Hypothyroidism alters diaphragm muscle development

GARY C. SIECK, LOUISE E. WILSON, BRUCE D. JOHNSON, AND WEN-ZHI ZHAN

Departments of Anesthesiology and Physiology and Biophysics, Mayo Clinic and Foundation, Rochester, Minnesota 55905

Although controversial, a correlation between muscle fiber phenotype and maximum specific force \( P_o \) has also been reported. For example, in skinned single fibers of the rat Dia, Eddinger and Moss (8) reported that histochemically classified type II fibers, putatively expressing adult fast MHC isoforms, generated \( \sim 1.5 \) times the force of type I fibers, putatively expressing the MHC-slow isoform. In agreement, Bottinelli and colleagues (1, 2) reported that, in rat skeletal muscle, single skinned fibers expressing adult fast MHC isoforms generated a greater \( P_o \) than fibers expressing the MHC-slow isoform. In the cat Dia, Sieck (25) also found that the \( P_o \) of fast-twitch motor units, comprising type II muscle fibers, was greater than that of slow-twitch motor units comprising type I fibers. Recently, in the developing rat Dia, we found a correlation between the relative expression of adult fast MHC isoforms and both the maximum shortening velocity and \( P_o \) of muscle fiber bundles (16). As the relative expression of adult fast MHC isoforms increased with early postnatal development, maximum shortening velocity became faster and \( P_o \) increased. Watchko and Sieck (31) also found a correlation between postnatal changes in Dia fatigue resistance and the relative expression of adult fast MHC isoforms.

The genes controlling MHC expression are responsive to thyroid hormone (15). Accordingly, altered thyroid status can affect the normal developmental transitions in MHC isoform expression (10, 11). For example, it has been reported that hypothyroidism (Hyp) delays the appearance of adult fast native myosin in developing rat Dia (7), but the effect of Hyp on the relative expression of the different individual MHC isoforms is unknown. In hindlimb muscles of the adult rat, Hyp increases the relative expression of the MHC-slow isoform, and there is a corresponding slowing of maximum shortening velocity (6). In the adult rat Dia, we found that, although Hyp induces a small but significant increase in the expression of the MHC-slow isoform, the changes in Dia contractile properties are very pronounced. This led us to conclude that, in the adult rat, Hyp-induced changes in Dia contractile properties cannot be solely attributed to altered MHC isoform composition (12). In developing muscle, Hyp may interact with both the ongoing transitions in MHC isoform expression and the changes in muscle contractile properties. Presently, there is very little information regarding the interactions of Hyp with developmental plasticity of either MHC isoform expression or contractile properties of the Dia.

The purpose of the present study was threefold: 1) to determine the impact of Hyp on the normal postnatal transitions of MHC isoform expression in the rat Dia; 2) to determine the impact of Hyp on the \( P_o \), fatigue...
resistance, and maximum shortening velocity of the developing Dia; and 3) to evaluate the relationships between the Hyp-induced alterations in MHC isoform expression and the changes in contractile properties.

**METHODS**

Pregnant adult Sprague-Dawley rats (10 days gestation) were assigned to either a control (Con) or a Hyp group. Thyroid hormone deficiency was induced by adding 6-n-propyl-2-thiouracil (PTU) to the drinking water (final concentration 0.05%) beginning at 10 days gestation and continuing until the pups were weaned at 21 days of age. Thereafter, the weaned rats were provided with food and water (0.05% PTU in the Hyp group) ad libitum. After parturition, the rats were weighed weekly. The animals were studied at 0, 7, 14, 21, and 28 days of age (days 0–28) because during this initial 4-wk period, there are marked developmental transitions in MHC isoform expression in the rat Dia (16, 18).

At selected ages, animals were anesthetized by an intraperitoneal injection of pentobarbital sodium and killed by exsanguination. The Dia was rapidly removed, and segments from the midcostal region were used for either MHC analysis or the study of contractile properties. Blood samples were analyzed for serum 3,5,3'-triiodothyronine (T3) and thyroxine (T4) levels by radioimmunoassay.

