New Meconium Biomarkers of Prenatal Methamphetamine Exposure Increase Identification of Affected Neonates

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BACKGROUND: Prenatal methamphetamine (MAMP) exposure is poorly reflected in neonatal meconium. Often, maternal self-reported MAMP use is not corroborated by positive results in amphetamines immunoassays of meconium, and even if initial test results are positive, they frequently are not confirmed by screening or amphetamine (AMP) by chromatographic analysis. The presence of the MAMP metabolites p-hydroxymethamphetamine (pOHMAMP), p-hydroxyamphetamine (pOHAMP), and norephedrine (NOREPH) in meconium may improve the identification of MAMP- and AMP-exposed neonates.

METHODS: Immunoassay-positive and -negative meconium samples were subjected to liquid chromatography–tandem mass spectrometric reanalysis for these recently identified metabolites.

RESULTS: pOHAMP and NOREPH were detected only when MAMP and/or AMP were present and thus do not appear to be promising biomarkers of prenatal MAMP exposure. pOHMAMP, in contrast, identified 6 additional neonates whose mothers reported MAMP exposure, yet had a meconium sample screened as negative; pOHMAMP was more likely to be present if maternal MAMP use continued into the third trimester. Although the pOHMAMP results for meconium samples corroborated the maternal self-reports, the confirmation rate for positive meconium screening results did not improve with the inclusion of these new biomarkers.

CONCLUSIONS: pOHMAMP identified additional MAMP-exposed neonates; therefore, MAMP, AMP, and pOHMAMP should be included in meconium chromatographic analyses. Maximizing the identification of MAMP-exposed children requires improvement in immunoassay screening tests to reduce false-negative and false-positive results. Additional research will help clarify which AMP-related compounds, if any, contribute to unconfirmed positive results in screening tests. Furthermore, nonamphetamine compounds endogenous to the complex meconium matrix also may cross-react, making chromatographic confirmation of screening results essential.

Infants prenatally exposed to methamphetamine (MAMP)10 are more likely to be small for their gestational age, have lower birth weights (1), and experience increased physiological stress (2). Identifying MAMP-exposed infants is imperative, not only to establish medical, behavioral, and social interventions, but also to characterize long-term effects. In the Infant Development, Environment, and Lifestyle (IDEAL) study, 71.0% of affected neonates were identified only through maternal disclosure during the postpartum interview, rather than from positive results in meconium tests; 25.2% were identified by self-report and positive meconium results, and 3.8% were identified by meconium analysis only (3).

We proposed possible explanations for the low rate of detection in meconium. First, most women in the IDEAL study stopped MAMP use in the first or second trimester (3, 4). Meconium begins forming in the second trimester, and data from our laboratory and others suggest that second-trimester drug exposure is poorly reflected in meconium (5, 6). Indeed, meconium samples were more likely to test positive when maternal MAMP use continued into the third trimester and exceeded once per week (3); yet, 54.3% of neonates

10 Nonstandard abbreviations: MAMP, methamphetamine; IDEAL, Infant Development, Environment, and Lifestyle; EMIT, enzyme-multiplied immunoassay technique; AMP, amphetamine; pOHMAMP, p-hydroxymethamphetamine; pOHAMP, p-hydroxyamphetamine; NOREPH, norephedrine; LOQ, limit of quantification; LC-MS/MS, liquid chromatography–tandem mass spectrometry.
Exposed during the third trimester had amphetamines-negative meconium.

Second, the testing procedure may contribute to the low number of amphetamines-positive meconium samples. Meconium samples were initially screened with the enzyme-multiplied immunoassay technique (EMIT) at a 500-ng/g amphetamines cutoff. If the results were positive, the presence of MAMP and amphetamine (AMP) was confirmed by GC-MS. It is possible that some meconium samples contained MAMP and/or AMP biomarkers at concentrations below the immunoassay cutoff. By directly analyzing these samples with a chromatographic procedure with improved detection limits, we could estimate the prevalence of false-negative immunoassay results within the IDEAL population.

Finally, nearly 70% of positive results in amphetamines screens were not confirmed. EMIT assays targeting MAMP/AMP cross-react with other sympathomimetic amines, including over-the-counter cold-medicine components, phenethylamines, and other illicit amphetamines. Additionally, endogenous substances, other exogenous compounds, or minor MAMP/AMP metabolites in meconium may produce positive immunoassay results. Previous research on cocaine and cannabinoids identified relatively minor adult metabolic products that occur in meconium in higher proportions and that markedly cross-react in immunoassays (7, 8).

Poor detection of MAMP exposure prompted our laboratory to investigate 3 potential alternative meconium biomarkers of prenatal MAMP exposure: \( p \)-hydroxymethamphetamine (pOHMAMP), \( p \)-hydroxyamphetamine (pOHAMP), and norephedrine (NOREPH) (Fig. 1). pOHMAMP and NOREPH were found in 86.0% and 25.6%, respectively, of MAMP-positive meconium samples not collected as part of the IDEAL study; the pOHAMP concentration was always below the limit of quantification (LOQ) (9). It was still not clear, however, whether these novel biomarkers would increase the identification of MAMP-exposed infants when MAMP and AMP were not present. The primary aim of this study was to determine whether the novel MAMP metabolites pOHMAMP, pOHAMP, and NOREPH could improve the identification of MAMP- and AMP-exposed neonates.

