

# FATTY ACID ETHYL ESTERS: QUANTITATIVE BIOMARKERS FOR MATERNAL ALCOHOL CONSUMPTION

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**Objective** To develop a laboratory marker to identify newborns exposed to alcohol.

**Study design** Meconium was collected from 30 infants from Jordan who were unexposed and from 248 Cleveland study infants of varying exposure status. Retrospective maternal alcohol histories were obtained. Fatty acid ethyl esters (FAEEs) were quantified with gas chromatography/flame ionization and compared between abstainers and non-abstainers to identify FAEEs of interest. The area under the receiver operating characteristic curve, sensitivity, specificity, and positive and negative predictive values were calculated by using definitions of drinking obtained from a graphical representation.

**Results** Six of 7 FAEEs were significantly different between the non-abstainers and at least 1 of 2 of the abstaining groups. FAEEs best predicted drinks per drinking day, and ethyl linoleate had the greatest area under the curve (76%), with a sensitivity rate of 88%, a specificity rate of 64%, a positive predictive value of 9%, and a negative predictive value of 99%. No combination of FAEEs was better than a single ester for identifying drinkers.

**Conclusion** Ethyl linoleate in meconium is a useful biological marker for identifying infants not exposed in utero to high levels of alcohol in a high-risk, substance-abusing, clinic-based sample. (*J Pediatr* 2005;■■■■:■■■-■■■)

Heavy drinking during pregnancy is the cause of fetal alcohol syndrome (FAS), the leading known cause of mental retardation.<sup>1</sup> Conservative estimates place the incidence of FAS at 0.33 of 1000 live births.<sup>1</sup> More prevalent are infants with a spectrum of outcomes, including alcohol-related birth defects, alcohol-related neurodevelopmental defects, and subtle effects on a variety of behavioral, educational, and psychological tests, resulting from low to moderate levels of drinking during pregnancy. Together, these effects are estimated to be present in 1% of all newborns<sup>2</sup> and to cost from \$75 million<sup>3</sup> to \$9.7 billion per year.<sup>4</sup>

There is a lack of clinical tools for assessing levels of drinking in pregnant women and identifying newborns who were exposed to alcohol. In one study, the diagnosis of FAS was missed in 100% of newborns in whom FAS was subsequently diagnosed in childhood.<sup>5</sup> Identification of affected newborns is desirable to facilitate early intervention, minimize secondary disabilities,<sup>6</sup> and identify mothers at high risk for drinking during gestation. Recent research has focused on developing biomarkers to identify maternal drinking during pregnancy. Maternal biomarkers showing promise include a combination of 4 maternal blood measurements: hemoglobin acetaldehyde adducts, gamma glutaryl transferase, mean corpuscular volume, and carbohydrate deficient transferrin.<sup>7</sup>

For identification of exposed neonates, fatty acid ethyl esters (FAEEs), non-oxidative metabolites of ethanol, in meconium are being investigated. In adults, FAEEs have been tested as a biological marker for alcohol-related fatalities.<sup>8</sup> Initially described in the cord blood of an infant born to an alcoholic mother,<sup>9</sup> FAEEs were subsequently

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Cynthia F. Bearer, MD, PhD, currently holds a patent for the measurement of fatty acid ethyl ester in meconium. Currently, there are no business relationships based on this patent.

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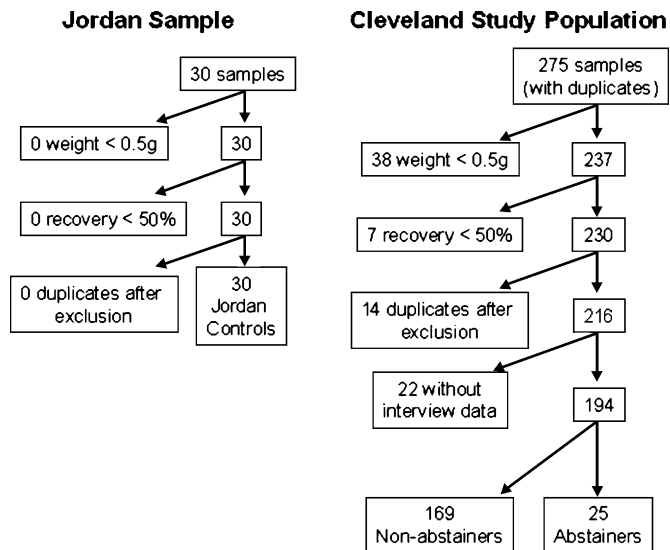
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AUC	Area under the receiver operating characteristics curve	GC/FID	Gas chromatography with flame ionization detection
DRDD	Drinks per drinking day	GC/MS	Gas chromatography with mass spectroscopy detection
DRWK	Drinks per week		
DYWK	Drinking days per week	NPV	Negative predictive value
FAS	Fetal alcohol syndrome	PPV	Positive predictive value
FAEE	Fatty acid ethyl ester	ROC	Receiver operating characteristics



