Prenatal Alcohol Exposure Patterns and Alcohol-Related Birth Defects and Growth Deficiencies: A Prospective Study

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Background: The physical features of fetal alcohol syndrome include smooth philtrum, thin vermillion border, short palpebral fissures, microcephaly, and growth deficiencies on weight and height. However, little is known about the specific quantities of alcohol exposure, pattern of drinking, timing of exposure, and magnitude of risk for each of these features.

Methods: Using data on 992 subjects collected prospectively in California between 1978 and 2005, we examined the patterns and timing of alcohol exposure in relation to these features. Structural features were assessed by a dysmorphologist who performed a blinded physical examination of all infants. Patterns of drinking were evaluated by drinks per day, number of binge episodes, and maximum number of drinks. Timing of exposure was evaluated 0 to 6 weeks postconception, 6 to 12 weeks postconception, first trimester, second trimester, and third trimester.

Results: Higher prenatal alcohol exposure in every pattern was significantly associated with the incidence of smooth philtrum but not with short palpebral fissures. The strongest associations were with timing of exposure in the second half of the first trimester (RR 1.25, 95% CI 1.14 to 1.36 for average number of drinks per day; RR 1.17, 95% CI 1.09 to 1.26 for maximum number of drinks in 1 episode). Similarly, thin vermillion border was most strongly associated with exposure in the second half of the first trimester. Findings with respect to timing of exposure were similar for microcephaly and reduced birth weight. However, reduced birth length was increased with exposure in any trimester. These associations were linear, and there was no evidence of a threshold.

Conclusions: Reduced birth length and weight, microcephaly, smooth philtrum, and thin vermillion border are associated with specific gestational timing of prenatal alcohol exposure and are dose-related without evidence of a threshold. Women should continue to be advised to abstain from alcohol consumption from conception throughout pregnancy.

Key Words: Fetal Alcohol Spectrum Disorders, Fetal Alcohol Syndrome, Prenatal Alcohol Exposure, Alcohol-Related Facial Features, Alcohol-Related Growth Deficiency.

OVER 35 YEARS AGO, fetal alcohol syndrome (FAS) was first identified by Jones and Smith (1973). Since that time, it has become clear that prenatal exposure to alcohol is associated with a spectrum of abnormalities, now referred to as fetal alcohol spectrum disorders (FASD). The characteristic physical features of FAS include growth deficiencies on height and weight, microcephaly, smooth philtrum, thin vermillion border, and short palpebral fissures.

Prenatal alcohol exposure during the first prenatal month has been associated with an increased risk for low birth weight (< 2,500 g), and birth length and head circumference below the 10th percentile (Day et al., 1989). Jacobson et al. (1994) also found lower birth weight (509 g less on average) and shorter length (4.0 cm shorter on average) in the infants of women who drank at least an average of 60 ml absolute alcohol per day during pregnancy compared to the infants of women who did not drink during pregnancy.

While research has demonstrated that prenatal alcohol exposure is associated with these characteristic physical features of FAS, there is a lack of clear-cut information on the risk for each of these specific features in relation to patterns of dose and timing of exposure. This may be due in part to inaccurate or imprecise measures of dose and timing because of reliance on maternal report, retrospective ascertainment, and/or recall bias. Lack of data on the magnitude of risk for exposure during specific gestational windows may also be due

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to the variability in how alcohol-related birth outcomes are assessed, particularly with respect to the characteristic facial features of FAS that are frequently subtle and difficult to recognize reliably.

The purpose of this study is to examine how specific prenatal alcohol exposure patterns relate to characteristic alcohol-related facial features and growth deficiencies using data drawn from an ongoing prospective study of pregnancy exposures and outcomes that includes a specialized dysmorphological examination for live-born infants.

MATERIALS AND METHODS

Source of the Sample

Data for this analysis were collected from women who enrolled in a cohort study conducted by the California Teratogen Information Service and Clinical Research Program (CTIS) between 1978 and 2005. CTIS is an educational counseling and research program funded by the State of California to provide confidential individualized risk assessments regarding any and all pregnancy exposures to pregnant women throughout the state. Women primarily initiate contact with CTIS by telephone, are interviewed by a Teratogen Information Specialist about all exposures, pregnancy history, and other risk factors, and are provided summary risk counseling free of charge. Information on exposures is collected in detail including specific dates, doses, and routes of administration. The counseling interview and subsequent risk assessment are provided confidentially, and if desired, anonymously, to ensure that women have access to accurate information without fear of repercussions when disclosing details about sensitive exposures or behaviors. Part of the initial intake interview involves establishing rapport with the woman including an explanation of the value of accurate and complete disclosure about exposures. Women who initially contact CTIS with questions about the risks of specific exposure(s) in pregnancy may or may not spontaneously ask for information about alcohol, but as part of the standard intake interview, alcohol exposure information is obtained from all callers.

