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Methadone, Cocaine, Opiates and Metabolite Disposition in Umbilical Cord and Correlations to Maternal Methadone Dose and Neonatal Outcomes

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Abstract

Objectives—To explore methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) umbilical cord disposition, correlate with maternal methadone dose and neonatal outcomes, and evaluate the window of drug detection in umbilical cord of *in utero* illicit drug exposure.

Methods—Subjects, 19 opioid-dependent pregnant women from two clinical studies, one comparing methadone and buprenorphine pharmacotherapy for opioid-dependence treatment, and the second examining monetary reinforcement schedules to maintain drug abstinence. Correlations were calculated for methadone and EDDP umbilical cord concentrations and maternal methadone dose, and neonatal outcomes. Cocaine- and opiate-positive umbilical cord concentrations were compared to those in placenta and meconium, and urine specimens collected throughout gestation.

Results—Significant positive correlations were found for umbilical cord methadone concentrations and methadone mean daily dose, mean dose during the 3rd trimester and methadone cumulative daily dose. Umbilical cord EDDP concentrations and EDDP/methadone concentration ratios were positively correlated to newborn length, peak neonatal abstinence syndrome (NAS) score and time-to-peak NAS score. Methadone concentrations and EDDP/methadone ratios in umbilical cord and placenta were positively correlated. Meconium identified many more cocaine and opiate positive specimens than umbilical cord.

Conclusion—Umbilical cord methadone concentrations were correlated to methadone doses. Also, our results indicate that methadone and EDDP concentrations might help to predict NAS

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severity. Meconium proved to be more suitable than umbilical cord to detect *in utero* exposure to cocaine and opiates; however, umbilical cord could be useful when meconium is unavailable due to *in utero* or delayed expulsion.

Keywords

methadone; cocaine; opiates; umbilical cord; *in utero* drug exposure

INTRODUCTION

Illicit drug consumption during pregnancy is an important public health problem. As revealed in the 2008 National Survey on Drug Use and Health, 5.1% of pregnant women aged 15 to 44 years reported illicit drug use in the month prior to the survey (1). Maternal drug-intake during gestation, in the context of other complex psychosocial stressors, may have negative effects on pregnancy outcomes, and fetal and child development (2-7).

Methadone-assisted therapy is recommended by the Center for Substance Abuse Treatment and The American Academy of Pediatrics for opioid-dependent pregnant women, reducing fetal exposure to maternal illicit drug use and other maternal risk behaviors, and improving obstetrical care and neonatal outcomes (8,9,9). However, there are consequences for the infant, with the most serious being neonatal abstinence syndrome (NAS). NAS is defined as a grouping of signs indicating alteration in CNS, ANS, gastrointestinal and respiratory functioning. NAS develops in 70-90% of methadone-exposed infants (10), with 45-60% requiring medication treatment (11-13).

Pharmacokinetics of methadone, heroin and cocaine can be explained, in part, by gene polymorphisms encoding drug-metabolizing enzymes and drug transporters. Methadone is biotransformed in the liver to the inactive metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine (EMDP), and minor metabolites methadol and normethadol that contribute to methadone's activity to a small extent (14). The main enzymes involved are cytochrome P450 (CYP) 3A4, and to a lesser extent, 2B6 (14-16), the later enzyme showing stereoselectivity towards the (S)-enantiomer (17). Genetic polymorphisms of CYP2B6 may be due to ethnic and sex differences (15,18). Heroin is rapidly metabolized to 6-acetylmorphine (6AM) and morphine by hepatic carboxylesterase-1 (hCE-1), carboxylesterase-2 (h C E-2) and plasmatic pseudocholinesterase (19). Morphine is further glucuronidated to morphine-3- and morphine-6-glucuronides by UDP-glucuronyltransferases, mainly UGT2B7, whose genetic polymorphism is implicated in the inter-individual variability in morphine response at a given dose (20,21). A minor morphine metabolite is formed by N-demethylation to normorphine mainly by CYP3A4, and CYP2C8 to a lesser extent (19). Codeine is O-dealkylated to morphine by CYP2D6. Genetic polymorphisms of CYP2D6 are responsible for abnormally high concentrations of morphine following codeine administration in some individuals, to low morphine concentrations in others (22,23). However, codeine's major metabolic pathway is N-demethylation to norcodeine by CYP3A4, which is further glucuronidated to codeine-6-glucuronide by UGT2B4 and 2B7 (19,24). Cocaine is biotransformed to its main metabolite benzoylecgonine (BE) by hCE-1, and to ecgonine methyl ester (EME) by pseudocholinesterase and hCE-2. Other important metabolites include norcocaine, which can follow two metabolic routes catalyzed by CYP3A4 or CYP3A4 and flavin containing monooxygenase, respectively, and cocaethylene, which is formed in the presence of ethanol and hCE-1 (19).