**Figure 1.** Flow chart showing selection scheme for meconium analysis.

identified in meconium.<sup>10</sup> In our initial study of a Cleveland population, mothers of infants whose meconium contained ethyl linoleate reported drinking an average of 10 drinks per week in the month before pregnancy.<sup>11</sup> The amount of reported drinking (3 drinks/week) by mothers whose infants did not have ethyl linoleate in their meconium was significantly different.<sup>11</sup> Four-fold higher levels of FAEEs were found in the meconium of an infant born to an alcoholic compared with that of a control infant.<sup>12</sup> FAEEs are also found in the meconium of infants born to mothers from abstaining populations.<sup>13</sup> Thus, there is a need to quantify FAEEs to determine the level of alcohol exposure. With a highly alcohol exposed mixed race/Cape Colored population from South Africa, the sensitivity and specificity rates of ethyl oleate for detecting women who drank an average of 3 or more drinks per drinking day was 84.2% and 83.3%, respectively.<sup>14</sup> These results prompted a quantitative reanalysis of our previous work in a Cleveland population.<sup>12</sup> With gas chromatography/flame ionization detection (GC/FID), we quantified the FAEEs from the meconium of the Cleveland population<sup>11</sup> and a comparison Jordan population to further investigate the relationship between FAEE quantity and maternal self-reported drinking. In addition, we investigated the clinical usefulness of this biomarker.

## METHODS

### Cleveland Study Subjects

Postpartum women were recruited from a large urban, teaching hospital in Cleveland, Ohio, to participate in a 2-year longitudinal study on the neurobehavioral effects of prenatal cocaine exposure.<sup>15-18</sup> Women were predominantly African-American (83%), of low socioeconomic status (99%), and identified from a population screened for risk of substance abuse during pregnancy. Informed consent was obtained as

approved by the institutional review board of MetroHealth Medical Center.

### Jordan Comparison Subjects

Postpartum Muslim women and their infants were recruited from a large urban hospital in Amman, Jordan. The meconium from Jordan was expected to have fewer false negative results than abstainers from the Cleveland study group because there is a strong religious, cultural, and societal prohibitive influence against drinking by Jordanian Muslim women.

### Meconium Collection

A total of 275 samples of meconium were obtained from 248 study infants in Cleveland and 30 samples from 30 comparison infants in Jordan. Meconium stool from each infant was scraped from the diaper, collected into falcon tubes (15 mL, polypropylene, Becton-Dickinson), and frozen at  $-70^{\circ}\text{C}$  until analysis.

### FAEE Analysis

The analyses were performed by investigators who were blinded to the questionnaire results. A total of 1 g wet weight of meconium was used for analysis. An a priori exclusion criteria was constructed to ensure the best representation of each meconium sample (Figure 1). For samples  $<1$  g (83 [F1] samples), the whole sample was used. Samples  $<0.5$  g (38 samples) were excluded. The analysis of FAEEs has been previously described.<sup>11,19</sup> In brief, 100  $\mu\text{L}$  of 1 mMol/L ethyl heptadecanoate was added to each meconium sample as an internal standard. Only samples with ethyl heptadecanoate percent recovery  $\geq 50\%$  were analyzed (230 samples). FAEEs were extracted with acetone/hexane and isolated with silica column chromatography. The isolated FAEEs were identified and quantified with gas chromatography using a flame ionization detector (HP5890 Series II). Peak areas were obtained by integration with HP Chemstation software and valley to valley baseline. Peaks with an area  $\geq 500$  and with retention times  $\pm 0.1$  minutes of authentic standards were used in the analysis. FAEEs from 10 samples were confirmed with gas chromatography with mass spectroscopy detection (GC/MS). The limit of detection was 2 pmole of ethyl heptadecanoate on column. For individuals with  $>1$  sample (14 individuals), the sample with greater weight and higher recovery was selected. Of these 216 samples, 22 had no maternal interview data. All the Jordanian samples were 1 g and had recovery  $\geq 50\%$ . The remaining 194 samples from Cleveland and all 30 samples from Jordan were used for further analyses (Figure 1).

