Dexamethasone treatment in the newborn rat: fatty acid profiling of lung, brain, and serum lipids

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Bruder, Eric D., Ping C. Lee, and Hershel Raff. Dexamethasone treatment in the newborn rat: fatty acid profiling of lung, brain, and serum lipids. J Appl Physiol 98: 981–990, 2005. First published November 12, 2004; doi:10.1152/japplphysiol.01029.2004.—Dexamethasone is used as treatment for a variety of neonatal syndromes, including respiratory distress. The present study utilized the power of comprehensive lipid profiling to characterize changes in lipid metabolism in the neonatal lung and brain associated with dexamethasone treatment and also determined the interaction of dexamethasone with hypoxia. A 4-day tapering-dose regimen of dexamethasone was administered at 0800 on postnatal days 3 (0.5 mg/kg), 4 (0.25 mg/kg), 5 (0.125 mg/kg), and 6 (0.05 mg/kg). A subgroup of rats was exposed to hypoxia from birth to 7 days of age. Dexamethasone treatment elicited numerous specific changes in the lipid profile of the normoxic lung, such as increased concentrations of saturated fatty acids in the phosphatidylcholine and cholesterol ester classes. These increases were more profound in the lungs of hypoxic pups. Additional increases in cardiolipin concentrations were also measured in lungs of hypoxic pups treated with dexamethasone. We measured widespread increases in serum lipids after dexamethasone treatment, but the effects were not equivalent between normoxic and hypoxic pups. Dexamethasone treatment in hypoxic pups increased 20:4n6 and 22:6n3 concentrations in the free fatty acid class of the brain. Our results suggest that dexamethasone treatment in neonates elicits specific changes in lung lipid metabolism associated with surfactant production, independent of changes in serum lipids. These findings illustrate the benefits of dexamethasone on lung function but also raise the potential for negative effects due to hyperlipidemia and subtle changes in brain lipid metabolism.

INHALED AND SYSTEMIC GLUCOCORTICOIDS have been widely used to treat syndromes of perinatal distress, many of which have an inflammatory component requiring pharmacological therapy (3, 7, 23, 25, 53). Dexamethasone, a highly potent glucocorticoid, has been the treatment of choice for neonates who are hypoxic due to cardiopulmonary conditions such as bronchopulmonary dysplasia (2, 13, 33, 46, 50). A major motivation for this therapy is the promotion of surfactant production and lung maturation and to induce closure of the ductus arteriosus (6, 34, 43, 45, 47). Dexamethasone decreases the duration of ventilatory support and lowers the incidence of chronic lung disease (13, 23, 33, 50).

Dexamethasone therapy, however, may lead to unfavorable long-term sequelae, including decreases in neuromotor and cognitive function (4, 15, 30, 36, 37, 55), and may also lead to left ventricular abnormalities and metabolic dysfunction (5, 14, 17, 24). A report of lipid intolerance in infants treated with dexamethasone highlights this potential for long-term metabolic dysfunction (2). It is for these reasons that the use of dexamethasone in neonates is decreasing (39) and that its use as a primary defense against the development of chronic lung disease is now being questioned (4). However, some of the short-term benefits of dexamethasone may outweigh the long-term risks (33).

The pathophysiology of and adaptation to neonatal hypoxia has been a subject of intense investigation (18–20, 31, 35, 41, 49). We have recently shown that hypoxia induces specific alterations in the lipid and fatty acid profiles of the adrenal gland and liver (8, 9). These studies have greatly benefited from the power of comprehensive lipid profiling, through which it is possible to analyze the concentrations of specific fatty acid metabolites in distinct lipid classes (51, 52). This method also allows the evaluation of the metabolic interaction of dexamethasone and hypoxia in the neonate (9).

The goal of the present study was to assess changes in lipid metabolism in the lung and brain associated with dexamethasone treatment, utilizing comprehensive lipid profiling. Furthermore, we used our model of neonatal hypoxia as a classic perturbation for which dexamethasone is used as treatment. We hypothesize that dexamethasone-induced changes in lung and brain lipid composition occur independently of changes in serum lipids. Our specific aims were to 1) analyze the effects of dexamethasone on the comprehensive lipid profile of the lung, 2) analyze the effects of hypoxia on lung lipid composition, 3) examine the effects of dexamethasone on the hypoxic lung, and 4) assess the effects of dexamethasone and/or hypoxia on the lipid composition of the brain and serum.