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The methods for separating and identifying different MHC isoforms have been previously described (16, 27). Briefly, segments of Dia (10–30 mg) were scissor minced on ice for 40 min in a high-salt solution (300 mM NaCl, 100 mM NaH2PO4, 50 mM Na2HPO4, 1 mM Na3P04, and 10 mM EDTA) at pH 6.5. The extracts were centrifuged (13,000 × g for 30 min) at 4°C. The supernatant was recovered and diluted 1:10 in 1 mM EDTA and 0.1% 2-mercaptoethanol and stored overnight at 4°C to allow precipitation of the myosin filaments. The sample was centrifuged (13,000 g for 30 min) at 4°C to form a myosin pellet. The supernatant was discarded, and the myosin pellet was dissolved directly in SDS sample buffer (at a final dilution of 1:200). The denatured sample was further diluted 1:200 with SDS sample buffer before it was loaded onto gels. Gel preparation was based on a modification of the procedure by Sugiuara and Murakami (28). A 3.5% acrylamide concentration (pH 6.8) was used in the stacking gel, whereas 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. (20). After staining, the gels were imaged on a computerized image-processing system, and the relative expression of different MHC isoforms was quantified densitometrically. Figure 1 shows an example of the electrophoretic separation of different MHC isoforms in the Dia at different ages.

Isometric and isotonic contractile measurements. The methods for determining isometric and isotonic contractile properties of the Dia have been previously described (16). Briefly, muscle segments (one for isometric and one for isotonic properties) were mounted in glass tissue chambers containing mammalian Ringer solution aerated with 95% O2 and 5% CO2 and maintained at either 26 or 15°C. The PO2 (~430 Torr), PCO2 (~38 Torr), and pH (~7.40) of the Ringer solution were periodically monitored throughout the experiment. D-Tubocurarine (0.012 mM) was added to the bath to prevent activation of intramuscular nerve fibers. The costal margin origin of the fibers was fixed with a surgical clamp mounted in series with a micropositioner near the base of the tissue chamber. A small piece of aluminum foil was glued to the central tendon and then attached to a force transducer (model 300B, Cambridge Technology) via a fine wire (isometric) or a glass pipette (isotonic). These connections provided a noncompliant attachment of the muscle bundle to the force transducer and prevented tearing of the central tendon. The muscle bundles were stimulated directly (Grass model S-88 stimulator and current amplifier) with rectangular current pulses (1.0-ms duration) delivered through platinum plate electrodes placed on either side of the muscle (~1 cm apart). To assure supramaximal stimulation, the current was increased by 25% over the current necessary to obtain peak twitch force responses (250–300 mA). Muscle fiber length was adjusted incrementally with a micropositioner until maximal

**Fig. 1.** Myosin heavy chain (MHC) isoform composition of diaphragm (Dia) was determined by densitometric analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. Representative gels for control (Con) and hypothyroid (Hyp) animals at different ages are shown. D0, D7, D14, D21, and D28, days 0, 7, 14, 21, and 28 of age, respectively; MHC-neo, neonatal isoform of MHC.
isometric twitch force responses were obtained (i.e., optimal fiber length \(L_o\)). \(L_o\) was then measured with digital calipers.

Isometric contractile properties were measured at 26°C. Muscle fiber bundles were stimulated at varying frequencies ranging from 1 to 100 Hz, delivered in 1-s-duration trains, to determine \(P_o\). Fatigue resistance of the muscle fiber bundle was evaluated with repetitive stimulation at 40 Hz in 330-ms-duration trains repeated each second for a 2-min period. A fatigue index (FI) was calculated as the ratio of the residual force after 2 min to the initial force.