### Maternal Prenatal Substance Abuse Assessment

The maternal postpartum interview<sup>20-22</sup> was used to estimate maternal alcohol use during the month before and for each trimester of pregnancy. For each period, mothers were requested to recall both the amount and frequency of alcohol use, and 3 drinking measures of alcohol intake were calculated:

**Table I. Characteristics of drinking of Cleveland non-abstainers (n = 169)**

Measure	Period	Mean ± SD	Median	Range
DRDD	Month before	2.54 ± 4.64	1.0	0-39.6
	1st trimester	2.00 ± 4.31	0	0-39.6
	2nd trimester	1.09 ± 2.72	0	0-22.0
	3rd trimester	1.02 ± 2.83	0	0-22.0
DYWK	Month before	2.56 ± 2.03	1.5	0.25-7.0
	1st trimester	2.75 ± 2.18	1.5	0.25-7.0
	2nd trimester	2.80 ± 2.28	1.5	0.25-7.0
	3rd trimester	2.07 ± 1.94	1.5	0.25-7.0
DRWK	Month before	8.19 ± 19.36	0	0-138.6
	1st trimester	6.71 ± 17.41	0	0-138.6
	2nd trimester	3.75 ± 23.12	0	0-110.0
	3rd trimester	3.41 ± 13.52	0	0-110.0

**Table II. Comparison between study groups**

FAEEs	Cleveland non-abstainers (median [range])	Jordan (median [range])	Cleveland abstainers (median [range])
N	169	30	25
Ethyl myristate*	52 <sup>‡§</sup> (0-21461)	32 (0-233)	35 (0-2697)
Ethyl palmitate*	166 <sup>‡§</sup> (0-27245)	72 (27.4-336)	65 (0-3524)
Ethyl palmitoleate*	695 (124-50241)	805 (290.5-2109)	574 (65-2172)
Ethyl oleate*	317 <sup>‡§</sup> (0-344047)	108 (0-569)	142 (0-55213)
Ethyl linoleate*	282 <sup>‡§</sup> (0-627705)	97 (0-1490)	118 (0-77056)
Ethyl linolenate*	143 <sup>§</sup> (0-173558)	79 (0-421)	80 (0-31389)
Ethyl arachidonate*	208 <sup>§</sup> (0-23560)	144 (0-2586)	0 (0-2349)
Score <sup>†</sup>	1.73 <sup>‡§</sup> (1.42-3.55)	1.58 (1.45-1.98)	1.60 (1.42-2.95)

\*ng/g wet weight.

†Unitless because it is the result of the principal component analysis.

‡Cleveland non-abstainers mean log (FAEE + 100) of meconium statistically significantly different than Jordan, with  $P < .0001$ .

§Cleveland non-abstainers mean log (FAEE + 100) of meconium statistically significantly different than Cleveland abstainers, with  $P < .05$ .

