Stereoselective analysis of methadone and EDDP in laboring women and neonates in plasma and dried blood spots and association with neonatal abstinence syndrome

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ABSTRACT:

Objective: This pilot study evaluated the relationship between maternal and neonatal R- and S-methadone and R- and S-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) exposure and the severity of neonatal abstinence syndrome. The use of dried blood spots (DBS) as an alternative for plasma in assessing methadone and EDDP was also assessed.

Study Design: Women receiving methadone for medication assisted treatment of opioid use disorder during pregnancy were eligible for recruitment. Plasma and dried blood spot samples were collected from mothers during labor, from cord blood, and from newborn during genetic screen. R-/S-methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) were measured by HPLC-MS/MS. Associations between methadone exposure, neonatal morphine requirements and severity of neonatal abstinence syndrome were examined.

Results: Twenty women and infants completed the study. Maternal methadone dose at delivery was 112 mg/d (range 60-180 mg/d). Sixteen neonates experienced NAS requiring morphine; 3 also required phenobarbital. Higher cord blood concentrations of R-methadone, R- and S-EDDP were associated with higher maximum doses of morphine ($p<0.05$).

Conclusion: Maternal methadone and cord blood concentration at delivery are variable and may be potential markers of neonatal abstinence syndrome.

INTRODUCTION

Opioid use has increased more than four-fold within the last decade,[1] leading to the declaration of a national public health emergency.[2] Misuse of opioids among pregnant women is common. Opioid misuse in pregnancy often leads to neonatal opioid withdrawal syndrome (NOWS) in the neonate. As a number of drugs, including antidepressants and other illicit substances, may contribute to clinical withdrawal in the neonates, we have employed the more broad term neonatal abstinence syndrome (NAS) in this study. NAS has increased nearly fivefold from 2000 to 2012. Every 25 minutes a baby is born with NAS in the U.S.[1,3]

NAS is a costly morbidity, most commonly caused by maternal opioid dependence during pregnancy. It is a self-limited constellation of signs and symptoms consisting of neurologic excitability, gastrointestinal dysfunction, and autonomic disturbances[4]. As a consequence of increased use of neonatal intensive care units and prolonged hospitalization for the treatment of NAS, average hospital charges for infants with NAS amount to more than five times those for all other infants[1,3,5].

To reduce the risk of neonatal morbidity and mortality associated with in utero exposure to heroin and other short-acting opioids, the American College of Obstetricians and Gynecologists (ACOG) and the American Society of Addiction Medicine (ASAM) recommend opioid-assisted therapy with methadone or buprenorphine during pregnancy.[6] While the use of buprenorphine has increased over the past few years, methadone is still widely used for medication assisted therapy. In 2018, 25% of outpatient opioid treatment programs only provided methadone.[7] Additionally, there are a number of women who are not candidates for buprenorphine

therapy, who may be treated with methadone. Although methadone treatment programs improve access to prenatal care and decrease some of the morbidities associated with illicit opioid use, a large number of infants exposed to methadone in utero develop NAS shortly after birth and require pharmacological treatment for withdrawal.[8]

Methadone is a synthetic opioid analgesic that has a long half-life of approximately 22 hours in adults. It is available as a racemic mixture of R- and S-methadone and undergoes N-demethylation to its main metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), which is inactive. R-methadone accounts for the majority of opioid effects (μ-opioid receptors) and has more potent analgesic effect than S-methadone[9,10]. Numerous studies have attempted to link the severity of NAS to maternal methadone dose, but the results have been inconsistent. In a meta-analysis, 19 studies found a correlation between maternal methadone dose and NAS severity, while 18 did not; when limited to those studies that used an objective scoring system to evaluate NAS, no association was found.[11] Interindividual variability in pharmacokinetics of methadone may lead to the discrepancy between methadone dose and NAS severity. Studies have reported up to 17-fold difference in plasma methadone concentration among individuals receiving the same dose.[12] This variability is explained, in part, by pharmacogenomic variants in drug metabolizing enzymes and transporters.[13,14] Metabolic changes associated with pregnancy may also contribute to variability in exposure to methadone.[15-17] Cord blood concentrations of methadone are lower than maternal concentrations due to the protective effects of the p-glycoprotein transporter in placenta.[18-21] There have been a few small studies examining the

relationship between cord blood methadone concentrations and the severity of NAS which suggest that lower cord blood concentrations and a faster rate of decline in neonatal plasma concentrations correlate to more severe withdrawal.[22,23] Similarly, in adults a more rapid decline in plasma methadone concentration correlates with increased withdrawal symptoms.[24] However, data examining the relationship between maternal and neonatal pharmacokinetics of methadone to the severity of NAS are lacking.

Current treatment paradigms for NAS base the need for pharmacotherapy on clinical assessment of the infant, e.g. by using the modified Finnegan score. Earlier identification of infants likely to experience severe NAS may allow for adjustment in clinical treatment algorithms for withdrawal, for example by reducing the threshold modified Finnegan score for initiating treatment, allowing the infant to begin treatment earlier. We hypothesize that maternal and neonatal concentration of methadone enantiomers at delivery may correlate with severity of NAS. Therefore, we undertook a pilot study to evaluate the relationship between maternal and fetal concentrations of individual enantiomers of methadone and its primary metabolite, EDDP, and the risk of neonatal NAS. In addition, we evaluated the use of dried blood spots (DBS) as an alternative to plasma for determining concentrations of methadone and EDDP.