In addition to genetic polymorphism in metabolic enzymes, variation in drug transporter activity also could play an important role in drug disposition. P-glycoprotein is a

transmembrane efflux transporter for methadone and other opioids including morphine, whose expression and function is influenced by physiological and environmental factors, induction or inhibition by other substrates, as well as genetic polymorphism in the gene encoding this protein, the multidrug resistance 1 (ABCB1) gene (25)

For safety and ethical reasons, it is difficult to study drug disposition in the maternal-fetal dyad. Controlled methadone administration to opioid-dependent women provides a model for evaluating drug deposition into umbilical cord and other matrices. We prospectively collected urine specimens three times a week throughout pregnancy to monitor relapse to opiates and cocaine, and collected matched meconium, umbilical cord, and placenta at birth. Positive urine test results defined the timing of illicit drug use, and percentage of positive urine specimens provided a relative measure of drug exposure frequency. These unique data defined the window of drug detection of opiates and cocaine in the different biological matrices, and permitted a comparison of the relative efficacy of these matrices for identifying prenatal drug exposure.

Detecting *in utero* drug exposure by testing biological matrices can be an important tool for assisting in the identification of women in need of drug treatment and neonates susceptible to NAS who may require specialized treatment and a longer hospital stay, and for initiating investigations into the welfare of all children in the home. Meconium is the matrix of choice for drug testing due to its easy, non-invasive collection and wide window of drug detection, reflecting prenatal exposure primarily from the 3rd trimester of pregnancy (26,27). However, in some circumstances, meconium collection is difficult, as expulsion can be delayed several days in premature babies and, in cases of fetal distress, discharge may occur before delivery. Umbilical cord was suggested as an alternative to meconium, due to its availability immediately after delivery, and sample amount. Montgomery et al. reported comparable sensitivity in identifying gestational drug exposure in matched meconium and umbilical cord specimens from women at high risk for illicit drug use (28). However, the window of drug detection in umbilical cord has not been adequately evaluated.

The aims of this research were to assess the disposition of methadone and its main metabolite, EDDP, in umbilical cord specimens from opioid-dependent pregnant women receiving methadone-assisted therapy, and to evaluate correlations between umbilical cord methadone and EDDP concentrations and methadone maternal dose and neonatal outcomes. In addition, cocaine and opiate results in umbilical cord were compared to those in matched placenta and meconium, and in urine specimens collected throughout pregnancy to evaluate the usefulness of this alternative matrix to detect *in utero* drug exposure.

MATERIALS AND METHODS

Human participants and methadone administration

Participants were recruited from two different clinical trials (A and B) conducted by the Center for Addiction and Pregnancy (CAP), Johns Hopkins Bayview Medical Center in Baltimore, Maryland. The aim of study A was to compare different schedules of monetary reinforcement for maintaining cocaine and opioid abstinence in opioid-dependent pregnant women (29). Study B compared methadone and buprenorphine pharmacotherapy for opioid-dependence treatment during pregnancy (30). These studies were approved by the Johns Hopkins Bayview Medical Center and the National Institute on Drug Abuse Institutional Review Boards, and all participants provided written informed consent.

Patients prescribed methadone generally received 30, 40, 50 and 60 mg on days 1-4, respectively. Additional increases of 5 or 10 mg were available based upon clinical indications.

Neonatal outcome measures

Estimated gestational age at birth (EGAB), physical birth parameters (weight, length and head circumference), Apgar scores at 1 and 5 minutes and NAS scores were collected at birth. NAS was systematically assessed for 10 days using a 19-item modified Finnegan Scale (30,31). The number of NAS observations varied from 6 to 8 per day while in the hospital (average±SD days in hospital 6.2±4.4; median 5), to 2 per day once discharged to the research unit. Time to NAS onset (h) was defined as the time from birth until the first score >4. A score of 4 was selected as the cutoff based on clinical experience and preliminary blinded-condition comparison data from drug-exposed and non-exposed neonates (26). Peak NAS score was defined as the highest score obtained, and time-to-peak (h) was calculated from time of birth to peak NAS score. NAS duration (h) was defined as the time from first score >4 to time after which all scores were <5. Infants were treated with morphine sulfate drops to reduce NAS if modified Finnegan score exceeded 9 on two consecutive measurements.

Umbilical cord collection, analysis and disposition of analytes in umbilical cord

Umbilical cord specimens were collected at delivery and stored at -20°C until analysis. Simultaneous methadone, EDDP, morphine, codeine, 6AM, cocaine and BE quantification in umbilical cord was performed with a fully validated liquid chromatography ion-trap mass spectrometry method (32). The distribution of analytes in umbilical cord at two locations, close to the fetus and close to placenta, was determined in seven placenta specimens prior to analysis of the remaining specimens.

