



Fetal Alcohol Exposure: Time to Know, Time to Act

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Population Baseline of Meconium Fatty Acid Ethyl Esters Among Infants of Nondrinking Women in Jerusalem and Toronto

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Summary: The detection of fatty acid ethyl esters (FAEE) in meconium may provide an objective estimate of prenatal alcohol exposure independent of maternal history. The authors report the results of the first population-based study conducted to investigate basal FAEE levels in the meconium of neonates not exposed to alcohol. Two hundred seven nondrinking women and their neonates were recruited from Toronto and Jerusalem. FAEE were extracted from meconium by solid-phase extraction and analyzed by GC / FID. Similar procedures were conducted in six neonates born to confirmed heavy drinkers. Low levels of meconium FAEE were detected from both cohorts (mean. 1,37 nmol/g vs. 2.05 nmol/g. Toronto vs. Jerusalem). Ethyl stearate, oleate, and linoleate were below the limit of detection in >80 of all samples, whereas ethyl laurate and palmitate were detected in >50% of the samples. Ethyl myristate was the FAEE most commonly detected (>80%). All six meconium samples with confirmed maternal drinking histories tested positive for FAEE at significantly higher levels (mean 11.08 nmol/g). The use of 2 nmol total FAEE/g meconium as the positive cutoff, when lauric and myristic acid ethyl esters were excluded yielded the greatest sensitivity (100%) and specificity (98.4%). The authors conclude that certain FAEE are present at measurable levels in the meconium of neonates not exposed to maternal drinking, and correction is needed to allow high specificity. **Key Words:** Baseline – Ethanol - Fatty acid ethyl esters – Meconium - Pregnancy.

Fetal alcohol spectrum disorders is the range of outcomes resulting from maternal alcohol abuse during pregnancy. Although there is no established safe drinking level during pregnancy, 20% of pregnant women continue to consume some alcohol at lower levels before they realize they have conceived, while 4% will continue to drink heavily throughout pregnancy (1).

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Presently, full-blown FAS occurs at an estimated rate of 4.3% among heavy drinkers (2), and the whole spectrum of alcohol-related damages can be as high as 1% (i.e., 9.1 per 1000 live births) in the general pediatric population (3). To date, detection of prenatal alcohol exposure is almost entirely dependent on maternal self-reporting, which may be affected by denial or underreporting (4). The use of

. They are synthesized primarily from ethanol and free fatty acids by the cytosolic FAEE synthase, or from ethanol and fatty acyl-CoA by the microsomal synthase (acyl-CoA: ethanol acyltransferase [ABAT]) (6). FAEE has been found at high concentrations (25–300 nmol/g tissue) in adipose tissue and in organs commonly damaged by chronic alcohol abuse, such as the liver, pancreas, heart, and brain, where FAEE synthase activity is highest (7).

Meconium analysis is often used to detect fetal exposure to commonly abused drugs due to higher sensitivity and specificity than tests that involve blood and urine (8). Its collection is simple and entirely noninvasive. Since deposition of drugs in meconium theoretically begins from the 13th week of gestation at the commencement of fetal swallowing and accumulates thereafter until birth, meconium testing may provide a detailed history of fetal exposure to various xenobiotics or substances taken by the mother during pregnancy (9).

The presence of FAEE in meconium has led to the development of a noninvasive neonatal screening method for the identification of intrauterine alcohol exposure. Two groups have previously quantified selected FAEE in meconium and found significant correlation between FAEE levels and maternal report of gestational alcohol consumption (10–12) or self-reported tolerance for alcohol (13). Bearer et al (14) focused their research on ethyl linoleate as the preferred biomarker for prenatal alcohol exposure in meconium. We have confirmed a case of heavy prenatal alcohol exposure, which yielded high FAEE accumulation in meconium. Recently, Moore et al (15) reported that ethyl palmitate, oleate, stearate, and linoleate were significantly elevated in the meconium of neonates exposed to excessive amounts of alcohol in utero.

However, to apply meconium FAEE measurements as a clinical tool, one must first define its sensitivity and specificity. Presently, a

single or multiple maternal blood markers of problem drinking is not effective in the identification of the majority of obstetric cases complicated by addiction disorders because of numerous ethical and legal concerns, in addition to variable sensitivity and specificity associated with different screening methods (5).

reliable baseline and clearly defined positive cutoff have not been established for meconium FAEE. The origins of endogenous ethanol and FAEE are uncertain, as are potential altered physiologic conditions that may modify their metabolism. The objective of the current study is to investigate whether FAEE are present in the meconium of neonates without gestational alcohol exposure and to determine a positive cutoff for clinical practice.