MATERIALS AND METHODS

Women ≥ 18 years of age with a singleton pregnancy who had been taking methadone beginning prior to the 28th week of gestation and who planned to deliver at Eskenazi or IU Health Methodist Hospitals were eligible for recruitment into this study. Women were excluded if they had a urine toxicology screen that was positive for other

opioids at the time of enrollment, liver or kidney dysfunction, or known fetal anomalies or genetic disorders. Enrollment occurred during an outpatient obstetric clinic visit, inpatient hospital stay, or upon admission to labor and delivery. The study was approved by the Indiana University Purdue University Indianapolis Institutional Review Board and written informed consent was obtained from each woman for herself and her infant.

Two blood samples were collected from each subject. Maternal samples were obtained upon admission to the labor and delivery unit for planned delivery and within 1 hour after birth. Infant samples were obtained from cord blood at the time of birth and in conjunction with the newborn screen. Maternal and cord whole blood samples were collected into EDTA tubes and aliquots transferred onto DBS cards (Whatman DMPK-903). The remaining blood was centrifuged to plasma and stored at -80ºC until analysis. The infant samples obtained during the newborn screen via heelstick were collected directly onto DBS cards. The DBS cards were allowed to dry completely and then placed into a sealed plastic bag with a desiccant packet and stored at room temperature until analysis.

Demographic and clinical data

Demographic and clinical data were obtained from the medical record. The modified Finnegan score[25] was used as standard of care to monitor withdrawal symptoms of infants. NAS was determined to be severe and warrant pharmacologic treatment when an infant had three consecutive Finnegan scores > 8 or one score ≥ 12. Treatment for NAS at both hospitals followed the same standard protocol which uses oral morphine as the primary medication, with the addition of phenobarbital as

adjunctive therapy for refractory symptoms. The recommended morphine starting dose was 0.04 mg/kg/dose every 4 hours. Morphine doses were escalated if needed and weaned according to physician discretion.

Analytical method

R- & S-methadone, R- & S-EDDP in plasma and DBS were quantified using HPLC-MS/MS (API 4000, Applied Biosystems) by the Indiana University Simon Cancer Center Clinical Pharmacology Analytical Core. Plasma samples and standards were prepared by adding diphenhydramine (internal standard, 20 ng) and 0.1 M phosphate buffer (200 µL, pH 7.4) to 200 µL plasma. For DBS samples, a single punch (6 mm) was placed in a micro-centrifuge tube and 10 µL of 1 ng/µL diphenhydramine and water were added to the sample. Plasma and DBS samples were extracted with ethyl acetate, the supernatant transferred to a clean tubes, evaporated to dryness, and reconstituted with 50 µL of 10% acetonitrile in 0.1% formic acid (pH 6.5). Samples (10 µL) were injected onto an Agilent 1200 HPLC equipped with a Leap CTC Autosampler, separated on a Chiral-AGP 150X4.6mm 5 µ column (Chiral Technologies, Daicel Group, West Chester, PA) over 15 minutes with acetonitrile (gradient from 10-34%) in 0.1% formic acid (pH 6.5). R and S Methadone, m/z 310.3/265.0; R and S EDDP, m/z 278.3/234.0; and diphenhydramine, m/z 256.2/167.0 were quantified on an ABSciex 4000 MS/MS. Based on a previous report, it was assumed the R-entantiomer EDDP elutes prior to the S-enantiomer.[26] The lower limit of quantification for R- and S-methadone was 0.05 ng/mL for plasma and 1 ng/mL for DBS, and for R- and S- EDDP was 0.025ng/mL for plasma and 0.5ng/mL for DBS.

Statistical analysis

Maternal and infant methadone and EDDP concentrations were determined in plasma and cord blood at delivery. Maternal concentration data were obtained at 2 timepoints. The elimination rate constant (k_e) was determined by linear extrapolation of log transformed concentration vs. time data. Maternal plasma concentrations were linearly extrapolated from 0 to 24 hours to estimate maternal area under the concentration time curve profile (AUC_{24h}). Infant k_e was calculated by linear extrapolation of the log-transformed DBS concentrations obtained from cord blood and at time of the newborn screen vs. time data. AUC_{inf} was estimated as the cord concentration/k_e. Maternal oral clearance was estimated as methadone dose/$AUC_{(0\text{-}24h)}$ and half-life as ln(2)/k_e. Due to timing of maternal blood draws and variability in plasma concentrations, maternal k_e could only be estimated for 7 of the 20 women in the study.

Associations between methadone dose, DBS or plasma concentrations of methadone and EDDP metabolites, total & peak neonatal morphine dose and neonatal length of stay (LOS) were evaluated by linear regression analysis (R 3.3.1).[27] This study was designed as a pilot to assess feasibility for a larger clinical study, so no formal power analysis was conducted.

