Effect of neonatal hypoxia on the development of hepatic lipase in the rat

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Lee, Ping C., Beth Jelinek, Mark Struve, Eric D. Bruder, and Hershel Raff. Effect of neonatal hypoxia on the development of hepatic lipase in the rat. Am J Physiol Regulatory Integrative Comp Physiol 279: R1341–R1347, 2000.—Increases in plasma lipids occur during hypoxia in sucking but not in weaned rats and may result from altered hepatic enzyme activity. We exposed rats to 7 days of hypoxia from birth to 7 days of age (suckling) or from 28 to 35 days of age (weaned at day 21). Hypoxia led to an increase in hepatic lipid content in the suckling rat only. Hepatic lipase was decreased to ~45% of control in 7-day-old rats exposed to hypoxia but not in hypoxic 35-day-old rats. Hypoxic suckling rats also had a 50% reduction in lactate dehydrogenase activity, whereas transaminase activity and CYP1A and CYP3A protein content were not different between hypoxic and normoxic groups. Additional rats were studied 7 and 14 days after recovery from hypoxic exposure from birth to 7 days of age; hepatic lipase activity had recovered to 85% by 7 days and to 100% by 14 days in the rats previously exposed to hypoxia. Administration of dexamethasone to neonatal rats to simulate the hyperglucocorticoid state found in hypoxic 7-day-old rats led to a moderate decrease (~75% of control) in hepatic lipases. Developmentally, in the normoxic state, hepatic lipases increased rapidly after birth and reached levels more than twofold that of the newborn by 7 days of age. Hypoxia delays the maturation of hepatic lipases. We suggest that the decrease in hepatic lipase activity contributes to hyperlipemia in the hypoxic newborn rats.

NEONATAL HYPOXIA IS A COMMON perinatal emergency and is often accompanied by serious morbidity (11, 25). Although considerable efforts have been directed at evaluating alterations in neurological, cardiopulmonary, and renal function (12, 25, 33), the metabolic response to hypoxia in the neonate has not been extensively examined. Neonatal hypoxia results in significant disorders in plasma lipids, particularly in nursing infants. This neonatal hypoxic dyslipidemia, observed over 25 years ago, has been attributed to direct effects of hypoxia on hepatic function (7, 15) although these studies were not definitive or complete.

We recently demonstrated that hypoxia led to marked increases in plasma cholesterol, triglycerides, and nonesterified fatty acids in the suckling neonatal rats but not in weaned 35-day-old rats (26). The liver is known to play an important role in lipid metabolism. Hepatic lipases modulate the uptake of cholesterol (31, 32) and the metabolism of many types of lipoproteins (9, 14, 17). In humans, hepatic lipase deficiency results in elevated plasma triglycerides and/or phospholipids (4, 6, 8, 24). Mice carrying the combined lipase deficiency mutations that affect the translation of lipoprotein lipase and hepatic lipase develop massive hypertriglyceridemia and die (30). In hepatic lipase knockout mice, increases in total circulating cholesterol, phospholipids, and high-density lipoprotein (HDL) cholesterol (but not triglyceride) are observed (16). Infusion of vectors expressing hepatic lipase into these knock-out mice normalizes the plasma levels of these components (1).

The aims of the present study were to 1) analyze changes in hepatic lipid content and lipase activity in neonatal suckling rats exposed to hypoxia for 7 days from birth compared with juvenile, weaned rats exposed to hypoxia for 7 days (from 28 to 35 days of age); 2) analyze the time course of recovery of neonatal rat from hypoxic exposure; and 3) determine whether the hypoxic effect on hepatic lipase can be mimicked by glucocorticoid administration.

METHODS

Animal Treatment

The animal protocol was approved by the Institutional Animal Care and Use Committees of the Medical College of Wisconsin and St. Luke’s/Sinai Samaritan Medical Center. Timed pregnant Sprague-Dawley rats (Harlan Sprague Dawley) were obtained at 14 days gestation and maintained on a standard sodium diet (Richmond Standard #5001) and water ad libitum in a controlled environment (0600–1800 lights on). Parturition

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usually occurred on the afternoon of gestational day 21 during which time rats were kept under observation.