METHODS AND MATERIALS

Subjects and Meconium Sample Collection

Two groups of women were recruited during pregnancy from the delivery wards of Mount Sinai Hospital (n = 104, Toronto, Canada) and Hadassah University Hospital (n = 103, Jerusalem, Israel). Women were excluded if they could not provide informed consent (verbal or written, as approved by either institution's Research Ethics Board). Two hundred fourteen neonates were born to these mothers. Meconium samples of at least 1g (wet weight) were available from 206 neonates (n = 102 Toronto, n = 104 Jerusalem) and stored frozen at –80°C until analysis. Six meconium samples (n = 3 Toronto, n = 3 Jerusalem) were excluded from subsequent analyses because of the failure to obtain a sufficiently clean extract for chromatographic analysis. Mothers admitting to social drinking during pregnancy (n = 15 Toronto, n = 2 Jerusalem) were separated from the rest of the cohort during data analysis. In addition, we analyzed meconium samples from six infants where there were confirmed reports of maternal alcohol abuse during pregnancy. Although alcohol use tends to be underreported in pregnancy, these six cases had been well documented with a long history of addiction to alcohol and had been confirmed by the referring agency or physician.

Maternal and Neonatal Characteristics

A questionnaire was administered verbally to all mothers either antepartum or postpartum to collect information regarding demographics, cigarette smoking and alcohol consumption during each of the three trimesters of pregnancy. Maternal age, gravidity, parity medical and obstetric history, infant birth weight, birth length, gestational age, head circumference, Apgar scores at 1 and 5 minutes, and perinatal complications were obtained from the hospital records.

Materials

All standards and solvents were obtained from Sigma (Toronto, Canada). Acetone and n-hexane were high-performance liquid chromatography (HPLC) grade or better. FAEE, including lauric acid (E12), myristic acid (E14), palmitic acid (E16:0), heptadecanoic acid (E17:0), stearic acid (E18:0), Oleic acid (E18:1), and linoleic acid (E18:2) ethyl esters, were diluted in hexane and stored at -20°C . Heptadecanoic acid (E17:0) ethyl ester in hexane was used as the internal standard. Silica-aminopropyl solid-phase extraction columns (BondElut) were obtained from Varian (Mississauga, Canada). Capillary GC column (ZB-WAX) was obtained from Phenomenex (Torrance, CA).

FAEE Analysis

Fatty acid ethyl esters were extracted from meconium according to a method modified from Bernhardt et al (16). Briefly, approximately 0.50g (wet weight) meconium was extracted by hexane:acetone (5.2, v/v). The two phases were separated via centrifugation at 3500 rpm for 15 minutes at 4°C , after which the hexane layer was removed and evaporated to dryness under a stream of nitrogen at 35°C . The residue was resuspended in 1mL hexane and loaded onto a silica-aminopropyl column previously conditioned with 1mL hexane. FAEE were eluted from the columns with 2mL hexane and evaporated to dryness. The residue was reconstituted with 50 μL hexane, and 2 μL were injected into a Varian 3400 gas chromatograph equipped with an 8200 autosampler and flame ionization detector (FID), with helium as the carrier gas at 1mL / min. A ZB-WAX

column (0.50 μm , 0.25 mm x 30 m) was used for the chromatographic separation of FAEE. The injector and detector were operated at 260°C and 300°C , respectively. The injection mode was splitless after 0.75 minutes, and the following temperature program was applied: 2 minutes at 50°C then $40^{\circ}\text{C}/\text{min}$ up to 215°C and hold for 15 minutes; finally, $30^{\circ}\text{C}/\text{min}$ up to 250°C and hold for 17.72 minutes, for a total run time of 40 minutes. The limit of detection (LOD) for the six FAEE ranged from 0.16 to 0.22 nmol/g (50 ng/g). and the limit of quantitation (LOQ) ranged from 0.32 to 0.44 nmol/g (100 ng/g). Meconium with FAEE levels detected between the LOD and LOQ were classified as containing trace levels. Results were expressed in nmol total FAEE/g meconium (i.e., sum of all six FAEE).

Statistical Analysis

Maternal and neonatal characteristics in the two populations were compared by Chi-squared test or Student's t-test wherever appropriate. Correlations between levels of FAEE and clinical variables were determined using Pearson correlation or Kendall's tau for nominal and categorical variables, respectively. The relationship between levels of FAEE stratified into three domains (below limit of detection, trace, above limit of quantitation), and clinical variables were analyzed by one-way repeated measures ANOVA. Analyses were performed using the SPSS software. Statistical tests were two tailed, and significance was defined as a *P* value of less than 0.05.

