

Fetal Exposure to Alcohol as Evidenced by Fatty Acid Ethyl Esters in Meconium in the Absence of Maternal Drinking History in Pregnancy

Daphne Chan, BSc,† Julia Klein, MSc,* Tatyana Karaskov, MD,* and Gideon Koren, MD*†*

Abstract: The detection of fatty acid ethyl esters (FAEE) in neonatal meconium has been proposed as a novel screening method for intrauterine exposure to alcohol. We investigated the potential use of meconium FAEE screening in a high-risk neonatal population in the absence of maternal drinking history. One hundred forty-two meconium samples of neonates suspected of intrauterine illicit substance exposure and referred to the Motherisk Laboratory were analyzed for the existence of drugs by enzyme-linked immunosorbent assay (ELISA) and FAEE by gas chromatography-flame ionization detection (GC-FID). A positive FAEE test was previously defined as a cumulative measurement of 7 individual FAEE \geq 2 nmol/g. Seventy-one percent of the samples tested positive for at least 1 illicit drug, with cannabis being the most prevalent (52.3%). Fourteen percent of all samples tested positive for prenatal alcohol exposure, as evidenced by cumulative meconium FAEE \geq 2 nmol/g. Ethyl oleate, linoleate, palmitate, and arachidonate were detected most often and at the highest levels. At least 3 individual FAEE were detected in 95% of all positive samples, and none could be identified by the use of 1 selected FAEE. Significantly elevated levels of FAEE above the baseline and the presence of multiple FAEE species in meconium are exclusive to neonates who have likely been exposed to excessive amounts of alcohol in utero. Babies born to mothers who are suspected to use illicit drugs in pregnancy are at elevated risk for exposure also to alcohol in utero. Meconium FAEE are emerging biologic markers that can potentially facilitate earlier diagnosis and intervention for less apparent forms of alcohol-related disabilities that cannot be confirmed in the absence of maternal drinking history.

Key Words: Alcohol, Fatty acid ethyl esters, Pregnancy, Meconium
(*Ther Drug Monit* 2004;26:474–481)

According to recent North American surveys, approximately 50% of women of reproductive age admitted to drinking regularly,^{1,2} and 12% to 16% of women reported using any amount of alcohol during pregnancy.^{2–4} Although the rate of any alcohol use during pregnancy has declined since 1995, the rates of binge drinking (ie, more than 5 drinks per occasion) and frequent drinking (ie, more than 7 drinks per week or binge drinking) remained unchanged among both pregnant women and nonpregnant women of reproductive age.^{2,3} Because most pregnancies are unplanned, many fetuses may be inadvertently exposed to excessive levels of ethanol in utero.

The adverse fetal effects of ethanol were first documented by Lemoine and colleagues in 1967.⁵ In 1973, Jones and Smith coined the term Fetal Alcohol Syndrome (FAS) to describe the extreme phenotypic expression of heavy prenatal alcohol exposure.⁶ It is now estimated that between 2 and 9 out of 1000 live births (~1%) are adversely affected by alcohol embryopathy, making it the most prevalent human teratogen.⁷ The estimated rates of FAS in the American population have been reported by the Centers for Disease Control and Prevention in 5 states (Alaska, Arizona, Colorado, New York, and Wisconsin) to range between 0.3 and 1.5 per 1000 over the period 1995 to 1997.⁸ Although the threshold of ethanol teratogenesis has not been clearly defined, virtually all cases of FAS described to date are among offspring of alcohol-dependent women.⁹ Because of shame, guilt, and fear of losing custody of the child, alcohol-dependent women often choose not to disclose the nature of their drinking patterns. This precludes the diagnosis of Fetal Alcohol Spectrum Disorder (FASD)¹⁰ in many cases, particularly Alcohol-Related Neurodevelopmental Disorder (ARND),⁷ because of the lack of pathognomonic presentation at birth and early infancy. In the absence of confirmed maternal drinking history, many affected neonates can not be diagnosed properly and receive appropri-

Received for publication March 11, 2004; accepted June 2, 2004.

From *The Motherisk Program, Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, Toronto, Ontario, Canada; and the †Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada.

Abbreviations: Confidence interval (CI), ethyl laurate (E12), ethyl myristate (E14), ethyl palmitate (E16), ethyl palmitoleate (E16:1), ethyl heptadecanoate (E17), ethyl stearate (E18), ethyl oleate (E18:1), ethyl linoleate (E18:2), ethyl linolenate (E18:3), ethyl arachidonate (E20:4), fatty acid ethyl esters (FAEE), fetal alcohol spectrum disorder (FASD), fetal alcohol syndrome (FAS), gas chromatography-flame ionization (GC-FID).

Address correspondence and reprint requests to Gideon Koren, MD, FABMT, FRCPC, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada (e-mail: gkoren@sickkids.ca).