Hypoxia from 0 to 7 days of age. As soon as a litter was completely delivered, the dam and her pups were immediately moved to an environment chamber and exposed to normobaric normoxia (21% O₂; room air) or hypoxia (12% O₂) as described previously (26, 27, 29, 33). We have previously shown that this exposure leads to arterial Po₂ levels in adults of 50–55 Torr with sustained hypocapnia and alkalosis (27, 29). Lactating dams were maintained with their litters for 7 days in a normoxic or hypoxic environment (28, 33). Chambers were briefly opened on day 4 to clean the cages. The experimental day was at the end of 7 days of exposure of dams and their litters to either normoxia or hypoxia. At 0800 of day 7, eight rat pups randomly selected from four different litters each from the normoxic and hypoxic groups were decapitated, and trunk blood was collected. Then, their livers were removed, cut into pieces, frozen immediately on dry ice, and stored at −80°C until analyzed.

A central group of eight litters of hypoxic pups were allowed to recover by returning them with their dams to a normoxic environment with eight litters of age-matched normoxic controls. After 7 and 14 days of recovery under normoxic conditions, seven pups randomly selected from four different litters each from the recovery and control normoxic groups were decapitated, and their livers were collected and processed as above. Trunk blood of rats from this experiment was collected for the measurement of plasma total cholesterol and triglycerides.

Hypoxia from 28 to 35 days of age. Male and female rats (n = 16) from randomly assigned litters raised under normoxic conditions were weaned at 21 days of age. At 28 days of age, they were placed in chambers, exposed to normoxia or hypoxia for 7 days, killed, and livers were collected as described above.

Treatment with dexamethasone. Female and male normoxic newborn rats (n = 18) were given single dose of dexamethasone daily (from days 0 to 6 by intraperitoneal injection at a dose of 13 ng/g body wt or vehicle (same volume of dimethyl sulfoxide, the solvent for dexamethasone). This dose of dexamethasone was chosen to mimic the hypercortisosteroid condition observed in the hypoxic 7-day olds (28). A noninjected group was also included as controls. All animals were killed at 7 days of age.

Development of hepatic lipase. Four litters of normoxic rats were used for this experiment. The day of birth was designated as age 0. Pups were allowed to suckle freely until the time of death. At 0, 1, 4, 5, 7, 21, and ~180 (adult) days of age, rats (~4–8/age) were killed and livers were removed and stored at −80°C as described until analyzed.

**Measurements**

Tissue homogenization. For the determination of lipase activity, a weighed amount of hepatic tissue was thawed and homogenized in ice-cold water with a Polytron for 30 s with the container immersed in ice. Triton X-100 was added to a final concentration of 0.08% and mixed. The homogenate was then centrifuged for 6 min at 1,500 g at 4°C. The supernatant fraction was collected and either analyzed immediately for enzyme assays or immediately frozen and stored at −80°C for later assays.

Hepatic lipid and protein measurement. Liver lipid contents were determined by the gravimetric method according to Folch et al. (10) with slight modifications. Briefly, 100- to 150-mg portions of liver fragments were homogenized in a 3.0-ml mixture of chloroform and methanol (2:1) using a motor-driven Teflon pestle homogenizer. The liquid portion was decanted, and the residual was rehomogenized in another 3 ml of chloroform-methanol mixture and allowed to sit at room temperature for 10 min with occasional stirring. Combined organic phases were filtered through glass wool, and 12 ml of distilled water were added to the filtrate. The mixture was stirred for 30 s and centrifuged at 500 g for 4 min at room temperature. The aqueous layer, up to the interphase, was discarded, and 3 ml of chloroform-methanol mixture were added to the remaining liquid and mixed for 30 s. The mixture was centrifuged at 500 g for 4 min. The bottom organic phase was carefully collected and transferred to preweighed vials. Evaporation of the organic solvent was carried out under a stream of N₂, and the vials were reweighed. The results are expressed as milligrams of lipid per gram of liver.