METHODS

Animal treatment. All experimentation was approved by the Institutional Animal Care and Use Committees of the Medical College of Wisconsin and St. Luke’s/Aurora Sinai Medical Center. Timed pregnant Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN; n = 8) were obtained at 14 days gestation and maintained on a standard sodium diet (Richmond Standard 5001, Brentwood, MO) and water ad libitum in a controlled environment (lights on, 0600–1800). Parturition usually occurred on the afternoon of gestational day 22, during which time rats were kept under observation. After litters were completely delivered, litter size was equalized by cross fostering, and the dam and pups (~13 per litter; mixed sexes) were immediately exposed to normobaric hypoxia (12% O2) or kept in room air as control (21% O2), as described previously (40, 41). We have previously shown that this exposure leads to arterial Po2 levels in...
adults of ∼50–55 Torr with sustained hypocapnia and alkalosis (40, 42).

Lactating dams were maintained with their litters for 7 days in a hypoxic or normoxic environment (49). Dexamethasone phosphate (Sigma, St. Louis, MO) was administered subcutaneously in a tapering regimen to normoxic and hypoxic pups at 0800 as follows: postnatal days 3 (0.5 mg/kg), 4 (0.25 mg/kg), 5 (0.125 mg/kg), and 6 (0.05 mg/kg) (15). Control pups were injected with saline. Pups were weighed on each day of injection. At 0800 on postnatal day 7, dams were removed from the chambers. Pups were quickly decapitated, and lung and brain tissue were quickly washed to remove any remaining blood and immediately snap frozen in liquid N2 (n = 4 pups/treatment group). Blood from each pup was immediately centrifuged for 5 s at room temperature, and the serum was quickly frozen on dry ice. Serum from two additional pups from the same treatment group was sequentially added to the previously frozen sample (on dry ice) to pool the serum from three pups (n = 4 pooled samples/treatment group). Samples were obtained from pups from four normoxic (with or without dexamethasone) and four hypoxic (with or without dexamethasone) litters.

Lipid profiling. A comprehensive assessment of lung, brain, and serum lipid profiles was performed (Lipomics Technologies, West Sacramento, CA). Brain and lung tissue (50 mg) and serum (200 µl) lipids were extracted in the presence of internal standards by the method of Folch et al. using chloroform:methanol (2:1 vol/vol) (16). Individual lipid classes from each extract were separated by preparative thin-layer chromatography, as described previously (51, 52). Authentic lipid class standards were spotted on the two outside lanes of the thin-layer chromatography plate to enable localization of the sample lipid classes. Lipid fractions were scraped from the plate and transesterified in 3 N methanolic-HCl in a sealed vial under a N2 atmosphere at 100°C for 45 min. The resulting fatty acid methyl esters were extracted with hexane containing 0.05% butylated hydroxytoluene and prepared for gas chromatography by sealing the extracts under N2. Fatty acid methyl esters were separated and quantified by capillary gas chromatography using a gas chromatograph (Hewlett-Packard model 6890, Wilmington, DE) equipped with a 30-m DB-225MS capillary column (J & W Scientific, Folsom, CA) and a flame-ionization detector, as described previously (51, 52). Intra-assay coefficients of variation were as follows: cholesterol ester (CE; 2.0%), diglyceride (DG; 5.5%), free fatty acid (FFA; 3.5%), lysophosphatidylcholine (LPC; 12.2%), phosphatidylcholine (PC; 5.0%), sphingomyelin (SM; 11.4%), phosphatidylethanolamine (PE; 13.0%), and triglyceride (TG; 0.4%). Assay sensitivity was set at 0.1 µmol/g because concentrations below this value increased the analytic variability to >20%.