After receiving counseling and referral, pregnant women who report exposure to 1 or more of 70 agents selected as a research focus and pregnant women who report no exposures to suspected or known teratogenic agents are asked whether they would like to participate in a follow-up study. Those who consent complete a more comprehensive telephone interview. Detailed data collected include information on last menstrual period, maternal age, gestational age when first contacted CTIS, pregnancy history, prenatal tests, and all exposures to prescription or over-the-counter medications, infections, acute or chronic diseases or illnesses, recreational drugs, alcohol, and tobacco. Participants are enrolled before the outcome of their pregnancy is known.

Depending on the gestational weeks at the time of enrollment, follow-up telephone interviews are conducted every 3 months during the remainder of the pregnancy to collect information on the continuation of exposures and if there have been any new exposures, prenatal tests, or pregnancy-related events. At the end of pregnancy, outcome information is obtained from the mother by telephone interview, including data on birth date, mode of delivery, Apgar scores, maternal and infant complications, birth weight, length, and head circumference. In addition, medical records are collected from the hospital of delivery and the child's pediatrician to provide and/or validate birth outcomes.

For all live-born infants, mothers are asked to participate in a standardized blinded dysmorphological examination of the child. These examinations are scheduled either in a clinic setting or in the participant's home or other location of her preference. The study dysmorphologist (KLJ) performs the examinations while blinded to the mother's exposure history and course of pregnancy. All mothers or caregivers are advised by study staff on multiple occasions prior to the physical examination that the examiner must remain blinded and therefore not to disclose any exposures until the examination has been completed. The dysmorphologist examines each infant for the presence or absence of minor structural defects using a standard checklist of 132 minor malformations (Chambers et al., 2001). Included on this list is an evaluation of the smoothness of the philtrum and the thinness of the vermillion border. In addition, a number of anthropometric parameters are documented including head circumference and palpebral fissure length.

The study was approved by the Institutional Review Boards at the University of California San Diego and San Diego State University.

For the purposes of this analysis, all live-born infants and their mothers who were enrolled in the study between 1978 and 2005 and who had completed a dysmorphology examination were selected. To thoroughly assess the relationship between prenatal alcohol exposure pattern and timing with the alcohol-related outcomes, we only included women who reported any alcohol consumption from the initial estimated date of conception to the time of delivery. Women were excluded from this analysis if they delivered twins or higher-order multiples, or if they were not residing in the state of California at the time of enrollment. For women who enrolled in the follow-up study with more than 1 pregnancy, only the first eligible birth was included in this analysis.

Classification of Exposure

Based on the published studies examining prenatal alcohol exposure, several categories representing patterns of exposure were developed and used to quantify the information the mother provided prior to delivery and at the pregnancy outcome interview regarding her consumption. At the intake interview and in all subsequent maternal interviews, women were asked about the specific dates, types of alcohol, and quantity consumed using guided interview techniques to help aid recall. Rules were created to define standard drinks and to quantify the number of drinks that were consumed based on maternal narrative reports. Drinks were standardized so the following amounts were considered as 1 serving: 4 ounces of wine, 1 and a half ounces of hard liquor, or 12 ounces of beer. If the mother reported fewer than 2 sips of alcohol on an occasion, this was classified as no exposure. Average daily volume, number of binge episodes (defined as 4 or more drinks per occasion), and greatest number of drinks in 1 occasion were quantified during the following periods: first half of the first trimester, second half of the first trimester, total first trimester, second trimester, and third trimester. The first trimester was defined as date of conception to 84 days postconception, the second trimester was defined as 85 to 168 days postconception, and the third trimester was defined as 169 days postconception to birth. The first half of the first trimester was defined as the first 42 days postconception, and the second half of the first trimester was defined as 43 to 84 days postconception.

The estimated date of conception and subsequent gestational age at delivery were calculated based on the first day of the last menstrual period and the length of the woman's regular cycle. For women who could not provide information or who had irregular menstrual periods, ultrasound dating was used. For women with regular periods, the estimated date of conception and due date were adjusted if ultrasound dates were discrepant based on standard obstetric guidelines.

Classification of Outcomes

Outcomes evaluated included the 3 characteristic facial features: short palpebral fissures, smooth philtrum, and thin vermillion border, and growth deficiencies on birth weight, birth length, and head circumference.

Palpebral fissure lengths were measured for the infant's left eye by the dysmorphologist using a rigid ruler marked in millimeters. Children were categorized as having short palpebral fissure length if they were at or below the 10th percentile using the Thomas growth curve (Thomas et al., 1987). Infants were categorized by the study dysmorphologist as having smooth philtrum or thin vermillion border based on qualitative judgment during the blinded physical examination because during most years of the study, no objective standards had been developed.

