Ethyl Linolenate is Elevated in Meconium of Very Low Birthweight Neonates Exposed to Alcohol *In Utero*

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Abstract

Background—The health implications of *in utero* alcohol exposure have been difficult to study in very low birth weight newborns (VLBW) because of an inability to identify maternal alcohol exposure. Fatty acid ethyl esters (FAEEs) are elevated in meconium of alcohol-exposed term newborns. We *hypothesized* that meconium FAEEs would be similarly elevated in alcohol-exposed VLBW premature newborns.

Methods—In a retrospective cohort study of 64 VLBW neonates, newborns were classified into Non-Exposed, Any Exposure, or Weekly Exposure groups based on an in-depth structured maternal interview. Meconium FAEE concentrations were quantified via gas chromatography mass spectrometry.

Results—Alcohol exposure during Trimester 1 (Any Exposure) occurred in ~30% of the pregnancies, while 11% of the subjects reported drinking ≥1 drink/week (Weekly Exposure). Meconium ethyl linolenate was higher in Any Exposure (p=0.01) and Weekly Exposure groups (p=0.005) compared to the Non-Exposed VLBW group. There was a significant positive correlation between Trimester 1 drinking amounts and the concentration of meconium ethyl linolenate (p=0.005). Adjusted ROC Curves evaluating ethyl linolenate to identify alcohol-exposed VLBW newborns generated areas under the curve of 88% with sensitivities of 86-89% and specificities of 83-88%.

Conclusions—Despite prematurity, meconium FAEEs hold promise to identify the alcohol-exposed VLBW newborn.

INTRODUCTION

Despite common knowledge of the negative effects of alcohol use during pregnancy, 20-45% percent of women report drinking during pregnancy (1,2). The impact of alcohol exposure on the newborn is particularly important for very low birth weight (VLBW) newborns since

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alcohol exposure has been linked to preterm delivery (3). Despite the potential for increased prematurity with *in utero* alcohol exposure, our understanding of alcohol's full impact on neonatal health of the VLBW premature population admitted to the Neonatal Intensive Care Unit (NICU) remains incomplete.

Clinical research has been hampered by the difficulty in identifying the alcohol-exposed premature newborn. Unfortunately, typical biomarkers used to help identify alcohol exposure in adults are not optimal for identifying *in utero* alcohol exposure (4). Blood alcohol or acetaldehyde levels cannot address long term intermittent fetal exposure because of such rapid elimination of these compounds from the body. Serum biomarkers routinely used to identify alcohol exposure in adults such as gamma-glutamyl transferase, mean corpuscular volume, hemoglobin associated acetaldehyde, and carbohydrate deficient transferrin are not optimal during pregnancy as they demonstrate only a 40-70% association with maternal alcohol consumption (5).

In non-pregnant adults, identification of patients who abuse alcohol has been standardized using the Alcohol Use Disorders Identification Test (AUDIT) and the Short Michigan Alcoholism Screening Test (SMAST). When tested in pregnant women, these tools are best at identifying heavy drinkers (5) whereas modest drinking is much more common in pregnancy (6). Maternal self-report of exposure through standardized questionnaires remains the mainstay for identification of the alcohol-exposed newborn, but these methods are known to be limited due to the stigma associated with the admittance of drinking alcohol while pregnant (5). Standardized questionnaires and in-person interviews improve upon the basic maternal self-report models, although they are time consuming. Therefore, in-depth interviews are useful in a research context as the closest approximation of a gold standard for comparison against new alternative markers of alcohol exposure. However, maternal self-reporting of alcohol consumption during pregnancy continues to underestimate the scope of exposure, thus ultimately allowing alcohol-exposed pregnancies and exposed premature newborns to remain clinically undetected. Although the majority of women stop or reduce alcohol consumption during pregnancy, consumption prior to recognition of pregnancy results in unrecognized exposure of the developing newborn to alcohol during the first trimester (7). This inability to rely on typical biomarkers or solely on maternal self-report continues to drive the search for neonatal biomarkers of *in utero* alcohol exposure

Fatty acid ethyl esters (FAEEs) are the non-oxidative metabolites of alcohol, and are promising biomarkers for *in utero* alcohol exposure (8-11). While alcohol can cross the placenta, FAEEs do not, thus neonatal FAEEs represent alcohol which was metabolized in fetal tissues (5). Both the concentration of alcohol and the duration of alcohol exposure contribute to the level of FAEEs. High concentrations of meconium FAEEs in full term neonates are associated with *in utero* alcohol exposure (11-15) and can be predictive of adverse neurological outcome in these children (16,17). To date, most studies in term neonates have focused on correlations between FAEE and heavy alcohol consumption late in pregnancy (5,15,18). However, alcohol use is most common early in pregnancy, particularly since many pregnancies are unplanned (19).