Copyright © 2004 by Lippincott Williams & Wilkins

ate treatment until later on in childhood, further increasing their risk for additional secondary disabilities.¹¹

Measurement of ethanol itself does not provide evidence of long-term fetal exposure because of its rapid elimination *in vivo*, and there is currently no other validated biological marker for fetal exposure to alcohol. Fatty acid ethyl esters (FAEE) are nonoxidative metabolites of ethanol that have been proposed as biological markers of alcohol consumption in adults.¹² Earlier studies on the potential use of meconium FAEE measurements in neonates with intrauterine ethanol exposure^{13–15} have been confirmed by recent reports in different study populations,^{16–20} ranking FAEE among emerging biomarkers of fetal exposure to alcohol.

We have recently established the first population baseline measurements of meconium FAEE among offspring of nondrinking women.¹⁹ In comparison to neonates born to mothers who drank heavily during pregnancy (ranged from 2 drinks per day to binge drinking 3 times per week), babies without prenatal alcohol exposure had significantly lower (at least 10-fold) cumulative levels of meconium FAEE (ie, the sum of all FAEE measured). Ethyl laurate (E12) and myristate (E14) were the predominant FAEE found in baseline meconium of neonates not exposed to alcohol. Neonates born to mothers who drank socially (ranged from 1 drink on an isolated occasion in the first trimester to 1 drink per month throughout pregnancy) were indistinguishable from the baseline cohort on the basis of meconium FAEE measurements. In contrast, neonates with heavy prenatal alcohol exposure accumulated significantly higher levels of longer-chain FAEE [ethyl palmitate (E16), stearate (E18), oleate (E18:1), and linoleate (E18:2)] in addition to those (E12 and E14) that were found in the baseline cohort. The mechanisms leading to the accumulation of selected meconium FAEE at low levels in the absence of gestational alcohol consumption remain to be investigated; however, it may be the combined result of diet and other physiological processes. A positive cutoff of cumulative meconium FAEE at 2 nmol/g (~600 ng/g), excluding E12 and E14, was established with 100% sensitivity and 98.4% specificity in this population. Although a sensitivity threshold for various levels of gestational alcohol consumption has not been clearly defined, the current positive cutoff accurately distinguishes between neonates born to nondrinkers and offspring of heavy drinkers.

The ultimate goal of an objective meconium FAEE screening test is to confirm prenatal alcohol exposure in the absence of maternal drinking history, which would in turn facilitate earlier diagnosis of FASD and the initiation of appropriate treatment and intervention. The potential use of meconium FAEE screening as a tool to identify at risk neonates with minimal maternal drinking history was investigated in the current study. The primary objective of the present study was to quantify the prevalence of prenatal alcohol exposure, as evidenced by positive meconium FAEE screening, among neo-

nates born to women suspected or proven to use other drugs of abuse. This population was chosen because of the known association between maternal alcohol and illicit substance use.²¹ The secondary objective of this study was to characterize the distribution and cumulative level of meconium FAEE in comparison to those reported in the literature.

METHODS

Subjects

The Motherisk Program at the Hospital for Sick Children is a counseling service for exposure to drugs, chemicals, radiation, and infections during pregnancy and lactation. Hundreds of samples are referred to our laboratory each year for drug testing to rule out or confirm prenatal exposures that are suspected clinically. The suspicion of maternal use of illicit substances and clinical histories comes typically from information gathered by Children's Aid Societies, clinicians, or other health care providers and community services caring for the mother or baby. The Research Ethics Board at the Hospital for Sick Children approves alcohol (FAEE) screening in meconium samples referred to our laboratory for routine illicit drug testing as part of a protocol (HSC2000/032) that investigates the potential use of meconium FAEE screening in suspected cases of prenatal alcohol exposure. For the purpose of the present analysis we quantified FAEE in meconium samples referred to our laboratory for the determination of prenatal exposure to illicit substances from March 2000 to March 2003. A total of 385 meconium samples were received for routine drug screening (ie, cannabis, cocaine, and opiates) during this period. Samples that retained a sufficient sample size (ie, at least 0.5 g wet weight) after drug screening were analyzed for FAEE (n = 142).

Analytic Methods

Meconium samples were collected and shipped frozen to the laboratory and aliquoted for illicit drug and FAEE screening. Nine individual FAEE (E12, E14, E16, E16:1, E18, E18:1, E18:2, E18:3, E20:4) were extracted and analyzed by gas chromatography with flame ionization detection (GC-FID) using a previously established method.¹⁹ A positive test is defined by a cumulative meconium FAEE measurement (ie, the cumulative sum of 7 individual FAEE) greater than 2 nmol/g (~600 ng/g) excluding E12 and E14. The limit of detection (LOD) of the validated assay is 0.16 to 0.22 nmol/g (~50 ng/g), and the limit of quantification (LOQ) is 0.32 to 0.44 nmol/g (~100 ng/g) for the individual FAEE. Illicit drugs of abuse including cannabis, cocaine and its metabolite benzoylecgonine, and opiates were quantified by enzyme-linked immunosorbent assay (ELISA) using established methods.²² The limit of detection for each drug is 50 ng/g when 0.2 g of meconium is used for testing. Samples were confirmed at random by gas chromatography-mass spectrometry (GC-MS) for validation purposes.

