Binge alcohol exposure in the second trimester attenuates fetal cerebral blood flow response to hypoxia

Dennis E. Mayock,1 Dana Ness,2 Robin L. Mondares,1 and Christine A. Gleason1
1Division of Neonatology, Department of Pediatrics, and 2Department of Comparative Medicine, University of Washington School of Medicine, Seattle, Washington

Submitted 29 August 2006; accepted in final form 17 November 2006

Mayock DE, Ness D, Mondares RL, Gleason CA. Binge alcohol exposure in the second trimester attenuates fetal cerebral blood flow response to hypoxia. J Appl Physiol 102: 972–977, 2007; doi:10.1152/japplphysiol.00956.2006.—Alcohol is detrimental to the developing brain and remains the leading cause of mental retardation in developed countries. The mechanism of alcohol brain damage remains elusive. Studies of neurological problems in adults have focused on alcohol’s cerebrovascular effects, because alcoholism is a major risk factor for stroke and cerebrovascular injuries. However, few studies have examined similar cerebrovascular effects of fetal alcohol exposure. We examined the effect of chronic binge alcohol exposure during the second trimester on fetal cerebrovascular and metabolic responses to hypoxia in near-term sheep and tested the hypothesis that fetal alcohol exposure would attenuate cerebrovascular dilation to hypoxia. Pregnant ewes were infused with alcohol (1.5 g/kg) or saline intravenously at 60–90 days of gestation (full term = 150 days). At 125 days of gestation, we measured fetal cerebral blood flow (CBF) and oxygen metabolism at baseline and during hypoxia. Maternal blood alcohol averaged 214 ± 5.9 mg/dl immediately after the 1.5-h infusion, with similar values throughout the month of infusion. Hypoxia resulted in a robust increase in CBF in saline-infused fetuses. However, the CBF response to hypoxia in fetuses chronically exposed to alcohol was significantly attenuated. Cerebral oxygen delivery decreased in both groups of fetuses during hypoxia but to a greater degree in the alcohol-exposed fetuses. Prenatal alcohol exposure during the second trimester attenuates cerebrovascular responses to hypoxia in the third trimester. Altered cerebrovascular reactivity might be one mechanism for alcohol-related brain damage and might set the stage for further brain injury if a hypoxic insult occurs.

The detrimental effects of alcohol on the developing fetus have been perceived since biblical times (15). Today, it is estimated that the combined incidence of fetal alcohol-related neurodevelopmental disorders and fetal alcohol syndrome (FAS) is ~1 in every 100 live births (46). Central nervous system features of FAS include mental retardation, neuronal migration defects (heterotopia), behavioral problems, and poor brain growth. Although considerable research has been focused on understanding alcohol’s detrimental effects on the developing brain, the mechanisms remain largely unknown. Much of this research effort has focused on alcohol’s direct neurotoxic effects (52, 53).

Studies in adult humans and animals suggest that, in addition to neurotoxic effects, chronic alcohol exposure impairs vascular function. Adult alcoholic patients are at high risk of stroke and cerebral ischemic injury (2, 4, 54). The neuropathy seen in adult alcoholics includes periventricular vessel sclerosis with perivascular gliosis (49). These changes suggest that vascular damage might lead to cerebral injury. In adult rats, chronic alcohol exposure is associated with impaired cerebral vasodilation, while vasoconstriction is preserved (34). Impaired endothelium-dependent vasodilation of cerebral arterioles has also been described in alcohol-fed rats due, in part, to impaired synthesis and/or release of nitric oxide (22, 35). Although a great deal of research effort has focused on the long-term vascular effects of alcohol exposure in the adult, little information is available regarding alcohol’s effect on the fetal vasculature. We previously reported that early-gestation fetal alcohol exposure resulted in significant blunting of the cerebral vasodilatory response to hypoxia in newborn sheep (21). We speculated that abnormal cerebral vascular function may be one mechanism for alcohol’s damaging effects.