RESULTS

Twenty-six women were enrolled in the study and 20 women (23-42 years) and neonatal (EGA 36^6-41^2) dyads completed the study. Six women did not complete the study due to inability to collect blood samples (n=5) or noncompliance with methadone therapy (n=1). Data from these six women were excluded from all analyses. The median maternal methadone dose at delivery was 112 mg/d (range 60-180 mg/d). Sixteen neonates (80%) experienced NAS requiring morphine treatment, and 3 (19%)

required adjunct therapy with phenobarbital. Demographic characteristics did not differ between those who did and did not develop NAS (Table 1). While the urine drug screens were negative for other opioids in all women, the meconium drug screen was positive for substances other than methadone in 6 infants (1 for amphetamines, 1 for cannabinoids, 1 for cocaine, 1 for barbiturates and opioids, 2 for other opioids). Five of these infants developed NAS ($p>0.05$). As expected, infants with NAS had significantly longer hospitalization than those without NAS (29 vs. 6 days, $p = 0.0029$).

DBS vs. Plasma Concentrations

The DBS:plasma ratios from cord blood were significantly higher than maternal ratios ($p \leq 0.05$, Figure 1). Therefore, maternal and cord blood DBS:plasma correlations were compared separately. In both maternal and cord blood samples R- and S-methadone were well-correlated (R-methadone maternal $R^2=0.70$, $p <0.001$ and cord blood $R^2=0.87$, $p<0.001$; S-Methadone maternal $R^2=0.76$, $p <0.001$ and cord blood $R^2=0.76$, $p<0.001$). However, R- and S-EDDP DBS and plasma concentrations were only weakly correlated (R-EDDP maternal $R^2=0.41$, $p <0.001$ and cord blood $R^2=0.30$, $p=0.03$; S-EDDP maternal $R^2=0.45$, $p <0.001$ and cord blood $R^2=0.12$, $p=0.13$).

Methadone Concentration and Dose

Maternal R- and S-methadone plasma concentrations at delivery were weakly correlated with maternal methadone dose ($R^2 = 0.55$, $p = 0.0006$ and $R^2 = 0.42$, $p = 0.004$, respectively, Figure 2A-B). In contrast, maternal concentrations of R and S-EDDP metabolites were not correlated with maternal methadone dose. Correlations between maternal dose and cord blood concentrations of parent drug and metabolite were significant, but weak ($R^2 \leq 0.35$, Figure 2C-D).

Neonatal Abstinence Syndrome

Maternal R-methadone concentrations were 146 ± 74 ng/mL vs. 202 ± 65 ng/mL and cord blood concentrations at delivery were 103 ± 63 ng/mL and 150 ± 71 ng/mL in infants who did and did not experience NAS, respectively (p=0.20). This trend towards neonates who experienced NAS having statistically non-significant lower maternal delivery and cord blood concentrations of methadone and EDDP metabolites than those who did not experience NAS was also observed with S-methadone, R-EDDP, and S-EDDP (Table S1). NAS was associated with faster maternal oral clearance of R-methadone, R-EDDP, and S-EDDP (p = 0.0086, 0.048, 0.035, respectively).

Neonatal withdrawal was assessed by Finnegan scores. Length of stay was positively correlated with maximum Finnegan score (p = 0.003; R^2 = 0.36). Although Finnegan score was not correlated with maternal concentrations at delivery or AUCs of R- or S-methadone or R- or S-EDDP, lower maternal AUC of R-methadone and S-EDDP were associated with longer length of hospital stay (p = 0.0029; R^2 = 0.74; and p = 0.0409; R^2 = 0.47, respectively). There was no association between neonatal AUC of methadone or EDDP with Finnegan scores or length of stay.

Among the 16 infants experiencing NAS, the peak dose of morphine per kg body weight required by neonates was associated with maternal concentration of R- and S-methadone, R-EDDP, and S-EDDP ($p<0.02$, $R^2>0.38$, Figure 3). Infants born to mothers with higher plasma concentrations had increased peak morphine doses. Similarly, higher R-methadone, R-EDDP, and S-EDDP cord blood concentrations were associated with higher maximum morphine doses/kg body weight ($p<0.01$, $R^2 > 0.33$).

While the trend was similar for S-methadone, the association with increased peak morphine doses was not significant ($p= 0.15$, $R^2 = 0.08$). Total morphine doses were not associated with maternal or cord blood concentrations of drug or metabolites at delivery.

Cord DBS to maternal plasma concentration ratios of drug and metabolites were highly variable. R- and S- methadone and S-EDDP had median cord DBS to maternal plasma ratios of 0.77 (range 0.47-1.21), 0.65 (0.004-1.15), and 0.78 (0.26-2.5), respectively. R-EDDP was more likely to have higher fetal exposure, with cord to maternal plasma ratios of 1.23 (0.49-3.36). There was no relationship between risk of NAS and cord to maternal ratios. Although trends were observed, there were no significant associations between half-life or AUC and severity of NAS as assessed by Finnegan score, total morphine dose, or peak morphine dose. Similarly, no significant correlations were observed for neonatal half-life and AUC_{∞} (N=17) of methadone or EDDP enantiomers and severity of NAS.