RESULTS

Baselin Population Without Gestational Alcohol Exposure

One hundred eighty-three meconium samples ($n = 84$ Toronto, $n = 99$ Jerusalem) were included in the baseline analysis (Table 1). In Toronto, total FAEE was below LOD in 7 (8.3%) and above LOQ in 27 (32.1%) meconium samples, respectively. Trace levels of FAEE were detected in the majority of samples (59.5%) in Jerusalem, total FAEE was below LOD in 15 (15.2%) and above LOQ in 46 (46.5%) meconium samples, respectively. Trace levels of FAEE were detected in 38 (38.4%)

samples. Myristic acid ethyl ester (E14) was most frequently detected in both populations (>80%), followed by lauric acid (E12) and palmitic acid (E16:0) ethyl esters (>50%). Stearic (E18:0), oleic (E18:1), and linoleic (E18:2) acid ethyl esters were undetectable in more than 80% of all samples in both populations. There were no significant differences in FAEE distribution between the two populations except for ethyl stearate, which was detected at higher concentrations in Jerusalem 1% above LOQ, 0.0 vs. 6.1). Significantly more Jerusalem samples had total FAEE detected above LOQ (32.1% vs. 46.5%, $P = 0.016$), at levels 1.5-fold greater compared with Toronto ($P = 0.053$). The corresponding mean total FAEE concentrations were 1.37 nmol/g (range, 0.26–5.25, 95%CI, 0.83, 1.91) in Toronto ($n = 27$) and 2.08 nmol/g (range, 0.34–10.2; 95% CI, 1.39, 2.77) in Jerusalem ($n = 46$).

Representative chromatograms from meconium samples with trace levels and elevated levels of E12 and E14 are shown in Figure 1A.

Baseline Population With Low Levels of Gestational Alcohol Exposure

Seventeen women reported drinking socially during pregnancy. The frequency of drinking ranged from I drink on an isolated occasion in the first trimester to I drink/mo itt each of the three trimesters (data not shown). As shown in Table 1, total FAEE was below LOD its three (17.6%) and above LOQ in six (35.3%) meconium samples. Trace levels were detected in eight (47.1%) samples. Myristic acid (E14) ethyl ester was most frequently detected (>80%), followed by lauric (E12) and palmitic (E16:0) acid ethyl esters (>40%). For samples with total FAEE levels above LOQ, the mean concentration was 0.42 nmol/g (range 0.32–1.40; 95%CI, 0.22, 0.46).

TABLE 1. Levels of FAEE detected in meconium samples among the different cohorts

FAEE*	Level	Social drinkers (n = 17)	Heavy drinkers (n = 6)	Toronto (n = 84)	Jerusalem (n = 99)	P
Lauric (E12)†	Below LOD	10 (58.8)	1 (16.7)	35 (41.7)	46 (46.5)	0.140
	Trace	5 (29.4)	1 (16.7)	38 (45.2)	32 (32.3)	
	Above LOQ	2 (11.8)	4 (66.7)	11 (13.1)	21 (21.2)	
Myristic (E14)†	Below LOD	3 (17.6)	3 (50.0)	7 (8.3)	17 (17.2)	0.156
	Trace	11 (64.7)	0 (0.0)	63 (75.0)	63 (63.6)	
	Above LOQ	3 (17.6)	3 (50.0)	14 (16.7)	19 (19.2)	
Palmitic (E16:0)†	Below LOD	9 (52.9)	0 (0.0)	35 (41.7)	52 (52.5)	0.149
	Trace	7 (41.2)	0 (0.0)	39 (46.4)	32 (32.3)	
	Above LOQ	1 (5.9)	6 (100.0)	10 (11.9)	15 (15.2)	
Stearic (E18:0)†	Below LOD	16 (94.1)	2 (33.33)	77 (91.7)	89 (89.9)	0.039
	Trace	1 (5.9)	0 (0.0)	7 (8.3)	4 (4.0)	
	Above LOQ	0 (0.0)	4 (66.7)	0 (0.0)	6 (6.1)	
Oleic (E18:1)†	Below LOD	17 (100.0)	2 (33.3)	82 (97.6)	94 (94.9)	0.497
	Trace	0 (0.0)	2 (33.3)	1 (1.2)	4 (4.0)	
	Above LOQ	0 (0.0)	2 (33.3)	1 (1.2)	1 (1.0)	
Linoleic (E18:2)†	Below LOD	17 (100.0)	3 (50.0)	79 (94.0)	96 (97.0)	0.455
	Trace	0 (0.0)	1 (16.7)	4 (4.8)	3 (3.0)	
	Above LOQ	0 (0.0)	2 (33.3)	1 (1.2)	0 (0.0)	
Total FAEE†	Below LOD	3 (17.6)	0 (0.0)	7 (8.3)	15 (15.2)	0.016
	Trace	8 (47.1)	0 (0.0)	50 (59.5)	38 (38.4)	
	Above LOQ	6 (35.3)	6 (100.0)	27 (32.1)	46 (46.5)	
Mean total [FAEE]‡	(nmol/g meconium (95% CI))	0.42 (0.22, 0.46)	11.08 (–0.14, 22.30)	1.37 (0.83, 1.91)	2.08 (1.39, 2.77)	0.053

* Total FAEE represents the sum of all FAEE measured.