Different times of alcohol exposure appear to yield different effects on the fetus. Third-trimester exposure to high doses of alcohol tends to result in microcephaly (reduced brain weight-to-body weight ratio) (45), while second- and third-trimester exposure is associated with low birth weight (24, 36). Brain regions affected by neuronal loss from first-trimester exposure are different from those affected by late-term exposure (44). In developing rats exposed to alcohol during the brain growth spurt, capillary diameter in the cerebellum and hippocampus was decreased (32). Although it is generally accepted that heavy drinking throughout pregnancy results in the FAS phenotype, the effects of alcohol exposure throughout pregnancy cannot be easily predicted from the effects of shorter periods of exposure (50).

We wondered whether chronic prenatal alcohol exposure would similarly attenuate fetal hypoxic cerebral vasodilation and whether this attenuated response could limit fetal cerebral oxygen delivery. We chose this second-trimester binge alcohol model for the following reasons. 1) Because the pattern of drinking in pregnant women is more commonly a binge pattern, it is more clinically relevant. 2) The second trimester is considered to be a particularly vulnerable time, since alcohol-induced neuronal loss in rats is reportedly greatest with prenatal alcohol exposure during the second trimester (38). In sheep, the brain growth spurt occurs in the second trimester (18). Taken together, the most vulnerable period with respect to growth of the developing fetal sheep brain is likely to be the second trimester.

We chose to study fetal sheep, since a wealth of information regarding fetal cerebral physiology is available for comparison.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: D. E. Mayock, Pediatrics, Box 356320, Univ. of Washington, Seattle, WA 98195-6320 (e-mail:mayock@u.washington.edu).
and we had previously used newborn sheep to study cerebral vascular effects of fetal alcohol exposure. This model also avoids the potential confounding effects of anesthesia (21).

MATERIALS AND METHODS

All experimental protocols were approved by Animal Care and Use Committees at the University of Washington and the University of Idaho. Mixed-breed ewes were used, and pregnancy was time dated. Full-term gestation is 150 days in the sheep. We studied 13 near-term (6 saline-control and 7 alcohol-exposed) fetal sheep at 125 ± 2 days of gestation.

Maternal infusion protocol. Pregnant ewes were randomized to receive daily (Monday–Friday) intravenous infusions of alcohol (n = 7) or saline (n = 6) from 60 to 90 days of gestation. Infusions were performed at the University of Idaho Animal and Veterinary Science Department farm (Moscow, ID). Jugular catheters were placed using sterile technique at 60 days of gestation. Catheter patency was maintained by heparin flush (1,000 U/ml). Alcohol (1.5 g of pure ethanol per kilogram of ewe weight, 5.7 ml/kg of 33% solution) or the equivalent volume of normal saline was infused over 1.5 h. Once the daily infusion protocol was complete, the jugular catheter was removed.

Maternal blood levels of glucose, lactate, and alcohol were measured before and after an infusion on the second and last day of each infusion week. Samples were obtained in this manner during all 4 wk of infusion. Blood glucose, lactate, and alcohol concentrations were measured enzymatically by glucose oxidase, lactate dehydrogenase, and alcohol dehydrogenase oxidation utilizing standard assay kits (Sigma Diagnostics, St. Louis, MO; Diagnostic Chemical, Oxford, CT).

Fetal surgical preparation. For 24 h before fetal surgery, food was withheld from the ewes, but the animals were allowed free access to water. Fetal surgery was performed using sterile technique at 120–123 days of gestation. The ewe was premedicated with atropine (0.2 mg/kg im) and xylazine (0.1 mg/kg im) and then anesthetized with inhaled isoflurane (0.5–3%). The trachea was intubated, and the ewe was mechanically ventilated. A 16-gauge venous catheter was placed percutaneously in a maternal jugular vein for fluid administration during surgery. After the abdominal skin was prepared, the uterus was exposed through a midline incision. The top of the fetal head and limbs were exposed one at a time through small uterine incisions for placement of catheters into the superior sagittal sinus, the brachiocephalic trunk (via axillary arteries), and the inferior vena cava (IVC) via pedal veins. A catheter (Tygon tubing) was sewn to a fetal hoof for measurement of amniotic fluid pressure. Fetal vascular catheters were filled with heparinized saline (10 U/ml). All incisions were closed with sutures. The catheters were exteriorized to the ewe’s flank, where they were secured in a pouch. The ewe received penicillin G benzathine (Bicillin, 1.2 × 10^6 U im) on completion of surgery. Ampicillin (500 mg) was administered into the amniotic fluid via the amniotic fluid catheter, and the estimated volume of lost amniotic fluid was replaced with warmed saline. Fetuses were studied 48 h after surgery. Maternal analgesia was maintained with buprenorphine (0.005 mg/kg h i.m.) administered to the ewe was carefully adjusted to maintain the desired 50% decrease in fetal SaO2 from baseline. Once two fetal arterial samples separated by 2 min were at the desired saturation level, fetal arterial and sagittal sinus venous samples were obtained for blood gas analysis and for blood flow calculations. CBF was then assessed with microspheres. These measurements were repeated in a similar fashion ~10 min later.

