Maternal dexamethasone treatment alters myosin isoform expression and contractile dynamics in fetal arteries

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The National Institutes of Health Consensus conference on the effects of corticosteroids for fetal maturation has recommended the use of antenatal steroids for women at risk for premature delivery (29). Unfortunately, many human preterm deliveries occur in the second trimester shortly after fetal exposure to maternal glucocorticoid treatment. Although randomized controlled trials support the use of antenatal steroids (7), the use of repeated courses of maternally administered antenatal steroids has not been adequately studied (3, 14). Nevertheless, it has become relatively common practice to treat women who are at continued risk for premature delivery with repeated weekly courses of antenatal steroids (3, 14).

Much of the information on the modulation of fetal development by glucocorticoid has been derived from the sheep model (29, 30). Derks et al. (8) found that infusion of betamethasone into fetal sheep increased blood pressure and femoral vascular resistance in ovine fetuses. Docherty et al. (9) found that infusion of dexamethasone into fetal sheep increased the endothelin ETα subtype receptors and contractility in fetal femoral arteries but decreased ETα receptors in fetal cerebral arteries. In contrast, Hegarty et al. (17) found that infusion of cortisol into fetal sheep had no effect on the density or affinity of angiotensin receptors or contractility of fetal carotid arteries. Anwar et al. (1) studied the effect of fetal glucocorticoid infusion on the contractility of fetal ovine femoral arteries and observed enhanced sensitivity to K+ depolarization but no difference in sensitivity to norepinephrine. To our knowledge, no studies have been published on the modulation of contractile protein expression in fetal ovine arteries by glucocorticoids.

Actin and myosin are the major contractile proteins in vascular smooth muscle. Smooth muscle myosin is a hexamer consisting of a pair of ~200-kDa heavy chains, a pair of 20-kDa regulatory light chains, and a pair of 17-kDa essential light chains. Unlike striated muscle, smooth muscle cells do not contain troponin, and phosphorylation of the 20-kDa regulatory myosin

MATERNALLY ADMINISTERED antenatal glucocorticoids have been widely used for the prevention of respiratory distress syndrome in low-birth-weight infants (7). This therapy has also been shown to facilitate the transition from fetal to neonatal life by beneficial effects on multiple organ systems in animal studies (4, 32, 34). However, recent evidence also suggests that prenatal exposure to glucocorticoids could trigger “fetal programming,” thereby predisposing the fetus later in adult life to diseases such as diabetes and hypertension (10, 22, 30).

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light chain is the predominant regulatory mechanism of contraction (15). The 17-kDa myosin light chains are not phosphorylatable but could potentially modulate the cycling of phosphorylated myosin cross-bridges by acting on the neck region of the myosin molecule (35).

Multiple isoforms of myosin heavy and light chains (LC) exist in various smooth muscle types (12, 27), and the isoform composition changes during embryonic development and in disease (36). The acidic (myosin LC17a) and basic (myosin LC17b) isoforms of the 17-kDa myosin light chain are products of alternative splicing of a single gene (28). Tissue-specific differential expression of the myosin LC17a and LC17b isoforms has been demonstrated in various smooth muscle types and found to correlate with shortening velocity and myosin ATPase activity in these tissues (18, 27). For example, the myosin LC17a isoform is the only isoform expressed in the phasic gastrointestinal smooth muscle. In contrast, both myosin LC17a isoform and LC17b isoform are expressed in the tonic arterial smooth muscle. Huang et al. (20) have shown that forced expression of myosin LC17 isoforms in single embryonic aortic and gizzard smooth muscle cells by transfection resulted in changes in the rate of force development. Arens et al. (2) observed developmental changes in the relative expression of the 200- and 204-kDa myosin heavy chain isoforms in fetal ovine aorta but could not measure contraction because ovine aorta does not develop contractility before birth. Ogut and Brozovich (31) have studied the developmental changes in the expression of the acidic (myosin LC17a) and basic (myosin LC17b) isoforms in phasic and tonic smooth muscles in the chick embryo. They found that expression of the myosin LC17a correlated with rate of force development in phasic smooth muscle during embryonic development. Therefore, myosin LC17 isoform expression appears to be a useful marker of smooth muscle development and growth.

