METHADONE POPULATION PHARMACOKINETICS: TOWARD UNDERSTANDING THE DOSE-RESPONSE RELATIONSHIP IN THE TREATMENT OF OPIATE ADDICTION

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Gavin Bryce-Samuel Bart

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Dedication

To Caprice, my number one wife.

Abstract

Methadone is a synthetic opiate agonist that is highly effective in the treatment of opiate addiction. When given as a long-term therapy, methadone maintenance reduces morbidity and mortality associated with opiate addiction. It is thus considered an "essential" medication by the World Health Organization.

The benefits of methadone maintenance in the treatment of opiate addiction are well established. Predicting treatment response for a given individual, however, remains difficult. While methadone dose is generally associated with treatment outcome, large interstudy and interindividual variability in plasma concentrations of methadone have made it difficult to link dose response to pharmacokinetic parameters. This thesis explores characteristics of methadone maintained patients and develops a population pharmacokinetic model that identifies variables associated with methadone pharmacokinetic parameters.

Chapter 1 provides a general review of the three Food and Drug Administration approved pharmacotherapeutic agents for the treatment of opiate dependence. Chapter 2 reviews the clinical pharmacology of methadone as used in the treatment of opiate dependence. Chapter 3 introduces us to the Hmong and their paradoxically exceptional treatment outcome in methadone maintenance on lower doses of methadone than their non-Hmong counterparts. This retrospective study helps form the hypothesis that their better treatment outcome is related to greater methadone exposure.

The results of this population pharmacokinetic study and the psychosocial differences between Hmong and non-Hmong are presented in Chapters 4 and 5, respectively. We found that the lower methadone dose requirement is explained by higher apparent bioavailability of methadone in Hmong. Other influences on methadone pharmacokinetics, more specifically clearance, include age, body mass index, and single nucleotide polymorphisms in the *ABCB1* and *CYP2B6* genes. While the potential for culture to influence methadone treatment outcome is acknowledged, there remain sufficient grounds to hypothesize a significant biological (i.e., pharmacokinetic and/or pharmacodynamic) influence.

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Introduction

Methadone (6-dimethylamino-4, 4-diphenyl-3-heptanone) was first synthesized by Bockmühl and Ehrhart in 1937. It was initially identified only by the company compound code 10820 and later the name amidon. Their work for I.G. Farbenindustrie was part of a larger pharmaceutical program synthesizing atropine derivatives as spasmolytics.[1] This program led to the development in 1939 of meperidine, which not only had spasmolytic properties but was an analgesic. Therefore, testing for analgesic properties of the amidon series compounds commenced. Little direct information on these experiments exists, however, following World War II, a U.S. Department of Commerce report on the "Pharmaceutical Activities at the I.G. Farbenindustrie Plant, Hochst am Main" indicates that amidon had 5-10 fold greater analgesic effect than meperidine.[1] Clinical development of this compound by I.G. Farbenindustrie was likely limited by wartime shortages affecting its production and potential adverse effects in a small series of human subjects.[1;2]

With the end of World War II, the Declaration Regarding the Defeat of Germany and Assumption of Supreme Authority by Allied Powers (June 5, 1945) gave Allied powers access to all German factories, research laboratories, and patents. The U.S. State Department's Technical Industrial Intelligence Committee identified compound 10820 as having analgesic potential heralding production and testing in the United States. Existing proprietary concerns over the name Amidone led the Council on Pharmacy and

Chemistry of the American Medical Association to declare methadon the generic designation for 6-dimethylamino-4, 4-diphenyl-3-heptanone.[2;3] The name was formally changed to methadone in 1948.[4]

Early clinical work with methadone focused on its analgesic potential. By 1946, human laboratory studies found methadone had greater analgesic properties per milligram than meperidine and morphine and a similar toxicity profile, with induction of lightheadedness, nausea, and decreases in pulse and respiratory rate.[5;6] When used in clinical pain management, significant relief of traumatic, post-operative, and malignant pain were noted.[7;8] Because methadone was a product of atropine analogs, it was not evident that it was even a narcotic. In fact IG Farbenindustrie scientists thought that the "well-being" post-operative patients experienced following methadone treatment was due to pain relief and that methadone itself did not have intrinsic euphorigenic properties.[1] However, they cautioned that while there had been no primary cases of addiction to methadone, both cocaine and morphine addicts may be at increased risk for having their "…addiction transferred to this group of compounds".[1]

Initial studies on the abuse liability of methadone were conducted at the US Public Health Services Hospital in Lexington, Kentucky. Single dose studies were conducted in non-addicts and non-dependent former heroin addicts with at least 6-weeks abstinence.[9] A 5 mg subcutaneous dose did not produce euphoria in either group. Larger doses were administered to the former addict group with consistent feelings of euphoria at 30 mg.

The subjects noted that the development of euphoria was slower than following morphine and that they would prefer morphine if given a choice but would use methadone if other opiates were not available. Finally a group of morphine addicted subjects were given methadone and were able to identify that they had received an opiate-like substance and did not request morphine instead of methadone. These initial findings led the authors to conclude that the dose of methadone used to treat pain should be limited in order to avoid development of euphoria and that methadone be regulated as a narcotic.

Chronic dosing studies provided insight to methadone's potential as a therapeutic agent in the treatment of opiate withdrawal. For example, significant reductions in Himmelsbach opiate withdrawal scale scores were observed when methadone 20-25 mg was administered thirty-six hours after the last dose of morphine in active morphine addicts.[10] Methadone was also substituted in a 1:4 ratio for morphine in active morphine addicts with no observed onset of withdrawal, no sedation, and without the patients noticing the change. After fourteen days of methadone substitution, its sudden cessation produced opiate withdrawal symptoms of slower onset, lesser intensity, and greater duration than following morphine cessation. The authors' concluded that:

> It is the unanimous opinion of all who have been concerned with the evaluation of the addiction liability that methadon, like morphine, is dangerous with respect to habituation. Since persons with known narcotic experience get a satisfactory subjective reaction from the drug, since the drug suppresses completely the morphine abstinence syndrome, since it can be substituted satisfactorily for morphine in cases of known morphine addiction and since it produces, in our opinion, a real, however mild, withdrawal picture, methadon must be classed as an

addicting drug.[10]

Methadone's cross-tolerance with morphine and ability to reverse opiate withdrawal symptoms was recognized as clinically important. By 1947 the American popular press touted methadone as a "New Drug for Breaking the Dope Habit."[11] And by 1948, there were even reports of addicts illegally obtaining methadone in order to relieve craving when "…not in the position to obtain morphine or heroin."[12] The US Public Health Service specialty hospital for opiate addiction in Lexington adopted methadone as the preferred treatment for opiate withdrawal in 1949.[11]

In 1964, in response to public health concerns over the increasing prevalence of heroin overdose deaths in New York City, researchers at the Rockefeller Institute for Medical Research studied chronic methadone administration as a means of maintenance treatment for opiate dependence.[13] As with the Lexington group, they replicated methadone's ability to relieve opiate withdrawal but also showed cross-tolerance to other opiates and reduced craving for opiates while patients received methadone.[14]

The initial Rockefeller studies set the foundation for methadone maintenance, which has become the standard of treatment for opiate dependence.[15] Methadone's long-acting properties and full mu opioid agonism reduce many pharmacologically and behaviorally reinforcing effects of short-acting opiates. Treatment response to methadone has been evaluated in the United States by the multisite Treatment Outcome Prospective Study

(TOPS). In TOPS, the average 1-year treatment retention in methadone maintenance was 34%.[16] The more recent Drug Abuse Treatment Outcome Study (DATOS) confirmed the variability in treatment retention (15%-76%) noted in TOPS, but indicated that with wider adoption of evidence-based treatment standards, treatment had improved with an average of 50% of all patients retained for 1-year.[17] These standards indicate that methadone is more effective than behavioral treatment alone and that the treatment response to methadone is dose dependent, with patients taking 60 mg – 120 mg of methadone daily having better treatment outcome than those taking less than 60 mg daily.[15;18]

The influence of methadone kinetics on treatment has been explored. Steady-state trough plasma methadone levels are dose dependent[19] and are inversely correlated to opiate withdrawal symptoms.[20;21] Methadone treatment outcome has also been correlated to trough, 2 hour, and 8 hour methadone levels, but the data are limited in scope. In one study, treatment outcome was determined in a cross-sectional sample and only in-treatment factors such as symptom relief and ongoing drug use could be assessed rather than retention in treatment.[22] In a second, prospective study, methadone levels were only measured during the initial 25-days of therapy but did correlate with reduced opiate positive urine toxicology.[23] Unfortunately, clinical retention was not assessed and generalizability of this study is complicated by selection bias due to the required 4-week abstinence-based inpatient lead-in prior to initiating methadone.

The plasma methadone concentration peak-to-trough ratio has also been used to evaluate the effectiveness of methadone in relieving withdrawal symptoms.[24;25] Prospective evaluation of this ratio, however, has not been explored in terms of treatment outcome. A cross-sectional study of methadone peak:trough did find that subjects with ongoing illicit substance use (a measure of treatment response) had higher ratios compared to those with less illicit drug use.[26] While peak:trough has correlated to the objective and subjective effects of methadone[25], there was no difference in peak:trough when a patient population complaining of early withdrawal symptoms (non-holders) was compared to patients without such a complaint (holders).[25] Interestingly, these studies found that the maximum rate of concentration decline following peak was greater in the non-holders and that this rate was positively correlated to composite total mood disturbance scores of the Profile of Mood States (POMS).[25;27] One study also evaluated methadone area under the curve (AUC) but did not find it to significantly differ between non-holders and holders.[25]

This brief introduction indicates that despite more than 45 years of methadone maintenance little is understood about the role of methadone pharmacokinetics in its dose-response effect. This dissertation will address the issue of methadone pharmacokinetics in an in treatment population drawing upon clinically observed differences in dose requirements between Hmong and non-Hmong methadone maintained patients. The use of population pharmacokinetic modeling will allow for real-world evaluation using sparse sampling strategies and, thus, a larger population enrollment and the creation of mixed effect models of pharmacokinetic parameters and exploration of a

variety of factors (e.g., gender, weights, age, ethnicity, methadone dose, etc.) that may influence these parameters.

Chapter 1 is a published invited peer-reviewed review article on the role of medications in the treatment of opiate dependence. This serves as background to why medications, and especially methadone, play a central role in the treatment of opiate dependence.

Chapter 2 is an in press book chapter on the pharmacokinetics and pharmacodynamics of methadone. This chapter will provide background to the basic clinical knowledge surrounding methadone in the treatment of opiate dependence.

Chapter 3 is a published research article showing significant differences in methadone treatment outcome and dose requirements between Hmong and non-Hmong patients enrolled in the Hennepin County Medical Center Addiction Medicine Clinic (formerly the Hennepin Faculty Associates Addiction Medicine Clinic). This observation laid the foundation for the current dissertation project, which hypothesizes that this difference in dose requirement is due to increased methadone exposure in Hmong compared to non-Hmong.

Chapter 4 describes the results of a population pharmacokinetic study of methadone in a methadone maintained population. The model supports a significant difference in methadone exposure between Hmong and non-Hmong.

Chapter 5 presents the descriptive statistics and psychometric analyses of non-pharmacokinetic data collected in the study described in Chapter 4.

Chapter 6 is a brief recapitulation of the dissertation findings. A brief list of future projects inspired by these findings is also presented.

Chapter 1

Maintenance Medication for Opiate Addiction: The Foundation of Recovery*

Opiate dependence (hereafter referred to as addiction) is a major public health problem with global reach.[28] The illicit use of opiates contributes to the global burden of disease and can result in premature disability and death.[29] Overdose is a significant cause of death and the incidence and prevalence of blood borne viruses (e.g., HIV, hepatitis B, and hepatitis C) are higher in illicit opiate users, especially injection drug users (IDU), than the general population.[30-34] In the United States, deaths related to opiate analgesic overdose now exceed those caused by both heroin and cocaine combined.[35] Access to, adherence to, and outcome for the treatment of general medical illness and infectious diseases such as HIV, viral hepatitis, and tuberculosis are reduced in opiate addicts.

Globally, between 24 and 35 million adults age 15-64 years used an illicit opiate in 2010.[36] Throughout Europe and Asia, opiate use is the primary reason for seeking treatment for illicit drugs.[36] While there has been relative global stability in prevalence of illicit opiate use, the United States has seen a significant increase in the illicit use of prescription opiates despite stable levels in heroin use.[36;37] In 2009 in the United States,

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opiates were second only to alcohol as the primary reason for treatment admission.[38] In fact from 1999 to 2009, annual treatment admissions for opiates increased from approximately 280,000 to 421,000 individuals.[38] The increase in prescription opiate use, overdose deaths, and treatment admissions parallels increases in production and distribution of prescription opiates.[35]

The addiction liability of opiates is high with 50% and 11% of people who used heroin or misused prescription opiates, respectively, last year meeting addiction criteria.[39] The focus of this review is on the pharmacologic treatment options for these opiate addicted individuals. A brief discussion of the neurobiology of opiate addiction will be followed by description of the role current FDA-approved treatments for opiate addiction have in facilitating recovery. Economic, ethical, and regulatory issues surrounding these medications are beyond the scope of this review and will not be discussed.

Neurobiology of opiate addiction

The risk for developing opiate addiction is a complex interaction between genetics, environmental factors, and the pharmacological effects of opiates. For example, selective breeding in rodents has produced strains prone to opiate self-administration; multiple genetic loci associated with opiate self-administration have been identified; and selective disruption of the gene encoding the mu opioid receptor, the principal target of opiates, can eliminate opiate self-administration and conditioned place preference.[(for a review see 40)]

Human family and twin studies have identified increased genetic risk for addiction in the first degree relatives of addicts but also that the genetic risk specific to opiate addiction is second only to that for alcoholism.[41;42]

Environmental factors such as availability of opiates, perceived risk of opiate use, psychosocial stressors, and learned coping strategies all influence the risk of developing opiate addiction. For example, the incidence of opiate addiction has paralleled the increased availability of opiates. While 75% of high school seniors perceive using heroin once or twice as dangerous, only 40% perceive similar use of prescription opiates as dangerous.[43] Traumatic lifetime experience may increase the risk for opiate addiction. In rodent models, maternal separation early in life increases vulnerability to opiate addiction for both the pup and the dam and, in humans, weak parental bonds increase the risk for illicit drug use during adulthood.[44-46] In humans, there is an association between post-traumatic stress disorder and opiate addiction with an over-representation in prevalence of this disorder in opiate addicts compared to the general population and to those with other substance use disorders.[47]

The euphoria and abuse liability of an opiate is related to both its pharmacokinetic and pharmacodynamic properties. The rapidity with which a drug enters and then exits the brain is positively correlated with its rewarding and reinforcing effects.[48-50] This principal is clinically apparent in the transition from oral or intranasal to smoked or intravenous routes of drug administration and through the practice of crushing extended release

tablets in order to achieve more immediate opiate absorption. Once in the brain, the primary target for abused opiates is the mu opioid receptor. This receptor is present throughout the brain with highest density in areas modulating pain and reward (e.g., thalamus, amygdala, anterior cingulated cortex, and striatum). Activation of mu opioid receptors inhibits GABA-mediated tonic inhibition of dopaminergic neurons in the ventral tegmental area.[51] This initiates a cascade of effects in diverse brain regions, including the striatum, amygdala, and prefrontal cortex, that are not only related to reward but influence the risk for repeated opiate use by heightening the saliency of drug related cues and the incubation of drug craving.[52;53]

Repeat opiate administration induces tolerance and imparts the potential for a withdrawal syndrome upon cessation. The unpleasant physical and psychological symptoms of withdrawal produce negative reinforcement whereby opiates continue to be used, often in escalating doses, in order to avoid their onset.[54] Furthermore, short acting opiates modulate stress responsive pathways causing dysregulation and further stress-induced negative reinforcement. This stress dysregulation can continue long after a person has discontinued opiates and, thereby, is a contributing factor to the risk for relapse during stress.

Long term approaches to the treatment of opiate addiction are required because of persistent alterations in dopaminergic, opioidergic, and stress responsive pathways. Human imaging studies have identified ongoing reductions in dopamine D2 receptor

binding potential in opiate addicts and that this reduction correlates with the duration of opiate use.[55;56] Animal models of opiate addiction and postmortem studies in human opiate addicts have identified alterations in opioid gene expression in specific brain areas related to reward and behavior.[(for review see 40)] Finally, stress response is exaggerated in former heroin addicts who are not taking methadone pharmacotherapy.[57] While pharmacotherapy may not correct alterations in dopamine receptor availability and opioid gene expression, it does appear to normalize several aspects of stress responsiveness.[58-60]

The advent of epigenetics has allowed us to gain understanding as to how genetic expression is modified by environmental and pharmacological inputs, thus linking all three main contributors to the risk for addiction. For example, environmental inputs such as maternal care or social hierarchy modulate expression of neuroreceptors that, in turn, influence drug self-administration.[61;62] Opiate administration also modulates expression of several genes including those in opioidergic, dopaminergic, and stress responsive pathways.[(for review see 40)] The details of how the interaction between genes, environment, and drugs contributes to the development, persistence, and relapse to addiction have yet to be elucidated. This interaction forms the hypothesized foundation for the persistence of addiction vulnerability even in those who have discontinued drug use and indicates that long term relapse prevention strategies need to include both environmental and pharmacological interventions beyond the immediate period of withdrawal.

Psychosocial intervention only

While this review does not comprehensively address non-pharmacological interventions for opiate addiction, when used alone, these approaches should be considered to lie outside the domain of first-line evidence based treatment. Historical data indicate poor outcome in patients provided only psychosocial interventions. Whether compelled or voluntary, return to opiate use approaches 80% within two years of intensive residential treatment.[63;64] While a systematic review by the Cochrane collaboration indicates some psychosocial interventions may be superior to others, a separate review found that psychosocial intervention alone was inferior to methadone maintenance for such outcomes as retention in treatment and reduction in opiate positive urine toxicology tests.[18;65] This later review also indicated a trend for greater mortality in psychosocial versus methadone treatment, a finding supported in other reports from populations that receive no treatment, psychosocial treatment only, or those who voluntarily discontinue pharmacotherapy.[66-70]

Medically assisted detoxification

The negative reinforcement of withdrawal is a primary driver of ongoing drug use. Several strategies to relieve opiate withdrawal symptoms have been evaluated. The short term (first 30 days) effect on relief of symptoms and return to illicit opiate use between alpha adrenergic agonists such as clonidine and lofexidine (and presumably dexmedetomidine) and opiate based regimens are similar.[71] Rapid withdrawal and

sedation assisted transition to opioid antagonist therapy has increased risk of serious adverse events when performed under heavy sedation and is too resource intensive to endorse given the limited benefit when performed under light sedation.[72;73] Longer period of detoxification (1-6 months) with methadone or buprenorphine are also ineffective in promoting abstinence beyond the initial stabilization period.[74;75]

Medications used to treat opiate addiction beyond the withdrawal period

Methadone, buprenorphine, and naltrexone are each FDA approved for the long-term treatment of opiate addiction (see Tables 1.1 and 1.2). Methadone has been used for the longest period of time and thus has a large body of research supporting its effectiveness. Buprenorphine is similar to methadone in mechanism of action (partial agonist versus full agonist) and effectiveness and thus will be discussed in a slightly abbreviated manner. Naltrexone, an opiate antagonist, has less of an historical basis for effectiveness but a newly evolving literature warrants attention.

