Published in final edited form as:

Clin Chem. 2008 December ; 54(12): 2018–2027. doi:10.1373/clinchem.2008.109173.

Meconium Nicotine and Metabolites by Liquid Chromatography–Tandem Mass Spectrometry: Differentiation of Passive and Nonexposure and Correlation with Neonatal Outcome Measures

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Abstract

BACKGROUND—Meconium analysis is a diagnostically sensitive and objective alternative to maternal self-report for detecting prenatal tobacco exposure. Nicotine and metabolite disposition in meconium is poorly characterized, and correlation of analytes' concentrations with neonatal outcomes is unexplored. Our objectives were to quantify nicotine, cotinine, *trans*-3'-hydroxycotinine (OH-cotinine), norcotinine, and glucuronide concentrations in meconium, identify the best biomarkers of in utero tobacco exposure, compare meconium concentrations of tobacco-exposed and nonexposed neonates, and investigate concentration–outcome relationships.

METHODS—We quantified concentrations of nicotine and 4 metabolites with and without hydrolysis simultaneously in meconium from tobacco-exposed and nonexposed neonates by liquid chromatography–tandem mass spectrometry. We compared meconium concentrations to birth weight, length, head circumference, gestational age, and 1- and 5-min Apgar scores.

RESULTS—Nicotine, cotinine, and OH-cotinine were the most prevalent and abundant meconium tobacco biomarkers and were found in higher concentrations in tobacco-exposed neonates. Whereas cotinine and OH-cotinine are glucuronide bound, performing the lengthy and costly enzymatic hydrolysis identified only 1 additional positive specimen. Unconjugated nicotine, cotinine, or OH-cotinine meconium concentration >10 ng/g most accurately discriminated active from passive and nonexposed neonates. There was no significant correlation between quantitative nicotine and metabolite meconium results and neonatal outcomes, although presence of a nicotine biomarker predicted decreased head circumference.

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Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures of Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.

Consultant or Advisory Role: None declared.

Stock Ownership: None declared.

Honoraria: None declared.

Expert Testimony: None declared.

CONCLUSIONS—Unconjugated nicotine, cotinine, and OH-cotinine should be analyzed in meconium to detect in utero tobacco exposure, as approximately 25% of positive specimens did not contain cotinine. Immunoassay testing monitoring cotinine only would underestimate the prevalence of prenatal tobacco exposure.

Tobacco exposure during gestation presents significant health concerns for pregnant women and fetuses. The 2004 Surgeon General's Report, *The Health Consequences of Smoking* (1), concluded that active smoking during pregnancy is causally linked to premature rupture of membranes, placenta previa, placental abruption, preterm delivery, shortened gestation, fetal growth restriction, and low birth weight. Supporting evidence has depended on maternal self-report to define smoking status; only 3 investigations confirmed smoking status by toxicological analysis (2–4). More recent studies relating maternal self-reported tobacco consumption to neonatal outcomes observed increased risk of congenital heart defects (5), postnatal infections in preterm infants (6), preterm birth (5, 7, 8), and lower birth weight (5, 7–10) among tobacco-exposed children. Maternal self-report is often unreliable, as pregnant smokers may deny or under-represent the extent of tobacco use (11–15). Boyd et al. (12) found that 26.2% of pregnant, self-identified non-smokers had saliva cotinine concentrations consistent with active smoking. Inaccurate self-report may influence exposure–outcome correlation results. England et al. (16) observed a misclassification bias of 14%–15% when exposure was determined by urinary cotinine concentrations vs maternal self-report.

Identifying nicotine biomarkers in biological specimens provides an alternative to self-reported gestational tobacco use. Meconium, the first neonatal feces, offers several advantages for detecting prenatal tobacco exposure. Meconium formation begins during the twelfth gestational week; therefore, meconium testing potentially reflects second- and/or third-trimester exposure. Accumulation over months yields higher concentrations and a longer detection window than other neonatal matrices, and collection from diapers is easy and noninvasive.

Until recently, only cotinine was identified in meconium (17–22). Generally, meconium cotinine is measured by immunoassay, often without chromatographic confirmation. Most cotinine immunoassays cross-react with *trans*-3'-hydroxycotinine (OH-cotinine),³ but not nicotine or nornicotine (23, 24). Nicotine and metabolite disposition in meconium is unknown, and relationships between biomarker concentrations and neonatal outcomes are unexplored. To date, no threshold to differentiate passive exposure and nonexposure from active tobacco smoking has been proposed.

