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The Role of Acidemia in Maternal Binge Alcohol-Induced Alterations in Fetal Bone Functional Properties

Onkar B. Sawant^a, Jayanth Ramadoss, PhD^c, Harry A. Hogan, PhD^b, and Shannon E. Washburn, DVM, PhD^a

^aDepartment of Veterinary Physiology and Pharmacology and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, Texas, 77843, USA

^bDepartment of Mechanical Engineering, Texas A&M University, College Station, Texas, 77843, USA

^cDepartment of Obstetrics and Gynecology, University of Texas Medical Branch, Galveston, Texas, 77555, USA

Abstract

Background—Heavy alcohol consumption during pregnancy negatively impacts the physical growth of the fetus. Though the deleterious effects of alcohol exposure during late gestation on fetal brain development are well documented, little is known about the effect on fetal bone mechanical properties or the underlying mechanisms. The purpose of this study was to investigate the effects of late gestational chronic binge alcohol consumption and alcohol-induced acidemia, a critical regulator of bone health, on functional properties of the fetal skeletal system.

Methods—Suffolk ewes were mated and received intravenous infusions of saline or alcohol (1.75 g/kg) over 1 hour on 3 consecutive days per week followed by 4 days without treatment beginning on gestational day (GD) 109 and concluding on GD 132 (term= 147 days). The acidemia group was exposed to increased inspired fractional concentrations of CO₂ to closely mimic the alcohol-induced decreases in maternal arterial pH seen in the alcohol group.

Results—Fetal femurs and tibias from the alcohol and acidemia groups were ~3-7% shorter in length compared to the control groups ($P<0.05$). Three point bending procedure demonstrated that fetal femoral ultimate strength (MPa) for the alcohol group was decreased ($P<0.05$) by ~24% and ~29% while the acidemia group exhibited a similar decrease ($P<0.05$) of ~32% and 37% compared to the normal control and saline control groups, respectively. Bone extrinsic and intrinsic mechanical properties including maximum breaking force (N) and normalized breaking force (N/kg) of fetal bones from the alcohol and acidemia groups were significantly decreased ($P<0.05$) compared to both control groups.

Correspondence concerning this article should be addressed to: Shannon E. Washburn, Department of Veterinary Physiology and Pharmacology and Michael E. DeBakey Institute, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4466, USA. swashburn@cvm.tamu.edu. Tel: 979-845-1993 Fax: 979-845-6544.

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Conclusions—We conclude that late gestational chronic binge alcohol exposure reduces growth and impairs functional properties of the fetal skeletal system and that the repeated episodes of alcohol-induced maternal acidemia may be at least partially responsible for these effects.

Keywords

Pregnancy; Fetal alcohol spectrum disorder; Teratogenicity; Acidemia; Bone strength

1. Introduction

Fetal alcohol spectrum disorders (FASD) is an umbrella term encompassing the full range of effects that can occur in an individual whose mother consumed alcohol during pregnancy. These include effects on physical, behavioral or cognitive development that can persist as lifelong disabilities, with the most severe end of the spectrum being fetal alcohol syndrome (FAS) (Abel, 1984; Streissguth et al., 1980; Warren et al., 2001). Facial abnormalities, growth deficits (whole body and bone), and central nervous system abnormalities are the primary defining diagnostic features of FAS (Riley et al., 2011). In spite of efforts to educate women about the teratogenic effect of alcohol, the prevalence of alcohol consumption in women of child-bearing age remains essentially unchanged over the last four decades (Caetano et al., 2006; CDC, 2004; NIAAA, 2000).