Contractile protein isoform expression is known to be developmentally regulated in vascular smooth muscle (36). However, it is not known whether glucocorticoid modulates contractile protein isoform expression in vascular smooth muscle cells in the fetus. Studying time course of contraction is important because extracellular and intracellular sources of Ca^{2+} are utilized at different times during vascular smooth muscle contraction. Initial force development is activated by Ca^{2+} released from the sarcoplasmic reticulum, whereas steady-state force is maintained by Ca^{2+} influx through calcium channels on the cell membrane (19). Therefore, differential changes in early or late time of a contraction would suggest different mechanisms. Given the above considerations, in this study, we tested the hypothesis that maternal glucocorticoid treatment modulates myosin LC17 expression and the contractile time course in fetal ovine carotid arteries.

**METHODS**

**Animal preparation.** This study was conducted after approval by the Institutional Animal Care and Use Committees of Brown University and Women and Infants Hospital of Rhode Island and according to the National Institutes of Health Guidelines for use of experimental animals. Surgery was performed under 1–2% halothane anesthesia on 11 timedated Eastern mixed breed pregnant ewes at 99–101 days of gestation as previously described in detail (37, 38). Singleton and twin pregnancies were included; however, when a twin gestation was present, only one fetus was catheterized, although the carotid arteries were obtained from both fetuses. The thoracic aorta was cannulated via the brachial artery for blood sample withdrawal. An amniotic fluid catheter was placed for pressure monitoring and to correct fetal arterial blood pressures. The fetuses were catheterized at 99–101 days of gestation after the ewes had received four courses of dexamethasone or placebo. The ewes were randomly assigned to one of four treatment groups: 1) single course dexamethasone or placebo, 2) five repeated courses of dexamethasone or placebo. The ewes were given a 6-mg intramuscular injection of dexamethasone (concentration = 4 mg/ml; 1.5 ml was given to each ewe for a total of 6 mg at each injection) at 12 h for 48 h starting on day 106 in the single course groups and the same dose was given on 76, 84, 91, 98, and 105 days of gestation in the repeated course groups. The rationale for choosing this treatment regime (i.e., 6 mg dexamethasone every 12 h for 48 h) was that the dose and treatment schedule of dexamethasone used in our study was similar to that currently recommended for fetal maturation in pregnant women with premature labor (29) and that it has become relatively common practice to treat women who are at continued risk for premature delivery with repeated weekly courses of antenatal steroids (3, 14). Dexamethasone was chosen for use in our studies, because it is one of the most extensively studied corticosteroids for accelerating fetal maturation, has been widely used in experimental studies of the central nervous system, and is also used to treat central nervous system disorders (29).

The plasma samples and carotid arteries for this study were obtained from animals enrolled in a larger series of studies to examine the effects of antenatal corticosteroids on the blood-brain barrier function in ovine fetuses (39). The studies on the carotid arteries had to be performed within 24 h after euthanasia of the ewes. Therefore, it was not always feasible to perform the studies on the carotid arteries from all of the fetuses in the original study (39). Therefore, in the present study, we only present contractile data from a few studies on the fetuses of ewes exposed to the single course of dexamethasone or placebo and present contractile data and contractile protein isoform expression from fetuses of ewes exposed to the repeated courses of glucocorticoids or placebo.

**Experimental protocol and methodology.** On days 106–108 of gestation, 18 h after the last dose of dexamethasone or placebo had been given to the ewes, fetal arterial samples were obtained while the ewes were standing quietly in a cart. After arterial samples for pH, blood gases, plasma osmolality, glucose, lactate, insulin, and cortisol concentrations and heart rate and mean arterial had been measured on the fetuses, the ewe was given intravenous pentobarbital (15–20 mg/kg) to achieve a surgical plane of anaesthesia. The fetuses were removed and weighed, and the carotid arteries were carefully dissected.

