J Korean Med Sci. 2018 Dec 10;33(50):e318
https://doi.org/10.3346/jkms.2018.33.e318
eISSN 1598-6357·pISSN 1011-8934

JKMS

Original Article
Pediatrics

Association of Fatty Acid Ethyl Esters in Meconium of Neonates with Growth Deficits at Birth: a Prospective, Single-Centre Cohort Study

Hyun-Seung Lee,[1] **Yeon Hee Kim**,[2] **Ho-Seok Kwak**,[3] **Jung-Yeol Han**,[4] **Sun-Jin Jo**,[5] **and Hae Kook Lee**[6]

[1]Department of Pediatrics, College of Medicine, The Catholic University of Korea, Seoul, Korea
[2]Department of Obstetrics and Gynecology, College of Medicine, The Catholic University of Korea, Seoul, Korea
[3]Department of Bioanalytical Mass Spectrometry, Sunin Bio Corporation, Seoul, Korea
[4]Department of Obstetrics and Gynecology, Cheil General Hospital and Women's Healthcare Centre, Dankook University College of Medicine, Seoul, Korea
[5]Department of Preventive Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea
[6]Department of Psychiatry, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

Received: Mar 24, 2018
Accepted: Aug 30, 2018

Address for Correspondence:
Hae Kook Lee, MD, PhD
Department of Psychiatry, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 271 Cheonbo-ro, Uijeongbu 11765, Republic of Korea.
E-mail: nplhk@catholic.ac.kr

ORCID iDs
Hyun-Seung Lee
https://orcid.org/0000-0002-5219-8384
Yeon Hee Kim
https://orcid.org/0000-0002-7689-3840
Ho-Seok Kwak
https://orcid.org/0000-0002-4569-2552
Jung-Yeol Han
https://orcid.org/0000-0001-5611-2392
Sun-Jin Jo
https://orcid.org/0000-0002-8465-9632
Hae Kook Lee
https://orcid.org/0000-0002-4985-001X

Funding
This research was supported by a research fund (HD15B2690) from Korea Centers for Disease Control and Prevention. The funders have no influence on the results.

ABSTRACT

Background: In this prospective cohort study, we investigated the association between fatty acid ethyl esters (FAEEs) in meconium as biomarkers of prenatal ethanol exposure and growth deficits, as birth outcomes, that constitute several of the key cardinal features of fetal alcohol syndrome.

Methods: A total of 157 meconium samples were collected from enrolled infants within 24 hours of birth, and nine FAEEs were quantified using liquid chromatography/tandem mass spectrometry. The relationships between cumulative concentrations of nine species of FAEEs in meconium and birth parameters of growth (age-sex-specific centiles of head circumference [HC], weight, and length) and respective and combined birth outcomes of growth deficits (HC ≤ 10th centile, weight ≤ 10th centile, and length ≤ 10th centile) were determined.

Results: Multivariate logistic regression analysis demonstrated that higher cumulative concentrations of meconium FAEEs correlated with elevated risks for HC and length, both, 10th percentile or less (adjusted odds ratio [aOR], 2.94; 95% confidence interval [CI], 1.12–7.74; $P = 0.029$) and HC and weight and length, all of them, 10th percentile or less (aOR, 3.27; 95% CI, 1.12–9.59; $P = 0.031$).

Conclusion: The elevated cumulative FAEEs in meconium were associated with combined growth deficits at birth, specifically HC and length, both, 10th percentile or less, which might be correlated with detrimental alcohol effects on fetal brain and bone development, suggesting a plausible alcohol-specific pattern of intrauterine growth restriction.

Keywords: Fatty Acid Ethyl Esters; Fetal Alcohol Syndrome; Fetal Alcohol Spectrum Disorders; Birth Outcomes; Growth Deficits, Meconium

Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Han JY, Lee HK. Data curation: Lee HS. Formal analysis: Lee HS, Jo SJ. Methodology: Kim YH, Kwak HS. Investigation: Lee HS, Kim YH. Writing - original draft: Lee HS. Writing - review & editing: Lee HS, Lee HK.

INTRODUCTION

Prenatal alcohol exposure of the developing fetus may lead to fetal alcohol spectrum disorders (FASD), associated with a wide range of adverse offspring outcomes representing the deleterious effects of ethanol.[1-4] Fetal alcohol syndrome (FAS), the most severe end of this continuum, is characterized phenotypically by growth retardation, microcephaly, and three specific facial dysmorphologies.[1,2,4] Fatty acid ethyl esters (FAEEs), a non-oxidative ethanol metabolites, have been used as direct biomarkers of gestational ethanol consumption analyzed in the meconium of neonates, and even in very-low-birth-weight infants, with the detection time window of the second and third trimesters of pregnancy, largely in the last 3 months.[5-10] The detection of FAEEs in meconium elucidates the patterns of ethanol exposure during the gestational period in contrast to women's hair, which cannot be guaranteed during pregnancy.[5]