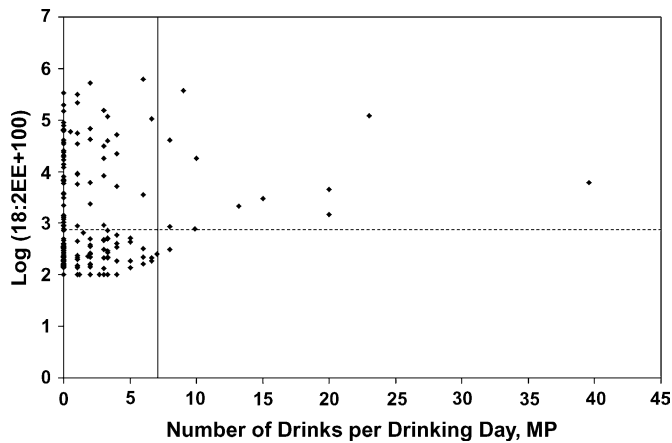
(1) number of drinks per drinking day (DRDD), (2) number of drinking days per week (DYWK), and (3) number of drinks per week (DRWK). To estimate the amount of alcohol used, the DRDD was computed on the basis of the amount of beer, wine, or hard liquor consumed, with 12 oz of beer, 4 oz of wine, or 1 oz of hard liquor assumed to be equivalent to 1 drink (0.5 ounces of absolute alcohol). DYWK was estimated on a Likert-type scale, ranging from 0 (less than once per month) to 7 (daily use). The DRWK was calculated by multiplying DRDD by DYWK. The postpartum interview was conducted as soon as possible after the birth of the child, generally within 1 month.

### Statistical Analysis

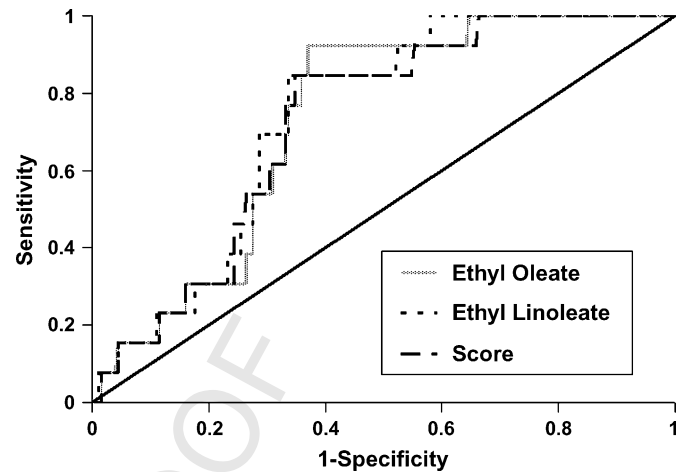
The purpose of the analyses was to develop a clinically useful tool to aid in screening infants for prenatal alcohol exposure. Analyses were carried out first to eliminate any FAEEs that lacked the potential to be predictive of alcohol intake, second to determine by a principal component analysis

whether some combination of FAEEs reduced noise and redundancy, third to define subjects as “drinkers” or “non-drinkers” on the basis of each of the 3 measures of alcohol intake, and fourth to use these definitions to estimate area under the receiver operating characteristics curve (AUC), sensitivity rate, specificity rate, and positive (PPV) and negative (NPV) predictive values.

Abstainers were strictly classified as Cleveland mothers who denied using alcohol, cocaine, tobacco or marijuana (n = 25) during the month before and during pregnancy. The remainder were classified as non-abstainers (n = 169). Subjects in the non-abstainer group reported the use of any drug, but not necessarily alcohol. This effectively created an internal comparison group, in addition to the external (Jordan) comparison group (Figure 1). This conservative approach to classification of abstainers and non-abstainers makes it less likely that we will be able to find significant associations, because undoubtedly our non-abstaining group includes some abstainers. There was no significant difference in the



**Figure 2.** Scattergram to define drinking. Example shows the  $\log_{10}(\text{ng/g} + 100)$  of ethyl linoleate versus DRDD in the month before pregnancy (MP). The solid line dichotomizes the population at  $<7$  or  $>7$  DRDD. The dotted line indicates a cutoff value of ethyl linoleate that gives 1 false negative result.



**Figure 3.** ROC curves for definition of drinking as  $>7$  DRDD in the month before pregnancy with ethyl oleate, ethyl linoleate, or the score from the principal component analysis.

201 proportion of African American mothers in the 2 groups (79%  
202 non-abstainers vs 92% abstainers,  $P = .18$  with Fisher exact  
203 test). The alcohol use of the non-abstainers is summarized in  
204 **Table I**.

205 To screen for the most predictive FAEE(s), the means  
206 of the  $\log_{10}$  transformed FAEE concentrations were compared  
207 with 2 sample  $t$  tests to compare Jordan control subjects,  
208 Cleveland abstainers, and Cleveland non-abstainers. The  
209 FAEE(s) with significant differences at a level of  $P < .05$   
210 between the Cleveland non-abstainers and at least 1 of  
211 the comparison groups were selected for further analysis  
212 **(Table II)**.