Placenta, meconium and urine analyses

Methadone, EDDP, morphine, codeine, 6AM, cocaine and BE were quantified in placenta by LC-ion trap-MS (33). Briefly, placenta was homogenized with 0.1% perchloric acid in a blender, centrifuged, and supernatant subjected to solid phase extraction with Strata™ XC cartridges. Limits of quantification (LOQs) concentrations were 10 ng/mL for methadone and 2.5 ng/mL for the other analytes. Method imprecision was <9.1% (n=20) and analytical recovery ranged from 84.4 to 113.3% (n=20) for all analytes. Quantification of methadone, EDDP, morphine, codeine, 6AM, hydromorphone, hydrocodone, oxycodone, BE, cocaethylene and m-OHBE in matched meconium specimens was performed by two previously published analytical methods (34,35) and at United States Drug Testing Laboratories (Des Plaines, IL, USA). Depending on the analytical method, meconium was homogenized by ultrasonication with methanol, or simply vortexed with methanol and 0.01% formic acid, centrifuged, and the supernatant extracted with Clean Screen ZSDAU020 cartridges. LOQs were from 1 to 5 ng/g, depending on the analyte. Method imprecision was <17% (n=20) and analytical recovery was within 85-123% (n=20). Thrice-weekly urine specimens were collected throughout pregnancy by CAP staff. On-site urine analysis for opiates (morphine) and cocaine (BE) was performed with the Abuscreen On-Track Rapid Assays for Drug Abuse (Roche Diagnostic Systems®, Indianapolis, Indiana, USA) (36), with cutoffs of 300 ng/mL. All specimens were stored at -20°C until analysis.

Statistical analysis

All statistical analyses were performed with SPSS 18.0 version for Macintosh. The Kolmorogov-Smirnov test was employed to evaluate normal data distribution. Pearson correlations evaluated relationships between umbilical cord methadone and EDDP concentrations and EDDP/methadone concentration ratios, and maternal methadone dose, neonatal outcomes (except for the Apgar score at 5 min, for which Spearman correlations were applied due to non-normality of data), and methadone and EDDP placenta and

meconium concentrations. Statistical probability *(p)* <0.05 was considered statistically significant.

RESULTS

Participant demographics and methadone dosing information

Table 1 shows maternal demographic characteristics and methadone dosing information. Of 19 women, 68% were African-American, 21% Caucasians, 5% biracial and 5% other race not specified. Mean±SD estimated gestational age at admission (EGAA) was 21±6 weeks. Mean maternal age was 29±5 years. Mean number of cigarettes smoked per day was 11±10. All were single births for a total of 19 infants. Mean (range; median) methadone daily dose throughout gestation was 68±17 mg (30-100; 70), and in the 3rd trimester 72±19 mg (30-103; 74). Cumulative dose throughout gestation and in the 3rd trimester showed higher inter-individual variability, ranging from 1715 to 13335 mg (7931±3463; 8320) and from 1200 to 8100 mg (5247±2047; 5205), respectively. Women were in the study an average of 118±38 days (53-200; 113).

Neonatal outcomes

Table 2 shows neonatal outcome measures, including EGAB, weight, head circumference, length, Apgar scores at 1 and 5 min, time to NAS onset, peak NAS score, time to peak NAS score, NAS duration and %NAS scores >4. Approximately half (52.6%) were delivered full-term (≥37 weeks), with a mean EGAB of 36.3±3.4 weeks. Three of four infants considered to be low birth weight (<2500 g) also had smaller head circumferences (<32 cm), and all infants of shorter than normal length (<45 cm) were pre-term. Smoking severity might have affected EGAB, but not other measured neonatal parameters. Only one out of the 5 newborns from non-smoking mothers (20%) was not delivered full-term and had birth weight, length and head circumference values below normal. The percentage of preterm newborns was increased to 64.3% (9 out of 14 newborns) within those whose mothers smoked during pregnancy; however, birth weight, length and head circumference were below normal values in only 4 of the newborns from smoking mothers (28.6%), being 2 of them prematurely delivered. All infants experienced NAS, although only 6 required treatment. Apgar scores at 1 and 5 min were normal (≥7 in all cases).

Umbilical cord methadone and EDDP disposition

Methadone and EDDP concentrations at the ends of the umbilical cord closest to the placenta (UC-placenta) and the fetus (UC-fetus) were quantified in 7 cases to determine if biomarkers were homogenously distributed (Table 3). Methadone and EDDP concentrations varied less than 31.3% in the two umbilical cord locations, suggesting similar concentrations throughout the cord. As a consequence, one intermediate location was analyzed for the remaining 12 specimens.

Methadone and EDDP were measurable in all umbilical cord specimens from participants receiving methadone pharmacotherapy. Methadone concentrations ranged from 29.7 to 262.2 ng/g (mean±SD 140.3±59.8; median 151), and EDDP from 8.2 to 240.8 ng/g (65.6±50.2; 52.8), as shown in Table 4. Methadone concentrations were, in general, 2-4 fold greater than EDDP's, with mean EDDP/methadone ratios 0.51±0.3, with the exception of participants 4 and 7, for whom similar concentrations were found for both analytes, and participant 8, for whom EDDP concentration was slightly higher than that of methadone. Table 4 also includes EDDP/methadone concentration ratios in umbilical cord specimens (n=19).

Correlations between methadone and EDDP concentrations in umbilical cord and maternal methadone doses

Pearson correlation coefficients were calculated for methadone and EDDP concentrations, and EDDP/methadone concentration ratios in umbilical cord and initial methadone dose, dose at delivery, mean daily dose, mean dose during the 3rd trimester, methadone cumulative daily dose and cumulative dose during the 3rd trimester. Statistically significant positive correlations were found for methadone concentrations in umbilical cord and methadone mean daily dose (r=0.515; p=0.024), mean dose during the 3rd trimester (r=0.563; p=0.012) and methadone cumulative dose (r=0.535; p=0.018).

