 3B). This DNV-related reduction in Dia m P o was comparable across the three time periods after DNV. There was also no correlation between the DNV-induced reduction in P o and any change in MHC composition of the Dia m (r² = 0.01, P > 0.05; Fig. 2B).

In CTL animals, V o of the Dia m nearly doubled between D-14 and D-28 (P < 0.05; Fig. 3A). In the DNV Dia m, V o was significantly lower than in CTL at each age (P < 0.05; Fig. 3A), and there was no age-related change. The progressive increase in Dia m V o in CTL animals correlated with the increase in relative composition of fast MHC isoforms (r² = 0.76; P < 0.05; Fig. 4A). In DNV animals, there was no correlation between Dia m V o and the relative composition of fast MHC isoforms (r² = 0.06, P > 0.05; Fig. 4A).

Fig. 1. The effects of denervation (DNV) on maximum tetanic force (P o) of the diaphragm muscle (Dia m) for postnatal days 14–28 (D-14 to D-28; A) and in adults (B). Open bars, values (means ± SE; n = 6–8 for each group) from control (CTL) animals; solid bars, values from animals exposed to unilateral DNV for 1–3 wk.
changes in Diám contractile properties induced by DNV. Thus, although alterations in MHC isoform expression may have contributed in part to the Diám 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 MHC\textsubscript{neo} gene expression (16, 18, 44). This may account for the reexpression of MHC\textsubscript{neo} that occurs after DNV in adult skeletal muscle (46). However, other studies have reported that the transition from MHC\textsubscript{neo} 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 Diám. However, DNV at D-7 did prolong the expression of MHC\textsubscript{neo} in the rat Diám, which lends support to a possible suppression of MHC\textsubscript{neo} 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 Diám, 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 Diám $P_o$ 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 Diám, 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 Diám, we found that 2 wk of unilateral Diám 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 Diám paralysis induced by spinal cord hemisection at C\textsubscript{2} resulted in little, if any, change in $P_o$ and $V_o$ (37). It has been suggested that Diám 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 Diám after C\textsubscript{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 Diám were quite different across these models. Furthermore, in another study in the rabbit, our laboratory found that the sternal region of the paralyzed Diám passively shortened while the midcostal region was passively shortened.
stretched by the continued inspiratory-related activation of the contralateral side. Despite these differences in passive strain between the two Dia\textsubscript{m} regions after transection of the contralateral side, the morphometric and contractile adaptations were comparable (61). Based on these combined results, we conclude that the passive mechanical effects of unilateral paralysis per se do not underlie the contractile changes induced by DNV.

It is more likely that the DNV-induced contractile changes of the Dia\textsubscript{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 rat soleus 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 $P_o$ than fibers expressing MHC\textsubscript{slow} or MHC\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{m} (29, 51, 58, 59, 64). The postnatal changes in Dia\textsubscript{m}, $P_o$, and $V_o$ were blunted by DNV; yet transitions in MHC isoform expression in the Dia\textsubscript{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\textsubscript{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 T\textsubscript{12}-T\textsubscript{13}, $V_o$ 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\textsubscript{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\textsubscript{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\textsubscript{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.

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and assistance with some of the studies.

experiments and Dr. Y.S. Prakash for comments on the manuscript

finally, the slowing of

Ca2+
sensitivity of force generation (11, 14, 47). Fi -

tions occurring during the postnatal development of the rat


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毫不可能。它也是可能的DNV 影响了收缩的Ca2+的敏感度，导致了force generation (11, 14, 47)。最后，从V_o变化中可以看到DNV 对收缩的影响。

REFERENCES