Materials

All standards and solvents were obtained from Sigma (Toronto, ON). Acetone and hexane were HPLC grade or better. Nine different FAEE, including ethyl laurate (E12), myristate (E14), palmitate (E16:0), palmitoleate (E16:1), heptadecanoate (E17:0), stearate (E18:0), oleate (E18:1), linoleate (E18:2), linolenate (E18:3), and arachidonate (E20:4), were diluted in hexane and stored at -20°C . Ethyl heptadecanoate (E17:0) in hexane was used as the internal standard. Silica-aminopropyl solid-phase extraction columns (BondElut®) were obtained from Varian (Mississauga, ON). Capillary GC column (ZB-WAX) was obtained from Phenomenex (Torrance, CA). Enzyme-linked immunosorbent assay (ELISA) kits for the analysis of cannabis, cocaine (benzoylecgonine), and opiates were obtained from Immulysis (Pomona, CA).

RESULTS

Prenatal Exposure to Illicit Substances

A total of 142 meconium samples of neonates born to women suspected of illicit drug abuse were analyzed for cannabis ($n = 130$), cocaine and benzoylecgonine ($n = 141$), and opiates ($n = 125$) (Table 1). Cannabis was the most common illicit drug detected in this cohort (52.3%, $n = 68$), followed by cocaine (27%, $n = 38$) and opiates (16%, $n = 20$). Seventy-one percent of all samples tested ($n = 101$) were positive for at least 1 illicit substance, whereas 29% ($n = 41$) of suspected samples tested negative.

Maternal and Neonatal History

Brief maternal and neonatal histories were available for only a minority of cases ($< 40\%$), and there was very limited information regarding prenatal exposure to illicit drugs and alcohol. Of the 142 meconium samples tested, only 18 had a history of maternal alcohol use that was indicated on an item-

TABLE 1. The Prevalence of Illicit Drug Use During Pregnancy

Screening Test (Total Cohort = 142)*	Positive	% of Samples Tested
Positive for at least 1 illicit drug	101	71.1
Cannabis ($n = 130$)	68	52.3
Cocaine or benzoylecgonine ($n = 141$)	38	27.0
Opiates ($n = 125$)	20	16.0
Negative for illicit drug	41	28.9
FAEE	20	14.1
Positive for illicit drug ($n = 101$)	12	11.9
Negative for illicit drug ($n = 41$)	8	19.5

*Most samples were assayed for multiple drugs of abuse.

TABLE 2. Meconium Samples that Tested Positive for Heavy Prenatal Alcohol Exposure as Evidenced by FAEE Screening

FAEE Positive Samples ($n = 20$)	Positive	% of Samples Tested
Positive for ≥ 1 illicit drugs	12	60.0
Cannabis	7	35.0
Cocaine or benzoylecgonine	4	20.0
Opiates	3	15.0

ized checklist as part of the hospital's admission record on arrival for delivery. It was not stated whether maternal alcohol use was present in the current or previous pregnancy, as well as the duration (eg, trimester of use or throughout pregnancy), extent (eg, consumption per occasion), and pattern of use (eg, daily, occasional binges). Gestational alcohol consumption history was not available for the rest of the samples.

Prenatal Exposure to Alcohol as Evidenced by Meconium FAEE

Among the 101 cases that tested positive for illicit drugs, 12 (11.9%) tested positive for FAEE, whereas among the 41 samples that tested negative for drugs of abuse, 8 tested positive for FAEE (19.5%) (Table 1). The most prevalent illicit substance detected among FAEE-positive samples was cannabis ($n = 7$), followed by cocaine ($n = 4$) and opiates ($n = 3$) (Table 2). There were no significant differences in the distribution of the various illicit drugs between positive and negative FAEE samples (Table 3). In 17 (12%) of the 142 meconium samples, FAEE were detected, but their cumulative levels (excluding E12 and E14) measured below the positive cutoff and therefore were referred to as "borderline positive." Among the 18 meconium samples accompanied by maternal history, only 5 (27.8%) tested positive for FAEE, and 4 (22.2%) were borderline positive. Overall, 20 of the 142 meconium samples were positive for FAEE (14.1%) (Table 4).

Distribution of Meconium FAEE

The rank order of FAEE detected in positive samples (from most to least prevalent) was E16 > E18:2 and E20:4 >

TABLE 3. The Prevalence of Illicit Drug Exposure Among Samples that Tested Positive or Negative for FAEE

Illicit Drug	FAEE Positive ($n = 20$)		FAEE Negative ($n = 122$)		P
	No. Tested	Positive (%)	No. Tested	Positive (%)	
Cannabis	18	7 (38.9%)	112	61 (54.5%)	NS
Cocaine	20	4 (20.0%)	121	34 (28.1%)	NS
Opiates	16	3 (18.8%)	109	17 (12.9%)	NS

TABLE 4. The Distribution of FAEE Among 20 Positive Meconium Samples (ie, Cumulative Meconium FAEE^a Levels (Excluding E12 & E14) Measured Above the 2 nmol/g Cutoff)

