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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Supported by the National Institute on Drug Abuse (R01-07957 [to L.S.]), the Association of Retarded Citizens of the United States (to C.B.), Cobey Chair in Neonatology (UMBF#40806) (to C.B.), the Mary G. Munroe Memorial Fund (CT#89), and the Mary G. Munroe Memorial Fund-PDIP (USMF#40261) (to C.B.). The authors declare no conflicts of interest.

Portions of the study were presented at the Annual Neurobehavioral Teratology Society Meeting, Bellevue, Washington, June 28-July 2, 2014.

http://dx.doi.org/10.1016/j.jpeds.2014.12.008

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]

Statistical Analyses

Data that were positively skewed were normalized via a log transformation (alcohol and drug use, maternal psychological distress) prior to analyses. Means and SDs were reported by the variables' original distribution, with transformations used in analyses. Concentrations for each FAEE were transformed by $\log_{10}$ (FAEE + 100) to correct skewed distribution.[16] Spearman correlation (r_s) was conducted to examine the association between specific FAEE and maternal, child, and caregiver characteristics. The relationship of each FAEE with cognitive outcomes was evaluated with a mixed linear model approach with maximum likelihood estimation procedures. Unstructured covariance matrix, estimating each variance and covariance uniquely from the data by making no assumptions regarding the nature of the residual correlations between the repeated measures, was used to account for correlated responses within a subject. We tested the homogeneity of FAEE effects, as well as the effects of sex and other covariates on children's cognitive outcomes over time by including an interaction term with time. If the interaction was not significant at $P < .10$, the interaction terms were removed from the model. Missing data were modeled using full-information maximum likelihood, which uses all available information from the observed data. Variables that were associated with FAEE analytes or with outcomes at $P \leq .20$ for at least one time point were entered into the model using backward elimination approach. Each FAEE was considered separately.

Results

The 191 mothers and children were primarily African American and of low socioeconomic status (Table I). Only 13% of mothers were married at the child's birth; 37% (n = 70) had not finished high school, with a mean (± SD) number of education years of 11.8 (±1.5). Sixty percent of the 191 birth mothers reported alcohol use during pregnancy, with 63% engaged in risk drinking (Tolerance, Worried, Eye-openers, Amnesia, and K/Cut down ≥2) with the mean (± SD) number of alcohol drinks per week of 6.5 (±11.9). Twenty-seven (14% of the total sample) mothers reported ≥7 drinks per week and 15 mothers ≥14 drinks per week, indicating that most of the women in this study were low-to-moderate drinkers. More than one-half of the sample (n = 117, 64%) smoked cigarettes, 46 (24%) used marijuana, and 87 (46%) used crack cocaine during pregnancy. The mean (± SD) gestational age was 38 (±3) weeks with a mean birth weight of 2994 (±697) g. Characteristics of caregivers and the home environment are presented in Table II. Between 8% and 10% of the children were cared for by nonrelative foster or adoptive parents at any given assessment. The mean (± SD) Full-Scale IQ (unadjusted) was 87 (±13) at age 9, 86 (±13) at 11, and 83 (±13) at 15.

Table III gives associations between 4 FAEE analytes (ethyl myristate, ethyl oleate, ethyl linoleate, and ethyl linolenate) and key maternal and infant characteristics. Because preliminary analyses indicated no relationship of 2 FAEE analytes (ethyl palmitate and ethyl arachidonate) with

Table I. Biological maternal and child characteristics (n = 191)

	n (%)/mean ± SD	Median (10%-90%)
Biological maternal		
African American	154 (80.6)	
Low socioeconomic status	191 (100)	
Married	24 (12.6)	
Age at birth	27.30 ± 5.28	26 (21-35)
Years of education	11.80 ± 1.49	12 (10-14)
Number of prenatal visits	7.49 ± 4.94	8 (1-14)
Parity	2.83 ± 1.69	3 (1-5)
Global Severity Index	0.67 ± 0.69	0.45 (0.08-1.51)
PPVT-R, standard score	76.76 ± 13.40	77 (60-93)
WAIS-R, Block Design	7.05 ± 2.11	7 (5-9)
WAIS-R, Picture Completion	6.92 ± 2.32	6 (5-10)
Measures of drinking during pregnancy (n = 114)*		
Number of drinks on drinking day	2.14 ± 2.79	1.5 (0.25-4.5)
Number of drinking days/wk	1.37 ± 1.49	0.75 (0.06-3.5)
Number of drinks/wk	6.51 ± 11.95	1.98 (0.06-18)
More than usual number of drinks	5.74 ± 8.83	3 (0-14)
Risk drinking (TWEAK ≥2)	72 (63)	
Other substance use during pregnancy*		
Cigarettes/day (n = 117)	12.06 ± 10.83	10.5 (1.2-20)
Marijuana joints/wk (n = 46)	2.91 ± 4.27	1.1 (0.1-7)
Cocaine units/wk (n = 87)	24.71 ± 51.83	5 (0.4-70)
Child		
Male	84 (44.0)	
Gestational age, wk	38.3 ± 2.9	39 (35-41)
Prematurity, <37 wk gestational age	41 (21.5)	
Hobel neonatal risk score	5.9 ± 15.3	0 (0-15)
Birth weight, g	2994 ± 679	3130 (2050-3710)
Birth length, cm	48.8 ± 3.9	49.0 (44.4-53.0)
Head circumference, cm	33.1 ± 2.4	33.0 (30.2-35.5)

