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Effects of heavy prenatal alcohol exposure and iron deficiency anemia on child growth and body composition through age 9 years

R. Colin Carter, MD¹, Joseph L. Jacobson, PhD^{3,4,5}, Christopher D. Molteno, MD⁵, Hongyu Jiang, PhD², Ernesta M. Meintjes, PhD⁴, Sandra W. Jacobson, PhD^{3,4,5,6}, and Christopher Duggan, MD, MPH^{2,6}

¹Divisions of Emergency Medicine, Children's Hospital Boston, Boston, MA, United States

²Gastroenterology and Nutrition, Children's Hospital Boston, Boston, MA, United States

³Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, United States

⁴Departments of Human Biology, University of Cape Town, Cape Town, South Africa

⁵Psychiatry and Mental Health, University of Cape Town, Cape Town, South Africa

Abstract

BACKGROUND—Prenatal alcohol exposure has been associated with pre- and postnatal growth restriction, but little is known about the natural history of this restriction throughout childhood or the effects of prenatal alcohol on body composition.

OBJECTIVE—To examine the effects of heavy prenatal alcohol exposure on longitudinal growth and body composition.

DESIGN—85 heavy drinking pregnant women (2 drinks/day or 4 drinks/occasion) and 63 abstaining and light-drinking controls (< 1 drink/day, no bingeing) were recruited at initiation of prenatal care in an urban obstetrical clinic in Cape Town, South Africa, and prospectively interviewed during pregnancy about alcohol, smoking, drug use, and demographics. Among their children, length/height, weight, and head circumference were measured at 6.5 and 12 months and at 5 and 9 years. Percent body fat was estimated at age 9 years using bioelectric impedance analysis.

RESULTS—In multiple regression models with repeated measures (adjusted for confounders), heavy alcohol exposure was associated with reductions in weight (0.6 SD), length/height (0.5 SD), and head circumference (0.9 cm) from 6.5 months to 9 years that were largely determined at birth. These effects were exacerbated by iron deficiency in infancy but were not modified by iron deficiency or measures of food security at 5 years. An alcohol-related postnatal delay in weight gain was seen at 12 months. Effects on head circumference were greater at age 9 than at other age points. Although heavy alcohol exposure was not associated with changes in body composition, children with fetal alcohol syndrome (FAS) and partial FAS (PFAS) had lower % body fat than heavy exposed nonsyndromal and control children.

Corresponding author (same for reprint requests): R. Colin Carter, MD Division of Emergency Medicine Children's Hospital Boston 300 Longwood Avenue Boston, MA 02115 RobertColin.Carter@childrens.harvard.edu Telephone: (617) 355-6624 Fax: (617) 730-0335.

⁶Drs. Christopher Duggan and Sandra W. Jacobson shared equally in the responsibilities of senior author of this paper.

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CONCLUSIONS—Heavy prenatal alcohol exposure is related to prenatal growth restriction that persists through age 9 years and an additional delay in weight gain during infancy. FAS and PFAS diagnoses are associated with leaner body composition in later childhood.

Keywords

fetal alcohol syndrome; intrauterine growth retardation; postnatal growth; body composition; bioelectric impedance analysis; prenatal alcohol exposure; iron deficiency; food security

INTRODUCTION

Three decades of human and animal research have identified a range of teratogenic effects of prenatal alcohol exposure, known broadly as fetal alcohol spectrum disorder (FASD). In addition to specific craniofacial dysmorphology, growth retardation is required for a diagnosis of fetal alcohol syndrome (FAS) and is one of the diagnostic criteria for a diagnosis of partial FAS (PFAS) (Stratton et al., 1996, Hoyme et al., 2005). Animal and human studies have consistently demonstrated intrauterine growth retardation (IUGR) among offspring exposed prenatally to alcohol, with smaller weight, length, and head circumference (Jacobson et al., 1994, Greene et al., 1991, Streissguth et al., 1981). These effects are independent of gestational age, indicating teratogenic effects on fetal growth that precede late gestation.

Although studies of the effects of fetal alcohol exposure on growth have consistently demonstrated deficits that persist through infancy, the data on long-term postnatal growth from prospective human studies have been inconsistent. In a prospective study of alcohol-exposed children in a low-income population, Day and colleagues found alcohol-related deficits in height, weight, head circumference, and skinfold thickness through age 14 years after controlling for other substance use during pregnancy (Day et al., 2002). Similar alcohol effects were seen on weight, height, and head circumference through age 8 years in a prospective study of impoverished children prenatally exposed to alcohol and cocaine (Lumeng et al., 2007). In a longitudinal, prospective cohort study in inner city Detroit, heavy alcohol exposure was related to smaller weight and length in infancy, and these deficits persisted through adolescence (Fuller et al., 2005). In this cohort, Jacobson et al. (1994a) also found that the magnitude of the effect of prenatal alcohol exposure on weight increased during the first year of life, suggesting a separate effect on postnatal growth, possibly mediated by a mechanism different from that responsible for IUGR. A few studies examining children exposed at moderate levels in more optimal socioeconomic circumstances have failed to show long-term differences in childhood anthropometrics (Streissguth et al., 1981, Streissguth et al., 1991, Sampson et al., 1994). Thus, beyond early infancy, it is unclear whether alcohol-related growth restriction gradually improves, worsens, or remains constant during childhood. The differences in longitudinal growth findings between cohorts may be partially explained by postnatal environmental influences that may mitigate or exacerbate alcohol-related growth restriction, but such influences remain largely unstudied. For example, we found that alcohol-related reductions in weight and length in infancy were markedly stronger among infants with iron deficiency anemia (IDA) (Carter et al., 2007) than infants without IDA. This interaction between iron status and the effects of alcohol on early growth has also been seen in a rat model (Rufer et al., 2010), but the impact of this and other postnatal factors on longitudinal growth is not known.