Birth weight and birth length were collected from the outcome interview with the mother after delivery and validated with medical records. Infants were categorized as having reduced birth weight and/or reduced length if their weight and/or length were in the 10th percentile or less using sex-specific 2000 CDC growth charts (National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion [NCHS], 2000c,d). Birth length and weight percentiles for preterm infants, defined as birth prior to 37 completed weeks' gestation, were assigned using the Lubchenco growth graph (Lubchenco et al., 1966).

Because head circumference at birth is frequently not known by the mother and/or because of missing medical records, head circumference measures were collected during the dysmorphological examination. Head circumference was measured with a flexible, nonstretchable tape, over the most prominent part of the forehead to find the widest circumference. Infants were categorized as having "microcephaly" using a more liberal definition of head circumference at or below the 10th percentile using the sex-specific 2000 CDC growth charts for children under the age of 3 (NCHS, 2000a,b). For children 3 years of age and older (n = 86), microcephaly was categorized based on plotting their measurements using the sex-specific Nellhaus growth graph (Nellhaus, 1968). For children examined under the age of 1 year and who were born before 37 weeks' gestation, chronological age of the child was adjusted for preterm delivery (Lubchenco et al., 1966).

Statistical Analysis

Descriptive statistics were computed for the predictive variable of interest—prenatal alcohol exposure pattern by gestational timing. Additional descriptive statistics were prepared for alcohol-related physical features and selected covariates. The covariates included sex of infant, maternal age, maternal education, maternal race, other known teratogen exposure (defined as exposed prenatally to anticonvulsants, cocaine, cyclophosphamide, disulfiram, angiotensin-converting enzyme inhibitors, fluconazole, diabetes or medications used to treat it, high fever, isotretinoin, lithium, varicella infection, and/or warfarin), weeks' gestation at enrollment, weeks' gestation at birth, year of birth, parity, cigarette use, and illicit drug use (amphetamines, heroin, cocaine, and marijuana). Access (Microsoft Office Access 2003; Redmond, WA) was used to build the data set. Growth percentiles for those plotted on CDC growth curves were determined by using Epi Info Nutrition (version 3.5.1; CDC, Atlanta, GA). All other analyses were performed with SAS (version 9.1.3; SAS Institute, Cary, NC).

The association between each alcohol exposure variable and each outcome was assessed using unconditional logistic regression modeling. To address potential confounding, each covariate was examined to determine whether the addition of the covariate into the unadjusted logistic regression model changed the association between the prenatal alcohol exposure and the outcome of interest by more than 15%.

Nearly one-third of subjects were missing data on maternal race and maternal education because that information was not collected as part of the standard maternal interview during the earlier years of data collection. Therefore, missing data for race and education are assumed to be missing at random. Crude risk ratios were estimated for the subset of the sample with data that included race and for the sample with data that included education. Then, race and education were evaluated as confounders within the subsample. As there was no evidence of confounding by these 2 covariates in the subset analysis, they were excluded from all further analyses.

Similarly, palpebral fissure lengths were missing for approximately 21% of the sample. Examination of the records indicated that missingness was because of the inability of the examiner to obtain an accurate measurement owing to a crying or uncooperative infant. Therefore, missingness was judged to be at random, and analyses were conducted with the subset for whom a measurement was available.

To determine whether there is a "safe" threshold amount of prenatal alcohol consumption, nonlinear relationships between the prenatal alcohol exposure and outcomes were evaluated by examining plots for clustering of outcomes and by building quadratic models. All statistical tests were 2 tailed and performed using an alpha level of 0.05.

RESULTS

A total of 992 women and their singleton infants were included in the analysis. As shown in Table 1, mean maternal age was 31 years, and the average weeks' gestation at the initial call to CTIS was 13 weeks. A total of 27% reported smoking cigarettes at some point in the pregnancy, 24% reported using at least 1 illicit drug during the pregnancy, and 29% of the women reported exposure to another agent classified as potentially teratogenic. The median age of the children at the time of the dysmorphology examination was 2.6 months.

As shown in Table 2, the median average number of drinks per day in the first trimester was 0.06 drinks with a 1st to 99th percentile range of 0.00 to 12.14, 0.00 drinks for the second trimester with a range of 0.00 to 6.86, and 0.00 drinks for the third trimester with a range of 0.00 to 4.00. The median number of binge episodes during each time period was 0 for the first trimester with a 1st to 99th percentile range of 0 to 84, 0 for the second trimester with a range of 0 to 84, and 0 for the third trimester with a range of 0 to 54. The median maximum number of drinks in 1 occasion during the first trimester was 1 with a 1st to 99th percentile range of 0 to 17, 0 for the second trimester with a range of 0 to 14, and 0 for the third trimester with a range of 0 to 11.