Statistical analyses. Fatty acid/lipid profile data obtained were quantitative (nmol fatty acid/g of tissue or serum). Significance of differences between vehicle-treated and dexamethasone-treated samples, normoxic and hypoxic samples, or a combination of the two interventions were assessed by unpaired Student’s t-tests (P < 0.05). This statistical approach has been validated for this type of metabo-

Fig. 1. Effects of dexamethasone treatment on the concentrations of individual fatty acid metabolites in lung tissue (top) and serum (bottom) of neonatal rats. Heat map showing individual fatty acid metabolite concentrations, as they appear in each lipid class, in lung tissue and serum of normoxic 7-day-old rats treated with a tapering dose regimen of dexamethasone (n = 4 rats/treatment). Quantitative data were used to calculate the percent increase (green squares) or decrease (red squares) of each measurement from the vehicle-treated control. Significance of differences was analyzed by t-test, with colors denoting P < 0.05. Differences not meeting P < 0.05 are shown in black. Cardiolipin and phosphatidylserine were only measured in the lung.
lomic analysis (51, 52). Quantitative data were visualized using the Lipomics Surveyor software system. The system creates a “heat map” graph for significant differences between samples. The heat map displays statistically significant increases from control values as green or yellow squares and statistically significant decreases as red or blue squares. The brightness of each individual square denotes the magnitude of the difference, as displayed with each of the heat maps. Differences not meeting $P < 0.05$ are shown in black. Data not presented in heat map form are expressed as means ± SE, and significance of differences was assessed by two-way ANOVA and Student-Newman-Keuls method for multiple comparisons ($P < 0.05$). Fatty acid ratios were calculated from individual sample measurements. The mean ratio, across all lipid classes, from each replicated treatment ($n = 4$ per group) was treated as one datum. Lipid class ratios were calculated from individual sample measurements, and the mean ratio from each replicated treatment ($n = 4$ per group) was treated as one datum.

RESULTS

Dexamethasone: effects on normoxic lung and serum lipid profiles. The effects of dexamethasone treatment per se on the lung and serum lipid profiles in normoxic pups are shown in Fig. 1. The column headers indicate fatty acid metabolites as they appear in each distinct lipid class (rows). First, notice the greater number of significant changes in the serum profile compared with that of the lung. Dexamethasone decreased the concentration of a number of fatty acid metabolites in the TG class ($P < 0.05$) of the lung. Treatment with dexamethasone increased the concentrations of several fatty acids in the PC class (e.g., 16:0, 18:3n3, 20:5n3, and 18:2n6), the cardiolipin (CL) class (e.g., 16:0 and 20:5n3), and the CE class (e.g., 18:3n3 and 20:4n6) ($P < 0.05$) of the lung. Dexamethasone also elicited a number of specific changes in the remaining lipid classes of the lung, including DG, FFA, LPC, PE, phosphatidylinerine (PS) and SM. Dexamethasone caused widespread increases in fatty acid concentrations in the serum of normoxic pups, with increases in at least three fatty acids in all lipid classes ($P < 0.05$) except FFA. Major fatty acid metabolites, such as 16:0, 18:0, 16:1n7, 18:1n9, 18:2n6, and 20:4n6, were increased in several lipid classes of the serum, including the CE, DG, LPC, PC, PE, and SM classes ($P < 0.05$). The concentration of 18:2n6 was increased in every lipid class analyzed ($P < 0.05$). Dexamethasone treatment had the fewest effects on the n3 fatty acids (across all classes). Differences not meeting $P < 0.05$ are shown in black.