Correlations between umbilical cord methadone and EDDP concentrations and neonatal outcomes

Pearson correlation coefficients were calculated for methadone and EDDP concentrations, and EDDP/methadone concentration ratios in umbilical cord, and neonatal weight, length, head circumference, Apgar scores at 1 and 5 min, time to NAS onset, peak NAS score, time to peak NAS score, NAS duration and %NAS score >4 (n=19). EDDP concentrations in umbilical cord and EDDP/methadone concentration ratios were statistically positively correlated to newborn length (r=0.536, p=0.018 and r=0.487, p=0.034, respectively), peak NAS score (r=0.596, p=0.007 and r=0.579, p=0.009, respectively) and time to peak NAS score (r=0.506, p=0.027 and r=0.589, p=0.008, respectively).

Correlations between concentrations of methadone and EDDP in umbilical cord, placenta and meconium specimens

Table 4 also contains methadone and EDDP concentrations and their ratios in matched placenta and meconium specimens. However, only 17 matched meconium specimens were available, and for two of them (participants 6 and 7) the amount of specimen only allowed methadone and EDDP determination (Tables 4 and 5). Methadone also was the primary analyte in placenta, while EDDP was present in much higher concentrations than the parent drug in meconium. Placental methadone and EDDP concentrations were higher than in umbilical cord, with placenta/umbilical cord methadone concentration ratios (mean±SD; median) of 10.7±3.6; 10.6 and for EDDP 2.7±2.3; 1.9. Much higher concentrations were found in meconium, with meconium/umbilical cord concentration ratios of 60.6±52.4; 52.0 for methadone and 762.1±468.3; 689.7 for EDDP. EDDP/methadone concentration ratio was 0.11±0.05; 0.10 in placenta, 0.51±0.30; 0.41 in umbilical cord, and 11.8±17.2; 6.3 in meconium.

Pearson correlation coefficients were calculated for methadone and EDDP concentrations, and EDDP/methadone concentrations ratios in matched umbilical cord, placenta and meconium specimens. Statistically significant positive correlations were observed for methadone concentrations and EDDP/methadone concentration ratios in umbilical cord and placenta (r=0.694, p=0.001 and r=0.639, p=0.003, respectively). A statistically significant positive correlation also was noted for EDDP concentrations in umbilical cord and methadone concentrations in meconium (r= 0.530, p= 0.029).

Cocaine, opiates and metabolites in umbilical cord, placenta, meconium and urine specimens

Matched placenta, meconium and thrice-weekly 2nd and 3rd trimester urine specimen results were available for 19, 15 and 17 participants, respectively (Table 5). Percentages of positive urine specimens in the 2nd and 3rd trimester, and time between the last positive urine specimen and birth also are included in Table 5. Only one matched umbilical cord and placenta specimen (participant 16) was positive for cocaine (7.3 ng/g in placenta) and/or its

metabolite benzoylecgonine (442.4 and 458.9 ng/g in umbilical cord and placenta, respectively); whereas, prenatal exposure to cocaine was confirmed in 11 matched meconium specimens by the presence of the cocaine metabolite, m-hydroxybenzoylecgonine (mOHBE). Urine tests were positive for cocaine in the 2nd or 3rd trimester in 8 of these women; however, for participant 8, all urine specimens were negative for cocaine. In all matrices, cocaine metabolite concentrations were higher than the parent drug.

Opiates were identified in only one umbilical cord and placenta specimen (participant 16) by the presence of morphine (40.3 and 39.8 ng/g in umbilical cord and placenta, respectively) and lower codeine concentrations (3.6 and 2.9 ng/g in umbilical cord and placenta, respectively), but no 6-acetylmorphine (6AM) was detected. Eight meconium specimens were positive for morphine, and one also for codeine (Table 5). Surprisingly, meconium from the infant with positive umbilical cord and placenta results was negative for opiates. Urine test results confirmed *in utero* opiate exposure in all cases.

Urine test results were negative for opiates and cocaine throughout gestation in one and nine participants, respectively. Seven participants had positive urine specimens for opiates in the 3rd trimester, and five for cocaine; however, none of the matched umbilical cord specimens verified *in utero* exposure to these analytes. Umbilical cord from participant 16 confirmed opiate and cocaine exposure during pregnancy, but the window of drug detection could not be determined, as urine data from the 3rd trimester were not available for this participant.

DISCUSSION

We describe for the first time methadone and EDDP disposition in umbilical cord from opioid-dependent pregnant women receiving methadone-assisted pharmacotherapy. In general, similar concentrations of methadone and EDDP were found in the maternal and fetal ends of the umbilical cords. High inter-individual variability in methadone and EDDP umbilical cord concentrations (%CV 59.8% and 50.2%, respectively) can be explained by differences in maternal methadone doses and duration of dosing; differences in maternal age and race also might play a role in the high inter-individual variability. In general, methadone concentrations were higher than EDDP, with a mean EDDP/methadone concentration ratio of 0.51±0.3.