As shown in Table 3, for the selected alcohol-related birth outcomes, and dichotomizing the sample into women who reported consuming 1 or more drinks per day on average in the first trimester and those who reported consuming less, there was evidence of higher risk with higher dose for microcephaly, thin vermillion border, smooth philtrum, and reduced birth length and weight, but also clear evidence that these outcomes did not occur exclusively in the higher dose category.

None of the covariates changed the association between any category of prenatal alcohol exposure and any alcohol-related physical feature by the prespecified criteria. Therefore, the final models remained unadjusted with no covariates.

As shown in Table 4, during the first trimester, there were several significant associations with drinking patterns.

Table 1. Descriptive Statistics (n = 992), CA Pregnancy Cohort, 1978 to 2005

Characteristics	Mean (standard deviation)
Maternal age	31.1 (5.7)
	Median (1st to 99th percentiles)
Weeks' gestation on entry	13.0 (4.4–37.9)
Weeks' gestation at birth	39.8 (33.1–42.6)
Parity	0 (0–4)
Age (months) at physical exam	2.6 (0.0–130.1)
	n (%)
Alcohol abstainers	
Conception to birth	32 (3.2)
Sixth week post-conception until birth	398 (40.1)
Race	
White	536 (54.0)
Other race	143 (14.4)
Unknown	313 (31.6)
Education	
≥1 year college	521 (52.5)
<1 year college	136 (13.7)
Unknown	335 (33.8)
Sex of infant	
Males	523 (52.7)
Females	469 (47.3)
Cigarette use	
Yes	268 (27.0)
No	724 (73.0)
Illicit drug use[a]	
Yes	234 (23.6)
No	758 (76.4)
Teratogen use[b]	
Yes	284 (28.6)
No	708 (71.4)
Infant year of birth	
Before 2000	715 (72.1)
2000 and later	277 (27.9)

[a]Amphetamines, heroin, cocaine, marijuana.
[b]Exposed prenatally to anticonvulsants, cocaine, cyclophosphamide, disulfiram, angiotensin converting enzyme inhibitors, fluconazole, diabetes or medications used to treat it, high fever, isotretinoin, lithium, varicella infection, and/or warfarin.

Breaking down first trimester exposure into the first and second halves, many of these significant findings were restricted to exposures during the second 6 weeks postconception. For example, for every 1 drink increase in the average number of drinks consumed daily, there was a 25% increased risk for smooth philtrum (95% CI 1.14, 1.36), a 22% increased risk for thin vermillion (95% CI 1.09, 1.35), a 12% increased risk for microcephaly (95% CI 1.02, 1.22), a 16% increased risk for reduced birth weight (95% CI 1.07, 1.27), and an 18% increased risk for reduced birth length (95% CI 1.08, 1.29). Findings were similar for each additional episode of binge drinking and each additional drink in the maximum number of drinks consumed in 1 occasion. Although there were some significant associations during the first 6 weeks postconception, effect sizes tended to be more modest.

During the second trimester, significant associations were found for smooth philtrum and for weight and length with selected drinking patterns. During the third trimester, only birth length was associated with average number of drinks per day (RR 1.20, 95% CI 1.02, 1.41) and maximum number of drinks consumed in 1 occasion (RR 1.12, 95% CI 1.01, 1.23). No drinking pattern or timing was significantly associated with short palpebral fissures.

Assessment for nonlinear relationships for each outcome did not reveal any evidence of clustering. Additionally, quadratic models were constructed and did not demonstrate any nonlinear relationships (data not shown). Therefore, there was no indication of a threshold amount of alcohol exposure that is safe with respect to the specific outcomes that demonstrated a significant association with exposure. Instead, the relationship was linear, with incrementally higher prenatal alcohol exposure resulting in incremental increased risks for the outcomes assessed.

DISCUSSION

This prospective study for the first time quantifies specific risks for carefully classified alcohol-related facial features and growth deficiencies with detailed information on specific windows of exposure in pregnancy. The number of binge episodes and number of daily drinks on average during the first trimester were significantly associated with all of the selected outcomes with the exception of short palpebral fissures. These findings are in agreement with other human and animal studies that have identified both first trimester and higher dose to be the highest-risk exposure pattern for alcohol-related facial features and growth deficiencies (Davies and Bledsoe, 2005; Maier and West, 2001a). Jacobson and colleagues (1994) reported a dose-dependent effect on birth weight and length among infants born to women who drank at least 60 ml per day during pregnancy. However, we found a linear increased risk with no safe threshold.