Isotonic contractile properties were measured at 15°C in a separate muscle fiber bundle from each animal. Maximum unloaded shortening velocity \(V_o\) was determined with the “slack” test in which 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. Due to the rapid length change (dL), the muscle bundle was unloaded, force fell to zero, and the muscle shortened at maximum velocity as the slack in the muscle was taken up. The time required for force to redevelop (dt) was then used to calculate \(V_o\) (dL/dt). \(V_o\) was normalized for \(L_o\) and expressed as the relative change in optimal muscle length per second (in ml/s). The lower bath temperature (i.e., 15 vs. 26°C) was used because of improved accuracy in measuring \(V_o\).

The stimulation paradigm and imposed length changes were controlled by a computer program. Force and length signals of the Cambridge dual-mode servo-control module were digitized at 1,000 Hz and stored on a computer disk file. After measurement of \(P_o\) and \(V_o\), the stimulated muscle segments were weighed, and CSA was estimated based on the following formula: CSA = muscle weight (in g)/(\(L_o\) (in cm)-density (1.056 g/cm³)). The estimated CSA of the fiber bundle was then used to determine specific force (i.e., force/CSA) of the muscle.

Statistics. All data are reported as means ± SE. A two-way analysis of variance was used to evaluate the data, with age and experimental condition as grouping variables. When appropriate, an unpaired t-test was used as a post hoc analysis to compare Con and Hyp groups. Correlations between postnatal changes in the relative expression of all adult fast MHC isoforms (percentage of total MHC expression) and changes in \(P_o\), F1, and \(V_o\) were calculated. These correlations are reported as \(r^2\) values instead of \(r\) values because both positive and negative correlations were observed. In addition, multiple stepwise linear regression was used to determine the contribution of each MHC isoform to the correlations between postnatal MHC isoform transitions and changes in contractile properties. Statistical significance of group differences and regressions were tested at \(P < 0.05\).

RESULTS

In the pups treated with PTU, Hyp was confirmed by the marked reduction in serum T3 and T4 levels, which were below detectable levels of the assay in the Hyp group (Table 1). In the Con animals, serum T3 levels progressively increased with postnatal development \((P < 0.05\); Table 1), whereas T4 levels peaked on day 14. From day 14 onward, the body weights of Hyp animals were significantly lower than those of Con animals \((P < 0.05\); Table 2). In the Hyp group, body weights stabilized on day 14 with no subsequent growth (Table 2). This compared with the rapid growth of Con animals that doubled their body weights during this 2-wk period \((P < 0.05\); Table 2).

### Table 1. Age-related changes in serum T3 and T4 levels in Con and Hyp rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Con T3</th>
<th>Con T4</th>
<th>Hyp T3</th>
<th>Hyp T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>&lt;15</td>
<td>3.0 ± 0.1</td>
<td>&lt;15</td>
<td>1</td>
</tr>
<tr>
<td>Day 7</td>
<td>32.5 ± 2.0</td>
<td>4.7 ± 0.2</td>
<td>&lt;15</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Day 14</td>
<td>35.2 ± 5.3</td>
<td>6.5 ± 0.4</td>
<td>&lt;15</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Day 21</td>
<td>70.1 ± 4.6</td>
<td>3.9 ± 0.1</td>
<td>&lt;15</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Day 28</td>
<td>90.8 ± 3.7</td>
<td>5.4 ± 0.1</td>
<td>&lt;15</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Values are means ± SE in ng/dl for 3,5,3′-triiodothyronine (T3) and in µg/dl for thyroxine (T4). Con, control; Hyp, hypothyroid. *Significant age-related difference.