Methadone

Methadone is a synthetic mu opioid receptor agonist originally synthesized in the late 1930's as a congener of atropine.[1] In the treatment of opiate addiction, methadone is administered orally in liquid, tablet, or dispersible tablet formulation and is a racemic mixture whose R-entantiomer is responsible for the opioid effect and both R- and S-

enantiomers are NMDA antagonists. Following oral administration, it is rapidly absorbed, undergoes little first pass metabolism, and has moderate bioavailability of 70%-80%. Methadone is approximately 90% bound to plasma proteins such as albumin, globulin fragments, and α_1-acid-glycoprotein. Methadone is also distributed throughout various tissues such as the liver, intestine, lung, muscle, and brain with an apparent volume of distribution during steady state of 3.6 L/kg. Following oral administration, peak plasma levels are reached within 2-4 hours and the elimination half-life at steady state is approximately 28 hours, allowing for once daily dosing. Methadone is hepatically metabolized into inactive compounds primarily by cytochrome P450 3A4 and 2B6 enzymes and is eliminated through both renal and fecal routes. The use of certain medications that induce (e.g., phenytoin, rifampicin, efavirenz) or inhibit (e.g., azole based antifungals) these enzymes may impact plasma methadone levels although the clinical effect in terms of precipitating withdrawal or inducing sedation are variable.[76]

Methadone safety is well established.[77] Like other opiate agonists, methadone has the potential to induce lethal respiratory suppression when given in doses that exceed an individual's tolerance. Recent increases in methadone associated deaths are primarily related to its minimally regulated use in the treatment of pain and not due to its use in the treatment of opiate dependence.[78] This may be due to too rapid dose escalations and a differential rate in development of tolerance to the analgesic and respiratory suppressive effects of methadone. In the setting of addiction treatment, higher levels of dosing supervision reduce mortality rates.[79] There has been recent concern regarding potential

cardiac safety of methadone. While methadone will increase the electrocardiographic QTc interval, this appears minimal in magnitude and rarely exceeds the 500 msec threshold associated with cardiac arrhythmia and sudden death in those with heart disease.[80;81] Evidence that preventing cardiac events through electrocardiographic monitoring or use of buprenorphine, which likely does not prolong the QTc, is lacking.[82] It appears, therefore, that the greatest risks in mortality associated with methadone maintenance occur during the induction period, because of multiple drug ingestion (e.g., benzodiazepines), or due to the loss of tolerance upon methadone discontinuation.[69]

Methadone's ability to relieve the opiate withdrawal syndrome was noted as early as 1947 and within two years it became the preferred medication for detoxification at the national narcotics hospital in Lexington, Kentucky.[10] Upon taking methadone, opiate addicts in withdrawal found their symptoms relieved; those with active addiction did not experience euphoria or request their usual and available doses of injected morphine; and, after chronic administration, sudden cessation of methadone produced a milder, albeit longer in duration, withdrawal syndrome than following morphine cessation.[11]

It was not until 1964 when scientists at the Rockefeller Medical Research Institute (now University) began to evaluate methadone maintenance as a means of long-term medication-assisted treatment for opiate addiction. This work helped to establish that not only did methadone relieve opiate withdrawal but, when at steady-state, it also blocked the euphoric and sedating effects of superimposed opiates.[13;14] Thus, with methadone,

major components of both the positive and negative reinforcing effects of short-acting opiates were reduced and craving subsided thus allowing the addict to concentrate on non-drug related activities.

Methadone response appears to be dose related with most patients stabilizing at doses between 60mg-120mg daily.[83] Response is most frequently measured in terms of retention in treatment and reduction in illicit opiate use, although improvements in psychosocial function and medical status have also been documented.[84] Mean 1-year retention in treatment is approximately 60% and can vary based on adherence to evidence-based dosing practices.[17;85-87] In terms of retention in treatment and adherence to treatment regimen, the results of methadone maintenance are similar to or exceed results for other medically managed diseases such as hypertension, dyslipidemia, and diabetes mellitus.[88] At any given time in treatment, approximately 15% of patients in methadone maintenance will have ongoing illicit opiate use. While there are some associations between treatment outcome and age, medical comorbidity, criminal justice involvement, ongoing non-opiate drug use, and patient satisfaction with treatment, predicting and then preventing treatment failure has not proved successful.[17;89-92] Providing intensive psychosocial services and counseling may improve treatment outcome during the initial 6 months of methadone maintenance but its benefit diminishes through time such that patients receiving intensive services have similar incidence of drug use at 1-year as those receiving standard counseling.[93]

Methadone maintenance is not the replacement of an illegally used opiate for a legally supervised opiate. Unlike abused opiates, once a stabilization dose is achieved (generally between 60mg-120mg daily), rarely is there need to increase dose due to development of tolerance. The reason for this is unknown but may be related to its NMDA antagonist properties.[94] In addition, at stabilization, methadone binds approximately 30% of mu opioid receptors allowing the remaining receptors to perform their usual physiological function in modulation of pain, reward, and mood.[95] Additionally, the psychosocial problems inherent in opiate addiction are also relieved upon methadone maintenance. Regulation of stress response is one such function that tends to normalize with methadone stabilization. For example, suppression of adrenocorticotropic hormone (ACTH) and cortisol caused by administration of short acting opiates, blunted diurnal variation in their release in active addicts, and the increase in these hormones during opiate withdrawal are all corrected during methadone maintenance (see Table 1.2)[77;96;97] Perhaps most importantly, many of the abnormal hormonal responses to stressors during addiction and even following abstinence based treatment are corrected once patients are stabilized on methadone.[(for review see 98)] Thus while methadone relieves withdrawal and blocks the effect of superimposed opiates, it may more importantly be thought of as a relapse prevention drug in that it normalizes many of the physiological stress-related responses that precede and contribute to relapse (Table 1.3).

Buprenorphine

Buprenorphine is a semi-synthetic mu opioid partial agonist with weak partial agonist effects at both delta and kappa opioid receptors. It was first synthesized in the late 1960's by Bentley et al. as part of analgesic explorations of thebaine congeners.[99] In the treatment of opiate addiction, buprenorphine is administered sublingually in tablet or film formulations. A new subdermal implant that delivers buprenorphine for 6 months is in development and showing promise in the treatment of opiate addiction.[100] Buprenorphine undergoes extensive first pass metabolism and oral administration results in poor bioavailability. Following sublingual administration, however, bioavailability is approximately 50%.[101] Buprenorphine is extensively protein bound to globulin fragments and is distributed to various tissues with an apparent volume of distribution during steady state of 3.7 L/kg. Following sublingual administration, peak plasma levels are reached within 1-3 hours and the elimination half-life at steady state is approximately 37 hours, allowing for once daily, and in some instances every other day, dosing. Buprenorphine is hepatically metabolized by cytochrome P450 3A4 and possibly 2C8 into the weak opioid partial agonist norbuprenorphine, which is eliminated through glucuronidation.[102] Both buprenorphine and norbuprenorphine are eliminated through renal and fecal routes. The use of certain medication that induce or inhibit cytochrome P450 3A4 may impact plasma buprenorphine levels although the clinical effect of this is minimal possibly due to buprenorphine's partial agonism, high receptor affinity, and/or because of the weak opioid effects of norbuprenorphine.[76]

The literature on safety evaluation of buprenorphine maintenance is less developed than

that of methadone, but phase III research reports indicate that buprenorphine maintenance is quite safe with equivalent adverse events to methadone and placebo.[85;87;103] Although buprenorphine is a partial agonist at mu opioid receptors, it may induce respiratory suppression but to a lesser extent than full agonists.[104] Additionally, as a partial agonist with high receptor affinity and modest efficacy, many of buprenorphine's effects plateau after approximately 16 mg, although doses of up to 32 mg are used clinically.[104] Thus, while it may have similar rewarding properties as methadone in non-tolerant opiate addicts, attempts to increase this effect or achieve intoxication through dose escalation beyond this ceiling are of little avail.[105] Nevertheless, deaths associated with buprenorphine have been reported following its more rapid delivery through injection or when combined with benzodiazepines.[106] In order to reduce the harm associated with buprenorphine injection, it is available in formulations that combine buprenorphine with the opioid antagonist naloxone in a 4:1 ratio. Naloxone undergoes extensive first pass metabolism and is not absorbed into the systemic circulation when taken orally or sublingually. Injection, however, allows naloxone to enter systemic circulation and compete with buprenorphine for receptor occupancy. This competition reduces initial effects of buprenorphine, thus lowering its rewarding properties and the risks of lethal respiratory suppression.[107] As with methadone, deaths in buprenorphine maintenance patients are more likely to occur during the initial induction period or due to loss of tolerance following its discontinuation.[69]

Buprenorphine's ability to both induce and relieve opiate withdrawal was observed by

Martin et al. in 1976 and within two years Jasinski et al. hypothesized that it may be used in the treatment of opiate dependence.[108;109] Because buprenorphine is a high affinity and moderate efficacy mu opioid partial agonist, it will displace other high efficacy opiates, if present, and induce withdrawal symptoms. On the other hand, when a patient has stopped using opiates and is in withdrawal, buprenorphine will bring relief through its partial agonist effect. Because of this dual effect, induction onto buprenorphine has the potential to precipitate withdrawal. It is, therefore, generally advised that the first dose of buprenorphine be given no sooner than 12 hours after the last use of a short-acting opiate and 24 hours after a long acting opiate, which may be difficult for many patients to achieve. Various induction protocols ranging from inpatient, to outpatient monitoring for withdrawal symptoms prior to first dose, to patient driven home-induction are available to help the clinician safely induce patients onto buprenorphine.[110;111] Following chronic administration, sudden cessation of buprenorphine produces a mild yet prolonged withdrawal syndrome.[109]

While there were several early reports that buprenorphine could relieve opiate withdrawal and block the effect of superimposed opiates, it was not used as a maintenance treatment until 1985.[109;112;113] As with methadone, upon relief of withdrawal and craving, patients on buprenorphine maintenance turned their focus to non addiction related activities. Unlike methadone, buprenorphine is not as highly regulated so most studies have evaluated buprenorphine maintenance in a primary care setting.

Buprenorphine response is dose related with most patients stabilizing on doses between 12 mg - 16 mg daily.[85;114] When adequate doses are used, treatment outcome in terms of retention and reduction in illicit opiate use is similar to that of methadone maintenance.[(for review see 115)] Unlike methadone, where dose can be increased to facilitate treatment response, buprenorphine's ceiling effect may limit its effectiveness in patients with ongoing opiate use.[116] In these individuals, transitioning from buprenorphine to methadone may allow for improved treatment outcome. Also, the role of intensive counseling does not appear to improve outcome of office based treatment compared to standard counseling.[116;117]

There is little difference in outcome between office based and opiate treatment program settings, although direct comparison of a randomized population has yet to be performed. A weakness in the buprenorphine literature is that most study follow-up periods are between 12-24 weeks. In these studies, retention rates are similar to that of methadone over the same period of time.[118] Whether this similarity persists for 1-year is uncertain. One small but dramatic program based placebo-controlled study found 1-year retention of 75% and 0% for buprenorphine and placebo, respectively.[67] Additionally, all patients receiving placebo dropped out by three months and four out of twenty had died by the end of the year whereas none receiving buprenorphine died.

Aside from ongoing opiate use, predictors of buprenorphine treatment outcome may include depression, income, and ongoing cocaine use.[89] There has been recent attention to

the use of buprenorphine for the treatment of prescription opiate addiction.[117] During maintenance treatment, patients have reduced illicit opiate use but following buprenorphine taper, more than 90% of patients return to illicit opiate use.[117] In another study comparing heroin addicts to prescription opiate addicts, the heroin addicted patients had more severe medical and addiction severity and did not do as well on buprenorphine as the less ill prescription opiate addicts.[119] Caution in interpreting this finding is warranted since these two populations are not comparable and the difference in outcome may be more related to addiction severity than to the patient's opiate of choice. There is no neurobiological or pharmacological reason why, after adjusting for these factors, heroin addicts and prescription opiate addicts would have different treatment outcomes and thus require separate consideration in medication choice.

The neurobiological effect of buprenorphine in the treatment of opiate addiction is presumed to be mediated through partial agonism of the mu opioid receptor. The effect at delta and kappa receptors is likely too weak to contribute to its treatment effectiveness. Buprenorphine binds extensively to mu opioid receptors with over 90% occupancy following doses of 16 mg or greater.[120] Buprenorphine can suppress stress responsive hormones such as ACTH and cortisol when administered acutely to healthy controls.[121] When stabilized methadone maintained patients were transitioned onto buprenorphine, basal levels of beta-endorphin remained normal.[122] It appears that most stress responsive markers are normalized in buprenorphine maintained patients and that failure to normalize correlates with craving and relapse.[123;124] Thus, as with methadone, the role of

buprenorphine in the treatment of opiate addiction is not simply replacement of an illicitly used opiate for a medically supervised opiate but rather as a medication that corrects many of the neurobiological processes contributing to relapse.

Naltrexone

Naltrexone is a semi-synthetic mu and kappa opioid receptor antagonist synthesized in the mid-1960's as a congener of oxymorphone.[125] In the treatment of opiate addiction, naltrexone is administered either orally in tablet formulation or intramuscularly in an extended release formulation. Following oral administration, it is rapidly absorbed but undergoes significant first pass metabolism with a bioavailability less than 50%.[126] Naltrexone has low protein binding capacity and an apparent volume of distribution of approximately 19 L/kg. Peak plasma levels following oral administration are reached within 4 hours and the elimination half-life at steady state is approximately 9 hours.[127] Naltrexone is reduced to the weak opiate antagonist 6β-naltrexol in the liver. Naltrexone, 6β-naltrexol, and their conjugates are renally eliminated with less than 3% recovered in the feces.[127] There are no known drug interactions that would alter naltrexone metabolism and thus limit its use.

Naltrexone safety is well established. There have been some reports of hepatotoxicity following high dose naltrexone and caution is advised in prescribing naltrexone in the setting of acute hepatitis or end stage liver disease.[128] Unlike methadone and

buprenorphine, naltrexone is not behaviorally reinforcing in individuals without opiate tolerance and does not induce respiratory suppression. Since it is an opiate antagonist, naltrexone may precipitate withdrawal in patients with physical dependence on opioids.

The initial hypothesis for the use of opioid antagonists in the treatment of opiate addiction was as a means of eliminating a conditioned response to use opiates.[129] Based on this hypothesis, return to opiate use following detoxification is caused by negative reinforcement of environmental stimuli (e.g., cues and social stressors) and if an antagonist prevented the addict from relieving this negative state through opiate use, then the behavior of turning to opiates in these situations would eventually cease. Indeed, naltrexone can block the effect of superimposed opiates for approximately 24-48 hours after oral dosing.[130] The plasma levels sufficient to block 25 mg of heroin are approximately 1-2 ng/ml, a level maintained for 21-28 days following 380 mg of the intramuscular extended release formulation.[131]

As early as 1976, NIDA convened a workgroup to study and promote the development of both oral and extended release naltrexone as a treatment for opiate addiction.[132] Early and successive work found that naltrexone was well tolerated with few adverse effects other than mild nausea. Patients taking naltrexone reported fewer days of heroin use and had few opiate positive urine drug tests.[133] Patient adherence and drop out has been a major stumbling block for oral naltrexone. In multiple studies of either daily or thrice weekly dosing, fewer than 20% of patients remain in treatment for 6 months.[133-135] A Cochrane

collaboration meta-analysis found that due to extensive drop-out rates, oral naltrexone maintenance with or without psychotherapy was no better than placebo treatment.[136]

Extended release naltrexone may improve treatment outcome because non-adherence to daily oral regimens is reduced by delivery of a once monthly injection. Currently there are limited data regarding the extended release intramuscular injection. In a two month randomized placebo controlled trial, only 70% of patients were retained for 8-weeks.[137] A larger trial in Russia retained 53% of patients at 6 months compared to 38% for placebo.[138] Patients receiving extended release naltrexone also had significantly fewer days of illicit opiate use. While intramuscular naltrexone is the only FDA approved extended release formulation, literature on subdermal implants capable of maintaining naltrexone plasma levels between 1-2 ng/ml for 6 months, may also contribute to our understanding of the role naltrexone may play in the treatment of opiate addiction. These studies have shown retention of approximately 60% at 6-months, exceeding that of oral naltrexone.[139;140] Illicit opiate use was also significantly reduced, however, in one study, patients receiving the implants had a higher rate of non-opiate drug use than those receiving methadone.[141]

The use of naltrexone in the treatment of opiate addiction is mechanistically quite different from that of methadone and buprenorphine. While each medication can block the effect of superimposed opiates and following steady-state oral administration, naltrexone achieves approximately 95% mu opioid receptor occupancy.[142] Unlike

methadone and buprenorphine, naltrexone is without intrinsic opiate activity and poses minimal risk abuse or diversion. What may be most compelling about naltrexone comes from the literature on its use in the treatment of alcoholism where it reduces craving, a frequent predecessor to relapse. In fact, reduction in base-line craving is correlated with its effectiveness.[143] Naltrexone's effect on craving in opiate addicts is less clear. Oral naltrexone may not reduce craving more than placebo and if it does, this reduction does not necessarily correlate with abstinence.[144;145] Failure of oral naltrexone to prevent relapse in opiate addicts may be related to ongoing stress dysregulation and is supported by animal research showing its failure to suppress stress-induced relapse.[146] Whereas both methadone and buprenorphine can normalize stress response, naltrexone maintenance may not. In fact, oral naltrexone administration stimulates ACTH and cortisol, even following chronic administration.[147] This stimulation mimics the hormonal response during opiate withdrawal. It also mimics the response to acute administration of alcohol, which may explain oral naltrexone's effectiveness for alcohol but not opiate addiction.[148] Whether extended release naltrexone has a similar effect on stress response remain unknown but its ability to reduce craving is promising.[138]

Methadone, buprenorphine, naltrexone direct comparisons

There are no randomized double-blind controlled trials comparing all three medications. One randomized trial comparing each of the medications found 24-week retention rates of 84%, 59%, and 21% for methadone 50 mg, buprenorphine 5 mg, and naltrexone 50

mg, respectively, despite suboptimal doses of methadone and buprenorphine.[149] A comparative study between buprenorphine and oral naltrexone found naltrexone response inferior.[150] There are no comparative outcome studies between either methadone or buprenorphine and extended release naltrexone. It has been observed that the 6-month retention rates following extended release naltrexone are similar to 1-year retentions in methadone maintenance and thus non-inferiority studies of extended release naltrexone are needed.[151]

Special populations

End stage liver disease: Decreased hepatic metabolism and plasma protein can lead to increased methadone clearance.[152] Increased methadone clearance may result in onset of withdrawal symptoms and can be prevented by increasing methadone dose. Since this will also increase methadone peak levels, it may result in sedation. If this occurs, the methadone dose may be split into two doses taken during the course of the day. There are no formal studies of buprenorphine pharmacokinetics in end stage liver disease. Given the long half-life and active metabolites of buprenorphine, it is unclear if dose adjustment is needed in end stage liver disease. FDA labels for both oral and intramuscular naltrexone recommended against their use in the setting of end stage liver disease.

