Association of Fatty Acid Ethyl Esters in Meconium and Cognitive Development during Childhood and Adolescence

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Objective To examine associations between amounts of fatty acid ethyl esters (FAEEs) in meconium and cognitive development in school-aged children exposed to alcohol and drugs in utero.

Study design A secondary analysis of a prospective cohort of children, primarily African American and of low socioeconomic status, that was recruited at birth. FAEEs were quantified with gas chromatography via a flame ionization detector. Meconium was analyzed for FAEEs in 216 newborns; 191 of these infants were assessed for IQ at ages 9, 11, and 15 years with the Wechsler Intelligence Scales for Children-Fourth Edition.

Results Longitudinal mixed model analyses indicated that, after we controlled for maternal and child covariates, greater concentrations of FAEEs (ethyl myristate, ethyl oleate, ethyl linoleate, and ethyl linolenate) were associated with lower Wechsler Intelligence Scales for Children-Fourth Edition Verbal Comprehension Index, Working Memory Index, and Full-Scale IQ scores. Associations of FAEEs with Verbal Comprehension Index, Working Memory Index, and Full-Scale IQ did not vary over time. No associations of FAEEs with Perceptual Reasoning and Processing Speed Indices were found.

Conclusion Elevated levels of FAEEs in meconium are potential markers for identifying newborns at risk for poor cognitive development related to prenatal alcohol exposure. *(J Pediatr 2015;166:1042-7)*.

Fetal alcohol spectrum disorders (FASDs) represent a wide range of developmental disabilities resulting from alcohol exposure in utero, including fetal alcohol syndrome (FAS) and alcohol-related neurodevelopmental disorder. FAS, characterized by growth restriction, a distinct pattern of facial features, and evidence of central nervous system dysfunction,[1] is the leading known preventable cause of intellectual disability.[2] Alcohol-related neurodevelopmental disorder refers to various neurologic abnormalities associated with prenatal alcohol exposure, such as problems with memory, learning ability, behavioral problems, and lower intelligence[3] without the facial dysmorphology. Even low-to-moderate levels of maternal drinking during pregnancy (<7 drinks per week), although less consistently, have been related to decreased cognitive ability in African American children,[4] poor memory,[5] and behavioral regulation problems.[6] It is estimated that as many as 2%-5% of younger school children in the US and Western Europe are affected by FASDs, with FAS affecting 2-7 per 1000 live births.[7] A recent meta-analysis[8] reported the overall prevalence of FAS and other FASDs among children in child welfare systems to be 6% and 17%, respectively.

Although early identification of infants at risk for alcohol-related problems is critical to reduce secondary disabilities,[9] the identification of such infants is quite challenging when the distinctive facial features of FAS are not present. Women often underreport drinking because of the stigma associated with drinking during pregnancy. Furthermore, although structured in-depth interviews given by trained professionals in research studies can elicit reliable information,[10] no reliable clinical tools for assessing levels of drinking in pregnant women and identifying newborns who were exposed to alcohol have been established.

Fatty acid ethyl esters (FAEEs), the nonoxidative metabolites of ethanol analyzed in meconium, have been investigated as biomarkers for identifying alcohol exposed neonates.[11,12] Increased concentrations of FAEEs in meconium correlate with fetal exposure to alcohol.[13-15] We previously reported the associations of FAEEs with mental and psychomotor development during the first 2 years of life.[16] To our knowledge, no studies have examined an association between FAEEs in meconium and cognitive outcomes during childhood and adolescence. The purpose of the present study is to extend our previous findings to examine relationships between FAEEs and cognitive development at ages 9, 11, and 15 years. We hypothesize that the relationship of the greater concentration of FAEEs in meconium with poorer cognitive outcomes will persist into older ages.

FAEE	Fatty acid ethyl ester
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorder

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Methods

This study included 191 children (84 boys, 107 girls) recruited at birth (September 1994 to June 1996) from a large, urban, teaching hospital for a longitudinal study on the neurobehavioral effects of prenatal cocaine exposure.[17] Women at high risk for drug use because of a lack of prenatal care, behavior suggesting intoxication, a history of involvement with the Department of Human Services, or self-admitted substance use had drug toxicology screenings at delivery. Women with a psychiatric history (major depression, bipolar disorder, or schizophrenia), low intellectual functioning (diagnosis of intellectual disability indicated in medical chart review), HIV-positive status, or chronic medical illness were excluded, as were infants with Down syndrome, FAS, or congenital heart defects. Random samples of meconium were collected from 248 newborns[14] after informed consent, and 216 had adequate analysis of meconium.[15] Of the 216 children, 14 had missing interview data, 2 children died, and 9 dropped out or were lost to contact, yielding the current sample of 191 (89% retention rate for living children with adequate analysis of meconium). Of the 191 children, 187 (98%) completed the 9-year assessment; 186 (98%) completed the 11-year assessment; and 176 (92%) completed the 15-year assessment. Eighty-nine percent (n = 170) had all 3 IQ assessments, with 98.5% having at least 2 assessments.

