OPIATES AND NEONATAL ABSTINENCE SYNDROME:
TOWARDS A BETTER UNDERSTANDING OF THE PHARMACOLOGY

by
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Abstract

Neonatal Abstinence Syndrome (NAS), either as a result of in utero opiate exposure or medical exposure in the intensive care unit, carries morbidity for neonates. There is no definitive research regarding optimal care for these infants. Thus, optimal medications, most effective dosing and strategies for mitigation of long-term developmental issues are unknown. In utero opiate exposure leads to chronic neuronal changes in opiate receptor signaling and the derangement of these pathways underlies the physical manifestations of NAS. Medical opiate exposure, as opposed to in utero exposure, involves high doses for shorter periods of time, but the resultant NAS is similar in manifestation and treatment. The introduction reviews the most current research on opiate tolerance and dependence mechanisms.

Understanding prescribing patterns of pain and sedation medications in the ICU sheds light on NAS risk. Chapter I describes a cohort study quantifying the cumulative medical opiate exposure in high risk neonates. Opiate exposure has significantly increased over a decade, and this new knowledge may lead to more scrutiny of pain protocols, more diligent use of pain scores and opiate weaning protocols to standardize pain and sedation treatment in the NICU.

In utero acquired NAS is treated with replacement opiate when symptoms are uncontrolled with non-pharmacologic interventions. In part due to a lack of pharmacologic research in these infants, there currently are multiple empiric morphine dosing regimens for NAS leading to widely different lengths of treatment and cost of care
between institutions. There is no clear understanding of how dose correlates with exposure and how exposure correlates with clinical outcomes. Chapter II describes a pharmacokinetic (PK) study in infants with NAS, quantifying bioavailability of enteral morphine and internally validating models for use in future studies.

Medicine is undergoing a paradigm shift away from blanket clinical approaches to individualized medicine. Maternal opiate maintenance therapy and resultant NAS must not be left behind. Chapter III reviews the potential for pharmacogenetics to vastly increase our understanding of opiate transfer across the placenta. The goals of my research program are to individualize maternal opiate medication choice and dosing, predict severity of NAS, and individualize NAS treatment.

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<td>AC</td>
<td>Adenylyl cyclase</td>
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<tr>
<td>BCRP</td>
<td>Breast Cancer Receptor Protein</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CDH</td>
<td>Congenital diaphragmatic hernia</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CREB</td>
<td>cAMP response element binding protein</td>
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<tr>
<td>CYP</td>
<td>Cytochrome p450</td>
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<tr>
<td>DOR</td>
<td>Delta Opioid Receptor</td>
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<tr>
<td>DORV</td>
<td>Double Outlet Right Ventricle</td>
</tr>
<tr>
<td>DTO</td>
<td>Diluted Tincture of Opium</td>
</tr>
<tr>
<td>ECMO</td>
<td>Extra Corporeal Membrane Oxygenation</td>
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<tr>
<td>EDDP</td>
<td>2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine</td>
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<td>EM</td>
<td>Extensive metabolizer</td>
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<td>GPCRs</td>
<td>G-protein coupled receptors</td>
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<td>GRK</td>
<td>G-Protein receptor kinase</td>
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<td>HLHS</td>
<td>Hypoplastic Left Heart Syndrome</td>
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<tr>
<td>ICU</td>
<td>Intensive Care Unit</td>
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<tr>
<td>Kir3</td>
<td>Inward rectifying potassium channel 3</td>
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<tr>
<td>KOR</td>
<td>Kappa Opioid Receptor</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi drug resistance</td>
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<tr>
<td>MOR</td>
<td>Mu opioid receptors</td>
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<td>NAS</td>
<td>Neonatal Abstinence Syndrome</td>
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<td>NMDAR</td>
<td>N-methyl-D-aspartate receptor</td>
</tr>
<tr>
<td>NPDE</td>
<td>Normalized prediction distribution error</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<td>P-gp</td>
<td>P-glycoprotein</td>
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<td>PD</td>
<td>Pharmacodynamics</td>
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<td>Pharmacokinetics</td>
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<td>PKA</td>
<td>Protein Kinase A</td>
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<td>Protein Kinase C</td>
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<td>PPHN</td>
<td>Persistent pulmonary hypertension of the newborn</td>
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<td>PM</td>
<td>Poor metabolizer</td>
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<td>RGS</td>
<td>regulators of G-protein signaling</td>
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<td>SNP</td>
<td>Single nucleotide polymorphism</td>
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<tr>
<td>UM</td>
<td>Ultra-rapid metabolizer</td>
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INTRODUCTION

Opiate medications are a critical part of medical care. Opiates are used in the neonatal ICU to provide analgesia and sedation to sick patients and opiates are used during pregnancy to treat dependent mothers in an effort to prevent fetal withdrawal. The mechanism of action of these drugs is well understood on the cellular level, but there is much to be desired regarding our understanding of their clinical use in specific populations, including neonates. In this introduction, we will review some of the general cellular physiology behind opiate therapy and the current leading theories behind the mechanisms of tolerance and withdrawal.

Pain Physiology and Opiate Receptors

The pain pathway involves both the peripheral and central nervous system. A peripheral pain signal starts with somatic nociceptors which respond to a noxious insult or tissue injury in organs such as the skin, muscle, visceral organs or bone. The afferent nerves in the periphery transduce the stimulus into impulses which travel along the faster A-delta fibers and then the slower C fibers. Certain afferent neurons respond specifically to inflammatory mediators. The afferent neurons converge in the dorsal root ganglion near the spinal cord. In the spinal cord, the pain signal is modulated and transmitted to higher central nervous system (CNS) levels via the spinothalamic tract through the thalamus to the somatosensory cortex.

This traditional understanding of the pain pathway is currently under scrutiny as there is increasing evidence that other very complex CNS interactions involving the stress response and autonomic system also play a distinct role on pain signaling. The parieto-insular cortex is important for pain processing. Distinct lamina I cell signaling pathways have been discerned
through advanced imaging. These newly described pathways are thought to relay certain types of pain messages including sharp, burning and cold.

Knowledge of the pain pathways gives rise to potential therapeutic drug targets. Binding of agonists to the opiate receptors on the presynaptic terminals of peripheral nociceptors leads to inhibition of ion channels and decreases the release of pain neurotransmitters. Binding of agonists to the opiate receptors on the dorsal root ganglia in the spinal cord leads to inhibition of neuronal firing and neurotransmitter release, modulating the transmission of pain signals through the spinothalamic tract. In addition, there are opiate receptors in the thalamus and somatosensory cortex. These more central opiate receptors are thought to lead to the unwanted side effects of opiate therapy including tolerance and dependence.

Four opiate receptor sub-types have been described, including Mu, Kappa, Delta and Nociceptin. Mu opioid receptors (MOR) are found on the peripheral processes of dorsal root ganglia neurons, and centrally in the brainstem and medial thalamus. Mu1 type receptors are responsible for analgesia while Mu2 type receptors are responsible for respiratory depression, pruritis and sedation. Kappa opiate receptors (KOR) are found in the limbic system, the brain stem and spinal cord and are thought to play a role in dependence and dysphoria. Delta receptors are largely isolated to the brain and their role in pain physiology is not well understood, although their potential role in tolerance and withdrawal will be discussed below. Nociceptin opiate receptors have been identified in brain tissue but their current contribution to opiate physiology is not well understood.

Current Theories of Opiate Tolerance and Dependence

Tolerance, physical dependence and subsequent withdrawal are phenomena which develop as adaptations to prolonged opiate receptor activation. Tolerance is a change in threshold drug dose or concentration needed to achieve the same sedative or analgesic effect, with the need
for increased doses over time to achieve the same clinical endpoint. In a study of escalation of opiate doses to achieve pain control, adult patients required up to a 10-fold dose increase in dose over a 15 month time frame, with younger adults needing a more rapid increase in dose. Withdrawal is the manifestation of physical dependence once the opiate is weaned from the system and plays a role in addiction behaviors in adults. Physical dependence is distinct from psychological addiction, with specific regard to neonates who cannot manifest the typical drug seeking behaviors and functional life disturbances classic of addicted adults. The adaptive changes of tolerance and dependence can take place on different levels ranging from protein receptor to intracellular pathways to neuronal network connectivity.

**Receptor Physiology**

All four opiate receptors have 7-transmembrane spanning proteins. The opiate receptors are G-protein coupled receptors (GPCRs) and their activation leads to a series of conformational changes and downstream intracellular effects. There are important nuances to the simple GPCR paradigm which might influence the propensity for a drug to cause tolerance and withdrawal. For example, the opiate receptors are stabilized in different conformations by agonists, inverse agonists and neutral antagonists. These different receptor conformations could very well lead to varying downstream signaling cascades.

Once bound by an opiate receptor agonist, the subunits of the G-Protein dissociate and each alpha, beta, and gamma units can lead to downstream cellular changes. The main cellular changes associated with opiate receptor signaling include alterations in adenylyl cyclase activity and intracellular cAMP concentrations and downstream modulation of both potassium and...
calcium ion channels. Both the up-regulation of potassium influx through the plasma membrane leading to overall hyperpolarization of the neuron and the decrease of calcium influx leads to decreased neurotransmitter release.

Through a series of experiments using selective ADP-ribosylation of subunits of the inhibitory G-Protein, it was found that one of the intracellular signaling mechanism of opiate receptor activation is down-regulation of adenylyl cyclase (AC) activity by the G-alpha subunit. AC inhibition remains the agonist effect most used in research to study the opiate receptors and ligand properties. The second important aspect of opiate signaling is the downstream modulation of calcium and potassium channels. Following receptor activation and G-Protein dissociation, the alpha subunit goes on to directly interact with Kᵢ₃, the inward rectifying potassium channel. This interaction leads to increased potassium influx and inhibition of tonic neural activity. This was shown in experiments using Kᵢ₃ knock-out mice, in whom enkephalin (an endogenous opioid agonist)-induced hyperpolarization was significantly reduced. Opiate receptor mediated modulation of calcium currents works via binding of the dissociated beta-gamma subunit of the G-protein directly to the calcium channel. In a comprehensive review by Tedford et al, the discovery history of this calcium modulating activity of GPCR and the many details determining the degree of calcium inhibition, including calcium channel alpha-subunit and G-Protein structural subunits are reviewed.

The remainder of this discussion around tolerance and dependence will focus on morphine and the MOR, as morphine is a very widely used analgesic in the neonatal population and is also the drug of interest in the pharmacology research undertaken as part of this thesis project.

**Mechanisms of Morphine Tolerance and Dependence**
Mu opioid receptors show basal constitutive activity in cell lines, and their basal activity is increased after chronic morphine exposure\(^9\). This increase in basal activity is thought to happen via receptor phosphorylation, as protein kinase inhibitors can prevent the transition in constitutive activity induced by morphine exposure. In addition to protein kinases, other proteins such as calmodulin have been studied for their effect of receptor function. When the MOR is unbound, calmodulin binds and competes for binding with the G-protein, preventing basal activity. Studies show that after prolonged morphine exposure, calmodulin is released even from unbound receptors, leading to ligand-independent receptor activity\(^10\).

In addition to increased basal activity, three other main mechanisms of tolerance have been proposed including receptor desensitization, receptor endocytosis and receptor downregulation. Desensitization is any process which unlink the opioid receptor from its G-Protein mediator or other typically modulated intracellular cascade messenger systems. Endocytosis is the process in which the receptor if taken from the plasma membrane into an intracellular compartment, making it inaccessible to ligand binding. Downregulation can be defined as any decrease in the number of active ligand binding sites, and mechanisms of downregulation include receptor degradation and decreased receptor protein transcription.

Early theories of opioid tolerance revolved mainly around downregulation of opiate receptors. Recent evidence suggests that different agonists have differing cellular effects and that a more nuanced explanation involving mechanisms such as desensitization and decoupling from downstream effect may be more physiologically relevant.

\(\text{Desensitization}\)

An example of desensitization is when the activation of the opiate receptor no longer produces the expected cellular events and this can be due to alterations in second messenger density or activity, or due to receptor modifications. The first and most rapid form of
desensitization involves receptor phosphorylation. G-Protein-coupled receptors kinases (GRKs) can phosphorylate the MOR and lead to recruitment of beta-arrestin, uncoupling the MOR from the G-Protein and inhibiting transmission of downstream signal. In neurons from the peri-acqueductal grey area in beta-arrestin knockout mice, tolerance (as measured by uncoupling of receptor activation and resultant calcium currents) was reduced following chronic morphine exposure when compared to neurons from control mice\textsuperscript{11}.

A second example of desensitization is alterations in second messenger signaling systems leading to a change in the typical cellular response to opiate agonist. Upregulation of proteins involved in opiate intracellular signaling pathways such as adenylyl cyclase, protein kinase A (PKA) and cAMP response element binding protein (CREB) have all been shown to occur after chronic opiate exposure. Chronic (5 day) morphine administration to rats led to an increase in basal and GTP-stimulated adenylyl cyclase in the locus ceruleus, and this effect was not observed with shorter morphine exposures of 2 hours and 1 day\textsuperscript{12}. Studies have shown that increased adenylyl cyclase activity is isozyme specific\textsuperscript{13}, with some AC enzymes upregulated and some downregulated after morphine exposure, further elucidating the complexity of intracellular alterations with chronic opiate exposure. In a study of mice using anti-sense message directed at PKA mRNA, it was shown that the tolerance induced by chronic morphine infusion could be diminished, presumably through inhibition of the transcriptional upregulation of PKA, independent of receptor-downregulation\textsuperscript{14}. Acute morphine exposure leads to decreased phosphorylation of CREB, which is a transcription factor that mediates the effects of cAMP on gene expression. This effect is lost with chronic morphine exposure, and CREB is hyperphosphorylated during precipitated opiate withdrawal, suggesting that epigenetic changes to this gene response element may mediate at least some of opiate tolerance after morphine exposure.
Another theory in the development of opiate tolerance involves the “protective effect” of agonist-induced endocytosis. Opiate receptor endocytosis was potentially originally designed to regulate the physiologic effects of endogenous opioid agonists such as enkephalin and endomorphin. The internalization and recycling to the cell surface of opiate receptors is in line with physiologic neurotransmitter release which is typically phasic or pulsatile. A hallmark of morphine activation of the MOR is that the typical cycle of endocytosis is broken, and this change in receptor physiology with exogenous opiate exposure has been linked to the development of tolerance.

When an agonist binds the MOR, the receptor is typically phosphorylated by the G-Protein coupled receptor kinase (GRK), leading to arrestin recruitment. Arrestin recruitment leads to uncoupling of the MOR from the G-Protein, rendering it non-functional and manifesting as desensitization. In addition, arrestin-bound G-protein uncoupled receptors are endocytosed and can be either recycled to the plasma membrane or trafficked towards degradation. A shift towards receptor degradation would result in overall downregulation of receptor density.

Morphine does not cause endocytosis of the MOR to the same extent that other agonists do, and because of this, the opiate receptors are activated for prolonged periods of time and lead to cAMP superactivation, a mediator of both tolerance and withdrawal. Recent reports suggest that unlike other agonist-induced endocytosis via arrestins, morphine produces desensitization via protein kinase C (PKC). In Chinese hamster ovary cells, morphine does not produce MOR endocytosis after 2 hrs (as compared to a typical opiate agonist). This lack of endocytosis was reversed by pre-treatment with a PKC inhibitor, suggesting that PKC is a mediator of resistance to endocytosis caused by morphine. This mechanism is known as heterologous desensitization
because the PKC actually acts on the alpha-subunit of the G-Protein complexed with the MOR and not the MOR itself\textsuperscript{6}.

Dr. Whistler and colleagues\textsuperscript{17} have proposed the RAVE hypothesis to describe opiate tolerance, in an effort to link the concepts of desensitization and endocytosis. The RAVE hypothesis involves a ratio which is calculated from “relative activity (RA)” of an agonist to activate potassium current and the “versus endocytosis (VE)” component. Each agonist can be assigned a ratio (compared to a standard ratio) based on its tolerance inducing mechanisms and agonists like morphine which induce very little endocytosis are assigned a high RA/VE number. This ratio was hypothesized to predict which type of tolerance would develop based on agonist properties. For example, in agonists with high RA/VE, intracellular compensatory mechanisms (AC, CREB, etc) would promote tolerance, and with low RA/VE fewer intracellular enzyme and protein changes would occur because endocytosis is the primary mechanism of tolerance. Opiates with a lesser RA/VE such as methadone and etorphine produce less tolerance when administered chronically than dose equi-analgesic doses of morphine.

In a subsequent study by the same group\textsuperscript{18}, the effects of morphine and methadone were tested on the wild-type and two mutated versions of the MOR (see Figure 2). In the D-MOR receptor, the entire cytoplasmic tail of the MOR was replaced by the cytoplasmic tail of the delta opioid receptor (DOR). This mutant was chosen because it would confer on the mutated cells the ability of morphine to cause endocytosis, and like the WT DOR, the endocytosed receptors would be targeted for degradation. The R-MOR is a variant of this D-MOR where particular residues of the WT MOR tail were left in place, such that the receptor would be endocytosed, but recycled back to the plasma membrane as opposed to degraded. Lastly, a MOR with alanine mutations (A-MOR) in the c-terminal tail was used as “loss of phenotype” construct because even agonists like methadone that typically induce endocytosis were unable to.
Using these four receptor types in a series of elegant experiments, the authors showed that activated receptors which were more readily endocytosed (low RA/VE numbers) were associated with less cAMP superactivation and these cells developed less in vitro tolerance. When receptors remain chronically activated on the surface, there is an upregulation of gene expression including adenylyl cyclase via CREB. Taking this line of experimentation a step further, the authors created a “knock-in” mouse which expressed a MOR with the added ability to be readily endocytosed upon morphine binding. Compared to controls, mice which expressed this mutant MOR showed enhanced morphine-induced antinociception, reduced morphine tolerance and reduced naloxone-precipitated withdrawal.

**Downregulation**

Receptor downregulation can occur as a result of either decreased transcription / production or enhanced degradation. The fate of endocytosed opiate receptors to plasma membrane recycling versus degradation in the lysosomal system is likely determined by specific protein interactions between the C-terminal end of the GPCR and the G-protein-coupled receptor associated sorting protein. Modulation of the c-terminal end of the MOR can affect tolerance mechanisms as described above.

There have also been studies looking at production of opiate receptors. Cell culture models have investigated mechanisms of translational downregulation of the MOR. MicroRNAs (miRNA) bind to post-transcriptional MOR mRNA and sequester it to P-bodies, leading to a decline in translation in a miRNA concentration dependent manner. Inhibitors of this miRNA
(allowing an increase in MOR translation) led to attenuation of opioid tolerance in a mouse model. Experiments in morphine exposed rats show brain-area specific downregulation of MOR transcription (mRNA levels) after 7 days of continuous therapy. Specifically, the hypothalamus shows decreased transcription, but not the locus ceruleus, the ventral tegmental area, or the nucleus accumbens\(^21\). Brain specific regulation of MOR is not currently well understood but is an active area of investigation. In mice, etorphine (but not morphine) therapy for 7 days led to a 30% decrease in spinal cord MOR density. Of note, treatment with either drugs produced tolerance with a shift in ED50 by approximately 7-fold\(^22\). So although morphine is known to cause tolerance, there absolute role of receptor downregulation is still an active area of study.

**Novel Understanding of the Role of N-methyl-D-aspartate receptor (NMDA) Receptors**

Recent increased molecular understanding of the intracellular events leading to tolerance suggest that most pharmacologic approaches used to attenuate morphine tolerance act somewhere on the pathway that links the activity or MOR and NMDA receptors (NMDARs). In mice, NMDA receptor antagonists prevent morphine tolerance and decrease the development of physical dependence as manifested by a naloxone precipitated withdrawal after chronic exposure to morphine\(^23\). Clinical evidence supports this hypothesis in clinical trials which co-treat post-operative pain with morphine and ketamine (an NMDA antagonist). Ketamine treated patients show decreased pain scores, decreased cumulative morphine consumption and decreased post-operative desaturations events compared to opiate monotherapy patients\(^24\). There are multiple case reports of ketamine used to rescue severely opiate tolerant patients with severe pain\(^25-27\).

Proteins involved in the cross-regulation between MOR and NMDARs include PKC, neuronal nitric oxide synthase (nNOS), G-alpha subunits and regulators of G-protein signaling (RGS) proteins. Sub-families of these proteins are expressed almost exclusively in neuronal tissue and are known to affect the MOR activity. At the intracellular c-terminal end of the MOR,
regulator proteins mediate the interaction between the NMDAR and MOR\textsuperscript{28} and also allow for binding of RGS proteins, recruiting neuronal nNOS and regulating NO production. Under the influence of zinc availability, regulated by NO, tolerance mediators such as PKC discussed above are controlled. Details of the molecular mechanisms known to facilitate the cross-talk between MOR and NMDARs are beyond the scope of this introduction, but a very thorough review has been presented by Dr. Garzon and colleagues in \textit{Current Drug Abuse Reviews}\textsuperscript{29}.