Postnatal MHC isoform transitions. The MHC-neo isoform comprised ~69% of all MHC isoforms expressed in the Con rat Dia at birth but completely disappeared by day 28 (Table 3). During the same period, there was an increase in the relative expression of the MHC-slow isoform from ~11 to ~28% and of the MHC-2A isoform from ~23 to ~35% \((P < 0.05\); Table 3). From day 0 to day 7 in Con animals, there was an abrupt decrease in the expression of the MHC-neo isoform with a concomitant increase in MHC-2A isoform expression \((P < 0.05\); Table 3). Thereafter, the expression of the MHC-neo isoform continued to decrease rapidly, but the expression of the MHC-2A isoform remained relatively stable. Between day 7 and day 14, the decrease in MHC-neo isoform expression was matched by an increase in the expression of the MHC-slow isoform \((P < 0.05\); Table 3), which stabilized after day 14. Between day 14 and day 28, the disappearance of the MHC-neo isoform was primarily matched by an increase in MHC-2X isoform expression, which initially appeared at day 14 and continued to increase until day 28 \((P < 0.05\); Table 3). Expression of the MHC-2B isoform did not emerge until day 21, and thereafter, the relative expression of the MHC-2B isoform remained relatively low (Table 3). As a result of these developmental transitions in MHC isoform expression, the relative expression of adult fast MHC isoforms in Con animals increased from ~23% on day 0 to ~72% on day 28 \((P < 0.05\).

The normal developmental transition of MHC isoform expression in the rat Dia was markedly altered in the Hyp group (Table 3). On days 0 and 7, the relative expression of the MHC-neo isoform did not differ between the Con and Hyp animals, but there were differences in the expression of the MHC-slow and MHC-2A isoforms. At both ages, the expression of the

### Table 2. Age-related changes in body weights of Con and Hyp rats

<table>
<thead>
<tr>
<th>Age</th>
<th>Con</th>
<th>Hyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>6.4 ± 0.2</td>
<td>6.1 ± 0.5</td>
</tr>
<tr>
<td>Day 7</td>
<td>15.5 ± 0.2</td>
<td>15.3 ± 0.3</td>
</tr>
<tr>
<td>Day 14</td>
<td>29.6 ± 0.5</td>
<td>21.5 ± 0.2</td>
</tr>
<tr>
<td>Day 21</td>
<td>48.1 ± 0.5</td>
<td>21.3 ± 0.8</td>
</tr>
<tr>
<td>Day 28</td>
<td>64.4 ± 1.4</td>
<td>23.7 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE in g. *Significant difference from Con.
MHC-slow isoform was greater in the Hyp animals compared with the Con animals (P < 0.05; Table 3), whereas the expression of the MHC-2A isoform was lower (P < 0.05; Table 3). This difference in the relative expression of the MHC-slow and MHC-2A isoforms progressively widened with age (P < 0.05; Table 3). The relative expression of the MHC-neo isoform was significantly greater on days 21 and 28 compared with the Con animals (P < 0.05; Table 3). The expression of the MHC-2X and MHC-2B isoforms was completely inhibited in the Hyp animals (Table 3). As a result of the effect of Hyp, the relative expression of adult fast MHC isoforms did not change (from ~15% on day 0 to ~18% on day 28).

Isometric and isotonic contractile properties. From day 0 to day 28 in Con animals, Po increased by approximately twofold (from ~8.4 to ~16.4 N/cm²; P < 0.05; Fig. 2). In Hyp animals, Po also increased from day 0 to day 14 (P < 0.05), but after day 14, Po declined (P < 0.05; Fig. 2). At each age, the Po generated by the Hyp Dia was much lower than that generated by the Con Dia (P < 0.05; Fig. 2). In Con animals, the increase in Po during early postnatal development correlated with an increase in the relative expression of adult fast MHC isoforms (r² = 0.50; P < 0.05; Fig. 3). In contrast, in the Hyp animals, there was no correlation between adult fast MHC isoform expression and the postnatal increase in Po (r² = 0.007; Fig. 3). Stepwise linear regression indicated that, in Con animals, the postnatal decrease in MHC-neo isoform expression provided the strongest correlation with the postnatal increase in Po (MHC-neo, r² = 0.61; P < 0.05), although the emergence of the MHC-2B isoform also contributed significantly (MHC-neo + MHC-2B, r² = 0.68; P < 0.05). Postnatal changes in the expression of other MHC isoforms did not further contribute individually to the overall correlation between MHC isoform expres-

Table 3. Age-related changes in relative expression of different MHC isoforms in Con and Hyp rats