Our findings with respect to the strongest effects in the second 6 weeks postconception for smooth philtrum, thin vermillion, microcephaly, and reduced birth weight are in contrast to some animal and human studies that have reported that an earlier stage in gestation is the critical period for FAS-like facial characteristics (Astley et al., 1999; Sulik, 1984). For example, Dunty and colleagues (2001) suggested that in the mouse model, the crucial window corresponds to the late second through early 6th week of human gestation. These investigators noted cell death in the brain and face following in utero alcohol exposure in the critical window and hypothesized that ethanol-induced cell death of specific progenitor cells leads to facial structure tissue mass loss that results in facial features associated with FAS. Similarly, Sulik and colleagues (1981) reported facial malformations in mice analogous to those seen in humans when mice were exposed to alcohol during the equivalent to the third week in human pregnancy. Our reduced birth length outcomes agree with Day and colleagues' (1989) reports of the first prenatal month exposure; however, we do not see the same associations during that same time period for most of our other alcohol-related outcomes.

Table 2. Descriptive Statistics (n = 992), CA Pregnancy Cohort, 1978 to 2005

Characteristics	Median (1st to 99th percentiles)	N (%) with any exposure
Drinks/day, avg, 1st trimester	0.06 (0.00–12.14)	883 (89.0)
Drinks/day, avg, 1st half 1st trimester	0.10 (0.00–15.00)	860 (86.7)
Drinks/day, avg, 2nd half 1st trimester	0.00 (0.00–10.00)	464 (46.8)
Drinks/day, avg, 2nd trimester	0.00 (0.00–6.86)	399 (40.2)
Drinks/day, avg, 3rd trimester	0.00 (0.00–4.00)	309 (31.1)
Number of binge episodes, 1st trimester	0 (0–84)	250 (25.2)
Number of binge episodes, 1st half 1st trimester	0 (0–42)	239 (24.1)
Number of binge episodes, 2nd half 1st trimester	0 (0–42)	109 (11.0)
Number of binge episodes, 2nd trimester	0 (0–84)	84 (8.5)
Number of binge episodes, 3rd trimester	0 (0–54)	40 (4.0)
Max number of drinks 1 episode, 1st trimester	1 (0–17)	890 (89.7)
Max number of drinks 1 episode, 1st half 1st trimester	1 (0–17)	871 (97.9)
Max number of drinks 1 episode, 2nd half 1st trimester	0 (0–16)	475 (47.9)
Max number of drinks 1 episode, 2nd trimester	0 (0–14)	420 (42.3)
Max number of drinks 1 episode, 3rd trimester	0 (0–11)	331 (33.4)

Table 3. Alcohol-Related Birth Outcomes CA Pregnancy Cohort, 1978 to 2005

Alcohol-related birth outcomes (n = sample size for each outcome)	≥1 Drink/day, average, 1st trimester n outcome/N exposed (%)	<1 Drink/day average, 1st trimester n outcome/N exposed (%)	Total number with outcome
Head circumference ≤10th percentile (n = 973)	11/127 (8.7)	53/846 (6.3)	64
Palpebral fissure length ≤10th percentile (n = 780)	13/89 (14.6)	102/691 (14.8)	115
Smooth philtrum (n = 992)	10/129 (7.8)	16/863 (1.9)	26
Thin vermillion border (n = 992)	6/129 (4.7)	11/863 (1.3)	17
Height ≤10th percentile (n = 958)	14/122 (11.5)	34/836 (4.1)	48
Weight ≤10th percentile (n = 983)	18/124 (14.5)	38/859 (4.4)	56

There are several possible explanations for these differences. First, our earliest gestational window of 0 to 6 weeks included the first 2 weeks' postconception, which is referred to by many teratologists as the "all or none" period, during the process of implantation and prior to the time when the placenta is fully functional, thereby limiting the transfer of alcohol from the maternal bloodstream to the developing embryo (Gilbert-Barness, 2010). Due to potential misclassification of embryonic exposure during that 0 to 6 week window, we could have biased the estimate of the effect toward the null. Second, our findings might have been influenced by spontaneous abortion. We included only live births in our sample. It is possible that women whose infants would have gone on to exhibit physical features because of alcohol exposure were more likely to be lost to spontaneous abortion following exposure in that first 6 week window.

However, it is also possible that the 6 to 12 week window is the embryologically sensitive period for development of some alcohol-related facial features. For example, in a study of development of the lateral philtral ridges in normal human embryos, distinct ridges were not detectable upon macroscopic examination of aborted tissue collected prior to 12 weeks' postconception, but were clearly evident in tissue collected after that gestational window. Furthermore, in that same study, the frenulum/associated connective tissue thought to be precursors to development of the lateral philtral ridges was not evident on microscopic evaluation until 9 weeks' postconception (Martin et al., 1996).

Short palpebral fissure length was not significantly associated with any pattern or timing of alcohol consumption, but approached significance for the maximum number of drinks consumed in 1 occasion during 6 to 12 weeks postconception. As palpebral fissure length is a continuous measure with 10% of the population expected to be categorized at or $<$10th percentile, it could be that the association with this feature is less specific than the associations with smooth philtrum and thin vermillion, and therefore, the study was underpowered to detect an effect.