† Student's two-tailed *t* test.

‡ Chi-square test.

Determination of Positive Cutoff

For the determination of a positive cutoff level in clinical practice, data from meconium samples containing cumulative FAEE levels greater than the LOQ were combined into one baseline population (n = 27, Toronto; n = 46, Jerusalem). Samples containing trace levels (between the analytical LOD and LOQ) of FAEE could not be included because such levels could not be accurately quantified. Also, data from six cases of confirmed maternal gestational alcohol abuse previously referred to our laboratory were included into the sensitivity and specificity calculations. The combined population mean total FAEE (n = 73) was 1.81 nmol/g (range, 0.26—10.21; 95%CI, 1.33, 2.30), FAEE distribution was similar to that of the individual populations as reported above. Among the confirmed alcoholic cases (n = 6), total FAEE was above LOQ in all meconium samples, and the corresponding mean was 11.08 nmol/g (range, 1.98—39.35; 95%CI, —0.14, 22.31; Table 1). In this population, palmitic acid (E16:0) and lauric acid (E12) ethyl ester were most frequently detected, in 100% and 80% of all samples, respectively. All other FAEE were detected to more than 50% of the samples. The chromatograms of a baseline meconium sample and an alcoholic sample are illustrated in Figure 1B. Figure 2 summarizes the distribution of various

FAEE detected in meconium samples among the different cohorts. A positive cutoff of 2 nmol/g, when lauric and myristic acid ethyl esters were excluded from the total FAEE sum, yielded the highest specificity (98.4%) and sensitivity (100%). The positive and negative predictive values were 62.5% and 100%.

Maternal and Neonatal Characteristics

Maternal and neonatal characteristics are summarized in Table 2. No significant differences in gestational age, delivery method, gender, Apgar-5, rates of perinatal complications, and birth defects were observed in the two populations. Mothers in Toronto were significantly older and had fewer births than mothers in Jerusalem. They were also more likely to smoke and use prenatal vitamins during pregnancy. While birth weight and head circumference were significantly greater among the Toronto neonates, Apgar score at 1 minute was significantly lower than the Jerusalem neonates. There was a significantly higher rate of neonatal distress and intrapartum meconium staining reported among the Toronto neonates.