PPVT-R, Peabody Picture Vocabulary Test-Revised; *TWEAK*, Tolerance, Worried, Eye-openers, Amnesia, and K/Cut down; *WAIS-R*, Wechsler Adult Intelligence Scale-Revised.
*Based on users (n) only.

Table II. Caregiver characteristics

	9-year (n = 187)	11-year (n = 186)	15-year (n = 176)
Nonkinship foster/adoptive care, n (%)	17 (9.1%)	15 (8.1%)	17 (9.7%)
HOME environment*	43.50 ± 6.53	46.21 ± 6.33	48.00 ± 6.74
Global Severity Index*	0.37 ± 0.45	0.42 ± 0.51	0.37 ± 0.46
PPVT-R, standard score*	80.01 ± 14.34	80.05 ± 14.07	79.74 ± 13.53
WAIS-R, Block Design*	7.14 ± 2.29	7.18 ± 2.19	7.19 ± 2.06
WAIS-R, Picture Completion*	7.16 ± 2.43	7.31 ± 2.46	7.40 ± 2.44
Substance use (past 30 d), n, median (10%-90%)†			
Alcohol drinks/wk	67, 1.5 (0.5-9)	62, 3.3 (0.5-10)	104, 1 (0.3-6)
Cigarettes/d	85, 10 (2-20)	80, 10 (3-20)	72, 10 (3-20)
Marijuana joints/wk	6, 4.3 (0.3-28)	4, 19 (1-35)	8, 1.5 (0.3-70)

HOME, Home Observation for Measurement of the Environment.
*Presented as mean ± SD.
†Median (10%-90%) is based on users (n) only. No caregivers reported cocaine use in the past 30 days, except one caregiver reporting 1.5 cocaine units per week at 9-year assessment.

Table III. Association (r_s) between FAEEs and maternal and infant characteristics

	Ethyl myristate	Ethyl oleate	Ethyl linoleate	Ethyl linolenate
Prenatal drinking (yes/no)	.08	.12*	.10	.04
Number of drinks on drinking day	.08	.10	.08	<.01
Number of drinking days/wk	.06	.09	.06	<.01
Number of drinks/wk	.08	.11	.09	.02
More than usual number of drinks	.07	.13*	.11	.04
Risk drinking (TWEAK ≥2)	<.01	.05	.05	−.02
Cigarettes per day	.04	.02	.03	−.01
Marijuana joints/wk	.10	.06	.08	.05
Cocaine units/wk	.08	.05	.03	.04
Maternal Global Severity Index at birth	.14*	.15†	.14*	.09
Parity	.13*	.18†	.14*	.08
Gestational age	.11	.08	.13*	.12
Birth weight	.13*	.12	.18†	.18†
Birth length	.13*	.13*	.20‡	.18†

*$P < .10$.
†$P < .05$.
‡$P < .01$.

outcomes, our analysis was focused on these 4 FAEEs. Maternal report of prenatal alcohol use (yes/no) was associated with ethyl oleate ($r_s = .13$, $P = .09$), which correlated weakly with self-reported amount of average alcohol use per week, which was averaged over the 3 trimesters and the month prior to pregnancy ($r_s = .11$, $P = .13$). More than the usual number of drinks was marginally associated with ethyl oleate ($r_s = .13$, $P = .086$), although a measure of the usual number of drinks on drinking day was not associated with FAEEs. No other drug variables (cigarettes, marijuana, and cocaine) were associated with FAEEs (all $P > .20$). Maternal psychological distress at birth and parity were related to a greater concentration of ethyl myristate, ethyl oleate, and ethyl linoleate. For infant measures, birth weight and birth length were associated with greater FAEEs.

Relationships of the 4 FAEE analytes with cognitive outcomes after adjustment for covariates are presented in Table IV. Covariates adjusted for each outcome are listed as footnotes, with significant covariates italicized. In the longitudinal analysis with mixed linear models, none of the interaction terms between the FAEEs and age/visits were significant, indicating that the association of FAEEs with cognitive outcomes did not vary over time. After we controlled for covariates, greater concentrations of ethyl oleate, ethyl linoleate, and ethyl linolenate were associated with lower Verbal Comprehension Index. For example, one unit (ng) change in $\log_{10}$ (ethyl oleate + 100) was related to a reduction of 2.05 points in Verbal Comprehension Index on average over time. Greater concentrations of ethyl myristate, ethyl oleate, and ethyl linolenate were associated with lower Working Memory Index. Also, increased concentrations of the 4 FAEE analytes were associated with lower Full-Scale IQ. No associations of FAEEs with the Perceptual Reasoning and Processing Speed Indices were found.