Heart rates, mean arterial blood, and amniotic fluid pressures in fetal sheep were measured with pressure transducers (model 1280 C; Hewlett-Packard; Lexington, MA) and recorded on a polygraph (model 17758 B Series, Hewlett-
Packard). Blood gases and pH were measured on a Corning blood gas analyzer (model 238, Corning Scientific; Medford, MA) at the temperature of fetal sheep (39.5°C). Plasma osmolality was measured in duplicate on a vapor pressure osmometer (Vapro model 5520, Wescon; Logan, UT) and glucose on a glucose/lactate analyzer (YSI 2300, STAT; Yellow Springs, OH). Insulin concentrations were measured in duplicate using Clinical Assays J GammaCoat J Cortisol 125I-radioimmunoassay (DPC; Los Angeles, CA). The Coat-A-Count Insulin antiserum exhibits 100% cross reactivity with insulin. The observed coefficients of variation for inter- and intra-assay precision were 6.1 and 6.3%, respectively. Cortisol concentrations were measured in duplicate using the Coat-A-Count Insulin, a solid-phase 125I-radioimmunoassay (DiaSorin; Stillwater, MN). The GammaCoat J antiserum exhibits 100% cross reactivity with cortisol. The observed coefficient of variation for inter- and intra-assay precision was 10.1 and 7.9%, respectively.

Contraction studies. Carotid arteries together with the vagus nerve were carefully dissected from the fetus with minimal stretching and then stored in cold (4°C) physiological salt solution (PSS) containing (in mM) 140.1 NaCl, 4.7 KCl, 1.2 Na2HPO4, 2.0 MOPS (pH 7.4), 0.02 Na2EDTA, 1.2 MgSO4, 1.6 CaCl2, and 5.6 d-glucose. Connective tissue and the vagus nerve were carefully removed by using microdissecting scissors under a dissecting microscope. Carotid arteries were then cut into 8-mm segments. Two stainless steel wire clamps were placed inside the lumen of each segment. One wire clamp was connected to a force transducer (Grass FT.03), and the other wire clamp was secured on a glass rod with Teflon tape. One wire clamp was placed inside the lumen of each segment. Arterial segments were then slowly thawed to room temperature, resulting in the dehydration of the tissue. Accordingly, the dehydration of the tissue was based on the difference in isoelectric pH for the two proteins. Myosin LC17a has an isoelectric pH of 4.19. Tissue homogenate was transferred to a slab gel for SDS-PAGE to separate the 17-kDa myosin light chains from other proteins by molecular weight. The myosin LC17a percentage was calculated from the ratio of LC17a/(LC17a + LC17b).

Statistical analysis. All results were expressed as means ± SE. Student’s t-test was used for the comparison of two means (Table 1 and Fig. 2B). Repeated measures ANOVA for two factors was used to compare serial measurements over time in the fetuses of the dexamethasone- and placebo-treated ewes (Fig. 1). When a significant interaction was present by ANOVA, the Newman-Keuls post hoc test. The least-squares regression analysis was also used (Fig. 2A). One-way ANOVA was used to compare the myosin LC17a percent among the fetuses of the dexamethasone- and placebo-treated ewes and the ewes (Fig. 3). If a significant difference was found by ANOVA, the Newman-Keuls post hoc test was used to identify specific differences among the groups. P < 0.05 was considered statistically significant.

RESULTS

Physiological profiles of the fetuses of ewes exposed to repeated courses of dexamethasone and placebo. The fetuses of the ewes exposed to repeated courses of dexamethasone weighed 17% less than those of the placebo-treated ewes (Table 1). Plasma osmolality, pH, P02, Pco2, insulin concentration, and lactate concentration did not differ between the fetuses of the ewes exposed to repeated courses of dexamethasone and placebo. The average plasma glucose concentration was 43% higher, and heart rate was 18% higher in fetuses of the ewes exposed to repeated courses of dexamethasone than placebo. Mean arterial pressure did not differ between the groups of fetuses. Although fetal plasma dexamethasone concentrations were not measured in this study, plasma cortisol concentrations were significantly lower in the ewes treated with repeated courses of dexamethasone (5.6 ± 0.2 ng/ml).