Alcohol is not only a neurotoxic teratogen and has the potential to cause damage in many regions of the brain but also has major detrimental effects on fetal bone development (Simpson et al., 2005; Snow and Keiver, 2007). In rats, maternal alcohol consumption (36% alcohol derived calories) throughout gestation increases maternal bone resorption (Keiver and Weinberg, 2003; Sawant et al., 2012; Washburn et al., 2012), decreases the fetal tibial diaphysis length, disrupts organization of the histological zones within the fetal tibial epiphyses, decreases the length of the resting zone, and increases the length of the hypertrophic zone (Snow and Keiver, 2007). Further, alcohol exposure throughout gestation decreases ovine fetal but not maternal bone strength (Ramadoss et al., 2006), and hampers rat fetal skeletal ossification independent of overall fetal growth restriction (Simpson et al., 2005). No study has yet quantified the effect of late gestational chronic binge alcohol exposure on fetal skeletal extrinsic and intrinsic properties such as maximum bone breaking force (N), normalized maximum bone breaking force (N/kg), cross sectional moment of inertia (CSMI) and ultimate bone strength (MPa). In essence, there is a paucity of studies on the cortical bone mechanical properties during critical windows of vulnerability.

We also wished to address the role of acidemia in alcohol-induced altered fetal cortical bone development, as alcohol exposure in human clinical cases is known to result in mixed respiratory and metabolic acidosis (Lamminpää and Vilska, 1990; 1991; Sahn et al., 1975; Zehtabchi et al., 2005). In animal models, acidemia has been described as a candidate mechanism for alcohol-induced developmental neuronal injury and altered amino acid homeostasis (Cudd et al., 2001; Horiguchi et al., 1971; Ramadoss et al., 2007; 2008b). Although the consequence of these repeated bouts of alcohol-induced acidemia on the skeletal growth of the offspring is unknown, acid base imbalance is known to hamper bone development by altering the bone minerals in order to maintain a stable physiological pH

(Arnett, 2003; Barzel, 1995; Bergstrom and Ruva, 1960; Bettice and Gamble, 1975; Bushinsky et al., 1986; 1996; 1999; Green and Kleeman, 1991). Therefore, we hypothesized that late gestation chronic binge alcohol exposure alters bone development as measured by mechanical properties and that these alterations are mediated by acidemia. The beginning of endochondral ossification, an initial process of appendicular bone chondrification, starts during the early gestational period whereas bone remodeling, a lifelong process of simultaneous bone formation and resorption, begins during the mid to late gestational period. This study will specifically evaluate the effect of late gestational chronic binge alcohol-induced acidemia on fetal body growth, and the physical and mechanical properties of the fetal bones.

2. Methods

2.1. Subjects

The experimental procedures were approved by the Institutional Animal Care and Use Committee at Texas A&M University. Suffolk ewes (aged 2–6 years) were mated on gestational day (GD) 0 and maintained outdoors until GD 90 as previously described (Cudd et al., 2001; West et al., 2001). Pregnancies of known date of conception were confirmed, as previously described (Cudd et al., 2001; Ramadoss et al., 2006). On GD 90, the ewes were transferred to an environmentally regulated facility (22°C and a 12:12 light-dark cycle), and they remained there for the duration of the experiments. Animals in all treatment groups were fed 2 kg/day of a “complete” ration (Sheep and Goat Pellet, Producers Cooperative, Bryan, TX) and complete ration was consumed each day by all subjects.

2.2. Surgery protocol

On GD 102 the ewes underwent surgery to implant femoral arterial and venous poly-vinyl chloride catheters (0.05” inner diameter, 0.09” outer diameter) as previously described (Cudd et al., 2001). In brief, anesthesia was induced by administering diazepam (0.2mg/kg intravenously; Abbott Laboratories, North Chicago, IL) and ketamine (4 mg/kg intravenously, Ketaset®; Fort Dodge, IA). The ewes were intubated and a surgical plane of anesthesia was maintained using isoflurane (0.5% to 2.5% IsoFlo®; Abbott Laboratories) and oxygen. Arterial and venous catheters were advanced into the aorta and vena cava via the femoral artery and vein, respectively. At the end of surgery, the ewe received an injection of flunixin meglumine (1.1 mg/kg intramuscularly, Banamine®; Scherring-Plough, Union, NJ), a prostaglandin synthase inhibitor to reduce postoperative pain. Ewes also received postoperative antibiotics (ampicillin trihydrate, Polyflex® (25 mg/kg administered subcutaneously for 5 days); Aveco, Fort Dodge, IA and gentamicin sulfate, Gentavet® (2 mg/kg administered intramuscularly twice daily for 5 days); Velco, St. Louis, MO.