With the exception of Day et al.'s (2002) finding of reduced skinfold thickness, none of the studies examining prenatal alcohol exposure and growth have evaluated whether alcohol exposure is associated with changes in body composition. Leaner body composition in

alcohol-exposed children would suggest deficiencies in nutritional intake, gastrointestinal absorption, nutrient utilization, and/or energy expenditure. Information about effects on body composition may also help elucidate the degree to which effects on growth are attributable to nutritional inadequacies or to physiologic effects resulting from fetal programming.

The current study examined data from a longitudinal, prospective cohort of 159 Cape Coloured (mixed ancestry (Southeast Asian, European, and indigenous South African)) maternal-offspring pairs recruited to assess the developmental effects of heavy prenatal alcohol exposure in Cape Town, South Africa (Jacobson et al., 2008b, Carter et al., 2005), where the incidence of FAS in the Cape Coloured population is 18 to 141 times greater than in the United States (May et al., 2000). The aims of this study were (1) to assess the relation of heavy prenatal alcohol exposure to longitudinal growth through age 9 years; (2) to examine whether the magnitude of these effects is constant or changes over time; (3) to examine whether these effects are stronger among children who were iron deficient as infants; and (4) to examine the relation of prenatal alcohol exposure to body composition at 9 years of age.

MATERIALS AND METHODS

Sample

Mothers were recruited between July 1999 and January 2002 from the antenatal clinic of a midwife obstetric unit that serves an economically disadvantaged Cape Colored community. Each mother was interviewed during her initial antenatal visit regarding her alcohol consumption both at the time of conception and the time of recruitment, using an interview derived from the timeline follow-back approach developed for the Detroit longitudinal study (Sokol et al., 1985, Jacobson et al., 2002). Any woman averaging at least 1.0 oz absolute alcohol (AA) per day, the equivalent of two standard drinks, or reporting at least two binge drinking episodes (≥ 2.5 oz AA per occasion, based on NIAAA criteria in effect at that time) during the first trimester of pregnancy was invited to participate in the study. Women initiating antenatal care at this clinic who drank < 0.5 oz AA/day and did not binge drink during the first trimester were invited to participate as abstainers/light drinkers. Women < 18 years of age and those with diabetes, epilepsy, or cardiac problems requiring treatment were not invited to participate. Religiously observant Muslim women were also excluded because their religious practices prohibit alcohol consumption, and they would, therefore, have been disproportionately represented among the controls. Nonobservant Muslim women were invited to participate in the study. Infant exclusion criteria were major chromosomal anomalies, seizures, neural tube defects, and product of twin or other multiple births. At the time the women were recruited into this study, there was a much lower incidence of HIV among the Cape Coloured population than in black African mothers (Makubalo et al., 2000), and HIV screening was not routine. One woman reported being HIV positive at the 5-year follow-up, and none of the children in the sample were reported by their mothers to be HIV-infected at the 9-year follow-up when interviewed about the child's health history. All women who reported drinking during pregnancy were advised to stop or reduce their intake, and women were invited to participate in a home visitor intervention program that incorporated principles of motivational interviewing.

Informed consent for recruitment and study participation were obtained from each mother. Consent and interviews were conducted in Afrikaans or English, depending on the mother's preference. Approval for human research was obtained from both the Human Investigation Committee at Wayne State University School of Medicine and the Ethics Committee at the University of Cape Town (UCT) Faculty of Health Sciences.

Prenatal Alcohol Exposure Assessments and FAS diagnosis

In the initial timeline follow-back interview administered at recruitment, each woman was asked about her drinking on a day-by-day basis during a typical 2-week period around time of conception, with recall linked to specific times of daily activities. If her drinking had changed since conception, she was also asked about her drinking during the past 2 weeks and when her drinking had changed. Each mother was subsequently interviewed at UCT using the timeline follow-back interview and asked about her alcohol, smoking, and drug use during the previous 2 weeks. If there were any weeks since the recruitment visit when she drank greater quantities, she was asked to report her drinking for those weeks as well. At the 1-month postpartum visit, the mother was asked about her drinking, smoking, and drug use during a typical 2-week period during the latter part of pregnancy, as well as her drinking during any weeks during that period when she drank greater quantities.

Volume consumed was recorded for each type of alcohol beverage consumed and converted to oz of absolute alcohol (AA) by using the weights proposed by Bowman, Stein, and Newton (liquor—0.4, beer—0.04, wine—0.2) (Bowman et al., 1975). Three summary measures were constructed—oz AA/day averaged across pregnancy and AA per occasion, and frequency of drinking. Heavy exposure was defined as either AA/day ≥ 1.0 or binge drinking, defined as average oz AA per occasion ≥ 2.0 (based on current NIH guidelines) at recruitment or across pregnancy. All infants whose mothers abstained, or drank < 0.5 AA/day and did not binge drink, were considered to have had no more than minimal exposure. Maternal report of drinking during pregnancy was previously validated in relation to levels of fatty acid ethyl esters, metabolites of alcohol that are trapped in meconium specimens, which were obtained from a subsample of newborns who participated in this study (Bearer et al., 2003). All postnatal assessments, including dysmorphology examinations, were performed by examiners who were blinded to prenatal alcohol exposure status.