Table 1. Physiological profiles of fetuses from ewes treated with placebo or dexamethasone

<table>
<thead>
<tr>
<th>Measure</th>
<th>Placebo</th>
<th>Dexamethasone</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>1.43 ± 0.05</td>
<td>1.18 ± 0.05</td>
<td>0.01*</td>
</tr>
<tr>
<td>Plasma osmolality,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mosmol/kg</td>
<td>296 ± 1</td>
<td>296 ± 1</td>
<td>0.52</td>
</tr>
<tr>
<td>Plasma pH</td>
<td>7.36 ± 0.01</td>
<td>7.31 ± 0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>Plasma Po2, mmHg</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
<td>0.22</td>
</tr>
<tr>
<td>Plasma Pco2, mmHg</td>
<td>47 ± 1</td>
<td>44 ± 1</td>
<td>0.06</td>
</tr>
<tr>
<td>Plasma lactate, mg/dl</td>
<td>8.29 ± 0.78</td>
<td>9.93 ± 1.49</td>
<td>0.36</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>28.5 ± 2.4</td>
<td>41.1 ± 4.2</td>
<td>0.03*</td>
</tr>
<tr>
<td>Plasma cortisol, ng/ml</td>
<td>6.99 ± 0.56</td>
<td>7.02 ± 0.32</td>
<td>0.96</td>
</tr>
<tr>
<td>Plasma insulin, IU/ml</td>
<td>9.82 ± 1.39</td>
<td>13.7 ± 1.47</td>
<td>0.07</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>184 ± 2</td>
<td>217 ± 9</td>
<td>0.01*</td>
</tr>
<tr>
<td>Mean arterial blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pressure, mmHg</td>
<td>39 ± 4</td>
<td>44 ± 3</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Data are shown in means ± SE; n = 9 to 10. *Significant difference.
than those treated with placebo (22.7 ± 8.4; \( P < 0.05 \)),
suggesting that dexamethasone had suppressed the
adrenocortical axis in the ewes exposed to repeated
courses of dexamethasone.

**Phenylephrine-induced contractions of fetal carotid
arteries derived from dexamethasone-treated and pla-
cebo-treated ewes.** Fetal carotid arteries from fetuses of
the ewes exposed to single and repeated courses of
dexamethasone (Fig. 1, A and B, closed circles) de-
veloped biphasic contractions in response to 1 \( \mu \text{M} \) phen-
ylephrine. The biphasic contraction was characterized
by an initial contraction, followed by a relaxation and
force redevelopment to steady state. The intermediate
phase of relaxation in response to phenylephrine was
either absent or less dramatic in the carotid arteries of
fetuses of the ewes exposed to single and repeated
courses of placebo (Fig. 1, circles, open circles).

Carotid arteries from fetuses of ewes exposed to a
single course of dexamethasone (Fig. 1A, closed circles)
 exhibited a contractile force that increased from a

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Phenylephrine-induced contractions of fetal carotid
arteries from fetuses of ewes exposed to a single course (A, closed circles) of
dexamethasone or repeated courses (B, closed circles) of dexameth-
asone. Open circles, fetuses of ewes exposed to placebo. Force values
at -1 and 0 min represent basal force in unstimulated arteries at the
times immediately before the addition of phenylephrine. Data are
presented as means ± SE (A, closed circles: \( n = 4 \) carotid artery
segments, and open circles, \( n = 3 \) carotid artery segments; B, closed
circles, \( n = 10 \) carotid artery segments, open circles, \( n = 13 \)).