Our laboratory recently validated the first method for simultaneous quantification of nicotine and 4 metabolites, cotinine, OH-cotinine, nornicotine, and norcotinine, in meconium (25). We employed this liquid chromatography–tandem mass spectrometry (LC-MS/MS) method to analyze 168 meconium specimens (125 specimens with and without enzymatic hydrolysis and 43 specimens without) to determine nicotine and metabolite presence in meconium and identify which analytes best detect prenatal tobacco exposure. Moreover, we hypothesized that meconium concentrations can accurately differentiate tobacco-exposed from non-exposed neonates and that meconium nicotine and metabolite concentrations can predict neonatal outcomes. Here we present the disposition of 5 nicotine biomarkers in meconium for the first time and compare the results to maternal self-report of tobacco exposure and birth weight, length, head circumference, gestational age, and 1- and 5-min Apgar scores. Furthermore, we propose a cutoff concentration for differentiating active maternal smoking from passive tobacco exposure during gestation.

³Nonstandard abbreviations: OH-cotinine, *trans*-3'-hydroxycotinine; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LOQ, limit of quantification.

Materials and Methods

PARTICIPANTS

Women in good postbirth health, age 13–45 years, at 2 major hospitals in Montevideo, Uruguay, were approached during a period of 45 days for study participation. Pereira Rossell Hospital, the main maternity center in the country, and Clínicas Hospital, a university hospital, account for 33% and 15% of births in Montevideo and Uruguay, respectively, and 50% of births in the low and mid-to-low socioeconomic sector. Women provided written informed consent to participate in this institutional review board–approved study. Interviews regarding previous pregnancies, lifestyle, and frequency and amount of alcohol, tobacco, and drug use were conducted within 48 h of delivery (an English translation of the questionnaire is available in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol54/issue12>). Neonates were evaluated for birth weight, head circumference, length, general health status, gestational age, and 1- and 5-min Apgar scores soon after birth. Maternal and neonatal demographics, medical history, and health status were obtained from hospital records.

In all, 215 meconium specimens and 61 interviews were available. Interviews may not have been conducted because of unavailability of the mother due to discharge, transfer, refusal, or other reasons. Insufficient meconium or accidents during transport eliminated 47 specimens for analysis.

MECONIUM ANALYSIS

Meconium from the first 48 h was scraped from diapers and stored at -20°C until shipment, transferred on ice to the National Institute on Drug Abuse, and maintained at -20°C until analysis. We quantified nicotine and metabolites using LC-MS/MS (25). Briefly, analytes of interest in 0.5 g meconium and deuterated internal standards were extracted by acidified methanol homogenization. We analyzed specimens with and without hydrolysis to quantify total and unconjugated drug concentrations and to estimate percent glucuronidation, if sufficient meconium was available. Overnight hydrolysis was achieved with *E. coli* type IX-A β -glucuronidase. We subjected acidified samples to solid-phase extraction to concentrate analytes and remove matrix components.

We evaluated 2 multiple reaction monitoring transitions per analyte and internal standard. Chromatographic separation was achieved using a binary mobile-phase gradient. The linear dynamic range was 1.25 [limit of quantification (LOQ) for cotinine, OH-cotinine, and norcotinine] or 5 (LOQ for nicotine and nornicotine) to 500 ng/g. Coefficients of determination (R^2) were >0.99 . Average hydrolysis efficiencies for nicotine-*N*-glucuronide, cotinine-*N*-glucuronide, and OH-cotinine-*O*-glucuronide were 90%, 80%, and 15%, respectively. Analytical recovery was $>76\%$ of nominal target concentrations; intra- and interday imprecision were $<20\%$.

STATISTICAL ANALYSES

We performed statistical analyses using SPSS for Windows, version 13.0. We used nonparametric analyses, Wilcoxon signed rank tests, and Mann–Whitney tests to compare concentration differences between groups, as concentrations were not normally distributed. Pearson correlations evaluated relationships between meconium concentrations and quantitative neonatal outcome parameters, and χ^2 tests examined relationships between categorical variables. *P* values <0.05 were considered statistically significant.