Interestingly, the only significant associations for head circumference were with first trimester exposure, and not with second or third. This may be consistent with the findings of Maier and West (2001b) who reported regional vulnerability of brain cells in rats to alcohol during the equivalent of the first 2 trimesters in human. However, Bonthius and West (1990) found high negative correlations between maximum blood alcohol concentration and brain weight as well as Purkinje cell number among rats exposed in a window corresponding to the third trimester in humans. Microcephaly may not have been significantly associated with the second and third trimesters in this analysis because the study was underpowered with insufficient numbers of women reporting high enough exposure in the second and third trimesters, or because the criteria of 10th percentile or less was nonspecific. Tenth percentile or less was selected a priori to define microcephaly based on Hoyme and colleagues (2005). Other classification criteria for features of FAS have used the 3rd percentile (Chudley et al., 2005).

Table 4. Prenatal Alcohol Exposure and Risk Ratio (95% CI) for Alcohol-Related Facial Features and Growth Deficiencies

Alcohol exposure category	Short palpebral fissure $n = 780$	Smooth philtrum $n = 992$	Thin vermillion $n = 992$
Drinks/day, avg, 1st trimester	1.00 (0.96, 1.05)	1.04 (1.00, 1.07)*	1.03 (0.99, 1.08)
Drinks/day, avg, 1st half 1st trimester	0.99 (0.96, 1.03)	1.01 (0.99, 1.04)	1.01 (0.99, 1.04)
Drinks/day, avg, 2nd half 1st trimester	1.06 (0.97, 1.16)	1.25 (1.14, 1.36)‡	1.22 (1.09, 1.35)‡
Drinks/day, avg, 2nd trimester	0.97 (0.81, 1.16)	1.19 (1.04, 1.37)†	1.09 (0.87, 1.38)
Drinks/day, avg, 3rd trimester	0.87 (0.58, 1.31)	1.09 (0.82, 1.44)	1.07 (0.75, 1.55)
Number of binge episodes, 1st trimester	1.01 (1.00, 1.02)	1.04 (1.02, 1.05)‡	1.03 (1.02, 1.05)‡
Number of binge episodes, 1st half 1st trimester	1.01 (0.99, 1.03)	1.05 (1.03, 1.08)‡	1.06 (1.03, 1.09)‡
Number of binge episodes, 2nd half 1st trimester	1.02 (1.00, 1.04)*	1.07 (1.05, 1.10)‡	1.06 (1.04, 1.09)‡
Number of binge episodes, 2nd trimester	1.00 (0.98, 1.02)	1.03 (1.01, 1.05)†	1.02 (1.00, 1.05)*
Number of binge episodes, 3rd trimester	0.99 (0.96, 1.02)	1.01 (0.98, 1.04)	1.01 (0.98, 1.04)
Max number of drinks 1 episode, 1st trimester	0.99 (0.95, 1.04)	1.01 (1.00, 1.03)	1.01 (0.99, 1.04)
Max number of drinks 1 episode, 1st half 1st trimester	0.99 (0.96, 1.03)	1.01 (1.00, 1.03)	1.01 (0.99, 1.04)
Max number of drinks 1 episode, 2nd half 1 trimester	1.03 (0.96, 1.09)	1.17 (1.09, 1.26)‡	1.13 (1.03, 1.24)†
Max number of drinks 1 episode, 2nd trimester	1.00 (0.94, 1.08)	1.11 (1.04, 1.19)†	1.06 (0.97, 1.17)
Max number of drinks 1 episode, 3rd trimester	0.99 (0.88, 1.12)	1.13 (1.00, 1.27)**	1.07 (0.89, 1.29)
	Microcephaly (OFC ≤10th percentile) $n = 973$	Reduced birth weight (≤10th percentile) $n = 983$	Reduced birth length (≤10th percentile) $n = 958$
Drinks/day, avg, 1st trimester	1.01 (0.97, 1.06)	1.03 (0.99, 1.06)	1.15 (1.06, 1.26)†
Drinks/day, avg, 1st half 1st trimester	1.00 (0.96, 1.04)	1.01 (0.99, 1.03)	1.10 (1.02, 1.20)**
Drinks/day, avg, 2nd half 1st trimester	1.12 (1.02, 1.22)**	1.16 (1.07, 1.27)‡	1.18 (1.08, 1.29)‡
Drinks/day, avg, 2nd trimester	1.06 (0.90, 1.23)	1.16 (1.03, 1.31)**	1.23 (1.09, 1.38)‡
Drinks/day, avg, 3rd trimester	0.65 (0.24, 1.80)	1.15 (0.97, 1.37)*	1.20 (1.02, 1.41)**
Number of binge episodes, 1st trimester	1.01 (1.00, 1.02)*	1.02 (1.01, 1.03)‡	1.02 (1.01, 1.03)‡
Number of binge episodes, 1st half 1st trimester	1.01 (0.99, 1.04)	1.04 (1.02, 1.06)‡	1.03 (1.01, 1.06)†
Number of binge episodes, 2nd half 1st trimester	1.03 (1.01, 1.05)**	1.04 (1.01, 1.06)†	1.04 (1.02, 1.07)‡
Number of binge episodes, 2nd trimester	1.01 (0.99, 1.03)	1.02 (1.00, 1.03)**	1.02 (1.01, 1.04)†
Number of binge episodes, 3rd trimester	0.98 (0.91, 1.05)	1.01 (0.99, 1.03)	1.01 (1.00, 1.03)
Max number of drinks 1 episode, 1st trimester	1.00 (0.97, 1.03)	1.01 (0.99, 1.03)	1.09 (1.02, 1.17)**
Max number of drinks 1 episode, 1st half 1st trimester	1.00 (0.97, 1.03)	1.01 (0.99, 1.03)	1.09 (1.02, 1.16)**
Max number of drinks 1 episode, 2nd half 1 trimester	1.08 (1.01, 1.16)**	1.11 (1.04, 1.18)†	1.12 (1.05, 1.19)‡
Max number of drinks 1 episode, 2nd trimester	1.05 (0.98, 1.12)	1.07 (1.00, 1.14)*	1.11 (1.04, 1.19)‡
Max number of drinks 1 episode, 3rd trimester	0.97 (0.83, 1.14)	1.08 (0.97, 1.20)	1.12 (1.01, 1.23)**