Limited published data are available to support the relationship between meconium FAEEs and birth outcomes of fetal growth. There are two studies addressing the correlation of higher amounts of specific meconium FAEE analytes with greater birth weight, greater birth length, and greater gestational age at birth under low-to-moderate prenatal alcohol exposure, without adjusting for age and sex and analyzing for birth outcomes of prenatal growth restriction.[11,12] Furthermore, the degree to which cumulative FAEEs in meconium are related to fetal growth restriction in occipital frontal circumference (OFC), weight, and length that constitute several of the key cardinal features of FAS is unknown. Most studies investigating the effects of ethanol exposure on prenatal growth restriction assessed individual outcomes or largely focused on birth weight ≤ 10th centile (intrauterine growth restriction or small-for-gestational-age). However, based on the FAS diagnostic criteria,[4] birth outcomes as a result of fetal growth restriction display combined manifestations of individual growth deficits. Accordingly, it is necessary to include the combined effects of growth deficits as well as the respective individual growth deficits in any correlation analysis. In Korea, risky alcohol consumption has been increasing among young females,[13,14] and the estimated prevalence of FAS was 4.2% among a sample of children receiving services in institutional settings.[15] A population-based survey revealed that alcohol abuse among Korean women showed the highest prevalence between ages 20–34 years and increased among the recent generations.[16] There has been an increasing interest among pediatricians, as the most likely practitioners to first encounter the neonates, for the early identification of infants with prenatal alcohol exposure who are potentially at risk for FASD, which can lead to reduced secondary disabilities, particularly in early infancy where facial dysmorphology assessment may be unavailable. We performed a prospective cohort study to examine the association between the accumulation of FAEEs in meconium and birth outcomes of growth deficits regarding OFC, weight, and length based on the FAS diagnostic growth criteria.

METHODS

Participants

This study was designed to include all infants born and receiving neonatal care in Uijeongbu St. Mary's Hospital, The Catholic University of Korea between 1st April and 31st August 2016. During the study period, 52 out of 209 infants born in the hospital were excluded: one with chromosomal disease, one with a congenital malformation, 19 due to multiple pregnancies, two with inaccurate gestational age, 11 who refused parental consent, and 18 with missed

specimen collections. In total, meconium samples from 157 singleton infants were obtained and successfully analyzed. Information related to perinatal characteristics was acquired from the medical records, encompassing gestational age, infant sex, maternal age, parity, delivery method, prematurity, maternal hypertension, maternal diabetes, infant head circumference (HC), infant weight, and infant length. Each anthropometric measurement (HC, weight, and length at birth) was transformed into an age-sex-specific centile using Fenton growth charts.[17] Birth outcomes of growth deficits were defined based on the key growth deficiency criteria for FAS as HC 10th percentile or less, weight 10th percentile or less, and length 10th percentile or less at birth. All possible combinations of each individual outcome were examined. Data pertaining to maternal drinking and smoking habits during pregnancy were acquired using standardized questionnaires and in-person interviews in the hospital during postpartum stay or within 1 month postpartum. All mothers were requested to provide details of the amount and frequency of their gestational alcohol consumption on an electronic form especially designed for the purpose of the study. In order to minimize the response bias and maximize the validity of self-reports,[18] trained investigators recorded the information using electronic files, which were immediately stored in a password-protected system with restricted access. The number of weekly drinks was estimated based on the alcohol content of beverages (beer, wine, soju, or hard liquor) consumed. As previously described by the United States National Institute on Alcohol Abuse and Alcoholism,[19] one standard drink contains 0.6 oz or 14 g of absolute ethanol. A FAEE concentration more than 2.00 nmol/g meconium has been recommended as positive for significant prenatal alcohol exposure.[6,7,20] For initial comparisons, all subjects were categorized into one of the three groups: undetectable FAEE (a group with meconium FAEE levels less than limit of quantitation (LOQ), under FAEE2 (a group with meconium FAEE levels greater than LOQ and 2.00 nmol/g or less), FAEE2 (a group with meconium FAEE levels greater than 2.00 nmol/g).

Meconium FAEE assessment

All meconium samples (> 500 mg per infant) were collected within 24 hours after birth, frozen and stored at −60°C for ≤ 6 months, and couriered to the Seoul Pharma Laboratories for the analysis of FAEEs. Quantification of FAEEs was performed by liquid chromatography/tandem mass spectrometry as described previously.[21] Nine FAEEs including ethyl laurate (E12:0), ethyl myristate (E14:0), ethyl palmitate (E16:0), ethyl palmitoleate (E16:1), ethyl stearate (E18:0), ethyl oleate (E18:1), ethyl linoleate (E18:2), ethyl linolenate (E18:3), and ethyl arachidonate (E20:4) were quantified. The limits of detection varied from 0.01 to 0.08 nmol/g, whereas the LOQ ranged from 0.02 to 0.27 nmol/g. Intra- and inter-assay precisions varied from 7% to 21% and from 10% to 17%, respectively. The intra-assay and inter-assay accuracies ranged from −17% to 15% and from −4% to 14%, respectively. The individual concentrations of the nine FAEEs were summed to obtain the cumulative concentration.