<table>
<thead>
<tr>
<th>Age</th>
<th>MHC-slow</th>
<th>MHC-neo</th>
<th>MHC-2A</th>
<th>MHC-2X</th>
<th>MHC-2B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>11.0 ± 0.6</td>
<td>65.9 ± 1.6</td>
<td>23.2 ± 2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 7</td>
<td>13.0 ± 2.2</td>
<td>51.0 ± 1.5</td>
<td>60.0 ± 0.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 14</td>
<td>25.2 ± 1.6</td>
<td>51.4 ± 1.2</td>
<td>43.7 ± 4.8</td>
<td>10.9 ± 0.7</td>
<td>0</td>
</tr>
<tr>
<td>Day 21</td>
<td>21.1 ± 1.7</td>
<td>8.4 ± 0.3</td>
<td>22.7 ± 1.2</td>
<td>21.8 ± 2.2</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>Day 28</td>
<td>28.2 ± 1.9</td>
<td>0</td>
<td>21.5 ± 1.8</td>
<td>31.1 ± 0.9</td>
<td>3.5 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SE in percentage of total myosin heavy chain (MHC) isoforms. MHC-neo, neonatal isoform of MHC. *Significant difference from Con.

Fig. 2. Maximum specific force (Po) increased progressively during early postnatal development in both Con (open bars) and Hyp (solid bars) animals. However, at each age, Po generated by Hyp Dia was significantly less than that of Con animals. Values are means ± SE. *Significantly different from Con animals.

Fig. 3. In Con animals (○), progressive increase in Po during early postnatal development correlated with progressive increase in relative expression of adult fast MHC isoforms. In Hyp animals (●), no such correlation existed. Values are means ± SE.
HYPOTHYROIDISM ALTERS MHC EXPRESSION

**DISCUSSION**

The results of the present study indicate that Hyp markedly alters the normal postnatal transitions in

![Fatigue Index](image1)

**Fig. 4.** Fatigue resistance (fatigue index) of Dia declined progressively during early postnatal development in Con animals (open bars). In contrast, Dia fatigue resistance remained high throughout early postnatal development in Hyp animals (solid bars). Values are means ± SE. *Significantly different from Con animals.

![% Fast MHC](image2)

**Fig. 5.** In Con animals (○), progressive decline in Dia fatigue resistance during early postnatal development correlated with progressive increase in relative expression of adult fast MHC isoforms. In Hyp animals (●), no such correlation existed. Values are means ± SE.

![Vo](image3)

**Fig. 6.** Maximum unloaded shortening velocity (V₀) of Dia increased progressively during early postnatal development in both Con (open bars) and Hyp animals (solid bars). However, at each age, V₀ of Hyp Dia was significantly less than that of Con animals. Values are means ± SE. *Significantly different from Con animals.

In Con animals, the postnatal decrease in MHC-neo isoform expression provided the strongest correlation with the postnatal decrease in the FI (MHC-neo, \( r^2 = 0.64; P < 0.05 \)). Postnatal changes in the expression of other MHC isoforms did not further contribute individually to the overall correlation between MHC isoform expression and FI. In the Hyp animals, there were no individual changes in MHC isoform expression that correlated with the postnatal changes in the FI.

In Con animals, the Dia FI decreased progressively from day 0 to day 28 (\( P < 0.05 \); Fig. 4). In contrast, in Hyp animals, the FI declined from day 0 to day 14 (\( P < 0.05 \)) but then increased thereafter (\( P < 0.05 \); Fig. 4). In Con animals, there was a correlation between the developmental decrease in the Dia FI and the age-related increase in the relative expression of adult fast MHC isoforms (\( r^2 = 0.59; P < 0.05 \); Fig. 5). In Hyp animals, no such relationship existed between the FI and the relative expression of adult fast MHC isoforms (\( r^2 = 0.01 \); Fig. 5). Stepwise linear regression indicated that, in Con animals, the postnatal decrease in MHC-neo isoform expression provided the strongest correlation with the postnatal decrease in the FI (MHC-neo, \( r^2 = 0.64; P < 0.05 \)). Postnatal changes in the expression of other MHC isoforms did not further contribute individually to the overall correlation between MHC isoform expression and FI. In the Hyp animals, there were no individual changes in MHC isoform expression that correlated with the postnatal changes in the FI.