Protein concentration was determined by the method of Bradford (3) using Bio-Rad reagents (Bio-Rad Laboratories). Biochemical measurements. Hepatic lipase was measured according to LEDFORD and ALAUPOVIC (20) with some modifications. Briefly, the lipolytic activity was determined by potentiometric titration (at a constant pH of 8.0) of ionized free fatty acids (FFAs) liberated from tributyrin (20 mM) in a citrate-phosphate buffer (1 mM) with 0.01% Triton X-100. Lipase activity was measured in the presence of 0.075 M and again in 2.4 M NaCl to distinguish between lipoprotein lipase and hepatic lipase, because the presence of a high-ionic strength environment inhibits lipoprotein lipase but not hepatic lipase (5). Units are expressed as micromoles of NaOH required to neutralize FFAs liberated per minute per gram of tissue or per gram of protein.

Lactate dehydrogenase (LDH) was determined by the “reverse” reaction in the presence of NADH using pyruvate as the substrate as previously described (34) and expressed as units per minute per gram of tissue or per gram of protein. Alkaline aminotransferase/glutamate pyruvate transaminase (ALT/GPT) was determined by the sample-start procedure with the ALT/GPT kit from Sigma (St. Louis, MO) and expressed as units per minute per gram of tissue or per gram of protein. Total plasma cholesterol and triglyceride were measured as previously described (26).

Cytochrome P-450 Westerns. Microsomes from the livers of hypoxic and normoxic 7-day-old rats were prepared by differential centrifugation as described previously (21). Western blots for the determination of cytochrome P-450 (CYP) 1A and CYP3A proteins were carried out as described previously (21, 23) using antibodies that react specifically with rat CYP1A or CYP3A proteins (from Xenotech). Protein concentration was determined by the method of Bradford (3) using Bio-Rad reagents (Bio-Rad Laboratories).

Statistics. Results are reported as means ± SD. Student’s t-test was used to compare the means between two groups with P ≤ 0.05 considered significant. Two-way ANOVA and Duncan’s multiple-range test were used for comparisons with more than two means.

**RESULTS**

Figure 1 shows the hepatic lipid and protein content from rat pups exposed to hypoxia from birth to 7 days of age and from juvenile rats weaned at 21 days of age and subsequently exposed to hypoxia from 28 to 35 days of age. Hypoxia from birth to 7 days of age resulted in a significant increase in lipid content and a decrease in hepatic protein content. There was no effect of hypoxia on hepatic lipid or protein content in 35-day-old rats that had been exposed to hypoxia for the prior 7 days.
Table 1 summarizes the hepatic enzyme levels from rat pups exposed to hypoxia from birth to 7 days of age and from juvenile rats weaned at 21 days of age and subsequently exposed to hypoxia from 28 to 35 days of age. Hypoxia from birth to 7 days of age resulted in significant decreases in total hepatic and lipoprotein lipase activity (assayed in the presence of 0.075 M NaCl) and hepatic lipase (assayed in the presence of 2.4 M NaCl). Hepatic LDH activity was also significantly decreased but not ALT/GPT activities. There was no effect of hypoxia on hepatic lipases, LDH, and ALT/GPT in juvenile rats weaned at 21 days of age and subsequently exposed to hypoxia from 28 to 35 days of age (Table 1). Liver microsomal CYP1A and 3A contents were similar in hypoxic and control 7-day olds (Fig. 2).

Table 2 shows the hepatic lipase activity of 7-day-old hypoxic rats and rats 7 and 14 days after return to normoxic environment. At 7 days of hypoxia, total hepatic lipase was decreased compared with age-matched normoxic controls. When the hypoxic 7-day-old rats were returned to normoxic conditions for 7 days (14 days of age), their hepatic lipases had recovered to 85% of control age-matched normoxic groups. At this stage, the hepatic protein concentration was also lower than the corresponding control level. At 14 days after the return of the 7-day-old hypoxic animals to normoxic condition (21 days of age), there were no differences in either the hepatic lipase level or protein concentration between the previously hypoxic group and the age-matched control normoxic group.