Children and their caregivers were seen by separate examiners at the developmental research laboratory for approximately 5 hours at each follow-up visit at ages 9, 11, and 15 years. Children were assessed by a clinical psychologist or master's level research assistant; caregivers were assessed by a social worker or trained research assistant. All research assistants were trained and supervised by a licensed clinical psychologist. Examiners were blinded to mother and infant alcohol and drug exposure status. All participants were given a monetary stipend, lunch, and transportation costs. This study was approved by the Institutional Review Board of the participating hospital. Parental written informed consent and child assent were obtained. A Certificate of Confidentiality (DA-98-91) was obtained from the Department of Health and Human Services.

Meconium was collected shortly after birth and stored at −70°C until analysis. The FAEEs were extracted with acetone/hexane and isolated via silica gel chromatography. Isolated FAEEs were identified and quantitated by gas chromatography with a flame ionization detector.[14,15] Six FAEE analytes were examined: ethyl myristate, ethyl palmitate, ethyl oleate, ethyl linoleate, ethyl linolenate, and ethyl arachidonate.[15] Ethyl stearate was not analyzed because of background noise on the chromatograms,[14] and ethyl palmitoleate was excluded as it did not correlate with alcohol exposure in humans[15] or sheep.[18] Meconium analyses were performed by investigators blinded to the infant's alcohol exposure status.

At the newborn visit, birth mothers were asked to recall frequency and amount of alcohol and drug use for the month prior to and for each trimester of pregnancy. Women were asked the number of drinks consumed per drinking day and what size serving they had. The number of standard drinks (0.5 oz. of absolute alcohol) of beer, wine, or hard liquor per drinking day was computed. Frequency of drinking was recorded on a Likert-type scale ranging from 1 (less than once a month) to 7 (daily use) and converted to reflect the average number of drinking days per week. Number of drinking days per week was multiplied by the number of drinks per drinking day to compute an average number of alcohol drinks per week in the month prior to pregnancy and in each trimester, which were then averaged to obtain a total average drinks per week over the 4 periods of time. Birth mothers also were asked to recall more than the usual number of drinks ("On the days that you drank more than the usual number of drinks, how many drinks do you have?"). Risk drinking during pregnancy was assessed with a total score of (T: Tolerance; W: Worried; E: Eye-openers; A: Amnesia; and K: K/Cut down [TWEAK])[19] ≥2 indicating pregnancy risk drinking.[20] Other substance use during pregnancy, number of tobacco cigarettes, marijuana joints smoked, and crack cocaine "rocks" consumed and the amount of money spent per day, also were collected along with the frequency of use, computing a total average score for each substance (cigarettes, marijuana, and cocaine). The alcohol and drug assessment was updated with the child's current caregiver at the 9-, 11-, and 15-year follow-up visits to measure recent (prior 30 day period) caregiver alcohol and drug use.

Demographic and medical characteristics, including maternal age at birth, gestational age, birth weight and length, head circumference, and Hobel Neonatal Risk score, were extracted from the medical records of the mothers and infants. Socioeconomic status was calculated using the Hollingshead Index.[21] Maternal vocabulary was assessed at birth via use of the Peabody Picture Vocabulary Test-Revised[22] and updated with its third edition[23] at later assessments. The Block Design and Picture Completion subtests of the Wechsler Adult Intelligence Scale-Revised[24] were used to estimate maternal nonverbal intelligence at infant birth. Maternal psychological distress was assessed with the Global Severity Index ($\alpha = 0.95$), a summary scale of the Brief Symptom Inventory,[25] at birth and at each follow-up visit. At each visit, the child's placement (with either biological mother/relative or adoptive/foster caregiver) was noted, and data on the current caregiver were updated to provide concurrent assessment of caregiver intelligence and psychological distress.

At 9, 11, and 15 years, children's intelligence was assessed using the Wechsler Intelligence Scales for Children-Fourth Edition,[26] which yields 4 summary indices (Verbal Comprehension, Perceptual Reasoning, Processing Speed, and Working Memory) and a Full-Scale IQ. The quality of the caregiving environment was assessed via interview at 9, 11, and 15 years with the Home Observation for Measurement of the Environment with the middle childhood version used at age 9 and the early adolescent version used at ages 11 and 15.[27]