Subject No.	Maternal History	Laurate (E12)	Myristate (E14)	Palmi-tate (E16)	Palmi-toleate (E16:1)	Stearate (E18)	Oleate (E18:1)	Linoleate (E18:2)	Linolenate (E18:3)	Arachi-donate (E20:4)	Total FAEE ^b	Total FAEE (Excluding E12 and E14) ^c
20	No	0.00	0.82	2.95	1.02	0.56	16.40	5.66	0.63	2.42	30.46	29.64
22	No	5.42	2.06	6.74	0.96	0.81	19.61	9.28	0.90	9.31	55.09	47.61
27	No	0.00	0.00	6.46	0.72	1.81	0.87	T	0.00	1.02	10.88	10.88
33	No	0.00	0.00	0.00	0.00	1.44	5.83	6.94	1.25	0.35	15.80	15.80
41	No	0.00	0.00	0.43	0.00	0.00	0.00	1.08	0.00	1.76	3.27	3.27
43	Yes	0.00	0.82	0.85	0.78	0.20	16.31	8.99	1.22	1.46	30.63	29.81
44	No	0.00	0.00	8.23	0.00	0.00	0.00	0.81	0.00	0.00	9.04	9.04
45	No	3.17	1.62	3.02	0.47	1.07	12.60	12.83	T	1.42	36.19	31.40
46	Yes	0.00	0.00	0.70	0.00	0.00	0.83	1.46	0.00	4.06	7.06	7.06
51	Yes	10.52	2.33	2.42	1.30	T	26.33	15.89	2.08	10.55	71.42	58.56
58	No	0.00	0.00	0.54	0.00	0.36	0.00	0.24	0.65	0.43	2.23	2.23
63	No	2.35	1.48	1.76	0.46	0.27	42.23	30.69	3.34	1.34	83.92	80.10
70	Yes	0.00	0.00	1.92	0.00	0.00	0.50	10.96	0.00	26.43	39.81	39.81
71	No	0.00	0.00	0.95	0.43	0.00	2.38	0.98	0.34	0.40	5.48	5.48
73	No	157.40	71.22	98.26	4.02	0.00	0.00	0.00	13.83	18.16	362.89	134.27
74	No	0.00	0.00	0.80	0.00	0.44	0.00	3.90	0.00	13.45	18.60	18.60
75	Yes	0.00	0.00	0.46	0.00	T	0.37	4.26	T	0.68	5.77	5.77
83	No	0.00	0.00	1.78	0.00	0.00	1.51	0.00	0.00	3.82	7.11	7.11
87	No	0.00	0.00	0.43	0.00	0.00	2.09	0.38	0.00	0.32	3.22	3.22
123	No	0.00	0.00	2.73	1.11	0.00	7.41	3.29	T	0.00	14.54	14.54
Mean		8.94	4.02	7.07	0.56	0.39	7.76	6.19	1.43	4.87	40.67	27.71
SD		35.04	15.84	21.59	0.93	0.55	11.36	7.63	3.33	7.19	79.39	32.70
95% CI		(-6.41, 24.30)	(-2.92, 10.96)	(-2.39, 16.53)	(0.16, 0.97)	(0.14, 0.63)	(2.78, 12.74)	(2.85, 9.53)	(-0.03, 2.88)	(1.72, 8.02)	(5.88, 75.46)	(13.38, 42.04)
Range		0-157.40	0-71.22	0-98.26	0-4.02	0-1.81	0-42.23	0-30.69	0-13.83	0-26.43	2.23-362.89	2.23-134.30
Below LOD (%)		15 (75.0)	13 (65.0)	1 (5.0)	10 (50.0)	9 (45.0)	5 (25.0)	2 (10.0)	8 (40.0)	2 (10.0)	0 (0.0)	0 (0.0)
Trace (%)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (10.0)	0 (0.0)	1 (5.0)	3 (15.0)	0 (0.0)	0 (0.0)	0 (0.0)
Above LOQ (%)		5 (25.0)	7 (35.0)	19 (95.0)	10 (50.0)	9 (45.0)	15 (75.0)	17 (85.0)	9 (60.0)	18 (90.0)	20 (100.00)	20 (100.00)

^aFAEE expressed in nmol/g meconium (wet weight).

^bTotal FAEE is the cumulative sum of 9 individual FAEE.

^cTotal FAEE (excluding E12 and E14) is the cumulative sum of the remaining 7 individual FAEE.

E18:1 > E18:3 > E18 > E16:1 (Fig. 1A). The majority of positive meconium samples (95%) contained at least 2 FAEE, and 85% of them tested positive for 4 FAEE or more (Table 5). None of the positive samples can be identified by the use of a single FAEE. In contrast, only 6% of borderline-positive samples and 28% of meconium samples accompanied by maternal drinking history contained 4 FAEE or more. In positive samples, cumulative FAEE concentrations (excluding E12 and E14) ranged from 2.2 nmol/g (685 ng/g) to 134.3 nmol/g (39,367 ng/g) [mean 27.7 nmol/g (8495 ng/g), 95% confidence interval (CI) 13.4 to 42.0 nmol/g (4,212 to 12,779 ng/g)]. Ethyl oleate (E18:1) was detected at the highest concentrations among positive samples [mean 7.8 nmol/g (2410 ng/g), 95% CI 2.8 to 12.7 nmol/g (864 to 3956 ng/g)]. Large interindividual variability was observed in the mean levels of E16, E18:1, E18:2, and E20:4 measured among positive samples (Fig. 1B).