DISCUSSION

This study assessed the plasma and DBS concentrations of R- and S-methadone and their primary metabolite R- and S-EDDP. Consistent with other studies,[12,20] we found a wide inter-individual variability in methadone concentrations in both pregnant women and their infants. It is widely recognized that a number of physiologic changes occur during pregnancy which alter drug disposition.[15-17] During pregnancy, there is a marked increase in methadone oral clearance, and many patients experience the need for a dose increase or split dosing of methadone in order to quell increased withdrawal symptoms.[17,28,29] This may be due in part to the fact that both CYP2B6 and CYP19

(aromatase) are induced by estradiol and progesterone.[30-32] In addition, CYP19, which is highly expressed in the placenta, has been shown to metabolize methadone.[33,34]

Cord blood concentrations of methadone were 65-77% of maternal plasma concentrations. A prior study of 15 mother-infant pairs measured maternal plasma and cord blood plasma concentrations of R-methadone using HPLC-MS and found a median cord blood:maternal ratio of 0.41 (range 0.19 – 0.56).[20] The differences in cord blood and plasma concentrations between studies may be related to our use of dried blood spot sampling. When comparing dried blood spot concentrations at delivery, the cord to maternal ratio for R-methadone was 0.6 and for S-methadone it was 0.4. Nanosvkaya et al. demonstrated that placental microsomes can metabolize methadone, although to a much lower extent than human liver microsomes[33]. Thus, the higher R-EDDP and S-EDDP cord to maternal ratios observed are likely due to placental metabolism of methadone.

Some studies have demonstrated that larger maternal methadone dosages in late pregnancy were associated with greater neonatal concentrations and increased risk of withdrawal,[18,19,22,35-40] but others refuted a correlation.[11,23,41-44] Cumulative fetal exposure can be expected to vary among infants born to mothers on equivalent methadone regimens. Methadone concentrations in cord blood and at 48 hours of age,[23] as well as the rate of decline in neonatal serum concentration,[22] appear to correlate with NAS signs. Kuschel et al.[23] found that infants who required rescue treatment had lower cord blood methadone concentrations and that, in all but one infant, methadone concentrations were undetectable in the serum at 48 hours. Doberczak noted that faster declines in postnatal blood methadone concentrations were associated

with more severe CNS withdrawal.[22] They also found a positive correlation between the rate of decline in neonatal plasma concentration between day 1 and days 3-4 after delivery and severity of CNS signs of withdrawal, as measured by the Lipsitz scoring system. We found that infants with greater exposure to R-methadone as measured by cord blood concentration had more severe NAS. However, we were unable to detect a correlation between methadone half-life and NAS severity.

Despite the long-standing use of methadone and experiences with NAS, there is virtually no data available on methadone pharmacokinetics in infants. A population pharmacokinetic study by Ward et al used pooled data from 4 studies of methadone metabolism in neonates and children and found that methadone clearance in neonates was similar to that in older children and adults.[45] The activity of most drug metabolizing enzymes begins to increase dramatically immediately following birth.[46] As we were only able to obtain a single sample from neonates following delivery in addition to cord blood, we could only estimate half-life by linear regression of drug concentrations at birth and the time of the newborn screen. However, it is likely that there is variability in the rate of enzyme development that is not captured by this approach. Such variability may also affect the risk and severity of NAS in neonates.

This study does have several limitations, most importantly a small sample size which precludes us from discerning any definitive associations and limits our ability to perform multiple comparisons to correct for known factors associated with NAS. For instance, three women whose infants experienced NAS were also being treated with antidepressants while none of the mothers whose infants were not diagnosed with NAS took antidepressants. We also included late preterm infants, which may have

introduced a confounding factor in terms of severity of NAS. It is known that preterm infants have overall less severe NAS compared to term infants. Doberczak et al. studied NAS in 178 term and 34 preterm infants and found that 81% of the term infants required pharmacotherapy for NAS, versus 59% of the preterm infants.[38] We only had three infants born less than 37 weeks gestation (one at 35.1 and two at 36.9 weeks), all of whom experienced NAS. The trends we observed in this pilot need to be further explored in larger studies. Neonatal sample collection was limited to cord blood and DBS samples at the time of neonatal screening. DBS collection appears to be an alternative to plasma collection for R- and S-methadone, as R^2 value is >0.74. However, there was only weak association between DBS and plasma concentrations for the EDDP metabolites (R^2 = 0.4). This may be due to a number of factors including collection technique and interindividual differences in hematocrit.[47,48] Unfortunately, hematocrits were not available from patients in this study. Thus, it is difficult to assess the relationship between neonatal EDDP concentrations and outcomes. We used Whatman-903 DBS cards; other DBS collection matrices may be more suitable for EDDP. Only two plasma or DBS samples were available from each woman. Time between methadone dosing and sample collection also varied between patients, which impacts interpretation of cord:maternal ratio. In women who labored more than 24 hours, methadone was often re-dosed between study samples, leading to difficulties in estimating terminal elimination rate. While the diagnosis of NAS and morphine dosing was set by standardized clinical protocols based on the Finnegan score, we recognize that this is a subjective assessment scale.

In spite of these limitations, this study demonstrates the feasibility of recruiting pregnant women with opioid abuse disorder and their neonates into a prospective pharmacokinetic-pharmacodynamic study. We also utilized a sensitive mass spectroscopy technique that allowed us to detect individual enantiomers of methadone and EDDP. Further studies assessing the relationship between *in utero* or postnatal exposure to methadone enantiomers may inform models to predict which infants will experience NAS, and the severity of NAS. These models could then enable earlier and more aggressive treatment of NAS, reducing length of neonatal stay and morbidities associated with severe withdrawal.