At age 5 years, children were examined in an FAS diagnostic clinic for growth and FAS anomalies using a standard protocol (Hoyme et al., 2005) by two expert U.S.-based FAS dysmorphologists, who initially examined each child independently and subsequently reached agreement regarding FAS diagnosis (Jacobson et al., 2008b). There was substantial agreement between the two U.S.-based dysmorphologists on their assessments of all dysmorphic features, particularly palpebral fissure length and philtrum and vermilion ratings ($r_s = 0.80, 0.84$, and 0.77 , respectively) based on the Astley and Clarren (Astley and Clarren, 2001) rating scales. There was also substantial agreement between them and a Cape Town-based dysmorphologist (median $r = 0.78$), who evaluated the 8 children who could not be scheduled for the clinic. A case conference was conducted by SWJ, JLJ, CDM, and the two U.S. dysmorphologists to determine FAS and PFAS diagnoses.

Anthropometry and Body Composition

Child weight, length/height, and head circumference were measured at each postnatal visit (6.5 and 12 months, 5 and 9 years) using standard protocols (CDC, 2007). Each measurement was obtained twice by trained research staff blind to exposure. In cases of disagreement between the initial two measurements (defined as ≥ 100 g for weight, ≥ 0.1 cm for length/height and head circumference), a third measurement was taken and the average of the two closest values was used for analyses. Using CDC norms, weight-for-age (WAZ), length/height-for-age (HAZ), weight-for-length (for infancy visits), and BMI (for the 5- and 9-year visits) Z-scores were calculated (Kuczmarski et al., 2002)). Given the lack of validated, continuous percentile references for head circumference after age 3 years, raw values (cm) were used for the 5- and 9-year visits. At the 9-year visit, multifrequency bioelectric impedance analysis was performed using a Quadscan 4000 (Bodystat Ltd., Douglas, UK) multifrequency bioelectric impedance analysis unit. Total body water (TBW)

and fat free mass (FFM) were estimated using standardized, validated equations created by Horlick et al. (2002) and Houtkooper et al. (1989), respectively. TBW was divided by body weight for each child to calculate % TBW. Body fat (BF) was calculated as body weight - FFM and then divided by body weight to calculate % BF.

Iron Status

Automated complete blood count analysis was performed on a subsample of the cohort during infancy, including white blood cell count, hemoglobin concentration (Hb), hematocrit, platelet count, and red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCH), and red cell distribution width (RDW). Samples were obtained at 6.5 months for 22 infants and 12 months for 74 infants. Infants were determined to have iron deficiency anemia (IDA) if they had (1) Hb < 10.9 g/L, (2) RDW > 15.0%, and (3) either MCV < 70.0 or MCH < 23.0 (Carter et al., 2007). Infants with Hb < 10.9 g/L who did not meet the other IDA criteria were determined to have anemia of other causes and were excluded from all analyses involving iron status. For statistical analyses, infants with IDA were compared to non-anemic infants. At the 5-year visit, complete blood count and serum ferritin assays were performed on venous blood samples. Children were classified as having iron deficiency (ID) if ferritin < 12 µg/L. IDA was defined as iron deficiency with Hb < 10.9 g/L (WHO/UNICEF/UNU, 1998). Children were considered iron sufficient (IS) if ferritin ≥ 12 µg/L and Hb ≥ 10.9 g/L. Children with Hb < 10.9 g/L and ferritin ≥ 12 µg/L were considered anemic from other causes and excluded from analyses.

Control Variables

Each mother was asked at both the antenatal and postnatal interviews how many cigarettes she smoked per day and how frequently (days/month) she used illicit drugs, including cocaine, marijuana, and methaqualone ("mandrax"), during pregnancy. Maternal parity, education, and marital status were determined during prenatal interviews. Birthweight was obtained from hospital medical records. Gestational age (GA) was calculated from early pregnancy ultrasound examination or expected date of confinement based on last menstrual period when ultrasound data were not available. At the 6.5- and 12-month infant visits, mothers were asked whether the infant was breastfed and, if so, for how many weeks or months. At the 5-year visit the USDA Core Food Security Module Questionnaire, a detailed questionnaire aimed at assessing food security during the preceding 12 months was administered to the mothers (Bickel et al., 2000). This module includes 16 detailed questions addressing anxiety regarding household food supply; experiences of running out of food without means to obtain more; adjustments to normal food use (e.g., substituting fewer and/or cheaper foods); and instances and consequences of reduced food intake for adults and children. Missing items within the 16-item questionnaire were imputed using the standardized method recommended by the USDA (Bickel et al., 2000) for 10 women (8 had 3 missing items; all 10 were missing 5 items). Households are given a Food Security Scale score and are then classified as Food Secure, Food Insecure without Hunger, Food Insecure with Hunger, or Food Insecure with Severe Hunger.

Statistical Methods

All quantitative variables were examined for normality of distribution. In cases of near normality, extreme outliers (mothers with values more than 3 SD beyond the sample mean) were recoded to one unit greater than the highest value observed within 3 SD above the mean as recommended by Winer (1971). This transformation was performed for average AA/drinking day (2 women) and cigarette smoking during pregnancy (1 woman).