2.3. Treatment groups

Four treatment groups were used in this study: 1. untreated normal control group (N= 13), 2. saline control group (received 0.9% saline; N= 7), 3. an alcohol group that received alcohol at a dosage of 1.75 g/kg body weight (40% w/v diluted in 0.9% saline; peak blood alcohol concentration 166.64 ± 12.45 mg/dl; N= 15), 4. an acidemia group, where the magnitude and pattern of decreases in maternal arterial pH produced by alcohol was mimicked for the

whole exposure period, independent of alcohol by manipulating the inspired fractional concentration of carbon dioxide (received saline in lieu of alcohol; N= 9). Normoxemic conditions were maintained throughout the experiment in the acidemic group. As we have previously reported that third trimester binge alcohol exposure at the dose used in the current study (1.75 g/kg) does not result in fetal hypoxemia (Cudd et al., 2001).

2.4. Experiment protocol

The experiment was conducted on three consecutive days per week, followed by 4 days without treatment, beginning on GD 109 until GD 132 to mimic a weekend binge drinking pattern common in women who abuse alcohol during pregnancy (Maier and West, 2001; Ramadoss et al., 2008a). In all treatment groups, the infusion solutions were delivered intravenously over one hour by peristaltic pump (Masterflex, model 7014-20 Cole Parmer, Niles, IL) and pumps were calibrated before each infusion. On the day of experiment, ewes in all treatment groups were placed in a modified metabolism cart, so that the animal's head was inside a Plexiglas chamber (Figure 1). A vinyl diaphragm attached to the open side of the chamber was drawn around the animal's neck to isolate the atmosphere in the chamber from ambient air (Ramadoss et al., 2011). In the acidemia group, subjects were exposed to increased inspired fractional concentrations of carbon dioxide for 6 hours, to create a similar pattern of reduction in maternal arterial pH (pH_a) as that produced by alcohol (Table 1) (Cudd et al., 2001). In all other groups, the bottom of the chamber was removed to allow them to breathe room air. Blood was drawn from the maternal femoral arterial catheter on all experiment days at 0, 0.5, 1, 1.5, 2, 3, 4, 5, and 6 hours from the start of the infusion for pH_a analysis using a blood gas analyzer (ABL 5; Radiometer, Weslake, OH). Fold change in the maternal pH_a from the baseline (baseline pH_a , saline control, 7.47 ± 0.00 ; alcohol, 7.50 ± 0.00 ; acidemia, 7.48 ± 0.01) is shown in Table 1. The saline control group was significantly different from the alcohol and acidemia groups at all the time points ($P < 0.001$). There were no differences between the alcohol and acidemia groups at any time point except at 1 and 4 hours. Previously we have shown that late gestational binge alcohol exposure from GD 109 to GD 132 results in maternal as well as fetal acidemia (Cudd et al., 2001), and hence we did not instrument the fetuses in the current study. Maternal blood alcohol concentrations (BACs) were determined as described previously (Cudd et al., 2001; Penton, 1985). On GD 133, the ewes were euthanized using pentobarbital sodium (75 mg/kg IV, Beuthanasia®; Schering-Plough Animal Health). Fetuses were removed from the uterus and fetal body weights and crown-rump lengths were measured and recorded. Fetal femurs and tibias were collected, cleaned, and stored at -20°C . Femoral and tibial lengths, anterior-posterior (AP) and mediolateral diameters (AP) were measured at middiaphyseal region using a precision caliper.