Data analysis/calculations. We utilized SSP as an estimate of intracranial pressure (ICP). Cerebral perfusion pressure (CPP) was calculated as the difference between mean arterial pressure (MAP) and SSP. CBF was calculated as follows: CBF = CPMbrain/CPMref × reference withdrawal rate (in ml/min), where CPMbrain and CPMref represent radioactivity counts per minute in brain and reference samples, respectively. Cerebral O2 consumption (CMRO2) was calculated as follows: CMRO2 = [CaO2 – CVO2] × CBF, where CaO2 and CVO2 represent cerebral arterial and sagittal sinus venous oxygen content, respectively. Cerebral oxygen delivery (OD) was calculated as follows: OD = CaO2 × CBF. Cerebral oxygen extraction was calculated as follows: (CaO2 – CVO2)/CaO2. Cerebral vascular resistance (CVR) was calculated as follows: CVR = (MAP – SSP)/CBF.

Measurements were calculated and data are reported as means ± SE for all study fetuses. Differences between groups were analyzed by two-way repeated-measures ANOVA. If the F test was significant, specific differences were sought with the Student-Newman-Keuls test. Significance was assumed at P < 0.05.

RESULTS

Maternal blood alcohol levels were measured on the 2nd day and the last day of each 5-day infusion period during all 4 wk of infusion. Blood alcohol levels, before and after infusion, were similar on days 2 and 5 of infusion over the 4 wk of infusion and, therefore, were averaged. Maternal blood alcohol levels averaged 214.2 ± 5.9 mg/dl immediately after the 1.5-h infusion. Maternal blood glucose and lactate concentrations were measured before and after infusion in both groups of
areas examined. However, flow to various brain regions was
increasing only 48%
exposed fetuses, the increase was significantly attenuated,
from baseline in the saline-control fetuses. In the alcohol-
hypoxia. In response to hypoxia, CBF increased by 90%
levels decreased significantly after the alcohol infusion. Blood
lactate levels increased after alcohol infusion but decreased in
the saline-control animals.

We examined CBF responses to hypoxia in 13 near-term
fetal sheep [6 saline-control (all male) and 7 alcohol-exposed
(5 male and 2 female) animals] at 125 ± 2 days of gestation.
Two baseline measurements were made in both groups of
fetuses. Since data from both of these assessments were similar
in both groups of fetuses, they were averaged for presentation
(Table 2). Two comparisons were not noted between groups for any
of the baseline measurements (Table 2). Because hypoxia
resulted in similar changes from baseline in both groups of
fetuses at both hypoxia points, averaged values are presented
(Table 2). As expected, arterial P O2 and C aO2 decreased during
hypoxia. In response to hypoxia, CBF increased by 90 ± 15%
from baseline in the saline-control fetuses. In the alcohol-
exposed fetuses, the increase was significantly attenuated,
increasing only 48 ± 10% from baseline (Fig. 1). Regional
brain blood flow increased significantly during hypoxia in all
areas examined. However, flow to various brain regions was
significantly attenuated in alcohol-exposed fetuses (Fig. 2).