T-tests and chi square analyses were used to identify which control variables differed between the heavily exposed and control groups. Any control variable that was related to a given outcome variable at $p < .20$ was considered a potential confounder (Maldonado and Greenland, 1993, Jacobson et al., 2008a). Covariates were chosen for inclusion in the final analysis of covariance (ANCOVA) and regression models using a forward selection approach, in which they were added sequentially to the analysis in order of the magnitude of their relation to the outcome variable. Covariates were retained in the model only if they resulted in a change of at least 10% in the magnitude of the regression coefficient relating alcohol exposure status to the outcome variable at step of entry (Greenland and Rothman, 1998, Jacobson et al., 2008a).

Multiple regression analyses with repeated measures (proc mixed; SAS v. 9.2, SAS Institute Inc., Cary, NC, USA) were performed to examine the relation between alcohol exposure and longitudinal growth outcomes (weight, length/height, weight-for-length or BMI *Z*-scores and head circumference (cm)). This method utilizes all outcomes observed for each subject (which may not correspond to the planned study visit schedule due to missed visits) and adjusts for within-subject variability. Unstandardized regression coefficients are reported.

ANCOVA was used to compare means for growth outcomes (weight, length/height, weight-for-length, and BMI *Z*-scores; head circumference in cm) between the exposed and control groups at each age using confounders selected from the multiple regression analyses for each outcome. To examine growth rates across the age intervals, change in *Z*-scores was calculated for each interval (e.g., 6.5 to 12 months, 12 months to 5 years, etc.) for weight, length/height, and weight-for-length or BMI *Z*-scores. For head circumference, change in head circumference raw values was calculated. ANCOVA was used to compare the change in *Z*-score and head circumference measures across each age interval between heavily exposed and control children, adjusting for confounders selected for each outcome measure.

To examine whether poor iron status exacerbated the effects of alcohol on growth, an interaction term was constructed (alcohol exposure $\times$ iron status) and added to the regression models. Given the sample size and the relatively low power of using interaction terms to detect moderator effects in non-experimental field studies, the regression models were also run separately for children in the different iron status groups (i.e., IDA vs. non-anemic) to examine whether the magnitude of the regression coefficients for prenatal alcohol exposure differed between groups, which would suggest that iron status may modify the effects of alcohol exposure on growth. Similar analyses with interaction terms and stratified regression analyses were used to examine whether food insecurity exacerbated the effects of alcohol on growth.

ANCOVA models were used to compare % TBW and % BF between heavily exposed and control children, after controlling for potential confounders chosen using the same forward selection method used in the regression models. In addition, separate ANCOVAs were performed to compare % TBW and % BF among children without FAS, those with PFAS and those with FAS.

RESULTS

Sample

Of the 159 women recruited, 84 initially reported heavy drinking and 75 reported abstaining or drinking at very low levels. Among the heavy drinkers, 4 moved away; there was 1 fetal death at 22 weeks gestation and 1 stillbirth, both of whom were found to have FAS at autopsy by H. Wainwright, MD (UCT Pathology Department); and 2 children died in early infancy. Among the abstainers/ light drinkers, 1 moved away, and 2 children died in infancy.

Six women initially recruited as light-drinkers reported heavy drinking in prospective interviews during pregnancy and were thus re-classified as heavy drinkers. Two women who initially denied drinking had children diagnosed with FAS; one of these acknowledged heavy drinking retrospectively, and the other had high meconium levels of fatty acid ethyl esters, indicating heavy exposure (Bearer et al., 2003). One mother of a child with PFAS who almost met binge criteria (oz AA per occasion = 1.9) later reported heavier drinking and bingeing retrospectively. All three mothers were then re-classified as heavy drinkers. Thus, the final sample included 85 heavily exposed children and 63 children with no or light exposure. Participation at each visit was similar between offspring of heavy drinkers and abstainers/light-drinkers (6.5-month visit: 87% vs. 94%, respectively; 12-month visit: 85% vs. 83%; 5-year visit: 95% vs. 95%; 9-year visit: 88% vs. 86%; for the 6.5-month visit $p = .091$; all p 's for other visits $> .4$). Sixty-six percent of the children came to all four visits; 26% missed one visit; 7% missed two visits; 1% missed three visits.

Sample characteristics are presented in Table 1. Heavy drinkers were less educated, more likely to be unmarried, and were recruited 3.2 weeks later in gestation than controls. Although heavy drinkers reduced the frequency of drinking after conception from 2.1 days/week on average to 1.5 days/week ($p < .001$), they continued to average over 8 standard drinks (4.1 oz AA) per occasion both at conception and across pregnancy. Although heavy drinkers were more likely to smoke than controls, smokers in both groups smoked similar numbers of cigarettes per day. Illicit drug use during pregnancy was rare in both groups, although more common among heavy drinkers. One control reported using marijuana (2.7 occasions/month), and none reported using cocaine or methaqualone ("mandrax"). Among heavy drinkers, 13 reported using marijuana (mean = 2.4 occasions/month), 4 methaqualone (mean = 1.6 occasions/month), and 1 cocaine (2.6 occasions/month). The heavy drinkers were more likely to report food insecurity, hunger, and severe hunger in their households than controls at the 5-year visit.

Children in the two groups were similar in sex and GA at birth. Children born to heavy drinkers had 4.1 times higher odds of having low birth weight ($\chi^2(1) = 7.81, p = .005$). During infancy, mean hemoglobin (Hb) concentrations were low-for-age for both heavily exposed and control infants. Three heavily exposed infants and one control infant who were not anemic (Hb: 10.9 g/L) and had borderline values for iron indices (Hb: 11–12 g/L; MCH: 20–23.4 pg; MCV: 65.1–70.7 fL, and RDW: 13.8%–18.8%) were classified as indeterminate and excluded from analysis. Heavily exposed infants had 2.1 times higher odds of IDA than those born to abstainers and light drinkers, but this finding did not achieve statistical significance ($\chi^2(1) = 2.44, p = .119$). At 5 years, children born to heavy drinkers had 6.5 times higher odds of having iron deficiency than those born to controls ($\chi^2(1) = 10.27, p = .001$). Four children born to heavy drinkers had IDA at this age, compared to none born to controls. At age 5 years, 17 children were diagnosed with FAS, and 22 children with PFAS.