The mean levels of individual and cumulative FAEE for the positive samples, borderline positive samples, and meconium samples that were accompanied by maternal history were summarized in Table 6.

DISCUSSION

The potential use of the meconium FAEE screening test as an objective tool to identify prenatal alcohol exposure among high-risk neonates in the absence of maternal drinking history was investigated in the present study.

Our study population consisted of neonates born to mothers suspected of illicit drug use during pregnancy, and meconium samples were referred to our laboratory for routine drug testing. The meconium test corroborated the clinical suspicion of illicit drug exposure in 71% (n = 101) of all cases. The use of cannabis was most prevalent in this study popula-

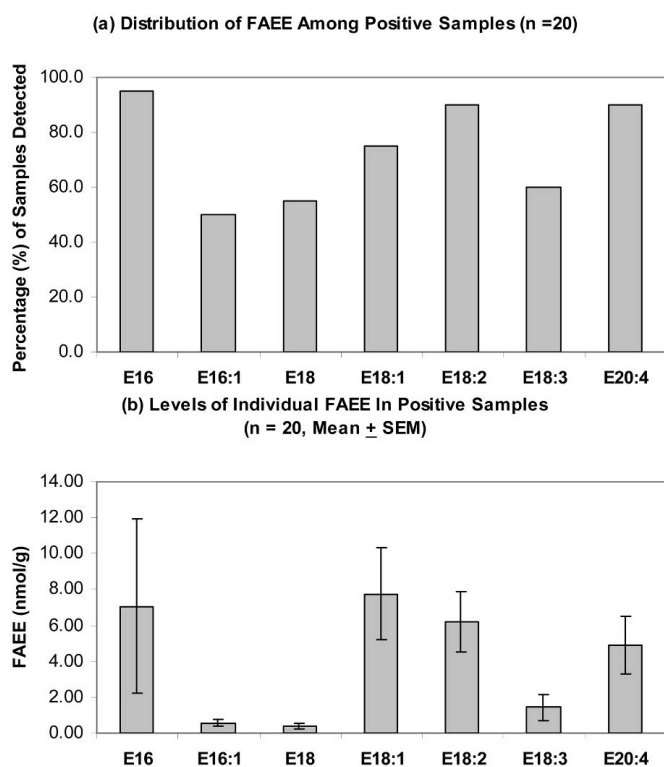


FIGURE 1. A, The distribution of FAEE among positive samples. B, Levels of individual FAEE [mean \pm standard error of the mean (SEM)] in positive samples.

tion (52.3%), followed by cocaine (27%) and opiates (16%). According to the results collected from Canada's Alcohol and Other Drugs Survey (1994), cannabis is the illicit substance most widely used by Canadians, particularly among those between 15 to 24 years.²³ Although Canadian population-based data regarding substance use during pregnancy are not avail-

able, 5% of women of childbearing potential (15 years and older) reported current use (last 12 months) of cannabis.²³ A national study conducted in the United States between 1996 and 1998 documented that 2.8% of pregnant women used illicit drugs.²⁴ The use of cannabis was most prevalent (75%), but cocaine accounted for 10% of all gestational illicit drug use. Of those pregnant women who used illicit drugs, more than 50% of them also used cigarettes and alcohol. In summary, the rates of substance use documented in our study correspond closely to those reported by North American studies.

Using an objective screening method, our study reveals that neonates born to mothers who are suspected or confirmed to use illicit drugs are at risk for prenatal alcohol exposure, as confirmed by positive meconium FAEE screening in 14% of all neonates tested. The reason for a higher rate of positive FAEE among drug-negative versus drug-positive meconium samples (19.5% versus 11.9%) remains to be investigated, but it may be attributed to the small study population. The pattern of FAEE distribution in positive samples was similar to those reported recently in meconium collected from neonates with prenatal alcohol exposure.^{18,19} Ethyl palmitate, oleate, linoleate, and arachidonate were the most prevalent FAEE found in positive meconium samples and were detected at the highest levels. The use of an expanded FAEE screening profile (ie, cumulative sum of 7 individual FAEE) in this study is more efficient in identifying neonates with alcohol exposure in utero. At least 3 individual FAEE were found in the majority of positive meconium samples (n = 19, 95%), whereas only 3 or fewer FAEE were detectable in 95% of the borderline-positive samples. The smallest number of individual species detected in a positive meconium sample was 2 FAEE (n = 1, 5%). Therefore, some cases may not be identified if any single FAEE were selectively measured or chosen as "the preferred biomarker," as suggested by previous investigations.^{16,20}

TABLE 5. Individual FAEE (E16, E16:1, E18, E18:1, E18:2, E18:3, E20:4) Detected in Positive Samples (n = 20), Borderline Positive Samples (n = 17), and Meconium Samples Accompanied by Maternal History (n = 18)