Table 2. Baseline fetal physical and
physiological measurements

<table>
<thead>
<tr>
<th></th>
<th>Saline (n = 6)</th>
<th>Alcohol (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>3.09 ± 0.21</td>
<td>3.38 ± 0.21</td>
</tr>
<tr>
<td>Brain wt, g</td>
<td>42.9 ± 1.7</td>
<td>42.9 ± 1.9</td>
</tr>
<tr>
<td>Twins or triplets, %</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>178 ± 4</td>
<td>167 ± 5</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>46 ± 2</td>
<td>40 ± 1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.37 ± 0.01</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td>P aO2, Torr</td>
<td>46 ± 1</td>
<td>44 ± 1</td>
</tr>
<tr>
<td>P aCO2, Torr</td>
<td>20 ± 1</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>S aO2, %</td>
<td>62 ± 4</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>CBF, ml 100 g⁻¹ min⁻¹</td>
<td>103.8 ± 11.5</td>
<td>116.0 ± 7.5</td>
</tr>
<tr>
<td>Hemoglobin, g/100 ml</td>
<td>10.34 ± 0.51</td>
<td>10.38 ± 0.20</td>
</tr>
<tr>
<td>CVR, mmHg/100 ml</td>
<td>0.43 ± 0.03</td>
<td>0.43 ± 0.04</td>
</tr>
<tr>
<td>C aO2, ml/100 ml</td>
<td>8.65 ± 0.83</td>
<td>8.37 ± 0.41</td>
</tr>
<tr>
<td>Cerebral O2 consumption, ml 100 g⁻¹ min⁻¹</td>
<td>2.69 ± 0.22</td>
<td>3.07 ± 0.16</td>
</tr>
<tr>
<td>Cerebral O2 delivery, ml 100 g⁻¹ min⁻¹</td>
<td>9.32 ± 0.94</td>
<td>9.70 ± 0.65</td>
</tr>
<tr>
<td>Cerebral O2 extraction</td>
<td>0.29 ± 0.02</td>
<td>0.30 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. P aO2 and P aCO2, arterial P O2 and P aCO2; S aO2, arterial O2 saturation; CBF, cerebral blood flow; CVR, cerebral vascular resistance; C aO2, arterial O2 content. There were no statistical differences between groups for any parameter.

Table 3. Fetal hypoxia responses

<table>
<thead>
<tr>
<th></th>
<th>Saline (n = 6)</th>
<th>Alcohol (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial pH</td>
<td>7.37 ± 0.01</td>
<td>7.33 ± 0.01</td>
</tr>
<tr>
<td>P aO2, Torr</td>
<td>46 ± 1</td>
<td>44 ± 1</td>
</tr>
<tr>
<td>P aCO2, Torr</td>
<td>20 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>C aO2, ml/100 g⁻¹ min⁻¹</td>
<td>8.7 ± 0.4</td>
<td>4.2 ± 0.2*</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>46 ± 2</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>178 ± 4</td>
<td>158 ± 7*</td>
</tr>
<tr>
<td>CMRO2, ml/100 g⁻¹ min⁻¹</td>
<td>2.69 ± 0.22</td>
<td>2.54 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± SE. Baseline and hypoxia values are averaged of 2 time points. CMRO2, cerebral metabolic rate. *P < 0.05 vs. baseline.

Despite the increase in CBF, cerebral oxygen delivery decreased during hypoxia in both groups of fetuses (Fig. 3). The decrease was greater in the alcohol- than in the saline-exposed fetuses (P = 0.04). There was no change in oxygen extraction in either group of fetuses from baseline to hypoxia.

CVR decreased significantly in both groups of fetuses during hypoxia (Fig. 4). The decrease was greater in the saline- than in the alcohol-exposed fetuses (P = 0.039). MAP increased significantly during hypoxia in the alcohol-exposed, but not in the saline-control, group (Table 3).

DISCUSSION

The major finding of this study is that binge fetal alcohol exposure in the second trimester attenuates fetal cerebral vasodilatory responses to hypoxia in the third trimester. This attenuation of hypoxic vasodilation limits fetal oxygen delivery to the brain during hypoxic episodes, which could contribute to fetal alcohol-induced brain injury. Our findings are consistent with previous studies in newborn lambs exposed to alcohol in utero (21).