2.5. Mechanical Testing

Prior to the mechanical testing using a three-point bending procedure, bones were thawed overnight to room temperature. For femur tests, the bones were placed on two lower stationary supports separated by 50 mm distance with the posterior surface of the bone contacting the upper moving contact; for tibia tests the supports were 65 mm apart. The upper moving contact was advanced at a slow, quasistatic displacement rate of 5.08 mm/min (0.2 in/min) until complete fracture of the bone specimen occurred. Three-point bending

procedures were conducted on an Instron (Norwood, MA, USA) 1125 load frame with force measured by a load cell attached to the upper contact. The maximum breaking force (N) during the test was recorded for each bone. Maximum breaking force for the individual fetus was normalized by the fetal weight on GD 133 (N/kg). After the three-point bending procedure, a thin cross section of bone was cut adjacent to the point of fracture and a digital image was made. Cross-sectional moment of inertia (CSMI) was calculated from these images using SigmaScan software (Systat Software Inc., San Jose, CA, USA). Rectangular CSMI was taken about the ML axis through the centroid of the cross-section and perpendicular to the plane of loading for the bending tests. Ultimate bone strength was estimated using classical beam bending theory equation. Ultimate bone strength (MPa) is $S = (FLd)/(8I)$ where F is the maximum breaking force (N), L the distance between two stationery supports (50 or 65 mm), d is the middiaphysis AP diameter (mm), and I is the rectangular CSMI (mm⁴).

2.6 Data Analysis

Physical and mechanical properties of the bones were measured using a one-way ANOVA. Further pairwise comparisons were done using Student-Newman-Keuls method. Statistical significance was determined *a priori* to be $P < 0.05$.

3. Results

3.1 Fetal Growth parameters

There was no significant effect of treatment on fetal whole body weight (kg) on GD 133 (Table 2). Fetal crown-rump length (cm) was significantly different among groups (ANOVA $P < 0.001$). The alcohol and acidemia groups had significantly shorter fetal crown-rump length than the saline control group ($\downarrow 9\%$, $P = 0.003$ and $\downarrow 11.5\%$, $P < 0.001$ respectively) but not the normal control group.

3.2 Bone Dimensions

3.2.1 Bone Length—Fetal femoral lengths were significantly different among groups (ANOVA, $P < 0.001$) (Table 3). Fetal femurs from the alcohol and acidemia groups were shorter than the normal control ($\downarrow 4\%$, $P = 0.004$ and $\downarrow 5\%$, $P = 0.003$ respectively) and the saline control groups ($\downarrow 6\%$, $P = 0.002$ and $\downarrow 7\%$, $P = 0.001$ respectively). Fetal tibial lengths were significantly different among groups (ANOVA, $P = 0.004$). Fetal tibias from the alcohol and acidemia groups were shorter than the normal control ($\downarrow 3\%$, $P = 0.043$ and $\downarrow 5\%$, $P = 0.010$ respectively) and the saline control groups ($\downarrow 4\%$, $P = 0.056$ and $\downarrow 6\%$, $P = 0.013$ respectively) groups. No differences were noted between the normal and saline control groups.

3.2.2 Bone Diameters—Fetal femoral mediolateral (ML) and anterior-posterior (AP) diameters (mm) were significantly different among groups (ANOVA, $P < 0.001$) (Table 3). Fetal femoral ML diameter for the alcohol and acidemia groups were smaller than the normal control group ($\downarrow 8\%$, $P < 0.001$ and $\downarrow 8\%$, $P = 0.001$ respectively) and the saline control group ($\downarrow 8\%$, $P = 0.009$ and $\downarrow 8\%$, $P = 0.015$ respectively). Fetal femoral AP diameter for the alcohol and acidemia groups were smaller than the normal control group ($\downarrow 8\%$, $P < 0.001$ and $\downarrow 9\%$, $P = 0.001$ respectively). Fetal tibial ML and AP diameters were significantly different

among groups (ANOVA, $P<0.001$) (Table 3). Fetal tibial ML diameter for the alcohol and acidemia groups were smaller than the normal control group ($\downarrow 9\%$, $P<0.001$ and $\downarrow 4\%$, $P=0.064$ respectively) and the saline control group ($\downarrow 11\%$, $P<0.001$ and $\downarrow 6\%$, $P=0.033$ respectively). Fetal tibial AP diameter for the alcohol and acidemia groups were smaller than the normal control group ($\downarrow 8\%$, $P<0.001$ and $\downarrow 4\%$, $P=0.082$ respectively) and the saline control group ($\downarrow 11\%$, $P<0.001$ and $\downarrow 7\%$, $P=0.019$ respectively). Fetal tibial ML and AP diameters for the alcohol group were thinner than the acidemia group ($\downarrow 5\%$, $P=0.011$ and $\downarrow 4\%$, $P=0.048$ respectively). No differences were noted between the normal and saline control groups.