**Morphine Dependence Mechanisms**

Tolerance can either be acute (with cellular responses observed after one dose of opiate) or chronic, with the latter likely reflecting a more profound and entrenched change in cellular function and intracellular networking after days to weeks of opioid administration. Physical dependence is manifested as withdrawal, with peak signs and symptoms appearing when opioid receptor occupancy declines to a minimum, but the symptoms can last for days to weeks in humans. Isolated desensitization or the “uncoupling” of the MOR from its downstream effectors is likely not the major culprit in dependence and withdrawal as discussed above. These more chronic phenomena are more likely the consequence of derangements in intracellular physiology, transcriptional and translational changes and adaptations / plasticity in the neuronal networks.

**General Caveats in Translating In Vitro Studies to Living Organisms**

At the end of this introduction, it must be discussed that there is no direct and simple relationship between what is observed in cell culture and what is clinically relevant for a human manifesting opiate tolerance or withdrawal. For example, it has been shown that the extent of uncoupling of MOR from G-protein mediated signaling differs among different neuron types\textsuperscript{30}, so within different areas of the central nervous system, different mechanisms are contributing to opiate effects. Secondly, most in vitro experiments focus on one pathway of tolerance (i.e. G-protein uncoupling or receptor internalization) when in reality, all of the mechanisms of opiate
tolerance are happening at all different rates and stages in various CNS regions, the composite of which manifests as a patient requiring increased opiate dosing or exhibiting signs of withdrawal secondary to physical dependence.

**Physical Withdrawal in the Newborn as a Result of Chronic Opiate Exposure**

The physiologic derangements described above manifest as withdrawal once the continuous opiate exposure is weaned or removed. This is true for neonates who have been exposed to chronic opiates either *in utero* or as a result of medical intensive care. The mechanisms of tolerance and dependence lead to cellular alterations which must be slowly re-adapted after opiate removal. After umbilical cord ligation at birth, or with rapid opiate weaning in the ICU, a large sympathetic outflow from the central nervous system results in the symptoms of neonatal opiate withdrawal known as NAS. These symptoms include tremors, hyper-reflexia, increased wakefulness and poor bonding. Additionally, autonomic symptoms including loose stools, tachycardia, tachypnea and lacrimation all are results of impaired central autonomic regulation that has been perturbed by continuous or semi-continuous activation of the central mu-opioid receptors. In extreme cases, the neuroexcitatory milieu of epinephrine and norepinephrine can lead to clinical seizures.

The treatment of these NAS symptoms revolves around opiate replacement and slow weaning, to allow the central nervous system the required time to reset the cellular changes caused by chronic exposure. Current research around optimal withdrawal therapy seeks a balance between treatment of symptoms versus unnecessary prolongation of opiate exposure. Non-opiate medications that work in the same cellular pathways of opiate withdrawal, namely clonidine and dexmedetomidine, are alpha-2 receptor agonists. These medications have the potential to augment or replace opiate therapy for NAS.
CHAPTER 1
PharmacoEpidemiology of Medical Opiate Use in the Neonatal ICU

Introduction

Neonates are commonly exposed to opiates and benzodiazepines for analgesic and sedative effects in the intensive care unit (ICU). Although their use is warranted, there are few evidence-based resources to guide initiation, maintenance and weaning of analgesic and sedative medications. Secular trends in opinions about need to treat pain in neonates have shifted over time, from thoughts that infants could not feel pain to a realization that neonatal pain perception is intact. Recently, the growing clinical perception is that the use of opiates in the Neonatal and Pediatric Intensive Care Unit is rapidly increasing and this increasing opiate exposure in the neonatal period is not without consequence.

There is a fine balance between treating pain and avoiding the adverse events associated with opiate exposure, both clinical and cellular. Animal evidence suggests that pain control with morphine attenuates long-term negative consequences such as hyperalgesia. A recent systematic review compiled multiple studies which associate painful procedures in the neonatal period to adverse neurologic outcomes; so there is indeed a need to minimize the experience of pain during a window of critical central and peripheral nervous system development. Conversely, animal data suggests that opiates given in the absence of pain may cause adverse cellular changes. In the rat, repeated morphine administration leads to long-term alterations in neurochemicals in the hippocampus. Morphine administration for six consecutive days in neonatal rats leads to increased supraspinal neuronal apoptosis in distinct anatomic brain regions, namely the cortex and the amygdala. The negative effects of long-term opiate treatment in the developing human brain are not currently understood outside of clinical manifestations of tolerance and physiologic dependence. Although it is understood that pain cannot go untreated in the Neonatal ICU, the
growing concern that long-term and high dose opiate therapy is likely not benign prompted our group to look more closely at trends in opiate exposure in a tertiary referral NICU over one decade. The aim of this study was to investigate changes in the use of analgesic-sedative therapy and the rates of iatrogenic NAS over time in critically ill infants over three time epochs: fiscal years 2003, 2007 and 2010.

Methods

Patients

This study was a retrospective cross sectional cohort study which included medical record extractions from fiscal years 2003-2004, 2007-2008 and 2010-2011 of all inborn infants admitted to Johns Hopkins Hospital with high risk diagnoses. After IRB review and approval, the billing office queried discharge diagnosis ICD-9 codes including 746.7 and 745.11 representing Hypoplastic Left Heart Syndrome (HLH) and Double Outlet Right Ventricle (DORV), 756.6 representing congenital diaphragmatic hernia (CDH), 747.83 representing persistent fetal circulation (PPHN), 756.73 and 756.72 representing gastroschisis and omphalocele (G/O), 765.21 and 765.22 representing 24 completed weeks of gestation and less than 24 completed weeks of gestation (<25 weeks). These diagnoses were chosen because they include infants who are likely to require longer duration of mechanical ventilation, have had major surgeries and multiple painful procedures and thus are likely to have received opiate treatment. The infants identified by diagnostic billing codes then underwent discharge summary review (in the electronic medical record (EMR)) to decide if they met inclusion criteria.

Inclusion criteria included all inborn infants at Johns Hopkins Hospital, carrying one of the afore-mentioned ICD-9 codes and living for a minimum of seven days. Infants who died before seven days of life were excluded because they represent an extreme form of clinical severity that is not representative of the typical ICU infant. In addition, infants who were
transferred to an outside hospital in less than seven days were excluded because the primary outcome of the study, chronic cumulative opiate exposure, could not be measured in these infants. Outborn infants were excluded because any opiate exposure at an outside institution or in transport would have been difficult to accurately quantify. In order to make the groups across time comparable, we also used disease-specific exclusion criteria. For HLH and DORV, the infant had to undergo open-heart surgery during their initial inpatient stay (including pulmonary artery banding). Infants discharged with no procedures or cardiac catheterizations alone were excluded. For PPHN, infants had to be full-term at birth, have cardiorespiratory failure requiring intubation but not necessarily nitric oxide therapy. Infants with only nasal canula, BiPAP or oxyhood therapy were excluded. Also, infants with PPHN associated with structural cardiac defect, genetic syndrome or pulmonary hypoplasia were excluded. The infants with severe secondary PPHN are not representative of the majority of this cohort who had transient PPHN precipitated by meconium aspiration syndrome, HIE, or sepsis.

Figure 3: Study Participant Selection Work-Flow
FY 2003
82 charts

19 Wrong year
13 No notes in EMR

9 Mild PPHN
5 Preterm PPHN
1 Unusual PPHN

1 DORV no surgery
4 complex cardiac

7 Lived < 7 days
1 Other

22 *
Final Cohort

FY 2007
68 charts

16 Wrong year
15 No notes in EMR

2 Mild PPHN
3 Preterm PPHN
3 Unusual PPHN

2 DORV no surgery

6 Lived < 7 days
6 at JHH < 7 days
1 Other

14 Final Cohort

FY 2010
88 charts

21 Wrong year
17 No notes in EMR

4 Mild PPHN
4 Preterm PPHN
4 Unusual PPHN

2 DORV no surgery

6 Lived < 7 days
1 at JHH < 7 days

29 *
Final Cohort

*One outlier excluded from final analysis from this group

† Unusual PPHN was a full-term infant with alveolar capillary dysplasia

‡ Unusual PPHN were one full-term infant with PPHN after arterial switch for Transposition of the Great Arteries, one full-term infant with diagnosis of severe surfactant deficiency, and one infant with long-standing anhydramnios

§ Unusual PPHN were one infant with severe GU anomaly, oligohydramnios and pulmonary hypoplasia, one infant with critical coarct, one infant with trisomy 21 and AV canal and one infant with severe Pulmonary Stenosis

Chart Review
Every eligible patient chart was thoroughly reviewed and demographic data, length of stay and need for transfer to step-down facility, pertinent secondary medical diagnoses, surgical procedures and need for Extra Corporeal Membrane Oxygenation (ECMO) were extracted. In addition, every dose of opiate written as either a one-time, standing intermittent, continuous infusion or continuous background infusion as part of Parent / Nurse-Controlled Analgesia (PNCA) orders was extracted and converted to morphine equivalents. Opiates extracted include morphine, fentanyl, hydromorphone, methadone and diluted tincture of opium (DTO). The conversion metrics used to convert non-morphine opiates to morphine equivalents are listed in Table 1. The medication orders were in electronic form for the 2010 cohort, but in paper form for two prior cohorts. One time or standing PRN orders or “as needed” opiate doses were excluded because the documentation was not always available in the paper charts.

In addition to opiate orders, orders for other types of medications were extracted. These included one-time or standing orders for paralytics (vecuronium and pancuronium) and one-time or standing orders for benzodiazepines (midazolam, diazepam, lorazepam). In an effort to identify a group of medications that would indicate overall degree of intensity of medical intervention, it was decided that antimicrobials, and specifically days on antimicrobials, would be a marker of changes in medical intervention intensity over time. We chose antimicrobials as a “control” for medicalization because their use is potentially more resistant to secular trends than other markers of medical intervention such as days of ventilation or days of intravenous nutrition. Orders for ampicillin, gentamicin, cefotaxime, vancomycin, cefepime, clindamycin, piperacillin, amoxicillin, metronidazole, acyclovir, fluconazole, and amphotericin were extracted. For paralytics, benzodiazepines and antibiotics, total days treated with these medications were calculated for each patient.

For the purposes of data analysis, NAS was defined in two ways: 1) the presence of a billing code or discharge diagnosis of iatrogenically acquired NAS in the patient record, or 2) the
need for weaning of opiate medications over > 1 day. NAS is measured with the Modified Finnegan Score in the NICU, and individual NAS scores were not extracted.

**Table 1: Opiate Conversions to Morphine Equivalents** Although there are multiple published opiate conversion algorithms, for the purpose of this study, we chose the conversions used clinically in our NICU. We used the same conversions in all three time epochs to make drug exposure comparable. It is possible that with other conversion metrics the absolute numbers would be different, but the trend would be similar.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Morphine : Opiate Equivalence Ratio</th>
<th>IV : Oral Conversion Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Fentanyl*</td>
<td>20:1</td>
<td>Always IV</td>
</tr>
<tr>
<td>Hydromorphone</td>
<td>5:1</td>
<td>Always IV</td>
</tr>
<tr>
<td>Methadone</td>
<td>10:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Diluted Tincture of Opium (DTO)¥</td>
<td>0.4:1</td>
<td>Always Oral</td>
</tr>
</tbody>
</table>

*Fentanyl conversions derived from Simons et al\(^\text{37}\) and Saarmenaa et al\(^\text{38}\)

¥ DTO conversion is 0.4 mg morphine for every 1 ml DTO

**Statistical Analysis**

The primary outcome is cumulative mg of morphine equivalent per infant. The per-infant result is then transitioned to a population measure such as mean or median per time epoch. The secondary outcomes are the need for opiate weaning (a surrogate marker for NAS) and a discharge diagnosis of NAS. STATA version 10.0 was used for all statistical analyses.

Exploratory data analysis was performed and then continuous variables were compared between groups with Kruskal Wallis ANOVA. Categorical variables were compared using chi squared test. Univariate regression was then used to test if time epoch was a statistically significant
predictor of cumulative Mg of morphine equivalents in both a linear (primary) and quintile (secondary) analysis. A p-value of less than 0.05 was considered statistically significant.

**Results**

Billing inquiry revealed 82, 68, and 88 unique patients for FYs 2003, 2007 and 2010 respectively. Please see Figure 1 for a flow diagram of inclusion and exclusion criteria. After multiple rounds of chart review, the final cohort for each year was finalized and the demographic information is presented in Table 1.2. The length of inpatient stay, the percentage of patients who underwent major surgery and who were placed on ECMO did not differ statistically between groups. Days treated with any antibiotics, a marker of overall medicalization, did not differ between groups (Figure 5). Although it did not reach statistical significance, there were more infants transferred to outside facilities while still treated with opiate medications in the later time period.

**Table 2: Infant Demographics**

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Total Number Infants</td>
<td>21</td>
<td>14</td>
<td>28</td>
<td>0.429</td>
</tr>
<tr>
<td>Primary Diagnosis, N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA &lt; 25 weeks</td>
<td>7 (33)</td>
<td>7 (50)</td>
<td>6 (21)</td>
<td></td>
</tr>
<tr>
<td>PPHN</td>
<td>6 (29)</td>
<td>2 (14)</td>
<td>6 (21)</td>
<td></td>
</tr>
<tr>
<td>HLH / DORV</td>
<td>5 (24)</td>
<td>4 (29)</td>
<td>6 (21)</td>
<td></td>
</tr>
<tr>
<td>Gastroschisis/Omphalocele</td>
<td>1 (5)</td>
<td>0</td>
<td>4 (14)</td>
<td></td>
</tr>
<tr>
<td>CDH</td>
<td>2 (9)</td>
<td>1 (7)</td>
<td>6 (21)</td>
<td></td>
</tr>
<tr>
<td>Birthweight (Median, IQR)</td>
<td>2860  (800,3331)</td>
<td>1450  (740,3045)</td>
<td>2675 (1998,3285)</td>
<td>0.554</td>
</tr>
<tr>
<td>Inpatient Stay, days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>56.2 (48.3)</td>
<td>60.6 (48.9)</td>
<td>68.9 (46.8)</td>
<td>0.412</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>36 (25,87)</td>
<td>43 (19,84)</td>
<td>50 (34,99)</td>
<td></td>
</tr>
<tr>
<td>Thoracic / Abdominal Surgery, N (%)</td>
<td>14 (67)</td>
<td>9 (65)</td>
<td>24 (86)</td>
<td>0.191</td>
</tr>
<tr>
<td>ECMO, N (%)</td>
<td>4 (19)</td>
<td>1 (7)</td>
<td>4 (14)</td>
<td>0.615</td>
</tr>
<tr>
<td>Days on Antibiotics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>26.8 (26.6)</td>
<td>23.1 (20.6)</td>
<td>31.9 (23.6)</td>
<td>0.308</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>15 (10,47)</td>
<td>16.5 (8,39)</td>
<td>22 (13,45)</td>
<td></td>
</tr>
</tbody>
</table>
The primary analysis of average cumulative opiate exposure modeled as a continuous variable in a linear regression did not reach statistical significance. On average, there was a 134 mg increase in opiate exposure for each subsequent time period (95% CI -12, 279, p-value 0.071). Because the data was not normally distributed with a strong right skew, we undertook a secondary analysis comparing the median exposures. This secondary analysis involved regression modeling of the median exposure per time epoch. The median cumulative opiate exposure per infant increased from 10 mg to 25 mg to 114 mg, and this was statistically significant with an average increase of 45 mg (95% CI 2.6, 88, p-value 0.038). Trends in cumulative opiate exposure are displayed in Figure 4.

The percentage of infants who carried a discharge diagnosis of iatrogenic NAS significantly increased over the three time periods from 9% to 36% to 50%, commensurate with the increased opiate exposure. There were no statistically significant increases in days of paralytic or benzodiazepine exposure over the three time epochs (Table 3), suggesting that opiate exposure was the main difference in sedato-analgesic use.

### Table 3: Iatrogenic Medication Exposure and Neonatal Abstinence Syndrome

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Cumulative Morphine Equivalents per Infant, Mg</td>
<td>134 (-12, 279)</td>
<td>104 (162)</td>
<td>175 (470)</td>
<td>367 (670)</td>
</tr>
<tr>
<td>Mean (SD) linear regression</td>
<td>10 (0-620)</td>
<td>25 (0-1794)</td>
<td>114 (0-2593)</td>
<td></td>
</tr>
<tr>
<td>Median (Range) quartile regression</td>
<td>45 (2.6, 88)</td>
<td></td>
<td></td>
<td>0.038</td>
</tr>
<tr>
<td>Days on Continuous Opiate Infusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td></td>
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<tr>
<td>-------------------------------</td>
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<tr>
<td>Days Paralytics</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.2 (27.5)</td>
<td>8 (0,18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.0 (13.8)</td>
<td>18.5 (4,25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.6 (22.3)</td>
<td>18 (7,38)</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.094</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Days Benzodiazepine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5 (2.1)</td>
<td>1 (0,2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.8 (5.0)</td>
<td>0 (0,1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.1 (4.7)</td>
<td>1 (1,4.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.434</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ever Required Weaning of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opiate, N (%)*</td>
<td>11 (53)</td>
<td>10 (71)</td>
<td></td>
<td></td>
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<tr>
<td>Discharge Diagnosis of NAS, N</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>2 (9)</td>
<td>5 (36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14 (50)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.012</td>
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</tbody>
</table>

*Statistically significant p-values are displayed in bold.

*There were three children who died before opiate weaning in the 2003-2004 cohort

There were two infants identified as outliers, both by statistical (undue leverage and Cooks D test) and clinical measures. These two infants both had cumulative exposures of over 5000 mg which is greater than 50 times the median in the highest exposure group. The first infant born in 2003 was a full-term infant with CDH and severe PPHN who required greater than two weeks of ECMO support, was treated for MRSA meningitis and received a Nissen/GT prior to discharge to step-down facility after an 102 day admission. The second infant born in 2010 was also a full-term with CDH, severe PPHN who was treated with ECMO for > 2 weeks, and was transferred to step-down facility after a 102 day admission with continuing treatment with methadone, valium and clonidine. As a comparison, all other infants on ECMO in the cohorts were canulated for fewer than 7 days.

**Figure 4:** Cumulative Morphine Equivalents by Time Period
Figure 5: Days on Antibiotics by Time Period
Discussion

This is the first study of which we are aware that attempts to quantify exact amounts of opiate exposure in the neonatal ICU population and to compare this exposure over time. The most important finding of this study is that medical opiate exposure is increasing over time in very high risk neonates in the ICU setting. Although Neonatal Infant Pain Scores (NIPS) were recorded during opiate therapy, they are not solely used to titrate medications because other factors including agitation, movement during wound healing with multiple invasive lines and tubes, endotracheal tube stability and synchronicity with mechanical ventilation, and oxygenation and ventilation status are often considered when titrating opiates. It is possible that pain control also improved concomitantly over this time period, but this is speculative as data on pain scores were not extracted from the charts for the reasons mentioned above. There were no systemic changes in pain management or pain protocols implemented during the time period studied. In addition, the practitioners in the NICU did not use pain treatment protocols or opiate weaning guidelines over the time period under study.

Although this study is limited to one tertiary care ICU, it is possible that this trend is more widespread than currently appreciated. The goal of this study is to provide a first glimpse into the cumulative amount of opiate received by an infant during an ICU stay. Because our study is limited in sample size and the data are not normally distributed, we were unable to perform meaningful regression analyses adjusting for all factors which might contribute to increasing opiate therapy, so definitive conclusions cannot be made until this study is replicated in a larger cohort. The sharp increase in cumulative opiate exposure is supportive of the impression among physicians that we are seeing an increase in the number and severity of iatrogenic Neonatal Abstinence Syndrome. These infants with prolonged, high dose opiate exposures become physiologically dependent and have withdrawal once weaning is initiated, leading to prolonged inpatient and, at times, outpatient weaning programs.
One prior study has investigated medical opiate exposure in neonates weighing less than 1500 grams who were ventilated from day of life one across six different NICUs. Unlike our study which aims to quantify cumulative exposure, this prior study addressed exposure as binary on three different hospital days. Opiate exposure varied by birth weight, illness severity and site. There was a 28-fold variation in opiate administration between the six study sites.

Sedato-analgesic medications are an important part of ICU care. Studies have shown decreased markers of physiologic stress, improved hemodynamic stability and improved synchrony with the ventilator, but these are all short-term outcomes and the long term effects of substantial opiate exposure in the immediate neonatal period are unknown. Given the lack of long-term studies, medical opiate exposure should be closely examined and efforts to curtail further increases may be warranted. The increase in exposure could be due to many clinical possibilities. For example, there could be secular trends in using more opiates for sedation, aggressiveness of opiate weaning, or tolerance of withdrawal symptoms vs markedly slow and prolonged weans in an effort to keep infants symptom free. Although we were unable to explain the etiology behind the increase in medical opiate exposure with this retrospective study, we still feel this is a trend worth discussing. Both clinical and research paradigms that are geared towards a lowest effective dose strategy and incorporate a multi-modal pain control approach might lessen the exposure to opiate medications. One barrier to weaning opiate medications is reliance on withdrawal scores which have not been well validated in sick, ventilated and post-operative infants. In addition, there are few well-validated sedation scores in the neonatal population, the use of which may prompt clinicians to recognize over-sedation or over-narcotization and wean opiate medications more readily.