213 Second, a principal component analysis with only the  
214 FAEEs with a significant difference between groups showed  
215 that only 1 linear combination of the FAEEs explained 91% of  
216 the variance of these highly correlated measures. A score for  
217 each subject was calculated by using this linear combination.

218 Third, each measure of alcohol intake (X-axis) was  
219 plotted against  $\log(\text{ng/g}+100)$  FAEE (Y-axis) for each  
220 period. On the basis of the graphical representation of the  
221 data, subjects were defined as “drinkers” or “non-drinkers” for  
222 the respective measure. **Figure 2** is an example of such  
223 a graphical representation with DRDD as the drinking  
224 measure. As can be seen, defining drinking as  $>7$  drinks per  
225 drinking day (solid line) allows a cutoff value of ethyl linoleate  
226 (dotted line) in which only 1 subject is misclassified. With  
227 these definitions, receiver operating characteristics (ROC)  
228 curves were generated to select the FAEE concentrations  
229 that yielded the best sensitivity and specificity and at which  
230 the values would be dichotomized into “positive test” and  
231 “negative test”. **Figure 3** shows a representative ROC curve for  
232 the 2 most useful FAEEs and the score from the principal  
233 component analysis using the value of  $\geq 7$  DRDD in the  
234 month before pregnancy to define drinkers. The results of the  
235 ROC analysis for select FAEEs are presented as AUC,  
236 sensitivity rate, specificity rate, PPV, NPV, and the cutoff  
point in both  $\text{ng/g}$  and  $\log(\text{ng/g}+100)$  **(Table III)**.

237 All analyses were done with  $\log_{10}$  transformations of the  
238 FAEE levels. Log transformations are routinely used to  
239 convert values that increase exponentially to a scale in which  
240 the increase is linear, thus allowing for standard statistical  
241 methods to be used. A constant value of 100 was added, which  
242 effectively set the value of the samples with values below the  
243 limit of detection at 100 to allow for log transformation. The  
244 alpha level was set at 0.05. All analyses were done with SAS  
245 software version 8.1 (SAS Institute, Cary, NC).  
246

## RESULTS

### Study Group Differences in FAEE

247 **Table II** represents the medians and ranges of each  
248 FAEE by study group. Four of the 7 FAEEs being studied  
249 (ethyl myristate, ethyl palmitate, ethyl oleate, ethyl linoleate)  
250 have statistically significant differences in the mean ( $\log_{10}$ )  
251 concentration between the Cleveland non-abstainers and both  
252 the Jordanian control subjects and the Cleveland abstainers. Ethyl  
253 linoleate is significantly different only between meconium  
254 samples of Cleveland non-abstainers and the Jordanians, and  
255 ethyl arachidonate is different only between the Cleveland  
256 non-abstainers and the Cleveland abstainers. Ethyl palmito-  
257 leate is the only FAEE with no significant differences among  
258 the study groups and is excluded from subsequent analysis.  
259 Although both control groups have limitations (Jordanian  
260 group may have different diets, genetic background, and  
261 environmental exposures; the Cleveland abstaining group may  
262 have more underreporting), the finding that both groups have  
263 FAEE levels that are significantly less than those of the  
264 Cleveland non-abstainers strengthens this finding.  
265

### Principal Component Analysis

266 Only 1 linear combination of FAEEs is required to  
267 explain the variance between the FAEEs. Scores for each  
268 subject using the 4 or 5 most heavily weighted FAEEs or all  
269 6 FAEEs were calculated. Further evaluation showed no  
270  
271  
272  
273