CONCLUSSIONS

This study demonstrates that DBS is a suitable alternative for methadone quantification, especially when sample volume is limited such as in neonates. However, the DBS method employed here is not sufficient for measuring EDDP metabolites. This pilot data indicates that maternal methadone AUC and cord blood concentration at delivery may be potential markers for NAS severity as measured by Finnegan score. However, EDDP metabolite concentrations do not appear to be correlated with NAS. Additional studies, with more objective measures of NAS, are needed to further understand the association between maternal and infant exposure to methadone and NAS.

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Table 1. Maternal demographics and neonatal outcomes in neonates with vs. without NAS diagnoses.

Characteristic	NAS (N=16)	No NAS (N=4)	P-value
Methadone dose (mg)	110 (60 - 170)	131 (95 - 180)	0.20
Maternal age (years)	28 (23 - 42)	35 (24 - 35)	0.25
Race			0.18
Black	0 (0%)	2 (50%)	
White	15 (94%)	2(50%)	
Other	1 (6%)	0 (0%)	
Ethnicity			1.0
Hispanic	1 (6%)	0 (0%)	
Non-Hispanic	15 (94%)	4 (100%)	
Height (cm)	164 (152 - 175)	160 (152 - 173)	0.48
Weight (kg)	86 (56 - 114)	79 (58 - 90)	0.51
BMI (kg/m^2)	31 (22 - 43)	29 (23 - 35)	0.60
Tobacco Smoker	15 (94%)	3 (75%)	0.74
Antidepressant Use	3 (19%)	0 (0%)	0.88
Gestational age at delivery (weeks)	38 (35 - 41)	39 (37 - 40)	0.81
Birth Weight (kg)	3 (2.3 - 3.7)	2.6 (2.4 - 2.9)	0.20
Male sex	8 (50%)	2 (50%)	1.0
Breastfeeding (Yes)	6 (38%)	3 (75%)	0.28

Meconium positive	5 (31%)	1 (25%)	0.70
1 minute Apgar score	9 (1 - 9)	9 (7 - 9)	0.32
Peak Finnegan Score	13 (10 - 20)	9 (7 - 10)	0.003*
Number of days in hospital	29 (19 - 55)	6 (5 - 6)	0.003*
Peak morphine dose (mg/day/kg)	0.05 (0.02 - 0.61)		
Total cumulative morphine dose (mg/kg)	5.64 (1.02 - 17.53)		

* $p < 0.05$ for Mann-Whitney test comparing “NAS” versus “no NAS”. Values are Median (Confidence Interval) or N (%). Groups were compared by χ^2, Fisher exact or Mann-Whitney test as appropriate. NAS, neonatal abstinence syndrome; BMI, body mass index

Figure 1. Correlation between plasma and DBS concentrations of methadone and EDDP. A) R-Methadone maternal (black) R^2=0.70, p <0.001 and cord blood (blue) R^2=0.87, p<0.001; B) S-Methadone maternal (black) R^2=0.76, p <0.001 and cord blood (blue) R^2=0.76, p<0.001, C) R-EDDP maternal (black) R^2=0.41, p <0.001 and cord blood (blue) R^2=0.30, p=0.03 and D) S-EDDP maternal (black) R^2=0.45, p <0.001 and cord blood (blue) R^2=0.12, p=0.13. Lines indicate linear regression fit of cord blood (blue dashed) and maternal (black solid) data.

Figure 2. Concentration of methadone (blue circles) and EDDP (red open squares) at delivery compared to maternal methadone dose in maternal plasma (A and B) and infant DBS (C and D). Lines indicate linear regression of A) Maternal plasma R-methadone (RM) concentration vs. dose (solid, R^2 = 0.55; p = 0.0006) and R-EDDP (RE) vs. dose (dashed, R^2 = 0.11; p = 0.13); B) Maternal plasma S-methadone (SM) concentration vs. dose (R^2 = 0.42; p = 0004) S-EDDP (SE) vs. dose (R^2 = 0.20; p = 0.053); C) Cord DBS R-methadone concentration vs. dose (solid, R^2 = 0.35; p =0.004) R-EDDP vs. dose (dashed, R^2 = 0.31; p = 0.007); and D.) Cord DBS S-methadone concentration vs. dose (R^2 = 0.32; p = 0.005) S-EDDP (R^2 = 0.32; p =0.006).

Figure 3. Correlations between peak dose of morphine/kg and maternal plasma (A and B) and cord DBS concentrations at delivery (C and D). Lines indicate linear regression of A) Maternal plasma R-methadone (RM) concentration vs. peak neonatal morphine dose (solid, R^2 = 0.54; p = 0.004) and R-EDDP (RE) vs. peak neonatal morphine dose (dashed, R^2 = 0.55; p = 0.008); B) Maternal plasma S-methadone (SM)

concentration vs. peak neonatal morphine dose (R^2 = 0.38; p =0.019) and maternal plasma S-EDDP (SE) concentration vs. peak neonatal morphine dose (R^2 = 0.48; p = 0.011); C) Cord DBS R-methadone vs. peak neonatal morphine dose (solid, R^2 = 0.33; p = 0.012) and cord DBS R-EDDP vs. peak neonatal morphine dose (dashed, R^2 = 0.39; p = 0.006); and D.) Cord DBS S-methadone concentration vs. peak neonatal morphine dose (solid, R^2 = 0.08; p = 0.15) and S-EDDP vs. peak neonatal morphine dose (dashed, R^2 = 0.36; p = 0.009).

Figure 1.