There are potential confounders to our study results. Although not statistically significant, there was an increased length of stay, increased use of paralytics to medically manage, and an increased rate of thoracic or abdominal surgery over time, all of which could partially account for the increase in exposure to opiates. Despite these potentially clinically meaningful trends, the
degree of increase in opiate exposure over time cannot be fully explained by these factors. A multimodal approach to pain and sedation therapy and to minimizing opiate withdrawal symptoms often includes use of a benzodiazepine. We considered that the decreased use of adjunct benzodiazepines for pain and sedation might explain the increase in opiate exposure we observed, but there was no difference in the days of benzodiazepine exposure over time in our data. The measure of benzodiazepine exposure we chose to extract from the charts was “days of exposure” as opposed to cumulative doses, and it is possible that if we had assessed benzodiazepines in a more granular fashion, the increase in opiate exposure would be explained by a decrease in cumulative benzodiazepine exposure.

There are limitations to our study. In regards to opiate, benzodiazepine and paralytic exposure, the charts were extracted for standing doses only – meaning that due to heterogeneity and missing data in charting of PRNs over the three time epochs, we chose not to include PRNs in our calculations of exposure. A potential interpretation of our results is that in the earlier time epochs, more PRN opiate doses may have been used instead of standing orders or infusions. In addition, other non-opiate medications used for pain control were not extracted (i.e. NSAIDs, Acetaminophen), so it is possible that a reverse trend in non-opiate use could explain the rise in medical opiate use. Lastly, the increase in opiate exposure could partially be explained by changes in prescribing patterns, i.e. doctors using more methadone in later epochs and using more lengthy opiate weaning strategies. Even if these potential scenarios were the case, such a sharp increase in exposure to opiates from any form of standing orders and infusions is still worth investigating.

Regarding secondary endpoints, there was no very clear way to retrospectively capture every infant who manifested signs of physical opiate dependence, so we chose to measure both rates of diagnosis of NAS and also the number of infants who required weaning of their medications. These are imperfect measures and we acknowledge that weaning of opiates may be a preventative strategy to mitigate signs of NAS. Additionally, because we did not analyze
cumulative benzodiazepine exposure, it is possible that the increase in NAS observed in these infants was a result of a combined withdrawal from increasing opiates and benzodiazepines. There are issues with current methods of quantifying withdrawal from medical opiate exposure in newborns. First, many scoring tools used in the NICU are only validated for withdrawal from in utero opiate exposure but are used because the clinical syndromes of withdrawal are thought to be sufficiently similar. Second, there are scoring tools validated for medical opiate and benzodiazepine withdrawal in pediatrics, but they are not well studied in infants less than 6 months of age.

It is also possible that opiate exposure is increasing over time because the care is “more intensive” overall. In an effort to measure intensity of care, we collected the number of days on the most commonly used antibiotics and antifungals. There was no significant change in antibiotic exposure in the time frame studied. This could imply that opiate exposure is increasing not as a function of overall increased medicalization, but as a unique entity.

Although the increase in discharge diagnosis of NAS could be due to changes in accuracy of billing or increased recognition of this syndrome, given the increase in opiate exposure, it is more likely secondary to a true increase in the incidence of physical withdrawal behaviors. The fact that the cumulative opiate exposure per infant during the inpatient stay rose despite the fact that an increasing number of infants were transferred to outside hospitals still treated with opiates is a testament to the overall increasing opiate exposure.

Conclusion

Although analgesia with opiates is important in the most critically ill neonates, it may be time to readdress our current sedative-analgesic practices and move towards more aggressive attempts to limit the amount and duration of opiate exposure in the ICU. Studies which address long-term neurodevelopmental outcomes stratified by opiate exposure are difficult due to many
confounding factors, but novel techniques for monitoring medical opiate exposure and identifying children with the highest cumulative doses might help inform future developmental studies.
Optimization of Morphine Dosing for NAS using Population Pharmacologic Modeling

An important step in understanding how to rationally dose medications involves understanding how the administered dose relates to exposure and response. Pharmacokinetics (PK) is the study of drug absorption, distribution, metabolism and elimination – and one of the main goals of PK studies is to understand how dose relates to exposure by estimating values such as clearance, volume of distribution, etc. Traditional PK approaches used in adult patients involve intense blood samples and creation of concentration – time curves for each individual, and then averaging these curves to understand the distribution of exposures controlling for dose. This traditional approach is often unrealistic in the smallest patients because the frequency of blood draws and the volume of blood required for this “rich data” type of analysis is not feasible.

Population pharmacokinetics or “PopPK” is a discipline which arose in the 1970s, and offers an alternative methodology for estimated PK parameters with more sparse and uneven concentration data. Both traditional PK studies and population PK studies have the same goals, but the statistics used are quite different and there is the potential for decreased clinical application due to the complexity of the analyses and inability of practicing clinicians to follow the logic.

Population analysis, also known as non-linear mixed effect modeling, is a method to integrate and model the data collected from more than one individual in an effort to understand both the average response, or drug concentration, and the variability in this response. This methodology takes into account variables that are known (time, dose) as “fixed effects” and can quantify random effects after accounting for patient specific traits. This approach allows for robust PK analyses with sparse and uneven sampling designs, giving vulnerable populations such as neonatal and pediatric ICU patients the ability to be part of clinical pharmacology research using novel sampling methods such as opportunistic sampling with clinical blood draws and
scavenged sampling. A very powerful tool of population analysis is that you can understand and quantify the variability due to specific patient factors or covariates, with the potential to individualize dosing.

Population PK parameters contain multiple layers and include the population mean, the interindividual variability, and the residual variability. The models built using the PopPK approach can be internally validated by assessing model goodness-of-fit, bootstrapping, visual predictive checks, and assessing the normalized prediction distribution error. In addition, models must ideally be externally validated by applying a different set of data (not used for model building) and testing model performance. Ultimately and most importantly, the model can be used to simulate exposures and the variability in exposures when different doses are given to future patients. George Box, one of the great statistical minds of the 20th century, once wrote “Essentially all models are wrong, but some are useful.” With this humbling reality in mind, the aim of the second portion of this thesis research was to prospectively develop a population PK model of enteral morphine and its glucuronide metabolites in neonates with moderate to severe Neonatal Abstinence Syndrome.

As displayed in the figure below, this model building step is the input for a future iterative process in which you can define clinically relevant endpoints and goals and use these goals to pose questions to the model. With a clinical goal and range of potential doses in mind, you can simulate the variability in responses within a population and then refine your questions and model until you feel confident moving forward with a clinical trial with new dosing guidelines which are model based.
Figure 6: Use of population modeling for clinical research

*adapted from “Expanding clinical applications of population pharmacodynamic modeling.” Minto et al.
*copyright request granted

CHAPTER 2
Population Pharmacokinetics of Enteral Morphine and its Glucuronide Metabolites in Neonatal Abstinence Syndrome

Introduction:

Morphine has been used for neonatal pain control and sedation for decades. In part due to a lack of a well defined pharmacokinetic-pharmacodynamic link, the prescribing practices between intensive care units is extremely variable\(^\text{46}\). In addition to pain control and sedation, morphine is one of two first line opiates for the treatment of neonatal opiate withdrawal syndrome, known as Neonatal Abstinence Syndrome (NAS). Infants who were exposed to opiates either \textit{in utero} or as part of medical care are at risk for the development of NAS. In a national survey of management of in- acquired NAS\(^\text{47}\), morphine sulfate was by far the most commonly used first line agent for both opiate and polysubstance withdrawal. Despite common first-line use, this study also found that there were 23 different treatment regimens reported from 211 neonatal units, with doses ranging from 10-400 mcg/kg administered 2-8 hourly. The 2010 Cochrane review of opiates for NAS found a significant benefit of treatment with opiate vs benzodiazepines, but there is insufficient research to determine the best opiate medication and at which dose\(^\text{48}\). Despite the widespread use of morphine as the first line agent in NAS, the pharmacokinetics of the enteral formulation have not been studied.

The pharmacokinetics of parenterally dosed morphine in infants and children have been well described\(^\text{49-51}\); as shown in an evaluation of currently published population models\(^\text{52}\), the greatest differences in predicted morphine clearance values between the models are observed in the first month of life. This is very pertinent to NAS treatment because by definition infants with NAS are treated with enteral morphine within the first month of life. One publication has assessed modeling morphine across all age ranges using a bodyweight dependent exponent for maturation
of clearance\textsuperscript{53}. Using an allometric exponent which decreased in a sigmoidal manner with bodyweight, there was no need to use additional covariates for size or age. This model drew upon a diverse range of patient ages and study designs and adds a novel approach for allometric scaling of morphine clearance.

In an effort to further understand the pharmacokinetics of enteral morphine in the first weeks of life, we undertook a prospective observational study of full-term neonates treated with enteral morphine for in and ICU acquired NAS. Our aim was to quantify the first pass metabolism by building upon prior published models of parental morphine data and to better understand the proportion of parent drug metabolized to varying glucuronide metabolites. In addition, we sought to confirm the prior identified patient covariates that influence morphine disposition in early infancy. By collecting the standardized pharmacodynamic marker of withdrawal scores in a controlled population, we hope in future studies to establish a PK-PD link in this population.

Methods

Patient Population

The study was approved by the Johns Hopkins Medical Institution IRB. Between the years 2012-2014, all mothers who delivered infants at risk for NAS due to in utero methadone or heroin exposure were approached for consent at a large multi-site academic hospital system. Once parental consent was obtained, infants were monitored closely for NAS using the Modified Finnegan Withdrawal Tool (Finnegan reference) and were only enrolled in the study if they required opiate therapy due to threshold severity of withdrawal symptoms. Once started on enteral morphine therapy, the daily times and doses of administration and the every three to four hourly NAS withdrawal scores were collected and entered into an electronic database. Plasma
samples were collected via heelstick puncture for capillary samples up to four times over the entire study period for quantification of drug and metabolite concentrations. In addition to in utero acquired opiate withdrawal, infants with ICU acquired NAS who were treated with enteral morphine were also eligible for study entry and parents were consented when infant was transitioned from IV opiates to enteral morphine during clinical care.

**Sample Processing and Quantitative Analysis**

Heelstick whole blood samples were refrigerated for less than 24 hours and then were centrifuged to isolate the plasma. Plasma samples were frozen at -80°C until batch analysis via HPLC (High-performance liquid chromatography )-MS/MS techniques.

Certified reference standards of morphine, morphine-3-β-glucuronide, and morphine-6-β-glucuronide, as well as a single lot of the isotopically labeled internal standards (IS) morphine-d₃, morphine-3-β-glucuronide-d₃, and morphine-6-β-glucuronide-d₃, were all purchased from Cerilliant Corporation (Round Rock, TX). Drug-free serum was obtained from BioRad Laboratories (Irvine, CA). HPLC grade water and methanol were purchased from Fischer Scientific (Pittsburgh, PA) and formic acid was acquired from Sigma Aldrich (St. Louis, MO). For selectivity, matrix effects and endogenous studies, remnant human serum was acquired from vacutainer serum separator tubes (SST) (BD, Franklin Lakes, NJ) and plasma for the plasma to serum comparison from lithium heparin tubes (BD, Franklin Lakes, NJ) via an Institutional Review Board (IRB)-approved protocol through The Johns Hopkins University School of Medicine. All certified reference standards previously mentioned were diluted in methanol (MeOH) to prepare separate working calibrator and quality control stock solutions using two different lot numbers of each compound to increase assay robustness. A methanol protein precipitation solution containing 50 ng/mL of each internal standard was prepared and stored at -20 °C until used.
During sample preparation, protein precipitation was accomplished by adding 500 µL of the methanol containing internal standard to 50 µL of each standard, control or sample. The resulting solution was vortexed for 30 seconds and centrifuged at 12,000 x g for 5 minutes. The supernatant was transferred to a 96-well plate, evaporated to dryness using a 60 °C air stream and reconstituted in 500 µL water containing 0.1% formic acid (mobile phase A). An aliquot of 15 µL was injected for high-performance liquid chromatographic-tandem mass spectrometric analysis.

A Thermo Scientific (San Jose, CA) Prelude system comprised of an Aria TLX1 system equipped with 1250 Transcend pumps and a CTC PAL was used for HPLC. Compounds were chromatographically separated using an AccuCore PFP (50 x 2.1 mm, 2.6 µm particle size) column that was maintained at 27 °C. Mobile phases consisted of water containing 0.1% formic acid (v/v) (mobile phase A) and methanol containing 0.1% formic acid (v/v) (mobile phase B). The total analytical run time for this assay was 4.2 minutes. Monitoring of the analytes and their respective internal standards was achieved using a TSQ Vantage tandem mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a heated electrospray ionization source (HESI). Mass spectrometric conditions were optimized by direct infusion of each of the five analytes at a flow rate of 10 µL/min and a mobile phase conditions of 50%:50% A:B into the mass spectrometer. The instrument was operated in selected reaction monitoring (SRM) and positive ionization mode. Product ions were selected based on both ion abundance and consistency in fragment ion formation over multiple infusions. The most highly abundant and consistent fragment ion was used for drug measurement. These ions matched other previously published articles [6, 8]. Quantification of drug concentrations was based on the specific analyte/IS peak area ratio.
Pharmacokinetic Modeling

Population pharmacokinetic model analysis was performed using Phoenix NLME 1.3 (Pharsight, Cary, North Carolina). The first order conditional estimation method with interaction (FOCE-I) was used in the modeling process. Using this estimation method, the interaction between BSV (between subject variability) and WSV (within subject variability) is taken into account. All the plots were generated using Phoenix or R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Model building was performed in four steps: (1) selection of structural model, (2) selection of statistical sub-model, (3) covariate analysis, and (4) model validation. The goodness-of-fit plots for the analysis including observed vs. individual predicted concentrations and vs population predicted concentrations were evaluated.

Because the number of data points used in model building was limited, we chose to analyze the morphine and glucuronide concentrations using multiple prior published structural models and pharmacokinetic profiles drawing on literature from infants, children and adults. We fixed the PK parameters of clearance and volume to known values based on prior information (see Table 6) and used the current neonatal data to estimate the fraction of an enteral morphine dose which is absorbed (a modified bioavailability).

(1) Structural model

A structural model after intravenous administration was built based on physiological consideration and previously published IV morphine pharmacokinetics models in adults. First, the compartmental PK disposition studies of morphine and its glucuronide metabolites based on rich sampling after intravenous administration in healthy adults were used as the base model\textsuperscript{54,55}. These two prior publications provided three separate PK models for parent drug morphine, M3G and M6G. To link these three models (displayed in boxes), we used concentration-time profiles
from a study in which IV morphine was administered to healthy adults, and the glucuronide metabolites were measured frequently. The PK profiles from this publication were digitized and the parameters fmM3G and fmM6G were estimated to link the three IV PK models. These steps led to a robust adult IV PK model.

Next, body weight based allometric scaling was used to extrapolate the adult parameters into pediatric estimates. We used a scaling factor of 1 for volume of distribution (V, V2 and V3) and a scaling factor of 0.75 for systematic clearance (CL) and inter-compartmental clearance (Q2 and Q3). The choice of scaling factor exponents was based on common acceptance of this scaling factor in the pediatric literature.

This pediatric IV PK model was then tested against pediatric morphine PK publications to test its performance to known published data in pediatric children treated with morphine for (1)
lumbar puncture in setting of leukemia treatment and (2) post-op pain. We found that the PK model for IV morphine administration based on allometric scaling alone did not perform well in these pediatric populations, and we postulated that this was due to inadequate controlling for renal clearance maturation. Using morphine pediatric PK data from various routes of administration and a function for maturation of extracellular water based on prior data, the final pediatric IV morphine administration model including precise formation fraction estimates for M3G and M6G was chosen.

Once the base structural model was chosen, we added a new gut compartment, and the corresponding absorption rate constants for parent drug (Ka), M3G (KM3G), and M6G (KM6G). Using the neonatal parent drug and metabolite data, these three absorption rate constants were estimated and added to the IV model. Using the final model, Fa (the total amount of morphine absorbed into the systemic circulation) was estimated.

\[
F_{\text{morphine}} = \frac{K_a}{(K_a+K_3+K_6) \times Fa} \\
F_{M3G} = \frac{K_3}{(K_a+K_3+K_6) \times Fa} \\
F_{M6G} = \frac{K_6}{(K_a+K_3+K_6) \times Fa}
\]

(2) Statistical Model

Between-Subject Variability (Interindividual Variability)

Between Subject Variability (BSV) for clearance and volume of distribution was modeled assuming a log-normal distribution:

\[ P = tvP \cdot e \] (1)

where P is the post hoc PK parameter (such as Clearance), tvP is the typical value of that PK parameter (such as tvC), and i is the corresponding between subject variability. Between subject
variability of clearance and volume of distribution were re-estimated from the data in this study using the prior described structural model as a base.

**Within-Subject Variability (Residual Error)**

Within Subject Variability (WSV) was modeled using a proportional error model: This proportional error model assumes that the residual error in the model is proportionally dependent on the corresponding drug concentration. For example, the higher the concentration, the higher the residual error.

\[
DV = C \cdot (1 + \epsilon)
\]  
(2)

In this model, DV is the dependent variable, in this case concentration.

**Bioavailability**

For interindividual variability in bioavailability, an additive between subject variability in the logit scale model was used as follows:

\[
\Theta = tv\Theta + \Theta
\]  
(3)

Where \( \Theta \) is the between subject variability for \( \Theta \). For consistency and interpretation purpose, the additive between subject variability for \( \Theta \) was transformed to between subject variability for bioavailability (\( F \)) in a multiplicative scale using the formula as follows:

\[
F = tvF \cdot (1 - tvF) \cdot \sqrt{\sigma^2_{\Theta}}
\]  
(4)

This equation includes the typical value for \( F \) (tvF) and OMEGA-squared \( \Theta \) which is the variance of between subject variability (BSV) on \( \Theta \).
(3) **Covariate Model**

Based on prior publications of morphine PK in infants, age was considered as a potentially significant covariate. The age effect on maturation of central volume of distribution and clearances were included in the final models. The maturation of morphine clearance was borrowed from a previous a publication directly\(^6\). However, the maturation of morphine distribution was newly modeled based on physiological considerations. Morphine is mainly distributed in extracellular water, which is represented by the central volume of distribution in the base three compartment model. The physiological maturation of extracellular water as a percentage of body weight was published in previous work\(^5\) and was used to further adjust the maturation of morphine distribution after considering the allometric scaling approach. A digitized extracellular water maturation curve was fitted by an exponential model as shown in Figure 7.

This exponential model was used to model the effect of age on distribution maturation.
Figure 7: Maturation of extracellular body water as a percentage of body weight. The red solid dots represent digitized data from previous work\textsuperscript{59}. The solid blue line represents model fit.

(4) Model Validation

*Goodness-of-Fit Plots*

Model selection was driven by the data and was based on various goodness-of-fit indicators, including comparisons based on the minimum objective function value (OFV), visual inspection of diagnostic scatter plots, and evaluation of estimates of population fixed and random effect parameters.

*Normalized Prediction Distribution Error*
Normalized Prediction Distribution Error (NPDE) analysis was performed in Phoenix and NPDE R package10 (version 2) in R 3.0.2. Two hundred replications of simulation were generated for each observation in the original dataset using the final model in Phoenix. A statistical test for normality was automatically generated by the R package. NPDE versus population predicted concentrations (PRED) and NPDE versus Time after Dose (TAD) were used to determine whether worrisome trends were present suggesting errors in bias or precision.

Bootstrap

Nonparametric bootstrap method using Phoenix NLME 1.3 was used to evaluate the precision of parameter estimation in the final model. Two hundred replications were generated by re-sampling from the observed morphine concentration dataset and PK parameters were estimated for each of the replication datasets separately. The median and corresponding 95% percentile interval (2.5th and 97.5th percentiles) obtained from the 200 sets of parameter estimations were compared to the estimations obtained from First Order Condition Estimation with Interaction (FOCE-I).