Pregnancy: The placenta is metabolically active and can increase clearance of both methadone and buprenorphine. Since methadone does not have active metabolites,

patients may experience early withdrawal and may require increases in or splitting of methadone dose during the second and third trimesters.[153] It is recommended that neither naloxone nor naltrexone be administered during pregnancy, although each are Category C, thus buprenorphine should be administered as the mono product and naltrexone should be avoided. Both methadone and buprenorphine are associated with improved maternal and fetal outcomes compared to abstinence based approaches. While the recent MOTHER trial found that the length, but not intensity, of neonatal abstinence syndrome and the neonates' need for morphine relief was lower in women taking buprenorphine compared to methadone, there was lower retention in the buprenorphine treated group.[154]

Adolescents: Opiate addiction is often a disease of pediatric onset. Early interventions can prevent the associated consequences of addiction such as HIV and hepatitis C.[155] Because adolescents often have shorter addiction history, it is not known whether they would require maintenance pharmacotherapy. Several reports comparing short-term detoxification to buprenorphine maintenance, however, show better results with longer periods of medication and high rates of relapse following discontinuation of medication.[(for a review see 156)] Comparative research to guide maintenance medication selection in adolescents is needed. Until such research is available the choice of maintenance medication should be based on available evidence and informed choice.

Chronic pain: A significant number of patients in maintenance pharmacotherapy complain of chronic pain.[157] Many of these patients may require daily or intermittent

opioid analgesics. Both methadone and buprenorphine have been used in the treatment of moderate to severe pain and their chronic use for opiate addiction does not preclude the regular use of opioid analgesics. Naltrexone can prevent the effectiveness of opioid analgesics. Its antagonist effect can be overridden in setting of acute pain but caution is advised.[158] It is not clear whether naltrexone maintenance can be recommended for the patient requiring ongoing opioid analgesia.

Criminal justice: Methadone and buprenorphine have been used with success in criminal justice populations.[159;160] Each can reduce recidivism and illicit opiate use. Oral naltrexone requires close supervision for adherence and trials of extended release naltrexone in criminal justice populations are forthcoming.[161] No direct comparisons of these medications have been performed in a criminal justice setting. While there is legal precedent for compulsory addiction treatment and medication in offenders, this precedent does not extend to a specific medication and the criminal justice system must avoid requiring one medication in favor of others and respect the informed choice of decisions made between a physician and patient.[162]

Health professionals: Opiate addicted health professionals have excellent treatment outcomes compared to the general population.[163] Behavioral interventions alone have retention rates approaching 80%.[164] While some have reported successful use of naltrexone as an adjuvant treatment in opiate addicted health professionals, in the absence of controlled trials it is difficult to know if it provides added benefit to behavioral

interventions alone.[165;166] Without clear benefit of naltrexone over methadone or buprenorphine, the selection of specific pharmacotherapy should be between a physician and patient and based on evidence and informed choice.

Conclusion: Medication and recovery

Extensive research shows that each of the three available medications used to treat opiate addiction have superior treatment outcomes to non medication based therapies. Increased retention reduces mortality, improves social function, and is associated with decreased drug use and improved quality of life. Thus, these medications help patients achieve "recovery" as it is currently defined.[167] While methadone and buprenorphine appear to have superior outcomes to both oral and intramuscular naltrexone, more direct comparisons are needed. Further work is needed to identify and predict treatment response to help individualize medication choice. Until such data are available, it is prudent, and within a patient's right to informed choice, for treatment professionals to provide information regarding these standard treatment options, their expected outcomes and potential adverse effects, and allow the patient to choose the medication that best suits his or her need.

Table 1.1 Clinical characteristics of methadone, buprenorphine, and naltrexone

	Methadone	**Buprenorphine**	**Naltrexone**
Controlled substance	Yes	Yes	No
Availability	OTP	OTP or DATA Waived practitioner	Any prescribing practitioner
1-year retention	60%	60%	20% (53% 6-months ER)
Direct expense	$	$$	$$-$$$$
Dosing frequency	Daily	Daily	Daily or monthly (ER)
Narcotic blockade	Yes, at steady-state	Yes, at steady-state	Yes
Can induce withdrawal	No	Yes	Yes
Overdose potential	Yes	Yes	No
Withdrawal upon cessation	Yes	Yes	No
Loss of tolerance on cessation	Yes	Yes	Yes
Complicates treatment of moderate-severe pain	No	No	Yes

OTP opiate treatment program; DATA Drug Addiction Treatment Act of 2000; ER extended release formulation

Table 1.2 Pharmacological profile of methadone, buprenorphine, and naltrexone

	Methadone	**Buprenorphine**	**Naltrexone**
Main effect	Mu full agonist, NMDA antagonist	Mu partial agonist	Mu antagonist
Bioavailability	70%-80%	50%	< 50% (~100% ER)
Half-life	28 hours	37 hours	9 hours (4.95 days ER)
Clinically apparent drug interactions	Rifampin, phenytoin, several ART	Select ART	Opioids NSAIDS (?)
Active metabolites	None	Nor-buprenorphine	6-beta-naltrexol

ART Antiretroviral therapy; NSAID Non-steroidal anti-inflammatory; ER extended release formulation

Table 1.3 Stress response hormones

	ACTH	Cortisol
Short-acting opiates	↓	↓
Opiate withdrawal	↑	↑
Methadone	↔	↔
Buprenorphine	↔	↔
Naltrexone (oral)	↑	↑
Naltrexone (ER)	?	?

ACTH adrenocorticotropic hormone; ER extended release

Chapter 2

Methadone Pharmacodynamics and Pharmacokinetics*

Gavin Bart, M.D.[1] and Sharon L. Walsh Ph.D.[2]

[1]Hennepin County Medical Center
701 Park Avenue, Mail Code G5
Minneapolis, Minnesota 55415
Phone: 612-873-4051
Email: bartx005@umn.edu

[2]Center on Drug and Alcohol Research
Department of Behavioral Science
University of Kentucky
515 Oldham Court
Lexington, KY 40502
Phone: 859-257-6485
Email: sharon.walsh@uky.edu

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Background

Methadone (6-dimethylamino-4, 4-diphenyl-3-heptanone) was first synthesized in 1937 by Bockmühl and Ehrhart as part of a larger pharmaceutical program synthesizing atropine derivatives as spasmolytics.[1] This program led to the development in 1939 of meperidine, which not only had spasmolytic properties, but also acted as an analgesic. Subsequent testing of methadone found it had a 5-10 fold greater analgesic effect than meperidine but its further development was limited by wartime supply shortages and potential adverse effects, such as nausea, experienced in a small group of human subjects.[1;168]

Following World War II, 6-dimethylamino-4, 4-diphenyl-3-heptanone was brought to the United States where it was soon given the generic designation methadone.[3;169] Methadone is a Drug Enforcement Agency Schedule II controlled substance approved for the treatment of severe pain and opiate dependence and is available in 5 mg and 10 mg tablets, 40 mg dispersible tablet, 5 mg / 5 ml, 10 mg / 5 ml, 10 mg/ml liquid, and 10 mg/ml injectable formulations. Because there are no significant differences in methadone pharmacokinetics or pharmacodynamics between the various oral formulations, they will be addressed as a single group.[170] When administered intravenously, the injectable formulation has 100% bioavailability and reaches peak plasma levels immediately following injection. No formal pharmacokinetic studies of subcutaneous or intramuscular methadone injection exist, although bioavailability is likely to approach 100%, and its pharmacokinetics are expected to be similar to intravenous or orally administered

methadone aside from the lag in absorption into the vascular space. Pharmacodynamic differences between the oral and intravenous routes of administration will be discussed.

Acute Pharmacodynamic Actions

Acute methadone administration, regardless of route of administration, produces dose-dependent physiological effects typical of mu opioid agonists, including pupil constriction (i.e., miosis), decreased gastrointestinal motility, and decreased respiratory rate (along with associated changes in other respiratory indices, such as increased expired CO_2 and decreased oxygen saturation). Acute effects of methadone on cardiovascular response (i.e., heart rate, blood pressure) are not generally of clinical significance (but see later chapter in this volume discussing evidence of methadone effects on cardiac QTc prolongation). Pupil constriction is a hallmark sign of mu opioid action and can be used to assess the time-action profile of methadone. Respiratory depression after treatment with methadone and other mu opioid agonists results from decreased chemoreceptor sensitivity to circulating CO_2 concentrations in the medullary brain stem. Thus, relatively low doses of methadone in opiate-naïve individuals and sufficiently high doses of methadone in opioid-tolerant individuals can lead to fatal overdose consequent to respiratory depression and cardiopulmonary failure. The risk for methadone (and opioid) overdose is exacerbated when used in combination with other opioids and/or sedatives, including alcohol and benzodiazepines.[171]

The onset of the pharmacodynamic action of intravenous methadone is evident within minutes of administration, and peak effects occur within the first hour after infusion.[172] Pharmacologic activity after parenteral administration of acute doses (intravenous or subcutaneous) persists for approximately 12 hours with complete dissipation by 24 hours post dosing [172;173] Observer ratings of opiate signs and study participant self-report of "liking" for the drug (and other associated abuse liability measures) generally follow this time-action curve when evaluated in experienced opioid abusers without physical dependence; however, miosis may be evident 24 hours after a single dose of methadone in the absence of other signs and symptoms. As with miosis, other studies have reported a longer duration of methadone action on respiratory depression after subcutaneous and oral administration. This can be a significant safety concern as illicit opioid users may perceive the absence of psychoactive effects and use additional opioids without recognizing the more persistent effect on respiratory depression. With respect to its qualitative subjective profile of mood effects, intravenous methadone was indistinguishable from morphine or heroin when all were tested within a group of opioid-experienced volunteers.[172]

When given parenterally, methadone and morphine (the prototypic euphorigenic comparator) are considered to be equipotent. However, because of the superior oral bioavailability of morphine, this ratio of methadone: morphine changes from 1:1; however, the relative potency estimates vary greatly from approximately 1:3 to as high as 1:30 for oral conversions and caution and clinical monitoring is recommended. Oral

methadone administration is associated with a slower onset of action compared to parenteral administration, with dose-dependent pharmacodynamic responses appearing within the first hour after ingestion and peak subjective responses occurring between 3 – 4 hours after oral dosing.[104;173;174] As with parenteral administration, the subjective effects of methadone (measures of euphoria and sedation) are generally undetectable at 12 hours post-dosing, while miosis may persist for 24 hours after a single acute dose.

Studies have examined the effect of methadone on a broad array of psychomotor performance and other cognitive function measures. Studies on the acute effects of methadone (and other opioids) generally have reported slowed response time in the absence of significant performance deficits (see Zacny et al., 1995 for critical review);[175] however, tolerance is reported to develop to some of these effects with chronic dosing. Numerous studies have reported no differences between methadone-maintained individuals when compared to control subjects on psychomotor and cognitive performance tasks, while others employing a broader range of tests have found some performance decrements in methadone-maintained patients in comparison to former heroin abusers[176] and non-drug using controls.[177] However, as these impairments are frequently described relative to incompletely matched control groups (e.g., non-drug using controls), the observed effects cannot be directly attributed to methadone alone because prior history of drug use, ongoing drug use, and other consequences of a drug-using lifestyle may play a role in the observed outcomes (e.g., prior and ongoing illicit

opioid and other drug use, nutritional status, prior head injury)[178] that differentiate the methadone-maintained patients from control subjects.

Pharmacodynamic Effects: Chronic Administration

Isbell and colleagues (1948) conducted the seminal human studies on the response to repeated or chronic administration of methadone and methadone physical dependence properties at the United States Public Health Service Administration Narcotics Hospital in Lexington, Kentucky.[179] In individuals with heroin use histories who were chronically maintained on morphine (four times per day) these studies demonstrated that parenteral methadone could suppress opioid abstinence signs and symptoms in physically dependent individuals during periods when their daily morphine injections were withheld. Moreover, the substitution of methadone for the regularly scheduled morphine dose prevented the emergence of withdrawal signs and symptoms. These authors also noted that methadone produced euphoria that persisted for a longer duration of action than that produced by morphine and that tolerance developed to the sedative and euphoriant effects with chronic dosing. Later studies of daily maintenance on methadone reported that chronic administration led to small but reliable reductions in respiratory rate, blood pressure and heart rate along with increased body temperature when compared to either the period prior to maintenance or to a control group.[173;176] During chronic treatment with methadone, pupil diameter will vary as a function of time since dosing, but typically there is some degree of miosis evident throughout the 24-hour dosing period.[180] Finally,

chronic administration of methadone with dosing for periods of 28 to 186 days at high daily doses up to 240 mg (60 mg four times daily, s.c.) led to a dependence profile very similar to that seen with morphine, including 1) development of tolerance with requests for dose escalation, and 2) emergence of an opioid withdrawal syndrome following cessation of dosing (but this emerged later and persisted longer than that seen after morphine discontinuation).

Pharmacodynamics in Treatment of Pain

Methadone is a racemic mixture whose R-entantiomer is a high affinity (Ki 0.6 nM) full agonist at the mu opioid receptor and both R- and S- enantiomers are N-methyl-D-aspartic acid (NMDA) receptor antagonists. It is commonly thought that both of these neuropharmacological actions may contribute to the efficacy of methadone as an analgesic because selective mu opioid agonists and NMDA antagonists produce pain relief in acute and chronic pain conditions. Affinity and/or efficacy at other receptors, such as delta and kappa opioid or monoamine receptors, are sufficiently low to rule out clinical significance. Methadone can be used by the oral, parenteral or rectal routes of administration for pain relief. Methadone has a long history of use as an analgesic since its early development; however, its use in clinical practice in the United States has increased significantly in recent years due to its 1) efficacy, 2) high oral bioavailability, 3) relative low cost for patients, and 4) an overall increase in prescribing of opioids for pain conditions subsequent to the mandate to assess pain as the fifth vital sign and

recommendations for broader usage of opioids for pain control by medical professional societies.

The onset of analgesia occurs within approximately 30 – 60 minutes after oral dosing and within the first half-hour after parenteral administration with peak analgesic responses occurring around 1 hour of dosing by both routes. While methadone is efficacious as an analgesic, its duration of action is less than would be predicted by its estimated half-life as the duration of pain relief is approximately 3 – 6 hours. This has been shown in the laboratory with experimental models of acute pain and clinical conditions of acute and chronic pain. Although the same principal of "start low and go slow" applies to initiating therapy with methadone for analgesia as induction onto methadone for opioid dependence, starting doses for pain relief are typically much lower than dosing for opioid dependence. Recommendations vary but contemporary clinical practice guidelines generally recommend starting with an initial low oral dose (e.g., 2.5 mg every 8 hours) for opiate-naïve patients and up to 5 mg for patients rotating from other opioids and titrating slowly. While numerous relative potency tables for opioid conversions are available, caution is recommended when rotating to methadone 1) because of its long half-life, and 2) because the conversion from some opioids onto methadone is not bidirectionally equivalent. Specifically, the mg/mg conversion from one opioid rotating onto methadone may not be equivalent to conversion from methadone rotating onto that opioid.[181] As with other responses to methadone, tolerance can develop to the analgesic effects faster than to the respiratory suppressive effects and, therefore, dosing may need

to be titrated upward under careful supervision in order to assure safe and appropriate clinical response. A lengthier discussion on methadone dosing recommendations for analgesia appears in another chapter of this volume.

Pharmacodynamics in Opioid Maintenance Treatment

The early observations by Isbell and others on the long duration of opioid action produced by methadone and its efficacy in suppressing withdrawal in opioid dependent individuals led to the seminal work of Dole and Nyswander in which they proposed and tested the use of methadone as a maintenance treatment for heroin addiction in the 1960's.[13] While methadone has now been in use for more than 40 years in the United States for the treatment of opioid dependence, its use for this indication occurs only under very the tight regulatory authority of the Federal government and in very restricted settings. Moreover, it is unlawful to prescribe methadone for opioid addiction outside of the context of specific inpatient circumstances or a federally licensed methadone outpatient treatment program. Most commonly methadone treatment is provided using oral liquid or the dispersible tablet formulations which may not be used for the treatment of pain.

Because of its long half-life and tendency to accumulate during the early stage of treatment (see below for further details), patients with opioid dependence are usually initiated onto methadone at a dose of 30 mg or less daily. Federal law provides an option

for an additional 10 mg on Day 1 of dosing for those patients who are not receiving relief from a 30-mg dose. Various induction schedules have been employed, and while none are mandated, the guiding principle is to "start low and go slow" in order to avoid adverse outcomes resulting from accumulation and overdose. As induction is initiated at relatively low doses, it is not unusual for patients with high levels of physical dependence to experience withdrawal symptoms during the period of induction and stabilization prior to reaching steady-state; these patients should be educated about the harmful risks of continuing illicit opioid use on top of their methadone dosing to avoid overdose. Fatal overdose in a patient receiving methadone is most likely to occur within the first two weeks of initiating treatment because of these risks.[182]

Once stabilized, most patients achieve adequate relief with once daily dose despite the rise and fall in plasma concentrations of methadone over the 24-hour dosing period. In certain instances, however, as with rapid metabolizers or in pregnancy, split dosing can be employed (but this is the exception rather than the norm). Its long duration of action accounts for the ability of methadone to suppress withdrawal signs and symptoms with only once daily dosing. In dose omission studies (or placebo substitution), a missed 24-hour dose can be detected through physiological changes but does not typically lead to frank withdrawal symptomatology under double-blind conditions across a range of methadone maintenance doses.[183;184]

In addition to suppression of withdrawal symptoms, another critical therapeutic benefit of methadone maintenance is the development of cross-tolerance to other opioids. Opioid cross-tolerance is the phenomenon whereby chronic maintenance on one opioid can lead to a diminished response to another opioid. The ability of methadone to produce cross-tolerance was reported in a seminal study in which subjects maintained on various doses of methadone were challenged under double-blind, placebo-controlled conditions with heroin, hydromorphone, morphine and methadone.[14] This study reported that there was significant "narcotic blockade" (i.e., cross-tolerance) to the euphoriant effects and observable signs of the various opioid challenges in the presence of methadone maintenance, and that the degree of blockade was greater with longer exposure to methadone, suggesting increased development of cross-tolerance over the course of stabilization. In another early study, inpatient subjects who had a history of opioid dependence but were not physically dependent at the start of the study were given the opportunity to work (e.g., by riding a stationary bicycle for a prescribed period of time) to earn injections of hydromorphone.[185] Over the course of the study, subjects were initiated onto daily methadone and the maintenance increased to successively higher daily doses such that at the end of the 6-week study 100 mg/day was given. This study demonstrated that the willingness to work for hydromorphone decreased as methadone maintenance dose increased and work effort corresponded to the degree to which the subjects reported "liking" for the hydromorphone, thereby indicating a link between the subjective response to the drug and self-administration behavior. Subsequent studies have expanded these findings on the dose-related effects of methadone cross-tolerance and have 1)

demonstrated that the degree of blockade is significantly less at 48 hours after methadone compared to 24 hours after methadone, supporting the need for daily dosing to achieve optimum response, and 2) revealed that, while lower doses of methadone (30 mg) may be sufficient to produce suppression of opioid withdrawal signs and symptoms, higher doses are needed to produce robust cross-tolerance and to suppress heroin self-administration in the laboratory.[184;186] Historically, average methadone maintenance doses in the United States were lower but have risen in response to a growing evidence base for the superior efficacy of higher maintenance doses (e.g., 80-120 mg daily)[187;188] that is likely attributable, in part, to greater cross-tolerance.