Results from Fig. 1B suggested that the intermediate
relaxation phase represents the major difference
between the carotid arteries from fetuses of ewes ex-
posed to repeated courses of dexamethasone and pla-
cebo. Therefore, we compared the rate constants of
this relaxation phase between the two groups by plotting
log (Force) against time and estimating the slopes by
linear regression analysis (Fig. 2A). We calculated the
slope of relaxation for each individual arterial segment
and then averaged the slopes for each group. The slope
of relaxation (Fig. 2B) was significantly more negative
in the carotid arteries from the fetuses of the ewes
exposed to repeated courses of dexamethasone than
placebo. A similar analysis was not performed for the
carotid arteries from the fetuses of the ewes exposed
to single courses of dexamethasone and placebo, because
the numbers of arteries examined were limited.

**Myosin LC17 isoform expression in fetal and mater-
nal carotid arteries.** The percentage of myosin LC17a
isoform expression in the carotid arteries from fetuses
of ewes exposed to repeated courses of dexamethasone
(42.6 ± 5.4\%) was significantly greater than in those of
placebo (23.9 ± 3.6\%)-treated ewes (Fig. 3A). In con-
The percentage of myosin LC17a in the carotid arteries of the ewes exposed to repeated courses of dexamethasone (77.9 ± 3.0%) did not differ from those exposed to placebo (82.5 ± 3.0%, Fig. 3B). The percentage of myosin LC17a in fetal carotid arteries was significantly lower than in the carotid arteries of placebo-treated ewes.

DISCUSSION

The physiological, biochemical, and weight changes in the fetuses of the ewes exposed to the repeated courses of dexamethasone (Table 1) are consistent with our previous work and those of others and suggest that the fetus exhibits growth retardation when the ewe is exposed to repeated glucocorticoid treatment and glucocorticoid-related glucose intolerance (21, 39). In contrast to findings with other antenatal and fetal corticosteroid regimens (4, 8) but consistent with our previous work (39), the fetuses did not exhibit elevations in systemic arterial blood pressure. The insulin concentrations were slightly but not significantly higher in the fetuses of the ewes exposed to repeated courses of dexamethasone than placebo (Table 1). It is well known that the control of plasma glucose concentration is relatively imprecise in the immature subject (6). This most likely accounts for the statistically significant increase in the plasma glucose concentration without a significant increase in the insulin concentrations in our study.

Although changes in the expression of contractile proteins have been examined during fetal development, the effects of maternal glucocorticoid treatment on fetal arterial contractile protein expression and dynamics have not been previously studied. Arens et al. (2) reported developmental changes in the expression of the 200- and 204-kDa myosin heavy chain in the fetal aorta throughout gestation but were not able to measure contractility, because the aorta did not respond to contractile stimuli until after birth. Anwar et al. (1) and Long et al. (23, 24) measured contractions in fetal ovine femoral and cerebral arteries in response to norepinephrine but did not measure contractile protein expression. In our study, we also found that fetal ovine carotid arteries generated a substantial contractile force in response to 1 μM phenylephrine (Fig. 1). Anwar et al. (1) compared norepinephrine-induced contractions between femoral arteries of fetal sheep infused intravenously with betamethasone and vehicle for 48 h but were not able to detect significant differences between the groups in the norepinephrine concentration-vascular response relationships. It is also important to point out that in contrast to the fetuses of dexamethasone-treated ewes in our study, the beta-
methasone-infused fetal sheep exhibited elevations in systemic arterial blood pressure (1). In our study, we observed significant differences in the time courses of phenylephrine-induced contractions between the carotid arteries from fetuses of dexamethasone- and placebo-treated ewes, although their steady-state forces did not differ (Fig. 1). In response to 1 μM phenylephrine, carotid arteries from fetuses of dexamethasone-treated ewes exhibited biphasic contractions, characterized by an intermediate phase of relaxation between initial force development and steady-state force maintenance. The rate constant of the relaxation phase was significantly higher in carotid arteries of the fetuses of the ewes exposed to repeated courses of dexamethasone than placebo (Fig. 2). Biphasic contractions induced by α-adrenergic receptor agonists have been observed in various arteries (26, 33) and are thought to represent temporal separations of an early phase of Ca$^{2+}$ release from the sarcoplasmic reticulum from a later phase of Ca$^{2+}$ influx through calcium channels on the cell membrane (19). Therefore, the observed biphasic contractions in our study suggest the existence of functional sarcoplasmic reticulum in the carotid arteries from the fetuses of the ewes exposed to repeated courses of dexamethasone. Developmental changes in the function of the sarcoplasmic reticulum in fetal ovine cerebral arteries have been previously reported. Long et al. (23) found that the sarcoplasmic reticulum is negligible in fetal cerebral arteries but plays a key role in adult cerebral arteries. Blood et al. (5) confirmed these findings by showing that fetal cerebrovascular smooth muscle contractions were dependent on extracellular Ca$^{2+}$ and L-type calcium channels. Therefore, the observed biphasic contractions in the carotid arteries of the fetuses of the dexamethasone-treated ewes were not expected and suggest that glucocorticoid accelerates the development of sarcoplasmic reticulum beyond the normal course of development in fetal carotid arteries. To our knowledge, our study represents the first report of glucocorticoid-mediated acceleration in the development of biphasic contractions in fetal arteries.