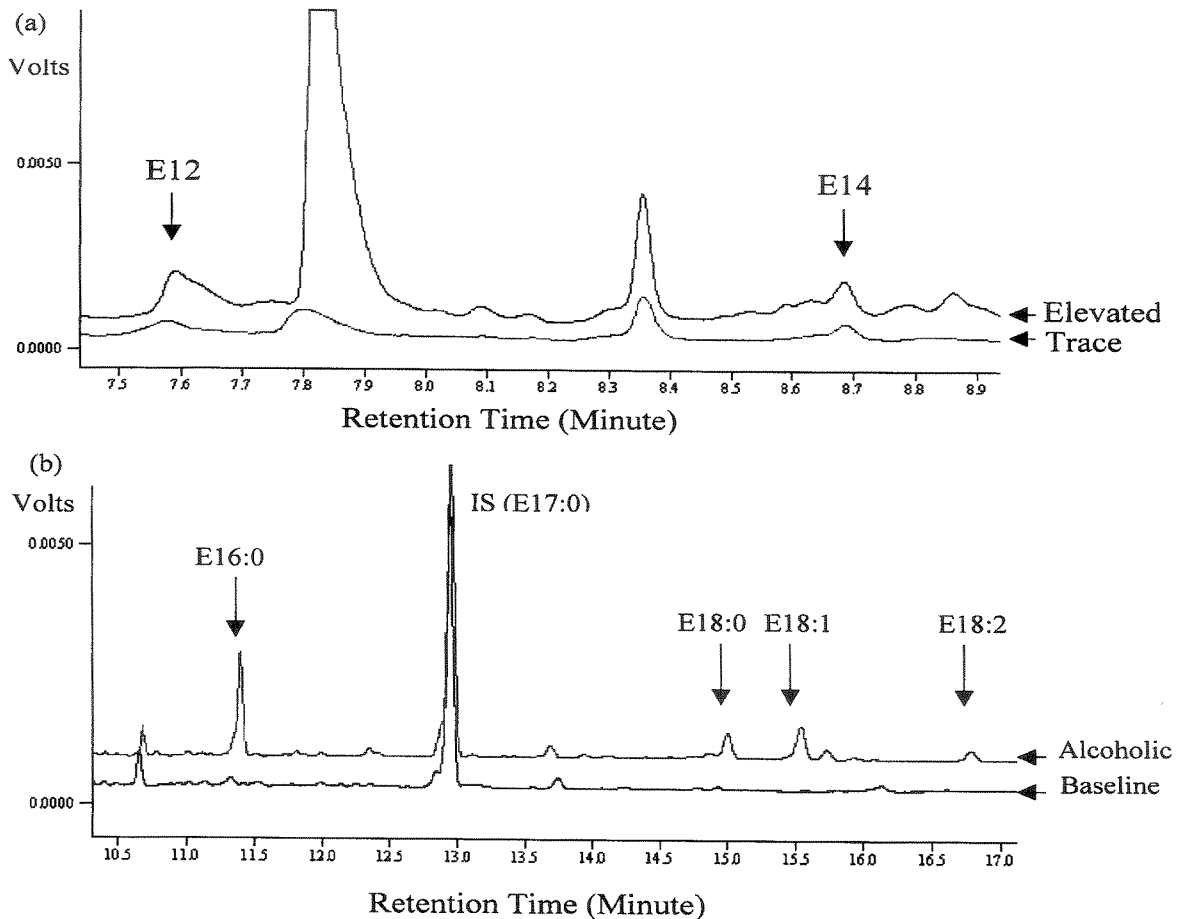


FIG. 1. Representative chromatograms from meconium samples (A) from the baseline cohort containing trace and elevated levels of E12 and E14 and (B) from a neonate born to an alcoholic mother and a neonate born to a nondrinking mother. The long chain FAEE (E16:0+) were detected at significantly higher levels in the alcoholic sample.

Relationship Between the Presence of FAEE and Clinical Variables

Ethyl myristate was found to increase with gestational age ($n = 32, r = 0.442, P = 0.011$), while ethyl palmitate decreased with advanced material age ($n = 25, r = -0.470, P = 0.018$). There was also a negative correlation between ethyl palmitate and birth weight ($n = 25, r = -0.404, P = 0.045$) and head circumference ($n = 24, r = -0.46, P = 0.023$). Prenatal vitamin use was associated with a reduction in total FAEE accumulation ($n = 181$; Chi-squared test = 12.33, $df = 2, P = 0.002$). In the Toronto cohort, there was an apparent association

between olive oil use by the mother and higher levels of total FAEE ($n = 84$, Chi-squared test = 8.43, $df = 2, P = 0.015$). FAEE accumulation was not associated with recurrent maternal microbial infection or gestational diabetes.

DISCUSSION

The FAEE meconium test holds substantial promise of becoming a screening test for heavy maternal drinking during pregnancy and, hence, for estimating the risk of

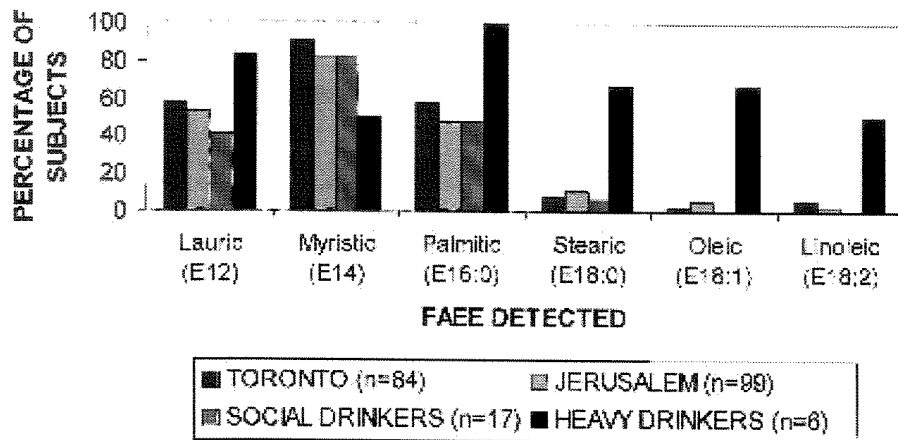


FIG. 2. Distribution of FAEE in meconium samples from (A) Toronto, (B) Jerusalem, (C) social drinkers, and (D) heavy drinkers. E12 and E14 were most frequently detected in the baseline cohorts and social drinkers. The longer chain FAEE (E16+) were detected mostly in meconium samples from neonates heavily exposed to alcohol in utero.

fetal alcohol spectrum disorder. However, it is critical to first define highly specific cutoff points that will separate true ethanol exposure from FAEE measurement in neonates born to nondrinking women. Our present study describes the first population baseline study for meconium FAEE conducted in neonates not exposed to alcohol. We found that FAEE are detected at low levels in the meconium of a substantial percentage of newborns without exogenous maternal alcohol consumption. On average, these basal levels were significantly lower (<six fold) than those measured in confirmed cases of heavy prenatal alcohol exposure (mean total FAEE, 1.82 nmol/g vs. 11.08 nmol/g). In addition, there were differences in the characteristic distribution of various FAEE species at baseline as

compared with alcohol exposed meconium. Whereas lauric (E12) and myristic (E14) acid ethyl esters predominated the baseline population, there was a trend toward increased presence of the longer chain FAEE (e.g., E16s and E18s) in the alcohol-exposed neonates. This is in agreement with data reported recently by Moore et al (15),