Results

MECONIUM RESULTS

We analyzed meconium from 168 neonates without enzymatic hydrolysis to quantify unconjugated drug concentrations; sufficient meconium remained for 125 specimens to undergo a second analysis with enzymatic hydrolysis, allowing direct comparison of unconjugated and total drug concentrations. The following results are from 125 paired meconium analyses (Table 1). Fifty-nine (47.2%) nonhydrolyzed and 60 (48.0%) hydrolyzed specimens had concentrations greater than the LOQs for at least 1 tobacco biomarker. Nicotine was the most prevalent analyte in both hydrolyzed and nonhydrolyzed specimens, followed by OH-cotinine, cotinine, nornicotine, and norcotinine. In 3 specimens, OH-cotinine concentrations exceeded the 500 ng/g upper LOQ. Determining exact concentrations was not possible for these specimens, as insufficient meconium remained for dilution; affected pairs were not included in quantitative comparisons and statistical analyses. In nonhydrolyzed specimens, cotinine was found in the highest concentrations, followed by OH-cotinine and nicotine, whereas in hydrolyzed specimens, OH-cotinine was followed by cotinine and nicotine. For the first time, nornicotine and norcotinine were detected in meconium, although in fewer specimens and at concentrations <25 ng/g. Analyte concentrations obtained before and after enzymatic hydrolysis were compared only in meconium specimens with paired unconjugated and total drug concentrations. Differences between unconjugated and total concentrations were significantly greater than zero for OH-cotinine and cotinine ($P < 0.001$), indicating substantial glucuronidation of these analytes.

Most specimens were positive for nicotine, cotinine, and/or OH-cotinine. No specimens were positive for nornicotine and/or norcotinine alone (Table 2). Nornicotine and norcotinine were found in specimens with high nicotine, cotinine, and OH-cotinine concentrations. Median (interquartile range) nicotine, cotinine, and OH-cotinine concentrations were 24.9 (9.1–78.7), 47.6 (18.9–106.8), and 37.4 (11.6–107.5) ng/g in nonhydrolyzed specimens, respectively, and 27.4 (10.3–84.1), 47.8 (18.3–116.9), and 49.8 (15.1–126.5) ng/g in hydrolyzed specimens.

Comparing concentrations from hydrolyzed and nonhydrolyzed specimens, median (interquartile range) percentages of glucuronide conjugates were 7.5% (2.1%–16.1%) for nicotine, 15.2% (6.0%–26.8%) for cotinine, and 28.9% (16.2%–46.1%) for OH-cotinine. Nonhydrolyzed concentrations were greater than hydrolyzed concentrations in 3 instances for OH-cotinine, 4 for cotinine, and 13 for nicotine. Wide variations in the degree of glucuronidation were observed for all 3 analytes.

We calculated unconjugated biomarker concentration ratios for each metabolite and nicotine, each metabolite and its direct precursor, and summed metabolites and nicotine. Median (interquartile range) ratios were 1.00 (0.72–1.60) cotinine:nicotine, 1.09 (0.51–2.25) OH-cotinine:nicotine, 0.02 (0.01–0.05) nornicotine:nicotine, 0.08 (0.06–0.10) nornicotine:cotinine, 0.04 (0.01–0.05) norcotinine:cotinine, 1.14 (0.64–1.78) OH-cotinine:cotinine, and 1.74 (1.41–2.78) total metabolites:nicotine.

MECONIUM NICOTINE BIOMARKER RESULTS VS SELF-REPORTED SMOKING STATUS

Maternal tobacco status questionnaires were available for 49 of 125 paired meconium analyses. Thirty-two women (65.3%) reported no smoking during pregnancy. In the nonsmoking group, no nicotine analytes were detected in 24 nonhydrolyzed (75.0%) and 23 hydrolyzed (71.9%) meconium specimens at the method's LOQ (Table 3). In the 8 positive specimens, only nicotine, cotinine, and OH-cotinine were observed, with unconjugated drug concentrations <10 ng/g in all but 1 specimen that contained 72.4 ng/g nicotine, 29.9 ng/g cotinine, and 27.0 ng/g OH-cotinine.

Seventeen women (34.7%) admitted smoking sometime during pregnancy. Only 3 of these (17.6%) were negative for tobacco biomarkers. Two women reported first-trimester smoking only; since meconium formation begins in the second trimester, detection of nicotine biomarker would not be expected. The mother of the third infant with negative meconium results self-reported smoking 1 to 5 cigarettes daily throughout pregnancy. Unconjugated and total cotinine, unconjugated and total OH-cotinine, and total nicotine concentration distributions were statistically different between smoking and nonsmoking groups, with larger concentrations observed in the smoking group. Norcotinine and nornicotine were quantified in approximately one-third of meconium specimens from the smoking group.

MATERNAL LIFESTYLE CHARACTERISTICS VS SELF-REPORTED SMOKING STATUS

Maternal interviews and meconium unconjugated drug concentrations were available for 61 neonates. Forty-one women denied smoking while pregnant, whereas 17 individuals reported smoking throughout gestation and 3 individuals during the first trimester only. Daily smoking of 1–5 and 6–10 cigarettes was reported by 73.7% and 26.3% of smokers, respectively. No woman reported smoking more than 10 cigarettes per day. Maternal age and number of children are presented in Table 4. Maternal lifestyle characteristics, grouped by meconium results, are presented in Table 5. More nonsmokers were married than smokers ($P < 0.05$), but there were no significant differences in educational level, working status, or alcohol use during pregnancy between groups. Thirteen women, 8 nonsmokers and 5 smokers, received tranquilizers during the first 3 months of pregnancy. One non-smoker and 3 tobacco users reported smoking cannabis during pregnancy, one of whom also consumed cocaine throughout pregnancy. No women reported gestational amphetamine, methamphetamine, hashish, inhalant, ecstasy, heroin, or morphine use.