Statistical analysis

The FAEE concentration groups were compared using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. Spearman rank order correlation was used to assess the association of cumulative FAEE concentrations with alcohol exposure (drinks per week) during all the trimesters and during second-to-third trimesters, and anthropometric parameters at birth. Logistic regression modelling was performed for individual and combined variables of growth deficits, and the odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. All baseline characteristics were included as possible confounders in the models. Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were used to determine the accuracy of the model in categorizing

https://doi.org/10.3346/jkms.2018.33.e318

the significant outcomes in multivariate logistic regression. Fisher's exact test and conditional logistic regression were conducted to determine adequate cut-offs. Statistical analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL, USA). A two-tailed $P \leq 0.05$ was considered statistically significant.

Ethics statement

The present study has been approved by the Institutional Review Board (IRB) of Uijeongbu St. Mary's Hospital (IRB No. UC110NMI0044). Written agreements were acquired from parents under the declaration of Helsinki.

RESULTS

Baseline characteristics and birth outcomes of growth deficits

Of the 157 meconium samples analyzed successfully, four (2.5%) tested positive for cumulative FAEE concentrations exceeding 2 nmol/g, 67 were detected at total FAEE concentrations ≤ 2 nmol/g, and 86 were undetected for quantifiable amounts of FAEEs (**Table 1**). The prevalence of gestational ethanol use was 8.9% (14/157 female) during all trimesters, and 2.5% (4/157 female) during second-to-third trimesters. Among the four mothers with infants testing positive for FAEEs, two reported ethanol use during the second-to-third trimesters (**Table 1**). Maternal ethanol ingestion during all the trimesters, HC and weight, both, 10th percentile or less, and HC and weight and length, all of them, 10th percentile or less were significantly associated with the cumulative FAEE concentrations (**Table 1**).

Relationships of meconium FAEEs with prenatal alcohol exposure, birth growth and birth outcomes of growth deficits

Correlation analyses revealed significant associations between cumulative meconium FAEE levels and drinks per week during all the trimesters ($\rho = 0.160$ and $P = 0.046$) and during second-to-third trimesters ($\rho = 0.174$ and $P = 0.029$). However, no correlation with birth growth parameters in the age-sex-specific centile (HC, $\rho = -0.128$ and $P = 0.111$; weight, $\rho = -0.018$ and $P = 0.825$; length, $\rho = 0.068$ and $P = 0.398$) was found. In logistic regression, for each 1 nmol/g higher FAEE concentration, the odds for HC and weight, both, 10th percentile or less increased by 1.84-fold ($P = 0.044$), the odds for HC and length, both, 10th percentile or less increased by 2.17-fold ($P = 0.015$), and the odds for HC and weight and length, all of them, 10th percentile or less increased by 2.37-fold ($P = 0.009$) (**Table 2**). After adjusting for potential confounders (gestational age, infant sex, maternal age, parity, caesarean section delivery, prematurity, maternal hypertension, maternal diabetes, and smoking during pregnancy), the odds of HC and length, both, 10th percentile or less increased by 2.94-fold ($P = 0.029$), and the odds of HC and weight and length, all of them, 10th percentile or less increased by 3.27-fold ($P = 0.031$) (**Table 2**).

Meconium FAEEs: analysis at different cut-off levels

ROC curve evaluating cumulative FAEE concentrations to identify newborns with HC and length, both, 10th percentile or less generated an AUC of 0.68 (95% CI, 0.44–0.93; $P = 0.162$). The sensitivity and specificity were 40.0% and 92.8%, respectively, at a cut-off of 0.5 nmol/g, and 20.0% and 98.0%, respectively, at a cut-off of 2 nmol/g. In the ROC analysis of cumulative FAEE concentrations distinguishing newborns with and without HC and weight and length, all of them, 10th percentile or less, the AUC was 0.79 (95% CI, 0.61–0.97; $P = 0.05$). The sensitivity and specificity were 50.0% and 92.8%, respectively, at a cut-off of 0.5 nmol/g and 25.0% and

https://doi.org/10.3346/jkms.2018.33.e318

Table 1. Baseline characteristics and birth outcomes of growth deficits in 157 infants based on meconium FAEE concentrations