Prenatal alcohol exposure and longitudinal growth

Table 2 examines the relation between heavy alcohol exposure and longitudinal anthropometric Z-scores. After controlling for potential confounders, average weights of children born to heavy drinkers were 0.6 SD lower than those born to light drinkers/abstainers; average heights were almost 1/2 SD lower. Average head circumference was 0.9 cm smaller among heavily exposed infants when compared to controls. When controlling for both food security at 5 years and potential confounders, average weights among heavily exposed children were 0.5 SD smaller than controls, average heights were 0.4 SD lower, and average head circumferences were 0.7 cm smaller. The magnitudes of these relations were markedly reduced after controlling for birthweight Z-score and gestational age at delivery. Heavy alcohol exposure was not related to differences in weight-for-length or BMI Z-scores.

Table 3 presents the relation between maternal alcohol consumption per occasion and longitudinal growth, controlling for potential confounders. Each increase in maternal drinking per occasion of 1 oz AA (~2 standard drinks) was associated with a decrease in both weight-for-age and length/height-for-age of 0.1 SD and a decrease in head circumference of 0.1 cm. The magnitudes of these relations were markedly reduced after controlling for birthweight *Z*-score and gestational age at delivery.

Figure 1 depicts the growth patterns of each exposure group over time. Mean *Z*-score values, adjusted for the potential confounders listed in Table 2, are charted separately for children born to heavy drinkers and controls at each visit. Alcohol-exposed infants had lower WAZ at birth and throughout childhood and lower HAZ at all visits. Weight-for-length *Z*-scores in infancy and BMI *Z*-scores at ages 5 and 9 years were similar between groups (all *p*'s > .20, ANCOVA). Head circumferences were smaller among alcohol-exposed infants when compared to controls.

ANCOVA analyses comparing changes in growth indices between visits revealed that WAZ among heavily exposed infants decreased from 6.5 to 12 months by 0.07 SD, whereas controls had an average increase in WAZ of 0.2 during this time period (*p* = .06, ANCOVA adjusting for infant sex). By 5 years, between-groups differences were similar to those seen at birth; between 12 months and 5 years, alcohol-exposed children had an increase in average WAZ of 0.1, whereas controls demonstrated a decrease of 0.2 (*p* = .09; ANCOVA adjusting for maternal parity and education and infant sex). Thus, a postnatal delay in weight gain was seen between 6.5 and 12 months but was no longer evident at age 5 years. Changes in WAZ over time among alcohol-exposed children were not significantly different from controls at all other age intervals (all *p*'s > .20, ANCOVA). Changes in HAZ over time were not different at all age intervals for both groups (all *p*'s > .20, ANCOVA). Changes in weight-for-length *Z*-scores over time followed the same trend as those for weight-for-age. From 6.5 to 12 months, controls increased by 0.17 *Z*-scores, whereas heavily exposed infants had a decrease of 0.4 (*p* = .036, ANCOVA); between 12 months and 5 years, heavily exposed caught up with an increase of 0.5 *Z*-scores compared to an increase of 0.1 among controls (*p* = .037, ANCOVA); from ages 5 to 9 years, there were no differences between the groups. From ages 5 to 9 years, heavily exposed children had smaller head growth (1.6 vs. 2.0 cm; *p* = .01, ANCOVA) than controls (adjusting for potential confounders and food security). Changes in head circumference at all other age intervals were similar between groups.

Interaction terms constructed by multiplying maternal alcohol consumption per drinking day by infant iron status (for infancy and age 5 years) were not significantly related to growth outcomes (*p*'s > .50, repeated measures multiple regression). However, in stratified analyses in which children who had IDA in infancy and those without anemia in infancy were examined separately, the effect of prenatal alcohol exposure on WAZ and HAZ was only seen among the children with IDA (Table 4). Thus, IDA in infancy appears to have exacerbated the effects of fetal alcohol exposure on growth. Stratified analyses comparing iron status groups at age 5 years were not performed because there were only four children with IDA at age 5. Of these four children, all were heavily exposed, and two had evidence of growth restriction. When comparing ID children to IS children at age 5, the effects of maternal alcohol consumption on postnatal growth did not differ.

The impact of food security was examined as a proxy for dietary insufficiency. Although food insecurity was associated with poorer longitudinal growth, an interaction term constructed by multiplying maternal alcohol consumption per drinking day by food security classification was not significantly related to growth outcomes (all *p*'s > .20). In stratified regression analyses, the effects of prenatal alcohol exposure on growth among children

whose mothers reported hunger or severe hunger were not different from the effects seen in the entire cohort.

Prenatal alcohol exposure and body composition

Although heavily exposed children had lower %TBW and %BF than controls in *t*-tests (Table 1), heavy alcohol exposure was not related to % TBW (56.9% vs. 57.5%; $p = .550$, ANCOVA) or % BF (18.4% vs. 17.6%; $p = .526$, ANCOVA), after controlling for confounders (child sex and maternal parity, smoking, and education). This is consistent with our finding that weight-for-length in infancy and BMI during childhood were not related to prenatal alcohol exposure. However, ANCOVA analyses demonstrated a relation between FAS diagnosis and higher % TBW and lower % BF (Table 5). *Post-hoc* analyses revealed that this effect was largely driven by differences between those with FAS or PFAS vs. nonsyndromal HE and control children (% TBW: 59.0% vs. 56.5%; $p = .009$ (ANCOVA); % BF: 15.6% vs. 19.1%; $p = .006$, ANCOVA).