Figure 3 shows total plasma cholesterol and triglyceride. Increases in total plasma cholesterol and triglyceride levels were evident in the 7-day-old hypoxic rats. After hypoxic animals were returned to normoxic condition, their total plasma cholesterol and triglyceride levels had returned to normal by 7 days after the termination of hypoxia.

Table 1. Hepatic enzymes in rats exposed to hypoxia for 7 days (0–7 or 28–35 days of age)

<table>
<thead>
<tr>
<th>Lipase</th>
<th>0.075 M NaCl</th>
<th>2.4 M NaCl</th>
<th>7 Day old</th>
<th>35 Day old</th>
</tr>
</thead>
<tbody>
<tr>
<td>U·g Prot−1·min−1</td>
<td>U·g liver−1·min−1</td>
<td>U·g Prot−1·min−1</td>
<td>U·g liver−1·min−1</td>
<td>U·g Prot−1·min−1</td>
</tr>
<tr>
<td>Control (8)</td>
<td>905 ± 50</td>
<td>98.8 ± 19.1</td>
<td>518 ± 28</td>
<td>56.4 ± 3.9</td>
</tr>
<tr>
<td>Hypoxic (8)</td>
<td>403 ± 23*</td>
<td>42.6 ± 12.8*</td>
<td>220 ± 10*</td>
<td>27.7 ± 8.5*</td>
</tr>
<tr>
<td>Control (8)</td>
<td>990 ± 75</td>
<td>116.7 ± 9.1</td>
<td>610 ± 42</td>
<td>69.8 ± 13.7</td>
</tr>
<tr>
<td>Hypoxic (8)</td>
<td>1,019 ± 108</td>
<td>112.9 ± 15.0</td>
<td>601 ± 55</td>
<td>66.6 ± 11.0</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different from corresponding control values with P ≤ 0.05. At 0.075 M NaCl, both lipoprotein lipase and hepatic lipase are active. At 2.4 M NaCl, only hepatic lipase is active. Numbers in parentheses are nos. of rats. Prot, protein; LDH, lactate dehydrogenase; ALT/GPT, alanine amino-transferase/glutamate pyruvate transaminase.
Table 3 summarizes the effect of a daily treatment of newborn rats from birth to 7 days of age with a low dose of dexamethasone (simulating the hypercortico-steroid condition in the hypoxic 7-day olds) (28) on hepatic lipase levels and protein concentrations. Treatment with the vehicle (dimethyl sulfoxide) alone did not result in a change in hepatic lipase (both total and hepatic lipase) and protein concentrations. Treatment with dexamethasone for the same duration resulted in a moderate decrease in hepatic lipase (both total and hepatic lipase) to \( \sim 75\% \) of control. Protein concentration, however, increased slightly but not significantly.

Figure 4 shows the developmental profile of hepatic lipase activity (total and hepatic lipase) from newborn to adult under normoxic conditions. At birth, hepatic lipase (assayed in 2.4 M NaCl) was relatively low and showed rapid increase during the first week of life. Thereafter, it plateaued at \( \sim 14–21 \) days of age and gradually declined to the adult level. Total lipase (assayed in 0.075 M NaCl) showed a similar pattern of rise from newborn to 7 days of age, leveled off, and declined to the adult level after weaning. Thus, by 7 days of age, most of the postnatal development of hepatic lipase had already occurred. There was no observable difference in the development profile between female and male rats (data not shown).

**DISCUSSION**

We have shown previously that hypoxia resulted in increases in plasma cholesterol, triglycerides, and non-esterified fatty acids in the suckling neonatal rats but not in weaned 35-day-old rats (26). Hepatic lipases play a critical role in lipid metabolism and modulate the plasma levels of many lipid metabolites (14, 17, 24). Hepatic lipase deficiency in humans results in the accumulation of serum HDL particles with elevated levels of triglycerides and phospholipids (4, 6, 8, 24). In animals, hepatic lipase enhances the uptake of cholesterol by hepatocytes in vitro (31). In vivo inhibition of hepatic lipase by antibodies to hepatic lipase decreases the rate of cholesterol uptake by the liver from chylomicron remnants (32). The same in vivo treatment with antihepatic lipase serum also resulted in increases in circulating triglycerides, phospholipids, and cholesterol content of lipoproteins (9, 18).