We have applied the above findings in proposing a positive cutoff level for the FAEE screening test in clinical practice. Bearer et al (12) did not report a cutoff value, but the presence of ethyl linoleate constituted a positive test. Moore et al (15) previously reported a LOQ and cutoff level of 50 ng total FAEE/g meconium. However, it was not justified in either study how these particular cutoffs were established and validated. In the current study

TABLE 2. Maternal and neonatal characteristics in Toronto and Jerusalem

Variable	Toronto (n = 84)	Jerusalem (n = 99)	P
Maternal age (yr) [‡]	32.31 ± 5.25	29.81 ± 5.44	0.002
Gravidity [†] (%)			
1	29 (34.5)	19 (19.2)	0.082
2	22 (26.2)	32 (32.3)	
>3	33 (39.3)	48 (48.5)	
Parity [†] (%)			
1	42 (50)	25 (25.3)	0.001
2	25 (29.8)	30 (30.3)	
>3	17 (20.2)	44 (44.5)	
Smoking [†] (%)			
Yes	13 (15.5)	6 (6.1)	0.037
No	71 (84.5)	93 (93.9)	
Vitamin use [†] (%)			
Yes	71 (85.5)	12 (12.2)	<0.001
No	12 (14.5)	86 (87.8)	
Gestational age (wk) [*]	39.43 ± 1.31	39.44 ± 1.34	0.964
Birth weight (g) [*]	3510.08 ± 547.89	3209.61 ± 474.56	<0.001
Head circumference (cm) [*]	35.02 ± 1.11	34.45 ± 1.34	0.006
Delivery [†] (%)			
Vaginal	60 (71.4)	80 (81.6)	0.103
Caesarean	24 (28.6)	18 (18.4)	
Gender [†] (%)			
Male	46 (54.8)	40 (41.2)	0.069
Female	38 (45.2)	57 (58.8)	
Apgar 1 [†] (%)			
0-4	2 (2.4)	0 (0.0)	0.029
5-7	6 (7.1)	1 (1.0)	
8-10	76 (90.5)	97 (99.0)	
Apgar 5 [†] (%)			
0-4	1 (1.2)	0 (0.0)	0.556
5-7	1 (1.2)	1 (1.0)	
8-10	82 (97.6)	96 (99.0)	
Perinatal complications [†] (%)			
Yes	20 (23.8)	16 (16.2)	0.195
No	64 (76.2)	83 (83.8)	
Neonatal distress [†] (%)			
Yes	15 (17.9)	1 (1.0)	<0.001
No	69 (82.1)	98 (99.0)	
Intrapartum meconium [†] (%)			
Yes	18 (21.4)	10 (10.1)	0.034
No	66 (78.6)	89 (89.9)	
Defects [†] (%)			
Yes	3 (3.6)	6 (6.1)	0.438
No	81 (96.4)	93 (93.9)	

* Student's two-tailed *t* test.

† Chi-square test.

when the cutoff was equivalent to the LOD (i.e., assuming that FAEE do not exist in the meconium of newborns not exposed to alcohol, thus, their presence constituted a positive screen), specificity of the FAEE test was as low as 12%. This indicates that many babies of nondrinking mothers have positive readings of some FAEE. When the cutoff level was increased at intervals from the LOD to 2 nmol/g (i.e., the lowest level of total FAEE measured in neonates born to confirmed drinkers in our laboratory), specificity improved from 12% to 91.8%. As discussed previously, ethyl laurate and myristate predominated the baseline population. Therefore, we repeated the calculation of specificity excluding these two FAEE. This led to significant improvement in specificity from 45.9% to 98.4%, suggesting that these particular esters contribute

mostly to the background noise in the baseline population. A subanalysis was done to compare the levels of these two esters between the baseline and drinking population, and they were not significantly different (data not shown). However, due to the small sample size of true positive cases, this finding needs to be verified in larger numbers of neonates with known prenatal alcohol exposure.

Since social drinking is an essential part of the North American culture, a second baseline population was chosen by us to serve as a negative control. A population from a teaching hospital in Jerusalem was chosen because of the known low prevalence of gestational alcohol consumption. There were no significant differences between the mean total FAEE levels detected in Toronto and Jerusalem. In addition, the characteristic FAEE profile was similar in both populations, with ethyl laurate and myristate being the predominant species detected. The remarkable similarity between these two baselines suggests that there was probably minimal dietary and genetic interference in endogenous FAEE metabolism of fetuses not exposed to alcohol.