In practice, it is commonly thought that patients are able to detect small variations in dose (as some clinic practices reduce doses for infractions - i.e., appearing intoxicated for daily dosing, etc.). Studies reveal that subjects are readily able to detect the effects of their regular methadone dose in comparison to a placebo substitute within the initial hours after dosing and discriminate active methadone from placebo.[180] When controlling for taste cues, it was found that subjects could detect both large increases and decreases in their regular dose but there was substantial inter-individual variability in the ability to detect these differences.[189] In another study, subjects maintained on either 30 or 60 mg methadone daily and challenged 40-hr after their last dose reported significant and dose-dependent increases on ratings of positive mood effects and opioid agonist-like symptoms after 30 and 60 mg (but not 15 mg) of oral methadone administered under

double-blind conditions; these effects were more robust for those maintained on the lower dose of methadone.[190]

General Pharmacokinetic Properties

Following oral administration, methadone is rapidly absorbed from the intestinal lumen with an absorption half-life of 15-60 minutes (Ka 1.4-3.4), with variability likely due to inter-individual differences in intestinal motility.[191] For example, patients already on opiates may have reduced gastrointestinal motility leading to slower methadone absorption than opiate naïve patients. While methadone is a substrate for P-glycoprotein transporters and cytochrome P-450 3A metabolizing enzymes, their presence in the intestine has little effect on methadone absorption.[192] Once absorbed across the intestinal lumen, methadone enters the portal circulation and then the liver. Although methadone is primarily metabolized in the liver, in humans it has a low hepatic extraction ratio meaning, in part, it is subject to little first-pass metabolism or alteration in bioavailability caused by changes in hepatic blood flow. Thus, methadone apparent oral bioavailability is between 80%-90%.[192] Methadone levels in bile are sufficiently low to exclude a significant effect of enterohepatic recirculation on plasma methadone levels.[193]

Methadone has a pKa of 8.25 and an n-octanol:water partition coefficient of 117 at a physiological pH of 7.4, making it highly lipophilic. Methadone is approximately 90% bound to plasma proteins such as albumin, globulin fragments, and α_1-acid-glycoprotein

and 10% is unbound and available for transit across tissue membranes (e.g., hepatic membranes for metabolism, glomerular membranes for urinary elimination, and across the blood-brain barrier where most of its pharmacodynamic effects are mediated).[194] Because α_1-acid-glycoprotein is an acute phase reactant, there has been concern that fluctuations in levels of this protein could increase levels of unbound methadone leading to increased adverse effects, such as sedation and respiratory suppression, but also to increased elimination and overall reduced methadone exposure. It does not appear, however, that differences in α_1-acid-glycoprotein binding result in clinically apparent symptoms.[195]

Methadone is distributed throughout various tissues such as the liver, intestine, lung, muscle, and brain with an apparent volume of distribution during steady state of 3.6 L/kg. The rate of distribution into and out of the tissue is different than that of elimination, thus methadone displays biexponential, or two compartment, pharmacokinetics. Some investigators have found a monoexponential model to be adequate in describing steady-state methadone pharmacokinetics. Aside from significant inter-individual variability in methadone pharmacokinetics, there is wide inter-study variability in estimated parameters that is due, in part, to methodological variability such as evaluation following single dose versus steady-state dosing, mono- versus bi-exponential models, frequency and length of plasma sampling, and clinical versus research setting.[196] Thus clinicians should be cautious in translating results of any one study into clinical practice.

Following oral administration, peak plasma levels are reached within 2-4 hours and the terminal half-life at steady state is 24-28 hours.[196] Achieving a steady state plasma level requires dosing over 4-5 half-lives of a drug and, therefore, is not approximated until day 5 of methadone dosing (see Table 2.1 and Figure 2.1). Increasing dose before steady state is achieved will result in an accelerated increase in plasma levels, which can contribute to the risk of methadone toxicity (e.g., excess sedation or respiratory suppression). Once steady state is achieved, the ratio of peak to trough methadone level is approximately 1.6-2.0.[24] Exceeding this ratio may be an indication of increased methadone clearance due to changes in elimination and/or metabolism.

Metabolism and Excretion

Methadone is metabolized in the liver predominately by a cytochrome P450 (CYP) mediated process of N-demethylation. Initial N-demethylation results in the inactive 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), which is N-demethylated into the inactive 2-ethyl-5-methyl-3,3-diphenylpyraline (EMDP). While at least seven other metabolites have been identified, they are produced in such small quantity that they will not be discussed. Both EDDP and EMDP are eliminated in the urine and approximately half of a methadone dose can be recovered in the urine over 96 hours as methadone, EDDP, and EDMP. Renal excretion of methadone is correlated to urinary pH, however, overall elimination of methadone in the urine is so small that manipulation of urinary pH is unlikely to have clinical impact or facilitate the treatment of methadone toxicity.[197]

Methadone is also eliminated in the feces, although mostly as metabolites and less than 5% as methadone. Small amounts of methadone can be detected in body fluids such as saliva, sweat, semen, and breast milk, but these fluids do not comprise a significant route of elimination and concentrations are generally very low and, especially in the case of breast milk, are not high enough to impart risk of methadone toxicity if consumed by others.

Several CYP enzymes are able to metabolize methadone including CYP 3A4, CYP 2B6, CYP 1A2, CYP 2C19, and CYP2D6. Unlike morphine or buprenorphine metabolism, methadone and its metabolites do not appear to undergo a secondary glucuronidation process. Isoenzymes other than CYP 3A4 and CYP 2B6 comprise a small percentage of methadone metabolism and medications or genetic variants known to induce or inhibit their function are not likely to have significant clinical effect on methadone pharmacokinetics or pharmacodynamics. CYP 3A4 is the most abundantly expressed CYP in the liver, comprising approximately 30% of all CYP isoforms, and is involved in the metabolism of over half of all prescribed medications. CYP 3A4 has traditionally been thought of as the main isoform responsible for methadone metabolism and prescribers have been cautioned about drug interaction when co-prescribing methadone with other drugs known to induce or inhibit CYP 3A4. For example, CYP 3A4 inducers, such as phenytoin, rifampin, and select antiretroviral therapies, increase methadone elimination and metabolism and can lead to clinically significant symptoms of opiate withdrawal (see Table 2.2). Azole antifungals inhibit CYP3A4 and increase peak plasma

levels and area under the time versus concentration curve but rarely do they result in increased sedation or respiratory suppression. However, withdrawal symptoms and increased methadone elimination/metabolism following nelfinavir, a known CYP 3A4 inhibitor, indicate that drug interactions are more complex than whether CYP 3A4 function is altered.[198] Indeed, there has been increased focus on the role CYP 2B6 plays in methadone metabolism. While *in vitro* studies support the role of CYP2B6 in methadone metabolism, *in vivo* studies have been more difficult because many of the medications known to interact with CYP 2B6 also interact with CYP 3A4. Thus prescribers should consider inducers and inhibitors of both CYP 3A4 and CYP 2B6 as having the potential to affect methadone levels with resultant clinical effects.

Because methadone is a racemic mixture, there has been interest in whether there is differential metabolism or pharmacokinetics between the opioid R-enantiomer and the non-opioid S-enantiomer. In human liver microsome studies, it appears that there is no stereoselectivity in CYP 3A4 methadone metabolism but that CYP 2B6 may metabolize S-methadone more rapidly than R-methadone.[199;200] Clinical studies have shown lower protein binding for R-methadone with subsequent increases in volume of distribution and renal clearance for the unbound enantiomer.[195] There was no difference between enantiomers and total renal clearance, however. It is not clear that enantiomer specific metabolism or pharmacokinetics translates into clinically significant differences but together they may explain part of the wide inter-individual variability in methadone pharmacokinetics.

Special Patient Populations

Several physiological states have the potential to affect methadone pharmacokinetics. For example, since a major component of methadone elimination is renal, there may be concerns that renal failure could result in accumulation of methadone and resultant toxicity. Evaluation of methadone pharmacokinetics in patients on either peritoneal or hemo-dialysis show that methadone pharmacokinetics remain unchanged, methadone is not removed by dialysis, and fecal elimination compensates for loss of renal function.[201] While large sample pharmacokinetic studies across a range of renal impairment have not been performed, there are no recommendations for dose adjustment in the setting of renal failure.

Patients taking methadone for the treatment of opiate addiction have a higher prevalence of hepatitis C and alcohol dependence than the general population and both conditions increase the risk for developing liver disease and cirrhosis. Although methadone is hepatically metabolized, methadone pharmacokinetics are unaltered in the absence of significant liver disease. In patients with biopsy-proven liver disease, methadone clearance was reduced only in those with decompensated cirrhosis and not those with milder forms of liver disease or cirrhosis.[202] Fluid shifts due to ascites, sodium retention, and decreased levels of circulating proteins increase methadone volume of distribution and decrease its apparent oral clearance. This is in contrast to patients with alcohol-

induced liver disease who were noted to have increased volume of distribution but increased apparent oral clearance.[203] Despite these contrasting findings, both studies found no difference in dose-adjusted area under the curve or dose adjusted mean plasma levels. Therefore, it is currently recommended that in the setting of severe liver disease, methadone dose be adjusted (up or down) based on assessment of symptoms (withdrawal or excess sedation) rather than the mere presence of disease.[202;203] There are no specific data to guide dosing strategies in the setting of acute liver disease or fulminant hepatitis.

Methadone pharmacokinetics are affected during the course of pregnancy. The placenta is a metabolically active organ with increased blood flow and expression of CYP isoforms as it grows. Therefore, by the second and third trimesters there may be increased methadone clearance via metabolism by placentally expressed CYP19 (aromatase).[204] Reduction in plasma protein levels and increased plasma volume during pregnancy also may contribute to alterations in methadone pharmacokinetics. In contrast to post pregnancy conditions, methadone clearance is greater and peak methadone levels lower as pregnancy progresses from weeks 20 through 40.[153] This can result in early onset of withdrawal symptoms and may require either an increase in methadone dose or splitting a single daily dose into twice daily dosing.

Conclusion: Relationship Between Methadone Pharmacodynamics and Pharmacokinetics

While randomized clinical trials have demonstrated a dose-dependent relationship between methadone maintenance dose and efficacy at reducing illicit opioid use, there is substantial inter-individuality in response to methadone, and, thus, a broad range of doses (e.g., from 30 – 150 mg, p.o.) can be used effectively to treat opioid dependence. The clinical observation of this wide variation in response has prompted numerous studies attempting to relate patient comfort and/or clinical response to circulating concentrations of methadone with the supposition that the inter-individual variability was related to individual pharmacokinetic differences. While past recommendations have suggested a therapeutic window for methadone maintenance between 100 - 400 ng/mL in plasma, studies have failed to demonstrate convincing evidence that clinical response to methadone is directly predicted by d,l methadone concentrations or the individual enantiomers (i.e., the concentration-effect relationship) or even methadone dose[20;205] (and this has been similarly difficult to model with regard to methadone analgesia).[206] Thus, therapeutic monitoring of plasma drug concentrations is not generally recommended.

Table 2.1. Development of steady-state drug levels. Assuming methadone terminal half-life is 24 hours and once daily dosing at the same dose level it takes 5 days to reach 93% of steady-state levels. Each subsequent day after the initial dose is represented in columns and rows. The percentage of each day's dose remaining in the body is in shown across columns. Cumulative dose levels are represented in rows.

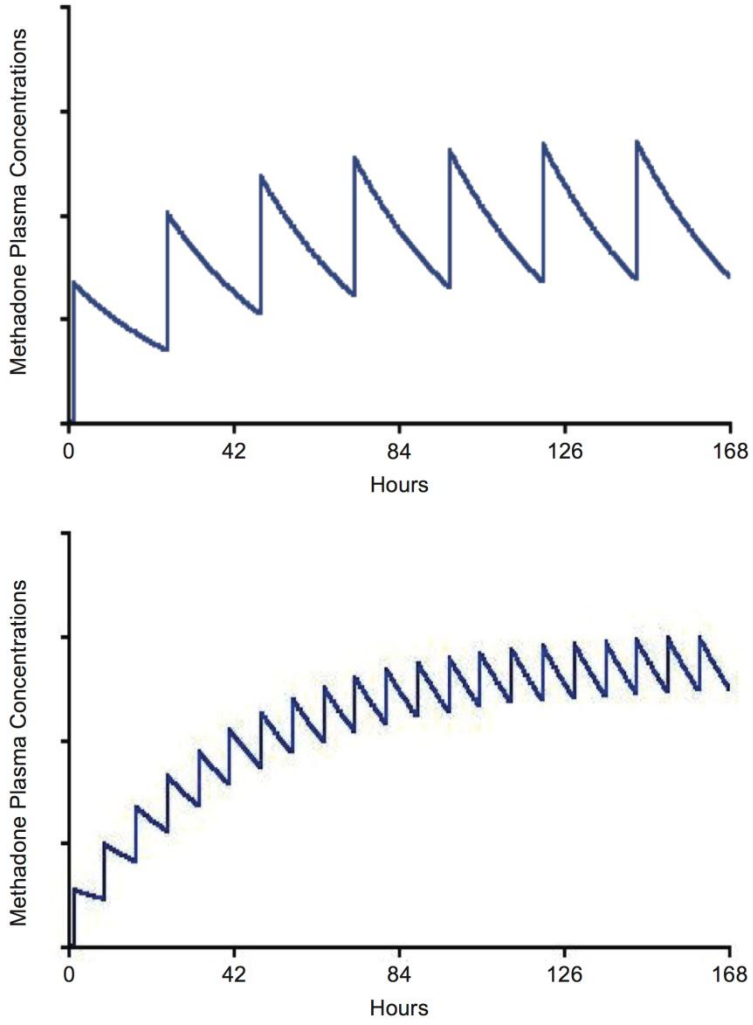
	Percent of Day's Dose Remaining					**Total**
Day	**2**	**3**	**4**	**5**	**6**	
2	**50**					**50**
3	**25**	**50**				**75**
4	**12.5**	**25**	**50**			**87.5**
5	**6.25**	**12.5**	**25**	**50**		**93.75**
6	**3.125**	**6.25**	**12.5**	**25**	**50**	**96.875**

Table 2.2. Common drug interactions with methadone. The clinical effect of a drug interaction is highly variable and, therefore, any methadone dose adjustment should be based on clinical response rather than preemptive change.

Interaction Effect	Drug	Effect	Clinical decision-making
Receptor antagonism/partial-agonism	Naloxone Naltrexone Nalmefene Buprenorphine Nalbuphine Nalorphine Butorphanol Pentazocine	Precipitation of opiate withdrawal, recrudescence of pain	Do not use in methadone patient unless specifically reversing acute life-threatening opiate overdose
Common Inhibitors	Ketoconazole Fluconazole Itraconazole Voriconizole	Inhibit methadone metabolism	Observe for clinical signs of opiate toxicity and adjust methadone dose accordingly
Common inducers	Rifampin Phenytoin Ritonavir boosted anti-retrovirals Nevirapine Efavirenz	Increase methadone metabolism	Observe for clinical signs of withdrawal and adjust methadone dose accordingly through increased or split dosing
Synergism	Benzodiazepines Barbiturates Tricyclic	Increase sedation Increase	Observe for signs of toxicity and adjust dose accordingly

	antidepressants Ethanol (other opiates prior to methadone cross-tolerance)	intoxication Decrease respiratory effort	Cautious dose increases when needed with frequent reassessment

Figure 2.1. Upper Panel: Plasma levels of methadone after initiation of daily oral dosing represented by monoexponential model. Lower Panel: Plasma levels of methadone following initiation of every 8-hour oral dosing. Total dose over 24 hours is the same in A and B.



Chapter 3

Superior methadone treatment outcome in Hmong compared to non-Hmong patients*

The Hmong are a distinct ethnic group from Laos. Little is known about how opiate addicted Hmong respond to methadone maintenance treatment. Therefore, Hmong attending an urban methadone maintenance program in Minneapolis, Minnesota were matched by gender and date of admission with non-Hmong attending the same program and both groups were evaluated for 1-year treatment retention, stabilization dose of methadone, and urine drug screen results. Hmong had greater 1-year treatment retention (79.8%) than non-Hmong (63.5%; $p<0.01$). Methadone dose was significantly associated with retention ($p=0.005$). Hmong required lower doses of methadone for stabilization (mean 49.0 mg versus 77.1 mg; $p<0.0001$). For both groups, positive urine drug screens were associated with stopping treatment. Determining the psychosocial and pharmacogenetic factors contributing to superior methadone treatment outcome in Hmong may provide further insight into opiate addiction and its treatment.

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Gavin Bart, MD[1], Qi Wang, PhD[2], James S. Hodges, PhD[2,3], Chris Nolan, Gregory Carlson, BA[1*]

1. Department of Medicine, Hennepin County Medical Center/Hennepin Faculty Associates, Minneapolis, MN 55415 USA
2. Biostatistical Design and Analysis Center, University of Minnesota, Minneapolis, MN 55455 USA
3. Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN 55455 USA

Corresponding author and reprint requests:
Gavin Bart, MD
Department of Medicine G-5
701 Park Avenue
Minneapolis, MN 55415
USA
Phone 612-873-4051
Fax 612-904-4299
Email bartx005@umn.edu

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1. Introduction

The Hmong are an ethnic minority from the mountains of Laos. They have been linked historically and linguistically to southern China but emigrated to Laos in the mid-18th century. The Hmong are a clan-based agrarian society and are known for their centuries old practice of opium cultivation [207]. Most traditional Hmong households in Laos have been involved in opium production and opium has played an important therapeutic role in the practice of Hmong medicine. While contact with opium through cultivation and/or traditional medical practice is widespread for Laotian Hmong, the number of Hmong who have used opium is unknown. Estimates from the 1960s and 1970s indicate that 8-12% of Hmong opium farmers were addicted to opium [208]. With intensive opium eradication strategies this number has decreased, with current prevalence of opium addiction in Laos estimated at 1% with household prevalence reaching 15% in some villages [209]. Current Hmong-specific prevalences are unknown.