Although meconium FAEEs have been well described in term neonates they have not been evaluated in VLBW neonates. Meconium is thought to begin accumulating at week 12 of gestation. Increased concentrations of FAEE upon alcohol exposure is well described in meconium that has accumulated during the second and third trimesters of pregnancy (10). However, it is unclear if meconium FAEE accumulation occurs with maternal consumption of alcohol in the VLBW infant born prematurely. The objectives of this study were to measure meconium FAEEs in VLBW newborns (1500 grams at birth) and to evaluate meconium FAEEs as potential biomarkers for *in utero* alcohol exposure in premature neonates. We *hypothesized* that FAEEs are detectable in the meconium of VLBW newborns and that meconium FAEEs would be elevated in the alcohol-exposed VLBW newborn.

RESULTS

Demographics of Study Population

During the study period, a total of 62 consenting subjects completed the full questionnaire via personal maternal interview. These women delivered 64 VLBW newborns (2 sets of twins) that were admitted to the NICU. The maternal and neonatal characteristics of the study population are described in Table 1. Overall, approximately thirty percent of the women reported consuming alcohol during pregnancy, while 63% of the subjects reported drinking during the 3 months prior to pregnancy. Of those who did report alcohol consumption during pregnancy, the majority (94%) reported alcohol consumption almost exclusively in Trimester 1. Of those who drank in Trimester 1, the average reported drinks/week (d/wk) were low [Median 0.75 d/wk (25th-75th: 0.5-1.4); Range: 0.20 – 8.0 d/wk]. However, 11% of the subjects (7/62) admitted to drinking weekly at levels 1 d/wk. The VLBW newborns in the overall study population were born at approximately 28 weeks of gestation and weighed approximately 1 kg at birth (Table 1).

Alcohol Exposure and Comparison Groups

The population of 62 maternal subjects were divided into a group of alcohol Abstainers (n=45) and alcohol Consumers (n=17), based on the answers provided during their extensive interview. Abstainers were defined as those mothers who reported zero alcohol consumption during Trimester 1 while Consumers were defined as those mothers who reported drinking any alcohol during Trimester 1. Of the Consumers, 41% (n=7) reported drinking during Trimester 1 at the level of 1 d/wk. We defined this subgroup as Weekly Consumers (Table 1). When comparing maternal characteristics between the groups, Consumers were more likely to have obtained education beyond high school compared to Abstainers, while both Consumers and the Weekly Consumer subgroups were more likely to consume alcohol in the 3 months before pregnancy compared to Abstainers. The Abstainers delivered 46 VLBW newborns defined as Not Exposed, while the consumers delivered VLBW newborns (n=18) that were classified as Any Exposure. Finally the Weekly Consumer's VLBW newborns were classified as Weekly Exposure (n=7). When comparing Neonatal characteristics, both gestational age and birth weight were significantly less in the Any Exposure group compared to Not exposed, while there were no statistical differences in gestational age or birth weight between the Weekly Exposure and Not Exposed groups (Table 1).