The origins of endogenous ethanol and FAEE are not well understood. Ethanol is a normal fermentation product of the gut's microflora, via anaerobic breakdown of carbohydrates in the large intestine. Nonmicrobial pathways have also been proposed, which include the final steps of carbohydrate metabolism, during which pyruvic acid is decarboxylated to acetaldehyde and then reduced further to ethanol by alcohol dehydrogenase (17). Endogenous ethanol production as a result of diet is unlikely, although it has been previously shown that endogenous methanol concentration increases after consumption of fruit and pure pectin (18). Recently, Perez-Camino et al (19) found that varying concentrations of fatty acid alkyl esters, the majority of which are ethyl and methyl esters, naturally exist in different types of olive oils. The concentrations of these esters were higher in altered olive fruit, olive pomace, and lampant oil (poor quality olive oil) compared with extra virgin olive oil. While olive oil was the most popular cooking oil in the Toronto cohort, it might have contributed to the basal levels of FAEE present in the meconium of neonates not exposed to alcohol in utero.

It is known that the anaerobic metabolism of certain yeasts and bacteria, such as *Candida* and

Saccharomyces. leads to the production of ethanol (20). Therefore, chronic maternal microbial infections can theoretically lead to an enhanced endogenous ethanol pool and, hence, greater production and accumulation of FAEE in neonates born to nondrinking mothers. However, no correlation was found between microbial infection and FAEE in our study cohorts because of small sample size. In theory, endogenous ethanol levels may also be higher with abnormal physiologic conditions such as metabolic disorders. However, similar endogenous ethanol levels have been detected in abstinent alcoholic patients, healthy persons, and those suffering from diabetes, hepatitis, and cirrhosis (21). Gestational diabetes is associated with alterations in fatty acid composition in maternal erythrocytes, placenta, and umbilical cord (22). Gestational diabetes was not associated with the accumulation of FAEE in our study cohorts; however, it may also be due to the small sample size. Whether these changes in lipid composition contribute to the metabolism of endogenous FAEE needs to be investigated in the future. It remains unclear whether a specific mechanism exists for the preferential accumulation of ethyl laurate and myristate in the meconium of neonates not exposed to alcohol. It seems unlikely that minor structural differences of various FAEE and enzymatic specificity would play a significant role in conveying such preferential accumulation. Moreover, C12 and C14 constitute only a minor fraction of all free fatty acids in maternal and cord plasma (<3%) (23), placenta (<1%) (22), and meconium (<3%) (24). The reason why ethanol preferentially conjugates with these acids and not the more abundant species remains unknown.

Myristic acid in maternal plasma increases throughout pregnancy, reaching its peak levels in the second trimester and sustains at this level until term (25). The correlation between ethyl myristate and gestational age coincides with this increase in myristic acid concentration. The negative correlation between ethyl palmitate and neonatal parameters (birth weight and head circumference) may be related to altered fatty acid metabolism in smaller fetuses, although it has been reported that levels of palmitic acid in maternal and cord plasma were not significantly different in IUGR and normal pregnancies (26). The most common saturated fatty acids found in natural fats are myristic (coconut oil), palmitic (animal/vegetable fat), and

stearic acids (animal/vegetable fat) (27). The negative correlation between ethyl palmitate and advanced maternal age may be explained by changes in dietary habits. The apparent association between olive oil (rich in oleic acid) use by the mother and higher levels of total FAEE may be related to the naturally existing FAEE is discussed above (19). The role of prenatal vitamins on FAEE metabolism is not known at this point.

It can be concluded that certain FAEE are present at basal levels in the meconium of neonates not exposed to alcohol. Although the mechanisms leading to this accumulation of FAEE without exogenous alcohol consumption remain to be investigated, the establishment of a baseline with a reliable positive screening cutoff is critical to the identification of prenatal alcohol exposure in clinical practice.

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HAIR AND MECONIUM TESTING FOR *IN UTERO* EXPOSURE TO ALCOHOL

The Hospital for Sick Children
Motherisk-Division of Clinical Pharmacology

BACKGROUND

Alcohol use during pregnancy is a significant public health problem. Approximately 60% of adult women in the USA report drinking at least occasionally and 20% of these women continue to drink while pregnant. Although the majority of women stops drinking during pregnancy, or at least reduce their drinking, there is a large number of pregnant women who continue drinking, and in doing so, knowingly or sometimes unknowingly damage the fetus. Presently, alcohol is the most widely used human teratogen. Fetal alcohol syndrome (FAS) is the most severe of fetal alcohol related abnormalities (FARA) which may be seen in the offspring of women who continue drinking heavily throughout during pregnancy.

As with any other drugs of abuse, the ascertainment of gestational exposure to alcohol is of utmost importance for the diagnosis of FARA. The most common methods for detection of alcohol use rely on maternal reports. However, it is widely recognized that history is likely to underestimate the true amount of alcohol consumed. At present time, physicians use a combination of 4 maternal blood markers of alcohol use in detecting alcohol-abusing pregnant women yet many cases are missed. A direct test in the newborn would potentially yield more information about the magnitude of true gestational exposure to alcohol which, in turn, could be used in the clinical diagnosis of the child.