NEONATAL OUTCOME MEASURES VS SELF-REPORTED SMOKING STATUS AND MECONIUM RESULTS

Neonatal outcome measures were stratified by maternal self-reported tobacco use (yes/no) and meconium nicotine and metabolite results (positive/negative) (Table 6). Meconium was considered positive if at least 1 analyte was greater than the LOQ. No statistically significant differences in neonatal birth parameters were observed when results were classified by maternal self-reported tobacco use. When grouped by nicotine biomarker status, mean head circumference was significantly smaller in children with positive results ($P = 0.003$), even after controlling for gestational age. The mean difference (95% CI) in head circumference was 1.48 (0.53–2.43) cm. Fifteen children born to 11 nonsmokers and 4 smokers had low birth weight (<2500 g). Ten were premature (<37 weeks gestation), 3 self-reported smokers gave birth at 30, 33, and 35 weeks, and 7 nonsmokers delivered at between 35 and 36 weeks. Gestational age, neonatal sex, and 1- and 5-min Apgar scores were similar for each group after stratifying by meconium nicotine results and maternal self-report status.

CORRELATING MECONIUM CONCENTRATIONS AND NEONATAL OUTCOME MEASURES

We calculated Pearson correlations for unconjugated nicotine, cotinine, OH-cotinine, norcotinine, and nornicotine concentrations and neonatal weight, head circumference, length, gestational age, and 1- and 5-min Apgar scores (Table 7). No significant correlations were found for unconjugated drug meconium concentrations and neonatal outcomes.

Discussion

These are the first data on the disposition of nicotine, cotinine, OH-cotinine, nornicotine, and norcotinine in meconium. We used a new LC-MS/MS method with sufficient LOQs for simultaneous selective analysis of these analytes. Although other methods have not reported nicotine and OH-cotinine in meconium, we found that these compounds were more

prevalent than and as abundant as cotinine (17, 19). Our results are consistent with a recent report identifying nicotine, cotinine, and OH-cotinine in meconium by HPLC with colorimetric detection. In that study, however, nicotine and cotinine were more abundant than OH-cotinine. Prevalence data were not provided (26).

Cotinine immunoassays are most commonly employed to determine tobacco exposure in meconium. In our study, however, 18.6% and 15.0% of positive specimens contained only nicotine after nonhydrolyzed and hydrolyzed analyses, respectively, whereas an additional 8% were positive for either OH-cotinine only or OH-cotinine and nicotine in combination. The low OH-cotinine concentrations in these specimens (range 1.3–7.8 ng/g) are not sufficient to generate positive results in a cotinine immunoassay with 30% OH-cotinine cross-reactivity at the standard 10 ng/g cutoff. These data suggest that immunoassays may fail to identify approximately 1 in 4 tobacco-exposed neonates.

We describe, for the first time, nicotine, cotinine, and OH-cotinine glucuronides in meconium. Cotinine and OH-cotinine are significantly glucuronide bound, whereas nicotine is not. Dempsey et al. (18) observed that cotinine detection rates after basic hydrolysis increased from 33% to 79% at a 50 ng/g cutoff using microplate enzyme immunoassay but attributed increases to reversing Schiff base bonds formed between free amino functions on meconium proteins and cotinine, not cotinine glucuronide cleavage. Interestingly, in our study, only 1 additional meconium specimen was identified as positive following hydrolysis. The low LOQ could contribute to the improved ability to detect unconjugated nicotine biomarkers. These data support that time-consuming and costly enzymatic hydrolysis can be eliminated without markedly compromising detection of tobacco-exposed infants.

Hydrolysis yielded higher analyte concentrations in 62.5%, 89.6%, and 92.0% of nicotine-, cotinine-, and OH-cotinine-containing specimens, respectively. In nearly all cases where unconjugated drug concentrations were higher than total drug values, the difference was within the method imprecision. Moreover, 2 separate meconium aliquots were analyzed for unconjugated and total drug concentrations, and despite extensive mixing before weighing, complete homogeneity may not have been achieved.