*$p < 0.10$, **$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

There are several limitations of this study. Women who call CTIS for counseling constitute a volunteer sample, may not be representative of the population, and therefore, these data may not be generalizable to all pregnant women who drink alcohol and their infants. While internal validity was maintained, external validity cannot be assumed because it is unknown to what extent self-selection of participants may have biased the sample. The use of maternal report for collection of alcohol consumption data could result in underreporting or misreporting (Ernhart et al., 1988). While the bias in reporting might be nondifferentially in the direction of underreporting leading to bias of the estimate of the relative risk toward the null, it is possible that there is differential under- or misreporting. Women who specifically called about alcohol exposure may have reported differently than those who called about something else (data not available). However, the interview protocol used for collecting consumption information from women who were motivated to call CTIS for counseling may have led to reduced bias in reporting. In addition, it is possible that there was bias in the women who elected to continue participation in the study up to the completion of the physical examination. However, it is unlikely that this bias was attributed to knowledge of outcome, as mothers would be highly unlikely to recognize in their infants the specific alcohol-related facial features evaluated in this study.

Strengths of this study include the collection of prenatal alcohol exposure during the pregnancy when more detailed exposure information could be collected prior to the known outcome of pregnancy. This may have helped to reduce recall bias (Jacobson et al., 2002). Additionally, a study dysmorphologist who was blinded to the entire history, including exposure(s) status of the mother, examined each infant in the context of a study that included exposures to more than 70 agents of interest as well as unexposed infants. Thus, the dysmophologist could not have been aware that certain structural features might be more or less likely to occur in any given infant. This helped reduce context and observer biases (Egglin and Feinstein, 1996; Harvey et al., 2003).

While some of the prenatal alcohol exposure patterns evaluated in this study were not found to be significantly associated with some of the selected alcohol-related birth outcomes, it is important to recognize that this study focused on physical features alone. It is thought that far more children with prenatal exposure to alcohol are affected neurobehaviorally than those who exhibit structural features of FAS (Sampson et al.,

1997). Future studies should address these same questions regarding gestational timing and dose relative to neurobehavioral outcomes.

Based on our findings, there is no safe threshold for alcohol consumption during pregnancy with respect to selected alcohol-related physical features. Women who are of childbearing age and who are contemplating or at risk for becoming pregnant should be encouraged to avoid drinking, and women who are pregnant should abstain from alcohol throughout pregnancy.

REFERENCES

Astley SJ, Magnuson SI, Omnell LM, Clarren SK (1999) Fetal alcohol syndrome: changes in craniofacial form with age, cognition, and timing of ethanol exposure in the macaque. Teratology 3:163–172.

Bonthius DJ, West JR (1990) Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. Alcohol Clin Exp Res 1:107–118.

Chambers CD, Braddock SR, Briggs GG, Einarson A, Johnson YR, Miller RK, Polifka JE, Robinson LK, Stepanuk K, Lyons Jones K (2001) Postmarketing surveillance for human teratogenicity: a model approach. Teratology 5:252–261.