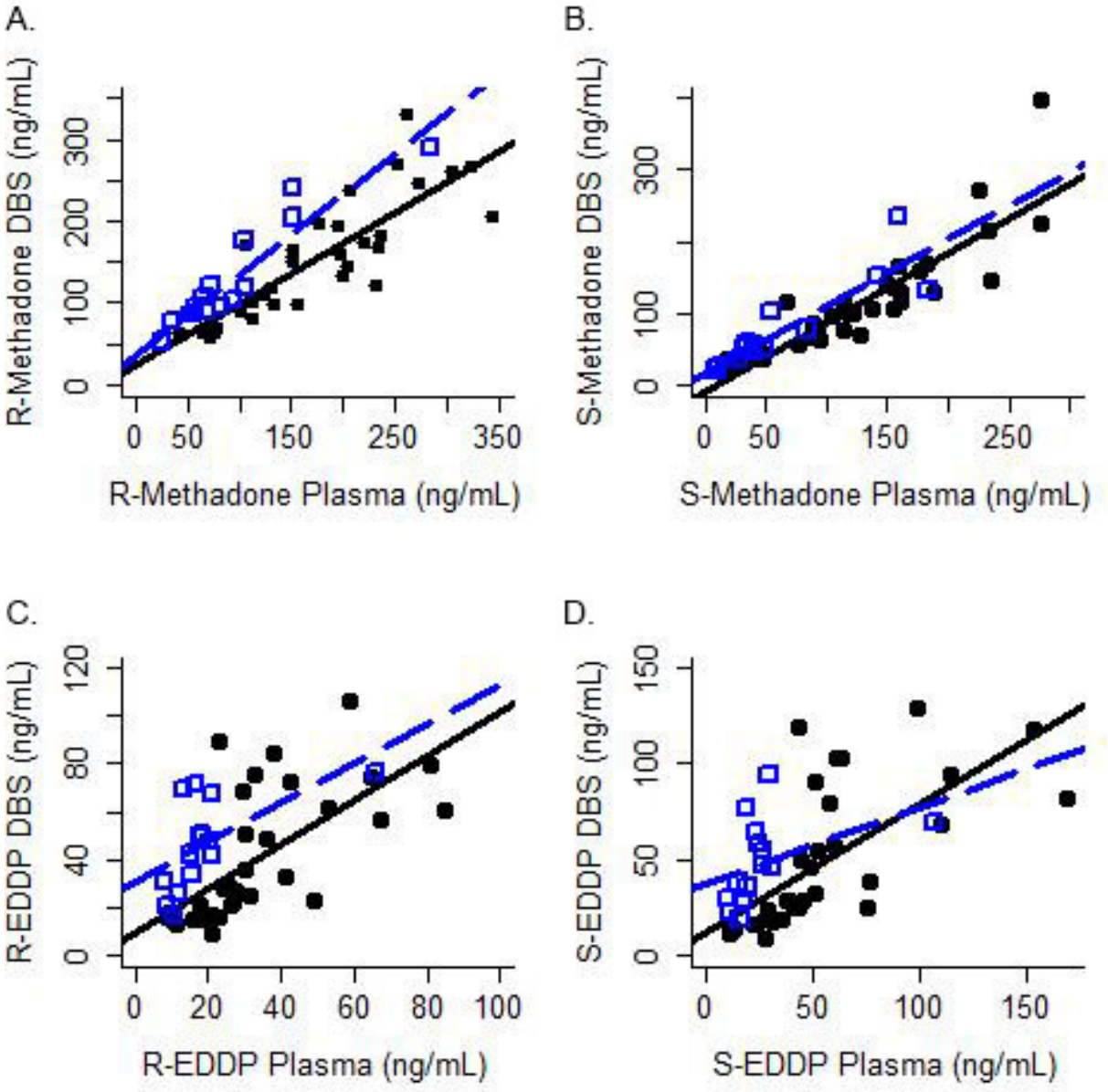
A.
R-Methadone DBS (ng/mL)
0
100
200
300
0
50
150
250
350
R-Methadone Plasma (ng/mL)
B.
S-Methadone DBS (ng/mL)
0
100
300
0
50
150
250
S-Methadone Plasma (ng/mL)
C.
R-EDDP DBS (ng/mL)
0
40
80
120
0
20
40
60
80
100
R-EDDP Plasma (ng/mL)
D.
S-EDDP DBS (ng/mL)
0
50
100
150
0
50
100
150
S-EDDP Plasma (ng/mL)

Figure 2.