Results

For the analysis, data from 18 prospectively enrolled fullterm infants, supplying a total of 51 drug concentrations, were used. The 18 infants included three with ICU acquired NAS and 15 with in utero acquired NAS. Gestational ages at birth were between 35 4/7 weeks to 41 0/7 weeks and birth weights were between 1920 grams and 3710 grams (see Table 4). These 18 infants provided 51 different samples on which parent drug morphine, morphine-3-glucuronide and morphine-6-glucuronide were measured.
Table 4: Patient Characteristics in Prospective Cohort (n=18)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median, Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational Age, weeks</td>
<td>37 2/7 (35 4/7 to 41 0/7)</td>
</tr>
<tr>
<td>Birthweight, grams</td>
<td>2778 (1920-3710)</td>
</tr>
<tr>
<td>ICU acquired NAS (%)</td>
<td>3/18 = 17%</td>
</tr>
<tr>
<td>In utero Methadone-only exposed (%)</td>
<td>11/18 = 61%</td>
</tr>
<tr>
<td>In utero poly-substance exposed</td>
<td>4/18 = 22%</td>
</tr>
<tr>
<td>Non-white race (%)</td>
<td>3/18 = 17%</td>
</tr>
</tbody>
</table>

Among the 18 prospectively collected patients, there were 11 samples in which parent drug morphine was < 5 ng/ml and there was a significant amount of M3G detected (median 25.4 ng/ml, range 9.8 to 316 ng/ml). In all 51 samples, there was more M3G detected than M6G. The ratio of M3G:M6G ranged from 3.0 to 5:1 in 44/51 samples. In the remaining seven samples, the ratio ranged from 5.8:1 to 28.5:1 (see Table 5).

Table 5: Individual Patient Drug Concentrations

<table>
<thead>
<tr>
<th>Pt ID</th>
<th>Dose</th>
<th>Time since Dose</th>
<th>Morphine (ng/mL)</th>
<th>Morphine-3-Glucuronide (ng/mL)</th>
<th>Morphine-6-Glucuronide (ng/mL)</th>
<th>Ratio M3G to M6G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>0.1 mg q3</td>
<td>2:46</td>
<td>11.56</td>
<td>76.64</td>
<td>19.6</td>
<td>3.91</td>
</tr>
<tr>
<td>1B</td>
<td>0.1 mg q3</td>
<td>3:00</td>
<td>5.85</td>
<td>45.22</td>
<td>12.28</td>
<td>3.68</td>
</tr>
<tr>
<td>1C</td>
<td>0.1 mg q3</td>
<td>3:00</td>
<td>&lt;5</td>
<td>13.45</td>
<td>&lt;5</td>
<td>13.45</td>
</tr>
<tr>
<td>2A</td>
<td>0.14 mg q3</td>
<td>2:43</td>
<td>8.43</td>
<td>78.41</td>
<td>24.67</td>
<td>3.18</td>
</tr>
<tr>
<td>2B</td>
<td>0.18 mg q3</td>
<td>2:19</td>
<td>13.14</td>
<td>103.21</td>
<td>33.03</td>
<td>3.12</td>
</tr>
<tr>
<td>2C</td>
<td>0.2 mg q3</td>
<td>3:00</td>
<td>11.25</td>
<td>116.39</td>
<td>37.33</td>
<td>3.12</td>
</tr>
<tr>
<td>2D</td>
<td>0.18 mg q3</td>
<td>3:00</td>
<td>5.8</td>
<td>79.62</td>
<td>22.75</td>
<td>3.50</td>
</tr>
<tr>
<td>3A</td>
<td>0.08 mg q3</td>
<td>2:25</td>
<td>32</td>
<td>67.63</td>
<td>21.17</td>
<td>3.19</td>
</tr>
<tr>
<td>3B</td>
<td>0.05 mg q3</td>
<td>0:23</td>
<td>&lt;5</td>
<td>26.2</td>
<td>6.73</td>
<td>3.89</td>
</tr>
<tr>
<td>3C</td>
<td>0.02 mg q3</td>
<td>4:00</td>
<td>&lt;5</td>
<td>24.52</td>
<td>6.56</td>
<td>3.74</td>
</tr>
<tr>
<td>4A</td>
<td>0.08 mg q3</td>
<td>1:02</td>
<td>9.46</td>
<td>48.06</td>
<td>16.14</td>
<td>2.98</td>
</tr>
<tr>
<td>4B</td>
<td>0.08 mg q3</td>
<td>3:00</td>
<td>6.85</td>
<td>43.09</td>
<td>14.07</td>
<td>3.06</td>
</tr>
<tr>
<td>4C</td>
<td>0.08 mg q3</td>
<td>3:04</td>
<td>&lt;5</td>
<td>13.89</td>
<td>&lt;5</td>
<td>13.89</td>
</tr>
<tr>
<td>4D</td>
<td>0.04 mg q3</td>
<td>2:00</td>
<td>&lt;5</td>
<td>19.26</td>
<td>5.73</td>
<td>3.36</td>
</tr>
<tr>
<td>5</td>
<td>0.14 mg q3</td>
<td>1:05</td>
<td>13.74</td>
<td>64.48</td>
<td>16.45</td>
<td>3.92</td>
</tr>
<tr>
<td>6A</td>
<td>0.18 mg q3</td>
<td>0:00</td>
<td>11.2</td>
<td>178.66</td>
<td>47.02</td>
<td>3.80</td>
</tr>
<tr>
<td>6B</td>
<td>0.16 mg q3</td>
<td>1:13</td>
<td>11.88</td>
<td>145.5</td>
<td>39.9</td>
<td>3.65</td>
</tr>
<tr>
<td>6C</td>
<td>0.08 mg q3</td>
<td>0:30</td>
<td>5.49</td>
<td>64.28</td>
<td>17.53</td>
<td>3.67</td>
</tr>
<tr>
<td>6D</td>
<td>0.04 mg q3</td>
<td>1:02</td>
<td>&lt;5</td>
<td>33.12</td>
<td>9.11</td>
<td>3.64</td>
</tr>
<tr>
<td>7A</td>
<td>0.16 mg q3</td>
<td>3:00</td>
<td>5.77</td>
<td>126.07</td>
<td>25.51</td>
<td>4.94</td>
</tr>
</tbody>
</table>
### Final Parent Drug Model

The final covariate models for clearance (CL), the central compartment for volume of distribution (V), inter-compartmental clearance (Q2, Q3) and peripheral compartments for volume of distribution (V2, V3) are shown as follows:

\[
\text{tvCL} = CL_{\text{std}} \cdot \left(\frac{\text{Wt}}{70}\right)^{0.75} \cdot \frac{\text{PMA}_{\text{HIIICL}}}{\text{PMA}_{\text{HIIICL}} + \text{CL}_{\text{mat50}} \cdot \text{HIIICL}} \text{ Liter/hour}^{-1} \quad (5)
\]

\[
\text{tvV} = V_{\text{std}} \cdot \left(\frac{\text{Wt}}{70}\right) \cdot \left(1 + \beta_{\text{vol}} \cdot e^{-\text{PNA}_{\text{HIIICL}} \cdot \frac{\text{Ln}(2)}}\right) \text{ Liter} \quad (6)
\]
tvQ2 = Q2std\(\left(\frac{\text{WT}}{70}\right)^{0.75}\) Liter/hour\(^{-1}\) \hspace{1cm} (7)

tvQ3 = Q3std\(\left(\frac{\text{WT}}{70}\right)^{0.75}\) Liter/hour\(^{-1}\) \hspace{1cm} (8)

tvV2 = V2std\(\left(\frac{\text{WT}}{70}\right) \cdot \left(1 + \beta_{\text{vol}} e^{-\text{PNA} \frac{\text{Ln}(2)}}{\text{Tvol}}\right)\) Liter \hspace{1cm} (9)

tvV3 = V3std\(\left(\frac{\text{WT}}{70}\right)\) Liter \hspace{1cm} (10)

where tvCL is the typical value of clearance and tvV is the typical value of volume of distribution. These typical values for total clearance (CL) and central volume of distribution (V) were estimated from the above equations using the covariates postmenstrual age (PMA), postnatal age (PNA) and body weight (WT) in kilograms. The rest of the parameters in the above equation were fixed to the values obtained from the previous publication\(^{54}\). Between and within subject variability were newly estimated during the modeling process for morphine clearance, volume of distribution and absorption fraction.

**Model for Absorption**

An absorption compartment and first order absorption rate constants were added to the prior discussed IV model. The final structure of the pharmacokinetic model is as follows:

\[
\frac{\text{d}A_a}{\text{d}t} = -K_a A_a \hspace{1cm} (11)
\]

\[
\frac{\text{d}A_1}{\text{d}t} = K_a A_a - \frac{\text{CL} A_1}{V} - \frac{Q_2 A_1}{V} + \frac{Q_2 A_2}{V_2} - \frac{Q_3 A_1}{V} + \frac{Q_3 A_3}{V_3} \hspace{1cm} (12)
\]

\[
\frac{\text{d}A_2}{\text{d}t} = \frac{Q_2 A_1}{V} - \frac{Q_2 A_2}{V_2} \hspace{1cm} (13)
\]

\[
\frac{\text{d}A_3}{\text{d}t} = \frac{Q_3 A_1}{V} - \frac{Q_3 A_3}{V_3} \hspace{1cm} (14)
\]
where Aa is the amount of morphine in the absorption compartment, A1 is the amount in the central compartment, A2 and A3 are the amounts in the peripheral compartments, and Q2 and Q3 are the intercompartmental clearance. Bioavailability was modeled logistically to theoretically constrain the value from zero to one using equation (15).

\[
F = \frac{e^{\theta_t}}{1 + e^{\theta_t}}
\]  

(15)

**Final Metabolite Model**

The same approach which was used to build the parent drug morphine population pharmacokinetic model was extended to describe the population pharmacokinetics of morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G), the two major metabolites of morphine. The formation of M3G and M6G as a result of parent drug morphine metabolism was added to the prior described morphine population pharmacokinetic model using two formation fraction factors (fmM3G and fmM6) that describe the proportion of the parent morphine converted to these two metabolites. Further, the first pass effect on morphine metabolism after oral administration was described by the addition of two absorption rate constants for M3G and M6G (KM3G and KM6G). The maturation of M3G and M6G elimination clearance (CLM3G and CLM6G) was borrowed from a previous publication\(^{61}\) which investigated the maturation of elimination clearance via GFR from neonates to adults.

\[
CLM3G = CLM3Gstd\cdot \left(\frac{WT}{70}\right)^{0.75} \cdot \frac{PMA_{GamR}}{PMA_{GamR} + CLR_{mat50GamR}} \text{Liter}
\]  

(16)

\[
CLM6G = CLM6Gstd\cdot \left(\frac{WT}{70}\right)^{0.75} \cdot \frac{PMA_{GamR}}{PMA_{GamR} + CLR_{mat50GamR}} \text{Liter}
\]  

(17)

The final integrated parent-metabolite model can describe the concentration time profile of morphine, M3G and M6G with an absorption fraction parameter Fa instead of bioavailability F.
Fa is the total percent of the morphine dose that is absorbed as morphine, M3G or M6G from the gut.

**Table 6:** Parameter Estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Meaning</th>
<th>Estimates</th>
<th>BSV</th>
<th>Bootstrap Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka</td>
<td>Hour$^{-1}$</td>
<td>Absorption rate constant parent drug</td>
<td>0.471</td>
<td>0.453</td>
<td>(0.208, 0.850)</td>
</tr>
<tr>
<td>Km3G</td>
<td>Hour$^{-1}$</td>
<td>Absorption rate constant M3G</td>
<td>0.0468</td>
<td>0.0601</td>
<td>(0.0131, 0.104)</td>
</tr>
<tr>
<td>Km6G</td>
<td>Hour$^{-1}$</td>
<td>Absorption rate constant M6G</td>
<td>0.0486</td>
<td>0.0511</td>
<td>(0.022, 0.093)</td>
</tr>
<tr>
<td>Fa</td>
<td>n/a</td>
<td>Absorption fraction</td>
<td>58.9%</td>
<td>8.5%</td>
<td>57.1% (50.8, 66.2)</td>
</tr>
<tr>
<td>Vstd$^\dagger$</td>
<td>L</td>
<td>Central volume of distribution</td>
<td>17.8</td>
<td>95.6%</td>
<td></td>
</tr>
<tr>
<td>CLstd$^\dagger$</td>
<td>L/hour</td>
<td>Parent drug clearance</td>
<td>75.3</td>
<td>68.6%</td>
<td></td>
</tr>
<tr>
<td>V2std$^\dagger$</td>
<td>L</td>
<td>Second compartment</td>
<td>87.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V3std$^\dagger$</td>
<td>L</td>
<td>Third Compartment</td>
<td>199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2std$^\dagger$</td>
<td>L/hour</td>
<td>Intercompartmental clearance</td>
<td>136</td>
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<td></td>
</tr>
<tr>
<td>Q3std$^\dagger$</td>
<td>L/hour</td>
<td>Intercompartmental clearance</td>
<td>19.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM6G1$^\dagger$</td>
<td>L</td>
<td>Central volume for M6G</td>
<td>9.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM6G2$^\dagger$</td>
<td>L</td>
<td>Peripheral volume for M6G</td>
<td>7.1</td>
<td></td>
<td></td>
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<tr>
<td>VM3G1*</td>
<td>L</td>
<td>Central volume for M3G</td>
<td>8.18993</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VM3G2*</td>
<td>L</td>
<td>Peripheral volume for M3G</td>
<td>11.9214</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QM6G$^\dagger$</td>
<td>L/hour</td>
<td>Intercompartmental clearance M6G</td>
<td>5.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QM3G*</td>
<td>L/hour</td>
<td>Intercompartmental clearance M3G</td>
<td>9.62188</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FmM6G$^{++}$</td>
<td>-</td>
<td>Formation fraction (from parent drug) of M6G</td>
<td>4.4 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FmM3G$^{++}$</td>
<td>-</td>
<td>Formation fraction (from parent drug) of M3G</td>
<td>32.9 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLM6G$^\dagger$</td>
<td>L/hour</td>
<td>Elimination Clearance of M6G</td>
<td>9.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLM3G*</td>
<td>L/hour</td>
<td>Elimination clearance of M3G</td>
<td>7.82924</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>-------------------------------</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>stdev0</td>
<td>-</td>
<td>Morphine</td>
<td>48.8%</td>
<td>40.5% (20.5, 58.8)</td>
<td></td>
</tr>
<tr>
<td>stdev1</td>
<td>-</td>
<td>M3G</td>
<td>23.1%</td>
<td>22.1% (15.1, 29.2)</td>
<td></td>
</tr>
<tr>
<td>stdev2</td>
<td>-</td>
<td>M6G</td>
<td>27.2%</td>
<td>27.2% (19.5%, 35.7%)</td>
<td></td>
</tr>
<tr>
<td>HillCL*</td>
<td>-</td>
<td>Maturation exponent</td>
<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GamR%</td>
<td>-</td>
<td>Steepness of clearance maturation curve</td>
<td>3.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLmat50+</td>
<td>Weeks</td>
<td>Age at which pediatrics reaches 50% of adult morphine clearance after adjusted by body Weight</td>
<td>58.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLRmat50%</td>
<td>Weeks</td>
<td>Age at which pediatrics reaches 50% of adult renal clearance after adjusted by body weight</td>
<td>55.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BetaV#</td>
<td>n/a</td>
<td>Fractional difference from Vstd at birth</td>
<td>0.613995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tvol†</td>
<td>Years</td>
<td>maturation half-life of the PNA-related changes of V</td>
<td>0.185467</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

± Fixed from model published by Holford et al. ⁶⁰
¥ Fixed from model published by Rhodin et al. ⁶¹
* Estimated from digitizing the PK profiles from Penson et al. ⁵⁵
§ Fixed from model published by Lotsch et al. ⁵⁴
++ Estimated from data in Stuart-Harris et al. ⁵⁶ and Lundeberg et al. ⁵⁸
# Estimated from extracellular body water maturation function

Model Validation

The goodness-of-fit plots showed that the population predicted and individual predicted morphine, M3G and M6G concentrations based on the final model were in agreement with the observed concentrations (Figure 8). Figure 9 depicts the distribution of NPDE vs time after dose (TAD) and vs log concentration for morphine and its metabolites. The dotted lines represent the 90% distribution of the NPDE.
Figure 8: Observed vs predicted concentrations

Observed vs population-predicted (upper panel) and individual predicted (lower panel) concentrations of (A) morphine-3-glucuronide, (B) morphine-6-glucuronide, and (C) morphine
**Figure 9:** Normalized Prediction Distribution Error (NPDE) is plotted vs Time after Dose and Population Predicted Concentration for (A) morphine-3-glucuronide, (B) morphine-6-glucuronide, and (C) morphine

**Discussion**

This is the first study to characterize the pharmacokinetics of enteral morphine and its glucuronide metabolites in young neonates with ICU and *in utero* acquired opiate withdrawal. We used sparse data to build upon prior published models from multiple populations in order to estimate the fraction of enterally dosed morphine absorbed in neonates. We hope to use this data as the first step in optimized dosing in this population. It was found that 59% of a morphine dose reaches the systemic circulation in these neonates, an estimate of bioavailability much higher than...
the 24% reported after oral morphine administration in adults\textsuperscript{62,63}. Although this PK study was performed in infants with NAS, this relatively high bioavailability can potentially be generalized to other neonates being treated with enteral morphine for post-operative pain or procedural pain.

Similar to prior published parenterally dosed morphine models, our data confirm the importance of maturation (age) and size (weight) on clearance of morphine. Strengths of this study include maximized use of prior published morphine PK models in different populations as prior data in parameter estimation and model building. Because the pediatric literature is so rich for IV morphine PK, it would have been unethical to perform a more traditional bioavailability study in these infants, meaning concurrent IV and enteral dosing. Additionally, we were able to measure morphine metabolites on very small samples and incorporate these into a universal structural model for morphine disposition.

Potential weaknesses to the study include error introduced in model building and PK parameter estimates due to inaccuracy of nursing record of morphine dosing times. All infants in the study were known to require exact documentation of doses by nursing staff, so we hope that medication and sample time recording error was minimized. Additionally, there is potential for model mis-specification given the strong emphasis on prior information, especially when the populations used do not exactly match that of the current study. The theory behind this approach is that the pharmacokinetics are universal for a given drug, “one drug one model”. The adult parameters, once externally validated and proven robust, can be used as prior information and applied to data collected in children to estimate the effects of pediatric specific covariates, in this case maturation and size.

An improved understanding of the disposition of enterally dosed morphine gives us the ability to simulate concentration – time profiles by varying dosing regimens in neonates with NAS. This is the first and necessary step in a larger process to optimize dosing of morphine in
this population. Further understanding of morphine distribution, including movement across the blood brain barrier and how this transport changes with age, will also further our ability to optimize morphine dosing. Other important yet to be studied factors include the ontogeny of the mu opioid receptor and its influence on clinical response, and the genetically derived variation in drug metabolism, disposition and receptor-ligand binding. Further research in these areas will add to our knowledge of morphine PK-PD interactions and will advance the care of infants with Neonatal Abstinence Syndrome.

**Figure 10**: Final Structural Model
The Variability in Placental Opiate Transfer and NAS

The current standard of care for opiate addicted pregnant women is to transition from illicit drugs or heavy prescription drug use into an opiate maintenance program, comprised of either methadone or buprenorphine. Women are started on standard doses and often require titration to mitigate withdrawal symptoms and prevent illicit drug relapse. Weaning off of opiate medications during pregnancy is generally not recommended due to the risk of fetal withdrawal and associated morbidity.

Once the opiate enters the mother’s system, there are many steps which are currently very poorly understood, which could have clinically meaningful impacts on fetal exposure to opiates during pregnancy and the development of significant NAS symptoms due to physical dependence in the neonate. One potentially large influence of maternal to fetal opiate transfer is the pharmacogenetic underpinnings of maternal PK, placental metabolism and transport, and neonatal metabolism and response. The published literature regarding genetic variability in opiate metabolism, placental function and opiate response are reviewed in this final chapter of this thesis. In Chapter III, I review the rationale behind the need to study these influences in opiate exposed mother-infant dyads.
CHAPTER 3

The Potential for Personalized Medicine in Maternal Opiate Dependence and Neonatal Abstinence Syndrome

Introduction

Opiate dependent pregnant women around the world are treated with opiate maintenance medications to prevent illicit use and withdrawal during pregnancy. Fetal exposure to opiates causes central nervous system alterations which manifest as physical withdrawal after birth. The extensive variability in postnatal withdrawal remains unexplained. Improved understanding of functionally significant allelic variants in pathways influencing placental opiate transfer during pregnancy can advance treatment of maternal opiate maintenance and lead to improved obstetric and perinatal care.

Background

Neonatal Abstinence Syndrome (NAS) is the physical manifestation of opiate dependence in a newborn who was chronically exposed to opiates during gestation via maternal use. Infants at risk for NAS must be monitored in the inpatient setting after birth, and a fraction require prolonged hospitalization for pharmacologic therapy and weaning. The most severely affected infants cannot orally feed, fail to gain weight, and have seizure activity related to opiate withdrawal. There is also long-term neurodevelopmental morbidity associated with in utero opiate exposure. When compared to controls, children who were in utero opiate exposed have deficits in language, motor and cognitive development64.