Meconium FAEEs in the VLBW population

Ethyl palmitate, stearate, oleate, linoleate, linolenate and arachidonate were readily detectable in the meconium samples by GC/MS. The values [median (25th-75th)] of each ethyl ester and the sum of all meconium FAEEs are presented in Table 2 for the Overall population, the Non-Exposed, Any Exposure and Weekly Exposure groups. As in other investigations of neonatal biomarkers of alcohol exposure, (20) we demonstrated a wide variability in FAEE concentrations across the exposure levels. This variability may reflect our small sample size and may potentially be compounded by prematurity as well as maternal underreporting of alcohol use during pregnancy. The concentrations of ethyl linolenate were significantly elevated in the Any Exposure group (median: 1,661 ng/gm) and the Weekly Exposure group (median: 19,952 ng/gm) compared to the Non-Exposed (median: 448 ng/gm). There was a significant positive correlation between Trimester 1 drinking amounts and the concentration of meconium ethyl linolenate (Spearman's rho, Correlation coefficient 0.345, p=0.005). Meconium FAEE levels did not significantly differ by neonatal sex, race, birth weight or gestational age (p=NS for each FAEE, data not shown). When both sets of twins were excluded, we found similar significantly elevated meconium ethyl linolenate values with Any and Weekly exposure (Supplemental Table S1, online).

ROC analyses of Meconium FAEEs

We generated ROC curves for meconium ethyl linolenate to assess whether this FAEE species or concentrations could significantly distinguish the Any Exposure group from Non-Exposed group. Since birth weight was significantly lower in the Any Exposure group while maternal drinking prior to pregnancy was significantly higher, we generated both unadjusted and adjusted ROC curves adjusting for these variables. Meconium ethyl linolenate alone was able to distinguish Any Exposure from Non-Exposed VLBW newborns with an AUC of 0.68 (p= 0.025, Figure 1, grey line). When adjusted for birth weight, gestational age and maternal drinking prior to pregnancy, meconium ethyl linolenate was able to distinguish Any Exposure from Non-Exposed VLBW newborns with an AUC of 0.88 (p= 0.000, Figure 1, black line). We also generated ROC curves for meconium ethyl linolenate to assess whether this FAEE could significantly distinguish Weekly Exposure from Non-Exposed. Indeed, ethyl linolenate alone was able to distinguish Weekly Exposure from Non-Exposed VLBW newborns with an AUC of 0.82 (p= 0.007, Figure 2, grey line). When adjusted for maternal drinking prior to pregnancy, meconium ethyl linolenate was able to distinguish Weekly Exposure from Non-Exposed VLBW newborns with an AUC of 0.88 (p= 0.001, Figure 2, black line). The AUC (95% CI), sensitivity, specificity, positive predictive value and negative predictive value for these ROC analyses are outlined in Table 3. When both sets of twins were excluded, generated unadjusted and adjusted ROC curves demonstrated ethyl linolenate' s ability to identify alcohol exposure (Supplemental Table S2, online).

DISCUSSION

Research into the neonatal health implications of alcohol exposure has been hampered by the continued difficulty surrounding identification of newborn babies that have been exposed *in utero*. This is of especially high importance for the VLBW population who are often born

prematurely. Although maternal drinking has been associated with an increased risk of extreme prematurity (3,21,22), limited data exists on the identification of alcohol-exposed newborns in the NICU. The current study is the first to report evaluation of FAEEs in meconium obtained from the VLBW NICU population.

This study found that FAEEs were quantifiable via GC/MS in the meconium of VLBW newborns despite their prematurity. GC/MS has been verified as a sensitive and reproducible method to measure FAEEs in meconium (18,23). Meconium FAEEs were detectable in both Non-exposed and exposed newborns. Low concentrations of FAEEs have been demonstrated in unexposed subjects, since ethanol is produced endogenously in small amounts as a by-product of normal gut physiology (5,12). We demonstrate that meconium ethyl linolenate is significantly elevated in the meconium of VLBW newborns with alcohol exposure. A positive relationship was identified between the amount of reported maternal alcohol consumption during Trimester 1 and accumulation of ethyl linolenate in the meconium of the VLBW infant. In adjusted analyses, ethyl linolenate in meconium successfully distinguished alcohol-exposed VLBW newborns exposed to any alcohol or weekly exposure from non-exposed with sensitivities ranging from 86-89% and specificities of 83-88%.

Identification of alcohol exposure using biomarkers such as FAEEs (15,24,25), ethyl glucuronide (12,26), and ethyl sulfate (27,28) has advanced our ability to identify the alcohol-exposed term newborn. The current study suggests that alcohol exposure *in utero* similarly increases FAEEs in VLBW premature newborn meconium, even when that alcohol consumption reportedly occurred during trimester one.