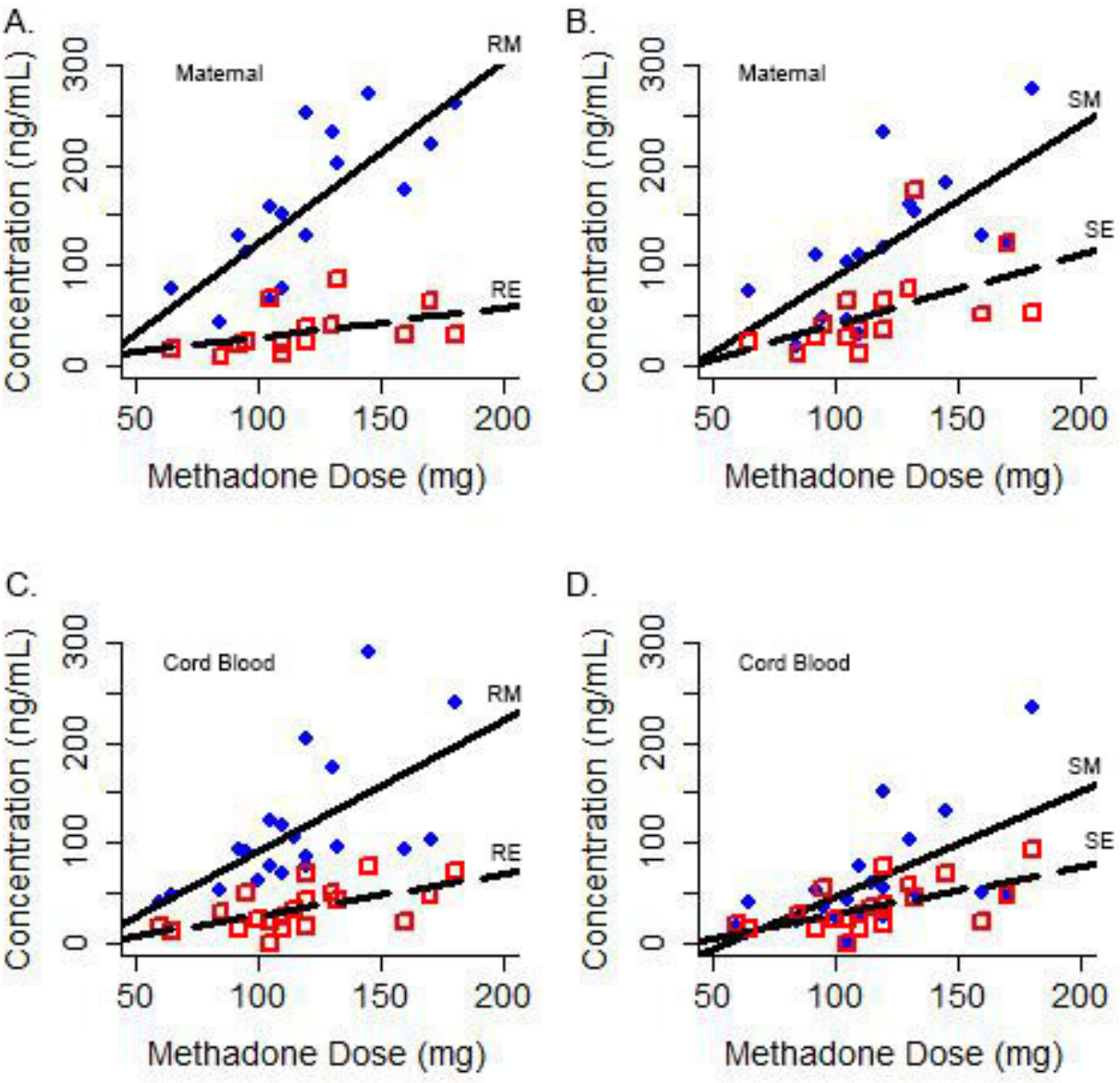
A.
Maternal
RM
RE
Concentration (ng/mL)
0
100
200
300
50
100
150
200
Methadone Dose (mg)
B.
Maternal
SM
SE
Concentration (ng/mL)
Methadone Dose (mg)
C.
Cord Blood
RM
RE
Concentration (ng/mL)
Methadone Dose (mg)
D.
Cord Blood
SM
SE
Concentration (ng/mL)
Methadone Dose (mg)

Figure 3.