The current standard of care for pregnant women with opiate addiction or dependence is not to attempt to wean them off their medications or illicit drugs, but instead to provide excellent prenatal care with the adjunct of a maintenance opiate program. The two maintenance medications most commonly used are methadone and buprenorphine. According to the Substance Abuse and Mental Health Services Administration, there were 1,739 programs in the United
States offering addiction treatment to pregnant women in 2011, comprising 12.7% of all treatment programs. There are no current data about absolute numbers of pregnant women admitted to methadone and buprenorphine treatment programs, but around 20% of pregnant women admitted to drug treatment programs report opiates as their primary substance of abuse.65. There have been many attempts to correlate maternal exposure, as measured by dose, to neonatal outcomes. A recent systematic review confirms the results of many smaller studies, namely that there is no currently known relationship between the maternal dose of methadone and the incidence or severity of NAS.66. This lack of association is likely because our current thinking on the maternal – fetal – neonatal transfer and effect of opiate medications is oversimplified and does not account for the potential impact of pharmacokinetics, pharmacodynamics and pharmacogenetics and how these are affected by gestational changes in maternal physiology. In alternate terms, a central issue is the lack of knowledge to explain the relationship between maternal dose and systemic fetal exposure at the level of the individual mother-infant dyad.

During pregnancy, opiate drugs available in the maternal system are passed to the fetus via the placenta, and drugs are actively effluxed back into the maternal circulation via transport proteins that are in both the basaolateral and apical sides of the syncytiotrophoblasts. In a small cohort of chronically treated women, the cord to maternal ratio at birth is 0.41 (range 0.19-0.56) for (R)-methadone and 0.35 (range .014-0.47) for buprenorphine.67. There are potential pharmacologic factors that could affect the amount of free drug in the maternal circulation available for fetal transfer at any given time. The factors include both physiologic changes in drug disposition during gestation and genetic variability in key maternal drug metabolizing pathways, both of which affect the quantities of parent opiate and active metabolites available for placental transfer.
In addition to changes in maternal drug metabolism, the pumping efficiency of placental transport proteins such as Multi-drug Resistant Protein 1 (MDR1)**1 and Breast Cancer Resistance Protein (BCRP) could alter the ratio of maternal to fetal drug exposures. Lastly, fetal characteristics, such as efficiency of drug metabolism and mu-opioid receptor sensitivity and function might alter the infant’s development of NAS given the exposure to maternal medications. In addition to gestational and ontogenic changes in these pharmacologic factors, genetic variation potentially adds a layer of complexity to placental opiate transfer (Figure 11).

The aim of this article is to review the pertinent literature addressing the effects of changing physiology during pregnancy and pharmacogenetic variation as sources of inter-individual variation in the extent and consequences of fetal/newborn exposure to opiates following maternal administration during pregnancy. This chapter will focus on pathways affecting opiate metabolism, opiate placental transport, and opiate receptor interactions. Data will be discussed as they pertain to the potential impact on our understanding and treatment of opiate dependence in pregnancy and Neonatal Abstinence Syndrome at the level of individual mother-infant pairs.

---

1 **The ABCB1 gene encodes Multi-Drug Resistant Protein 1, otherwise known as Permeable-glycoprotein (P-gp). For the purpose of this review article, we will refer to the official gene name ABCB1, and the protein will be referred to as MDR1.
Figure 11: Factors in maternal to fetal opiate transfer with known genetic variability (original figure)

Maternal Circulation

Placental Syncitiotrophoblasts

Fetal / Neonatal Circulation

Parent Opiate Drug

Metabolizing Enzymes

Inactive Metabolite

Active Metabolite

=modulated by genetic changes

Liver Transport Proteins

Metabolizing Enzymes

Renal Elimination

LIVER

Mu-opioid receptor

Maternal Metabolism of Opiates

Methadone

There are known clinically significant genetic modifiers of opiate metabolism in humans. Alterations in maternal metabolism of opiate medications could affect the amount of active drug available for fetal transfer during pregnancy and modify the degree of central nervous system adaptation which later manifests as NAS after birth.
Methadone is primarily N-demethylated to 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in the liver, predominantly by CYP2B6\textsuperscript{68}. There are studies which show large interindividual variability in methadone pharmacokinetics. A study in peripartum women treated with methadone has shown no correlation between dose and plasma methadone concentrations\textsuperscript{69}. There is evidence that a portion of this variability can be attributed to allelic variation in drug metabolizing enzymes.

In vitro studies have assessed altered enzyme function leading to changes in metabolite formation as compared to wild type enzymes. In human liver microsomes, \textit{N}-demethylation of methadone by variant CYP2B6 enzymes is reduced to 25-33\% of the wild-type enzyme.\textsuperscript{70} In addition, CYP2B6 genetic variants preferentially metabolize the S-methadone enantiomer,\textsuperscript{71} resulting in a net reduction of R-methadone \textit{N}-demethylation and different enantiomers of methadone may have differential placental transport.

A human study has shown that haplotype blocks describing commonly co-occuring CYP2B6 single nucleotide polymorphisms (SNPs) in both intronic and exonic regions are predictive of plasma concentrations of methadone and the concentration to dose ratio in 366 patients undergoing chronic methadone maintenance therapy\textsuperscript{72}. A re-analysis of phenotypic extremes in a different cohort of methadone maintenance patients has shown that certain CYP2B6 SNPs are more or less common in patients with high and low methadone concentrations, strengthening the etiologic link between genetic changes in the metabolizing enzyme and differences in methadone plasma concentrations\textsuperscript{73}. In a systematic review, it was shown that patients homozygous for the CYP2B6*6 allele have higher trough (R) and (S) methadone plasma concentrations, suggesting that methadone metabolism is significantly slower in *6 homozygous carriers\textsuperscript{74}. Genetic polymorphisms in CYP2B6 are potential predictors of methadone clearance and concentrations and could prospectively be used to understand which fetuses are going to be exposed to more parent methadone.
**Oxycodone**

Oxycodone is metabolized to oxymorphone, its active metabolite, by CYP2D6. There are four phenotypic groups based on CYP2D6 activity: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM). Ratios of oxymorphone to oxycodone significantly differ by metabolizing enzyme genotype in 121 adult post-operative patients and PMs had the highest oxycodone consumption, suggesting a clinical consequence of poor metabolism to the active form of the drug. Differential metabolism to oxymorphone in the maternal system can alter the concentrations of active metabolite available for placental transfer.

**Placental Transport of Drugs**

The placenta is a major line of defense for the developing fetus against pathogens and potentially harmful endogenous and exogenous substances in the maternal system. Placental transporters are known to modulate fetal exposure to maternal medications and other environmental exposures. Multi drug resistant (MDR1), also known as P-glycoprotein (P-gp) is encoded by ABCB1. Breast cancer resistance protein (BCRP) is encoded by ABCG2. Both of these transporters are part of a larger subgroup of ATP binding cassette (ABC) transporters known to control efflux of drug across the placenta. There are comprehensive reviews about transporters and the placenta, but the breadth and scope of their content are beyond the focused discussion of the studies in vitro, in animal models and in human placenta which confirm the role of these transporters is modulating drug concentrations between the maternal and fetal circulations, and the genetic variation which effect their function in regard to Neonatal Abstinence Syndrome.

In vitro studies have shown that inhibitors of normal transport protein function increase drug concentrations in fetal circulation as compared to controls. Type II diabetes medications
have been studied extensively given the risk of hypoglycemia in the fetus. BCRP is postulated to play a role in effluxing glyburide at the apical membrane of the syncytiotrophoblasts, thus protecting the fetus from hypoglycemia. In a dual perfusion placental model, a BRCP inhibitor caused an almost doubling of the fetal-to-maternal concentration ratio of glyburide (0.56 +/- 0.06 for inhibitor treated perfusions vs. 0.32 +/- 0.06, p-value 0.04)\textsuperscript{80}. In cell lines over expressing BCRP, addition of transport protein inhibitors increased the intracellular concentrations (analogous with fetal circulation) of glyburide 3-fold\textsuperscript{81}. Outside of the realm of diabetes therapy, antibodies against P-gp in monolayer cell culture dual chamber experiments increased the basolateral (fetal) concentrations of drugs such as dexamethasone and ritonavir by as much as two fold compared with baseline levels\textsuperscript{82}.

Animal studies have confirmed the importance of P-gp in protecting the fetus in vivo from exogenous substances. In an experiment where multiple fetal genotypes were created in the same mother by mating males and females heterozygous for the MDR1a/1b genes which encode P-gp, IV drugs were administered to the pregnant mice and the fetal mice were sacrificed and labeled drug was quantified in the fetal plasma at various time points after maternal dosing\textsuperscript{83}. For digoxin, saquinivir and paclitaxel, the fetal-to-maternal plasma concentration ratios were significantly higher for the P-gp deficient fetal mice (Figure 1). In addition, this group dosed the dams with enteral P-gp inhibitors and found that they could increase fetal concentrations of saquinivir by up to 7-fold. This study showed that presence or absence of functional P-gp can profoundly limit the passage of therapeutic or toxic drugs to the fetus.
Figure 12: Ratio of fetal concentration to maternal plasma concentrations.


CF-1 mice strains contain a spontaneous mutation in the mdr1a gene, leaving them P-gp deficient in multiple organs, including the placenta. In a study of these mice, males and females were selectively mated to create a range of litter genotypes including P-gp homozygote normal, heterozygote and homozygote null. The dams were treated with a radiolabeled toxin known to induce cleft palate and the neonatal mice were examined for birth defects (see Table 7) and have drug concentrations measured at birth. Zero percent of homozygous normal mice had cleft palate but 30% of heterozygous and 100% of homozygous null mice had cleft palate, showing a gene-
dose effect for the role of P-gp in protecting fetal mice from this teratogen. Correspondingly, neonatal levels of toxin were 87-112, 124-180, and 542-678 pmol/g in the aforementioned genotypic groups\(^{84}\).


<table>
<thead>
<tr>
<th>Female × Male</th>
<th>Vehicle control</th>
<th>L-652,280 (1.5 mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+/-) × (+/-)</td>
<td>(-/-) × (-/-)</td>
</tr>
<tr>
<td>Litters examined</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Fetuses examined</td>
<td>108</td>
<td>141</td>
</tr>
<tr>
<td>Cleft Palates(^{5})</td>
<td>1 (9)</td>
<td>0</td>
</tr>
<tr>
<td>FETuses (%)</td>
<td>1 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Litters (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{5}\)Foregenotyped mice were mated and females received vehicle or 1.5 mg/kg/d L-652,280 on Gestation Days 6 through 15. On Gestation Day 18, animals were euthanized, and the fetuses were examined for cleft palate.

P-glycoprotein genotypes are (+/+), homozygous positive; (+/-), heterozygous; (-/-), homozygous negative.

Number of affected fetuses or litters with at least one affected fetus. Number in parentheses is affected fetuses or litters/total × 100.

\(^{*}\)The one unaffected litter had only one fetus.

In addition to the known drug transporting properties, the ontogeny of placental transporters has been investigated using human placental tissue. In a study of homogenized placental tissue comparing 13-14 weeks via chorionic villus sampling and 38-41 weeks via vaginal and c-section deliveries, it was shown that the density of P-gp as measured by western blot was twice as high in early placentas as compared to late (p-value 0.0004)\(^{85}\). A second study confirmed these findings by comparing ABCB1 mRNA levels and P-gp expression by western blot between 6-13 week placentas from therapeutic terminations, 24-35 week placentas from preterm births and term placentas. Results (seen in Figure 13) show that there is statistically significantly more mRNA in early placentas when compared to term\(^{86}\).
Placental Metabolism and Transport of Opiates

Due largely to the work of the Obstetric-Fetal Pharmacology Research Unit at University of Texas, we have in vivo and in vitro data of placental opiate metabolism and transfer. Key findings from various studies in these realms are summarized and synthesized in the following sections.

Placental Opiate Metabolism

Studies in placental microsomal preparation have given insight into the placental metabolism of methadone both in preterm\textsuperscript{87} and term placentas\textsuperscript{88}. Using placentas from term healthy pregnancies, microsomal fractions of trophoblast tissue were studied using selective
inhibitors for different metabolizing enzymes and measuring biotransformation of methadone to EDDP. CYP19 / aromatase chemical inhibitors and neutralizing monoclonal antibodies were found to have the most profound effect on methadone metabolism, with 70-88% reduction in EDDP formation. The apparent Km and Vmax values are summarized in Table 8. Furthering this work by investigating placentas from earlier in gestation, late second trimester, early third trimester and late third trimester placental tissue was similarly used to measure enzyme kinetics. The apparent Km values for the different gestations were similar (Table 8) but the metabolism potential increased with gestation showing a statistically significant doubling of intrinsic clearance between late second trimester and late third trimester. In addition, there was marked interindividual variability in metabolizing enzyme activity even within the same gestational ages, four to six fold, suggesting that aromatase function could be one of the important factors in fetal exposure to active methadone and resultant NAS development.

Buprenorphine is also metabolized by CYP19 / aromatase in the placenta and, similar to methadone, placental microsome studies have confirmed that enzyme activity in biotransformation of buprenorphine to norbuprenorphine (norBUP) increases with gestational age (Table 8).
Table 8: Placental Opiate Metabolism Table (compiled from multiple publications) Experimental model is microsomal fraction of trophoblasts for all experiments represented.

<table>
<thead>
<tr>
<th>Opiate</th>
<th>Enzyme System</th>
<th>Gestational Age</th>
<th>Km (microM)</th>
<th>VMax (pmol/min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methadone</td>
<td>CYP19/aromatase</td>
<td>Term (?)</td>
<td>424 +/- 92</td>
<td>420 +/- 89</td>
<td>Nanovskaya 2004</td>
</tr>
<tr>
<td>Methadone</td>
<td>CYP19/aromatase</td>
<td>17-27 wks (n=12)</td>
<td>514 +/- 187</td>
<td>192 +/- 88</td>
<td>Hieronymus 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28-33 wks (n=12)</td>
<td>519 +/- 154</td>
<td>271 +/- 88</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>34-40 wks (n=12)</td>
<td>503 +/- 120</td>
<td>385 +/- 129</td>
<td></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>CYP19/aromatase</td>
<td>Term (n=6)</td>
<td>13 +/- 4</td>
<td>2.9 +/- 0.7</td>
<td>Deshmukh 2003</td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>CYP19/aromatase</td>
<td>17-26 wks (n=7)</td>
<td>15 +/- 9</td>
<td>1.9 +/- 0.4</td>
<td>Fokina 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>27-33 wks (n=17)</td>
<td>16 +/- 0.2</td>
<td>2.4 +/- 0.8</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>34-37 wks (n=6)</td>
<td>24 +/- 8</td>
<td>2.8 +/- 0.7</td>
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</tr>
</tbody>
</table>

**Placental Opiate Transport**

P-gp is a known transporter for methadone. Using a single layer of syncytiotrophoblasts lineage cells in a dual perfusion model, Nanovskaya and colleagues showed that methadone transfer to the fetal circuit was increased by 30% by different P-gp inhibitors\(^91\). The authors concluded based on this experiment that the concentration of methadone in the fetal circulation is likely affected by the expression and activity of P-gp.

From ex vivo experiments in a dually perfused placental model, it appears that buprenorphine transport across the placenta is not mediated by P-gp\(^92\), but rather via passive diffusion. Buprenorphine crosses placental cells into the fetal circuit less than methadone, with only 8.6 +/- 1.3% of initial maternal circuit concentrations detected in the fetal circuit after a four hour equilibration\(^93\). This decreased transfer of buprenorphine is thought to be secondary to its
highly lipophilic nature and significant tissue accumulation within the placenta as compared to both the maternal and fetal compartments. These ex vivo studies assess short time frames of placental pharmacokinetics and the applicability to chronic buprenorphine dosing in human pregnancies may not follow similar patterns.

In addition to ontogenic changes discussed prior, evidence suggests that placental transporter expression can be induced by exposure to certain illicit substances, potentially impacting the maternal-fetal balance of methadone. In 24 term placentas tested in a dual perfusion model, experiments showed that the addition of methadone to control medium significantly increased the expression of P-gp (as measured by western blot analysis) by $49 \pm 41\%$ (p-value 0.03). Addition of cocaine and methadone, or heroine and methadone, increased the P-gp expression by $75 \pm 63\%$ (p-value 0.01) and $59 \pm 51\%$ (p-value 0.03) respectively. Given that P-gp is known to transport methadone, this ex vivo evidence suggests that methadone induces P-gp expression, potentially adding to the mechanisms of protecting the fetus from high opiate exposures.

**Genetic Alterations in Placental Transport**

*ABCB1 (P-gp)*

There is known genetic variation in the *ABCB1* gene which encodes P-gp. Among various different populations, the most common single nucleotide polymorphisms in ABCB1 are 1236C>T (synonymous), 2677G>T/A/C (non-synonymous) and 3435C>T (synonymous). The non-synonymous 2677G>T linked in combination with 1236C>T and 3435C>T (MDR1*2) occurs in 62% of European Americans and 13% of African Americans and is shown to have enhanced in vivo P-gp activity, with the AUC for a prototype drug in non-pregnant humans being 40% greater in MDR1*1 homozygotes as compared to MDR1*2 homozygotes with heterozygotes having intermediate values.
Human placental in vitro studies into the effects of genetic variation are many and have shown that genetic polymorphisms in genes which encode placental transporters have an effect on mRNA and protein expression and activity\textsuperscript{96,97}. The biggest concerns in the field is the generally small number of placentas under study, the substrate specific effects of genetic changes and the inability to fully control for environmental factors which can alter transporter expression and function. A recent comprehensive review of the genetic implications for placental transport by Dr. Daud and colleagues proposes grouping the SNPs in placental proteins by similar functional in vitro effect or suspected phenotype in order to move from individual allele frequency analysis to cumulative phenotype frequency analysis\textsuperscript{98}. The potential for fetal teratogenicity has been studied in mothers carrying the MDR1 3435C>T SNP and there is increased risk of cleft lip and palate\textsuperscript{99} and congenital heart disease\textsuperscript{100} in mothers with the minor allele.

\textit{ABCG2 (BCRP)}

Common SNPs in ABCG2 include 421C>A, 34G>A and 376C>T. One study in human placentas showed that homozygotes for the A421 allele had significantly lower protein levels than those for the C421 allele and heterozygotes had intermediate values\textsuperscript{101}. The clinical implications of SNPs in ABCG2 have not yet been investigated in pregnancy.

\textbf{Neonatal Metabolism and Drug Effector Compartment}

The current standard therapy for moderate to severe NAS revolves around opiate replacement therapy post-natally. The two most commonly used opiates are enteral morphine and enteral methadone solutions. Between the years 2004 to 2011, morphine was used as first line therapy in 49-53\% of 14 large US hospitals and methadone used as first line therapy declined from 44\% to 11\%\textsuperscript{102}. Genetic modifiers to morphine metabolism include polymorphisms in both OCT1, a gene which encodes a liver transporter, and UGT2B7, the enzyme responsible for morphine glucuronidation. For methadone, SNPs in CYP2B6 are known to correlate to clearance.
of methadone in adults. In addition to genetic modifiers of the pharmacokinetics, data suggests that polymorphisms in the mu-opiate receptor (MOR1) can have clinically important implications in response to opiate treatment for pain and these same SNPs may play a role in an infant’s clinical response to morphine or methadone therapy for NAS.

*Neonatal Opiate Disposition*

In order for morphine to be metabolized and cleared, it must first be transported from the plasma to the hepatocyte and then metabolized via glucuronidation. OCT1 facilitates hepatic morphine uptake\textsuperscript{103}. In humans, carriers of a loss of function allele in *OCT1* had a mean AUC of morphine 56% higher than non-carriers\textsuperscript{103}. Race appears to correlate with clinical response to morphine for pain, adverse events and morphine clearance\textsuperscript{104}. Because this correlation is not fully explained by SNPs in drug metabolizing enzymes, liver uptake proteins including OCT1 are being investigated. Morphine clearance was compared among 146 post-operative children, stratified by haplotype for loss-of-function *OCT1* variants. Using population modeling and *post hoc* Bayesian estimates, morphine clearance was 17% lower in homozygotes for loss-of-function alleles\textsuperscript{105}. In this population, it was confirmed that the genetic contribution of *UGT2B7* -900A>G to morphine clearance was low compared to the contribution of *OCT1* genetic variability. The OCT1 transporter may not only affect PK of postnatally administered opiates, but also the clearance of maternally acquired drug in the first few days after delivery. The rapidity of clearance of maternally acquired opiate may impact onset and severity of NAS symptoms.

*Neonatal Drug Effector Compartment*

In addition to the genetic influences on PK, an important aspect of response to clinical treatment with morphine is the effector site. For NAS, this involves opiate moving across the blood brain barrier (BBB) and acting upon the opiate receptor. *ABCB1* encodes the BBB transporter P-glycoprotein. There is animal data linking genetic variation in this gene to opiate
induced hyperalgesia, tolerance and dependence\textsuperscript{106}. The observations in this mouse study were especially powerful because the investigators were able to measure opiate concentrations in the brain and further validate the underlying physiology of genetic modifiers of BBB transport function. In human adults, SNPs in ABCB1 are correlated with need for rescue medication in chronic pain therapy\textsuperscript{107} and opiate consumption in immediate post-operative pain\textsuperscript{108}.