Currently established FAEE cut offs have been well described to identify heavy drinking in 2nd and 3rd trimesters in term newborns with high sensitivity and specificity (29). However, given that these cut-off were established after multiple investigations in the term newborn population, it is unknown and highly doubtful that they are applicable to the current understudied VLBW population. In these reports, accumulation of ethyl linolenate is rarely cited with alcohol exposure. It is also possible that different types of FAEEs accumulate at different stages of pregnancy with *in utero* alcohol exposure. Indeed, the initial studies pioneered by Bearer et al demonstrated that accumulation of ethyl oleate in term newborns strongly related with drinking in 2nd and 3rd trimesters (8).

There are several limitations to our study. As with other studies evaluating *in utero* alcohol exposure, there is no gold standard to identify alcohol consumption against which to compare the FAEE measurements (11) and we relied on maternal self-report through a structured interview. Maternal interviews carry inherent risks of multiple types of bias including the stigma associated with reporting alcohol consumption during pregnancy. The maternal structured interview, although time-consuming, increases the reliability of maternal self-report. Thus retrospective self-report of alcohol use via a structured maternal interview remains a mainstay for identifying alcohol use during pregnancy (30). We suspect, as have others reported, that alcohol exposure per maternal self-report may significantly under-represent the true prevalence of exposure (29,31).

Our small sample size also limited our analyses, particularly in our evaluation of the relationship between concentration of FAEE and amount of alcohol consumption. The small sample had inadequate power to verify our model in a different subset of the sample population. Finally, our study had a primarily African-American population, which mirrored our NICU population. Our population may not be representative of the general population in other NICUs across the United States. This may be important since ethnicity may impact the type of FAEE that is most increased in alcohol exposed neonates (5).

Despite the limitations of this initial study, meconium ethyl linolenate is proposed as a potential biomarker for trimester one alcohol exposure in the VLBW NICU population. Further studies with larger numbers are necessary to validate our findings and fully access FAEE accumulation in the alcohol-exposed neonate born prematurely. Proposed models to identify alcohol exposure in this population could be improved by combining meconium FAEEs with other biomarkers (20) in other matrices including newborn blood, placenta (32) and hair (12,33). As such, a biomarker panel would be ideal to increase sensitivity and avoid false negatives (11). Ultimately, a multi-step approach combining prenatal screening and maternal interview after delivery with biological screening of neonatal matrices for FAEE and other biomarkers such as ethyl glucuronide will advance our ability to identify alcohol exposure in this population.

CONCLUSION

In summary, we have demonstrated for the first time that *in utero* alcohol exposure during the 1st trimester was associated with increased meconium ethyl linolenate in the VLBW newborn born prematurely. Additional investigations are warranted to examine the premature population, who, similar to term newborns, are often exposed to alcohol *in utero*. The ability to identify the alcohol-exposed VLBW baby remains the crucial first step to fully understanding the pathophysiology of the effects of alcohol exposure in the vulnerable NICU population.

METHODS

Study Population

Mothers who delivered premature newborns weighing   1,500 grams who were admitted to the NICUs of Grady Memorial Hospital or Emory University Hospital Midtown were eligible for enrollment into the study. The population at Grady Memorial Hospital is primarily urban, while that at Emory University Hospital Midtown is a mix of suburban and urban. Exclusion criteria included maternal refusal to participate, multiple congenital anomalies on neonatal physical exam, and clinically suspected or confirmed chromosomal abnormality of the newborn. Mothers with a history of HIV were excluded for the safety of our laboratory workers. This study was approved by the Emory University IRB (IRB00000976, Gauthier, PI). The VLBW newborns were under the care of Attending Emory Neonatologists in the NICU unrelated to this research study to ensure that participation did not alter patient care.

Study Design

This was a retrospective cohort design, with the cohort being all women enrolled at the two sites from May 2009 to November 2013. Each hospital had a dedicated research nurse to enroll subjects and conduct structured maternal interviews. Mothers underwent an in-depth structured interview while at the hospital, within two weeks of delivery, to determine the amount (drinks/week) and timing of alcohol consumption before and during pregnancy. This interview information defined the comparison groups of maternal subjects and the comparison groups of VLBW newborns.