3.2.3 Bone Cross Sectional Moment of Inertia (CSMI)—Neither fetal femoral nor tibial CSMI showed any significant changes among groups (Table 4).

3.3 Mechanical Properties

3.3.1 Extrinsic Properties

3.3.1.1 Maximum Breaking Force: There were significant differences among groups for the fetal femoral and tibial maximum breaking force (ANOVA $P<0.001$) (Figure 2). Fetal femoral maximum breaking forces for the alcohol and acidemia groups were lower compared to the normal control group ($\downarrow 26\%$, $P<0.001$ and $\downarrow 31\%$, $P<0.001$ respectively) and the saline control group ($\downarrow 25\%$, $P=0.001$ and $\downarrow 30\%$, $P=0.001$ respectively). Fetal tibial maximum breaking forces for the alcohol and acidemia groups were lower than the normal control ($\downarrow 40\%$, $P<0.001$ and $\downarrow 44\%$, $P<0.001$ respectively) and saline control groups ($\downarrow 43\%$, $P<0.001$ and $\downarrow 47\%$, $P<0.001$ respectively). No differences were noted between the normal and saline control groups.

3.3.2 Intrinsic Properties

3.3.2.1 Maximum breaking force per unit fetal body weight: Upon normalization by fetal body weight, fetal femoral and tibial maximum breaking force per unit fetal body weight (N/kg) showed significant differences among groups (ANOVA, $P<0.001$) (Figure 3). Normalized fetal femoral breaking force for the alcohol and acidemia groups were lower than the normal control group ($\downarrow 21\%$, $P=0.003$ and $\downarrow 13\%$, $P=0.078$ respectively) and were also reduced compared to the saline control group ($\downarrow 30\%$, $P<0.001$ and $\downarrow 23\%$, $P=0.017$ respectively). Normalized fetal tibial maximum breaking force for the alcohol and acidemia groups were observed to be lower than the normal control group ($\downarrow 29\%$, $P<0.001$ and $\downarrow 27\%$, $P=0.002$ respectively) and the saline control group ($\downarrow 32\%$, $P<0.012$ and $\downarrow 30\%$, $P=0.018$ respectively). No differences were noted between the normal and saline control groups.

3.3.2.2 Ultimate Bone Strength: Fetal femoral and tibial ultimate strengths (MPa) were significantly different among groups (ANOVA, $P<0.001$) (Figure 4). Fetal femoral ultimate strengths for the alcohol and acidemia groups were lower than the normal control group ($\downarrow 24\%$, $P<0.001$ and $\downarrow 32\%$, $P<0.001$ respectively) and the saline control group ($\downarrow 29\%$, $P=0.003$ and $\downarrow 37\%$, $P<0.001$ respectively). Similar analysis indicated that the fetal tibial ultimate strength for the alcohol and acidemia groups were lower than the normal control ($\downarrow 37\%$, $P<0.001$ and $\downarrow 53\%$, $P<0.001$ respectively) and saline control groups ($\downarrow 53\%$, $P<0.001$ and $\downarrow 65\%$, $P<0.001$ respectively). The normal control group exhibited lower fetal

tibial strength than the saline control group ($\downarrow 25\%$, $P=0.001$). No differences were noted between the normal and saline control groups for the fetal femoral ultimate strength.

4. Discussion

The current study clearly demonstrates the detrimental effects of late gestational chronic binge alcohol consumption on mechanical properties and growth of the fetal bones. Four major findings can be gleaned from this study, the first being that late gestational chronic binge alcohol exposure decreased fetal femoral and tibial ultimate strength. The second notable finding from this study is that developmental alcohol exposure targets both intrinsic and extrinsic bone mechanical properties, leading to decreased fetal femoral and tibial maximum bone breaking force and normalized bone breaking force. Third, the fetal bone growth parameters were compromised after late gestational chronic binge alcohol exposure. Fourth, a decrease in pH in the acidemia group similar to that produced by alcohol results in comparable deficits in growth, extrinsic and intrinsic properties of fetal bones.