Characteristics	Undetectable FAEE	Under FAEE2	FAEE2	*P* value
No. of infants	86	67	4	
Total concentration of FAEEs, nmol/g				< 0.001[a]
Mean ± SE	-	0.22 ± 0.04	3.57 ± 0.64	
Range	< LOQ	0.01–1.63	2.14–5.03	
95% CI	-	0.14–0.29	1.52–5.61	
Baseline characteristics				
Gestational age, wk	37.5 (24.7–40.9)	37.4 (29.0–40.6)	38.6 (34.9–39.1)	0.759[a]
Sex, male	38 (44.2)	34 (50.7)	1 (25)	0.525[b]
Maternal age, yr	32.5 (20–45)	34.0 (17–46)	27.0 (24–39)	0.320[a]
Parity	1.0 (0–4)	0.0 (0–4)	0.0 (0–2)	0.494[a]
Caesarean section delivery	50 (58.1)	38 (56.7)	4 (100)	0.276[b]
Prematurity	32 (37.2)	26 (38.8)	1 (25.0)	0.951[b]
Maternal hypertension	6 (7.0)	11 (16.7)	1 (25.0)	0.089[b]
Maternal diabetes	7 (8.1)	11 (16.7)	1 (25.0)	0.145[b]
Smoking during pregnancy	1 (1.2)	1 (1.5)	1 (25.0)	0.075[b]
Ethanol ingestion (all trimesters)	6 (7.0)	6 (9.0)	2 (50.0)	0.048[b]
Drinks per week	0.25 (0.25–2.50)	1.25 (0.50–3.00)	3.50 (2.00–5.00)	0.085[a]
Ethanol ingestion (2nd and 3rd trimesters)	1 (1.2)	1 (1.5)	2 (50.0)	0.067[b]
Drinks per week	0.25	0.50	3.50 (2.00–5.00)	0.259[a]
Birth parameters of growth				
HC, cm	33.5 (22.8–38.0)	32.8 (25.5–38.5)	34.0 (29.9–36.0)	0.474[a]
HC, percentile	64.5 (0–100)	54.0 (0–100)	81.0 (0–90)	0.113[a]
Weight, g	3,035 (740–4,000)	2,880 (900–5,530)	3,075 (2,070–3,525)	0.841[a]
Weight, percentile	49.0 (3–100)	47.0 (1–100)	63.0 (0–90)	0.636[a]
Length, cm	47.0 (32.0–53.0)	47.5 (34.1–54.0)	48.0 (43.0–50.0)	0.750[a]
Length, percentile	37.5 (0–99)	41.0 (0–99)	35.5 (0–93)	0.645[a]
Birth outcomes of growth deficits				
HC ≤ 10P	7 (8.1)	11 (16.4)	1 (25.0)	0.144[b]
Weight ≤ 10P	6 (7.0)	9 (13.4)	1 (25.0)	0.172[b]
Length ≤ 10P	15 (17.4)	8 (11.9)	1 (25.0)	0.343[b]
HC and weight ≤ 10P	1 (1.2)	6 (9.0)	1 (25.0)	0.022[b]
HC and length ≤ 10P	1 (1.2)	3 (4.5)	1 (25.0)	0.055[b]
Weight and length ≤ 10P	4 (4.7)	4 (6.0)	1 (25.0)	0.221[b]
HC and weight and length ≤ 10P	0 (0.0)	3 (4.5)	1 (25.0)	0.011[b]
Weight and/or length ≤ 10P	17 (19.8)	13 (19.4)	1 (25.0)	1.000[b]
HC and weight and/or length ≤ 10P	2 (2.3)	6 (9.0)	1 (25.0)	0.052[b]

Values are presented as median (range) or number (%).
FAEE = fatty acid ethyl ester, SE = standard error, CI = confidence interval, LOQ = limit of quantitation, HC = head circumference; Undetectable FAEE = a group with meconium FAEE levels less than LOQ, Under FAEE2 = a group with meconium FAEE levels greater than LOQ and 2.00 nmol/g or less, FAEE2 = a group with meconium FAEE levels greater than 2.00 nmol/g, HC ≤ 10P = head circumference 10th percentile or less, Weight ≤ 10P = weight 10th percentile or less, Length ≤ 10P = length 10th percentile or less.
[a]Kruskal-Wallis test; [b]Fisher's exact test.

Table 2. Association of cumulative meconium FAEE concentrations with birth outcomes of growth deficits

Outcomes at birth, ≤ 10P	Unadjusted			Adjusted		
	OR[a]	95% CI	*P* value	OR[a,b]	95% CI[b]	*P* value[b]
HC	1.45	0.84–2.52	0.184	1.53	0.81–2.89	0.187
Weight	1.47	0.84–2.59	0.182	1.26	0.67–2.38	0.471
Length	1.22	0.68–2.17	0.503	1.38	0.72–2.63	0.330
HC and weight	1.84	1.02–3.32	0.044	1.76	0.84–3.67	0.134
HC and length	2.17	1.16–4.04	0.015	2.94	1.12–7.74	0.029
Weight and length	1.74	0.97–3.14	0.064	1.94	0.91–4.13	0.087
HC and weight and length	2.37	1.25–4.52	0.009	3.27	1.12–9.59	0.031
Weight and/or length	1.13	0.64–2.00	0.668	1.09	0.59–2.01	0.784
HC and weight and/or length	1.76	0.98–3.16	0.061	1.79	0.87–3.72	0.116