\textit{OPRM1} encodes the mu-opioid receptor 1 which is the main receptor target for morphine therapy in NAS. The \textit{OPRM1} 118A.G SNP is associated with less need for pharmacologic therapy and shorter length of treatment in infants with \textit{in utero} acquired NAS\textsuperscript{109}. Although data about MOR1 genetic variability in NAS limited, extensive recent literature in adults shows that common variants in \textit{OPRM1} are associated with post-op opiate response to pain\textsuperscript{110-112} clinical severity of opiate drug overdose\textsuperscript{113,114} and tolerance to experimentally induced pain\textsuperscript{115}. Given a similar receptor target for pain treatment and NAS treatment, suffice to say that genetic variability in \textit{OPRM1} may contribute to clinical response to opiate therapy for NAS and that this is a field which requires further study.

Combination genotypes on ABCB1 and \textit{OPRM1} (i.e. wild type for both vs. mutant for either or both) are associated with oxycodone clinical effects and adverse drug reactions in adult humans. In addition to single gene effects, the combination effect of genetic variability in BBB transport and opiate receptor function may be synergistic or additive in clinical response to treatment of NAS.

\textbf{Summary}

In summary, there are knowledge gaps in maternal opiate maintenance therapy and fetal opiate exposure. If we can more fully understand how maternal dose relates to maternal exposure, how the placenta regulates the maternal exposure to the fetus, and how the fetus and neonate are variable in their pharmacodynamic effects, therapy for these mother-infant dyads could be more
tailed than current. For example, the gestational changes in drug metabolizing enzyme function are not adequately understood. Although there are current PBPK models for pregnant women, certain enzymatic pathways require more data to understand the complete picture of drug disposition. The interplay of relative upregulation and downregulation of multiple hepatic elimination pathways, and how these interact with increased renal clearance must be taken into account. Secondly, we need further research to understand the influence of genetic variants on placental transporter and enzyme expression and function with regards to fetal opiate exposure. It is possible that with improved knowledge of genotype-phenotype correlation, the most at risk fetuses could be identified prenatally and appropriate maternal medication adjustments could be made. Lastly, interindividual variability in response to postnatal treatment is multifactorial. The severity of in utero dependence, neonatal opiate disposition, the dynamic postnatal clearance and pharmacodynamic effect all play a role in an individual infant’s clearance of maternal medication and response to postnatal opiate therapy. As discussed in a recent commentary on changes in drug disposition during pregnancy, the most promising path forward is to integrate findings from in vitro studies, animal studies, and in vivo human clinical studies to create a more complete understanding of maternal disposition and fetal exposure (Figure 3).

Neonatal Abstinence Syndrome carries long term morbidity, and the current empiric approaches used to choose maternal drug and dose, decisions to wean, identifying the infants most at risk, and postnatal treatment paradigms could all use improvements towards more personalized therapies in order to potentially mitigate these morbidities. A recent article compares the early neurodevelopmental outcomes of infants exposed to heroin, methadone and other opiates in utero with age matched Bayley-III standard controls. Mean scores for language (82.12 vs. 100), motor (96.25 vs. 100) and cognition (90.18 vs. 100) were all statistically significantly lower in the opiate exposed group. In addition, infants exposed prenatally to opiates are shown to have alterations in attention and increased childhood behavioral problems. Infants of
opioid dependent women are exposed to varying levels of drug during a critical window of brain
development, and a more tailored understanding of placental opioid transfer and a more
individualized approach may improve long term outcomes.

There are recent and rapidly progressing advances in non-invasive fetal genotyping\textsuperscript{119},
and the fetal genotype largely encodes the placenta. This technology has potential to change the
field of Obstetric and Perinatal Pharmacology. If we can understand genetic alterations in
placental metabolism and transport, and this understanding is complemented with the ability to
know a placental genotype early in pregnancy, maternal medications can be modified to minimize
fetal exposure to harmful exogenous compounds.

Prenatal opioid exposure is increasing and the genetic underpinnings of placental opioid
transfer and neonatal susceptibility and response to therapy for NAS are currently very poorly
understood. In order to move the field of maternal opioid therapy and medical management of
NAS forward, a deeper understanding of the variability in propensity to develop NAS and its
resultant severity are necessary. The mechanisms which underlie maternal opioid metabolism,
placental opioid metabolism and transport, and neonatal opioid metabolism and effector
compartment physiology likely all play some role in the complex process of an infant
withdrawing from opiates in the first weeks of life after gestational opioid dosing. Given the
evidence presented in this review of the known clinically significant polymorphisms in the genes
involved, understanding the genetic underpinnings of these mechanisms is an important next step
in clinical research for the field of NAS and perinatal pharmacology. In the future, all of these
factors could be combined into a comprehensive systems-based model to predict risk and
individualize treatment of NAS in affected newborns.
CONCLUSION

This thesis encompasses three studies, all of which further our understanding of neonatal opiate exposure and treatment. Medical opiate exposure and resultant NAS are increasing, and the awareness of this will lead neonatologists to reconsider pain and sedation protocols and consider standardized approaches to escalation and weaning of opiates in the NICU. Building upon prior knowledge, we have shown that enteral morphine is 59% bioavailable in a cohort of infants with NAS, the first step in future drug simulation studies aimed at optimizing opiate dosing for NAS. Lastly, the current knowledge as it pertains to the potential for genetic variability to influence placental opiate transfer is reviewed. A deeper understanding of these intricate processes will aid in design of studies aimed at personalizing care for maternal opiate dependence and resultant Neonatal Abstinence Syndrome.

Neonates are considered an “orphan population” by many in pharmacology and drug development. These three bodies of work are the preliminary knowledge for the promise of future research. Drug prescription monitoring will continue to shed light on the pharmacoepidemiology of neonatal drug exposure. Elegantly designed studies with sparse sampling and advanced modeling techniques, maximizing use of prior data, can expand the pharmacokinetics and pharmacodynamics knowledge base of current and future drugs in neonates. And lastly, in order to keep up with the rapidly evolving pace of modern medicine, pharmacogenetic underpinnings of variability in placental opiate transfer holds the promise of individualizing the approach to maternal drug therapy and fetal/neonatal exposures.
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107. Takashina Y, Naito T, Mino Y, Yagi T, Ohnishi K, Kawakami J. Impact of CYP3A5 and ABCB1 gene polymorphisms on fentanyl pharmacokinetics and clinical responses in

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APPENDIX I: IRB Protocol for PharmacoEpidemiology Study

JHM IRB - eForm A – Protocol

- Use the section headings to write the JHM IRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.
- When submitting JHM IRB eForm A (new or revised), enter the date submitted to the field at the top of JHM IRB eForm A.

******************************************************************************

1. Abstract
   a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

   Neonatal abstinence syndrome (NAS), the syndrome of narcotic withdrawal in neonates has been well studied1. NAS can be the results of either discontinuation of narcotic exposure when an infant is born to a drug-abusing mother or the discontinuation of narcotic in the medical setting after need for narcotic therapy in the management of pain and sedation. Symptoms of NAS include tremors, irritability, vomiting, trouble sleeping, incessant crying, problems feeding and other behavioral disturbances. Although NAS is a predictable side effect of narcotic use in the ICU setting, recently practitioners have begun to realize its true impact on hospital length of stay. There is an increasing effort to document iatrogenically induced Neonatal Abstinence Syndrome (NAS) when narcotics are used by health practitioners for pain control and sedation.

   The hypothesis of this study is that infants born in fiscal year 2011 with the same primary diagnoses have greater exposure to narcotics and that the duration of treatment of iatrogenic NAS is longer in more recent years. It is also hypothesized that there is a duration or cumulative dose of narcotic that predicts iatrogenic NAS, which would be different for premature and mature infants.

   This study will give health practitioners a better understanding of the trends in infant exposure to narcotics and iatrogenic NAS over time, and will bring the need for more in depth study of optimizing prevention and treatment for iatrogenic NAS to attention among researchers in pediatrics and neonatology.

   In determining the duration or cumulative dose of narcotic that predicts iatrogenic NAS, this study can serve as an initial guide for health practitioners in maximizing the benefits of hospital use of narcotics while minimizing its associated risks in the neonatal population.
2. **Objectives** (include all primary and secondary objectives)

**Specific Aim 1:** Compare cumulative exposure of narcotic (by measures such as cumulative dose or duration, mean mg per day of admission) between 3 time epochs: fiscal year (FY) 2004, FY 2008 and FY 2011 in the Johns Hopkins NICU and PICU.

**Hypothesis:** After controlling for confounding factors related to narcotic exposure, infants born in 2011 will have greater cumulative exposure to narcotics as part of ICU care.

**Specific Aim 2:** Describe trends in the incidence of iatrogenic NAS over time.

**Hypothesis:** Because of increasing exposure to narcotics over time, infants born in the latter time strata are more likely to experience iatrogenic NAS.

**Specific Aim 3:** Identify a critical cumulative narcotic exposure that predicts well a diagnosis of NAS in a majority of infants. **Hypothesis:** The predictor of five days of narcotic exposure as predictive of NAS currently in the literature is accurate.

3. **Background** (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

There is a general sense among practitioners that the therapeutic use of narcotics in neonates has been increasing over time. To our knowledge, there has been no study assessing the trends over time in neonatal narcotic exposure in the Intensive Care Unit. While there have been studies showing infants exposed to more than five days of continuous narcotics are at increased risk of NAS, other factors such as gestational age and cumulative dose may also contribute to their responses. Our research team has approached the neonatal pharmacist Carol Wesolowski to consult with her about the best possible way to capture total narcotic exposure and she recommends a combination of hand calculation from orders in the chart and then secondary confirmation of narcotic exposure via pharmacy records of narcotic doses dispensed. She has confirmed that the electronic pharmacy records go back beyond FY 2004 and can be queried by patient medical record number.

Our research team has consulted with the department of Pediatric Billing located at the Johns Hopkins White Marsh location and have developed a search strategy to successfully identify all infants with the diagnoses discussed via billing records. By fiscal year, they can query all billing records (including inpatient/outpatient/radiology/laboratory) for the top five billing diagnoses associated with a charge. They feel confident that through casting this wide net, we will be able to capture all infants admitted to Johns Hopkins Hospital with these diagnoses through their search results.
Our research team (which includes neonatology, pharmacy, clinical pharmacology) has experience with retrospective chart review and calculation of cumulative narcotic exposure. Please see below for brief example.

**Retrospective chart review: Use of opioids for treatment of postoperative pain in neonates**

In order to determine the number and characteristics of potential subjects for a separate trial, we have retrospectively reviewed the charts of 23 consecutively admitted infants who were ≥35 weeks at the time of surgery between the dates of 3/1/10-5/1/10 (non-cardiac diagnosis) and 7/1/11-12/30/11 (cardiac diagnosis). We determined the cumulative opioid dosage (in morphine equivalents) during the first 4 post-operative days, the duration of opioid therapy, the number of postoperative days before the start of enteral feeds, and the total number of postoperative ventilator days. All the cardiac and non-cardiac infants were managed in the PICU or NICU, respectively.

Table 1. **Characteristics of patients who would meet entry criteria for the proposed study.**

<table>
<thead>
<tr>
<th>Surgery</th>
<th>Cumulative opioid during first 4 PO days (mg morphine equivalents)</th>
<th>Duration of opioid therapy in days</th>
<th>Number of PO days on Mech. Vent</th>
<th>Time (in days) to start of enteral feeds after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiac</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N=9</strong></td>
<td>M±SD 71±40 Median 70 (11.3-140)</td>
<td>11.6 ±9.5 7.5 (3-34)</td>
<td>4±2.5 3 (1-7)</td>
<td>3.6±1.6 3 (2-7)</td>
</tr>
<tr>
<td><strong>Non-Cardiac</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>N=9</strong></td>
<td>M±SD 6.3±10 Median 2.4 (0.44-8.8)</td>
<td>4.6±1.8 5 (2-7)</td>
<td>2.7±2.1 2.5 (0-6)</td>
<td>4.7±2.7 4 (1-10)</td>
</tr>
</tbody>
</table>

4. **Study Procedures**
   a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).
This study will be a retrospective chart review of infants with diagnoses prone to high or prolonged narcotic exposure chosen from three time strata: fiscal years 2004, 2008 and 2011. These diagnoses will include 1) hypoplastic left heart/double outlet right ventricle, 2) congenital diaphragmatic hernia, 3) meconium aspiration syndrome/persistent pulmonary hypertension of the newborn, 4) gastroschisis/omphalocele and 5) extreme prematurity (born at less than or equal to 25 weeks completed gestation).

These infants will be identified via billing records and once the infant is deemed eligible via brief NICU/PICU discharge summary review (look at diagnoses listed, surgical procedures and length of stay), a more extensive chart review will be conducted (see below).

For each infant, the chart will be reviewed and the following data will be collected:

**Demographic data:** medical record, date of birth, gender, race, gestational age at birth, date of admission, date of discharge

1. primary clinical diagnosis requiring ICU admission

2. total duration of narcotic therapy

3. cumulative narcotic dose in milligrams of morphine equivalents

   Equations for conversion to morphine equivalents:

   1 mcg/kg fentanyl equivalent to 20 mcg/kg morphine

   1 mcg/kg dilaudid equivalent to 5 mcg/kg morphine

   1 mg methadone equivalent to 10 mg morphine

4. Diagnosis of NAS in chart (yes/no)

5. Did infant need slow wean of narcotic therapy although never diagnosed with NAS officially?

6. Confounder variables collected which would affect the estimate of association between year of admission and narcotic exposure:

   a. SNAP-II score\(^3\): every infant will have a disease severity score calculated 1) at 12 hours of life and 2) on the day that narcotic therapy was initiated. This severity score will be accounted for in the analysis of narcotic exposure

   b. Other major medical diagnoses (resp, CV, GI)
c. All surgical procedures

d. Infant exposed to paralytic (Yes/No)

e. Length of stay (cumulative narcotic exposure will be standardized by length of stay for certain parts of the analysis).

7. Was infant exposed to benzo and what was total benzo dose?

8. Time from start of narcotic to extubation

9. Time from start of narcotic to full enteral feeding (off IV nutrition)

10. Number of days on antibiotic therapy (this will be used as an overall measure of “intensive care” – i.e. a control measure to compare the increasing narcotic exposure. For example, if someone wonders if narcotic exposure is increasing because overall intensity of ICU care is increasing, we will have antibiotic exposure as a comparison “control” outcome.

b. Study duration and number of study visits required of research participants. N/A
c. Blinding, including justification for blinding or not blinding the trial, if applicable. N/A
d. Justification of why participants will not receive routine care or will have current therapy stopped. N/A
e. Justification for inclusion of a placebo or non-treatment group. N/A
f. Definition of treatment failure or participant removal criteria. N/A
g. Description of what happens to participants receiving therapy when study ends or if a participant’s participation in the study ends prematurely. N/A

5. **Inclusion/Exclusion Criteria** Infants will be included if they are identified through billing records as having been at JHH NICU/PICU during the above-mentioned fiscal years and they carry one of the five diagnoses: 1) hypoplastic left heart/double outlet right ventricle, 2) congenital diaphragmatic hernia, 3) meconium aspiration syndrome/persistent pulmonary hypertension of the newborn, 4) gastroschesis/omphalocele and 5) extreme prematurity (born at less than or equal to 25 weeks completed gestation). Infants will be excluded if they were not born at Johns Hopkins or if they were transferred to another facility before narcotic therapy was stopped.

6. **Drugs/ Substances/ Devices N/A**
a. The rationale for choosing the drug and dose or for choosing the device to be used. N/A
b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed. N/A
c. Justification and safety information if non-FDA approved drugs without an IND will be administered. N/A

7. Study Statistics
   a. **Primary outcome variable:** cumulative narcotic exposure in mg of morphine equivalents (this will be modeled as a continuous outcome)
   b. **Secondary outcome variables:** length of therapy with narcotics, length of hospitalization, time to full enteral feeds, time to extubation
   c. Statistical plan including sample size justification and interim data analysis. From preliminary billing data, we estimate that we will have 40 infants per year that fulfill the inclusion criteria listed above (majority are meconium aspiration/PPHN and primary cardiac diagnoses).
   d. Power analysis: With 10 patients in each time strata for a certain primary diagnosis, we will have an 88% power to predict a 20% change in narcotic exposure using a significance value of .05. (Used for power calculation: Average narcotic exposure 100 mg morphine equivalents, standard deviation 20 mg).
   e. The exposure of time will be modeled as disjoint categories by year strata and we will use linear regression to model narcotic exposure, controlling for covariates discussed above. A type-1 error cutoff of .05 will be used for statistical significance.
   f. For comparing incidence of NAS in the three strata, we will use analysis of variance techniques to compare the groups, also considering a type-1 error cutoff of .05 for statistical significance.
   g. Early stopping rules. N/A

8. Risks
   a. Medical risks, listing all procedures, their major and minor risks and expected frequency. N/A
   b. Steps taken to minimize the risks. N/A
   c. Plan for reporting unanticipated problems or study deviations. N/A
   d. Legal risks such as the risks that would be associated with breach of confidentiality. All data will be de-identified in the study database with a master file linking patient ID to study number kept locked in the PI office. All members of the study will be thoroughly trained in HIPAA compliance and data management.
   e. Financial risks to the participants. N/A

9. Benefits
   a. Description of the probable benefits for the participant and for society. This study will give health practitioners a better understanding of the trends in infant exposure to narcotics and iatrogenic NAS, and its possible correlation with the increasing duration of treatment.
In determining the duration or cumulative dose that predicts iatrogenic NAS, this study can serve as a guide for health practitioners in maximizing the benefits of hospital use of narcotics and minimizing its associated risks.

10. **Payment and Remuneration**  
a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol. N/A

11. **Costs**  
a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them. N/A

**REFERENCES**

1 Carnevale F, Ducharme C. Adverse reactions to the withdrawal of opioids and benzodiazepines in paediatric intensive care. Intensive and Critical Care Nursing. 1997; 13: 181-188.


APPENDIX II: IRB Protocol Morphine PK Study

JHM IRB - eForm A – Protocol

- Use the section headings to write the JHMIRB eForm A, inserting the appropriate material in each. If a section is not applicable, leave heading in and insert N/A.
- When submitting JHM IRB eForm A (new or revised), enter the date submitted to the field at the top of JHMIRB eForm A.

1. Abstract
   a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

   Neonatal Abstinence Syndrome is a complex physiologic result of in utero or ex-utero iatrogenic exposure to opiate medications. The in utero exposure is a result of maternal use of opiates, usually methadone or heroine, during the pregnancy. The iatrogenic exposure is a result of medical treatment with opiates for the management of pain and sedation in the Neonatal and Pediatric Intensive Care Unit. The cellular mechanisms of analgesia, tolerance and dependence to opiates are increasingly clear, but the clinical management of Neonatal Abstinence Syndrome is yet imprecise. The goals of this research project include: SA1 To develop a population pharmacokinetic model for oral morphine and its glucuronide metabolites in neonates undergoing therapy for either ICU or in utero acquired Neonatal Abstinence Syndrome. These two cohorts will include 40 fullterm infants, each prospectively recruited at the time of treatment for Neonatal Abstinence Syndrome. SA2 To develop a population pharmacokinetic/pharmacodynamic model for the relationship between morphine and its metabolites with the clinical outcome of behavioral withdrawal scores. This model will allow a quantitative link between morphine, morphine metabolite serum concentrations and clinical symptoms of NAS. SA3 Compare the pharmacodynamic relationship from SA2 between two populations of infants with NAS – those in utero exposed and those ICU exposed in order to identify key differences in the morphine concentration – clinical outcome relationship. Taking into account differences in the
pharmacologic relationships in the two populations, we will predict appropriate dosing regimens based on identified important clinical covariates for goal withdrawal scores during treatment of NAS.

Relevance: Neonatal Abstinence Syndrome is a common and challenging clinical problem faced by pediatricians and neonatologists, and a disease which carries great morbidity for neonates. Understanding the pharmacologic mechanisms which underly therapy will greatly advance the field of opiate tolerance and withdrawal in this vulnerable population.

2. Objectives (include all primary and secondary objectives)
The response to morphine therapy for the management of pain and sedation during acute illness and the response to weaning of narcotics during detoxification is clinically variable and unpredictable in the neonatal population. While biologic and genetic factors likely contribute to this response variability, little is known about factors that contribute to response variability to opiate detoxification in infants. Infants are exposed to opiates either in utero via maternal drug use, or during medical therapy in the intensive care units (ICU), and Neonatal Abstinence Syndrome (NAS) is a the manifestation of opiate withdrawal when these medications are weaned or discontinued. Heterogeneity in practice for treatment of NAS including starting doses, rapidity of escalation and de-escalation of opiates, and the duration of therapy lead to either unnecessary symptoms in infants, or unnecessary opiate exposure. The overall goal of this research is to better understand the physiology of Neonatal Abstinence Syndrome and to maximize medical therapy for NAS using a population pharmacology approach to describe the relationship between serum morphine and the clinical syndrome of NAS.