Formalized treatment outcome research for opium addicted Hmong in Laos has been limited by low sample sizes and non-uniformity of treatment approaches (e.g., herbal remedies, detoxification, Buddhist monastery, "reeducation" camps) [210;211]. Estimates indicate a poor long-term treatment outcome for abstinence-based treatment, with 80-100% of patients returning to opium use after discharge [210-213]. Pharmacotherapy for opiate addiction in Laos is limited and mostly consists of detoxification rather than maintenance. Therefore, evaluation of treatment outcome in Hmong may best be

conducted in settings with a more established treatment infrastructure such as the United States.

Following the end of the Vietnam War, communist persecution of Hmong in Laos became untenable and an exodus began. Since the mid-1970's more than 180,000 Hmong have arrived in the United States with major concentrations in Minnesota, California, and Wisconsin. Among these immigrants, an estimated 2-5% are addicted to opium [214]. The Twin Cities of Minneapolis and St. Paul are home to the largest urban Hmong population in the world. Of the 60,000 Minnesotan Hmong, more than 200 are currently enrolled in methadone maintenance treatment for opium addiction.

Since 1964, methadone maintenance has become the standard of treatment for opiate addiction [83]. Methadone's long-acting properties and full mu-opioid agonism reduce many pharmacologically and behaviorally reinforcing effects of short-acting opiates. This results in general improvements in illicit drug use, criminality, and quality of life [84]. One of the best predictors of achieving these positive clinical outcomes is retention in treatment [215]. In the multisite Treatment Outcome Prospective Study (TOPS), the average 1-year treatment retention in methadone maintenance was 34% [16]. The more recent Drug Abuse Treatment Outcome Study (DATOS) confirmed the variability in treatment retention (15%-76%) noted in TOPS, but indicates that with wider adoption of evidence-based treatment standards, treatment has improved with an average of 50% of all patients retained for 1-year [17]. Treatment response to methadone is also dose

dependent, with patients taking 60 mg – 120 mg of methadone daily having better treatment outcome than those taking less than 60 mg daily [18;83;85;86;216]. In addition to methadone dose, other positive predictors of treatment retention include age older than 35 years, lower frequency of daily drug injections at admission, counseling session attendance, rapport with counselor, and desire for help [17;89;90]. Studies evaluating ethnicity as a predictor of treatment outcome in methadone patients have not found this to be a significant influence although Asian, let alone Hmong, populations were not specifically included in the analyses [89;217].

Because methadone treatment data on Hmong are lacking we conducted the current retrospective chart-review study to evaluate treatment outcome (measured as 1-year retention in treatment and urine drug screen results) and dose requirement in Hmong compared to non-Hmong attending a single urban methadone maintenance program.

2. Methods

We conducted a retrospective chart review to compare 1-year retention in treatment, stabilization dose of methadone, and urine drug screen results between Hmong and non-Hmong patients enrolled in the Hennepin Faculty Associates (HFA) Addiction Medicine Program. This study was approved by the Human Subjects Research Committee of the Hennepin County Medical Center and, as a chart review study, was exempted from consent requirements.

In June 1994, the Hennepin Faculty Associates Addiction Medicine Program opened a methadone maintenance clinic to serve the needs of the opiate-addicted population of the greater Twin Cities community. Based in the Department of Medicine of the Hennepin County Medical Center in downtown Minneapolis, the HFA program is an academically affiliated non-profit clinic serving as a safety-net resource.

All admissions from clinic inception through March 31, 2005 were reviewed (n=1411). Patients who transferred from other methadone programs were excluded; otherwise all patients receiving at least one dose of methadone were eligible for inclusion. In instances where a subject had multiple admissions to the HFA program, the earliest admission was chosen for review. To ensure that the two groups had the same fraction of males and were treated contemporaneously, each Hmong patient (n=104) was matched with a non-Hmong patient (n=104) based on gender and date of admission (in most cases matched

admission dates were no more than one week apart). However, age matching could not be performed as the Hmong tended to be older than the non-Hmong patients. Therefore, analyses (described below) did not use the matched pairs. Charts were reviewed for date of admission, date of and reason for discharge (or retention through April 1, 2006 if they were still in the program on that date), stabilization dose of methadone (defined as the dose received on the majority of days during the first year or the highest dose achieved for patients retained less than one year), and urine drug screen results. Specimens from random and clinically indicated urine drug screens were analyzed by a commercial laboratory (Hennepin County Medical Center or Hennepin Faculty Associates) using a standard commercial immunoassay kit capable of detecting the presence of methadone, methadone metabolites, amphetamine, cocaine, benzoylecgonine, barbiturates, benzodiazepines, opiates, and alcohol. Urine drug screen results were categorized as negative, positive for opiates, positive for non-opiate drugs, and positive for both opiates and non-opiates. Cannabinoids were not routinely tested for and therefore were not included in the analysis.

Hmong and non-Hmong groups were compared according to age and stabilization dose using a two-sample t-test. Urine drug screen data were aggregated by quarter for each person. Differences between Hmong and non-Hmong and changes over the 4 quarters of follow-up were tested, separately for opiates and non-opiate drugs, using generalized linear mixed models (GLMM), specifically a logistic regression conditional on person and a person-specific random effect (a 4-variate normal with AR(1) correlation structure).

For each person and quarter, the outcome was a pair of numbers, that person's total number of urine tests and total number of positive tests in that quarter. A variant analysis added subject age as a continuous covariate. An alternative analysis weighted each person in each quarter to account for subjects who were no longer in treatment, using weights proportional to the reciprocal of the probability of having data in that quarter (estimated using logistic regression, depending on group, age, sex, stable dose and, for non-Hmong, ethnicity). Results of the weighted analysis were identical to those presented here, to the table's accuracy. Other alternative analyses used generalized estimating equations (GEE) with an AR(1) working correlation, both weighted and unweighted. These results are very similar to those presented here and are not shown. Treatment retention of Hmong and non-Hmong at each follow-up time were estimated using the Kaplan-Meier procedure and compared using the log-rank test. The association of retention with urine drug screen results was analyzed using Cox regression with time-varying covariates describing fraction of positive tests for opiates and for non-opiate drugs. For each person, these were the logits of their estimated probabilities of testing positive in each quarter, from the GLMM analysis. For all retention analyses, patients transferring to another methadone program were censored; all other patients not retained for 365 days were considered to have stopped treatment.

3. Results

3.1 Patient characteristics

Non-Hmong patients were matched to a Hmong patient by gender and date of admission. The Hmong were significantly older in age than non-Hmong (Table 3.1), making age matching difficult.

Table 3.1

	Hmong (n=104)	Non-Hmong (n=104)	Significance
Male (%)	78 (75%)	78 (75%)	Identical by matching
Mean age in years (range, SD)	49.6 (24-88, 14.0)	41.0 (20-58, 8.3)	P<0.0001
Ethnicity (%)	Hmong 104 (100%)	Caucasian 52 (50%) African American 40 (38%) Native American 4 (4%) Hispanic White 3 (3%) Hispanic Black 2 (2%) Asian 2 (2%) Mixed 1 (1%)	

The mean (SD) stabilization dose of methadone for all patients was 63 (25.8) mg. The Hmong patients were stabilized on a significantly lower methadone dose than non-

Hmong patients: 49.0 (17.4) mg versus 77.1 (25.1) mg (p<0.0001). The groups did not differ in methadone dose when adjusted for body weight (Hmong average 0.95 mg/kg, SD 0.43; non-Hmong average 0.98 mg/kg, SD 0.37).

3.2 Urine drug screen

Table 3.2 shows comparisons of estimated fraction of positive urine drug screens in each quarter of treatment. The chance of an opiate-positive drug screen decreased after the first quarter of treatment (main effect of quarters, X^2=24.50 on 3 df, p<0.0001), but Hmong did not differ significantly from non-Hmong (main effect of ethnicity X^2=0.73 on 1 df, p=0.39). The Hmong group's fraction of positive tests started somewhat higher in the first quarter of treatment (0.37 vs. 0.26) but the two groups had similar fractions of positive tests thereafter; the two groups' time paths did not differ significantly (interaction of ethnicity and quarter X^2=1.51 on 3 df, p=0.21). Comparing Hmong vs. non-Hmong in the first quarter alone gave P = 0.046, but this is not significant after adjusting the significance threshold for multiple comparisons (one comparison for each of the four quarters). Regarding non-opiate drug screens, Hmong had far fewer positive screens in all quarters (main effect of ethnicity X^2=87.34, p<0.0001 overall and in each quarter individually), but there was no effect of time in treatment (X^2=0.65, p=0.58 and X^2=0.49, p=0.69, for the main effect of quarters and the interaction, respectively). In analyses adjusting for age, age was not significant (P > 0.14 for both opiates and non-opiate drugs)

and tests comparing ethnicities and quarters of treatment were nearly unchanged after adjusting for age (data not shown).

Table 3.2: Urine test results: Fractions using opiates and other drugs, Hmong and non-Hmong, by quarter

		Fraction using opiates				Fraction using other drugs			
Quarter	Ethnicity	Estimate	P*	Lower 95% CL	Upper 95% CL	Estimate	P*	Lower 95% CL	Upper 95% CL
1	Hmong	0.37	0.046	0.29	0.46	0.01	<0.0001	0.01	0.02
	Non-Hmong	0.26		0.19	0.34	0.24		0.16	0.34
2	Hmong	0.18	0.64	0.13	0.24	0.01	<0.0001	0.01	0.03
	Non-Hmong	0.16		0.11	0.23	0.30		0.20	0.42
3	Hmong	0.13	0.85	0.09	0.18	0.01	<0.0001	0.01	0.03
	Non-Hmong	0.13		0.09	0.19	0.31		0.20	0.45
4	Hmong	0.15	0.67	0.10	0.22	0.01	<0.0001	0.00	0.02
	Non-Hmong	0.13		0.08	0.19	0.34		0.22	0.49

*Comparing Hmong vs. non-Hmong in each quarter

3.3 1-year retention in treatment

Hmong had significantly greater 1-year retention than non-Hmong, respectively 79.8% (95% CI 72.1%-87.5%) versus 63.5% (95% CI 54.1%-72.8%) (p=0.006; see Figure 3.1). Among those who did leave treatment before 1 year, reasons for leaving did not differ between Hmong and non-Hmong: loss to follow-up (38% versus 40%), discharge for behavioral reasons (24% versus 28%), transfer to another clinic (10% versus 14%),

patient request for taper (14% versus 2%), incarceration (5% versus 9%), and other reasons (9% vs. 7%).

Considering the influence of ongoing drug use on retention, positive drug screens were significantly associated with risk of stopping treatment for opiate positive results (hazard ratio for stopping treatment 1.44, 95% CI 1.22-1.70, $p<0.0001$) and for non-opiate positive results (hazard ratio for stopping treatment 1.32, 95% CI 1.16-1.49, $p<0.0001$). After adjusting for the effect of positive drug screens, Hmong patients no longer differed significantly from non-Hmong patients (hazard ratio for stopping treatment for Hmong vs. non-Hmong 1.31, 95% CI 0.63-2.71, $p=0.47$).

Finally, there was a significant association between methadone dose and retention, with hazard ratio for stopping treatment 0.85 for each 10 mg increase in methadone dose (95% CI 0.76-0.95; $P = 0.005$). After adjusting for methadone dose, Hmong were still significantly less likely than non-Hmong to stop treatment (hazard ratio for stopping treatment 0.30, 95% CI 0.16-0.55; $p<0.0001$).

4. Discussion

This is the first comparison of methadone-maintained Hmong and non-Hmong populations in the literature and one of the few reports from the United States that includes an Asian methadone population. We found a significant difference in treatment retention between Hmong and non-Hmong patients enrolled in a single methadone maintenance program. Furthermore, while methadone dose was generally associated with clinical retention, the Hmong had significantly greater retention despite requiring significantly lower doses of methadone.

Retention in treatment may be one of the strongest predictors of long term outcome in methadone maintenance [215]. Identification of factors associated with clinical retention may help in tailoring treatment approaches to reduce the risk of leaving treatment. For example, psychiatric comorbidity has predicted poor outcome in some studies and the provision of on-site psychotherapy or treatment for depression has improved treatment outcome [218-220]. Using evidence-based dosing regimens rather than set ceiling doses has also resulted in improved retention [84;216]. Factors such as age, gender, and ethnicity which have been weakly associated with retention are not modifiable but do inform us of the importance of tailoring treatment approaches to specific populations [221]. Besides patient factors, other programmatic and community variables impact treatment outcome [92].

In this study, ongoing drug use predicted stopping treatment, whether the drug used was an opiate or a non-opiate. Several studies have found that ongoing cocaine, benzodiazepine, and alcohol use predict stopping treatment [222-224]. We did not include marijuana use in our analysis, but a retrospective meta-analysis by Epstein and Preston did not find positive urine screens for cannabinoids to predict clinical retention [225].

While we found both opiate and non-opiate drug use predicted stopping treatment, the probability of non-opiate drug use did not appear to change through the first year of treatment. It could be argued that the persistence of high non-opiate drug use in the test samples is due to selection bias, so that patients with positive tests are more likely to receive future tests. However, in this study the number of urine tests in a quarter was not associated with the fraction of tests that were positive for non-opiate drugs, either for all subjects combined ($p = 0.16$) or for Hmong and non-Hmong considered separately ($p = 0.10$ and 0.39, respectively). This indicates that it may not be the ongoing drug use itself that predicts stopping treatment but rather that ongoing drug use is a surrogate marker for unidentified destabilizing factors (e.g., medical psychiatric, legal, psychosocial) that also predict stopping treatment [226]. It is unknown whether addressing these factors alone can improve treatment retention independent of any effect on ongoing drug use. Elucidating this may help appropriately orient therapeutic priorities towards an emphasis on the contributing psychosocial factors of which ongoing drug use is but a marker, rather than on the drug use itself.

After adjusting for urine drug screen results, Hmong was no longer a predictor of treatment outcome. Their extended retention in treatment, therefore, may be a reflection of lower frequency of ongoing drug use, especially non-opiate drugs. We do not have medical or psychosocial data that could help further predict treatment outcome differences between ethnicities. In a previous report of the first forty Hmong patients enrolled in our methadone program, however, we found a high level of baseline psychiatric symptomatology: Hamilton Depression Scale (HAM-D) mean score 28.6 (range 17-44), Hamilton Anxiety Scale mean score 26.19 (range 16-41), Zung Depression Scale mean score 40.5 (range 22-65), and a Global Assessment of Functioning (GAF) mean of 56.6 (range 40-70) [227]. These levels of symptomatology are consistent with or more severe than previous reports in non-Hmong populations entering methadone maintenance [228-230] and, therefore, it is less likely that our observed difference in treatment outcome is related to ethnic differences in psychosocial distress. Finally, the Hmong and non-Hmong patients were all cared for within a single clinical setting, thereby reducing potential differences in treatment approach that could affect outcome.

A relatively novel finding is that the Hmong required lower doses of methadone than the non-Hmong. Retention in methadone maintenance is generally dose related, as was found here; however, in several studies retention is greatest for doses above 60 mg daily [84-86;216]. Our nearly 80% 1-year retention for the Hmong at an average of 49 mg daily is remarkable. While the Hmong do have lower body mass than non-Hmong and the milligram per kilogram methadone dose was similar in the two groups, there is no clinical

or pharmacokinetic precedent to use weight-based rather than absolute milligram amount when interpreting dose-outcome data.

Variants in genes related to drug metabolism or effect may account for the superior outcome on lower doses in Hmong. This is not unprecedented in that for nicotine dependence, East Asians are more likely to have a genetic variant resulting in reduced nicotine metabolism that correlates to their lower level of cigarette consumption [231;232]. Additionally, those with this variant are more likely to respond to nicotine replacement therapy [233]. Whether a similar pharmacogenetic effect exists for genes involved in methadone pharmacokinetics is unknown and worth pursuing given our results.

There are several limitations to this study. First, this is a retrospective chart review rather than a prospective study. We have attempted to control for a cohort effect by matching patients for date of admission. We were unable to control frequency of urine drug screening but we reduced its effect to some extent by aggregating tests by quarters. Second, there are likely several psychosocial and clinical process factors that contribute to treatment outcome that could not be assessed in this retrospective study. Future studies would benefit from considering other patient characteristics such as socioeconomic status, education, medical and psychiatric comorbidity, Addiction Severity Index composite scores, treatment motivation, and treatment satisfaction. Third, all of the Hmong were opiate dependent from smoking opium whereas none of the non-Hmong were opium smokers. The extent to which the difference in type of opiate used and route

of administration could have influenced our findings is unknown. Finally, there may be distinct cultural differences that influence motivation for treatment and family/cultural supports for recovery that were not measured. Prior reports of culturally oriented detoxification or abstinence-based approaches to opium addiction in Hmong found results to be as poor as those of non-Hmong [210], thereby reducing the potential that cultural differences were the sole or major mediator of our findings.

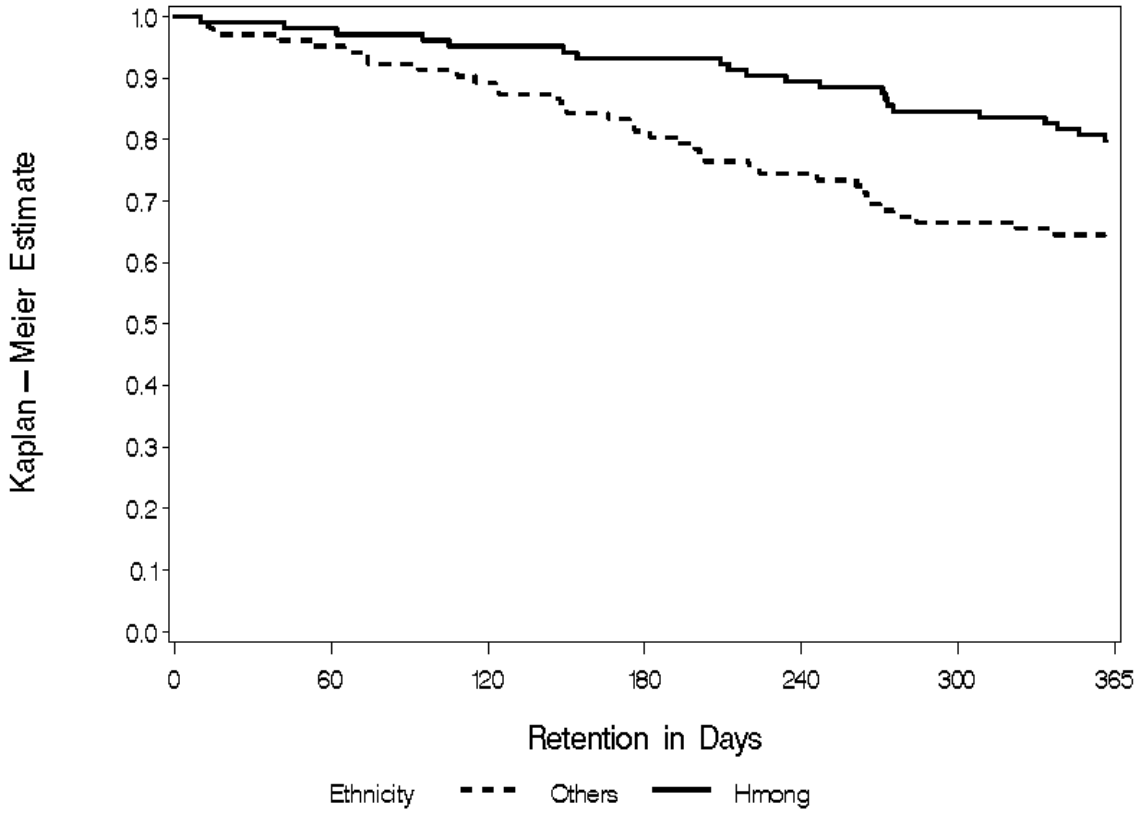
Conclusion

We have identified a significant difference in methadone treatment retention despite lower dose requirements in Hmong versus non-Hmong patients attending a single clinical site. Identification of factors that appear to make methadone more effective at low doses in Hmong compared to non-Hmong may ultimately lead to more generalizable approaches to dose optimization and treatment improvement.

