Computed tomography assessment of peripubertal craniofacial morphology in a sheep model of binge alcohol drinking in the first trimester

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ABSTRACT

Identification of facial dysmorphology is essential for the diagnosis of fetal alcohol syndrome (FAS); however, most children with fetal alcohol spectrum disorders (FASD) do not meet the dysmorphology criterion. Additional objective indicators are needed to help identify the broader spectrum of children affected by prenatal alcohol exposure. Computed tomography (CT) was used in a sheep model of prenatal binge alcohol exposure to test the hypothesis that quantitative measures of craniofacial bone volumes and linear distances could identify alcohol-exposed lambs. Pregnant sheep were randomly assigned to four groups: heavy binge alcohol, 2.5 g/kg/day (HBA); binge alcohol, 1.75 g/kg/day (BA); saline control (SC); and normal control (NC). Intravenous alcohol (BA; HBA) or saline (SC) infusions were given three consecutive days per week from gestation day 4–41, and a CT scan was performed on postnatal day 182. The volumes of eight skull bones, cranial circumference, and 19 linear measures of the face and skull were compared among treatment groups. Lambs from both alcohol groups showed significant reduction in seven of the eight skull bones and total skull bone volume, as well as cranial circumference. Alcohol exposure also decreased four of the 19 craniofacial measures. Discriminant analysis showed that alcohol-exposed and control lambs could be classified with high accuracy based on total skull bone volume, frontal, parietal, or mandibular bone volumes, cranial circumference, or interorbital distance. Total skull volume was significantly more sensitive than cranial circumference in identifying the alcohol-exposed lambs when alcohol-exposed lambs were classified using the typical FAS diagnostic cutoff of ≤10th percentile. This first demonstration of the usefulness of CT-derived craniofacial measures in a sheep model of FASD following binge-like alcohol exposure during the first trimester suggests that volumetric measurement of cranial bones may be a novel biomarker for binge alcohol exposure during the first trimester to help identify non-dysmorphic children with FASD.

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Introduction

The teratogenic effects of alcohol abuse during pregnancy were formally identified as fetal alcohol syndrome (FAS) about four decades ago (Jones & Smith, 1973). FAS is diagnosed primarily based on three criteria: 1) presence of at least two of three characteristic dysmorphic facial features (smooth philtrum, thin upper lip, and short palpebral fissures); 2) growth deficits in height and/or weight; and 3) structural, neurologic, or functional central nervous

system (CNS) abnormalities (Bertrand, Floyd, & Weber, 2005; Wattendorf & Muenke, 2005). It is now recognized that the large majority of children with untoward effects from prenatal alcohol exposure do not fully meet the criteria for a diagnosis of FAS, but still express a broad range of skeletal, neurological, developmental, behavioral, or learning abnormalities (Aase, Jones, & Clarren, 1995; Astley & Clarren, 2000; Sokol & Clarren, 1989). In 2004, the designation fetal alcohol spectrum disorders (FASD) was adopted as an umbrella term to encompass the full range of effects resulting from prenatal exposure to alcohol, with FAS being the most severe (Riley & McGee, 2005).

In the United States, FASD is the leading cause of preventable birth defects and developmental disorders (NIAAA, 2012), with a prevalence ranging from 1% of all births (Hoyme et al., 2005; Leibson, Neuman, Chudley, & Koren, 2014) to as high as 2–5% (May et al., 2009). FASD places a large burden on the economy, with estimates of the cost as high as $4 billion annually due to health care, lost productivity, and special education (Hoyme et al., 2005; Lupton, Burd, & Harwood, 2004). Despite extensive efforts to inform the public about the risks of alcohol use during pregnancy, 7% of approximately half a million pregnant women surveyed had consumed alcohol in the previous 30 days and 1.4% reported binge drinking (CDC, 2012). Over a span of nearly 20 years, binge drinking among pregnant women has not significantly declined (CDC, 2009).

Optimal management of children with FASD depends on accurate identification of the full spectrum of children adversely affected by prenatal alcohol exposure. Diagnosis of FAS through clinical screening relies on trained recognition or anthropometric measurements of dysmorphic features of soft tissue structures of the face, yet children with FAS constitute fewer than 10% of all children with FASD. Reliable indicators of adverse effects of prenatal alcohol exposure are needed to help identify the much larger number of children with FASD who do not meet the facial dysmorphology criterion. Quantitative analysis of craniofacial bones, which are derived from embryonic neural crest cells that are sensitive to alcohol exposure during neurulation (Smith, Garic, Flentke, & Berres, 2014), provides a potential approach for identifying a more sensitive indicator. Relatively little work has been done to evaluate the underlying craniofacial bones, and its potential use as a quantitative indicator to identify children with FASD is largely untapped.