OH-cotinine total and glucuronide results should be interpreted with caution, as hydrolysis efficiency of authenticated OH-cotinine-*O*-glucuronide was determined to be 15% during method validation. Possibly, the high degree of OH-cotinine glucuronidation observed in authentic meconium could be due to the presence of *N*-glucuronide or diglucuronide species, as well as OH-cotinine-*O*-glucuronide. Human liver microsomes incubated with OH-cotinine are capable of forming both *N*- and *O*-glucuronides, although OH-cotinine-*N*-glucuronide has not been reported in adult urine (27, 28).

Statistically significant differences in meconium cotinine and OH-cotinine concentrations were observed between exposed- and nonexposed neonates. These results are consistent with studies showing differences in meconium cotinine concentrations from neonates born to smoking and nonsmoking mothers. Ostrea et al. (19) reported significantly higher mean nicotine metabolite concentrations in meconium of neonates born to passive (31.6 $\mu\text{g/L}$), light (34.7 $\mu\text{g/L}$), and heavy (54.6 $\mu\text{g/L}$) smokers compared to non-smokers (10.9 $\mu\text{g/L}$) after cotinine RIA analysis with 38%–55% OH-cotinine cross-reactivity. Similarly, Sherif et al. (20) differentiated nonsmokers [mean (SD), 185.0 (24.2) $\mu\text{g/L}$] from passive [263.4 (52.5) $\mu\text{g/L}$] and active [367.2 (143.7) $\mu\text{g/L}$] smokers based on meconium cotinine concentrations by RIA. Employing liquid chromatography with colorimetric detection, Kohler et al. (26) reported mean (SD) nicotine, cotinine, and OH-cotinine concentrations of 105.3 (90.0), 111.2 (68.8), and 53.2 (43.8) ng/g, respectively, in meconium of 38 neonates exposed throughout gestation as confirmed by maternal urinalysis, but detected no analytes in

nonexposed neonates' meconium. Similarly, Pereg et al. (21) reported a mean (SD) meconium cotinine concentration of 114.1 (2.9) ng/g in 17 tobacco-exposed neonates, and Baranowski et al. (17) observed a mean (SD) meconium cotinine concentration of 34.3 (20.0) ng/g in 20 neonates born to active and/or passive smokers, but neither detected cotinine in meconium from nonexposed neonates (26). Differences in meconium concentrations may reflect differences in study populations, smoking habits, cultural attitudes toward gestational smoking, nicotine cigarette content, and/or analytical variability, including cross-reactivity with other nicotine analytes.

Approximately 30% of participating mothers provided self-reported tobacco smoking histories. This subset of matched meconium specimens and self-reported histories was evaluated for correlations with neonatal outcomes. Additional maternal interviews could have strengthened concentration–outcome relationships. Also, smoking history was used to derive a proposed nicotine and metabolite cutoff to differentiate active from passive and nonexposed neonates.

Establishing a meconium cutoff concentration to identify maternal smoking status is desirable, as self-report is often unreliable. We investigated the presence of nicotine, cotinine, or OH-cotinine >10 ng/g in meconium as an effective cutoff, based on the concentration distribution between smoking and nonsmoking groups. Prediction efficiency of prenatal smoking exposure or nonexposure was determined at a variety of cutoff concentrations by ROC curve analysis. The 10 ng/g cutoff had the highest predictive value (93.3%) of potential cutoff concentrations based on maternal self-report. At a 10 ng/g cutoff, the sensitivity and specificity were 82.4% and 97.0%, respectively. Thirty-two mothers reported no tobacco use during pregnancy, but nicotine biomarkers were measurable in 8 meconium specimens. In 7, analyte concentrations were <10 ng/g and considered negative. The eighth specimen contained 72.4 ng/g nicotine, 30.0 ng/g cotinine, and 26.6 ng/g OH-cotinine, concentrations consistent with tobacco exposure. Maternal self-report indicated previous tobacco use but no active smoking during pregnancy. It is possible that this woman did not accurately report her smoking status or that she was exposed to high levels of environmental smoke. Three neonates in the smoking group had negative meconium results; in 2 cases, mothers reported smoking only during the first trimester, before meconium begins to form. In the third case, the woman reported smoking 1–5 cigarettes per day throughout pregnancy. It is unclear why nicotine and/or metabolites were not detected.

The cutoff concentration determination has several limitations. First, maternal self-report was defined as the gold standard. Given that self-reported tobacco consumption by pregnant women may be unreliable (as suspected in at least 1 specimen in this study), the number of false positives could increase due to under-reporting of tobacco smoking. Additionally, reported tobacco consumption at any time during pregnancy constituted “exposed,” although meconium that begins forming in the second trimester would not detect first-trimester smoking. In this instance, false negatives could be increased. These two biases could decrease, not increase, diagnostic sensitivity, specificity, and effectiveness. The 10 ng/g proposed cutoff achieved a high effectiveness of 93.3% for differentiating non-smokers and passive smokers from active smokers. Other limitations include unknown extent of environmental participant exposure. Additional evaluation of the proposed cutoff concentration is needed to confirm the effectiveness of the 10 ng/g cutoff.