![Heat map of lung and serum lipid profiles](image-url)
Hypoxia: effects on lung and serum lipid profiles. Figure 2 depicts the effects of hypoxia per se on lipid profiles in lung tissue and serum. Again note the abundance of changes in the serum profile compared with the lung; hypoxia had minimal effects on the lipid profile of the neonatal lung. The concentration of 20:5n3 was decreased in the FFA and TG classes, whereas the concentration of 18:3n3 was decreased only in the FFA class in the lung (P < 0.05). In the serum, hypoxia caused significant increases in most major fatty acid metabolites of the TG class, encompassing all fatty acid families measured (P < 0.05). Several metabolites in the DG class of serum were increased by hypoxia (e.g., 16:0, 16:1n7, 18:1n9, 22:6n3, and 20:4n6) (P < 0.05). Hypoxia also increased the concentrations of several fatty acids in the PC class (e.g., 14:0, 16:1n7, 18:1n9, and 18:2n6) (P < 0.05). Decreases in fatty acid concentrations in the serum of hypoxic pups were measured in the CE class (e.g., 16:0, 20:5n3, and 20:4n6) (P < 0.05). Several metabolites in the DG class of serum were increased by hypoxia (e.g., 16:0, 16:1n7, 18:1n9, 22:6n3, and 20:4n6) (P < 0.05). In the serum, hypoxia caused significant increases in most major fatty acid metabolites of the TG class, encompassing all fatty acid families measured (P < 0.05). Several metabolites in the DG class of serum were increased by hypoxia (e.g., 16:0, 16:1n7, 18:1n9, 22:6n3, and 20:4n6) (P < 0.05). Decreases in fatty acid concentrations in the serum of hypoxic pups were measured in the CE class (e.g., 16:0, 20:5n3, and 20:4n6) (P < 0.05). Decreases in fatty acid concentrations in the serum of hypoxic pups were measured in the CE class (e.g., 16:0, 20:5n3, and 20:4n6) (P < 0.05). Decreases in fatty acid concentrations in the serum of hypoxic pups were measured in the CE class (e.g., 16:0, 20:5n3, and 20:4n6) (P < 0.05).

Dexamethasone: effects on hypoxic lung and serum lipid profiles. The effects of dexamethasone on the lipid profiles of lung tissue and serum of hypoxic pups compared with vehicle-treated hypoxic pups are depicted in Fig. 3. Although hypoxia by itself had little effect, dexamethasone treatment in hypoxic pups had profound effects on the lung lipid profile. Dexamethasone elicited increases in many of the fatty acid metabolites in the CL class (e.g., 16:0, 18:0, 18:1n9, 22:6n3, and 18:2n6) in addition to those in the PC class (e.g., 16:0, 18:3n3, 22:6n3, and 20:4n6) (P < 0.05). The lungs of hypoxic pups treated with dexamethasone also exhibited decreases in the TG class (e.g., 16:0, 18:1n9, 20:5n3, and 20:4n6) (P < 0.05). Other isolated changes were measured in the CE, FFA, PE, PS, and SM classes (all P < 0.05). Treatment of hypoxic pups with dexamethasone increased the serum concentration of numerous metabolites in the CE class (e.g., 16:0, 16:1n7, 18:1n9, 20:5n3, and 20:4n6) and elicited various increases in the LPC, PC, and SM classes (P < 0.05). Dexamethasone treatment in hypoxic pups also decreased the concentrations of various fatty acid metabolites in the TG, DG, PC, and PE classes (all P < 0.05).

Dexamethasone: effects on major fatty acids in the normoxic and hypoxic lung. Figure 4 depicts the effects of dexamethasone on the concentrations of major fatty acid metabolites, across all lipid classes, in lung tissue of normoxic and hypoxic pups. Dexamethasone elicited a decrease in the concentration of 18:1n9 in normoxic (P < 0.02) and hypoxic (P < 0.05) lung...
Hypoxia by itself caused significant decreases in 18:2n6 (P < 0.008), 20:3n6 (P < 0.006), and 20:4n6 (P < 0.04). Dexamethasone treatment in hypoxic pups resulted in further decreases in 18:2n6 (P < 0.006) and 20:3n6 (P < 0.01) in lung tissue compared with hypoxic pups treated with vehicle. The concentrations of four major n3 fatty acids are depicted in Fig. 4, bottom. Dexamethasone decreased the concentrations of 22:5n3 (P < 0.001) and 22:6n3 (P < 0.001) in lung tissue of normoxic pups. Hypoxia also caused decreases in the concentrations of 22:5n3 (P < 0.02) and 22:6n3 (P < 0.02), as well as in the concentration of 20:5n3 (P < 0.05). Dexamethasone treatment in hypoxic pups further decreased the concentrations of 22:5n3 (P < 0.009) and 22:6n3 (P < 0.001).