Maternal Questionnaire Data

Mothers were interviewed in-person by one of two trained research nurses who completed our structured questionnaire. Our standardized questionnaire, which incorporates the AUDIT and Time-Line Follow Back (TLFB), was modeled after those used by the Centers for Disease Control and Prevention (34,35) and in studies we have performed on term (36) and premature newborns (32). The AUDIT questionnaire is an indirect line of questioning based around the negative effects of excessive drinking (37). It was validated in pregnant African American women (38). During this in-depth interview, questions about alcohol were embedded within more than 50 questions and generally took over 30 minutes to complete.

Meconium FAEE Determination

First passed meconium samples from enrolled neonates were saved in the diaper, labeled with a de-identified study number, placed on ice, and transported immediately to the Gauthier laboratory. Each meconium sample was scraped from the diaper and stored at $-80°$ C until batch analysis by GC/MS using methods we have previously described (32,39). FAEEs of interest included ethyl palmitate, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl linolenate and ethyl arachidonate, or the total sum of all FAEES. Briefly, thawed meconium samples (1 gram of wet weight meconium/sample) were homogenized and spiked with a non-biological surrogate standard (SS) of pentadecanoic acid ethyl ester (MP Biomedicals, LLC, Santa Ana, CA). FAEEs were extracted using methanol/chloroform as we have previously published (40), filtered across extraction columns (UCT, Bristol, PA), dried under nitrogen gas, and then reconstituted in methanol. The concentrations (ng/g) of the individual FAEEs were normalized to the dry weight of a corresponding thawed meconium sample (grams) obtained after drying (48 h at 50°C). The lower limit of detection (LOD) for each FAEE type was: 233 ng/gm for ethyl palmitate, 312 ng/gm for ethyl stearate, 315 ng/g for ethyl oleate, 305 ng/g for ethyl linoleate, 448 ng/g for ethyl linolenate, and 618 ng/gm for ethyl arachidonate. Undetectable concentrations were assigned the lower LOD.

Statistical analyses

Meconium FAEE concentrations were compared between the exposure groups (Non-Exposed, Any Exposure or Weekly Exposure). Since the study population included two sets of twins, analyses were also repeated in a second population which excluded both sets of twins. Continuous FAEE concentrations between two groups were compared using the non-parametric Mann Whitney-U test. A multivariate logistic regression model was developed to most accurately identify the alcohol exposed VLBW newborn. The log transformation of the

drinking amounts and FAEE concentration was used in order to account for non-normality. The models were developed using backward regression and clinical reasoning and practicality. Univariate analysis of demographic and survey data was used to determine variables that should be included in the model.