Aim 1: Develop a population pharmacokinetic model to describe changes in parent drug: metabolite ratios for multiple metabolic pathways of morphine. Prospectively collected morphine and glucuronide metabolite concentrations measured in serum samples from 40 ICU and 40 in utero exposed full term infants will be combined to model serum concentrations of these drugs based on important clinical covariates including postnatal age, gender, ventilation status and end organ function. This pharmacokinetic model will be used in S.A. #2 to make a clinically useful link between morphine dosing/metabolism and the clinical syndromes of NAS.

Aim 2: Develop a population pharmacodynamic (PD) model describing the relationship between validated withdrawal scores (WS) for NAS and morphine/morphine metabolite concentrations in full term infants with NAS. To begin to explore factors that contribute to response variability during detoxification in hospitalized infants, we propose to determine the pharmacodynamic relationship between WS and serum morphine levels in two cohorts of infants who are undergoing
detoxification from opioids, either as a result of ICU exposure or maternal use during pregnancy. We hypothesize that changes in morphine metabolism with age and differences in end organ function will account for some of the interindividual variability in response to weaning of morphine during treatment for NAS.

**Aim 3:** Compare the pharmacodynamic relationship between morphine and WS in two populations of infants undergoing treatment for NAS: those prenatally exposed and iatrogenically exposed. Using “cohort status” as a covariate in the PK/PD model, we will investigate clinically meaningful differences in the morphine concentration – withdrawal score relationship which would warrant different dosing or treatment thresholds for the ICU population. We hypothesize that morphine concentrations and WS will have a different PD relationship in the prenatally exposed cohort than in the ICU cohort because infants in the ICU have complex illnesses that affects their pharmacologic response to morphine therapy.

With the accomplishment of these three aims, we will gain a much greater understanding of oral morphine pharmacology in this complex population. The pharmacokinetic/pharmacodynamic properties which underlie current therapy for NAS will aid us in more rational dosing of morphine for NAS in sub-populations of infants, leading to more appropriate narcotic exposure, better control of symptoms and improved prognostic abilities regarding length of therapy. In the future, these models could be prospectively validated and used to create guidelines which would globally impact the care of infants with NAS.

### 3. **Background**
(briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

**Opiates are an integral part of Neonatal and Pediatric Intensive Care.** Pediatric and neonatal patients in the Intensive Care Unit are subject to multiple painful procedures and are at high risk for chronic discomfort from mechanical ventilation and indwelling catheters and other devices; in addition their typical pain responses may be immature and lead to undertreatment. The necessity of adequate analgesia came to the forefront in the 1970s and there are multiple reasons to treat pain including decreased stress response, decreased neuronal cell death, and improved clinical outcomes. Opiates are the most commonly used analgesic in the ICU setting. Multiple behavioral scales which incorporate both vital sign and clinical/physical data to measure pain in children and neonates have been validated and are used to guide opiate administration.
Opiate therapy leads to tolerance and physical dependence. Morphine is the most commonly used drug for analgesia in ventilated neonates.\textsuperscript{126} Morphine is a mu-opioid receptor agonist and when given peripherally, the cumulative exposure at the effector site in the CSF is 18\% that in the plasma in adults.\textsuperscript{127} Opiate induced hyperpolarization of the neuronal membrane leads to decreased neurotransmitter release leading to clinical analgesia and sedation. Therapy with morphine leads to tolerance and dependence in neonates. Cellular mechanisms of tolerance and dependence include supersensitization of adenylyl cyclase and altered coupling of opiate receptors to excitatory g-proteins. Via protein kinase signaling systems, the duration of opiate receptor occupancy can influence opiate receptor internalization, down-regulation and desensitization.\textsuperscript{128} Multiple predictors of the development of tolerance and dependence have been identified and include duration of therapy, continuous vs intermittent therapy and cumulative dosing.

Morphine metabolism to M3G and M6G is developmentally variable. Morphine is metabolized via hepatic enzyme UGT2B7 to morphine 3-glucuronide and morphine 6-glucuronide, both of which are highly hydrophobic molecules. At birth, the median glucuronidation activity is low with a postmenstrual age and postnatal age-dependent increases to an adult levels.\textsuperscript{129} Preterm neonates primarily metabolize to M3G which has anti-analgesic properties and this predisposes them to rapid morphine tolerance.

The population pharmacokinetics of IV morphine has been studied in neonates and infants. The population pharmacokinetics of morphine and its metabolites have been modeled and validated in infants.\textsuperscript{130} Dr Knibbe and colleagues studied 248 infants and their resultant 2159 morphine and glucuronide concentrations to build a non-linear mixed effects population pharmacokinetic model. The formation and elimination clearance of the glucuronides were estimated and variation was found to be correlated with infant bodyweight. In addition, a postnatal age of 10 days was found to be associated with glucuronide formation clearance, independent of birthweight or postnatal age. Using this model, simulations were able to show that in newborns and infants less than three years old, a loading dose of 100 mcg/kg followed by a continuous infusion of 10 mcg/kg/hour resulted in a narrow and predictable range of serum morphine and metabolite concentrations. Developmental pharmacodynamics is the study of maturation of biologic systems and the changing drug-clinical target relationship.

The population pharmacokinetic (morphine metabolism in the body) to pharmacodynamic (the clinical effect of morphine) link has not been explored in Neonatal Abstinence Syndrome. Neonatal Abstinence Syndrome (NAS) is prevalent in the NICU and PICU.\textsuperscript{124,131} There have been multiple validated withdrawal symptom scales published and they are routinely used for diagnosis and management of NAS which result from both in utero and ICU exposure to opiates. There are currently no studies that investigate the therapeutic serum concentrations of morphine and
metabolites which correspond to “well managed” NAS which could be defined as a threshold withdrawal score. There are many barriers to this type of research, but there are examples in the literature of important insight into PK/PD relationships using pain scales.

**Population pharmacodynamic models have been used successfully in adults to correlate drug data with clinical outcomes in adults and children.** A population kinetic/dynamic model has been developed to investigate morphine titration in the immediate post-operative period using visual analog pain scales in adults. In this model, important clinical covariates predicting response to morphine therapy included decreasing delay between extubation and titration, intra-operative NSAIDs, and decreasing initial postoperative pain. Somma et al described the PD of midazolam using sedation scores and using mixed effect modeling, was able to predict the sedation score within 1 level with 83% accuracy. Anderson et al used population pharmacodynamic modeling to recommend acetaminophen dosing guidelines post-tonsillectomy, with a goal to achieve a visual analog pain score <4/10.

There are important clinical covariates unique to the neonatal ICU population which would need to be measured and accounted for in the PK/PD modeling of morphine for **Neonatal Abstinence Syndrome.** The most striking aspect of neonatal and pediatric PK/PD parameters is the rapid rate of change early in life secondary to changes in body composition, major organ function, and in the case of CNS-active drugs, the maturation of the blood brain barrier and the neuronal connections and receptor density themselves. Parameters that have been previously considered in this realm of modeling include: postmenstrual age, postnatal age, weight. Given the correlation of age and weight attainment, pharmacologic models use allometric scaling methods to account for changes in weight with age in order to investigate other important covariates. In the ICU population, other important influences such as derangement in normal kidney (elimination) and hepatic (metabolism) function could influence the effect of morphine on NAS. Of note, it has been documented in both adults and neonates, mechanical ventilation can cause changes in hepatic blood flow and thus the rate of glucuronidation of certain drugs. Unique to the pharmacodynamics of NAS include measures of previous narcotic exposure including cumulative dose and duration of therapy with opiates in the ICU exposed.

It has been assumed that in utero acquired and ICU acquired NAS are the same biologic entity, thus they are measured and treated similarly. The medical literature includes a multitude of studies on the risk factors for, diagnosis, and treatment of in utero acquired NAS, but there has been little direct investigation of ICU acquired NAS and the majority of this limited research has been epidemiologic in nature. There is clinical concern that the traditional signs and symptoms of NAS are altered in a medically labile population. Directly comparing the PD relationship between
morphine/morphine metabolites in these two cohorts will provide insight into any potential differences in the underlying pathophysiology.

Pharmacodynamic modeling of morphine for NAS – what are the clinical implications?

The current management NAS is imprecise and extremely variable regarding starting doses, titration methods and length of therapy. Understanding the pharmacodynamic link between morphine, morphine metabolites and clinical withdrawals scores would allow simulations based on patient characteristics to rationally dose and titrate morphine therapy for Neonatal Abstinence Syndrome.

4. Study Procedures
a. Study design, including the sequence and timing of study procedures (distinguish research procedures from those that are part of routine care).

b. Study duration and number of study visits required of research participants.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.

d. Justification of why participants will not receive routine care or will have current therapy stopped.

e. Justification for inclusion of a placebo or non-treatment group.

f. Definition of treatment failure or participant removal criteria.

g. Description of what happens to participants receiving therapy when study ends or if a participant’s participation in the study ends prematurely.

Overview: This is a prospective cohort study involving two groups of infants: a) fullterm well infants in the Newborn Nursery with Neonatal Abstinence Syndrome (NAS) acquired in utero who are being treated with oral morphine, and b) fullterm infants less than 12 months old in the Neonatal Intensive Care Unit who are being treated with oral morphine for NAS acquired via iatrogenic exposure. Both cohorts will be identified via pharmacy prospective POE ordering of intermittent oral morphine. Blood samples will be collected to measure morphine, glucuronide metabolites of morphine, and sulfation metabolites of morphine – these will be used for the population pharmacokinetic analysis. Saliva samples will be collected, DNA extracted and SNP genotyping of three relevant genes performed and these SNP results will be used in the pharmacodynamics analysis. NAS scores will be extracted from the electronic record and used as the clinical outcome for the pharmacodynamic analysis. The PK/PD relationship of morphine for NAS will be compared between the two cohorts, and model based simulations will be used to recommend rational morphine dosing for NAS based on relevant clinical covariates identified is SA2 and SA3.

Patient population:

a) In utero cohort: 40 consecutive infants with a diagnosis of NAS secondary to in utero exposure to either methadone, heroine or both will be identified via automated oral morphine order entry recognition in POE. The current standard of
care for NAS at the Johns Hopkins fullterm nursery is oral morphine, So every infant with NAS would be eligible for the study.

b) NICU cohort: Infants with NAS from iatrogenic exposure will be identified via a screening process which includes: automated recognition of any POE order for “NAS scores” – these infants will be evaluated, and if they are receiving oral morphine for NAS, they will be eligible for the study cohort. We will limit to infants >35 weeks GA as this groups is the most likely to receive oral morphine as standard of care for NAS. Infants >12 months postnatal age at time of eligibility will be excluded.

-GA at birth will be defined by admission note to NICU, postnatal age will be calculated as number of days since birth

-Exclusion criteria: Infants with major chromosomal anomalies (trisomies), infant with CNS disease which would make NAS scoring inaccurate (hypoxic ischemic encephalopathy, stroke, major brain malformation) will be excluded.

NAS scoring: Infants at risk for NAS, either from in utero or iatrogenic exposure, receive every 4 hours Modified Finnegan scores and these are recorded in the electronic health record at the time of collection by a trained NICU/Newborn nursery RN. From the start of morphine therapy for NAS, all scores will be recorded for every enrolled infant in an electronic database which pulls the data directly from the patient record (this mechanism is already established for an ongoing clinical trial). The NAS scores will be collected until 48 hrs after morphine is discontinued or until infant is transferred to another facility.

Blood sampling: In order to build a population pharmacokinetic model of oral morphine, blood samples will be collected for measurement of morphine and its metabolites. Several reference papers have modeled the PK of IV morphine in the neonatal population and these are used to determine approximate sample sizes for morphine and morphine metabolite levels.

Collection times after q3 or q4 morphine dose: 15, 30, 45, 60, 120, 180, 240 minutes post dose

Newborn nursery: 40 infants, 4 samples collected per infant at any of the above times, 12-18 samples at each of the above time points after combining data from all infants. These infants do not have routine blood draws, thus the 200 microliter samples will be collected via heelstick (3-4 drops) in capillary tubes, each on a separate treatment day to decrease risk of pain to participants.

NICU population: 40 infants, 4 samples collected per infant at any of the above times, 12-18 samples at each of the above time points after combining data from all infants. These infants have routine bloodwork and coinciding with clinical blood draws, we will have 200 microliters placed in a capillary tube
**Saliva sampling:** After enrollment and once infant has started enteral morphine, so will definitely be included in study population, a buccal swab will be used to collect saliva from the infant’s cheek. The saliva will be stored at room temperature and batch shipped to Tufts University CTRC Core Laboratory for DNA extraction and SNP genotyping. The OPRM1, COMT and ABCB1 genes will be analyzed for a total of six single nucleotide polymorphisms. These genotype results will be used as clinical covariates in the pharmacodynamic modeling process.

**After each enrolled infants, the collection times post dose that still require samples will be continually updated, and we will ensure that over the entire study population, we have an adequate number of samples at each timepoint.**

**Morphine and morphine metabolite assay**

a. Samples will be centrifuged and serum will be frozen at -70°C until mass spectrometry assay

b. High performance liquid chromatography, mass spectrometry will be performed as described by Bouwmeester et al.\textsuperscript{137} This assay method is currently being reproduced at the JHH Applied Clinical Pharmacology laboratory.

c. Morphine and morphine metabolite data will be kept in online database on secure server.

**Non-linear mixed effect modeling**

NONMEM software will be used for both the population pharmacokinetic and pharmacodynamic modeling. Based on prior IV morphine and metabolite modeling\textsuperscript{130,137}, multiple covariates will be considered for the final model:

- **clinical covariates for PK model:** All available data will be collected at the time of sample collection

  1. gestational age at birth and postnatal age; 2. birth weight and daily weight; 3. total bilirubin, serum creatinine; 4. positive pressure ventilation status (dichotomized, 0 for ventilated, 1 for extubated); 5. Treatment with known inducers of hepatic glucuronidation (phenobarbital, rifampicin, and carbamazepine)\textsuperscript{145}

- **clinical covariates for PD model:** 1. length of narcotic therapy prior to NAS treatment; 2. cumulative exposure to morphine equivalents prior to narcotic exposure; 3. subcohort status (\textit{in utero} vs ICU acquired NAS will be treated as a binary variable); 4. Co-treatment with other CNS active medications (i.e. benzos, clonidine) 5. SNP category (major vs minor alleles) for OPRM1, COMT and ABCB1 genes.
Simulation of morphine doses for target NAS scores using PK/PD model

NONMEM software will be used to simulate children which vary by the final model covariates to estimate dosing schemes which will result in target morphine concentrations and NAS scores.

5. Inclusion/Exclusion Criteria

Inclusion Criteria:

a. In utero exposed cohort: >35 weeks gestational age at birth, exposed to heroine and/or methadone prenatally via maternal report, diagnosed with Neonatal Abstinence Syndrome via Modified Finnegan Withdrawal scores collected by newborn nursery RNs, treated with oral morphine for NAS.

b. ICU exposed cohort: >35 weeks gestational age at birth, exposed to either fentanyl, morphine, or hydromorphone as part of medical care, diagnosed with Neonatal Abstinence Syndrome by clinical team and being treated with oral morphine for NAS.

Exclusion Criteria: Infants with major chromosomal anomalies (trisomies), infant with CNS disease which would make NAS scoring inaccurate (hypoxic ischemic encephalopathy, stroke, major brain malformation) will be excluded.

6. Drugs/Substances/Devices

a. The rationale for choosing the drug and dose or for choosing the device to be used.

b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.

c. Justification and safety information if non-FDA approved drugs without an IND will be administered.

There are no drugs/substances/devices which will be used as part of study protocol. Oral morphine is currently the standard of care for Neonatal Abstinence Syndrome and infants will only be eligible for the study if their primary clinical providers have diagnosed them with NAS and started oral morphine therapy.

7. Study Statistics

a. Primary outcome variable.

b. Secondary outcome variables.

c. Statistical plan including sample size justification and interim data analysis.

d. Early stopping rules.

This is a clinical pharmacology study, so typical outcome variables to do apply. We aim to accurately model with population estimates for clearance, volume of distribution and
to quantitatively estimate the interpatient variability around these estimates. We will consider multiple clinical covariates in the model (outlines in Study Design) in order to decrease this interpatient variability. The sample size of 40 infants per arm is based on prior publications investigating the clinical pharmacology of IV morphine and its metabolites, and oral morphine in older children with cancer. There will not be any early stoppage rules.

8. **Risks**
   a. Medical risks, listing all procedures, their major and minor risks and expected frequency.
   b. Steps taken to minimize the risks.
   c. Plan for reporting unanticipated problems or study deviations.
   d. Legal risks such as the risks that would be associated with breach of confidentiality.
   e. Financial risks to the participants.

**Material to be collected as part of study procedures:**

**Demographic information:** From the infants chart, we will collect information about infant sex, race, basic medical history.

**Details of drug exposure:** In the in utero exposed cohort, mothers will complete a verbal one page questionnaire at the time of consent which will identify substances used during the pregnancy, the duration and frequency of use. For the ICU exposed cohort, we will collect drug information from the patient chart which quantifies duration and cumulative exposure to opiates.

**NAS scores:** The Modified Finnegan score is a non-invasive behavioral withdrawal score which is collected by the primary nurse every four hours while infant in being treated for NAS. These scores will be remotely extracted from the patient chart of an enrolled infant and stored in a database to be analyzed with the serum measurements of morphine.

**Peripheral blood draws:** Each infant enrolled in the study will have maximum of 4 (depending on how long they are treated for NAS) heelstick blood draws (total of 50 microliters or 3 drops of blood per draw). This equates to less than 1 ml/kg over the entire study period. Blood draws will never be more frequent than two times per week. These sample collections will be timed with routine bloodwork whenever possible. This is most likely to happen in the ICU population when even convalescing infants are ordered for bloodwork on a regular basis (usually once per week). With the exception of total biliurbin concentrations and blood for the state newborn screen, infants in the newborn nursery do not routinely undergo blood draws.

**Buccal saliva samples:** There are no known risks of collecting buccal saliva from neonates.

**Risks to Subjects** The collection of demographic information and NAS scores does not subject an infant to increased risk. Specifically for the newborn nursery cohort, the detailed maternal drug history could increase the risk of social sequelae for the mother.
– infant dyad. The study information collected will be kept confidential and will be immediately de-identified after collection. In regards to the heelstick blood draw of 3 drops - This is the standard method used to obtain blood from infants for routine hospital laboratory tests. The infant will experience some pain when the lancet goes into his/her heel. Other than this momentary pain, the discomfort of heel stick should be minimal. However, in about 10% of cases a small amount of bleeding under the skin will produce a bruise (hematoma). A small scar will form on the heel. The risk of local infection in this procedure is less than 1 in 1,000. Although venipuncture is an option for blood collection in neonates, given the extremely small volume required for the study, it seems that heelstick is the more reliable and least painful option. The specific SNP analysis that we are doing has limited clinical implications outside of the research realm at this point in time. The results of the SNP analysis will not be shared with the infant’s families and will be used solely for the purpose of the current research project.

Minimizing risks Maternal drug use information collected at the time of consent will be kept separate from infant clinical chart after collection and will only be accessible to the primary investigator and co-investigators who need this information for later data analysis. Regarding heelstick blood draws, pain will be minimized with the following measure: prior heel warming, blanket swaddling, pacifier administration (with or without oral glucose solution), ambient light and noise reduction, and developmentally appropriate positioning. The nurses who are certified in heelstick technique will be the ones collecting the study samples. Of note, performing a POPULATION PK and PD analysis is a known method to limit number of samples required per subject and overall to estimate pharmacologic parameters.

There are no anticipated financial risks to participants.

9. Benefits
   a. Description of the probable benefits for the participant and for society.

Risk benefit analysis The infants enrolled in the study will not experience any personal health benefit. Their medical management will not be altered by the results of the blood work or study recording of NAS scores. The blood draw is considered “minimal risk” as it is not outside of the realm of a procedure that many normal newborns have during a newborn nursery stay for purposes of measuring bilirubin or sending the legally mandated state newborn screen at 1-3 and 10-14 days of age. The minimal risk of blood draw is balanced by the overall population knowledge gained by the information gleaned from the study results, namely the clinical pharmacologic basis of morphine as treatment for NAS.

10. Payment and Remuneration
a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

Families will be given a $20 Baby R Us gift card for their son/daughters participation in the study. The gift card would be transferred to the mother or father upon study consent (The gift card will only be given to the parent that signs the consent form). The parent consenting must complete and sign all payment and remuneration forms before the gift card can be transferred.

11. Costs
a. Detail costs of study procedure(s) or drug(s) or substance(s) to participants and identify who will pay for them.