Binge drinking during the first trimester (including prior to pregnancy recognition) is the most common pattern of risk drinking in women of childbearing age (Conover & Jones, 2012; Maier & West, 2001; Ramadoss, Hogan, Given, West, & Cudd, 2006). Craniofacial bone changes are an appropriate candidate as a structural biomarker for binge drinking, in part because of the spatiotemporal links between embryonic CNS and craniofacial development during the first trimester. Bones that form the neurocranium (frontal, parietal, and occipital bones) are derived principally from the neural crest and paraxial mesoderm, whereas bones that form the viscerocranium (mandible, maxilla, nasal, lacrimal, and jugal bones) originate primarily from the first two pharyngeal arches (Moore, 2013). During neurulation, neural crest cells originate at the junction of the neural folds and eventually the cells migrate to various locations throughout the embryo to form body structures (Smith et al., 2014). A subset of neural crest cells, cranial neural crest cells, forms structures of the face (bones, cartilage, and cranial nerves) (Sulik, Cook, & Webster, 1988), and are highly sensitive to alcohol. Damage to these cells results in dysmorphic craniofacial features and some associated brain abnormalities associated with FAS (Cartwright & Smith, 1995; Rovasio & Battiato, 1995; Smith, 1997).

In humans, key events over the first trimester of pregnancy (O'Rahilly & Müller, 1996) include neurulation (spanning postfertilization days 22–31), which involves formation of the neural tube and neural crest, with anterior neuropore closure by day 30. Emergence of the full five-vesicle stage is evident at 5 weeks, along with formation of the pontine flexure, optic cup, and nasal pit. Formation of the telencephalic cortical plate begins at 7–8 weeks with the appearance of the five zones of the emerging cerebral cortex (marginal, cortical plate, subplate, subventricular, and ventricular zones). By comparing the timing of the same events during embryonic development in sheep (that have a 147-day gestation), it is possible to establish temporal equivalence in sheep to the first 8 weeks of human development. In sheep, neural tube formation extends into the 3rd gestational week and the anterior neuropore closes by gestational day (GD) 21. The five-vesicle stage is evident in the 4th gestational week, while the cortical plate begins forming in the lateral wall of the telencephalon at GD35. By GD40, the lateral wall has formed a defined cortical plate and the five zones of the emerging cerebral cortex are evident (Reynolds & Møllgård, 1985). Comparatively, the first 40 days of embryonic development in the sheep are roughly equivalent to the first 8 weeks of human development.

Experimental animal models, primarily mouse models, have shown that binge-like alcohol exposure during the first trimester equivalent is sufficient to induce abnormal craniofacial development. These studies have identified key roles of dose and timing of prenatal alcohol exposure in producing craniofacial effects that model some of those seen in FAS (Sulik, 1984; Sulik et al., 1988). Specifically, they have shown quantitatively that measurable craniofacial changes do occur (Anthony et al., 2010; Hernandez-Guerrero, Ledesma-Montes, & Loyola-Rodriguez, 1998; Lipinski et al., 2012; Robin & Zackai, 1994). A major goal of the current study was to extend this approach to a well-defined sheep model of binge alcohol exposure during a portion of the first trimester equivalent, to assess whether quantitative analyses of craniofacial bone volumes using computed tomography (CT) in 6-month-old (peripubertal) lambs could accurately predict exposure.

In this study, sheep were evaluated at 6 months of age because we wanted a target age comparable to middle school-aged children about to enter puberty during early adolescence. Sheep reach puberty when they attain 50–70% of their mature body weight, typically between 7 and 8 months of age, depending on breed (The Merck Veterinary Manual, 2005). Therefore, the developmental status of these lambs may be analogous to children in their middle-school years with undiagnosed FASD that have developmental, behavioral, and/or learning deficits often identified by educators. Additionally, we sought to compare the relative accuracy of classifications of prenatal alcohol exposure through a single CT scan, which allowed measurement of both cranial bone volumes and linear distance measures taken from bone (cranial) and soft tissue (facial) landmarks, along with cranial circumference. If confirmed, these quantitative approaches in this sheep model could provide new indices of the effects of prenatal alcohol exposure that may prove more sensitive than traditional FAS diagnostic indices (head circumference; facial morphometrics). This could translate into improved identification of children with FASD.

Measures evaluated in the current study include the volume of the bones of the neurocranium and viscerocranium, along with linear measurements of the skull, face, and cranial circumference. CT was utilized because it is non-invasive, readily available in most hospitals, produces high-resolution, three-dimensional images, and one scan allows measurement of bony and soft tissue structures of the face. CT has been effectively used to study fixation artifacts in embryologic studies (Schmidt et al., 2010) and recently in studies of craniofacial dysmorphology in mouse models of FASD (Kaminen-Ahola et al., 2010; Shen et al., 2013). Repeated weekend binge drinking (3 consecutive days per week) over the first 8 weeks of