Chudley AE, Conry J, Cook JL, Loock C, Rosales T, LeBlanc N, Public Health Agency of Canada's National Advisory Committee on Fetal Alcohol Spectrum Disorder (2005) Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. CMAJ 5 (Suppl):S1–S21.

Davies JK, Bledsoe JM (2005) Prenatal alcohol and drug exposures in adoption. Pediatr Clin North Am 5:1369–1393.

Day NL, Jasperse D, Richardson G, Robles N, Sambamoorthi U, Taylor P, Scher M, Stoffer D, Cornelius M (1989) Prenatal exposure to alcohol: effect on infant growth and morphologic characteristics. Pediatrics 84:536–541.

Dunty WC Jr, Chen SY, Zucker RM, Dehart DB, Sulik KK (2001) Selective vulnerability of embryonic cell populations to ethanol-induced apoptosis: implications for alcohol-related birth defects and neurodevelopmental disorder. Alcohol Clin Exp Res 10:1523–1535.

Egglin TK, Feinstein AR (1996) Context bias. A problem in diagnostic radiology. JAMA 21:1752–1755.

Ernhart CB, Morrow-Tlucak M, Sokol RJ, Martier S (1988) Underreporting of alcohol use in pregnancy. Alcohol Clin Exp Res 4:506–511.

Gilbert-Barness E (2010) Teratogenic causes of malformations. Ann Clin Lab Sci 2:99–114.

Harvey EA, Coull BA, Holmes LB (2003) Anticonvulsant teratogenesis 5: observer bias in a cohort study. Birth Defects Res A Clin Mol Teratol 6:452–456.

Hoyme HE, May PA, Kalberg WO, Kodituwakku P, Gossage JP, Trujillo PM, Buckley DG, Miller JH, Aragon AS, Khaole N, Viljoen DL, Jones KL, Robinson LK (2005) A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 institute of medicine criteria. Pediatrics 1:39–47.

Jacobson JL, Jacobson SW, Sokol RJ, Martier SS, Ager JW, Shankaran S (1994) Effects of alcohol use, smoking, and illicit drug use on fetal growth in black infants. J Pediatr 124:757–764.

Jacobson SW, Chiodo LM, Sokol RJ, Jacobson JL (2002) Validity of maternal report of prenatal alcohol, cocaine, and smoking in relation to neurobehavioral outcome. Pediatrics 5:815–825.

Jones KL, Smith DW (1973) Recognition of the fetal alcohol syndrome in early infancy. Lancet 7836:999–1001.

Lubchenco LO, Hansman C, Boyd E (1966) Interuterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 42 weeks. Pediatrics 37:403–408.

Maier SE, West JR (2001a) Drinking patterns and alcohol-related birth defects. Alcohol Res and Health 3:168–174.

Maier SE, West JR (2001b) Regional differences in cell loss associated with binge-like alcohol exposure during the first two trimesters equivalent in the rat. Alcohol 1:49–57.

Martin RA, Jones KL, Benirschke K (1996) Absence of the lateral philtral ridges: a clue to the structural basis of the philtrum. Am J Med Genet 2:117–123.

National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (NCHS) (2000a) Birth to 36 months: boys head circumference-for-age and weight-for-length percentiles. Available at: http://www.cdc.gov/growthcharts/data/set1clinical/cj41l019.pdf. Accessed November 15, 2010.

National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (NCHS) (2000b) Birth to 36 months: girls head circumference-for-age and weight-for-length percentiles. 2000. Available at: http://www.cdc.gov/growthcharts/data/set1clinical/cj41c020.pdf. Accessed November 15, 2010.

National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (NCHS) (2000c) Birth to 36 months: boys length-for-age and weight-for-age percentiles. Available at: http://www.cdc.gov/growthcharts/data/set1clinical/cj41c017.pdf. Accessed November 15, 2010.

National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (NCHS) (2000d) Birth to 36 months: girls length-for-age and weight-for-age percentiles. Available at: http://www.cdc.gov/growthcharts/data/set2clinical/cj41c068.pdf. Accessed November 15, 2010.

Nellhaus G (1968) Head circumference from birth to eighteen years: practical composite international and interracial graphs. Pediatrics 41:106–114.

Sampson PD, Streissguth AP, Bookstein FL, Little RE, Clarren SK, Dehaene P, Hanson JW, Graham JM Jr (1997) Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. Teratology 5:317–326.

Sulik KK (1984) Critical periods for alcohol teratogenesis in mice, with special reference to the gastrulation stage of embryogenesis. Ciba Found Symp 105:124–141.

Sulik KK, Johnston MC, Webb MA (1981) Fetal alcohol syndrome: embryogenesis in a mouse model. Science 4523:936–938.

Thomas IT, Gaitantzis YA, Frias JL (1987) Palpebral fissure length from 29 weeks gestation to 14 years. J Pediatr 2:267–268.