To our knowledge, this is the first study to comprehensively evaluate the mechanical properties of fetal bones after late gestational *in-utero* chronic binge alcohol exposure. We observed significant decreases in both extrinsic and intrinsic properties of fetal skeletal system including fetal femoral and tibial ultimate strength and normalized maximum breaking force after prenatal binge alcohol consumption during late gestation. In the context of this study, extrinsic properties refer to the whole bone structural properties and intrinsic properties refer to the tissue level material properties (Hogan et al., 1997; Ramadoss et al., 2006). Maximum breaking force and ultimate strength represent different physical attributes of the fetal bones such as, load carrying capacity and material (tissue) level integrity, respectively. Decrease in these mechanical properties of functional fetal bones indicates that alcohol is responsible for disturbing the cortical tissue quality. We did not observe an effect of alcohol on CSMI. These findings support our earlier report that chronic binge alcohol consumption throughout gestation reduces fetal ultimate bone strength and normalized maximum breaking force but not CSMI (Ramadoss et al., 2006).

While the present study evaluate the effect of prenatal alcohol exposure on the functional properties of the fetal bones after *in utero* alcohol exposure during late gestation, studies in the rodent model highlight potential causes by which alcohol exposure during the developmental period might affect tissue integrity in fetal bones. Alcohol exposure in non-pregnant, four-week-old actively growing rats decreased ultimate femoral cortical strength and maximum breaking force (Hogan et al., 1997), reduced bone density and bone mass in femora and tibiae (Sampson et al., 1996) and stopped tibial epiphyseal growth rate and proliferation (Sampson et al., 1997). Broulik et al. administered alcohol to eight week old rat pups daily for three months, then tested femoral strength and found an approximate 12% reduction (Broulik et al., 2010). Maternal alcohol exposure throughout gestation in the rat disrupted organization of fetal tibial histological zones within the epiphysis (Snow and Keiver, 2007) and a decreased number of ossification centers in the fetal skeleton (Keiver et al., 1997; Simpson et al., 2005). Similarly, In another study, maternal alcohol exposure both prior to and during gestation caused a deprivation in the number of ossification centers in the fetal skull, sternum, limbs and vertebral centra on day 20 of gestation in the rat (Lee and

Leichter, 1983). The findings from our study add to this body of works in the rodent model by evaluating the functional properties of fetal bone and a candidate mechanism for damage from in-utero alcohol exposure using a highly translational animal model in which all three human trimester-equivalents of pregnancy occur *in utero*.