FAEE = fatty acid ethyl ester, OR = odds ratio, CI = confidence interval, HC = head circumference.
HC ≤ 10P = head circumference 10th percentile or less, Weight ≤ 10P = weight 10th percentile or less, Length ≤ 10P = length 10th percentile or less.
[a]ORs are estimated per 1 nmol/g higher FAEE concentration; [b]Values are adjusted for gestational age, infant sex, maternal age, parity, caesarean section delivery, prematurity, maternal hypertension, maternal diabetes, and smoking during pregnancy.

https://doi.org/10.3346/jkms.2018.33.e318

Table 3. Meconium FAEE concentrations at different cut-offs and HC and length, both, 10th percentile or less (HC and length ≤ 10P) at birth

FAEE cut-offs, nmol/g	HC and length ≤ 10P, No. (%)		*P* value[a]	OR[b] (95% CI)[b]	*P* value[b]
	Positive (n = 5)	Negative (n = 152)			
> 0.2	2 (40.0)	21 (13.8)	0.156	3.88 (0.65–23.25)	0.137
> 0.3	2 (40.0)	17 (11.2)	0.111	4.84 (0.81–28.98)	0.084
> 0.4	2 (40.0)	14 (9.2)	0.081	5.88 (0.98–35.16)	0.052
> 0.5	2 (40.0)	11 (7.2)	0.055	7.39 (1.23– 44.19)	0.029
> 2.0	1 (20.0)	3 (2.0)	0.123	9.56 (1.07–85.56)	0.043

FAEE = fatty acid ethyl ester, HC = head circumference, OR = odds ratio, CI = confidence interval.
[a]Fisher's exact test; [b]Conditional logistic regression.

Table 4. Meconium FAEE concentrations at cut-offs and HC and weight and length, all of them, 10th percentile or less (HC and weight and length ≤ 10P) at birth

FAEE cut-offs, nmol/g	HC and weight and length ≤ 10P, No. (%)		*P* value[a]	OR[b] (95% CI)[b]	*P* value[b]
	Positive (n = 4)	Negative (n = 153)			
> 0.2	2 (50.0)	21 (13.7)	0.103	5.83 (0.82–41.36)	0.078
> 0.3	2 (50.0)	17 (11.1)	0.072	7.26 (1.02–51.56)	0.047
> 0.4	2 (50.0)	14 (9.2)	0.052	8.81 (1.24–62.56)	0.030
> 0.5	2 (50.0)	11 (7.2)	0.035	11.08 (1.56–78.64)	0.016
> 2.0	1 (25.0)	3 (2.0)	0.099	12.75 (1.33–122.57)	0.027

FAEE = fatty acid ethyl ester, HC = head circumference, OR = odds ratio, CI = confidence interval.
[a]Fisher's exact test; [b]Conditional logistic regression.

98.0%, respectively, at a cut-off value 2 nmol/g. In conditional logistic regression, significant ORs were found at cut-offs of 0.5 and 2.0 nmol/g for HC and length, both, 10th percentile or less (**Table 3**) and at cut-offs of 0.3, 0.4, 0.5 and 2.0 nmol/g for HC and weight and length, all of them, 10th percentile or less (**Table 4**). Fisher's exact test revealed the only significant cut-off of 0.5 nmol/g for HC and weight and length, all of them, 10th percentile or less ($P = 0.035$) (**Table 4**).

DISCUSSION

The current study demonstrated that higher cumulative concentrations of nine FAEEs in meconium were associated with birth outcomes of combined growth deficits, including HC and length, both, 10th percentile or less and HC and weight and length, all of them, 10th percentile or less (**Table 2**), despite lack of associations with individual birth outcomes of growth deficits and growth parameters (age-sex-specific HC, weight and length centiles) in multivariate logistic modelling. The association was even more robust with the combination of all three individual outcomes representing symmetric intrauterine growth restriction in unadjusted and adjusted models. These findings suggest that the combined growth deficits at birth were significantly correlated with ethanol exposure relative to the respective outcomes. The common denominator of HC and length, both, 10th percentile or less of the significant variables suggests that OFC and length reflecting fetal brain and bone growth were more significant than weight largely contributed to by other soft tissues in fetal growth restriction related to ethanol exposure, indicating the existence of a plausible alcohol-related pattern of intrauterine growth restriction. To date, numerous studies involving animal models have documented detrimental effects of alcohol on fetal brain and bone development. A variety of mechanisms investigated in experimental models revealed significant deficits in neural development following embryonic ethanol exposure, including rapid cerebral vasoconstriction,[22] abnormal neural maturation,[23] neurodegeneration by reactive oxygen species[24] and neuroapoptosis[25] in the developing brain. A chick embryo model demonstrated that ethanol exposure represses development of craniofacial and long bones by inhibiting

https://doi.org/10.3346/jkms.2018.33.e318

the generation of cranial neural crest cells, chondrogenesis and ossification via excessive production of reactive oxygen species and altered osteogenesis-related gene expression.[26] A mouse micro-computed tomography study documented growth retardation of fetal craniofacial bone following alcohol exposure, with the neurocranium (upper skull) more significantly affected than the viscerocranium (face).[27] Studies involving sheep and rat fetuses revealed that fetal bone growth was more sensitive to alcohol than overall growth as measured by body weight, particularly at moderate exposure levels.[28,29] Taken together with our results, it is postulated that OFC and length are more significant predictors of developmental ethanol exposure than weight that has been focused on in previous studies.