The only cost associated with the study is measurement of morphine and morphine metabolites serum concentrations, and the DNA extraction and SNP analysis - this will clearly be a research procedure and will be go to a non-clinical lab and be paid by the research grant.
APPENDIX III: IRB Approved Consent Form for Morphine PK Study
RESEARCH PARTICIPANT INFORMED CONSENT AND PRIVACY AUTHORIZATION FORM

Protocol Title: Clinical Pharmacology of Oral Morphine for Neonatal Abstinence Syndrome
Application No.: NA_00073443
Sponsor: National Institute of Health (NIH)
Principal Investigator: Estelle Gauda, MD
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1. What you should know about this study:
   - You are being asked to join a research study.
   - This consent form explains the research study and your part in the study.
   - Please read it carefully and take as much time as you need.
   - Please ask questions at any time about anything you do not understand.
   - Ask your study doctor or the study team to explain any words or information in this informed consent that you do not understand.
   - You are a volunteer. If you join the study, you can change your mind later. You can decide not to take part or you can quit at any time. There will be no penalty or loss of benefits if you decide to quit the study.
   - During the study, we will tell you if we learn any new information that might affect whether you wish to continue to be in the study.
   - If you receive routine medical treatment (including medical or laboratory tests) in the study or if you are taking part in the study at the Clinical Research Unit, information about your research study participation will be included in your medical record, which is used throughout Johns Hopkins. Doctors outside of Johns Hopkins may not have access to this information. You can ask the research team to send this information to any of your doctors.
   - When Johns Hopkins is used in this consent form, it includes The Johns Hopkins University, The Johns Hopkins Hospital, Johns Hopkins Bayview Medical Center, Howard County General Hospital,
Johns Hopkins Community Physicians, Suburban Hospital, Sibley Memorial Hospital and All Children’s Hospital.

- The Johns Hopkins School of Medicine Institutional Review Board (IRB) sometimes reviews studies that are conducted at other institutions. These other institutions are solely responsible for conducting the study safely and according to the protocol that the Johns Hopkins IRB has approved. Information about how to contact the investigator at the institution that is responsible for the study is included in this form. When another institution is conducting the study, the word “we” in this consent form may include both Johns Hopkins and the participating institution.

- Biospecimens will be collected in this study. Biospecimens may include any of the following: blood, tissue, saliva, urine, bone marrow, cells, etc. Most biospecimens contain DNA, which is the genetic code for each person.

- If children and adults can join this study, the word “you” in this consent form will refer to both you and your child.

- During this study, you will not have access to certain medical information and test results collected for study purposes. If an emergency occurs while you are in the study, medical information needed for your treatment can be made available to your study physician and other physicians who treat you. When the study is completed, all the information in your medical record will be available to you.

2. **Why is this research being done?**

This research is being done to better understand the treatment of infants with Neonatal Abstinence Syndrome (NAS).

NAS is an illness which is the result of babies being exposed to opiate medications. Infants are exposed to opiates in two ways: 1. when the mother takes substances such as methadone or heroin while she is pregnant, or 2. when the infant is sick at birth and needs opiate medications for pain control and sedation as part of their care.

Infants with NAS are watched closely and treated with oral morphine for their symptoms of withdrawal. The morphine is weaned (gradually lowered) until the infant no longer needs it. The goal is this study is to measure morphine and morphine metabolites (break-down products) in the infants’ blood and relate these levels to withdrawal symptoms. By understanding this connection between drug levels and symptoms, we hope to improve the dosing of morphine for future infants with Neonatal Abstinence Syndrome.

All infants born at over 35 weeks gestation who are at risk for NAS or who have NAS are eligible for the study. Your infant will only be enrolled in the study if he/she requires oral morphine therapy for NAS as part of their regular care.

Infants with Neonatal Abstinence Syndrome (NAS) may join.

**How many people will be in this study?**

About 40 infants from the full term newborn nursery and 40 infants from the Neonatal Intensive Care Unit will be in this study.

3. **What will happen if you join this study?**

If you agree to allow your child to be in this study, we will ask you to allow your child to do the following things:
• If your infant was exposed to opiates during pregnancy:
  1. We will collect information from you about your drug use during pregnancy. This information is for study purposes only and will help us understand the way your infant responds to treatment for NAS.
  2. Your infant will have withdrawal scores measured every four hours by the nurse (this is routine patient care). As part of this research study, the withdrawal scores will be collected for the study records.
  3. If your infant has high withdrawal scores, they will be treated will oral morphine (this is routine patient care).
  4. No more than twice per week and as part of this research study, we will perform a heelstick to collect blood from the infant’s foot. This will be about eight drops of blood (less than 1/5 of a teaspoon). The infant will never have more than a total of four blood draws while in the study.
  5. At the start of the study, we will use a swab to collect a small amount of saliva from inside your infant’s cheek. This saliva will be used to make a DNA sample and look at three genes that affect how a baby responds to morphine when being treated for NAS. The DNA will only be used to look at these three genes and no other genetic testing will be done.
  6. The results of the blood work (morphine and morphine metabolite levels) and the saliva (DNA results) are only used for the research study and the infant’s doctors will not know the results.

• If your infant was exposed to opiates in the Intensive Care Unit:
  1. We will collect information on how much opiates the infant received.
  2. Your infant will have withdrawal scores measured every four hours by the nurse while he/she is being treated with oral morphine (this is routine patient care). As part of this research study, the withdrawal scores will be collected for the study records.
  3. No more than twice per week and as part of this research study, we will perform a heelstick to collect blood from the infant’s foot. This will be about eight drops of blood (less than 1/5 of a teaspoon). The infant will never have more than a total of four blood draws while in the study.
  4. At the start of the study, we will use a swab to collect a small amount of saliva from inside your infant’s cheek. This saliva will be used to make a DNA sample and look at three genes that affect how a baby responds to morphine when being treated for NAS. The DNA will only be used to look at these three genes and no other genetic testing will be done.
  5. The results of the blood work (morphine and morphine metabolite levels) and the saliva (DNA results) are only used for the research study and the infant’s doctors will not know the results.

The Genetic Information Nondiscrimination Act (GINA) may help protect you from health insurance or employment discrimination based on genetic information.

The law provides that health insurance companies and group health plans
  • may not ask for genetic information from this research and
  • may not use genetic information when making decision about eligibility or premiums

The law will not stop health insurance companies from using genetic information to decide whether to pay claims. The law also will not help you get other types of insurance (such as: life, disability or long-term care).
How long will you be in the study?
Your infant will be in the study while he/she is being treated with oral morphine for NAS. This amount of time is different for every infant.

You son/daughter will be in this study until they are discharged from the hospital.

4. What are the risks or discomforts of the study?

Heelstick blood draw: This is the standard method used to obtain blood from infants for routine hospital laboratory tests. The infant will experience some pain when the lancet goes into his/her heel. Other than this momentary pain, the discomfort of heel stick should be minimal. However, in about 10 out of 100 cases, a small amount of bleeding under the skin will produce a bruise. There is also an extremely low (less than 1 in 1,000) chance of bacteria from the skin entering the site and causing an infection.

The study team will do everything in our power to decrease the pain associated with the heelstick. This includes swaddling the infant, giving a pacifier, and warming the heel prior to the stick so that the blood flows more freely.

Oral swab for saliva sample to collect DNA: There are no known risks for collection of saliva with a swab. Despite the GINA protections and the best efforts of the research team, there may still be a risk if information about you were to become known to people outside of this study.

Oral morphine: All infants in the study will be treated with oral morphine for Neonatal Abstinence Syndrome as part of their routine care. This medication may need to be increased or decreased based on your infant’s symptoms. It can be associated with constipation or itching (rarely) and in high doses, can cause decreases in a baby’s breathing or blood pressure. The morphine doses used for the treatment of NAS have not been related to these side effects.

Loss of confidential information: There is the risk that information about your child may become known to people outside this study. To protect against this, the study team will keep all records with study information securely protected, but there is always a small risk that someone outside of the study could gain access to the study information.

Despite the GINA protections and the best efforts of the research team, there may still be a risk if information about you were to become known to people outside of this study.

Genetic information is unique to you, even without your name or other identifiers. For this reason, genetic information like DNA may be used to identify you and possibly your family members. We have procedures (such as, labeling your biospecimens with a password protected code known only to select research staff) to prevent people working with your DNA from discovering if it belongs to you. However, there is the risk this can happen as new ways of tracing genetic information are being developed that may make re-identification of genetic information possible.

There may be side effects and discomforts that are not yet known.

5. Are there benefits to being in the study?

There is no direct benefit to your infant from being in this study.
If your child takes part in this study, your child may help others in the future. The information we gain by studying the infants in this study will help us better take care of infants with Neonatal Abstinence Syndrome in the future.

6. **What are your options if you do not want to be in the study?**
   If you decide not to allow your child to join this study, there is no change in the care or treatment of your infant. You do not have to allow your child to join this study. If your child does not take part in the study, your child’s care at Johns Hopkins will not be affected.

7. **Will it cost you anything to be in this study?**
   No. The only study procedure that costs money is measuring morphine levels in the blood collected from your infant and this is paid for by the study team.

8. **Will you be paid if you join this study?**
   Once you have decided to enroll your infant, you will be given a $20 gift card to Babies R Us to buy supplies needed for your baby. Only one gift card will be given to the consenting parent for this study.

   You may be required to provide your social security number to be paid for taking part in this study. Federal tax law requires that you report your research payments when you file your taxes. If your total payments from Johns Hopkins exceed $600 per year, Johns Hopkins will report these payments to the Internal Revenue Service and you will receive a 1099-MISC form from us.

9. **Can you leave the study early?**
   - You can agree to allow your child to be in the study now and change your mind later.
   - If you wish to end your child’s participation, please tell us right away.
   - Leaving this study early will not stop your child from getting regular medical care.
   - If your child leaves the study early, Johns Hopkins may use or give out your child’s health information that it already has if the information is needed for this study or any follow-up activities.

10. **How will your privacy be protected?**
   We have rules to protect information about you. Federal and state laws and the federal medical Privacy Rule also protect your privacy. By signing this form you provide your permission, called your “authorization,” for the use and disclosure of information protected by the Privacy Rule.

   The research team working on the study will collect information about you. This includes things learned from the procedures described in this consent form. They may also collect other information including your name, address, date of birth, and information from your medical records. This could include information about HIV and genetic testing, or treatment for drug or alcohol abuse or mental health problems.

   The research team will know your identity and that you are in the research study. Other people at Johns Hopkins, particularly your doctors, may also see or give out your information. We make this information available to your doctors for your safety. If you think this study might affect your clinical care, please inform your doctor.
People outside of Johns Hopkins may need to see or receive your information for this study. Examples include government agencies (such as the Food and Drug Administration), safety monitors, other sites in the study and companies that sponsor the study.

We cannot do this study without your authorization to use and give out your information. You do not have to give us this authorization. If you do not, then you may not join this study.

We will use and disclose your information only as described in this form and in our Notice of Privacy Practices; however, people outside Johns Hopkins who receive your information may not be covered by this promise or by the federal Privacy Rule. We try to make sure that everyone who needs to see your information keeps it confidential – but we cannot guarantee that your information will not be re-disclosed.

The use and disclosure of your information has no time limit. You may revoke (cancel) your permission to use and disclose your information at any time by notifying the Principal Investigator of this study by phone or in writing. If you contact the Principal Investigator by phone, you must follow-up with a written request that includes the study number and your contact information. The Principal Investigator’s name, address, phone and fax information are on page one of this consent form.

If you do cancel your authorization to use and disclose your information, your part in this study will end and no further information about you will be collected. Your revocation (cancellation) would not affect information already collected in the study, or information we disclosed before you wrote to the Principal Investigator to cancel your authorization.

11. Will the study require any of your other health care providers to share your health information with the researchers of this study?

As a part of this study, the researchers may ask to see your health care records from your other health care providers. This includes the results of any drug screens that you took during your pregnancy if your infant was exposed to opiates during pregnancy.

12. What treatment costs will be paid if you are injured in this study?

Johns Hopkins does not have a program to pay you if you are hurt or have other bad results from being in the study. However, medical care at Johns Hopkins is open to you as it is to all sick or injured people.

- **If you have health insurance:** The costs for any treatment or hospital care you receive as the result of a study-related injury will be billed to your health insurer. Any costs that are not paid for by your health insurer will be billed to you.

- **If you do not have health insurance:** You will be billed for the costs of any treatment or hospital care you receive as the result of a study-related injury.

By signing this form you will not give up any rights you have to seek compensation for injury.

13. What other things should you know about this research study?

a. What is the Institutional Review Board (IRB) and how does it protect you?

The Johns Hopkins Medicine IRB is made up of:

- Doctors
• Nurses
• Ethicists
• Non-scientists
• and people from the local community.

The IRB reviews human research studies. It protects the rights and welfare of the people taking part in those studies. You may contact the IRB if you have questions about your rights as a participant or if you think you have not been treated fairly. The IRB office number is 410-955-3008. You may also call this number for other questions, concerns or complaints about the research.

b. What do you do if you have questions about the study?
Call the principal investigator, Dr. Estelle B Gauda at 410-614-0151. If you wish, you may contact the principal investigator by letter or by fax. The address and fax number are on page one of this consent form. If you cannot reach the principal investigator or wish to talk to someone else, call the IRB office at 410-955-3008.

c. What should you do if you are injured or ill as a result of being in this study?
If you think you are injured or ill because of this study, call Estelle B Gauda at 410-614-0151 during regular office hours.

If you have an urgent medical problem related to your taking part in this study, call Dr. Estelle B. Gauda at 410-614-0151 during regular office hours and 410-748-3218 after hours and on weekends.

d. What happens to Data and Biospecimens that are collected in the study?
Johns Hopkins and our research partners work to understand and cure diseases. The biospecimens and/or data you provide are important to this effort.

If you join this study, you should understand that you will not own your biospecimens or data, and should researchers use them to create a new product or idea, you will not benefit financially.

With appropriate protections for privacy, Johns Hopkins may share your biospecimens and information with our research sponsors and partners.
14. **What does your signature on this consent form mean?**
   
   Your signature on this form means that:
   
   - you understand the information given to you in this form
   - you accept the provisions in the form
   - you agree to join the study
   
   You will not give up any legal rights by signing this consent form.

   **WE WILL GIVE YOU A COPY OF THIS SIGNED AND DATED CONSENT FORM**

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<th>Signature of Participant</th>
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Curriculum Vitae

Tamorah Lewis, MD
6015 Westwood Court
Parkville, MO 64152
Cell: 443-803-4882
Email: trlewis@cmh.edu

PROFESSIONAL EXPERIENCE

Assistant Professor
7/2014-Present
Children’s Mercy Hospital
University of Missouri - Kansas City School of Medicine
Department of Pediatrics
Divisions of Neonatology and Pediatric Clinical Pharmacology

POSTGRADUATE TRAINING

Combined Neonatology/Clinical Pharmacology Fellow
07/2010-6/2014
Johns Hopkins Hospital
Baltimore, MD

Johns Hopkins Harriet Lane Pediatric Residency
06/2007-06/2010
Johns Hopkins School of Medicine
Baltimore, MD

Board Certification:
American Board of Pediatrics 10/18/2010

EDUCATION

Johns Hopkins Bloomberg School of Public Health
06/2011 – current
Baltimore, MD
Candidate for PhD in Clinical Investigation

Johns Hopkins School of Medicine
08/2003-05/2007
Baltimore, MD

USMLE Scores:
Step 1 (12/15/05): 246
Step 2 CK (12/19/06): 260
Step 2 CS (3/30/07): Pass
Step 3 (4/1/08): 229
Boston College
08/1999-05/2003
Presidential Scholar, Bachelor of Science in Biology
Cum laude GPA 3.646
Chestnut Hill, MA

PRE-FACULTY PROFESSIONAL EXPERIENCE

2004 – 2006
Johns Hopkins Hospital Department of Pediatrics   Baltimore, MD
Coinvestigator
- Co-research coordinator for study: The effect of clonidine in the standard treatment of neonatal abstinence syndrome
- Responsibilities included enrolling infants (consenting mothers) during weekends on-call, following NAS scores and adjusting meds per protocol, drawing and cataloguing blood samples, nurse in-services regarding study protocol

2002 – 2003
Tufts University Physiology Dept.   Boston, MA
Student intern
- Assisted in developing a high-throughput assay to characterize the pumping activity of NPC, the protein mutated in Niemann Pick C disease, a cholesterol storage disorder. At the end of the summer 2002, I made and presented a poster about this research.

PUBLICATIONS


Submitted for Review: Pharmacoepidemiology of Medical Opiate Exposure in the Neonatal Intensive Care Unit; Journal of Opioid Management

ABSTRACTS
1) Population Pharmacokinetics of Enteral Morphine to Aid Dosing Strategy in Neonatal Abstinence Syndrome; Lewis, T; Liu, T; Ezell, T; Gauda, E; Sartori, D; Ivaturi, V.
-Presented as poster at ASCPT 2014

PROFESSIONAL SERVICE
AWARDS AND HONORS
1) National Institutes of Health – Loan Repayment Program awardee
2) Food and Drug Administration (FDA) ORISE Fellowship, Office of Clinical Pharmacology, 3/2014-6/2014

INVITED TALKS / ATTENDANCE
1) Lecture “Neonatal Abstinence Syndrome for the Primary Practitioner”, Pediatric Trends Conference, Baltimore, MD; April 2014
2) Lecture “Pulmonary Arterial Hypertension in Infants and Young Children”, FDA Office of Clinical Pharmacology Scientific Rounds; May 2014
3) Moderator, “Pediatric Pharmacology”, Pediatric Academic Society (PAS) meeting Vancouver, BC; May 2014

MEMBERSHIPS AND ASSOCIATIONS
- American Academy of Pediatrics, Section on Perinatal Pediatrics
- American Society of Clinical Pharmacology and Therapeutics

RESEARCH
7/2010 – 2013 Johns Hopkins Hospital, Baltimore, MD
“Efficacy of Clonidine for Iatrogenic Neonatal Abstinence Syndrome”
PI: Estelle Gauda, MD
IRB-approved, randomized, controlled trial of IV / enteral clonidine vs placebo in infants less than six months of age exposed to prolonged opiate infusions and at risk for Neonatal Abstinence Syndrome. Primary outcome is length of therapy.

5/2012 – 6/2014 Johns Hopkins Hospital, Baltimore, MD
“Clinical Pharmacology of Oral Morphine for Neonatal Abstinence Syndrome”
PI: Estelle Gauda, MD
IRB-approved, sparse sampling population pharmacokinetic study of oral morphine, morphine-3-glucuronide and morphine-6-glucuronide in neonates with both in utero and iatrogenically acquired Neonatal Abstinence Syndrome. This model will then be used to build a PK/PD model with Modified Finnegan Scores in an effort to improve accuracy of morphine dosing for NAS.

3/2012 – 2014 Johns Hopkins Hospital, Baltimore, MD
“Trends in Narcotic Use and Neonatal Abstinence Syndrome”
PI: Estelle Gauda, MD
IRB-approved retrospective cohort study of high risk infants admitted to NICU in three time epochs: 2004, 2008 and 2011. The goal is to compare narcotic exposure in similar infants over time and to investigate trends in rates of iatrogenic NAS.

TEACHING PRESENTATIONS
2013 Lewis, Tamorah “Neonatal Clinical Pharmacology.” Baltimore City-Wide Conference, Johns Hopkins Hospital Baltimore, MD

MENTORING ACTIVITIES
1) Betty Erfe: I met Ms Erfe as an undergraduate interested in participating in clinical research during a Post-Bachelaureate program prior to applying for medical school. I mentored her in a chart review research project and co-wrote a letter of recommendation for her medical school application. She started Harvard Medical School in July 2014.

ORGANIZATIONAL ACTIVITIES
2010-2012  Interviewer - Pediatric Residency Program
Johns Hopkins University School of Medicine
Baltimore, MD
2011-present  NRP Instructor – Pediatric Residency Program Annual Intern NRP Training
Johns Hopkins University School of Medicine
Baltimore, MD

CONFERENCES AND SEMINARS ATTENDED
2014
- Pediatric Academic Society, Vancouver, BC, 5/2/14-5/5/14

2013
- NICHD T32 Clinical Pharmacology Fellow Conference, Rockville MD 10/21-10/22/2013

2012
- Facilitating Pediatric Research with Modeling and Simulation, Children’s Hospital of Philadelphia 4/23-4/25/2012
- American Society for Clinical Pharmacology and Therapeutics Annual Meeting, National Harbor Maryland, 3/14-3/17/2012
- Second Annual Updates and Advances in Neonatal Care for the Practitioner: Neonatal Brain Injury and Necrotizing Enterocolitis, 10/11/2012, Bethesda, MD
- SAAM PK Modeling Workshop, 10/17-10/18/2012, NIH campus Bethesda MD

2010
- Updates and Advances in Neonatal Neurological Care for the Practitioner: Neonatal Hypoxia-Ischemic Brain Injury and Related Topics, 9/22/2010, Bethesda, MD