The present study evaluated the effects of 7 days of hypoxia on hepatic lipid contents and hepatic lipases in neonatal (suckling) and juvenile (weaned) rats. Hyp-
Hepatic lipase activity (assayed in the presence of 2.4 M NaCl). The lipase activity (assayed in the presence of 0.075 M NaCl); solid bars, number of animals varies from 4 to 8 for each age. Open bars, total sent the means

6

Fig. 4. Developmental profile of hepatic lipases under normoxic conditions. The date of birth was considered as day 0. Values represent the means ± SD from all animals killed at each time point. The number of animals varies from 4 to 8 for each age. Open bars, total lipase activity (assayed in the presence of 0.075 M NaCl); solid bars, hepatic lipase activity (assayed in the presence of 2.4 M NaCl). The vertical lines above the bars represent SD of the means.

Table 3. Effects of low dose of dexamethasone treatment from birth to 7 days of age on hepatic protein concentration and lipase activity

<table>
<thead>
<tr>
<th>Protein Concentration, mg/g liver</th>
<th>Lipase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.075 M NaCl</td>
</tr>
<tr>
<td></td>
<td>U·g liver⁻¹·min⁻¹</td>
</tr>
<tr>
<td>Control (6)</td>
<td>112.7 ± 8.1</td>
</tr>
<tr>
<td>Vehicle (6)</td>
<td>113.9 ± 7.2</td>
</tr>
<tr>
<td>DX (6)</td>
<td>120.9 ± 3.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. Numbers in parentheses represent the nos. of animals used. *Significantly different from corresponding values in the control group with P < 0.05. Newborn rats were given dexamethasone (DX) at a dose of 13 ng/g body wt or vehicle (same volume of dimethyl sulfoxide) daily from 0–6 days. Treated rats were killed at 7 days of age together with age-matched controls. At 0.075 M NaCl, both lipoprotein lipase and hepatic lipase are active. At 2.4 M NaCl, only hepatic lipase is active.

oxia induced significant increases in liver fat content with concomitant decreases in hepatic lipases in the 7-day-old hypoxic rats but not in the 35-day-old (weaned) rats. Both total (assayed in the presence of low ionic strength) and hepatic lipases (assayed in the presence of high ionic strength) were affected. These results coupled with the observed increases in plasma total cholesterol, triglycerides, and FFAs in the 7- but not 35-day-old hypoxic rats (26) and the reported involvement of hepatic lipases in the formation of dyslipidemia (16, 30) suggested a correlation between the decrease of hepatic lipase levels and the hyperlipidemia in the 7-day-old hypoxic rats. The decrease in hepatic and lipoprotein lipase may also contribute to the increase in lipid content in the liver of 7-day-old hypoxic rats.

Hypoxia also resulted in a slight but significant decrease in hepatic protein concentration in the 7- but not 35-day-old rats. This might reflect the decrease in enzyme proteins and, therefore, reduced lipase activities in the 7-day-old hypoxic animals. Other hepatic marker enzymes were evaluated to see whether this was the case. LDH activity showed >50% reduction in the 7-day-old hypoxic group but a slight increase (+15%) in the 35-day-old hypoxic group. Liver level of transaminase activity (ALT/GPT) showed no difference between the hypoxic and normoxic 7-day-old rats but a slight increase in the hypoxic 35-day olds. Microsomal CYP1A and 3A content were not different between control and hypoxic 7-day olds. Thus not all hepatic enzymes were reduced in the 7-day-old hypoxic group, and, therefore, the lower hepatic lipase found was relatively selective. The observed lowering of hepatic LDH activity might be partly responsible for the increase in plasma lactate level in the 7-day-old pups (unpublished results).