Methadone concentrations in umbilical cord were positively correlated to methadone mean daily dose, mean dose during the 3rd trimester and cumulative dose. To our knowledge, dose-concentration relationships for maternal methadone dose and methadone and EDDP umbilical cord concentrations were never reported. However, correlations between maternal methadone dose and methadone concentrations in maternal plasma (37-41) and umbilical cord blood (38,41) were previously evaluated, reporting discrepant results.

A significant positive correlation also was noted for EDDP umbilical cord concentrations and EDDP/methadone concentration ratios and neonatal length. As previously described, greater maternal methadone doses were associated with higher umbilical cord methadone and EDDP concentrations. We suggest that the positive correlation between EDDP and neonatal length is reflecting an improvement in neonatal outcome in babies from pregnant women receiving appropriate methadone-assisted therapy, and perhaps lower exposure to illicit drugs. Umbilical cord EDDP/methadone ratio also was positively correlated with neonatal length. Methadone placenta accumulation was much higher than for EDDP; therefore, higher methadone doses will also produce greater umbilical cord EDDP/methadone concentration. Supporting our hypothesis, neonates from pregnant women receiving methadone-assisted therapy had improved neonatal growth parameters as compared to those whose mothers abused heroin and did not receive this treatment (42-44).

Almost a third (31.6%) of newborns were treated for NAS with morphine sulfate drops, which could have influenced NAS scoring for these newborns. Despite treatment potentially lowering peak NAS and altering time to NAS peak, umbilical cord EDDP concentrations and EDDP/methadone concentration ratios were significantly correlated to peak NAS score and time-to-peak NAS score. Therefore, it may be possible to predict NAS intensity based on umbilical cord concentrations.

Umbilical cord concentrations were not previously correlated to neonatal parameters; however, others investigated correlations between neonatal plasma methadone concentrations and NAS intensity. Mack et al. did not find a relationship between neonatal methadone plasma concentrations and NAS intensity (39). In contrast, Harper et al. (41) reported a positive correlation between methadone dose or plasma concentration and NAS severity. Our results supported findings of others, where NAS intensity associated with lower neonatal methadone plasma concentrations (38,45) a higher rate of decline in neonatal methadone levels (37), or both (46).

Methadone and EDDP concentrations in umbilical cord were compared to matched placenta and meconium concentrations. As in umbilical cord, methadone was the predominant placenta analyte; however, mean placenta methadone concentrations were 10.7±3.6 times as great as those in umbilical cord. Mean EDDP/methadone ratio in placenta was only 0.11±0.05. Umbilical cord EDDP concentrations were about half those in placenta (placenta/umbilical cord EDDP ratio 2.7±2.3). Methadone placental metabolism was reported to be approximately 1% (47). Although methadone metabolism in umbilical cord may have occurred, it is more likely that the much lower umbilical cord methadone concentrations were due to low placental transfer. This also is supported through *in vitro* experimentation by Nekhayeva et al., suggesting placental methadone accumulation (47). Furthermore, methadone plasma concentrations (70-660 ng/mL) in pregnant women receiving 5 to 100 mg daily methadone doses (37-39,41,48) were higher than those reported in umbilical cord blood (17-250 ng/mL) (38,39,41,48,49), and umbilical cord tissue in our study (29.7-262.2 ng/mL). These methadone concentrations were much lower than those we found in placenta (308-2647 ng/mL), reflecting placental accumulation. As opposed to umbilical cord and placenta, EDDP was the predominant biomarker in meconium. Moreover, concentrations of both analytes in meconium were much higher than those in the other matrices, reflecting meconium accumulation throughout gestation, and subsequent metabolism to the inactive metabolite.

Correlations between methadone and EDDP concentrations in matched umbilical cord, placenta and meconium specimens also were evaluated. Although methadone transfer from placenta appears to be low, placenta and umbilical cord methadone concentrations, and EDDP/methadone ratios were strongly correlated. A statistically significant positive correlation also was found for EDDP umbilical cord concentrations and methadone meconium concentrations. The reason for this correlation is not clear. We hypothesize that higher methadone meconium concentrations result from higher methadone doses and, therefore, higher methadone (and EDDP) concentrations in umbilical cord. Methadone meconium concentrations were significantly correlated to EDDP, but not to methadone umbilical cord concentrations, most likely due to limited methadone placental transfer.

In addition, cocaine and opiate umbilical cord concentrations were determined. BE was found in only one umbilical cord specimen, confirming *in utero* cocaine exposure. Likewise, Moore et al. (50) reported significant BE concentrations in the umbilical cord of a woman with a positive urine cocaine test at delivery. Cocaine was not detected in this umbilical cord. Winecker et al. (51) analyzed umbilical cord tissue from pregnant women admitting cocaine consumption during gestation, and found BE as the predominant analyte, followed

by norcocaine, mOHBE and ecgonine methyl ester; trace amounts of cocaine were only found in one specimen. In the present study, similar BE concentrations were observed in matched placenta specimens, but low cocaine concentrations also were noted. Thus, placental cocaine transfer also appeared to be low. Opiate and metabolite disposition in umbilical cord were not previously quantified. We found morphine concentrations 10 times higher than those of codeine in the only positive opiate umbilical cord specimen, at concentrations comparable to those in the matched placenta specimen.