Figure legend

3.1 Kaplan-Meier estimator curve for 1-year retention in treatment. Hmong retention is significantly greater than non-Hmong retention (log-rank test $X^2_{df1}=7.56$, $p=0.006$).

Figure 3.1


Kaplan – Meier Estimate
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
60
120
180
240
300
365
Retention in Days
Ethnicity
Others
Hmong

Chapter 4

Altered methadone pharmacokinetics in a South East Asian population requiring low doses during maintenance therapy: A population pharmacokinetic study

The misuse of and dependence on opiates is a growing problem within the United States and worldwide.[35;36] It is associated with significant morbidity and mortality through overdose and infectious diseases transmitted by injection drug use.[34] For more than 40 years, the long-acting synthetic opioid methadone has played a central role in the treatment of opiate dependence.[234]

Despite its effectiveness in the treatment of opiate dependence, methadone is often difficult to use due to its highly variable pharmacokinetics.[196] This difficulty has recently become apparent in the significant rise in methadone associated mortality seen primarily when prescribed for pain by physicians who likely are less familiar with this variability than physicians within highly regulated methadone maintenance settings.[78] In fact, training in safe prescribing strategies for methadone has resulted in reduced mortality.[79] While this education has saved lives, we are still faced with a medication which has highly variable pharmacokinetics making it difficult to devise standard dosing regimens informed by therapeutic drug monitoring.

The influence of methadone pharmacokinetics on treatment outcome has been explored. Steady-state trough plasma methadone levels are dose dependent[19] and are inversely correlated to opiate withdrawal symptoms.[20;21] Methadone treatment outcome has also been correlated to trough, 2 hour, and 8 hour methadone levels, but the data are limited in scope. In one study, treatment outcome was determined in a cross-sectional sample and only in-treatment factors such as symptom relief and ongoing drug use could be assessed rather than retention in treatment.[22] In a second, prospective study, methadone levels were only measured during the initial 25-days of therapy and did correlate with reduced opiate positive urine toxicology.[23] Unfortunately, clinical retention was not assessed and generalizability of this study is complicated by selection bias due to the required 4-week abstinence-based inpatient lead-in prior to initiating methadone.

The plasma methadone concentration peak to trough ratio has also been used to evaluate the effectiveness of methadone in relieving withdrawal symptoms.[24;25] Prospective evaluation of this ratio, however, has not been explored in terms of treatment outcome. A cross-sectional study of methadone peak:trough did find that subjects with ongoing illicit substance use (a measure of treatment response) had higher ratios compared to those with less illicit drug use.[26] While peak:trough has correlated to the objective and subjective effects of methadone[25], there was no difference in peak:trough when a patient population complaining of early withdrawal symptoms (non-holders) was compared to patients without such a complaint (holders).[25] Interestingly, these studies found that the maximum rate of concentration decline following peak was greater in the non-holders and that this

rate was positively correlated to composite total mood disturbance scores of the Profile of Mood States (POMS).[25;27] One study also evaluated methadone area under the curve (AUC) but did not find it to significantly differ between non-holders and holders.[25] This raises an interesting point in that if there is no difference in peak, trough, peak:trough, or AUC in holders and non-holders but there is a significant difference in concentration decline, then there may be a difference in distribution and/or disposition between methadone's active R- and inactive S- enantiomers that analysis of total methadone levels may have missed.

While methadone is a racemic mixture, most pharmacokinetic studies have not investigated the enantiomers separately. Since R-methadone provides the main therapeutic effect, it may be important to evaluate the pharmacokinetics of each enantiomer separately. Evaluations of the separate enantiomers have found little difference between enantiomers in area under the plasma concentration curve (AUC_τ) at steady-state.[235-237] Both enantiomers maintain a significant dose and plasma AUC_τ relationship.[235] Interestingly, R-methadone has a longer terminal half-life.[238;239] Therefore, therapeutic monitoring of racemic methadone, which assumes a constant 1:1 ratio of R- and S-methadone may fail to detect important differences in enantiomer-specific kinetics. For example, Mitchell et al. evaluated methadone AUC for each enantiomer and found that the ratio of S-methadone AUC to R-methadone AUC correlated to the pharmacodynamic response to methadone (total mood disturbance score of the POMS) in a methadone maintained population.[240]

These approaches are limited by their use of single or sparse plasma levels without linking these to pharmacokinetic parameters such as clearance (CL). By linking these to pharmacokinetic parameters, the variability due to pharmacodynamic influences such as tolerance on dosing can be minimized as these would be unlikely to influence pharmacokinetics.

Population pharmacokinetics (POPPK) is a useful approach towards quantifying drug exposure-clinical response relationships.[241] The POPPK approach is extensively used, valid, and in some instances, preferred to the more intensive traditional pharmacokinetic studies.[242] POPPK studies the sources and correlates of variability in drug concentrations and pharmacokinetics within and between individuals and populations. Unlike traditional pharmacokinetic studies, which gather dense data that assess individual variability in drug kinetics, POPPK can use sparse data to model measures of drug exposure and identify factors (e.g., ethnicity, gender, age, weight) that influence variability in drug concentrations across populations.[243] Several measures of exposure potentially can be used as either independent variables or as covariates to model exposure-response relationships. The area-under-the-concentration-time curve (AUC), pre-dose concentration (trough or C_{min}), maximum concentration (peak or C_{max}), steady state concentration (C_{ss}), and the time above a certain plasma drug concentration are typical examples.

POPPK allows identification of important determinants of pharmacokinetics in target populations, quantification of the variability in pharmacokinetic parameters, and enables testing of the relationship of these pharmacokinetic estimates to a suitable pharmacodynamic marker (e.g., for methadone maintenance, 1-year clinical retention and urine toxicology results).[241] Thus we can model not only the time-course of drug in the body but also use exposure metrics from these analyses to investigate exposure-response relationships. The sparse sampling of POPPK renders the method especially useful for populations in which intensive blood sampling is problematic, such as people with poor venous access (e.g., injection drug users), individuals with schedule conflicts preventing dense sampling, individuals in outpatient settings, and populations with cultural prohibitions on the volume of blood that can be drawn. This is of particular relevance to this study as the Hmong believe blood is imbued with vital power and are, therefore, reluctant to have the volumes required for dense sampling removed in a single day.[244]

Through sparse sampling, POPPK allows larger populations to be studied than with inpatient dense sampling studies. A few small n (range n = 30-59) POPPK studies of methadone exist.[191;239;245] A POPPK validation study, which compared traditional dense pharmacokinetic sampling with sparse modeling using only 2 time points within the same cohort, found POPPK clearance estimates to be highly accurate (1%) and precise (<4%) when compared to those measured by the traditional pharmacokinetics approach.[239] In addition, the POPPK approach has been validated (for racemic methadone as well as each

of the enantiomers) in a population that participated in both dense and sparse data sampling.[239]

While the POPPK approach has been validated for methadone maintained individuals there are no studies from large or diverse populations. In fact, over 90% of the subjects in POPPK studies of methadone were Caucasian and none of the studies were conducted within a United States population. With larger more diverse sample sizes variables that contribute to methadone pharmacokinetics (e.g., ethnicity) and treatment outcome may be identified. Based on our previous observations that methadone maintained Hmong are on a lower mean dose of methadone yet achieve greater rates of 1-year retention in treatment than do non-Hmong[246], we hypothesized that POPPK could detect decreased methadone clearance in Hmong compared to non-Hmong.

Methods

Methadone maintained patients enrolled in a single urban safety-net hospital outpatient addiction medicine clinic were invited to participate in this study via posted flier and word of mouth. Initially, patients were recruited into two separate studies: a cross-sectional study requiring patients to have been on methadone for at least two months without dose change during the previous five days and a prospective study that recruited patients during their first week on methadone and followed them at 1, 3, 6, and 12 months. The prospective study was closed to enrollment after the first 14 subjects due to

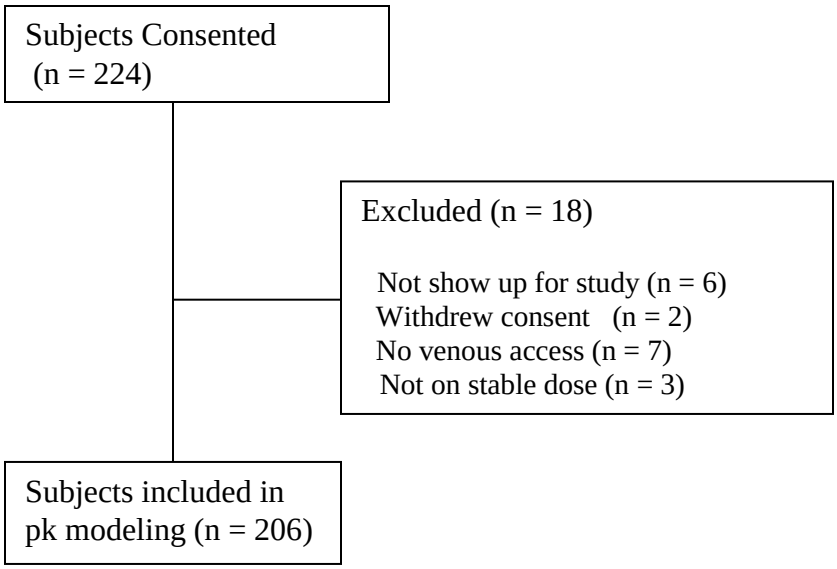
slow accrual and these subjects were followed as per protocol but their data were combined with the cross-sectional subjects in creating the population pharmacokinetic model.

As per Federal criteria, all subjects on methadone maintenance were at least 18 years of age and had met Diagnostic and Statistical Manual of Mental Disorders criteria for opiate dependence of at least one year duration prior to initiating methadone. Subjects were excluded if they were unable or unwilling to provide informed consent, had decompensated liver disease, were in the second or third trimester of pregnancy, or were taking medications known to alter methadone pharmacokinetics (e.g., phenytoin, rifampin, or highly active antiretroviral therapy for HIV). The study was approved by the Human Subjects Research Committee of the Hennepin County Medical Center and conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983). Because data included sensitive psychiatric and drug related information a Certificate of Confidentiality was obtained from the National Institutes of Health National Institute on Drug Abuse. The Hmong speaking population were not literate in English or written Hmong, however, consent forms were translated into Hmong and then read to patients by native Hmong speakers in the presence of a study coordinator who could answer questions about the protocol.

Most subjects recruited into the study were used in the data analysis (see Figure 4.1). Six subjects did not show up for their study date, two withdrew consent, seven had poor

venous access, and three were excluded because their methadone dose had changed within five days of study.

Figure 4.1



Procedures

After signing informed consent, patients had 10 ml of venous blood drawn into lithium heparin blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) approximately 22-24 hours after (trough) the previous day's dose of methadone and then again 2-4 hours after (peak) taking their daily methadone dose. With separate consent, two 10 ml venous blood samples (15% EDTA tubes, Tyco Healthcare Group LP, Mansfield, MA) were collected for DNA isolation and analysis. In a few instances when

venous access for one of these time points could not be obtained, a peak or trough sample was taken on a later date. Dates and times of methadone dosing were obtained from a real-time medication dispensing software system (Methasoft, Netalytics, Greer, SC) and dates and times of all blood samples were maintained in a Microsoft Access database. All doses of methadone were consumed under direct supervision on the day of study and more than 95% of the previous day doses were also directly observed (exceptions were for Sunday doses when patients were studied on a Monday).

Subject weight and height were collected on a standard clinical scale (Seca Model 700, Seca Corporation, Hanover, MD) for body mass index (BMI) calculation as weight in kilograms/height in meters^2. Subjects were asked to provide information such as ethnicity and a list of medications they were taking. Subjects also provided a urine specimen for drug testing and completed semi-structured interviews and questionnaires such as the Addiction Severity Index, Fifth Edition[247], Structured Clinical Interview for DSM-IV[248], and Symptom Checklist-90.[249] All interviews were conducted by a single trained bachelor level research coordinator, thus eliminating inter-rater variability. Interviews were not recorded for later fidelity audits, however. For Hmong subjects, interviews were conducted with the assistance of an interpreter knowledgeable in medical and drug use terminology.

Assays

Blood samples were placed on ice and, within 45 minutes of being drawn, were centrifuged at 2000 g for fifteen minutes at 4^{o} centigrade for plasma separation. Plasma was immediately stored in 2.0 mL Nunc Cryotubes (Thermo Fisher Scientific, Rochester, NY) at -80^{o} centigrade until analyzed. Plasma levels of each methadone enantiomer were determined using an LC-MS/MS protocol adapted from Foltz et al.[250] Briefly, 100 ul of human plasma was extracted with 400 ul of hexane at pH 11.0 (200 ul of 500 mM sodium bicarbonate). After 10 minutes of vigorous shaking the samples were centrifuged at 12500 rpm for 5 minutes. A 250 ul aliquot of the supernatant was collected. Supernatant was dried at ambient temperature under a stream of nitrogen for 5 minutes. Dried sample were reconstituted with 100 ul of mobile phase for LC-MS/MS.

LC-MS/MS was performed using a TSQ Quantum Classic LC-MS/MS (Thermo Scientific, Waltham, MA) with Agilent 1200 HPLC (Agilent, Santa Clara, CA) and a Chiral-AGP column (5 cm x 2.0 mm, 5 um particle size, Regis Technologies, Morton Grove, IL). Calibration and quality control using R- and S- methadone and their tritiated counterparts (Cerilliant, Round Rock, TX) revealed an assay lower level of quantitation of 2.75 ng/ml for R-methadone and 2.25 ng.ml for S-methadone and a linear range of detection measured between 2.75-687 ng/ml and 2.25-565 ng/ml, respectively. Although not tested beyond these ranges, Liang et al. found this methodology to be linear up to 1000 ng/ml for each enantiomer.[250] Between and within assay variability percent coefficient of variation were below 6% for both enantiomers. Assay variability and calibration curve data are presented in Appendix A.

Urine specimens collected on day of study were analyzed for amphetamine, benzodiazepine, barbiturates, cocaine, and opiates using a commercial immunoassay (EMIT, Beckman, Brea, CA). The presence of methadone metabolite (EDDP) in urine was also determined (CEIDA, Microgenics, Fremont, CA). All urine drug screening was performed on site in a CLIA and College of American Pathologists certified laboratory at the Hennepin Faculty Associates (Minneapolis, MN).

Genetic analysis

Whole blood drawn for genetic analysis was immediately shipped to the Rutgers Cell and DNA Repository for DNA extraction on an AutoPure LS automated DNA extractor using the Puragene Reagent System (GENTRA Systems, Qiagen, Valencia, CA). In short, RNase is added to the WBC lysis stage with isopropanol precipitation of the DNA and resuspension in 1X TE buffer (pH 8.0). This methodology routinely provides high molecular weight DNA of 20 kb or greater with an average yield of 30 μg/ml whole blood.

Purified DNA (mean concentration 116 ng/μl) was shipped to the University of Minnesota for sequencing of twenty-one single nucleotide polymorphisms across a number of genes whose products are implicated in methadone pharmacokinetics and/or

opiate dependence (see table 4.1). Primer design and DNA sequencing was performed using Sequnom's (San Diego, CA) iPLEX Gold reaction and MassARRAY System for MALDI-TOF (Matrix-assisted laser desorption ionization - time of flight) mass spectrometry based sequencing. Primer design and allele/genotype frequency tables are presented in Appendix B.

Table 4.1 Selected Polymorphisms for genetic analysis

Gene	SNP	Functional Effect	Nucleotide Change	Amino Acid Change	Citation(s)
CYP3A4	rs2740574	↓ activity	-392A>G	NA	251‡
	rs28371759	↑ activity	20070T>C	L293P	252
	rs4986909	↓ activity	22026C>T	P416L	253
CYP2B6	rs3211371	↑ activity	25505C>T	R487C	254‡
	rs3745274	↓ activity	516G>T	Q172H	255‡
	rs2279343	↑ activity	785A>G	K262R	256
	rs8192709	unknown	64C>T	C22R	257
CYP2D6	rs1065852	splice defect	100C>T	P34S	258
	rs5030656	↓ activity	2615-2617delAAG	K281del	259†
CYP2C19	rs3758581	↑ activity	80161A>G	I331V	260
ABCB1	rs1045642	↑expression	3435C>T	NA	251‡
	rs6949448	unknown	86979750A>G	NA	261‡
	rs2235067	unknown	86987858G>A	NA	261‡
	rs2032582	↓ activity	2677G>A/T	S893A/T	261‡
	rs1922242	unknown	87011603A>T	NA	261‡
	rs1128503	↑ activity	1236C>T	NA	262
	rs2520464	unknown	87039022 A>G	NA	261‡
	rs3789243	unknown	87058822C>T	NA	261‡
	rs9282564	↑ activity	61A>G	N21D	251‡
CYP1A2	rs762551	↑ inducibility	-163C>A	NA	263

‡implicated in methadone dose, level, or effect

Population Pharmacokinetic analysis

For determination of population pharmacokinetics, a nonlinear mixed-effects modeling (NONMEM) approach was used with a sparse sampling design including two time points, roughly 2-4 hours and 22-24 hours after once daily methadone dosing. A one compartment model with first order absorption and elimination (ADVAN2 TRANS2) was used with the NONMEM 7.2.0 and PDx-Pop 5.0 (both Icon Development Solutions, Ellicott City, MD) software packages. Based on existing literature, the absorption constant Ka was fixed at 1.5,[264] thus the model estimated CL/F, V/F, and F. The interindividual variability (IIV) and residual unexplained variability (RUV) were modeled using proportional error models for CL/F, V/F, and F. The model estimation of IIV for V/F did not converge, thus it was fixed assuming a normal distribution with a mean of 0. A first-order conditional estimation with interaction (FOCE-I) was used for all analyses.