DISCUSSION

In this prospective longitudinal cohort, heavy prenatal alcohol exposure was related to reductions in weight, length/height, and head circumference. Our findings of persistent longitudinal growth deficits in children exposed prenatally to alcohol are consistent with other prospective studies examining children from economically-disadvantaged populations (Day et al., 2002, Lumeng et al., 2007) and support the use of growth restriction at any age as one of the diagnostic criteria for FAS and PFAS. Our use of longitudinal regression models using repeated measures for each outcome adjusts for within-subject variability and avoids multiple testing issues that may arise with comparisons of values at discrete time points. The effects on weight were substantially diminished when controlling for birth weight, and the magnitude of the effect of prenatal alcohol exposure on length/height remained remarkably consistent across the four age points. Thus, the longitudinal effects of alcohol on growth were largely determined at the time of birth, as has been demonstrated for other disease processes with fetal origins (Barker, 2004). Prenatal alcohol exposure may have caused epigenetic changes *in utero* that resulted in permanent effects on growth. Such changes may occur in the mother, placenta, and/or fetus. Animal models have demonstrated altered gene expression profiles of mRNA and proteins associated with growth and metabolism among ethanol-exposed fetuses including maternal IGF-1 (Shankar et al., 2006), an important effector of somatic growth, and other maternal and fetal proteins involved in energy production and protein translation (Xu et al., 2008). This altered gene expression may result from epigenetic changes, as alcohol exposure has been associated with alterations in DNA methylation in both the fetus (Liu et al., 2009) and placenta (Haycock and Ramsay, 2009).

Although these findings indicate that the effects of alcohol on growth are mainly determined prenatally, postnatal influences may have an impact on these effects over time. Consistent with Jacobson et al.'s (1994a) findings in a Detroit inner-city cohort, heavy prenatal alcohol exposure was related to a postnatal delay in weight gain during infancy that was no longer evident at 5 years. The cause of this delay remains unclear. After age 6 months, infants generally begin to rely more on sources of nutrition other than breast- and/or bottle-feeding, and prenatal alcohol exposure may cause postnatal developmental delays that affect food intake (e.g., in oromotor function) and/or physiologic nutrient utilization. The growth delay seen in infancy occurs during a critical time of development, during which growth delays in other disease models (e.g., prematurity) have been shown to cause long-term neurodevelopmental deficits. Thus, future studies are needed to examine the cause of the delay and whether interventions involving macro- or micronutrient supplementation in infancy might ameliorate this growth delay as has been seen for other fetal alcohol outcomes

in animal models (Xu et al., 2006, Thomas et al., 2009). We also identified a worsening of alcohol-related reductions in head circumference between ages 5 and 9 years. What the pattern of this delay will be after age 9 years is unknown, and the implications of this delay for cognitive function are unclear.

Effects of alcohol exposure on growth were similar for children whose mothers reported differing degrees of hunger and access to food, suggesting that food insecurity does not exacerbate the effects of alcohol on growth. Moreover, we found that heavy alcohol exposure *per se* was not related to lower weight-for-length or BMI Z-scores or to changes in % TBW or % BF at age 9 years, suggesting that prenatal alcohol leads to proportionate reductions in body fat, lean body mass, and water. However, children with clinical diagnoses of FAS or PFAS did have significantly lower body fat compared to children without these diagnoses, indicating lower fat stores. Among children with FAS or PFAS, mean % BF for males was at the 9th percentile, whereas for females it was below the 2nd percentile (using the references set provided by McCarthy et al.(2006)). Among children without FAS or PFAS, the means for males and females were both at the 50th percentiles. Our food security data suggest that the difference in % BF between children with FAS or PFAS and those without these diagnoses is not due to inadequate food availability. This difference could be attributable to decreased intake (e.g., total energy or protein), increased metabolic needs (e.g., from increased catabolism or physical activity), or a deficit in nutrient utilization in alcohol-exposed children. Moreover, it is not clear whether the low body fat seen in these most affected children might be amenable to interventions that bolster nutrition, which are effective in other diseases, e.g., cystic fibrosis, metabolic disorders, and intestinal diseases. Further understanding of the etiology of this alteration in body composition is warranted. These findings underscore the importance of a full medical evaluation for potentially correctable medical or environmental etiologies of growth restriction in all children, regardless of prenatal alcohol exposure or FAS diagnosis.