Number of Individual FAEE Detected	Positive Samples (n = 20)	Borderline Positive Samples (n = 17)	Samples with Maternal History (n = 18)
0	0 (0.0)	0 (0.0)	9 (50.0)
1	0 (0.0)	6 (35.3)	2 (11.1)
2	1 (5.0)	5 (29.4)	1 (5.6)
3	2 (10.0)	5 (29.4)	1 (5.6)
4	5 (25.0)	1 (5.9)	2 (11.1)
5	3 (15.0)	0 (0.0)	0 (0.0)
6	3 (15.0)	0 (0.0)	1 (5.6)
7	6 (30.0)	0 (0.0)	2 (11.1)
≥ 4 individual FAEE	17 (85.0)	1 (5.9)	5 (27.8)

TABLE 6. Comparison of Individual and Cumulative FAEE Concentrations Among Positive Samples, Borderline Positive Samples, and Samples Accompanied by Maternal History

FAEE (nmol/g) (Mean ± SD)	Positive Samples ^a (n = 20)	Borderline Samples ^b (n = 17)	Samples with History ^c (n = 18)
Laurate (E12)	8.94 ± 35.04	0.00 ± 0.00	0.58 ± 2.48
Myristate (E14)	4.02 ± 15.84	0.37 ± 0.54	0.28 ± 0.62
Palmitate (E16)	7.07 ± 21.59	0.55 ± 0.44	0.40 ± 0.73
Palmitoleate (E16:1)	0.56 ± 0.93	0.02 ± 0.09	0.12 ± 0.35
Stearate (E18)	0.39 ± 0.55	0.17 ± 0.27	0.01 ± 0.05
Oleate (E18:1)	7.76 ± 11.36	0.06 ± 0.16	2.46 ± 7.07
Linoleate (E18:2)	6.19 ± 7.63	0.02 ± 0.10	2.31 ± 4.71
Linolenate (E18:3)	1.43 ± 3.33	0.13 ± 0.29	0.29 ± 0.60
Arachidonate (E20:4)	4.87 ± 7.19	0.16 ± 0.29	2.42 ± 6.52
Total FAEE ^d	40.67 ± 79.39	1.42 ± 0.76	9.91 ± 20.23
Total FAEE (excl. E12 and E14) ^e	27.71 ± 32.70	1.05 ± 0.45	8.94 ± 17.72

^aPositive samples were defined as samples having a total FAEE (excluding E12 and E14) measurement ≥ 2 nmol/g.

^bBorderline samples were defined as samples having a total FAEE (excluding E12 and E14) measurement below the positive cutoff.

^cSamples with history were defined as samples in which maternal drinking history was indicated (see Maternal and Neonatal History).

^dTotal FAEE is the cumulative sum of 9 individual FAEE.

^eTotal FAEE (excluding E12 and E14) is the cumulative sum of the remaining 7 individual FAEE.

There was significant interindividual variability in the levels of individual or cumulative FAEE detected among positive samples. One significant outlier (Subject 73, Table 4) in the positive group achieved a cumulative level of FAEE (constituted of 6 individual species) that was significantly higher than all other positive samples. This is concordant with a previous finding by Moore et al, who identified a distinctive group of meconium samples in which total FAEE levels detected were significantly elevated when compared with the entire group of positive samples.¹⁸ Such observed variabilities may be attributed to different drinking habits (eg, social, heavy, episodic binge drinkers), duration of exposure (1 trimester, 2 trimesters, or throughout pregnancy), and extent of exposure (eg, number of standard drinks consumed per drinking occasion). This may also explain why only 5 of the 18 samples accompanied by limited maternal drinking history tested positive. Maternal history accompanying these 18 samples only indicated evidence of drinking without further details. It is highly probable that most women stopped or reduced their drinking significantly after the first trimester, when FAEE begins to deposit into meconium. The objective of our study was to evaluate the potential of the FAEE screening test in identifying neonates at risk for prenatal alcohol exposure independent of maternal drinking history. Neonates tested in the current study were referred to our laboratory based on clinical suspicion for prenatal exposure to illicit substances and not prospectively recruited based on history of maternal alcohol use. Therefore, it is not possible to associate a quantitative level of drinking

with a positive FAEE test at this time because there is currently no established correlation between cumulative meconium FAEE and maternal alcohol consumption over the course of pregnancy.

A recent report by Bearer and colleagues provided evidence that 1 particular FAEE in meconium, ethyl oleate (E18:1), correlated well with maternal self-reported drinking in a dose-response manner with a threshold of 1.5 oz (ie, 3 standard drinks) average absolute alcohol per drinking day in a small group of South African pregnant women and their neonates, who were prospectively recruited based on drinking history.²⁰ A positive cutoff for E18:1 at 32 ng/g meconium (dry weight) was established with 84.2% sensitivity and 83.3% specificity. Meconium is approximately 70% \pm 10% water in content (mean \pm SD, unpublished data); therefore, this translates to approximately 9.6 ng/g (0.03 nmol/g) meconium (wet weight). In contrast, the cumulative FAEE positive cutoff of 2 nmol/g in our laboratory was validated in a large population of nondrinking women and their newborns and a small group of neonates with confirmed heavy prenatal alcohol exposure. To compare the efficiencies of the 2 positive cutoffs established by the 2 groups, we reanalyzed our data using both cutoffs and summarized this comparison in Table 7. A total of 24 meconium samples were considered positive using either cutoff. Fifteen samples were identified as positive using both cutoffs, and 9 samples were identified as positive using 1 of the 2 cutoffs (Chan, n = 5; Bearer, n = 4). The 5 samples that were missed by Bearer's cutoff all contained at least 3 individual