A detailed description of the IDEAL study has previously been published (10); each site’s institutional review board approved the study. After providing informed, written consent, mothers were interviewed about the amount and frequency of MAMP, Ecstasy (3,4-methylenedioxymethamphetamine, or MDMA), and AMP consumption during pregnancy.

Meconium was collected from diapers until the appearance of milk stool. Samples remained refrigerated until overnight transport to the United States Drug Testing Laboratories (in Des Plaines, IL) for analysis. Syva EMIT II Plus (Dade Behring/Siemens Healthcare Diagnostics) screens designed for urine amphetamines testing were used for meconium samples after they had been subjected to methanol homogenization and solid-phase extraction. If samples had screening results \( \geq 500 \) ng/g, GC-MS analysis confirmed the presence of MAMP and AMP (5-ng/g cutoff). All samples were frozen at \(-20^\circ C\).

After analysis, meconium samples were shipped frozen to the National Institute on Drug Abuse for further evaluation. Meconium samples from 3 IDEAL participant groups were chosen: (a) 48 women who denied amphetamines use yet had positive results in the

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**Fig. 1.** Metabolic pathway of MAMP, AMP, NOREPH, pOHAMP, and pOHMAMP.
meconium screen; (b) 62 women who self-reported amphetamines use yet had negative results in the meconium screen; and (c) 22 women who self-reported amphetamines use and had positive results in the meconium screen. Reanalysis included methanol homogenization, solid-phase extraction, and liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis (11, 12). The first 72 samples were analyzed for MAMP, AMP, pOHMAMP, pOHAMP, and NOREPH; a 12.5-ng/g LOQ cutoff was used except for pOHMAMP, for which an 8-ng/g cutoff was used (11). The remaining 60 samples were analyzed with an LC-MS/MS procedure with lower LOQs for MAMP (2.5 ng/g), AMP (5 ng/g), and pOHMAMP (1 ng/g) (12); pOHAMP and NOREPH were excluded because of their low prevalence in the initial data set.

SPSS version 16.0 for Windows (SPSS) and Microsoft Excel were used for data analysis and statistical evaluation. P values < 0.05 were considered statistically significant.

Of the 132 meconium samples, 43 (32.6%) contained one or more MAMP biomarkers by LC-MS/MS analysis. Most positively testing samples (62.7%) contained MAMP, AMP, and pOHMAMP; MAMP and AMP were found in only 16.3% of the samples. MAMP and pOHMAMP were identified in 4.7%, and MAMP only was observed in 2.3%. Surprisingly, 6 (14.0%) of 43 samples were positive for pOHMAMP without positive results for MAMP or AMP. pOHAMP and NOREPH were identified in few samples and only when MAMP and AMP also were present.

The 48 women who denied any amphetamines use during pregnancy had meconium samples that screened as positive. The results for most meconium samples were not confirmed in the LC-MS/MS reanalysis; only 2 samples contained MAMP, AMP, and/or pOHMAMP, including 1 sample containing 6.2 ng/g MAMP (Table 1). We evaluated meconium samples from the neonates of 62 women who reported amphetamines consumption during pregnancy, yet their infants’ meconium samples screened as negative; 48 of these women stopped amphetamines use in the first or second trimester (early exposure), whereas the remaining 14 women continued abuse into the third trimester (late exposure). Twenty (32.3%) of the 62 meconium samples contained one or more biomarkers exceeding the LC-MS/MS LOQ, including 6 samples with pOHMAMP only (Table 1). These 6 samples were from 2

<table>
<thead>
<tr>
<th>Negative maternal self-report and positive result in immunoassay screen (n = 48)</th>
<th>Samples analyzed, n</th>
<th>Positive, n (%)</th>
<th>Median, ng/g</th>
<th>Range, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAMP</td>
<td>48</td>
<td>2 (4.2)</td>
<td>2479</td>
<td>6.2–4952</td>
</tr>
<tr>
<td>AMP</td>
<td>48</td>
<td>1 (2.1)</td>
<td>1106</td>
<td></td>
</tr>
<tr>
<td>pOHMAMP</td>
<td>48</td>
<td>1 (2.1)</td>
<td>28.9</td>
<td></td>
</tr>
<tr>
<td>pOHAMP</td>
<td>19</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOREPH</td>
<td>19</td>
<td>1 (5.3)</td>
<td>31.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive maternal self-report and negative result in immunoassay screen (n = 62)</th>
<th>Samples analyzed, n</th>
<th>Positive, n (%)</th>
<th>Median, ng/g</th>
<th>Range, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAMP</td>
<td>62</td>
<td>14 (22.6)</td>
<td>29.3</td>
<td>5.1–10370</td>
</tr>
<tr>
<td>AMP</td>
<td>62</td>
<td>12 (19.4)</td>
<td>23.1</td>
<td>5.1–1600</td>
</tr>
<tr>
<td>pOHMAMP</td>
<td>62</td>
<td>15 (24.2)</td>
<td>23.0</td>
<td>1.3–435</td>
</tr>
<tr>
<td>pOHAMP</td>
<td>34</td>
<td>1 (2.9)</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>NOREPH</td>
<td>34</td>
<td>2 (5.9)</td>
<td>26.8</td>
<td>13.7–39.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Positive maternal self-report and positive result in immunoassay screen (n = 22)</th>
<th>Samples analyzed, n</th>
<th>Positive, n (%)</th>
<th>Median, ng/g</th>
<th>Range, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAMP</td>
<td>22</td>
<td>21 (95.5)</td>
<td>1455</td>
<td>188–10250</td>
</tr>
<tr>
<td>AMP</td>
<td>22</td>
<td>21 (95.5)</td>
<td>285</td>
<td>20.3–1012</td>
</tr>
<tr>
<td>pOHMAMP</td>
<td>22</td>
<td>19 (86.4)</td>
<td>140</td>
<td>27.2–402</td>
</tr>
<tr>
<td>pOHAMP</td>
<td>19</td>
<td>0 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOREPH</td>
<td>19</td>
<td>8 (42.1)</td>
<td>17.0</td>
<td>14.2–96.9</td>
</tr>
</tbody>
</table>