The prenatal ethanol exposure levels of our infants studied considered low-to-moderate depending on the maternal self-reports. Maternal alcohol intake, especially at relatively higher levels during pregnancy, results in fetal growth restriction in weight, OFC and length.[30-32] However, inconsistent results have been observed in infants prenatally exposed to light-to-moderate maternal drinking, including lack of effect on fetal growth restriction and greater birth weight.[33-35]

The prevalence of positive FAEE analyses for significant prenatal alcohol exposure in our cohort was 2.5%. This is lower than the 3.1%–4.4% (39–56 of 1,271 samples) reported in the Prince Edward Island population-based study[36] with the greater observed maximum FAEE level than ours, and higher than the 2% (11 of 505 specimens) in a cohort of Southwestern Ugandan women with the maximum FAEE level similar to ours.[37] The relatively low prevalence in our cohort may result from sample collection in a low-risk community hospital setting, as opposed to high-risk settings such as special institutional clinics offering structured services. The high opt-out rate of 25% and the short study period of 5 months in our study may have biased the sample towards the lower prevalence of positive FAEE analyses as well as the lower maximum FAEE concentration. This result may be attributed to the differential decline in participation rate by high-risk women, and the possible effect of seasonal variation in drinking habits.

Meconium FAEEs have been accepted as established biomarkers of prenatal alcohol exposure in the updated clinical guideline for the diagnosis of FASD.[4] The clinical use of meconium FAEE analysis has been attempted to identify children at risk for future alcohol-related problems,[11,38] and was advocated by a meta-analysis complementing maternal self-reports increasing the risk of under-reporting of gestational ethanol intake.[39] It is likely that more robust data correlate prenatal alcohol exposure with the cumulative sum of FAEEs rather than individual FAEEs in meconium.[7] Our study showed a positive correlation between cumulative FAEE concentrations in meconium and maternal self-reported drinking, as reported previously.[6,7,40] The cut-offs for positive FAEE analyses in meconium indicating significant prenatal alcohol exposure vary widely from 0.17 to 33 nmol/g.[6,7,20,40] Our study found that a cut-off of > 0.5 nmol/g in meconium was significant for HC and weight and length, all of them, 10th percentile or less at birth in both Fisher's exact test and logistic regression analysis (**Table 4**). However, this finding was limited by the results from ROC analyses showing the low sensitivity of 50.0% at the 0.5 nmol/g cut-off despite an AUC of 0.79 ($P = 0.05$) with a cut-off specificity of 92.8%.

The study has limitations. The small size of the sample collected in one setting limits generalization of the results and warrants further investigations. Another limitation is the detection window of meconium restricted to the latter half of gestation. However, a cohort

https://doi.org/10.3346/jkms.2018.33.e318

study of premature newborns proposed ethyl linolenate, a meconium FAEE analyte, as a potential biomarker for the detection of trimester one ethanol exposure.[10]

In conclusion, this study suggests that elevated cumulative FAEEs in meconium are associated with combined growth deficits at birth, specifically HC and length, both, 10th percentile or less, which might be related to detrimental effects of alcohol on fetal brain and bone growth, suggesting a plausible alcohol-specific pattern of intrauterine growth restriction. It additionally suggests that elevated meconium FAEEs may be an informative biomarker for the early detection of infants potentially at risk for FASD. Our results reinforce other studies recommending the complementary use of meconium FAEE assay in a multi-step approach involving maternal self-reporting and screening with a panel of other biomarkers in neonatal and maternal matrices.[5,6,10]

ACKNOWLEDGEMENTS

We would like to thank the labor and delivery nursery and neonatal nursery staffs of Uijeongbu St. Mary's Hospital of The Catholic University of Korea. Special thanks are due to the mothers and children who participated in this study.