TABLE 7. Comparison of 2 Established Positive Cutoffs in Identifying Neonates with Suspected Prenatal Alcohol Exposure

Sample No.	Positive by Bearer's Cutoff: ^a [E18:1] > 0.03 nmol/g	Positive by Chan's Cutoff: ^b Total [FAEE] > 2 nmol/g	Comment (FAEE Detected)
20	Yes	Yes	
22	Yes	Yes	
26	Yes	No	Trace E18:2
27	Yes	Yes	
33	Yes	Yes	
41	No	Yes	E16, E18:2, E20:4
43	Yes	Yes	
44	No	Yes	E16, E18:2, E20:4
45	Yes	Yes	
46	Yes	Yes	
51	Yes	Yes	
58	No	Yes	E16, E18, E18:2, E18:3, E20:4
63	Yes	Yes	
70	Yes	Yes	
71	Yes	Yes	
73	No	Yes	E12, E14, E16, E16:1, E18:3, E20:4
74	No	Yes	E16, E18, E18.2, E20:4
75	Yes	Yes	
83	Yes	Yes	
87	Yes	Yes	
103	Yes	No	E14, E16, E18, E18:1
114	Yes	No	E16, E16:1, E18:1, E20:4
123	Yes	Yes	
136	Yes	No	E16, Trace E18:1 & 18:3

^aPositive cutoff adapted from Bearer et al.²⁰ A cutoff of ethyl oleate (E18:1) greater than 32 ng/g meconium (dry weight) translates to approximately 9.6 ng/g (0.03 nmol/g) meconium (wet weight), assuming 70% water content.

^bPositive cutoff adapted from Chan et al.¹⁹ A positive cutoff of total FAEE at 2 nmol/g meconium (wet weight) is the cumulative sum of 7 individual FAEE, excluding E12 and E14.

FAEE at significantly elevated concentrations, except for the chosen biomarker, ethyl oleate (E18:1), and therefore could be identified as positive only using the cumulative positive cutoff. All four samples that were classified as positive only by Bearer's cutoff contained E18:1 as well as several other FAEE species that were detected by our method. However, their cumulative levels were very low and measured below our established positive cutoff of 2 nmol/g. The technologies used by our group and Bearer's group in the isolation (solid-phase extraction versus silica gel chromatography) and analysis (GC-FID versus GC-MS-MS) of FAEE may have contributed to the differences observed. But overall, there is reasonable agreement between positive cutoffs established by 2 laboratories in different study populations, further validating the meconium FAEE screening method.

Based on these findings, we conclude that although a threshold has not been established for the dose-response char-

acteristics of the cumulative meconium FAEE test (ie, the number of drinks required to constitute a positive screen), the positive cutoff of cumulative FAEE at 2 nmol/g established previously from our population baseline study is efficient in distinguishing neonates who are not exposed to alcohol during pregnancy from those who are exposed heavily. Broad-spectrum FAEE screening has also demonstrated higher efficiency than selective FAEE testing in the identification of neonates with suspected prenatal alcohol exposure. Based on the evidence that a dose-response relationship may exist for particular individual FAEE with maternal drinking,²⁰ it is reasonable to speculate that the cumulative sum of individual FAEE would also correlate with gestational exposure to alcohol. Therefore, those neonates identified by positive cumulative meconium FAEE screening are likely to be more heavily exposed to alcohol in utero. For those samples in which cumulative meconium FAEE was greater than the LOQ but below the

positive cutoff level (borderline positive, $n = 17$), gestational exposure to heavy maternal drinking is unlikely; however, prenatal exposure to lower levels of ethanol can neither be absolutely ruled out nor confirmed in the absence of maternal drinking history at this time. As discussed previously, the current cutoff does not distinguish between neonates born to non-drinkers and those of social drinkers. This is one of the main limitations of the meconium FAEE test currently, but this can be resolved in the future by conducting properly designed prospective studies and recruiting pregnant women with a wide spectrum of drinking histories.

Despite limitations of the present study, we identified pregnant women with addiction problems to be at risk for heavy drinking using an objective and noninvasive test in their neonates. The Centers for Disease Control and Prevention has recently surveyed alcohol use among women of childbearing age for the years 1991 to 1999.⁸ The rates of binge drinking (more than 5 standard drinks per occasion) and frequent drinking (more than 7 drinks per week or binge) remained largely unchanged at an average of about 3% over this period. Most relevant for the present study, the prevalence of heavy drinking during pregnancy (more than 14 drinks per week) was estimated between 0.1% and 0.3%.³ Our present study found that 14% of women suspected to be illicit drug users in pregnancy were heavy drinkers, rendering a relative risk of 47 to 140 of drinking heavily (14% versus 0.1% to 3%) as compared with the general population of pregnant women.