This study had limitations common to other longitudinal studies of development. Some children were unable to attend all visits. However, attrition was very low overall, and most children missed no more than one visit. Although the heavy drinkers and abstainers in this cohort were recruited from the same impoverished community, numerous unmeasured environmental, dietary, and genetic influences on postnatal growth and body composition may exist in this sample, making some degree of residual confounding possible. Noise surrounding estimates of true alcohol exposure to the fetus may also obscure some of the group differences in growth outcomes. For example, individual variation in maternal and fetal alcohol metabolism due to factors, such as body size, metabolic activity, hydration, and genetic influences may affect the accuracy and precision of estimates of prenatal alcohol exposure. Differences between true and estimated exposure are likely small, however, given the validity of maternal reports demonstrated in relation to meconium assays (Bearer et al., 2003) in this cohort and the validity of the interviewing techniques shown in the Detroit Longitudinal Cohort Study (Jacobson et al., 2002). This study was not powered to detect statistically significant interaction effects of iron status and prenatal alcohol exposure, given the sample size of the cohort. However, the stratified analyses comparing the magnitude of the effects of prenatal alcohol exposure on growth between iron status groups suggest possible interaction effects between iron status in infancy and prenatal alcohol exposure, as noted above. Although the USDA Food Security Questionnaire has been used in varying cultural groups and geographic locations (Webb et al., 2006), it has not been validated in studies with Cape Coloured subjects, and the categorizations used in this study may not be accurate. At the time of measurement, to our knowledge, no validated food security measure for the peri-urban Cape Coloured community existed. Although equations for bioelectric impedance analysis have not been validated in Cape Coloured children and thus may not estimate true % TBW and % BF values for individuals, this method can be used to make

comparisons within an ethnically homogeneous population such as the cohort in this study (Sun et al., 2003).

This study confirms prior findings of alcohol-related growth restriction that persist through childhood and provides novel insight into the patterns of this growth restriction. To our knowledge, this study is the first to examine the effects of heavy prenatal alcohol exposure on growth over time using a single model with repeated measures for each outcome. The effects of alcohol exposure on weight were largely determined at birth, and the effects on length/height were relatively constant over time, suggesting that the long-term growth deficits seen are the result of teratogenic processes that occurred prenatally. We also found separate postnatal effects of alcohol on growth, including a delay in weight gain at 12 months, as seen previously in Detroit, and a novel finding of worsening alcohol-related reductions in head circumference between 5 and 9 years. The effects of alcohol exposure on longitudinal growth were stronger among children who had IDA as infants but were neither mediated nor modified by postnatal nutrition at age five, as indicated by iron status and food security. Heavy alcohol exposure was not related to changes in body composition, as measured by weight-for-length *Z*-scores, BMI *Z*-scores, or body fat measurements. However, children with FAS and PFAS had lower percent body fat than children without these diagnoses, indicating leaner body composition in the most affected children, the cause of which remains unclear. Future studies are needed to examine the mechanisms underlying the alcohol-related growth retardation and changes in body composition observed in this cohort.

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Figure 1.

Estimated marginal means for child growth indices by prenatal alcohol exposure status (with 95% CI; adjusted for confounders selected in multiple regression models (see Table 2); Z-scores based on CDC (2000) reference standards)

▲ Controls
■ Heavy Exposure

[†]*p* .10. **p* .05. ***p* .01

Table 1

Sample characteristics

	Controls (n = 63)		Heavy exposure (n = 85)		p
	N	M ± SD or n (%)	N	M ± SD or n (%)	
Maternal characteristics					
Weeks gestation at recruitment	63	23.4 ± 7.8	85	26.6 ± 10.1	.037
Age at delivery	63	26.6 ± 5.7	85	26.9 ± 6.6	.799
Years of school completed	63	9.6 ± 2.1	85	7.8 ± 2.6	< .001
Married (%)	63	30 (47.6)	85	16 (18.8)	< .001
Parity	63	2.0 ± 1.2	85	2.3 ± 1.5	.117
Average oz AA per day					
At conception	63	0.0 ± 0.1	85	1.4 ± 1.6	< .001
During pregnancy	63	0.0 ± 0.1	85	0.9 ± 1.0	< .001
Average oz AA per drinking day					
At conception	63	0.1 ± 0.4	85	4.1 ± 2.8	< .001
During pregnancy	63	0.1 ± 0.4	85	4.1 ± 2.5	< .001
Number of drinking days/week					
At conception	63	0.1 ± 0.3	85	2.1 ± 1.5	< .001
During pregnancy	63	0.1 ± 0.2	85	1.5 ± 1.1	< .001
Cigarette users (%)	63	30 (47.6)	85	74 (87.1)	< .001
No. per day among users		8.5 ± 8.2		7.7 ± 5.2	.651
Marijuana users (%)	63	1 (1.6)	85	13 (15.3)	.005
Occasions per month among users		2.7 ± N.A.		2.4 ± 2.1	.872
Food security					
Food insecure (%)	58	27 (46.6)	81	56 (69.1)	.008
Hunger (%)	58	12 (20.7)	81	44 (54.3)	< .001
Severe hunger (%)	58	8 (13.8)	81	32 (39.5)	< .001
Child characteristics					
Sex (% male)	63	28 (44.4)	85	51 (60.0)	.061
Gestational age (wk)	63	39.0 ± 2.0	85	38.6 ± 2.7	.340
Birthweight (g)	63	3094.0 ± 466.6	85	2731.9 ± 592.8	< .001
Birthweight Z-score	63	-0.7 ± 0.9	85	-1.3 ± 1.0	< .001