Dexamethasone: effects on normoxic and hypoxic lung and serum lipid classes. Table 1 summarizes the interaction of dexamethasone treatment with hypoxia on lipid class concentrations in lung tissue and serum. The lungs of normoxic pups treated with dexamethasone showed increases in CE and PC (P < 0.02), as well as a large decrease in TG (P < 0.001). Hypoxia by itself had no effect on any of the measured lipid classes in the lung (P > 0.05). Dexamethasone treatment in hypoxic pups increased the concentrations of PC and CL (P < 0.001) in the lung but decreased the TG concentration more than twofold (P < 0.001) compared with hypoxic pups treated with vehicle. Nearly all serum phospholipid classes measured were increased by dexamethasone treatment in normoxic pups. These include increases in LPC (P < 0.001), PE (P < 0.001), PC (P < 0.001), and SM (P < 0.005). Dexamethasone also caused increases in the serum concentrations of TG (P < 0.008) and CE (P < 0.001) in normoxic pups. Hypoxia alone caused increases in the serum concentrations of DG and TG (P < 0.001). Dexamethasone treatment in hypoxic pups caused increases in serum CE (P < 0.001), LPC (P < 0.004), and SM (P < 0.05) concentrations and decreases in DG (P < 0.02) and TG (P < 0.03) compared with hypoxic pups treated with vehicle.

Dexamethasone: effects on normoxic and hypoxic PC- and TG-associated fatty acids in the lung. Table 2 highlights the effects of dexamethasone on specific fatty acid concentrations in the PC and TG classes of normoxic and hypoxic lung tissue. In the PC class of normoxic lung tissue, dexamethasone increased the concentrations of 16:0 (P < 0.005), 18:2n6 (P < 0.02), and 20:5n3 (P < 0.04) and also increased the total concentration of saturated fatty acids (P < 0.02) in this lipid class. Hypoxia alone had no significant effects on any fatty acid concentrations in the PC class (P > 0.05). However, dexamethasone treatment in hypoxic pups increased the concentrations of 14:0 (P < 0.03), 16:0 (P < 0.001), 16:1n7 (P < 0.02), total saturated fatty acids (P < 0.001), 18:2n6 (P < 0.003), and 20:5n3 (P < 0.02) in the PC class. In the TG class of normoxic lung tissue, dexamethasone decreased the concentrations of 14:0 (P < 0.02), 16:0 (P < 0.001), total saturated fatty acids (P < 0.001), 18:2n6 (P < 0.001), and 20:5n3 (P < 0.001). Hypoxia by itself decreased the concentrations of 18:2n6 (P < 0.01) and 20:5n3 (P < 0.001) in the TG class. Treatment with dexamethasone elicited significant decreases in the concentrations of 14:0 (P < 0.01), 16:0 (P < 0.001), total saturated fatty acids (P < 0.001), 18:2n6 (P < 0.001), and 20:5n3 (P < 0.003) in the TG class of hypoxic lung tissue compared with hypoxic pups treated with vehicle.
Dexamethasone: effects on lipid and fatty acid ratios in the normoxic and hypoxic lung. Table 3 shows the effects of dexamethasone on lipid and fatty acid ratios in lung tissue. In normoxic pups, dexamethasone decreased the TG-to-DDA (P < 0.005) and TG-to-PC (P < 0.001) ratios and also increased the CL-to-DG (P < 0.03) and CE-to-DDA (P < 0.02) ratios. Dexamethasone also increased the ratios of CE to DG (P < 0.002), 16:0 to 18:0 (an indicator of elongase activity; P < 0.04) and SFA to MUFA (P < 0.05). The brains of hypoxic pups treated with dexamethasone also had decreased concentrations of 20:3n9 in the DG class (P < 0.05).