CONCLUSION AND FUTURE DIRECTIONS

This is the first study that evaluated the potential of meconium FAEE screening in identifying high-risk neonates suspected of prenatal alcohol exposure in the absence of maternal history. Our study confirmed previous findings that significantly elevated levels of cumulative FAEE and the presence of more than 4 individual FAEE species in meconium are exclusive to neonates who have likely been exposed to excessive amounts of alcohol in utero. Our findings also indicate that pregnant women suspected of using illicit drugs have an extremely elevated risk of drinking in pregnancy and may have a significantly elevated risk of having an offspring affected by FASD. These results strongly support the potential use of an objective and noninvasive meconium FAEE screening test among high-risk neonates. More research is needed to establish a definitive threshold for the meconium test and to correlate distinct drinking behaviors with different test results. Because confirmed maternal drinking history is usually lacking in the majority of children affected by less apparent forms of alcohol-related disorders, meconium FAEE are emerging biological markers that can potentially facilitate earlier diagnosis and interventions in affected children and indirectly lead to more favorable outcomes.

ACKNOWLEDGMENTS

Supported by a grant from the Canadian Institutes for Health Research (CIHR) and by a New Emerging Team (NET) Grant from CIHR. D.C. is supported by the Research Training Centre, The Hospital for Sick Children. G.K. is a Senior Scientist of CIHR.

REFERENCES

1. Roberts G, Nanson J. *Best Practices: Fetal Alcohol Syndrome/Fetal Alcohol Effects and the Effects of Other Substance Use During Pregnancy*. Ottawa: Canada's Drug Strategy Division, Health Canada, 2000.
2. Alcohol use among women of childbearing age—United States, 1991–1999. *MMWR Morbid Mortal Wkly Rep*. 2002;51:273–276.
3. Alcohol consumption among pregnant and childbearing-aged women—1991 and 1995. United States. *MMWR Morbid Mortal Wkly Rep*. 1997;46:346–350.
4. Canadian Perinatal Health Report (2000). Ottawa: Minister of Public Works and Government Services Canada, Health Canada, 2000.
5. Lemoine P. The history of alcoholic fetopathies. *J FAS Int*. 2003;1:e2.
6. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet*. 1973;2:999–1001.
7. Sampson PD, Streissguth AP, Bookstein FL, et al. Incidence of fetal alcohol syndrome and prevalence of alcohol-related neurodevelopmental disorder. *Teratology*. 1997;56:317–326.
8. Fetal alcohol syndrome—Alaska, Arizona, Colorado, and New York, 1995–1997. *MMWR Morbid Mortal Wkly Rep*. 2002;51:433–435.
9. Abel E. *Fetal Alcohol Syndrome: From Mechanism to Prevention*. Boca Raton, FL: CRC Press, 1996.
10. Barr HM, Streissguth AP. Identifying maternal self-reported alcohol use associated with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res*. 2001;25:283–287.
11. Olson HC, Morse BA, Huffine C. Development and psychopathology: fetal alcohol syndrome and related conditions. *Semin Clin Neuropsychiatry*. 1998;3:262–284.
12. Laposata M. Fatty acid ethyl esters: short-term and long-term serum markers of ethanol intake. *Clin Chem*. 1997;43:1527–1534.
13. Mac E, Pacis M, Garcia G, et al. A marker of fetal exposure to alcohol by meconium analysis. *Pediatr Res*. 1994;35:238A.
14. Bearer CF, Swick A, Singer L. FAEE: Biomarker for prenatal alcohol use. *Alcohol Clin Exp Res*. 1996;20:139A.
15. Bearer CF, Yamashita T, Lee SC, et al. Meconium FAEE and maternal ethanol use. *Alcohol Clin Exp Res*. 1997;21:119A.
16. Bearer CF, Lee S, Salvator AE, et al. Ethyl linoleate in meconium: a biomarker for prenatal ethanol exposure. *Alcohol Clin Exp Res*. 1999;23:487–493.
17. Klein J, Karaskov T, Koren G. Fatty acid ethyl esters: a novel biologic marker for heavy in utero ethanol exposure: a case report. *Ther Drug Monit*. 1999;21:644–646.
18. Moore C, Jones J, Lewis D, et al. Prevalence of fatty acid ethyl esters in meconium specimens. *Clin Chem*. 2003;49:133–136.
19. Chan D, Bar-Oz B, Pellerin B, et al. Population baseline of meconium fatty acid ethyl esters among infants of nondrinking women in Jerusalem and Toronto. *Ther Drug Monit*. 2003;25:271–278.
20. Bearer CF, Jacobson JL, Jacobson SW, et al. Validation of a new biomarker of fetal exposure to alcohol. *J Pediatr*. 2003;143:463–469.
21. Stewart DE, Streiner D. Alcohol drinking in pregnancy. *Gen Hosp Psychiatry*. 1994;16:406–412.
22. Bar-Oz B, Klein J, Karaskov T, et al. Comparison of meconium and neonatal hair analysis for detection of gestational exposure to drugs of abuse. *Arch Dis Child Fetal Neonatal Ed*. 2003;88:F98–F100.
23. Canada's Alcohol and Other Drugs Survey (1994). Ottawa: Division of Special Surveys, Statistics Canada, 2003.
24. Ebrahim SH, Gfroerer J. Pregnancy-related substance use in the United States during 1996–1998. *Obstet Gynecol*. 2003;101:374–379.