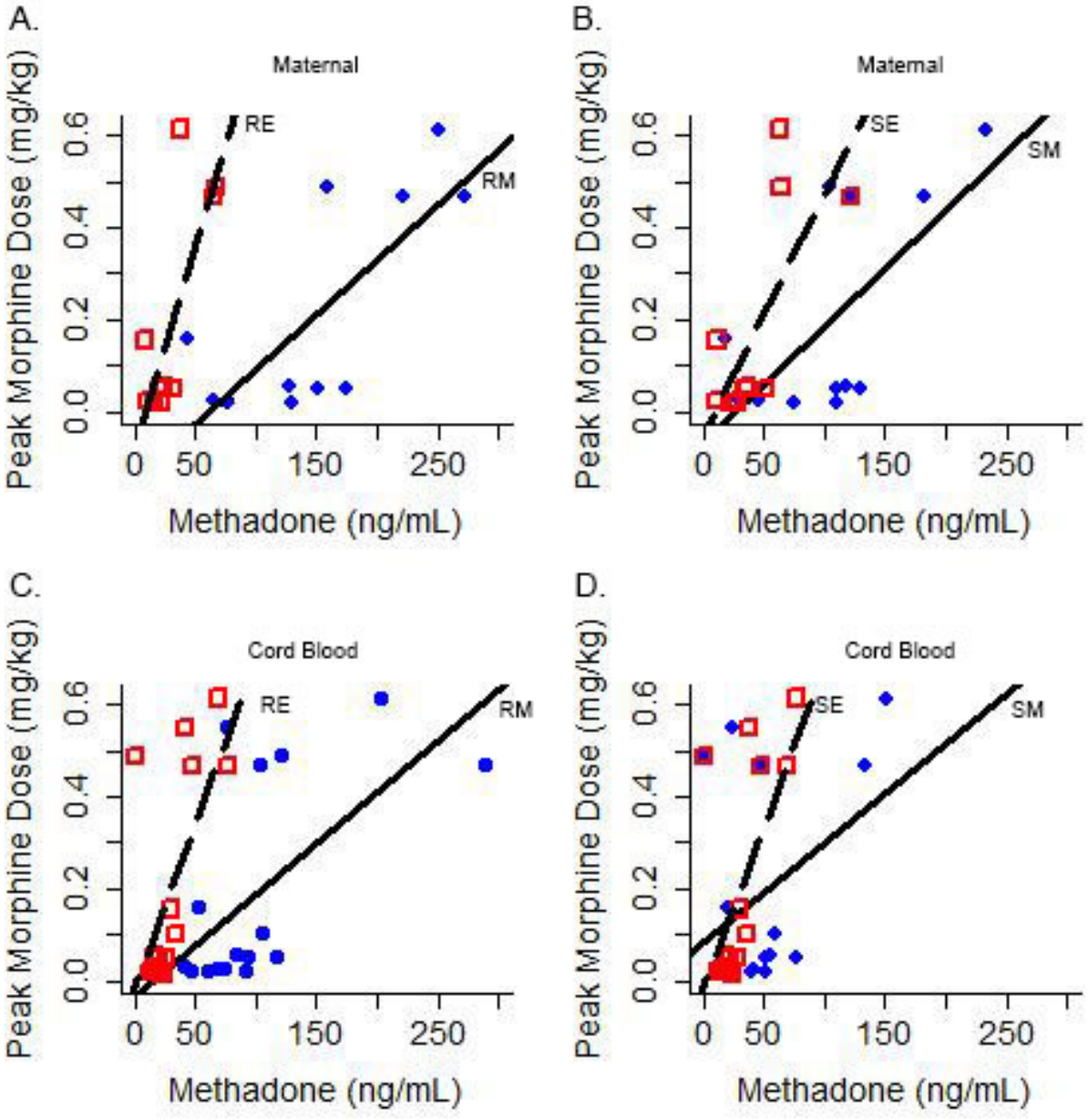
A.
Maternal
RE
RM
Peak Morphine Dose (mg/kg)
0.0
0.2
0.4
0.6
0
50
150
250
Methadone (ng/mL)
B.
Maternal
SE
SM
Peak Morphine Dose (mg/kg)
0.0
0.2
0.4
0.6
0
50
150
250
Methadone (ng/mL)
C.
Cord Blood
RE
RM
Peak Morphine Dose (mg/kg)
0.0
0.2
0.4
0.6
0
50
150
250
Methadone (ng/mL)
D.
Cord Blood
SE
SM
Peak Morphine Dose (mg/kg)
0.0
0.2
0.4
0.6
0
50
150
250
Methadone (ng/mL)

Table S1. Concentration at delivery

Subject	NAS	Daily dose of Methadone (mg)	Time since last Methadone dose (h)	R-Methadone (ng/ml)		S-Methadone (ng/ml)		R-EDDP (ng/ml)		S-EDDP (ng/ml)	
				Maternal Plasma	Infant DBS	Maternal Plasma	Infant DBS	Maternal Plasma	Infant DBS	Maternal Plasma	Infant DBS
1	Yes	65	12.2	75.93	47.39	75.02	40.65	16.42	11.76	23.30	13.28
2	Yes	92	8.3	129.63	92.96	109.73	52.43	20.95	13.98	27.36	13.67
3	Yes	110	23.8	77.20	69.14	29.49	27.04	11.59	14.25	11.58	13.07
4	Yes	120	7.4	128.29	85.61	118.33	55.50	23.43	16.89	36.28	18.91
5	Yes	105	5.3	64.43	75.54	45.74	42.38	NA	20.34	27.64	22.12
6	Yes	60	10.0	NA	41.00	NA	17.00	NA	16.20	NA	18.90
7	Yes	100	4.3	NA	61.00	NA	27.00	NA	23.10	NA	24.30
8	Yes	85	21.5	43.70	53.00	18.20	21.00	9.04	30.40	11.69	29.40
9	Yes	160	8.5	174.69	94.00	128.65	51.00	31.33	20.40	51.13	21.70
10	Yes	110	6.6	151.83	118.00	110.36	77.00	20.47	25.40	35.00	28.40
11	Yes	115	28.5	NA	106.00	NA	60.00	NA	33.80	NA	35.30

12	Yes	145	7.3	271.50	290.00	182.20	133.00	0.00	76.80	0.00	69.50
13	Yes	170	1.6	220.50	104.00	121.70	47.00	64.90	47.90	121.89	47.20
14	Yes	120	47.7	NA	77.00	NA	25.00	NA	42.20	NA	37.80
15	Yes	120	1.5	251.60	204.00	233.70	151.00	37.70	69.30	63.40	77.30
16	Yes	105	17.2	158.54	122.00	103.42	0.41	67.50	NA	64.00	NA
17	No	132	1.4	202.60	96.00	154.50	51.00	86.13	41.90	175.50	45.50
18	No	130	8.2	232.40	176.00	160.90	103.00	41.01	50.50	76.42	58.50
19	No	95	10.4	111.40	91.00	48.30	35.00	24.42	49.20	41.24	54.60
20	No	180	6.8	261.60	240.00	276.50	235.00	29.76	71.80	51.63	94.10