Dexamethasone: effects on the normoxic and hypoxic lipid profile of the brain. The effects of dexamethasone on the brain lipid profile of normoxic and hypoxic pups are shown in Table 4. Dexamethasone treatment in normoxic pups elicited increases in FFA (e.g., 14:0 and 20:4n6), PE (20:2n6), and PS (20:2n6) classes (P < 0.05). Hypoxia alone increased the concentration of 20:2n6 in the PE class, increased 22:5n3 and 20:3n6 in the TG class, and decreased the concentration of 18:1n7 in the PS class (P < 0.05). Dexamethasone treatment in hypoxic pups elicited increases in DG (18:1n7), FFA (e.g., 22:6n3 and 20:4n6), PC (20:2n6), PE (18:1n7 and 20:3n9), and PS (20:3n9 and 20:3n6) (P < 0.05). The brains of hypoxic pups treated with dexamethasone also had decreased concentrations of 20:3n9 in the TG class (P < 0.05).

**DISCUSSION**

The use of comprehensive lipid profiling has led to the development of a highly detailed metabolic characterization of lung and brain in the newborn rat.
Significant effects of dexamethasone and/or hypoxia on fatty acid concentrations of brain lipid classes

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Fatty Acid</th>
<th>Normoxia-Dex</th>
<th>Hypoxia-Dex</th>
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<tr>
<td>DG</td>
<td>18:1n7</td>
<td>ns</td>
<td>+32%</td>
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<tr>
<td>FFA</td>
<td>14:0</td>
<td>+75%</td>
<td>ns</td>
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<tr>
<td></td>
<td>20:3n6</td>
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<td>20:3n6</td>
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<td>+119%</td>
</tr>
<tr>
<td></td>
<td>18:1n7</td>
<td>+117%</td>
<td>+57%</td>
</tr>
<tr>
<td></td>
<td>22:6n3</td>
<td>ns</td>
<td>+121%</td>
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<tr>
<td>PC</td>
<td>20:2n6</td>
<td>ns</td>
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<td>+38%</td>
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<td></td>
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<td></td>
<td>22:5n3</td>
<td>+582%</td>
<td>ns</td>
</tr>
</tbody>
</table>

All values were considered significant ($P < 0.05$), except ns (not significant; $P > 0.05$). †Increased; ‡decreased.
hypoxic neonate alters lipid metabolism in an attempt to adapt to the environment. Hypoxia only tended to increase the concentration of PC and the saturation of PC-associated fatty acids and tended to decrease the concentration of lung TG, which could also be part of an adaptive response.

The pulmonary benefits of dexamethasone treatment in hypoxic neonates have been extensively studied (7, 13, 23, 46). Our results indicate that many of the effects of dexamethasone measured in the normoxic lung were qualitatively similar to those found in the hypoxic lung. Moreover, these effects were often more pronounced in the hypoxic lung and highlight the benefits of dexamethasone treatment in neonatal respiratory distress. Dexamethasone increased the concentration of PC and CL in the hypoxic lung and further decreased the concentration of TG (compared with vehicle-treated hypoxic pups). In the PC fraction of the hypoxic lung, dexamethasone increased the concentrations of numerous fatty acid metabolites (e.g., 16:0, 16:1n7, and 18:2n6). Perhaps the most notable change in the dexamethasone-treated hypoxic lung was the increased concentration and saturation of CL. As stated above, CL has been implicated in a variety of mitochondrial functions and is a key component of the inner mitochondrial membrane (26, 28). Increased mitochondrial CL concentrations could confer advantages at the cellular level by increasing the efficiency of oxidative phosphorylation under nonoptimal conditions, such as hypoxia (22, 28). Our findings suggest another possible beneficial effect of dexamethasone treatment in the lungs of hypoxic neonates.

Effects of dexamethasone on the serum lipid profile. Treatment of normoxic pups with dexamethasone resulted in global increases in serum lipids. We have previously reported increased serum concentrations of TG and total cholesterol after dexamethasone treatment (9). The present results extend the characterization of serum lipids and fatty acids affected by dexamethasone. Another study, which examined the effects of dexamethasone on serum lipids in neonates with bronchopulmonary dysplasia, measured increases in plasma TG and FFA (2). Interestingly, the FFA class was the least affected in our study. The dexamethasone-induced increases in serum TG and CE are likely due to the stimulation of very-low-density lipoprotein synthesis and secretion by the liver (38), which may have contributed to changes in CE concentrations in the lung (21). Dexamethasone also induced increases in serum PC concentrations, which may have contributed to the increase in lung PC. However, the twofold-greater concentration of PC in the lung (compared with the serum) suggests a direct effect on the lung itself, as described previously (6, 43, 45, 47).