REFERENCES

1. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet* 1973;302(7836):999-1001.
PUBMED | CROSSREF
2. Astley SJ, Clarren SK. Diagnosing the full spectrum of fetal alcohol-exposed individuals: introducing the 4-digit diagnostic code. *Alcohol Alcohol* 2000;35(4):400-10.
PUBMED | CROSSREF
3. Cook JL, Green CR, Lilley CM, Anderson SM, Baldwin ME, Chudley AE, et al. Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. *CMAJ* 2016;188(3):191-7.
PUBMED | CROSSREF
4. Hoyme HE, Kalberg WO, Elliott AJ, Blankenship J, Buckley D, Marais AS, et al. Updated clinical guidelines for diagnosing fetal alcohol spectrum disorders. *Pediatrics* 2016;138(2):e20154256.
PUBMED | CROSSREF
5. Cabarcos P, Álvarez I, Tabernero MJ, Bermejo AM. Determination of direct alcohol markers: a review. *Anal Bioanal Chem* 2015;407(17):4907-25.
PUBMED | CROSSREF
6. Burd L, Hofer R. Biomarkers for detection of prenatal alcohol exposure: a critical review of fatty acid ethyl esters in meconium. *Birth Defects Res A Clin Mol Teratol* 2008;82(7):487-93.
PUBMED | CROSSREF
7. dos Santos FS, de Martinis BS, Furtado EF. The detection of fetal alcohol exposure by FAEEs meconium analysis. *Curr Dev Disord Rep* 2016;3(4):235-41.
CROSSREF
8. Goh YI, Hutson JR, Lum L, Roukema H, Gareri J, Lynn H, et al. Rates of fetal alcohol exposure among newborns in a high-risk obstetric unit. *Alcohol* 2010;44(7-8):629-34.
PUBMED | CROSSREF
9. Bearer CF, Santiago LM, O'Riordan MA, Buck K, Lee SC, Singer LT. Fatty acid ethyl esters: quantitative biomarkers for maternal alcohol consumption. *J Pediatr* 2005;146(6):824-30.
PUBMED | CROSSREF
10. Gross TS, Harris F, Brown LA, Gauthier TW. Ethyl linolenate is elevated in meconium of very-low-birth-weight neonates exposed to alcohol in utero. *Pediatr Res* 2017;81(3):461-7.
PUBMED | CROSSREF

11. Min MO, Singer LT, Minnes S, Wu M, Bearer CF. Association of fatty acid ethyl esters in meconium and cognitive development during childhood and adolescence. *J Pediatr* 2015;166(4):1042-7.
PUBMED | CROSSREF

12. Peterson J, Kirchner HL, Xue W, Minnes S, Singer LT, Bearer CF. Fatty acid ethyl esters in meconium are associated with poorer neurodevelopmental outcomes to two years of age. *J Pediatr* 2008;152(6):788-92.
PUBMED | CROSSREF

13. Lee HK, Chou SP, Cho MJ, Park JI, Dawson DA, Grant BF. The prevalence and correlates of alcohol use disorders in the United States and Korea--a cross-national comparative study. *Alcohol* 2010;44(4):297-306.
PUBMED | CROSSREF

14. Lee SH, Shin SJ, Won SD, Kim EJ, Oh DY. Alcohol use during pregnancy and related risk factors in Korea. *Psychiatry Investig* 2010;7(2):86-92.
PUBMED | CROSSREF

15. Lee HS, Jones KL, Lee HK, Chambers CD. Fetal alcohol spectrum disorders: Clinical phenotype among a high-risk group of children and adolescents in Korea. *Am J Med Genet A* 2016;170A(1):19-23.
PUBMED | CROSSREF

16. Choe SA, Yoo S, JeKarl J, Kim KK. Recent trend and associated factors of harmful alcohol use based on age and gender in Korea. *J Korean Med Sci* 2018;33(4):e23.
PUBMED | CROSSREF

17. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. *BMC Pediatr* 2013;13:59.
PUBMED | CROSSREF

18. Connors GJ, Volk RJ. Self-report screening for alcohol problems among adults. In: Allen JP, Wilson VB, editors. *Assessing Alcohol Problems: a Guide for Clinicians and Researchers.* 2nd ed. Bethesda, MD: Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute on Alcohol Abuse and Alcoholism; 2003, 21-35.

19. Chiung M, Chen MA, Yi H, Dawson DA, Stinson FS, Grant BF, et al. Alcohol use and alcohol use disorders in the United States, a 3-year follow-up: main findings from the 2004–2005 wave 2 national epidemiologic survey on alcohol and related conditions (NESARC). In: U.S. Alcohol Epidemiologic Data Reference Manual. Volume 8, Number 2. NIH Publication No. 10-7677. Bethesda, MD: National Institute on Alcohol Abuse and Alcoholism; 2010, 4-7.

20. Chan D, Bar-Oz B, Pellerin B, Paciorek C, Klein J, Kapur B, et al. Population baseline of meconium fatty acid ethyl esters among infants of nondrinking women in Jerusalem and Toronto. *Ther Drug Monit* 2003;25(3):271-8.
PUBMED | CROSSREF

21. Kwak HS, Kang YS, Han KO, Moon JT, Chung YC, Choi JS, et al. Quantitation of fatty acid ethyl esters in human meconium by an improved liquid chromatography/tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010;878(21):1871-4.
PUBMED | CROSSREF

22. Raghunathan R, Wu C, Singh M, Liu CH, Miranda RC, Larin KV. Evaluating the effects of maternal alcohol consumption on murine fetal brain vasculature using optical coherence tomography. *J Biophotonics* 2018;11(5):e201700238.
PUBMED | CROSSREF