Developmentally, there is a rapid increase in hepatic lipase immediately after birth. Hepatic lipase activity reached a level more than twice that of the newborns by 7 days of age. It is feasible that hypoxia during this critical stage of ontogeny interferes with the normal development of hepatic lipases and results in abnormally low level of these enzymes. If this hypothesis is correct, then returning the hypoxic 7-day-old rats to normoxic conditions should free them from the physiological impairment (hypoxia) and allow them to develop normally and subsequently acquire the normal levels of lipase. This was indeed the case as hepatic 7-day olds, when returned to normoxic environment, regained a large portion of their hepatic lipase activity (85% of control) by 7 days and were completely recovered by 14 days posthypoxia. Interestingly, the plasma levels of total cholesterol and triglycerides were already normalized by 7 days posthypoxia. It is likely that the levels of hepatic lipases in these once hypoxic animals, although not reaching the levels found in age-matched controls, had reached a level that was sufficient for the maintenance of normal levels of plasma lipids. Furthermore, there are other factors, such as milk lipid contents from the dam, besides hepatic lipases that also play a role in modulating the plasma level of these lipid compounds. These factors might be affected by neonatal hypoxia but take less time than hepatic lipases to recover.

Although the exact mechanism for the effects of hypoxia on hepatic lipase development is not known, we speculated that hypoxia might act, at least in part, through modulation of the endocrine system. One of the observations that is more relevant to the present
study is that of a marked increase in plasma corticosterone during neonatal hypoxia (28). Glucocorticoids are known to have important functions in modulating the ontogeny of many enzyme systems, notably in the exocrine pancreas and the gastrointestinal tract (19), the liver (13), and the lung (2). In addition, we have shown previously that glucocorticoids inhibit the development of lipases in the rat lingual gland (22). In the present study, we attempted to simulate the increase in plasma glucocorticoid as observed in hypoxia by daily administration of a low dose of dexamethasone (a synthetic glucocorticoid with a longer biological half-life and higher potency than corticosterone) for the same duration as the hypoxic treatment in newborns from birth to 7 days of age. The dexamethasone-injected animals showed a decrease in hepatic lipases (75% of control) but to a lesser degree than in hypoxia (<45% of control). Liver protein concentration, unlike that of a reduction in the hypoxic rats, was not affected with dexamethasone. Thus the increase in glucocorticoid that resulted from hypoxic treatment might have contributed, in part, to the decrease in hepatic lipases but could not account for the entire action of hypoxia. A more definitive experiment is, however, required to establish a link between hypoxia, steroid hormones, and hepatic lipase activities.

In conclusion, the present study demonstrated that hypoxia in neonates causes dyslipidemia due, at least in part, to disturbance of lipid metabolism in the liver. Hypoxic conditions lead to lowering of hepatic lipases whose activities are important in maintaining the normal levels of plasma lipid components. Hypoxia might, in part, act through increases in plasma glucocorticoids in its effects on hepatic lipases.

**Perspectives**

Hypoxia is recognized as one of the most common neonatal syndromes. Conditions that result in hypoxia frequently occurred in the pre- and postnatal period. Fetal distress is the most common cause of prenatal hypoxia. Asphyxia at birth due to prematurity, trauma, blood loss, or obstruction of airway accounts for varying duration of hypoxia in infants. Hypoxia causes considerable morbidity and mortality in the neonate. Although the primary concerns of neonatal hypoxia are the detrimental effects on neurological, cardiopulmonary, and renal changes, the observed dysfunction in lipid metabolism might play an important role in the nutritional adaptation of the hypoxic infants, particularly during the recovery phase. Infants have a high rate of metabolism to provide for rapid growth (and repair if necessary). Lipid is a major source providing the necessary fuels for metabolism and building blocks for essential metabolites in growth and repair. Dysfunction of lipid metabolism in neonatal hypoxia will compromise the tissue uptake and utilization of nutrients. This might have a more profound effect on the health of the infant than previously appreciated.

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