**Table III. Receiver operating characteristics curve analysis**

FAEE prenatal period	AUC (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	FAEE cutoff point (ng/g)	FAEE cutoff point Log(ng/g+100)
<b>Drinker <math>\geq</math> 7 drinks/drinking day</b>							
Ethyl oleate							
Month before	74	92	61	14	99	676	2.83
1st trimester	73	88	61	9	99	837	2.92
2nd trimester	70	78	59	8	98	445	2.65
3rd trimester	68	80	58	5	99	445	2.65
Ethyl linoleate							
Month before	73	85	65	14	98	680	2.83
1st trimester	76	88	64	9	99	761	2.88
2nd trimester	72	89	58	9	99	383	2.58
3rd trimester	70	80	57	5	99	390	2.59
Ethyl arachidonate							
Month before	71	92	59	13	99	214	2.33
1st trimester	74	88	58	8	99	213	2.33
2nd trimester	71	78	54	7	98	202	2.31
3rd trimester	71	80	62	5	99	306	2.49
Score							
Month before	72	85	63	14	98	74*	1.87
1st trimester	75	88	62	9	99	74*	1.87
2nd trimester	71	78	60	8	98	68*	1.83
3rd trimester	69	80	59	5	99	68*	1.83
<b>Drinker <math>\geq</math> 21 drinks/week</b>							
Ethyl oleate							
Month before	69	79	63	18	97	802	2.90
1st trimester	59	61	61	13	94	836	2.92
2nd trimester	60	64	58	8	97	443	2.65
3rd trimester	62	75	58	7	98	445	2.65
Ethyl linoleate							
Month before	71	84	58	17	97	361	2.56
1st trimester	59	67	57	13	95	361	2.56
2nd trimester	59	64	58	8	96	384	2.58
3rd trimester	68	75	58	7	98	390	2.59
Ethyl arachidonate							
Month before	70	84	60	18	97	213	2.33
1st trimester	63	72	58	14	96	213	2.33
2nd trimester	64	73	63	10	98	306	2.49
3rd trimester	70	88	63	9	99	306	2.49
Score							
Month before	71	84	51	15	97	49*	1.69
1st trimester	60	72	50	12	95	49*	1.69
2nd trimester	64	73	54	8	97	54*	1.73
3rd trimester	66	75	60	7	98	68*	1.83

\*Unitless value.

274 difference in predictive value of these 3 scores. Thus, only  
 275 scores calculated with the 4 FAEEs are shown. Comparison  
 276 of these scores between study groups is shown in Table II.  
 277 The scores are significantly different between the Cleveland  
 278 non-abstainers and both the Cleveland abstainers and the  
 279 Jordanian groups for all FAEEs except ethyl palmitoleate.

### Definition of “drinking” for Each Measure of Exposure

The graphical analyses of the data indicate that not only are the Cleveland abstainers different from the non-abstainers in overall FAEE concentrations, but that within the non-abstaining group, the concentrations are dependent on the

level of drinking. An example of a graphical analysis for DRDD versus ethyl linoleate is shown in Figure 2. As can be seen in the figure, defining drinking as  $>7$  DRDD (solid line), and using a cutoff value of 2.8 (631 ng/g; dashed line) yields only 1 false negative result. This pattern was the same for all periods studied. Therefore, to analyze the predictive values of the FAEE to detect "drinking," we chose the definition of "drinking" as  $>7$  DRDD. Using the same approach, we defined "drinking" for each of the other measures. For DRWK, "drinking" was defined as  $\geq 21$  DRWK, and for DYWK, "drinking" was defined as  $\geq 5$  DYWK for all prenatal periods.

### ROC Analysis

The first 2 analyses aforementioned established a relationship between reported drinking and FAEE concentrations in meconium. The ROC analyses were used to determine which of the measures of alcohol exposure was best associated with FAEE concentration, for which period(s) the concentrations were the most predictive, and which of the individual FAEEs or the score from the linear combination of FAEEs was the most useful in the clinical setting. In general, values of AUC, sensitivity rate, specificity rate, PPV, and NPV for each of the 3 drinking measures were consistent across periods. The FAEEs and the score were most predictive of DRDD (Table III). For DRDD, all the FAEEs had AUCs between 63% and 76% during all prenatal periods. The FAEEs with the best performances for DRDD were ethyl oleate, ethyl linoleate, and ethyl arachidonate (Table III).

The ROC analysis for DRWK is reported in Table III. Using  $>21$  DRWK as the definition of "drinking," the AUC ranged from 54% to 71% for all periods, FAEEs, and score values. Ethyl arachidonate showed the most consistent result with AUCs of 63% to 70%, sensitivity rates of 72% to 88%, specificity rates of 58% to 63%, PPV of 9% to 18%, and NPV of 96% to 99%.