23. Zhu Y, Wang L, Yin F, Yu Y, Wang Y, Shepard MJ, et al. Probing impaired neurogenesis in human brain organoids exposed to alcohol. *Integr Biol* 2017;9(12):968-78.
PUBMED | CROSSREF

24. Nakhoul MR, Seif KE, Haddad N, Haddad GE. Fetal alcohol exposure: the common toll. *J Alcohol Drug Depend* 2017;5(1):257.
PUBMED | CROSSREF

25. Lotfullina N, Khazipov R. Ethanol and the developing brain: inhibition of neuronal activity and neuroapoptosis. *Neuroscientist* 2018;24(2):130-41.
PUBMED | CROSSREF

26. Li ZY, Ma ZL, Lu WH, Cheng X, Chen JL, Song XY, et al. Ethanol exposure represses osteogenesis in the developing chick embryo. *Reprod Toxicol* 2016;62:53-61.
PUBMED | CROSSREF

27. Shen L, Ai H, Liang Y, Ren X, Anthony CB, Goodlett CR, et al. Effect of prenatal alcohol exposure on bony craniofacial development: a mouse MicroCT study. *Alcohol* 2013;47(5):405-15.
PUBMED | CROSSREF

28. Ramadoss J, Hogan HA, Given JC, West JR, Cudd TA. Binge alcohol exposure during all three trimesters alters bone strength and growth in fetal sheep. *Alcohol* 2006;38(3):185-92.
PUBMED | CROSSREF

29. Simpson ME, Duggal S, Keiver K. Prenatal ethanol exposure has differential effects on fetal growth and skeletal ossification. *Bone* 2005;36(3):521-32.
PUBMED | CROSSREF

30. Carter RC, Jacobson JL, Molteno CD, Dodge NC, Meintjes EM, Jacobson SW. Fetal alcohol growth restriction and cognitive impairment. *Pediatrics* 2016;138(2):e20160775.
PUBMED | CROSSREF

31. Carter RC, Jacobson JL, Sokol RJ, Avison MJ, Jacobson SW. Fetal alcohol-related growth restriction from birth through young adulthood and moderating effects of maternal prepregnancy weight. *Alcohol Clin Exp Res* 2013;37(3):452-62.
PUBMED | CROSSREF

32. Lehikoinen A, Ordén MR, Heinonen S, Voutilainen R. Maternal drug or alcohol abuse is associated with decreased head size from mid-pregnancy to childhood. *Acta Paediatr* 2016;105(7):817-22.
PUBMED | CROSSREF

33. Strandberg-Larsen K, Poulsen G, Bech BH, Chatzi L, Cordier S, Dale MT, et al. Association of light-to-moderate alcohol drinking in pregnancy with preterm birth and birth weight: elucidating bias by pooling data from nine European cohorts. *Eur J Epidemiol* 2017;32(9):751-64.
PUBMED | CROSSREF

34. Lundsberg LS, Illuzzi JL, Belanger K, Triche EW, Bracken MB. Low-to-moderate prenatal alcohol consumption and the risk of selected birth outcomes: a prospective cohort study. *Ann Epidemiol* 2015;25(1):46-54.e3.
PUBMED | CROSSREF

35. Henderson J, Gray R, Brocklehurst P. Systematic review of effects of low-moderate prenatal alcohol exposure on pregnancy outcome. *BJOG* 2007;114(3):243-52.
PUBMED | CROSSREF

36. Bryanton J, Gareri J, Boswall D, McCarthy MJ, Fraser B, Walsh D, et al. Incidence of prenatal alcohol exposure in Prince Edward Island: a population-based descriptive study. *CMAJ Open* 2014;2(2):E121-6.
PUBMED | CROSSREF

37. English LL, Mugyenyi G, Nightingale I, Kiwanuka G, Ngonzi J, Grunau BE, et al. Prevalence of ethanol use among pregnant women in Southwestern Uganda. *Matern Child Health J* 2016;20(10):2209-15.
PUBMED | CROSSREF

38. Zelner I, Shor S, Lynn H, Roukema H, Lum L, Eisinga K, et al. Clinical use of meconium fatty acid ethyl esters for identifying children at risk for alcohol-related disabilities: the first reported case. *J Popul Ther Clin Pharmacol* 2012;19(1):e26-31.
PUBMED

39. Lange S, Shield K, Koren G, Rehm J, Popova S. A comparison of the prevalence of prenatal alcohol exposure obtained via maternal self-reports versus meconium testing: a systematic literature review and meta-analysis. *BMC Pregnancy Childbirth* 2014;14:127.
PUBMED | CROSSREF

40. Kwak HS, Han JY, Choi JS, Ahn HK, Kwak DW, Lee YK, et al. Dose-response and time-response analysis of total fatty acid ethyl esters in meconium as a biomarker of prenatal alcohol exposure. *Prenat Diagn* 2014;34(9):831-8.
PUBMED | CROSSREF