The accuracy of the model in categorizing alcohol exposure was determined using receiver operating characteristic (ROC) curves and area under the curve (AUC). A p 0.05 was considered statistically significant. All analyses were performed using SPSS Statistics Version 21 (IBM, Armonk, NY) and SAS Statistics Software version 9.4 (SAS Institute, Cary, NC).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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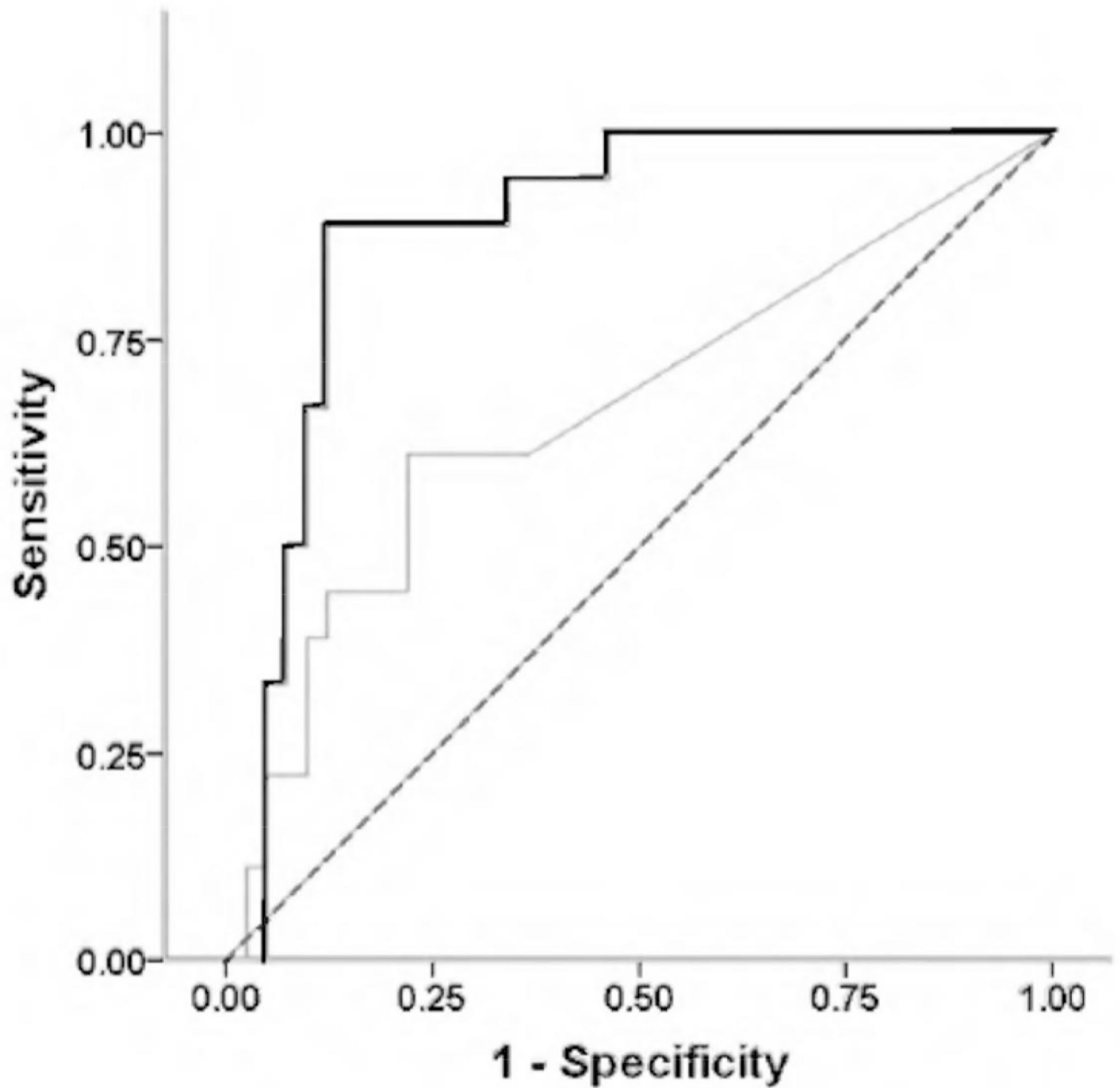
Figure 1. Receiver Operating Characteristic curves for Any Exposure
Unadjusted and adjusted ROC curves were generated for meconium Ethyl Linolenate. The Y axis denotes Sensitivity and the X axis denotes 1- Specificity. The grey line denotes the unadjusted