A base pharmacokinetic model was developed without covariate effects. A stepwise generalized additive model (GAM) was evaluated using Xpose 4.3.2 (xpose4_4.3.2) (http://www.xpose.sourceforge.net) running within R version 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria) to identify candidate covariate effects was used. Covariates considered in the GAM analysis were chosen based on biological plausibility and included gender, ethnicity (categorical as Caucasian, African American,

American Indian, Hmong, Hispanic), age (scaled to 40 years), weight (scaled to 70 kg), body mass index (scaled to 25 kg/m^2), prospective study participation with repeat measures, Global Severity Index score of the SCL-90, urine toxicology result, and genotype of twenty-one different single nucleotide polymorphisms across the *ABCB1*, *CYP3A4*, *CYP2B6*, *CYP2D6*, *CYP2C19*, and *CYP1A2* genes (see Table 4.1). Because the use of medications known to interact with methadone was an exclusion criteria and inspection of enrolled subject medication lists did not include any specific classes of medication that would plausibly alter methadone pharmacokinetics, co-medication was not included as a covariate.

The GAM regressed each covariate separately and in combination on CL and F using both linear and nonlinear models. The model with the lowest Akaike information criteria (AIC) was considered the full model. Covariates identified through GAM were then incorporated into a mixed-effects model (full model). Covariates in the full model were next deleted stepwise using NONMEM. In each step each covariate was left out of the model one at a time. Any covariate whose deletion from the model resulted in a change in objective function value (OFV) greater than 6.63 (corresponding to a $p < 0.01$) was retained in the model. The reduced final model was defined when no additional covariates could be deleted from the model.

Model building was performed separately for each enantiomer. Results from these separate models are presented below.

Model qualification

Final model parameter estimates and their 95% confidence intervals were qualified by reestimation using a nonparametric bootstrap approach. NONMEM was used to generate 1000 bootstrap datasets. Parameter estimates were rank ordered and the values at the 2.5% and 97.5% of the rank order were used as the lower and upper bounds of the bootstrap 95% confidence interval.

Results

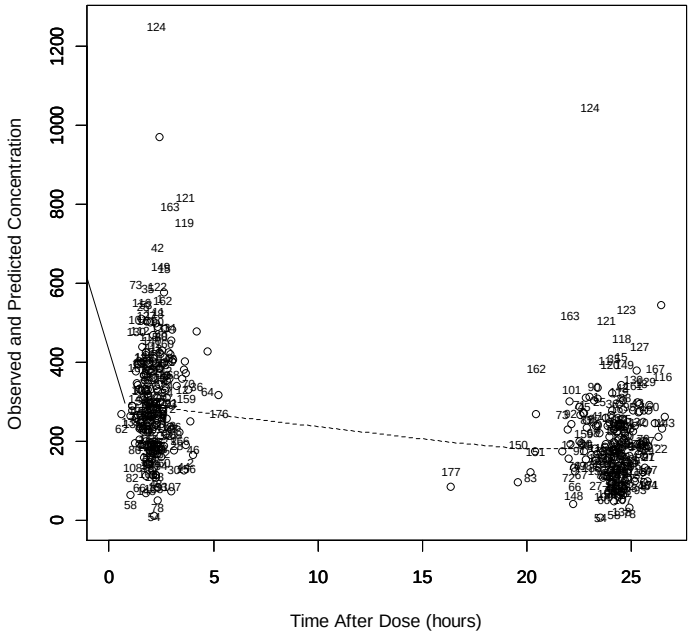
Data from 206 methadone maintained subjects and 441 methadone plasma concentrations were included in this study. Baseline characteristics between Hmong and non-Hmong subjects are presented in Table 4.2. Plasma methadone concentrations were generally measured 2-4 hours and 24-25 hours after methadone dosing (Figure 4.2).

Table 4.2

	Hmong (n=76)	**Non-Hmong (n=130)**	**P value**
Male, N (%)	54 (71.1)	72 (55.4)	$< 0.05^{A}$
Age, years (SD)	56.6 (11.6)	41.7(10.7)	$< 0.001^{B}$
Methadone dose, mg (SD)	54.0 (19)	82.4 (31.2)	$< 0.001^{B}$
Weight, kg (SD)	61.9 (12.6)	88.9 (20.6)	$< 0.001^{B}$
BMI, kg/m^2 (SD)	25.7 (4.9)	30.5 (6.6)	$< 0.001^{B}$
Time on methadone, yrs (SD)	6.0 (3.9)	1.9 (2.8)	$< 0.001^{B}$

A χ^2 B t-test

Figure 4.2 Time after dose

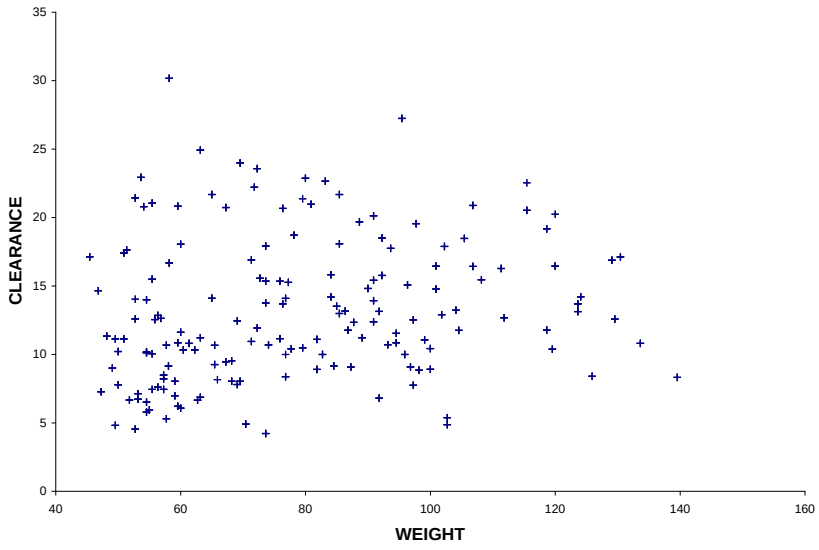


R-Methadone

Base model parameter estimates for CL/F and V/F were 8.6 L/h and 345 L, respectively. R-methadone half-life, therefore, was 27.8 hours. Following forward inclusion of covariates using GAM, informative covariates on CL/F included age, BMI, ethnicity, and the *ABCB1* 2677 G>A/T and *CYP2D6* 100C>T polymorphisms. The only covariate on V/F was ethnicity. Covariates on F included ethnicity, the *CYP1A2* -163 C>A polymorphism, and the *ABCB1* 87058822 C>T polymorphism.

After backward elimination in NONMEM, the lowest objective function value was obtained with covariates on CL/F of age, BMI, and the *ABCB1* 2677 GG genotype. The remaining covariates on F were Hmong ethnicity and the *CYP1A2* -163 CC genotype. There were no covariates on V/F. Due to concern that Hmong race may be collinear with weight and BMI and thus bias CL estimates the following diagnostic plot was generated and shows that there is no correlation between weight and clearance in the study population (Figure 4.3). There was also no difference in the milligram per kilogram dose requirement between Hmong and non-Hmong, 0.89 mg/kg (SD 0.32) and 0.98 mg/kg (SD 0.45), p = 0.1, respectively.

Figure 4.3 Clearance by weight



The model estimation for variance of V/F did not converge, thus it was fixed at a mean of 0 in the final model.

Model Qualification

From 1000 bootstrap simulations, 98% had successful minimization and were included in the analysis (see table 4.3 for results). Mean parameter estimates from the bootstrap were comparable to estimates from NONMEM and varied by less than 10% from those in the final model.

Visual Predictive Check

The 10^{th}, 50^{th} (median), and 90^{th} percentile R-methadone concentrations obtained from bootstrap simulation are shown in Figure 4.5. The results indicate that greater than 90% of the observed R-methadone concentrations fit within the 10^{th} and 90^{th} percentiles of the model predicted concentration and are symmetrically distributed around the median.

The final model for R-methadone is represented in the following equations:

$CL/F = 8.6\ L/h \times (Age/40)^{-0.316} \times (BMI/25)^{-0.272} \times 0.851$ (if *ABCB1* 2677 GG)

$V/F = 345\ L$

Relative F = 1.50 (if Hmong) or 1.19 (if *CYP1A2* -163 CC)

$Ka\ (h^{-1}) = 1.5$ (fixed)

We halved and doubled our estimate of Ka and did not obtain significantly different parameter estimates (data not shown).

Table 4.3 shows final parameter estimates for R-Methadone and bootstrap analysis.

Parameter	NONMEM		Bootstrap Analysis	
	Estimate (θ)	**95% CI**	**Estimate (θ)**	**95% CI**
CL/F (L/h)	8.6	7.8-9.3	8.5	7.9-9.4
(Age/40)$^{\theta 2}$	-0.316	-0.480--0.152	-0.319	-0.481--0.140
(BMI/25)$^{\theta 3}$	-0.272	-0.497--0.0466	-0.277	-0.524--0.0441
***ABCB1* 2677 GG**	0.851	0.768-0.934	0.853	0.774-0.940
V/F (L)	345	309-381	345	309-381
F non-Hmong (reference)	1.00	--		
Hmong	1.50	1.32-1.68	1.50	1.32-1.69
***CYP1A2* -163 CC**	1.19	1.01-1.37	1.20	1.01-1.40
IIV of CL (%CV)	0.0392 (19.8)	-0.0108-0.0892	0.0397	0-0.0904
IIV of F (%CV)	0.0941 (30.7)	0.0443-0.144	0.0906	0.0378-0.134
RV, proportional (%CV)	0.0348 (18.7)	0.0186-0.0510	0.0343	0.0218-0.0522

IIV interindividual variability; RV residual variability

Goodness-of-fit plots are presented below.

Figure 4.4 Goodness of fit plots R-methadone

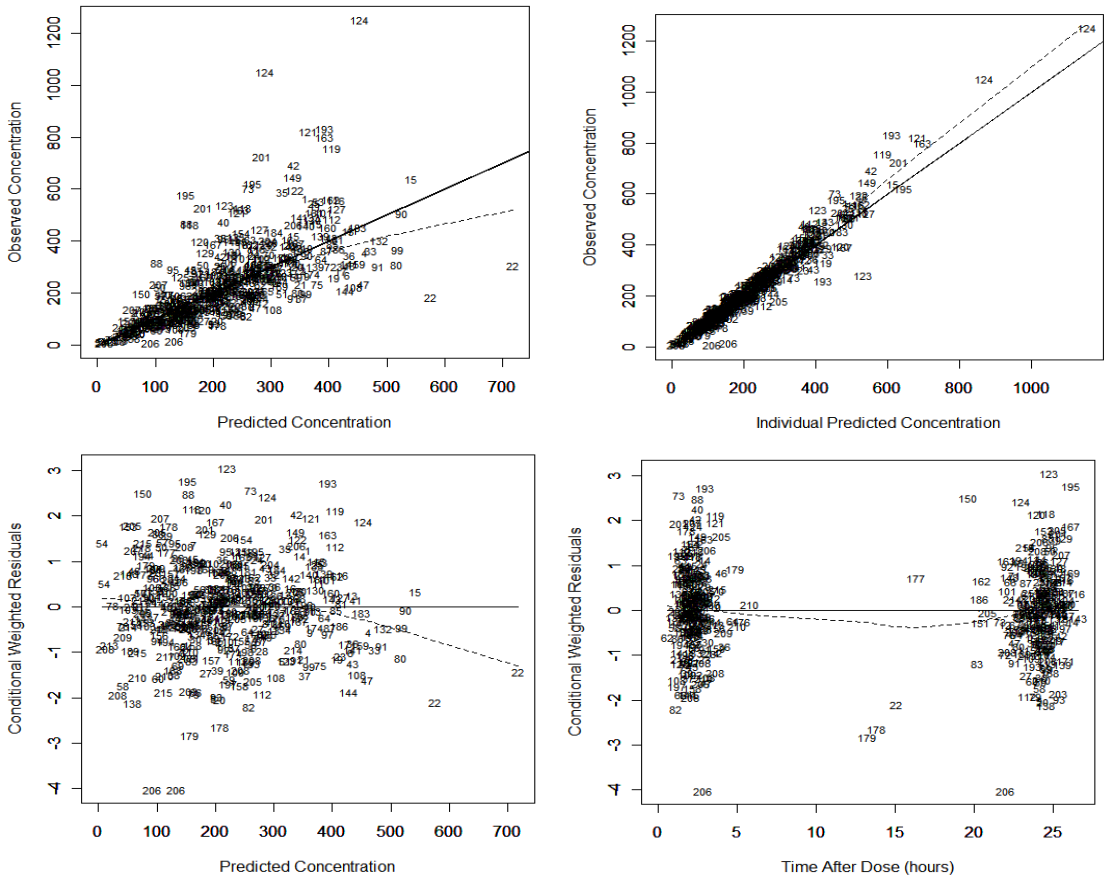
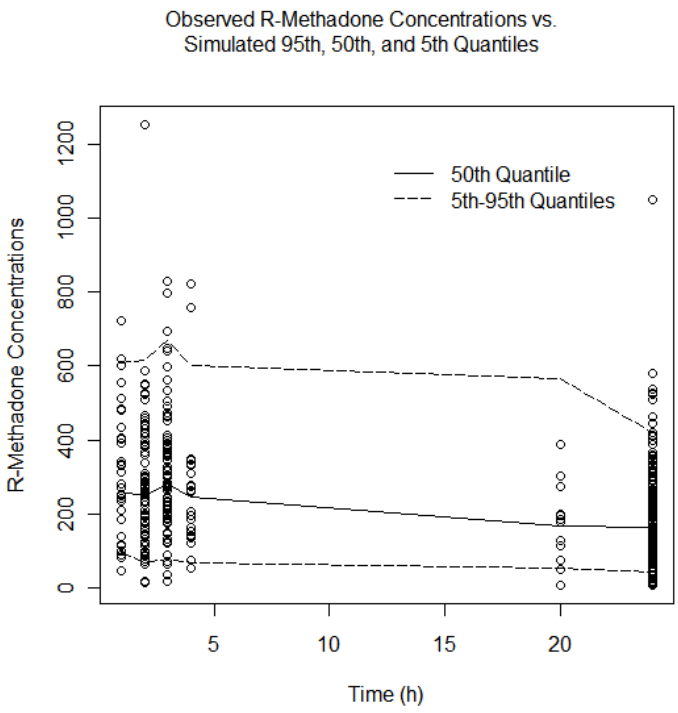


Figure 4.5 Visual Predictive Check R-methadone



S-Methadone

Base model parameter estimates for CL/F and V/F were 10.6 L/h and 252 L, respectively. S-methadone half-life, therefore, was 16.5 hours. Following forward inclusion of covariates using GAM, informative covariates on CL/F included age, BMI, ethnicity, the *ABCB1* 2677G>A/T, *ABCB1* 86979750C>T, *CYP2D6* 100C>T, *CYP2B6* 516G>T, and *CYP1A2* -163C>A polymorphisms. Covariates on V/F included the *CYP2B6* 792A>G, *CYP2B6* 516G>T, and *CYP2B6* 64C>T polymorphisms. Covariates on F included ethnicity and the *ABCB1* 87058822C>T, *ABCB1* 86979750C>T, *CYP2B6* 516G>T, *CYP2B6* 64C>T, and *CYP1A2* -163C>A polymorphisms.

After backward elimination in NONMEM, the lowest objective function value was obtained with covariates on CL/F of age, BMI, the *ABCB1* 2677 GG genotype, and the *CYP2B6* 516G>T SNP. Hmong ethnicity was the only covariate on F. There were no covariates on V/F.

As with R-methadone, the model estimation for variance of V/F did not converge, thus it was fixed at a mean of 0 in the final model.

Model Qualification

From 1000 bootstrap simulations, 97% had successful minimization and were included in the analysis (see table 4.4 for results). Mean parameter estimates from the bootstrap were comparable to estimates from NONMEM and varied by less than 10% from those in the final model.

Visual Predictive Check

The 10^{th}, 50^{th} (median), and 90^{th} percentile S-methadone concentrations obtained from bootstrap simulation are shown in Figure 4.7. The results indicate that greater than 90% of the observed S-methadone concentrations fit within the 10^{th} and 90^{th} percentiles of the model predicted concentration and are symmetrically distributed around the median.

The final model for S-methadone is represented in the following equations:

CL/F = 10.6 L/h x $(Age/40)^{-0.255}$ x $(BMI/25)^{-0.304}$ x 0.844 (if *ABCB1* 2677 GG) x 0.846 (if *CYP2B6* 516GT) x 0.666 (if *CYP2B6* 516TT)

V/F = 252 L

Relative F = 1.51 (if Hmong)

Ka (h^{-1}) = 1.5 (fixed)

We halved and doubled our estimate of Ka and did not obtain significantly different parameter estimates (data not shown).

Table 4.4 shows final parameter estimates for S-Methadone and bootstrap analysis.

Parameter		NONMEM	Bootstrap Analysis	
	Estimate (θ)	**95% CI**	**Estimate (θ)**	**95% CI**
CL/F (L/h)	10.6	9.3-11.9	10.6	9.3-12.1
(Age/40)$^{\theta 2}$	-0.255	-0.422--0.0878	-0.257	-0.431--0.0725
(BMI/25)$^{\theta 3}$	-0.304	-0.555--0.0531	-0.312	-0.579--0.0715
***ABCB1* 2677GG**	0.844	0.755-0.933	0.846	0.761-0.936
***CYP2B6* 516GT**	0.846	0.764-0.928	0.850	0.767-0.940
***CYP2B6* 516TT**	0.668	0.526-0.810	0.671	0.535-0.845
V/F (L)	252	227-278	252	227-280
F non-Hmong (reference)	1.00	--		
Hmong	1.51	1.32-1.70	1.51	1.31-1.72
IIV of CL (%CV)	0.0408 (20.2)	-0.0164-0.0980	0.0402	0.0-0.0899
IIV of F (%CV)	0.150 (38.7)	0.0777-0.222	0.149	0.0852-0.2170
RV, proportional (%CV)	0.0458 (21.4)	0.0246-0.0670	0.0449	0.0268-0.0683

IIV interindividual variability; RV residual variability

Goodness-of-fit plots are presented below.