Nornicotine and norcotinine are biomarkers of active maternal smoking; however, these analytes were present in only one-third of meconium specimens from tobacco-exposed neonates and never in nicotine-, cotinine-, and OH-cotinine-negative specimens, negating their value as better nicotine biomarkers. Nonetheless, it is possible that these analytes could be important predictors of other neonatal nicotine toxicities.

Few studies have correlated cotinine meconium concentrations and adverse neonatal events. Nuesslein et al. (22) associated high cotinine concentrations in meconium (that is, greater than the 36.8 $\mu\text{g/L}$ population median, as determined by RIA) with lower birth weight and increased risk of upper and lower respiratory infections during the first 6 months of life. In our study, we evaluated for the first time meconium concentrations of nicotine, cotinine, OH-cotinine, nornicotine, and norcotinine as predictors of birth weight, head circumference, length, gestational age, and 1- and 5-min Apgar scores. There were no statistically significant relationships between nicotine and/or metabolite concentrations in meconium and neonatal birth parameters, although the presence of a nicotine biomarker in meconium was associated with decreased head circumference. A limitation of these findings is the sample size of 61 neonatal outcomes. Despite the sample size, these preliminary data are important, as this is the first time that relationships between meconium nicotine and OH-cotinine concentrations and neonatal outcome parameters have been investigated. Women participating in this study were relatively light smokers (<5 cigarettes daily), which may also explain why no correlation was observed. This level of self-reported cigarette consumption is consistent with the WHO estimate of per capita cigarette consumption in Uruguay (approximately 1300 cigarettes annually or 3.4 cigarettes daily) (29). Although stronger associations between heavy smoking and adverse outcomes have been observed, Jaddoe et al. (7) noted significantly lower birth weight in neonates whose mothers smoked <5 cigarettes per day in late pregnancy compared to nonsmokers. Multiple factors affect fetal growth, including maternal health, nutrition, and drug and alcohol exposure. This study investigated only one potential influence on intrauterine development.

Whereas nicotine and metabolites were identified in meconium, metabolite origin is undetermined. Nicotine, cotinine, OH-cotinine, nornicotine, and norcotinine and glucuronides may cross the placenta and deposit in meconium. Alternatively, nicotine could cross the placenta, be biotransformed by the fetal liver, and deposit in meconium. To date, only nicotine has been experimentally shown to cross the placenta, with 90% entering fetal circulation and 1% converted to cotinine (30, 31). Placental transfer and metabolism of cotinine, OH-cotinine, nornicotine, and norcotinine should be further evaluated, since such data could highlight placental and fetal liver protective mechanisms.

In addition to cotinine, we suggest nicotine, the most prevalent analyte, and OH-cotinine, the most abundant analyte, as effective biomarkers in meconium for monitoring prenatal tobacco exposure.

Acknowledgments

Research Funding: This research was funded by the Intramural Research Program of the NIH, National Institute on Drug Abuse, and by the Generalitat Valenciana.

Role of Sponsor: The funding organizations played a direct role in the final approval of the manuscript.

We gratefully acknowledge the efforts of David Darwin from the National Institute on Drug Abuse, Hugo Míguez from CONICET, Argentina, Verónica Parodi, Adriana Menéndez Silvera, and Raúl Bustos, and all those at the Universidad de la República Uruguay who made this study possible.

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Table 1

Nicotine and metabolite concentrations in 125 paired nonhydrolyzed and hydrolyzed meconium specimens.

Analyte	LOQ, ng/g	n	% positive	Median, ng/g	Range, ng/g
Nonhydrolyzed specimens					
Nicotine	5	54	43.2	24.9	5.0–316.1
Cotinine	1.25	43	34.4	47.6	1.5–310.0
OH-cotinine	1.25	47	37.6	37.4	1.3–249.7 ^a
Norcotinine	1.25	12	9.6	3.8	1.4–13.5
Nornicotine	5	14	11.2	10.0	5.0–24.8
Hydrolyzed specimens					
Nicotine	5	53	42.4	27.4	5.2–330.6
Cotinine	1.25	46	36.8	47.8	1.3–376.0
OH-cotinine	1.25	48	38.4	49.8	1.9–336.2 ^b
Norcotinine	1.25	13	10.4	4.3	1.4–14.8
Nornicotine	5	16	12.8	10.8	5.4–24.9

^aOne concentration exceeded upper LOQ and insufficient specimen for reanalysis; data not included in median and range calculations.