For DYWK, all the FAEEs had AUCs ranging from 52% to 67% (data not shown). Because of the low values of the AUCs, no further analysis with this measure of drinking was done.

We conclude that FAEE concentrations in general are related to prenatal alcohol exposure, particularly at high levels of DRDD and DRWK. With NPVs  $>97\%$  and  $>95\%$ , respectively, FAEE levels in meconium are particularly well suited for identifying those babies who have not been exposed to these high levels of drinking in utero. In addition, the principal component score had no better predictive value than the single most predictive FAEE for each alcohol measure.

### Discussion

Screening of newborns for prenatal ethanol exposure has been done routinely by history-taking alone. The identification of a biological marker that better represents the amount of prenatal drinking to which an infant has been exposed is a challenging task. This study showed that, in a high-risk, predominately African American Cleveland population using

a GC/FID assay, FAEE in meconium was most related to the self-reported DRDD. These findings are consistent with our published findings from a cohort of women in South Africa.<sup>23</sup> In that study, we found that ethyl oleate correlated best to DRDD and that the correlation of ethyl oleate to maternal history increased with stage of pregnancy. However, there are important differences between these 2 studies. First, women in the South African population were heavier drinkers. The DRDD, DYWK, and DRWK medians were  $5.2 \pm 4.0$  (range, 0-15),  $1.3 \pm 1.5$  (range, 0-6.7), and  $12.6 \pm 21.0$  (range, 0-102.8), respectively. In the Cleveland non-abstaining population, these values were  $2.54 \pm 4.64$  (range, 0-39.6),  $2.56 \pm 2.03$  (range, 0.25-7.0), and  $8.19 \pm 19.36$  (range, 0-138.6). Having a broader range of drinking in the South African population would result in better correlation.

Second, the degree of error or noise in the South African reports of drinking may be less than that of the Cleveland population. The South African population was followed prospectively, whereas the Cleveland group had retrospective alcohol histories. Previous studies have shown that antenatal self-reported drinking more accurately predicts outcome than retrospective reports.<sup>24</sup> The South African population is expected to more accurately report drinking because of a greater acceptance of drinking during pregnancy among this population.<sup>23</sup> In addition, there may be cultural differences in the amount of alcohol reported in a drink. One report has shown that women markedly underestimate the amount of alcohol they consume because of the volume of their drinks.<sup>25</sup> This study, although converting type of drink to standard drink, did not try to estimate the size of each reported drink.

Third, the sensitivity and specificity rates of the measurement of the FAEE were better in the South African study. The South African meconium was analyzed with a GC tandem mass spectrometer, an instrument with greater sensitivity and specificity than the GC/FID used to analyze the samples from the Cleveland and Jordan populations. Thus, higher predictive values in the South African study may be caused by lower noise in both the questionnaire data and the meconium data.

Fourth, the meconium analyzed in this study was from 1 meconium stool selected arbitrarily from all the meconium excreted by the infant. Preliminary data from other laboratories suggest that meconium is formed sequentially, with earlier gestational ages represented in the first meconium passed, and subsequent ages represented in subsequent meconium stools.<sup>26</sup> Alternatively, FAEEs could accumulate uniformly throughout meconium and not in concurrently forming meconium at the time of exposure. The samples used in our study, which were unsystematically scraped from the neonate's diaper, may not represent accurate levels of FAEEs corresponding to a particular gestational age. Future studies will need to serially collect all meconium to determine whether FAEE amounts vary within samples from the same infant.

Fifth, the 2 populations studied were culturally and ethnically distinct. Thus both genetic and dietary differences may account for the different findings in these 2 studies.

Several important questions need to be addressed in future studies. Do FAEEs distribute evenly throughout meconium? Animal studies would permit both control over the timing and quantity of alcohol consumption and the sequential collection of meconium. Do FAEEs identify a high-risk population of children? Studies on FAEE and outcome should be pursued to determine how well the biomarker can predict future adverse events in infants exposed to prenatal alcohol, establish a gold standard test, and implement early intervention programs for exposed children. FAEE in meconium show promise as a useful biological marker for determining alcohol exposure in utero.

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