In Con animals, the V₀ of the Dia increased nearly fourfold between day 0 and day 28 (\( P < 0.05 \); Fig. 6). In Hyp animals, the V₀ also increased with age (\( P < 0.05 \)), but the age-related change in V₀ was only 2.5-fold (Fig. 6). Therefore, while V₀ of the Hyp Dia was much slower than that of the Con muscle at each age, this difference became more pronounced with age (\( P < 0.05 \); Fig. 6). In Con animals, the progressive increase in Dia V₀ with early postnatal development correlated with the increase in the relative expression of adult fast MHC isoforms (\( r^2 = 0.79; P < 0.05 \); Fig. 7). In Hyp animals, there was no correlation between the postnatal increase in Dia V₀ and the relative expression of adult fast MHC isoforms (\( r^2 = 0.002; P < 0.05 \)). Stepwise linear regression indicated that, in Con animals, the postnatal decrease in MHC-neo isoform expression provided the strongest correlation with the postnatal increase in V₀ (\( r^2 = 0.75; P < 0.05 \)). Postnatal changes in the expression of other MHC isoforms did not contribute individually to the overall correlation between MHC isoform expression and V₀. In the Hyp Dia, the progressive increase in V₀ correlated only with the decrease in MHC-neo expression (MHC-neo, \( r^2 = 0.58; P < 0.05 \)).
MHC isoform expression in the rat Dia. Expression of adult fast MHC isoforms was inhibited by Hyp, whereas MHC-slow isoform expression was increased and disappearance of MHC-neo expression was delayed. In normal Con animals, the $P_o$ and $V_o$ increased progressively during the first 4 wk of life, whereas the $F_l$ progressively declined. In Hyp animals, the $P_o$ and $F_l$ displayed no consistent change with postnatal development, whereas the $V_o$ increased progressively, albeit at a slower rate, compared with Con animals. In Hyp animals, the Dia $P_o$ and $V_o$ were significantly lower than those in Con animals at each age, whereas the $F_l$ remained higher. In Con animals, postnatal changes in the $P_o$, $F_l$, and $V_o$ correlated with transitions in MHC isoform expression, but this was not generally the case in Hyp animals. In Con animals, the progressive decrease and eventual disappearance of the MHC-neo isoform provided the strongest predictive correlations for postnatal changes in both the Dia $P_o$ and $F_l$. Such relationships were not observed in Hyp animals where MHC-neo isoform expression persisted until day 28. However, in Hyp animals, the gradual decline in MHC-neo expression, albeit attenuated in comparison with Con animals, contributed to the progressive increase in $V_o$. We conclude that Hyp markedly affects the postnatal transitions in MHC isoform expression in the Dia and that these alterations in MHC isoform expression contribute, at least in part, to the dramatic changes in contractile properties that also occur with Hyp. However, the altered MHC isoform expression in the Hyp Dia provides no predictive power for the changes in contractile properties that occur.

In a recent study in the adult rat Dia, Gosselin et al. (12) found that a 3-wk exposure to Hyp caused only a slight increase in MHC-slow isoform expression but caused a marked reduction in both the $P_o$ and $V_o$. In the adult, they concluded that, although alterations in MHC isoform expression do occur with Hyp, these alterations could not completely account for the changes in contractile properties induced by Hyp. These results contrasted with those of Caiazzo et al. (6), who found that a 20-wk exposure to Hyp induced alterations in MHC isoform expression in the rat plantaris and soleus muscles that they concluded fully accounted for the decrease in $P_o$ and $V_o$ that were also observed. As in the present study, these authors reported that Hyp appeared to suppress the expression of adult fast MHC isoforms in both muscles while increasing the relative expression of the MHC-slow isoform. It is possible that the apparent discrepancies between the effects of Hyp on the adult rat Dia that we observed and those reported by Caiazzo et al. for the plantaris and soleus muscles might relate to the duration of exposure to Hyp. In the present study, the developing rats were exposed to Hyp throughout the embryological and early postnatal periods of muscle differentiation. Accordingly, compared with adults, the effects of Hyp on MHC isoform expression were much more pronounced in the developing rats. However, the effects of Hyp on Dia contractile properties were comparable between adults and younger animals (12).