In February 1999, the Motherisk program launched a new, toll-free Alcohol **and Substance Abuse Helpline (1-877-3274636)** which provides fact-based information for all Canadian women during and after pregnancy. An important aspect of this service is the possibility of hair and meconium testing for drugs of abuse including alcohol.

What procedures are used?

Hair: a minimum of 10mg. Of hair should be collected from a few different sites by cutting the hair as close to the scalp as possible. All the hair should be dropped in a small, paper envelope that should be mailed to Motherisk.

Meconium : a minimum of 1 gram should be collected in clean, appropriately labeled small plastic bottles(urine collection bottle) and kept frozen. The sample should be sent as soon as possible, on dry ice, to the following address:

MOTHERISK
Attn: Julia Klein
Hospital for Sick Children
Toronto, Ontario
M5G 1X8

If the first meconium sample is collected on the weekend, it is best to freeze it and send it as soon as possible on Monday morning.

Attached to the hair and/or meconium sample, should be a letter stating why is alcohol use suspected. Also, **it should be clearly indicated** were should we send the results and the invoice.

Once we receive the sample, the alcohol adducts (fatty acid ethyl esters) are extracted from the hair and/or meconium and analyzed using a gas chromatographic method.

How long does it take ?

It takes two days to complete the analysis of a single hair or meconium sample. However, samples are tested in batches. Depending on the number of samples received, a new batch may be tested once every week, or two weeks.

What are the advantages of meconium testing?

Since meconium starts forming during the second trimester of pregnancy, it provides information about the second and third trimester alcohol exposure.

What are the advantages of hair testing?

Hair starts to form at the end of the second trimester of pregnancy; therefore it provides information about alcohol exposure in the last trimester. Unlike meconium, neonatal hair is available for up to three months after birth.

What are the limitations of testing for alcohol adducts in neonatal hair and meconium?

It can not pinpoint to a certain time (day, week or month) during the pregnancy when the drinking occurred, and at present time we don't know the correlation between the number of drinks the mother had and the concentration of FAEE in hair or meconium.

What does it cost?

Fees may vary according to the number of samples submitted, For information please contact FASINFO at 1-877-327-4636 or:

For details, contact

Julia Klein, Director, Fetal Toxicology Lab

Phone: 416-813-5780

Fax: 416-813-5189

Email jklein@sickkids.on.ca



MOTHERISK

Population Baseline of Meconium Fatty Acid Ethyl Esters Among Infants of Nondrinking Women in Jerusalem and Toronto

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Summary: The detection of fatty acid ethyl esters (FAEE) in meconium may provide an objective estimate of prenatal alcohol exposure independent of maternal history.

The authors report the results of the first population-based study conducted to investigate basal FAEE levels in the meconium of neonates not exposed to alcohol. Two hundred seven nondrinking women and their neonates were recruited from Toronto and Jerusalem. FAEE were extracted from meconium by solid-phase extraction and analyzed by GC/FID. Similar procedures were conducted in six neonates both to confirmed heavy drinkers. Low levels of meconium FAEE were detected from both cohorts (mean, 137 nmol/g vs. 2.08 nmol/g, Toronto vs. Jerusalem). Ethyl stearate, oleate, and linoleate were below the limit of detection in >80% of all samples, whereas ethyl laurate and palmitate were detected in >50% of the samples. Ethyl myristate was the FAEE most commonly detected (80%). All six meconium samples with confirmed maternal drinking histories tested positive for FAEE at significantly higher levels (mean, 11.08 nmol/g). The use of 2 nmol total FAEE/g meconium as the positive cutoff, when lauric and myristic acid ethyl esters were excluded, yielded the greatest sensitivity (100%) and specificity (98.4%). The authors conclude that certain FAEE are present at measurable levels in the meconium of neonates not exposed to maternal drinking, and correction is needed to allow high specificity. **Key Words:** Baseline—Ethanol—Fatty acid ethyl esters—Meconium—Pregnancy.

Fetal alcohol spectrum disorders is the range of outcomes resulting from maternal alcohol abuse during pregnancy. Although there is no established, safe drinking level during pregnancy, 20% of pregnant women continue to consume some alcohol at lower levels before they realize they have conceived, while 4% will continue to drink heavily throughout pregnancy (1). Presently, full-blown FAS occurs at an estimated rate of

4.3% among heavy drinkers (2), and the whole spectrum of alcohol-related damages can be as high as 1% (i.e., 9.1 per 1000 live births) in the general pediatric population (3). To date, detection of prenatal alcohol exposure is almost entirely dependent on maternal self-reporting which may be affected by denial or underreporting (4).

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Once we receive the sample, the alcohol adducts (fatty acid ethyl esters) are extracted from the hair and/or meconium and analyzed using a gas chromatographic method. Final calculations are based upon established standards