Developmental changes in contractile protein expression have been studied in the fetus, but the effects of glucocorticoid on changes in the fetal carotid arteries have not been previously examined. Ogut and Brozovich (31) have reported that myosin LC17 isoform expression is an important marker of fetal development in chick embryos. Fisher et al. (13) found that the percentage of myosin LC17a isoform in the phasic smooth muscle was low early in embryonic development and then increased later in development to become the dominant isoform by the time of hatching (13). Consistent with their findings, we also observed lower myosin LC17a expression in carotid arteries of fetuses of placebo-treated ewes than the adult ewes. A novel finding in our study is that maternal administration of repeated courses of dexamethasone induced an almost twofold increase in the percentage of myosin LC17a isoform in fetal carotid arteries (Fig. 3A), thus bringing the percentage of myosin LC17a isoform closer to that observed in adult carotid arteries (Fig. 3B). In contrast, the percentage of myosin LC17a isoform in maternal carotid arteries was not affected by treatment with dexamethasone. These observations indicate that dexamethasone accelerates myosin LC17a expression in fetal carotid arteries beyond the normal course of development. These observations also suggest that the underlying mechanism(s) of phenotypic modulation triggered by glucocorticoid is lost in adult carotid arterial smooth muscle cells. To our knowledge, this study represents the first report of dexamethasone-induced modulation of contractile protein isoform expression in fetal arteries. Our findings are particularly striking because, although we administered repeated courses of dexamethasone to the ewes, the exposure of the fetuses would have been considerably lower than that of the ewes.

The differential sensitivity of fetal and adult arteries to glucocorticoid treatment is intriguing in view of the known heterogeneity in contractile protein expression in single smooth muscle cells. Eddinger et al. (11) have measured myosin LC17a and LC17b mRNA isoforms in single vascular smooth muscle cells derived from rabbit aorta and carotid arteries. They found that the percentage of myosin LC17a mRNA isoform in individual single cells varied over a wide range from 50 to 100%, and average mRNA expression correlated with average protein expression at the tissue level. The heterogeneity in myosin LC17 isoform expression in individual smooth muscle cells suggests different stages of differentiation in these cells. We speculate that the observed effect of dexamethasone on increasing myosin LC17a isoform expression in fetal carotid arteries could represent accelerated differentiation of a subpopulation of vascular smooth muscle cells to the adult phenotype. If these fully differentiated vascular smooth muscle cells possess limited or no potential for phenotypic modulation later in life as suggested by the finding in the ewes (Fig. 3B), then the vascular system in the fetuses of the dexamethasone-treated ewes is left with a reduced population of vascular smooth muscle cells that have the potential of phenotypic modulation in response to mechanical and chemical stimuli later in life.

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