The cerebral vasodilatory response to hypoxia has been demonstrated in preterm and near-term fetal sheep, newborn lambs, and adult sheep (30). Similar vascular responses have been described in most other species, including humans (14). Cerebral vasodilation occurs as the brain tries to maintain
adequate cerebral oxygen delivery, despite reduced oxygen content. In earlier studies using radiolabeled microspheres, even during hypoxia, the near-term fetal sheep brain maintained its baseline cerebral oxygen delivery by increasing CBF and, if necessary, increasing cerebral oxygen extraction to meet tissue oxygen needs (25, 30). In the preterm fetal sheep, however, similar levels of hypoxia result in significant compromise of cerebral oxygen delivery and limited ability to maintain cerebral oxygen needs (20). The present study demonstrates that alcohol attenuates the cerebral vasodilatory response to hypoxia in near-term fetuses, and this response is similar to that noted in preterm fetuses during hypoxia. However, as with most fetal sheep studies, our relatively small number of animals means that our results may not have been powered enough to exclude the possibility of type II error. We could speculate that fetal alcohol exposure delays normal cerebrovascular maturation.

Recent investigations utilizing a new technique based on cerebral heat production and laser-Doppler flowmetry have suggested that increased CBF during moderate hypoxia may not maintain adequate regional oxygen delivery in the fetal sheep. In their study of near-term fetal sheep, Hunter and colleagues (28) found that cerebral metabolic rate fell significantly during moderate hypoxia, as reflected by cerebral tissue temperature changes. They concluded that the increase in CBF did not result in maintenance of cerebral oxygen delivery. However, the increase in fetal CBF in their investigation with similar levels of hypoxia was less than predicted from other studies (20). Indeed, Bishai et al. (11) found that the change in CBF measured by laser-Doppler flowmetry during hypoxia significantly underestimated that measured by the microsphere technique. Further studies by these same investigators utilizing this technique demonstrated that adenosine may mediate the increase in CBF and the decrease in cerebral metabolic rate during hypoxia (12). On the basis of this new technique, more careful studies of fetal regional cerebral responses to various interventions would be possible and might yield results that are different from those previously reported.

In our alcohol-exposed near-term fetuses, the attenuated cerebral vasodilatory response to hypoxia was associated with a similarly attenuated decrease in CVR (Fig. 3). Although both groups demonstrated a significant decrease in CVR from baseline normoxic levels, the decrease was significantly less in the alcohol-exposed than in the saline-control animals. Moreover, MAP increased in alcohol-exposed fetuses during hypoxemia, a response that should have resulted in a further increase in CBF if cerebral autoregulatory capability was compromised. Despite the increase in CPP and the decrease in CVR, cerebral oxygen delivery was still compromised. These changes indicate that prenatal alcohol exposure alters fetal vessel reactivity to hypoxia, although the mechanism is yet to be determined.

The attenuation of fetal hypoxic cerebral vasodilation associated with prior alcohol exposure we observed may be due to one or more factors. Prenatal alcohol exposure has been demonstrated to cause retardation of central nervous system development (17, 19, 37). If alcohol stunts vascular growth (32), this may account for the attenuated response to hypoxia, similar to that seen in more immature fetuses (20). However, because the cerebrovascular response to hypercapnia is not altered by alcohol exposure, this explanation is unlikely (21). Alcohol may indirectly affect developing fetal vessels by causing intermittent fetal hypoxia and/or placental insufficiency. High concentrations of alcohol produce spasm of umbilical vessels (3, 41), and chronic alcohol consumption decreases placental blood flow in rats (31). However, a study in sheep demonstrated that, during late-term gestation, exposure to high amounts of alcohol in binge-type dosing did not induce acute hypoxia in the fetal sheep (16).