Discussion

We demonstrated that the level of cognitive functioning during childhood and adolescence was inversely associated with increased concentrations of FAEEs in low SES, primarily African American, urban children who were poly-drug exposed prenatally. Greater levels of the 4 FAEE analytes were associated with poorer Verbal Comprehension, Working Memory, and Full-Scale IQ, supporting our hypotheses. The negative association between greater concentration of FAEEs in meconium and cognitive development noted during the first 2 years of life continued at ages 9, 11, and 15 years. These findings are consistent with our previous studies based on maternal report of alcohol use. Maternal self-report of average alcohol use during pregnancy was related to lower vocabulary scores and working memory at 9 years[28] and to lower Verbal Comprehension and Working Memory Indices at 15 years.[29] Despite weak correlations between maternal reported alcohol use and FAEEs, their relationships with cognitive development converged. However, neither study in which the authors used maternal self-report found associations with Full-Scale IQ, suggesting greater sensitivity of FAEEs in identifying alcohol-exposed children.

Some findings related to FAEEs are noteworthy. Greater birth length and birth weight were correlated with increased concentration of FAEEs. Although heavy drinking during pregnancy is related to restricted growth, the effects of

Table IV. Adjusted association of FAEEs

	Verbal Comprehension*		Working Memory†		Full-Scale IQ‡	
	b ± SE	*P* value	b ± SE	*P* value	b ± SE	*P* value
Ethyl myristate	−3.26 ± 1.67	.053	−4.17 ± 1.96	.035	−3.60 ± 1.79	.046
Ethyl oleate	−2.05 ± 0.91	.025	−2.31 ± 1.06	.032	−2.07 ± 0.97	.035
Ethyl linoleate	−1.81 ± 0.83	.031	−1.90 ± 0.98	.054	−1.77 ± 0.88	.047
Ethyl linolenate	−2.31 ± 1.02	.024	−2.63 ± 1.19	.029	−2.62 ± 1.09	.017

Note. b = regression coefficient.
Significant ($P < .05$) covariates are listed in italics:
*Adjusted for age/visit, birth length, birth mother's education and psychological distress at birth, current caregiver's PPVT-R scores, HOME score, and child race.
†Adjusted for age/visit, birth length, birth mother's education and psychological distress at birth, current caregiver's Block Design scores, and child sex.
‡Adjusted for age/visit, birth length, birth mother's education and psychological distress at birth, current caregiver's Block Design scores, HOME score, and child race.

low-to-moderate drinking during pregnancy on birth outcomes are not consistent, with some studies reporting greater birth weight and less prematurity among infants with low-to-moderate prenatal alcohol exposure.[30] In this study, infants with FAS diagnosed at birth were excluded on the basis of the selection criteria of the parent study.[17] In addition, greater maternal psychological distress was correlated with increased concentration of FAEEs, suggesting that mothers experiencing greater distress also drank more.

Limitations in our study should be noted. For FAEE determination, use of the gas chromatography with a flame ionization detector is less sensitive and may falsely identify peaks as FAEE when gas chromatography tandem mass spectroscopy would correctly identify them as FAEE or not. Self-reported alcohol consumption during pregnancy was collected retrospectively, producing possible recall bias. Furthermore, underreporting or denial because of the stigma associated with pregnancy drinking may be responsible for the weak correlations between various measures of alcohol use during pregnancy and FAEEs observed in this study. Also, birth length and head circumference from medical records may not be reliable. The effects of behavioral, educational, cognitive, or social skills intervention that children may have received were not controlled because of a lack of such data.

Despite these limitations, this study has significant strengths. A comprehensive set of covariates and confounders, including other prenatal substance exposures (cigarette, marijuana, cocaine) and caregiving environments, were evaluated and controlled statistically when necessary. Assessment at multiple time points, combined with a high rate of retention for follow-up, allowed repeated observations of an association between FAEEs and cognitive outcomes at different developmental stages, increasing confidence in the findings. Longitudinal analyses allowed us to test whether the relationship of FAEEs on cognitive outcomes changed over time. The present study extends previous studies of FAEEs by demonstrating that poor cognitive development noted in infancy persists into childhood and adolescence. It provides additional evidence that increased levels of FAEEs in meconium may be an informative biological marker for the early identification of children at risk for cognitive developmental delay, which will serve as guidance for early intervention. It also establishes predictive validity of FAEEs for determining alcohol exposure in utero. ■

Submitted for publication Sep 11, 2014; last revision received Nov 19, 2014; accepted Dec 3, 2014.

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