curve and the black line denotes the curve adjusted for gestational age, birth weight and maternal drinking prior to pregnancy. A dashed diagonal reference line (line of no discrimination) corresponds to an area under the curve (AUC) of 0.5.
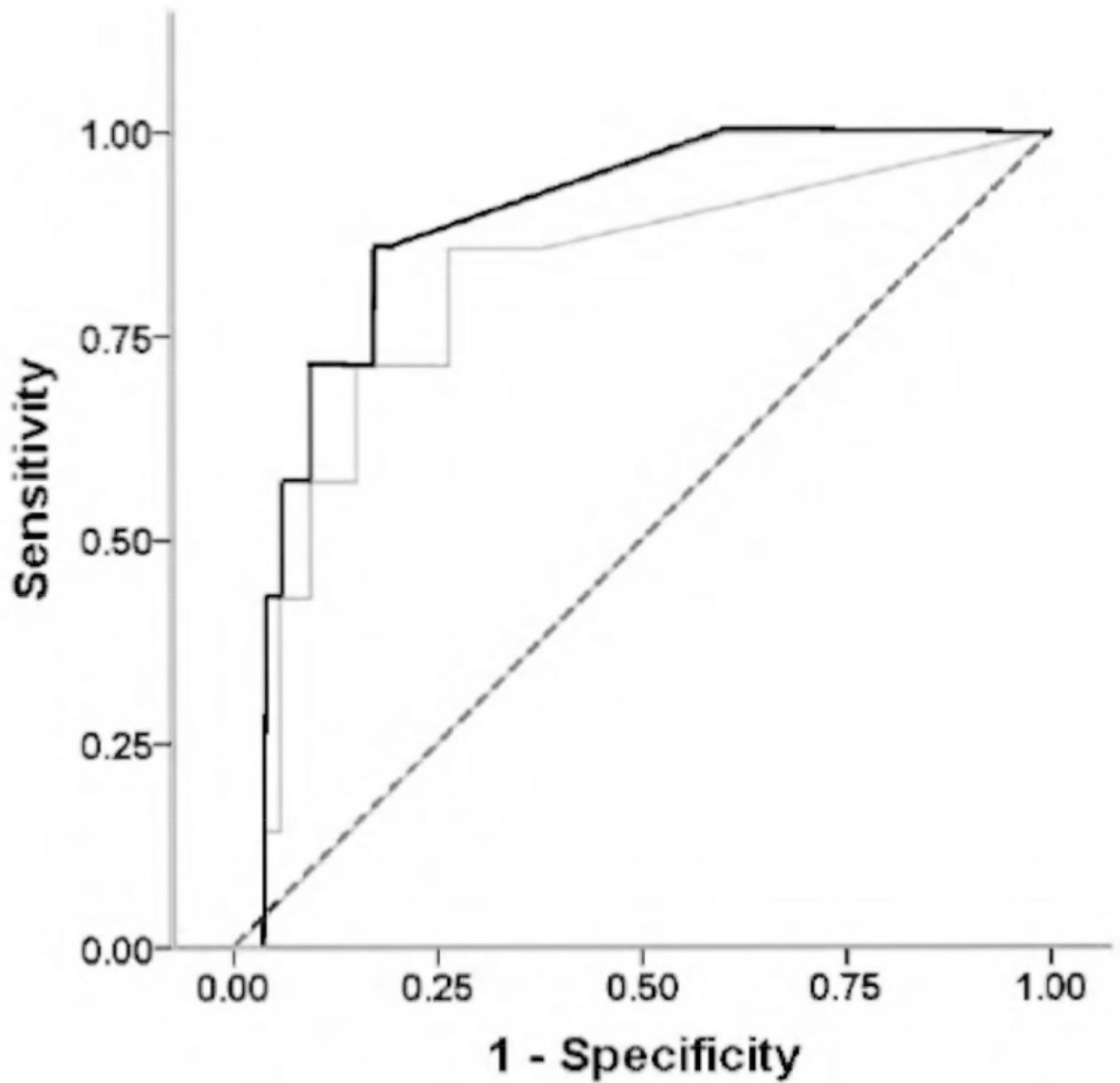
Figure 2. Receiver Operating Characteristic curves for Weekly Exposure
Unadjusted and adjusted ROC curves were generated for meconium Ethyl Linolenate. The Y axis denotes Sensitivity and the X axis denotes 1-Specificity. The grey line denotes the unadjusted curve and the black line denotes the curve adjusted for maternal drinking prior to pregnancy. A dashed diagonal reference line (line of no discrimination) corresponds to an area under the curve (AUC) of 0.5.