Alcohol might influence cerebral vasomotor tone by altering sensitivity to neurotransmitter levels or receptor function.
Chronic alcohol exposure leads to potentiated epinephrine vasoconstriction in rats (1). In the alcohol-exposed fetus, the increased sympathetic sensitivity during hypoxia could counteract hypoxic vasodilation. Altered vasomotor tone could also be due to altered neurotransmitter levels. Serotonin neuron development is altered in alcohol-exposed fetal mice (55). Additionally, receptor function could be impaired. In young guinea pigs exposed to alcohol as fetuses, GABA receptors are altered (10). In preliminary work, we demonstrated that alcohol exposure early in gestation was associated with fewer vasoactive intestinal peptide-producing neurons in fetal sheep (9). Decreased expression of vasoactive intestinal peptide, a potent cerebral vasodilator, might be responsible, in part, for impaired hypoxic cerebral vasodilation. Ethanol induces lipid peroxidation and nuclear factor-κB activation in cerebral vascular smooth muscle, changes that may trigger proinflammatory events and apoptosis (5, 7, 33). Cerebral vessel alcohol exposure recruits leukocytes with vessel damage and inflammation (6). Brain alcohol exposure also decreases intracellular magnesium and high-energy metabolites (8). Finally, alcohol infusion led to decreased maternal blood glucose levels and increased lactate levels in our study. These changes certainly must have a negative effect on the fetus, but the extent of this compromise is not known. In preliminary work, we have demonstrated increased levels of glucose transporter protein 1 in fetal brain tissue after binge alcohol exposure. This change suggests that blood glucose levels were decreased in fetuses exposed to alcohol in utero (39). These factors, separately or in combination, could contribute to abnormal vasomotor tone or vasoactivity in the alcohol-exposed fetus. Vaso dilatory responses have been shown to be altered in studies of chronic alcohol exposure in adult animals. Mechanisms for this altered response could include abnormal synthesis of, release of, or response to endothelium-derived vasodilatory substances, such as adenosine or acetylcholine. Hypoxic cerebral vasodilation is mediated by adenosine in adult (40) and fetal brain (12, 23, 28) in direct proportion to the severity of hypoxia. Cerebral vessels from adult rats chronically exposed to alcohol do not vasodilate normally in response to adenosine, (34, 48) and actually vasoconstrict in response to acetylcholine (34). Fetal sheep studies implicate adenosine in the increase in CBF and the decrease in cerebral metabolism during hypoxia (12). Activation of the adenosine A1 receptor during asphyxia appears to have a neuroprotective effect in the fetal sheep by suppressing neural activity (27). Endothelium-dependent vasodilation of cerebral arterioles is impaired in alcohol-fed rats and is believed to be due in part to impaired synthesis and/or release of nitric oxide (22, 35).

We chose our study model because of our previous experience and that of others and because of the wealth of cerebral physiological data available in the sheep (21, 43). Additionally, we chose a high level of infused alcohol to replicate binge drinking, since most investigators believe that FAS is caused by heavy maternal alcohol abuse (50). The blood alcohol levels achieved in our study (214.2 ± 5.9 mg/dl) greatly exceed the legal limit for driving while intoxicated in most states. In humans, significant narcosis occurs at blood alcohol levels of 200 mg/dl; stupor and coma occur at 300 mg/dl. A recent survey by the Centers for Disease Control and Prevention found that 10% of pregnant women used alcohol and 2% engaged in binge drinking (51).

In conclusion, the results of this study demonstrate a significant attenuation of hypoxic cerebral vasodilation in the late-gestation fetal sheep chronically exposed to alcohol in the second trimester. This diminished ability of the alcohol-exposed fetal cerebral circulation to vasodilate when confronted with hypoxia limits cerebral oxygen delivery. This attenuation of hypoxic cerebral vasodilation places the fetus at increased risk of brain injury during such a stress. The inability of the alcohol-exposed sheep fetus to adequately increase CBF during hypoxia might explain the similar brain injury pattern in fetuses exposed to hypoxia or alcohol alone (13). Secondary insults such as maternal smoking and drug (e.g., cocaine) abuse may result in brain injury in a fetus previously rendered more vulnerable to hypoxia or ischemia by alcohol exposure. Cumulative effects of the direct toxic effects of alcohol, in addition to hypoxic injury, may explain the wide range of pathological findings in alcohol-related neurodevelopmental disorder and alcohol-related behavior disorder (29).

GRANTS

This study was supported by National Institute of Alcohol Abuse and Alcoholism Grant R01 AA-012403.

REFERENCES