Chronic prenatal binge alcohol consumption paradigm used in this study during late gestation hampered fetal body and bone growth as evidenced by the reduction in fetal crown-rump length, femoral length, femoral MI diameter, and tibial ML and AP diameters in the alcohol and acidemia groups compared to the saline control group. Our findings are consistent with human clinical cases in which prenatal alcohol exposure leads to a delay in mean bone age (Habbick et al., 1998) and growth restriction in children up to 14 years of age (Day et al., 2002). Studies in rats have also demonstrated that prenatal alcohol exposure reduces fetal body weight and length (Abel and Dintcheff, 1978; Keiver et al., 1997; Keiver and Weinberg, 2004; Simpson et al., 2005). Our results are also in agreement with previous findings in sheep (Ramadoss et al., 2006) and with reports that maternal alcohol consumption throughout the 21 days of gestation in the rat decreases fetal bone and diaphysis length (Snow and Keiver, 2007). In addition, McMechan et al. (2004) observed a decrease in rat forepaw digit length on postnatal day 31 following prenatal alcohol exposure from GD 8 to 20. Collectively, all of these studies in the human, sheep and rat support our current findings that prenatal alcohol exposure during late gestational period decreases fetal crown rump length and bone growth parameters. Our findings show that a decrease in pH in the acidemia group similar to that produced by alcohol leads to comparable deficits in mechanical properties of the fetal skeletal system. Bone dimensions, and extrinsic as well as intrinsic parameters of the fetal bones from the acidemia group were reduced compared to both the saline and normal control groups, but no differences were observed between the alcohol and acidemia group. Both *in-vivo* and *in-vitro* studies have demonstrated that in response to acidosis, bones play a vital role in the maintenance of a stable pH, but it is at the expense of bone mineral content (Arnett and Spowage, 1996; Barzel, 1995; Bushinsky et al., 1996; Green and Kleeman, 1991). In response to an extracellular acidic environment, the bone surface loses intracellular sodium and potassium in exchange for hydrogen ions to restore normal extracellular H⁺ concentration (Bergstrom and Ruva, 1960; Bettice and Gamble, 1975; Bushinsky et al., 1986). Metabolic acidosis also causes the loss of calcium, phosphate and bicarbonate from femoral midcortical region in exchange for H⁺ to exhibit the proton buffering capacity of bone, as demonstrated in postweanling mice (Bushinsky et al., 1999). Renal tubular acidosis affects bone development in infants and children (McSherry, 1978; McSherry and Morris, 1978), and inhibition of growth hormone secretion may be one of the mechanisms underlying acidosis-induced restriction of bone development (Challa et al., 1993). Acidosis also increases the osteoclast activity by stimulating the release of osteoclastic enzyme β -glucuronidase and inhibits osteoblastic activity by inhibiting osteoblastic collagen synthesis and blocking alkaline phosphatase (Krieger et al., 1992). The findings from these studies on the effects of acidemia on bone together with the results from our study make a compelling case that acidemia is an important candidate mechanism for prenatal alcohol-induced bone deficits.

Perspectives and Significance

Although the overall conformation of adult bone is determined by number of factors, fetal programming during gestation may affect bone adaptation during adulthood in response to the mechanical stress of weight and applied load. Effects of prenatal alcohol exposure on the long-term biomechanical properties of human bones are unknown. The current report demonstrates that late gestational binge alcohol exposure hampers growth and mechanical properties of the fetal skeletal system and these alterations are mediated, at least in part, by alcohol-induced decreases in pH. In addition to shedding light on the effects of prenatal alcohol exposure on the developing bone functional properties, these data also guide further scientific exploration of early developmental origins of adult bone disorders.

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Figure 1. Illustration of the ventilation chamber

Maternal blood gases in the acidemia group were manipulated by placing the ewe in a chamber and manipulating the inspired gases to mimic the change in maternal arterial pH produced by alcohol. The front half of the subject was confined inside the plexiglass chamber, while the rear half was accessible to the investigator for sampling blood. The fractional concentrations of oxygen and carbon dioxide in the chamber were measured using gas monitors.

Figure 2. Binge alcohol and acidemia exposure effects on Fetal Femoral and Tibial Maximum Breaking Force (N)

Fetal femoral and tibial maximum breaking force (N) was significantly reduced in the alcohol and acidemia groups compared to the normal control (*) and saline control (#) groups. Values are means $\pm$ SEM.

Figure 3. Binge alcohol and acidemia exposure effects on Fetal Femoral and Tibial Max Breaking Force per Unit Fetal Body Weight (N/kg)

Fetal femoral and tibial maximum breaking force per unit fetal body weight for the alcohol group was significantly lower than the normal control (*) and saline control (#) groups. For the acidemia group, fetal femoral maximum breaking force per unit fetal body weight was significantly lower than the saline control (#) group, and tibial maximum breaking force per unit fetal body weight was significantly lower than the normal control (*) and saline control (#) groups. Values are means $\pm$ SEM.

Figure 4. Binge alcohol and acidemia exposure effects on Fetal Femoral and Tibial Ultimate Strength (MPa)

Fetal femoral and tibial bone strength was significantly lower in the alcohol and acidemia group compared to the normal control (*) and saline control (#) groups. Fetal tibial bone strength was significantly different between the normal control and saline control groups. Values are means $\pm$ SEM.