To evaluate the usefulness of umbilical cord to detect *in utero* cocaine and opiate exposure, umbilical cord concentrations were compared to those in matched meconium and urine specimens. Meconium testing identified *in utero* cocaine exposure in 11 participants, with 63.7% containing only mOHBE. Although this metabolite was not included in the umbilical cord analytical method, it is unlikely that this analytical difference contributed to fewer umbilical cord positive findings based on Winecker et al. results showing BE as the primary analyte (51). With regard to opiates, morphine was detected in 8 meconium specimens, one also positive for codeine, while none of the matched umbilical cord specimens were positive. Surprisingly, the matched meconium specimen for the only morphine- and codeine-positive umbilical cord specimen (participant 16) tested negative for opiates. All participants with the last cocaine or opiate positive urine test in the 3rd trimester had positive meconium results. The drug detection window in umbilical cord could not be established, as matched urine data from the third trimester were not available for the only cocaine and opiate positive umbilical cord specimen (participant 16). Umbilical cord specimens were negative even when the last positive urine test was just 3 or 13 days before delivery (participants 9 and 11), while meconium results (participant 11) indicated maternal cocaine and opiate consumption. These results might reflect a shorter window of drug detection in umbilical cord than in meconium. Sensitive umbilical cord analytical LOQs should have compensated for the much lower drug concentrations in this biological matrix.

Our results demonstrate that meconium detects more easily *in utero* drug-exposed neonates than umbilical cord, in contrast to results reported by Montgomery et al. (28). These authors screened for cocaine, opiates, amphetamines, cannabinoids and phencyclidine in matched meconium and umbilical cord specimens from infants of pregnant women suspected of illicit drug-intake, finding >90% agreement in these matrices for all analytes. This could be due to a short period of time from last maternal drug consumption to delivery for Montgomery's study participants, higher limits of quantification, or few positive specimens. In addition, the authors did not report any data on metabolites analyzed or measured concentrations in each matrix. The authors also extended their findings by screening and confirming 500 umbilical cord specimens from women suspected of illicit drug consumption (52), obtaining negative and positive predictive values >98% and >70%, respectively, for methamphetamine, cocaine, opiates, cannabinoids and phencyclidine. Their data supported the efficacy of this alternative matrix for detection of fetal drug exposure; however, data were not available to establish the window of drug detection in umbilical cord. Marin et al. (53) determined nicotine and metabolites in matched meconium and umbilical cord specimens from women with first or second-hand tobacco exposure history during pregnancy. The authors concluded that both specimens could be applied for the detection of *in-utero* exposure to nicotine in the third trimester; however, concentrations of all analytes were generally greater in meconium and, specifically, nicotine concentrations were 3.7 to 60.7-fold higher in meconium (mean= 150.8 ng/g; median= 114 ng/g) than in umbilical cord (mean= 4.3 ng/g; median= 3.8 ng/g). These much higher concentrations of parent drug in meconium suggest accumulation of nicotine in this matrix and, therefore, a longer window of detection than in umbilical cord may be expected.

CONCLUSION

These data describe for the first time methadone and EDDP concentrations in umbilical cord specimens from women receiving methadone-assisted therapy, and a statistically significant correlation for methadone umbilical cord concentrations and maternal methadone doses. Also, we report preliminary data suggesting that umbilical cord methadone and EDDP concentrations predict NAS severity. Finally, our results indicate that meconium is better suited to confirm *in utero* drug exposure to cocaine and opiates compared to umbilical cord due to the lower concentrations found in this biological matrix and, probably, to its shorter window of drug detection.

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Table 1

Maternal demographics and methadone dosing data (n=19).

Subject	Age	Race	# cigarettes/day	EGAA (weeks)	Days in study	Initial dose (mg)	Dose at delivery (mg)	Mean daily dose (mg/day)	Mean 3rd trimester daily dose (mg/day)	Cumulative dose (mg)	Cumulative 3rd trimester dose (mg)
1	32	AA	20	25	53	50	30	29.6	30.0	1,715	1,200
2	23	O	10	28	76	20	60	37.9	37.9	3,070	3,070
3	34	AA	10	27	89	60	85	69.9	69.9	3,215	3,215
4	35	C	0	29	65	60	80	67.5	67.5	4,590	4,590
5	33	AA	8	25	104	30	45	44.7	45.0	4,695	4,230
6	32	AA	20	14	102	30	75	71.5	75.0	6,010	1,800
7	19	C	20	20	121	30	65	63.1	64.5	6,565	6,195
8	31	AA	8	24	81	50	90	79.2	86.8	6,815	5,205
9	21	C	40	20	113	40	70	70.0	73.7	7,980	4,495
10	30	AA	0	23	124	50	70	65.5	68.6	8,320	6,650
11	25	AA	14	22	102	40	95	80.5	85.7	8,450	5,910
12	19	AA	10	17	176	30	60	59.5	60.0	8,980	4,800
13	34	AA	3	24	127	50	80	70.5	74.7	9,300	7,990
14	31	AA	0	10	132	30	90	73.6	89.7	9,795	3,945
15	34	AA	10	23	111	90	110	100.4	102.5	11,245	8,100
16	25	B	20	20	141	40	70	81.3	86.1	11,540	7,405
17	26	C	20	17	139	40	100	86.5	97.0	12,110	6,690
18	30	AA	0	16	176	40	85	74.1	81.2	12,964	8,040
19	32	AA	0	11	200	30	70	66.3	70.0	13,335	6,160
Mean	28.7	-	11.2	20.8	117.5	42.6	75.3	68.0	71.9	7,931.3	5,246.8
SD	5.3	-	10.4	5.5	38.4	15.9	19.0	16.6	19.0	3,462.7	2,046.7
Median	31.0	-	10.0	22.0	113.0	40.0	75.0	70.0	73.7	8,320.0	5,205.00