Table 1

Subject Characteristics

Maternal Characteristics	Overall (n=62)	Abstainers (n=45)	Consumers (n=17)	Weekly [a] Consumers (n= 7)
Maternal Age				
Years, median (25th- 75th)	28 (22-33)	25 (22-33)	31 (24-35)	31 (21-38)
Black Race, n (%)	54 (87%)	36 (80%)	15 (88%)	7 (100%)
Gravidity, median (25th- 75th)				
Total	2 (1-4)	2 (1-4)	3 (1-7)	1 (1-6)
Livebirth	2 (1-3)	2 (1-3)	1 (1-3)	1 (1-2)
Trying to get Pregnant, n (%)	13 (21%)	11 (24%)	1 (6%)	0 (0%)
Prenatal Care- Yes, n (%)	55 (89)	43 (84%)	17 (100%)	7 (100%)
Education beyond High School n (%)	29 (47%)	15 (33%)	12 (71%) [*]	5 (71%)
Married or Living with Partner (n=60) n (%)	13 (22%)	11 (24%)	2 (11%)	1 (14%)
Illicit Drug Use, n (%)				
3 months before pregnancy	12 (19%)	9 (20%)	3 (18%)	2 (29%)
During pregnancy	9 (14%)	6 (13%)	3 (18%)	2 (29%)
Tobacco Smoking during pregnancy n (%)	14 (22%)	9 (20%)	5 (29%)	2 (29%)
Consumed alcohol, n (%)				
3 months before pregnancy	39 (63%)	21 (47%)	17(100%) [**]	7 (100%) [*]
During Pregnancy	18 (29%)	-	-	-
During Trimester 1	17 (27%)	-	-	-

Neonatal Characteristics	Overall (n=64)	Non-exposed (n=46)	Any Exposure (n=18)	Weekly [a] Exposure (n=7)
Gestational Age, Weeks, Median (25th-75th)	28 (26-30)	28 (27-30)	27 (25-29) [*]	28 (26-30)
Weight, Grams, Median (25th-75th)	1102 (785– 1248)	1123 (950-1273)	802 [*] (633-1231)	1095 (800-1235)
Male Child, n (%)	34 (53%)	21 (46%)	13 (73%)	2 (29%)

[a] Defined as consuming/exposed to 1 drink/week during Trimester 1.

[*] p 0.05 versus Abstainers or Not exposed, respectively

[**] p 0.001 versus Abstainers or Not exposed, respectively

Table 2

Fatty Acid Ethyl Ester Profile in VLBW Meconium

FAEE Type	Overall (n=64)	Non-Exposed (n=46)	Any Exposure (n=18)	Weekly Exposure (n=7)
Ethyl Palmitate	5,491 (2,359-8,704)	5,830 (2,371-8,424)	4,219 (2,206-10,315)	6,680 (2,365-8,481)
Ethyl Stearate	3,648 (312-8,092)	3,374 (312-7,926)	4,190 (312-8,546)	769 (312-17,173)
Ethyl Oleate	10,254 (2,876-17,471)	8,353 (3,010-17,035)	14,006 (2,335-31,006)	15,973 (1,388-204,153)
Ethyl Linoleate	5,277 (322-13,269)	4,560 (801-9,809)	8,959 (305-67,209)	44,943 (305-210,413)
Ethyl Linolenate	448 (448-3,107)	448 (448-993)	1,661 (448-31,230)[*]	19,952 (448-78,448)[**]
Ethyl Arachidonate	27,391 (13,053-62,867)	27,391 (13,514-49,299)	25,943 (12,665-103,177)	11,539 (8,633-169,100)
Total FAEEs	63,823 (36,264-132,938)	57,159 (28,541-116,590)	96,204 (39,180-204,458)	87,548 (16,873-794,767)

Values are ng/gm dry weight expressed as Median (25th-75th). Non parametric comparisons by Mann-Whitney U Test.

[*] p=0.01 versus Non-Exposed

[**] p= 0.005 versus Non-Exposed

Table 3

Analysis of ROC Curves for Alcohol Exposure

	AUC (95%CI)	p Value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Any Exposure						
Unadjusted	0.682 (0.525-0.838)	0.025	61	80	54	84
Adjusted [a]	0.883 (0.794-0.973)	<0.001	89	88	74	95
Weekly Exposure						
Unadjusted	0.816 (0.639-0.992)	0.007	86	75	30	98
Adjusted [b]	0.881 (0.767-0.996)	0.001	86	83	38	98

AUC- area under curve; PPV- positive predictive value; NPV-negative predictive value.

[a]Model to identify Any Exposure to alcohol during Trimester 1 by Ethyl Linolenate adjusted for birth weight, gestational age and maternal drinking prior to pregnancy.

[b]Model to identify Weekly Exposure to alcohol (1 drink/week) during Trimester 1 by Ethyl Linolenate adjusted for maternal drinking prior to pregnancy.