^bTwo concentrations exceeded upper LOQ and insufficient specimen for reanalysis; data not included in median and range calculations.

Table 2

Prevalence of nicotine and metabolites in paired nonhydrolyzed and hydrolyzed specimens (n = 125).

Analyte	Nonhydrolyzed specimens		Hydrolyzed specimens	
	n	% of positives	n	% of positives
No analytes above the LOQ (negative specimen)	66	0	65	0
Any analyte (positive specimen)	59	100	60	100
Nicotine + cotinine + OH-cotinine	25	42.4	24	40.0
Nicotine only	11	18.6	9	15.0
All analytes	10	16.9	11	18.3
Nicotine + cotinine + OH-cotinine + nornicotine	4	6.8	5	8.3
OH-cotinine only	3	5.1	3	5.0
Nicotine + cotinine + OH-cotinine + norcotinine	2	3.4	2	3.3
Nicotine + OH-cotinine	2	3.4	2	3.3
Cotinine only	1	1.7	3	5.0
Cotinine + OH-cotinine	1	1.7	1	1.7
Nornicotine only	0	0	0	0
Norcotinine only	0	0	0	0

Table 3

Nicotine and metabolite concentrations in 49 meconium specimens grouped by maternal smoking self-report.

Analyte	Nonsmoking group (n = 32)				Smoking group (n = 17)			
	n	%	Median, ng/g	Range, ng/g	n	%	Median, ng/g	Range, ng/g
Nonhydrolyzed specimens								
No analyte	24	75.0			3	17.6		
Any analyte	8	25.0			14	82.4		
Nicotine	6	18.8	8.9	8.4–72.4	13	76.5	42.9	5.3–172.8
Cotinine	4	12.5	6.0	1.5–29.9	14	82.4	42.6	8.0–179.8
OH-cotinine	5	15.6	3.1	1.3–27.0	14	82.4	37.4	7.2–249.7
Norcotinine	0	0.0			5	29.4	4.4	1.4–4.8
Nornicotine	0	0.0			5	29.4	10.2	5.0–12.2
Hydrolyzed specimens								
No analyte	23	71.9			3	17.6		
Any analyte	9	28.1			14	82.4		
Nicotine	6	18.8	8.3	5.4–76.9	13	76.5	45.2	6.2–184.3
Cotinine	6	18.8	2.3	1.3–31.6	14	82.4	52.8	8.2–198.5
OH-cotinine	6	18.8	5.9	1.9–37.5	14	82.4	70.9	12.0–336.2
Norcotinine	0	0.0			6	35.3	3.5	1.9–5.5
Nornicotine	0	0.0			6	35.3	10.2	5.4–12.8

Table 4

Maternal age and parity classified by meconium analysis results.

Maternal parameters	Meconium results		<i>t</i> or χ^2 (<i>df</i>)	<i>P</i> value
	Negative	Positive ^a		
Age, years				
Mean (SD)	24.9 (7.0)	24.7 (7.1)	0.136 (58)	0.892
Median	24.0	23.0		
Range	13–39	15–41		
Number of children				
Mean (SD)	2.5 (1.8)	3.1 (2.5)	2.711 (46)	0.308
Median	2	2		
Range	1–8	1–10		

^aSpecimens with an analyte concentration greater than the LOQ were considered positive.

Table 5

Maternal characteristics classified by meconium analysis results.

Maternal parameters	Meconium results		<i>t</i> or χ^2 (df)	<i>P</i> value
	Negative, n (%)	Positive, n (%) ^a		
Total	32 (52.5)	29 (47.5)		
Civil status				
Married	11 (34.4)	1 (3.4)	11.085 (3)	0.011 ^b
Separated	1 (3.1)	0 (0.0)		
Not married, lives with partner	13 (40.6)	18 (62.1)		
Single	6 (18.8)	10 (34.5)		
Not available	1 (3.1)			
Educational level				
Primary school			5.363 (6)	0.498
Completed	8 (25.0)	14 (48.3)		
Not completed	3 (9.4)	1 (3.4)		
Secondary school				
Completed	1 (3.1)	1 (3.4)		
Not completed	17 (53.1)	12 (41.4)		
Tertiary studies				
Completed	1 (3.1)	1 (3.4)		
Not completed	1 (3.1)	0 (0.0)		
Other	1 (3.1)	0 (0.0)		
Working status				
Employed	3 (9.4)	3 (10.3)	0.083 (2)	0.959
Unemployed	8 (25.0)	8 (27.6)		
Housewife	21 (65.6)	18 (62.1)		
Alcohol use during pregnancy				
Yes, throughout pregnancy	0 (0.0)	1 (3.4)	5.895 (3)	0.117
Only during first trimester	2 (6.2)	3 (10.3)		
Only during second and third trimester	12 (37.5)	3 (10.3)		
No	15 (46.9)	17 (58.6)		
Not available	3 (9.4)	5 (17.2)		
Self-reported tobacco use during pregnancy				
Yes, throughout pregnancy	1 (3.1)	16 (55.5)		
Only during first trimester	2 (6.3)	1 (3.4)		
No	29 (90.6)	12 (41.4)		
Daily cigarette consumption				
1–5	1 (33.3) ^c	13 (76.5)		
5–10	1 (33.3)	4 (23.5)		