In the present study, Hyp was associated with a significant reduction in body weight beginning on day 14, similar to a previous report (11). It is likely that the protein synthesis rate was reduced in the Hyp animals and that this might have affected the $P_o$ by a reduction in myofibrillar density. In the present study, it was not possible to use weight-matched untreated rats as control animals because a decrease in caloric intake itself alters thyroid status in neonatal rats and induces changes in MHC isoform expression (3).

The genes controlling myosin expression are known to respond to thyroid hormone but in a muscle-specific manner (19). For example, it has been previously reported that in the rat the transition from MHC-neo to fast MHC isoform expression is delayed in the extensor digitorum longus (EDL) muscle (11), but the transition from MHC-neo to MHC-slow isoform expression in the soleus muscle occurs more rapidly (4, 11). The EDL and soleus muscles in the rat are predominantly composed of type IIb (MHC-2B) and type I (MHC-slow) fibers, respectively, and the effects of Hyp on genetic control of MHC isoform expression in these muscles may not reflect that occurring in a mixed muscle such as the Dia. However, as in the EDL, we found that the early postnatal transition between MHC-neo and adult fast MHC isoform expression was inhibited by Hyp while the transition from MHC-neo to MHC-slow was promoted. These results are in general agreement with previous work (7, 19), where it was found that Hyp delays the appearance of adult fast isoforms in the developing rat Dia. However, in these previous studies, the developmental transitions in the expression of individual MHC isoforms were not directly examined.

Changes in thyroid hormone levels may play a role in the normal sequential transition of MHC isoform expression in the rat Dia because, in Con rats, $T_4$ serum levels peaked around the same time as the rapid transition...
from MHC-neo to adult fast MHC isoforms. This possible correlation between serum T3 levels and developmental myosin transitions was also observed in hind-limb muscles of the rat (11). It should be noted, however, that the serum T3 levels progressively increased with postnatal development. T3 has a more potent effect on target cells than T4, and elevated T3 levels are associated with increased energy requirements and rapid growth. It is possible that peripheral conversion of T4 to T3 is required to support the rapid transitions of MHC isoforms. Accordingly, the divergence ofDia contractile properties between Hyp and Con animals became more pronounced after day 14.

The mechanism(s) by which thyroid hormone influences the developmental transitions in MHC isoform expression is unknown. Alterations in the activation of the myogenic helix-loop-helix transcription factors Myo-D and myogenin may occur (13). The changing innervation patterns during early postnatal development may also contribute to the postnatal transitions in MHC isoform expression. During the first 2 postnatal wk in the rat, innervation of the Dia transforms from polyneuronal innervation, where a muscle fiber may be innervated by more than one motoneuron, to the adult pattern of innervation, where each muscle fiber is innervated by only one motoneuron (26).

In summary, Hyp markedly altered the early postnatal transitions in MHC isoform expression in the rat Dia, resulting in a marked increase in MHC-slow isoform expression, a decrease in the expression of adult fast MHC isoforms, and a persistent expression of the MHC-neo isoform. Hyp also dramatically affected Dia contractile properties, causing a substantial reduction in both the P0 and Vp. It is likely that the Hyp-induced alterations in MHC isoform expression during early postnatal development contributed, at least in part, to the dramatic changes in Dia contractile properties.

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Address for reprint requests: G. C. Sieck, Anesthesia Research, Mayo Clinic, 200 First St. SW, Rochester, MN 55905 (E-mail: gcs@Siecklab.Mayo.edu).

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