Table 1. Prevalence and concentrations of amphetamines and metabolites in meconium as assayed by LC-MS/MS.
women who used amphetamines in the first and/or second trimesters and 4 women who continued their use into the third trimester. MAMP, AMP, and pOHMAMP individual and total biomarker concentrations by LC-MS/MS analysis were below the immunoassay cutoff in 59 (95.2%) of 62 meconium samples, including all samples from neonates exposed early in gestation. Among the 22 women with a positive self-report and positive meconium-screening results, the results of all but 1 of the meconium samples were confirmed by both GC-MS and LC-MS/MS (Table 1).

We compared pOHMAMP presence and its concentrations in meconium samples with maternal patterns of MAMP use and estimated gestational age to investigate factors possibly influencing pOHMAMP disposition. pOHMAMP was identified more frequently, and it occurred at higher concentrations (median, 138 ng/g; range, 3.5–345 ng/g) when exposure continued into the third trimester (23 of 34 neonates, 67.6%) than if drug use stopped earlier (11 of 50 neonates, 22.0%; median, 23.0 ng/g; range, 1.2–343 ng/g). Exposure frequency was not related to pOHMAMP presence or concentration. Younger maternal age and decreased birth weight were associated with pOHMAMP presence but were not linearly correlated with concentrations in meconium; similar results were observed for MAMP and AMP (see Table 1 in the Data Supplement that accompanies the online version of this Brief Communication at http://www.clinchem.org/content/vol56/issue5).

Monitoring pOHAMP and NOREPH did not increase the identification of affected infants; these analytes were found only in conjunction with MAMP and AMP. Six additional neonates, however, all of whom had negative screening results, were identified by only pOHMAMP presence in meconium. Further research is necessary to determine the identity of other immunoassay-reactive biomarkers and to reduce the immunoassay’s cross-reactivity with other endogenous and exogenous analytes. Moore et al. identified a large proportion of meconium samples testing positive in the amphetamines immunoassay as being positive for pseudoephedrine or phenylethylamine (13).

Nearly 1 in 3 neonates with maternal self-reported MAMP use but immunoassay-negative meconium results had positive LC-MS/MS results, although most of the concentrations were low. Decreasing the immunoassay cutoff concentration might have identified more true-positive samples, but given the already low confirmation rate, it would likely also have identified additional unconfirmed samples. Marin et al. directly compared the EMIT assay (200-ng/g cutoff) with an ELISA (20-ng/g cutoff) (14). Despite the lower ELISA cutoff, both immunoassays demonstrated equivalent false-positive rates when compared with the results of chromatographic confirmation tests. In cases of maternal self-reported MAMP abuse, it may be advisable to perform chromatographic testing directly or to include neonates in the MAMP-exposed group on the basis of positive results in meconium tests or the maternal self-report, as was done in the IDEAL study.

pOHMAMP was more often present when maternal amphetamines consumption continued into the third trimester, but its presence was not influenced by the frequency of MAMP use. It is still not clear what other factors may contribute to pOHMAMP formation and/or disposition. MAMP is biotransformed to AMP and pOHMAMP by CYP2D6 (15), which has >70 allelic variants (16) and increased activity during pregnancy (17); the participants’ pharmacogenomics are unknown. In urine, unchanged MAMP, AMP, and pOHMAMP concentrations predominate (18, 19), similar to the disposition in meconium. Fetal liver produces CYP2D6 early in gestation (20), but its metabolic efficiency for MAMP is unknown. Additional studies are necessary to elucidate which factors influence biomarker disposition in meconium.

In summary, pOHMAMP alone identified additional amphetamines-exposed neonates, whereas pOHAMP and NOREPH did not. Confirmation rates of amphetamines immunoassay–positive meconium did not increase. Further research is necessary to identify cross-reactive species that contribute to unconfirmed positive results in immunoassays of meconium.

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