Maternal parameters	Meconium results		<i>t</i> or χ^2 (<i>df</i>)	<i>P</i> value
	Negative, n (%)	Positive, n (%) ^a		
Not available	1 (33.3)			

^a Specimens with an analyte concentration greater than the LOQ were considered positive.

^b Statistically significant.

^c Percentage of reported smokers.

Table 6
Neonatal outcomes grouped by maternal self-reported smoking status and positive meconium results.

Neonatal birth parameter	Maternal self-reported smoking status			Meconium testing results		
	Nonsmokers	Smokers	<i>t</i> or χ^2 (df)	<i>P</i> value	Negative	Positive ^a <i>t</i> or χ^2 (df) <i>P</i> value
Weight, g						
Mean (SD)	2941 (591)	2829 (609)	0.683 (58)	0.497	3006 (576)	2783 (593) 1.488 (59) 0.142
Median	2910	2898			2972	2860
Range	1945–4115	1470–3700			1945–4115	1470–3725
Head circumference, cm						
Mean (SD)	34.2 (1.8)	33.3 (2.3)	1.66 (58)	0.102	34.6 (1.7)	33.1 (2.0) 3.109 (59) 0.003 ^b
Median	34.0	34.0			34.0	33.0
Range	31.3–39.0	28.0–37.0			32.0–39.0	28.0–37.0
Length, cm						
Mean (SD)	47.5 (2.8)	47.3 (3.0)	0.192 (58)	0.848	47.9 (2.6)	46.8 (3.0) 1.536 (59) 0.130
Median	47.5	48.0			47.5	48.0
Range	42.0–52.0	40.0–50.0			42.0–52.0	40.0–50.0
Gestational age, weeks						
Mean (SD)	37.8 (1.6)	37.6 (2.6)	0.318 (26)	0.753	37.8 (1.7)	37.6 (2.2) 0.320 (59) 0.750
Median	38.0	39.0			38.0	39.0
Range	35.0–40.0	30.0–40.0			35.0–40.0	30.0–40.0
Apgar score						
1 min						
Mean (SD)	7.7 (1.9)	8.1 (1.5)	–0.915 (58)	0.634	7.8 (1.6)	7.8 (2.0) 0.200 (58) 0.841
Median	8	9			8	9

Neonatal birth parameter	Maternal self-reported smoking status				Meconium testing results		
	Nonsmokers	Smokers	<i>t</i> or χ^2 (<i>df</i>)	<i>P</i> value	Negative	Positive ^a	<i>t</i> or χ^2 (<i>df</i>)
Range	1–10	3–9			1–9	6–10	
5 min							
Mean (SD)	8.8 (1.2)	9.4 (0.9)	–1.682 (57)	0.098	9.0 (1.0)	9.1 (1.2)	–0.361 (57)
Median	9	10			9	9	
Range	5–10	7–10			6–10	5–10	

^aSpecimens with an analyte concentration greater than the LOQ were considered positive.

^bStatistically significant.

Table 7

Meconium concentration and neonatal outcome measure correlations [r (P)].

Neonatal outcome	Analyte			
	Nicotine	Cotinine	OH-cotinine	Normicotine
Weight	0.188 (0.369)	0.296 (0.181)	0.261 (0.230)	-0.541 (0.346)
Head circumference	-0.069 (0.743)	-0.028 (0.902)	0.063 (0.776)	-0.716 (0.174)
Length	0.157 (0.454)	0.277 (0.213)	0.213 (0.329)	-0.502 (0.389)
Gestational age	0.269 (0.194)	0.285 (0.198)	0.292 (0.176)	-0.365 (0.546)
Apgar score				
1 min	0.257 (0.225)	0.335 (0.138)	0.237 (0.289)	0.212 (0.731)
5 min	0.315 (0.143)	0.333 (0.140)	0.153 (0.508)	0.043 (0.945)