MECONIUM FATTY ACID ETHYL ESTERS: AN EMERGING MARKER
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A CRITICAL REVIEW of "Prevalence of fatty acid ethyl esters in meconium specimens."

Due to the relatively short half-lives of ethanol and its major metabolite, acetaldehyde, there has been intense search of more stable and reliable biological markers. Fatty acid ethyl esters (FAEE) are a group of ethanol metabolites that result from the enzymatic conjugation of free fatty acids or fatty acyl-CoA to ethanol. FAEE have been proposed as markers of recent and chronic ethanol consumption in adult drinkers and in post mortem specimens. Recently, the presence of FAEE in neonatal meconium samples stimulated the development of a novel neonatal screening test for prenatal alcohol exposure, which is often determined based on maternal self-reports in the past.

The study presented by Moore et al. investigated the presence of FAEE in meconium specimens collected from 2 different geographic and cultural populations, using a detailed analytical method involving solid phase extraction and gas chromatography-mass spectrometry (chemical ionization). The authors have chosen an expanded FAEE screening profile which included ethyl laurate (E12), myristate (E14), palmitate (E16:0), palmitoleate (E16:1), stearate (E18:0), oleate (E18:1), linoleate (E18:2), linolenate (E18:3), and arachidonate (E20:4).

Analyses of meconium specimens from the 2 populations revealed similar FAEE distribution, with E16:1 and E18:2 predominating as the FAEE detected most often and at the highest levels, despite differences in the percentage of positive samples. This finding is in agreement with a previous report and with on-going research in our laboratory. A positive cumulative cut-off level of FAEE greater than 50 ng/g was chosen by Moore and colleagues because it was the laboratory’s analytical limit of detection above which accurate detection of FAEE was possible. However, the authors did not clarify whether this limit has been validated and associated with prenatal alcohol exposure. E12 and E14 were detected in most meconium samples, independent of prenatal alcohol exposure, and therefore were selectively eliminated from the screening profile by the authors. The reason why these particular FAEE were excluded was not explained in the current report. In the authors’ previous work, they reported negative meconium samples to contain less than 50 ng/g total FAEE when E12 and E14 were excluded. However, samples may contain much higher levels of E12 and E14, and further justification is required prior to the exclusion of these FAEE from the screening profile.

In our laboratory, we investigated the presence of FAEE in the meconium of neonates not exposed to alcohol from 2 different populations (Jerusalem and Toronto), in comparison to neonates that were exposed to alcohol heavily in utero. The objective of our study was to define a population baseline for FAEE in meconium and to establish a reliable positive cut-off in clinical practice. FAEE distribution was similar among samples tested in both populations, E12 and E14 being the species detected most often while the rest of the esters were undetectable in greater than 80% of all samples tested. In comparison, a wide range of FAEE including the longer chain FAEE (E16+) were detected in meconium samples from neonates with confirmed prenatal alcohol exposure at significantly higher levels. On average, the levels of FAEE detected from baseline meconium samples were significantly lower (10 fold) than those measured in neonates with confirmed heavy prenatal exposure (mean total FAEE, 1.82 vs. 11.08 nmol/g). Due to the presence of certain FAEE in meconium samples of neonates without prenatal alcohol exposure,
specificity of the FAEE screening test was determined when the positive cut-off was varied between 0 to 2 nmol/g, which was the lowest cumulative FAEE level detectable in a sample with confirmed exposure. Since E12 and E14 were found to predominate in the baseline population and independent of prenatal alcohol exposure, the same exercise was repeated when these FAEE were excluded. Based on the data collected from our population, we concluded that the use of 2 nmol cumulative FAEE/g, when E12 and E14 were excluded yielded the highest specificity (98%).

The paper by Moore et al. also discussed for the first time the stability of FAEE in meconium samples. Ethyl arachidonate (E20:4) was undetectable in one population while it was found in 26% of samples in another population. The authors suspected that exposure might have occurred earlier on in pregnancy because Refaai et al. previously showed that the presence of E20:4 is associated with recent alcohol consumption. However, it is inconceivable that over 400 samples tested in random from one population shared similar histories of exposure. Also, the authors suspected that difference in storage and shipping conditions prior to analysis might have contributed to the overall stability of FAEE in meconium. In vitro stability data suggested that the best storage and shipping conditions are frozen, however, it was not discussed whether individual FAEE differ in stability within the matrix under different handling conditions. It should be noted that the stability of FAEE in other matrices (e.g. blood and various tissues) has not been reported to be an issue in the literature, and we have a similar experience in our laboratory.

The use of an expanded screening profile of FAEE by Moore et al. ensures high clinical sensitivity. Moreover, empirical analysis of total FAEE concentrations in meconium samples from both populations divided into 4 quartiles revealed findings with potential clinical implications. The highest quartile included samples with mean total FAEE concentrations significantly higher (6 to 60 folds) than the other three quartiles in both study populations, even though the prevalence of individual FAEE was different. Although a correlation between the extent of prenatal alcohol exposure to cumulative FAEE level in meconium has not been established to date, such relationship was shown for ethyl linoleate. Hence the high levels shown by Moore et al. may be indicative of significant exposure and therefore an elevated risk of fetal alcohol spectrum disorders (FASD). It would be interesting also to conduct in the future multivariate analysis to determine whether certain combinations of FAEE from the screening profile predicts prenatal alcohol exposure with higher sensitivity and specificity.

Prenatal alcohol exposure is gaining an increasingly higher profile as a public health issue. While other non-invasive neonatal screening methods are being sought, FAEE meconium measurement appears to become the first to reach the clinical finish line.

REFERENCES