Table 1

Mean ($\pm$ SEM) fold change in maternal arterial pH at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours from the baseline (saline control, 7.47 ± 0.00 ; alcohol, 7.50 ± 0.00 ; acidemia, 7.48 ± 0.01).

Time (hour)	Saline	Alcohol	Acidemia
0.5	1.003 ± 0.002	0.990 ± 0.001^a	0.991 ± 0.001^a
1.0	1.004 ± 0.001	0.986 ± 0.001^a	0.979 ± 0.001^{ab}
1.5	1.000 ± 0.001	0.987 ± 0.001^a	0.989 ± 0.001^a
2.0	1.006 ± 0.001	0.988 ± 0.001^a	0.988 ± 0.001^a
3.0	1.002 ± 0.001	0.989 ± 0.001^a	0.989 ± 0.001^a
4.0	1.003 ± 0.001	0.992 ± 0.001^a	0.988 ± 0.001^{ab}
5.0	0.998 ± 0.001	0.991 ± 0.001^a	0.990 ± 0.001^a
6.0	1.004 ± 0.001	0.995 ± 0.001^a	0.992 ± 0.001^a

Table 2

Mean ($\pm$ SEM) fetal whole body weight (kg) and crown-rump length (cm) on GD 133. ^a and/or ^b indicate that group is statistically different than the normal control group and/or the saline control group, respectively.

Group	Fetal Body Weight (kg)	Fetal Crown-Rump Length (cm)
Normal Control	4.83 $\pm$ 0.14	52.27 $\pm$ 0.65 ^b
Saline Control	4.96 $\pm$ 0.62	55.50 $\pm$ 0.98 ^a
Alcohol	4.78 $\pm$ 0.52	50.50 $\pm$ 0.86 ^b
Acidemia	4.19 $\pm$ 0.2	49.12 $\pm$ 0.71 ^{a b}

Table 3

Mean ($\pm$ SEM) fetal femoral and tibial length, mediolateral (ML) and anterior620 posterior (AP) diameter. ^a and/or ^b indicate that group is statistically different than the normal control group and/or the saline control group, respectively.

Group	Femoral			Tibial		
	Length (mm)	ML Diameter (mm)	AP Diameter (mm)	Length (mm)	ML Diameter (mm)	AP Diameter (mm)
Normal Control	95.49 $\pm$ 0.62	10.59 $\pm$ 0.12	10.11 $\pm$ 0.09	112.33 $\pm$ 0.91	10.00 $\pm$ 0.09	8.49 $\pm$ 0.09
Saline Control	97.59 $\pm$ 1.79	10.62 $\pm$ 0.26	9.76 $\pm$ 0.25	114.08 $\pm$ 1.37	10.27 $\pm$ 0.18	8.80 $\pm$ 0.16
Alcohol	91.64 $\pm$ 0.75 ^{a b}	9.73 $\pm$ 0.15 ^{a b}	9.34 $\pm$ 0.14 ^a	109.34 $\pm$ 1.12 ^a	9.14 $\pm$ 0.13 ^{a b}	7.84 $\pm$ 0.11 ^{a b}
Acidemia	90.35 $\pm$ 1.59 ^{a b}	9.76 $\pm$ 0.18 ^{a b}	9.19 $\pm$ 0.22 ^a	106.99 $\pm$ 1.50 ^{a b}	9.64 $\pm$ 0.16 ^b	8.18 $\pm$ 0.14 ^b

Table 4

Mean ($\pm$ SEM) fetal femoral and tibial cross sectional moment of inertia (CSMI). No differences were noted among groups.

Group	Femoral CSMI (mm ⁴)	Tibial CSMI (mm ⁴)
Normal Control	310.54 $\pm$ 15.40	197.53 $\pm$ 7.71
Saline Control	303.87 $\pm$ 13.92	156.93 $\pm$ 8.37
Alcohol	278.38 $\pm$ 17.19	177.28 $\pm$ 12.06
Acidemia	293.25 $\pm$ 15.51	208.77 $\pm$ 15.98