We and others have previously shown that hypoxia in neonates leads to increased serum TG concentrations (9, 12, 18, 20, 29). Hypoxia may modulate the function of enzymes associated with TG synthesis and degradation (12, 38). The only significant effect of hypoxia on serum lipids, besides increased TG, was an increase in DG. A small component of the increase in DG could be explained by changes in dietary intake (10), since our experimental approach requires hypoxic exposure of the dam. Dexamethasone treatment in hypoxic pups did not mimic the effects observed in serum of normoxic pups. The concentrations of CE, LPC, and SM were increased by dexamethasone in serum from normoxic and hypoxic pups. Dexamethasone decreased the concentration of serum TG in the hypoxic pups (compared with vehicle-treated hypoxic pups), an effect opposite of that found in normoxic pups. This finding is at variance with previous results from our laboratory, which found serum TG in the hypoxic pup to be further increased by dexamethasone (9). This discrepancy between studies is likely the result of sample handling and/or assay methodology; the previous study utilized a colorimetric assay to measure TG (9).

Effects of dexamethasone on brain lipid profile. Hypoxia by itself also had a minimal effect on brain lipids. This study, to our knowledge, is the first to provide a highly detailed profile of brain lipid and fatty acid concentrations after dexamethasone treatment in neonates. It is now generally accepted that neonatal dexamethasone treatment is detrimental to neurodevelopment and brain function in the long term (4, 15, 30, 36, 37, 55). The exact mechanisms through which this dysfunction develops remain uncertain. A recent study (32) found that neurite growth is dependent on increased internalization of 20:4n6 and 22:6n3, which are eventually incorporated into phospholipids. These two fatty acids have also been implicated in the proper developement of the visual system (27). Although we did not detect increased concentrations of these two fatty acids in any phospholipid class, we did measure an increase in 20:4n6 in the FFA class of dexamethasone-treated normoxic pups. Likewise, hypoxic pups treated with dexamethasone had increased concentrations of 20:4n6, as well as 22:6n3, in the FFA class. The significance of these findings and how they may relate to future neural dysfunction remain unclear. Because we analyzed the brain as a whole, we could not evaluate potential changes in lipid composition in specific nuclei or brain regions.

Summary and perspectives. Dexamethasone is used to treat neonatal lung disease, which is often due to bronchopulmonary dysplasia or other inflammatory processes (2, 13, 33, 46, 50). These syndromes are often the result of premature birth. In the United States, ~12% of live births annually are preterm, providing a large population of potential candidates for dexamethasone treatment (1). The benefits of dexamethasone use in treating lung disease of prematurity are well characterized, and the main function of this treatment is to promote the production of surfactant (6, 43, 45, 47). Our results extend these findings and offer additional insight into the beneficial role dexamethasone treatment has on lung function. As with any animal model, caution must be taken when extrapolating results to human pathophysiology. For example, alveolarization of the rat lung occurs postnatally, and surfactant phospholipid metabolism differs from species to species (11, 44). Dexamethasone therapy has been shown to decrease the time sick infants require ventilation, but its overall effects on morbidity and mortality remain a point of argument (4, 39).

Although the use of dexamethasone for the treatment of neonatal illness is on the decline, there are still situations in which the benefits of treatment far outweigh the short- and long-term risks (33). A number of recent studies have shown that dexamethasone use in the neonate leads to neurological dysfunction later in life (4, 15, 30, 36, 37, 55). It is now a general consensus that, when required, the dose of dexamethasone should be reduced to the smallest effective dose (4, 42, 50). Our results indicate that the detrimental effects of dexamethasone on brain function likely do not involve short-term changes in lipid metabolism. This suggests a more subtle effect
on neurodevelopment and helps to explain why these developmental defects often do not present until adolescence (36, 55).

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