	Controls (<i>n</i> = 63)		Heavy exposure (<i>n</i> = 85)		<i>p</i>
	<i>N</i>	<i>M</i> ± <i>SD</i> or <i>n</i> (%)	<i>N</i>	<i>M</i> ± <i>SD</i> or <i>n</i> (%)	
Birthweight < 2500g (%)	63	5 (7.9)	85	22 (25.9)	.005
Breastfeeding (%)	63	59 (93.7)	85	74 (87.1)	.189
No. of weeks among those breastfed		32.3 (20.4)		32.7 (21.2)	.924
Ages at visits					
6.5-month	60	7.1 (0.6)	74	7.1 (0.7)	.978
12-month	52	12.3 (0.6)	72	12.3 (0.7)	.477
5-year	60	4.7 (0.7)	81	5.1 (0.8)	.002
9-year	54	8.7 (0.8)	75	9.1 (0.8)	.002
Iron status					
Infancy					
Hemoglobin (g/dL)	48	10.8 ± 1.3	48	10.5 ± 1.2	.254
Iron deficiency anemia (%) ^a	34	13 (38.2)	37	21 (56.8)	.119
Age 5 years					
Hemoglobin (g/dL)	55	12.2 ± 0.7	78	12.0 ± 0.8	.130
Iron deficiency (%)	55	3 (5.5)	77	21 (27.3)	.001
Iron deficiency anemia (%)	55	0 (0.0)	77	4 (5.2)	.086
Diagnosis (age 5 years)					
FAS (%)	58	0.0 (0.0)	81	17 (21.0)	< .001
PFAS (%)	58	0.0 (0.0)	81	22 (27.2)	< .001
Bioelectric impedance analysis					
Percent total body water	54	56.1 (5.7)	74	58.1 (4.7)	.024
Percent body fat	54	19.6 (7.5)	74	16.8 (6.2)	.024

^aExcluding 21 children with anemia of other causes (13 controls, 8 heavily exposed) and 4 infants who were not anemic (Hb: 10.9 g/L) and had borderline values for iron indices (Hb: 11–12 g/L; MCH: 20–23.4 pg; MCV: 65.1–70.7 fL, and RDW: 13.8%–18.8%) and were classified as indeterminate (1 control, 3 heavily exposed).

Table 2

Relation of heavy prenatal alcohol exposure to child anthropometric indices

	Univariate		Multivariable adjusted for potential confounders		Multivariable adjusted for potential confounders and food security group		Multivariable adjusted for potential confounders, food security group, and gestational age and weight-for-age Z-score at birth	
	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)
Weight-for-age Z-score ^a	-.66***	(-1.0, -.22)	-.60***	(-.93, -.27)	-.49**	(-.83, -.15)	-.21	(-.53, 0.11)
Height-for-age Z-score ^b	-.60***	(-.90, -.30)	-.49**	(-.80, -.18)	-.43**	(-.75, -.11)	-.21	(-.52, 0.10)
Weight-for-length or BMI Z-score ^b	-.29*	(-.59, .01)	-.22	(-.54, .10)	-.14	(-.46, .18)	-.14	(-.46, 0.18)
Head circumference (cm) ^c	-.44	(-1.08, .20)	-.88***	(-1.32, -.44)	-.74**	(-1.22, -.26)	-.39 [†]	(-.86, 0.08)

[†] *p* .10.

* *p* .05.

** *p* .01.

*** *p* .001.

^a Potential confounders: maternal parity.

^b Potential confounders: maternal education (years of school completed).

^c Potential confounders: maternal parity, infant sex, and child's age at measurement.

Table 3
Relation of average maternal alcohol consumption per drinking day to anthropometric indices

	Univariate		Multivariable adjusted for potential confounders		Multivariable adjusted for potential confounders and gestational age and weight-for-age Z-score at birth	
	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)
Weight-for-age Z-score ^a	-.13***	(-.20, -.06)	-.11***	(-.18, -.04)	-.05	(-.11, 0.01)
Height-for-age Z-score ^b	-.12***	(-.18, -.06)	-.09***	(-.15, -.03)	-.04	(-.10, 0.02)
Weight-for-length or BMI Z-score ^b	-.04	(-.10, .02)	-.01	(-.07, .05)	0.04	(-.02, 0.10)
Head circumference (cm) ^c	-.11 [†]	(-.23, .01)	-.13**	(-.23, .03)	-.06	(-.16, 0.04)

[†] *p* .10.

* *p* .05.

** *p* .01.

*** *p* .001.

^a Potential confounders: maternal parity, food security.

^b Potential confounders: maternal parity, food security, maternal smoking, and maternal education.

^c Potential confounders: maternal parity, food security, and child's age at measurement.

Table 4

Comparison of relation of maternal alcohol consumption per drinking day to anthropometric indices between infant iron status groups

	Non-anemic (<i>n</i> = 37)		Iron deficiency anemia (<i>n</i> = 34)	
	Beta	(95% CI)	Beta	(95% CI)
Weight-for-age Z-Score ^a	-.08	(-.23, .07)	-.14 [*]	(-.26, -.02)
Height-for-age Z-Score ^b	-.08	(-.20, .04)	-.12 [*]	(-.22, -.02)
Weight-for-length or BMI Z-Score ^b	-.07	(-.22, .08)	-.06	(-.18, .02)
Head circumference (cm) ^c	-.15 [†]	(-.31, .01)	-.06	(-.26, .14)

[†]
p .10.

^{*}
p .05.

^aAdjusted for maternal parity.

^bAdjusted for maternal parity, maternal smoking, and maternal education.

^cAdjusted for maternal parity and child's age at measurement.

Table 5

Mean % total body water and % body fat by FAS diagnosis

	Not FAS or PFAS				<i>p</i>
	Controls (<i>n</i> = 53)		Heavy Exposure (<i>n</i> = 37)		
	<i>M</i> (95% CI)		<i>M</i> (95% CI)		
% Total body water	56.5 (55.2, 57.8)		56.4 (54.9, 57.9)		.026
% Body fat	19.0 (17.3, 20.7)		19.2 (17.2, 21.2)		.023
	</				

Note. Means are adjusted for maternal parity and child sex.