Table 2

Neonatal outcome measures for the 19 *in utero* methadone-exposed infants. Normal estimated gestational age at birth (EGAB) is ≥ 37 weeks, normal weight≥2,500 g, normal head circumference≥ 32 cm and normal length≥ 45 cm. Bolded text indicate below normal parameters.

Subject	Mother smoker	EGAB (weeks)	Weight (g)	Head circumference (cm)	Length (cm)	1 min Apgar Score	5 min Apgar Score	Time to NAS onset (h)	Peak NAS score	Time-to-peak NAS score (h)	NAS duration (h)	%NAS Scores>4
1	YES	**32.0**	**2,080**	32	45.0	9	9	6.3	10	119.6	113.3	66.7
2	YES	**34.0**	2,745	33	48.0	8	8	-*	4	16.5	-*	-*
3	YES	**32.0**	2,995	33	49.0	8	9	3.5	14	89.5	341.0	89.7
4	NO	37.0	3,110	34	50.0	7	9	30.0	15	75.8	58.5	12.7
5	YES	40.4	4,370	36	51.0	8	9	12.7	6	30.7	45.0	28.6
6	YES	**30.4**	**1,505**	**26**	**41.0**	9	9	13.0	10	13.0	96.0	31.6
7	YES	40.0	4,025	35.5	49.0	9	9	26.0	9	69.7	59.5	47.8
8	YES	**36.0**	3,650	33	52.5	9	9	28.5	19	194.0	289.5	66.7
9	YES	**35.0**	2,740	32	50.0	8	8	54.0	6	54.0	22.0	18.5
10	NO	39.0	3,760	34.5	51.0	9	9	23.5	11	30.5	86.7	55.8
11	YES	**31.0**	2,850	33	47.0	8	9	60.7	8	80.7	154.5	51.4
12	YES	38.3	**2,385**	**30.5**	**43.5**	8	9	19.4	8	61.1	61.7	69.6
13	YES	40.0	2,675	34	48.0	8	9	127.8	7	166.3	49.0	6.3
14	NO	**33.3**	**2,125**	**30**	**43.5**	8	9	40.4	8	40.4	12.0	25.0
15	YES	38.0	2,515	**30**	48.0	9	9	23.2	6	23.2	48.0	13.6
16	YES	39.3	2,865	32	46.0	8	8	19.0	7	30.0	556.0	84.5
17	YES	**35.0**	3,180	34	52.0	7	9	13.3	14	77.3	320.5	90.1
18	NO	41.0	3,140	34	51.0	8	9	16.0	8	16.0	25.0	66.7
19	NO	38.9	4,810	36	52.0	9	9	38.2	11	60.0	22.2	22.2
Mean	-	36.3	3,027.6	32.8	48.3	8.3	8.8	30.9	9.5	65.7	131.1	47.1
SD	-	3.4	816.3	2.4	3.3	0.7	0.4	28.6	3.8	49.8	148.5	28.0
Median	-	37.0	2,865.0	33.0	49.0	8.0	9.0	23.4	8.0	60.0	60.6	49.6

NAS: neonatal abstinence syndrom

* No data on time to NAS onset, NAS duration and %NAS scores >4 for participant 16.

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Table 3

Mean, standard deviation and coefficient of variation (%CV) for methadone (MTD) and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) concentrations in the umbilical cord closest to the placenta (UC-placenta) and closest to the fetus (UC-fetus) in 7 participants.

Subject	Analyte	UC-placenta (ng/g)	UC-fetus (ng/g)	Mean±SD	%CV (n=2,%)
1	MTD	24.0	35.3	29.7±8.0	27.0
	EDDP	10.3	6.5	8.2±2.6	31.3
2	MTD	127.8	133.3	130.6±3.9	3.0
	EDDP	75.2	107.3	89.5±22.3	24.9
3	MTD	87.7	114.5	101.1±18.9	18.7
	EDDP	67.7	77.5	71.2±6.8	9.6
4	MTD	52.7	54.4	53.5±1.2	2.2
	EDDP	57.9	49.8	52.8±5.6	10.7
10	MTD	174.1	127.9	151.0±32.6	21.6
	EDDP	62.2	49.0	54.5±9.1	16.8
11	MTD	96.3	120.9	108.6±17.4	16.0
	EDDP	49.7	40.9	44.4±6.1	13.7

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Table 4

Methadone (MTD) and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) concentrations and EDDP/MTD ratios in 19 umbilical cord and matched placenta and meconium specimens from pregnant women receiving methadone-assisted pharmacotherapy.

	Umbilical Cord (ng/g)			Placenta (ng/g)			Meconium (ng/g)		
Subject	**MTD**	**EDDP**	**EDDP/MTD**	**MTD**	**EDDP**	**EDDP/MTD**	**MTD**	**EDDP**	**EDDP/MTD**
1	29.7	8.2	0.28	308.0	35.9	0.12	2,555	10,546	4.1
2	130.6	89.5	0.69	1,553.9	147.8	0.10	4,997	28,628	5.7
3	101.1	71.2	0.70	537.3	78.6	0.15	7,281	57,600	7.9
4	53.5	52.8	0.99	549.2	96.1	0.18	5,481	34,753	6.3
5	118.1	74.4	0.63	1,092.4	105.6	0.10	6,373	44,369	7.0
6	193.2	45.4	0.24	2,647.0	517.4	0.20	2,184	53,142	24.3
7	62.2	51.3	0.83	405.2	71.7	0.18	85	6,375	75.0
8	202.6	240.8	1.19	1,538.2	312.3	0.20	11,334	53,800	4.7
9	262.2	42.9	0.16	1,372.1	105.5	0.07	-	-	-
10	151.0	54.5	0.36	1,511.6	120.7	0.08	5,785	32,826	5.7
11	108.6	44.4	0.41	1,048.6	90.4	0.09	5,009	64,800	12.9
12	98.0	40.3	0.41	1,198.6	95.9	0.09	5,235	80,503	15.4
13	87.7	63.3	0.72	1,724.3	213.5	0.12	18,840	62,600	3.3
14	168.0	21.6	0.13	1,784.2	78.4	0.04	2,548	22,900	9.0
15	169.2	58.1	0.34	2,627.0	302.0	0.11	-	-	-
16	185.9	45.9	0.25	1,441.6	86.9	0.06	2,492	13,188	5.3
17	192.1	138.6	0.72	2,643.5	189.0	0.07	21,902	57,200	2.6
18	184.6	54.6	0.30	1,909.0	142.2	0.07	5,047	38,675	7.7
19	167.8	48.4	0.29	1,985.9	103.9	0.05	9,864	33,352	3.4
Mean	140.3	65.6	0.51	1,467.2	152.3	0.11	6,883.1	40,897.5	11.8
SD	59.8	50.2	0.30	720.4	115.4	0.05	5,804.6	20,922.8	17.2
Median	151.0	52.8	0.41	1,511.6	105.5	0.10	5,235.0	38,675.0	6.3

A dash indicates that the testing was not done

Table 5

Results for cocaine (COC), benzoylecgonine (BE), meta-hydroxybenzoylecgonine (mOHBE), morphine (MOR) and/or codeine (COD) in meconium specimens, and percentage thrice-weekly urine specimens' results in the second and third trimester (T).

	Meconium (ng/mL)					Urine					
						Opiates			Cocaine		
Subject	COC	BE	mOHBE	Morphine	Codeine	[a] 2nd T % positive	[b] 3rd T % positive	[c] day last positive	[a] 2nd T % positive	[a] 3rd T % positive	[c] day last positive
1	N	N	24.3	10.2	N	100	0.0	42	33.3	0.0	46
2	N	4.3	200.3	43.0	N	STT	6.7	27	STT	63.3	25
3	N	2.4	155.1	65.9	N	STT	11.5	31	STT	7.7	31
4	N	N	26.0	10.0	N	STT	10.7	61	STT	0.0	AN
5	N	N	N	N	N	25.0	0.0	104	25.0	0.0	104
6	-	-	-	-	-	3.0	0.0	99	0.0	0.0	AN
7	-	-	-	-	-	0.0	0.0	AN	0.0	0.0	AN
8	N	N	12.5	14.0	N	33.3	0.0	75	0.0	0.0	AN
9	-	-	-	-	-	16.7	63.6	3.0	0.0	0.0	AN
10	N	N	N	N	N	18.2	0.0	120	0.0	0.0	AN
11	4.4	62.0	57.3	2,555.0	16.3	25.0	7.1	15	31.2	25.0	13
12	N	N	119.0	37.0	N	17.6	6.7	34	5.9	6.7	34
13	N	N	12.2	N	N	55.5	0.0	118	0.0	4.4	62
14	N	N	N	2,281	N	2.8	0.0	128	0.0	0.0	AN
15	-	-	-	-	-	0.0	14.7	18	0.0	0.0	AN
16	233.0	969.0	254.0	N	N	94.1	-	-	88.2	-	-
17	N	N	N	N	N	18.7	0.0	100	0.0	0.0	AN
18	N	N	104.0	N	N	3.0	0.0	176	3.0	0.0	176
19	N	N	68.0	N	N	-	-	-	-	-	-

N negative results (<limit of quantification); - data unavailable

STT: participant started in 3rd trimester; AN: always negative.

[a] % positive urine specimens in 2nd trimester

[b] % positive urine specimens in 3rd trimester

[c] days from last positive urine specimen to birth