Figure 4.6 Goodness of fit plots S-methadone

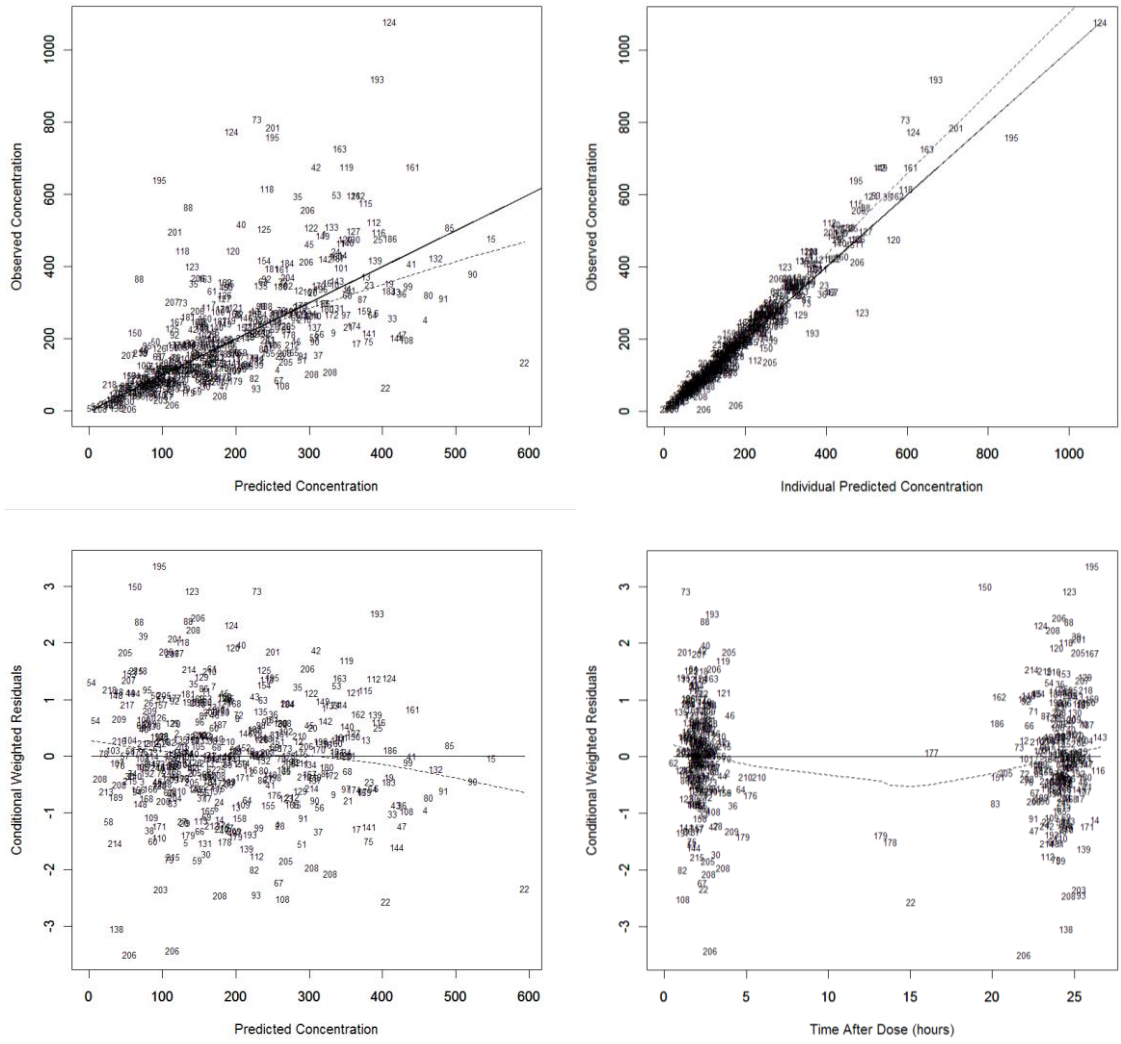
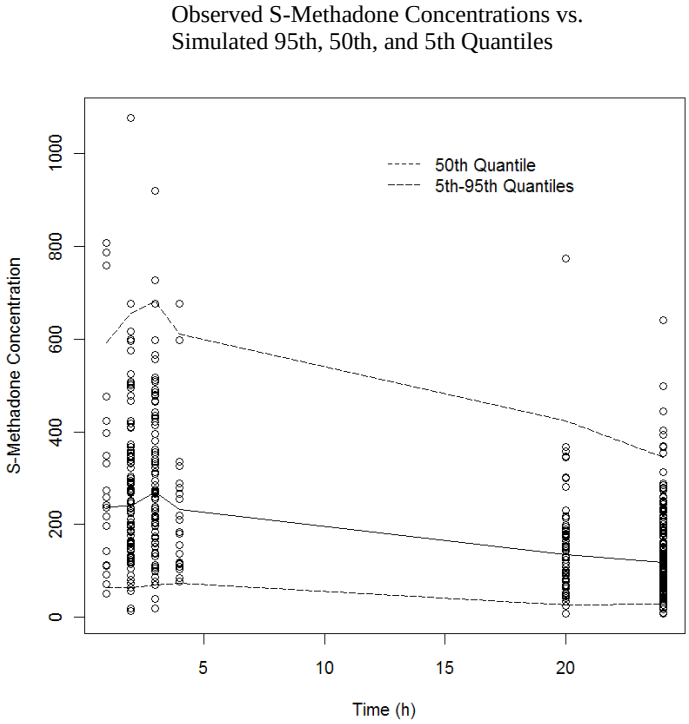


Figure 4.7 Visual Predictive Check S-methadone



Discussion

This study used population pharmacokinetics to show that, compared to non-Hmong, Hmong have greater relative apparent bioavailability of methadone. Whether this difference explains previous findings for lower methadone dose requirements and better treatment outcome in Hmong is unknown. We also found a minor influence of age, BMI, and the *ABCB1* 2677 GG genotype on R-methadone clearance. Age, BMI, the *ABCB1* 2677 GG genotype and the *CYP2B6* 516 SNP influenced S-methadone clearance. For

unclear reasons the *CYP1A2* -163 CC genotype slightly increased R-methadone apparent bioavailability.

Our overall parameter estimates are similar to previous population pharmacokinetic studies of methadone (Table 4.5).[239;245] Rostami-Hodjegan et al. and Foster et al. both used biexponential models whereas we used a monoexponential model. Importantly, one such study performed parallel analysis of selected sparse samples with a full set of dense sampling data to validate population pharmacokinetic parameter estimates against traditional pharmacokinetic measures within a single population.[239] The sparsity of time points obtained in this study limited our ability to converge the estimate of V and to test a biexponential model. Despite our inability to confirm a biexponential model, if we are subject to model misspecification then an estimate of Ka, which we fixed, would be impacted but estimates of clearance and volume of distribution would remain unbiased.[265]

Table 4.5 Previous population pharmacokinetic studies of methadone

Study	N	CL/F L/h (95%CI)	V/F L (95%CI)
Bart et al.			
R-Methadone	206	8.6 (95%CI 7.8-9.3)	345 (95%CI 309-381)
S-Methadone	206	10.6 (95%CI 9.3-11.9)	252 (95%CI 227-278)
Rostami-Hodjegan et al.[245]			
Racemic methadone	35	10.3 (±SD3.4; 5.2)	123 (±SD 43; 67)
Foster et al.[239]			
R-Methadone	59	8.7 (±SD7.9; 9.6)	597 (±SD 538; 663)
S-Methadone	59	8.3 (±SD7.3; 9.5)	345 (±SD 312; 382)

Our finding that pharmacokinetic parameters were influenced by genetic polymorphisms is of particular interest. For example, the *ABCB1* gene encodes the P-glycoprotein efflux transporter located in the intestinal lumen, blood brain barrier, and kidney. Methadone is a substrate for this transporter and, therefore, functional variants of this gene could influence methadone pharmacokinetics. In fact, knockout studies in mice lacking *ABCB1* show higher brain levels of methadone and heightened methadone-induced analgesia compared to wild-type mice.[266;267] In humans, P-glycoprotein inhibition with quinidine increased methadone levels and effect on pupil size following oral administration but had no effect on methadone levels or pupil size following intravenous methadone, indicating

that in humans P-glycoprotein does not significantly influence methadone access to the brain.[268]

Genetic variants of *ABCB1* have been associated with methadone dose requirements, although results are mixed. Levran et al. did not find a significant effect of *ABCB1* 2677 genotype and methadone dose in stabilized methadone patients but when this SNP was included with the *ABCB1* 3435 and 1236 SNPs in a haplotype analysis, patients with a TT or GT genotype required higher methadone doses than those without these genotypes.[261] An Australian study found that a five SNP haplotype that included the *ABCB1* 2677 SNP was significantly associated with methadone dose, with patients having one or two haplotypes containing the *ABCB1* 2677G allele requiring higher methadone doses.[269] A study in Han Chinese found that carriers of a three SNP haplotype that includes an *ABCB1* 2677 G or T allele also require higher methadone doses than non-carriers.[270] While these studies looked only at methadone dose, Crettol et al., also evaluated methadone plasma levels in relation to the *ABCB1* 2677 variant.[251] Carriers of the G allele had significantly higher trough levels of R-methadone with no effect on S-methadone or peak levels of either enantiomer. A haplotype analysis of the same three SNP block as Hung et al., however, found that the *ABCB1* 2677 G allele was associated with a 1.2 fold increase in plasma levels of both R- and S- methadone.

Crettol et al. normalized for patient weight and methadone dose, thus increases in plasma level could represent pharmacokinetic influence, although the potential influence on

bioavailability instead of clearance cannot be ruled out. If bioavailability were the main effect, then we might expect a difference in methadone peak levels rather than the difference in trough levels observed by Crettol. In our study, we found the *ABCB1* 2677 GG genotype to be associated with reduced methadone clearance a result that may be consistent with Crettol. Additionally, this polymorphism did not influence estimates of apparent bioavailability, also indirectly consistent with Crettol. Our findings are limited, however, in that we did not perform a haplotype analyses to determine whether they are due to the *ABCB1* 2677 variant or other variants in linkage disequilibrium with this polymorphism.

It is of particular interest that in addition to small influences of age and BMI, the *CYP2B6* 516G>T variant had a large effect on S-methadone clearance. S-methadone is stereoselectively metabolized by CYP2B6.[200;271-273] There are several reports of differential pharmacokinetic parameters for R- and S-methadone. Kharasch et al. have noted reduced apparent oral clearance and volume of distribution for S-methadone compared to R-methadone.[192;198;274] Foster et al., did not identify a difference in clearance between the enantiomers but did find that the apparent volume of distribution was significantly lower for S-methadone (see Table 4.5).[239] Boulton et al., found opposite results with clearance and volume of distribution of S-methadone significantly greater than R-methadone.[195]

The *CYP2B6* 516G>T polymorphism encodes the CYP2B6*6 variant and imparts a poor metabolizer phenotype. *In vivo* studies show that the variant results in aberrant splicing and reduced protein expression.[255] Although this variant is in strong linkage disequilibrium with at least one other variant (*CYP2B6* 792A>G), we did not do haplotype analyses. In this instance this may not be a limitation in that using a minigene construct, Hofmann et al. determined that despite linkage disequilibrium the decrease in protein expression is specifically attributable to the *CYP2B6* 516G>T variant.[255;257]

A number of studies have linked the *CYP2B6* 516G>T variant to alterations in pharmacokinetic parameters. For example, efavirenz, a non-nucleoside reverse transcriptase inhibitor used in the treatment of HIV, exposure is increased in an allele-responsive manner with 516TT > 516GT > 516GG similar to our allele-responsive decrease in S-methadone clearance.[275] In addition to greater exposure, those with a 516T allele were more likely to experience efavirenz induced neurotoxicity.[275;276] Clearance of nevirapine, another non-nucleoside reverse transcriptase inhibitor, is reduced by 15% in 516GT and by 30% in 516TT carriers.[277] In methadone maintained populations this variant has been associated with lower dose requirements, with higher trough levels of S-methadone, and a greater peak trough ratio for S-methadone compared to R-methadone.[26;251;270]

Since S-methadone is the inactive enantiomer, we do not expect a direct clinical effect of the 15% and 33% decrease in S-methadone clearance for the 516GT and 516TT carriers,

respectively. In patients whose methadone levels are being measured for clinical correlates, however, an assay that is not stereoselective would be anticipated to give a misleadingly high level for carriers of the 516T allele due to over representation of S-methadone. This may explain, in part, the wide interindividual variability seen in several studies of methadone levels. Thus for future developments in methadone therapeutic drug monitoring, a stereoselective assay would be important as 30%-40% of Caucasian and African populations carry at least one 516T allele.

It is difficult to explain the small influence we found of the *CYP1A2* -163 CC genotype on increasing R-methadone apparent bioavailability. Inclusion of *CYP1A2* in this study was based on case reports of clinical effects following administration of medications known to inhibit or induce CYP 1A2 (and other isoforms).[196] For example, fluvoxamine, a CYP 1A2 inhibitor, may increase sedation in patients taking methadone.[278] Fluvoxamine is also an inhibitor of CYP 2D6 and CYP 2C19, thus it is difficult to determine whether these case reports are specifically attributable to CYP 1A2 inhibition. There are no *in vitro* data to support CYP 1A2 as being significantly involved in racemic or stereoselective methadone metabolism.[199;279] *In vivo* assessment of CYP 1A2 activity as measure by salivary caffeine half-life, failed to show an effect of this isoform on steady-state methadone metabolism.[280] Additionally, Crettol et al. did not find the *CYP1A2* -163C>A variant to influence plasma levels of methadone.[251] Finally, because methadone is a low hepatic extraction drug and CYP 1A2 is minimally expressed in the small intestine[281], any hypothesized effect of this variant would be on methadone

clearance and not apparent bioavailability as we found. We cannot rule out the potential that the *CYP1A2* -163 SNP is in linkage disequilibrium with other polymorphisms that do affect R-methadone bioavailability. The clinical implications of this finding are likely small as the 19% increase in R-methadone apparent bioavailability the *CYP1A2* -163CC genotype confers is within the acceptable 80%-125% of reference F range set forth in FDA guidelines on bioavailability.[282]

Another limitation was our small set of hypothesis driven genetic data which prevented us from doing larger haplotype analyses or evaluating the role of other genes that could explain the difference in F between Hmong and non-Hmong (e.g., genes encoding methadone binding proteins like alpha 1-acid glycoprotein or CYP 3A5). Since the Hmong remain a non-admixed ethnic minority, their ethnicity may serve as a surrogate marker for genetic influences on methadone pharmacokinetics. The unique genetic background of the Hmong is supported by microsatellite marker studies performed in other minorities from the same geographic region.[283;284] Since ethnicity and genetics may be collinear, we cannot rule out and did not evaluate for gene x ethnicity interactions. Future attempts to do this might be of further benefit but would likely be limited by sample size of the various cells.

A further limitation was that we did not have a large prospectively assessed population that would allow us to detect possible methadone autoinduction. Finally, we used limited pharmacodynamic data such as ongoing drug use or SCL-90 scores but did not perform

pharmacokinetic-pharmacodynamic (pk-pd) studies using opiate related measures such as pupillary response or symptoms of clinical withdrawal or intoxication.

Summary

Population pharmacokinetic modeling of methadone maintenance is a feasible means of assessing covariates of pharmacokinetic parameters and to test for pharmacokinetic bases to clinically apparent differences in populations receiving methadone.

Our hypothesis of decreased methadone clearance in Hmong was not upheld. Instead, the finding of increased methadone apparent bioavailability in the Hmong appears to allow them to stabilize on lower doses of methadone than non-Hmong. This may indicate unique pharmacogenetic, dietary, or plasma protein binding influences on methadone in this population. Despite significant impact of ethnicity on F, there was not a difference in methadone CL/F or V/F between Hmong and other races. There were however, minor (< 20%) influences on CL for age, BMI, and for R-methadone, the *ABCB1* 2677G>A/T polymorphism. A larger (33%) reduction in S-methadone CL was noted for the *CYP2B6* 516TT genotype. Pharmacodynamic models exploring these covariates (including genes associated with methadone pharmacodynamics) will need to be evaluated before the efficacy of therapeutic dose monitoring in methadone maintenance can be determined.

Chapter 5

Ethnic Differences in Psychosocial Factors in Methadone Maintenance: Exploratory and Descriptive Analyses

The primary effects of methadone in the treatment of opiate dependence are reductions in opiate withdrawal symptoms, drug craving, and continued illicit opiate use. These effects result from methadone's combined pharmacokinetic and pharmacodynamic properties. Because psychosocial instability and psychiatric diagnoses may have somatic and other subjective manifestations (generalized aches, fatigue, irritability, etc.) which may be confused for opiate withdrawal symptoms or may trigger craving, these behavioral-psychiatric domains could influence the perceived pharmacodynamic effect of methadone and thus lead to higher methadone doses.

In Chapter 3, we identified differences in methadone dose requirements between Hmong and non-Hmong patients showing that the Hmong require significantly lower doses. In Chapter 4, we found that not only is there a difference in dose requirement between Hmong and non-Hmong, but that Hmong have higher apparent oral bioavailability (F) for methadone compared to non-Hmong. Despite this difference, we cannot exclude the role that psychosocial factors may play in influencing dose differences between Hmong and non-Hmong.

The purpose of this chapter is to explore psychosocial differences between Hmong and non-Hmong patients receiving methadone maintenance. There were no *a priori* hypotheses being tested but rather we sought to gather data that could help improve understanding of a Hmong and non-Hmong population participating in a population pharmacokinetic modeling study of methadone and to help inform future studies.

Methods and Procedures

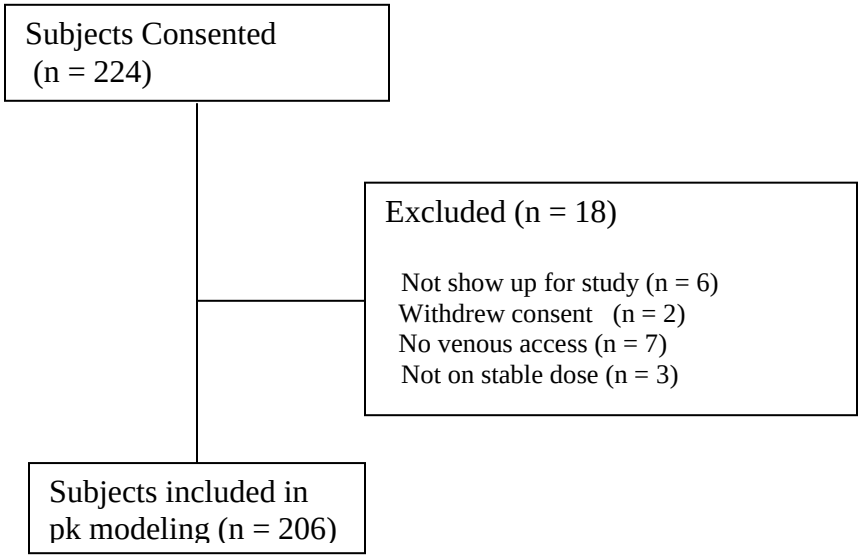
Methadone maintained patients enrolled in a single urban safety-net hospital outpatient addiction medicine clinic were invited to participate in this study via posted flier and word of mouth. Initially, patients were recruited into two separate studies: a cross-sectional study requiring patients to have been on methadone for at least two months without dose change during the previous five days and a prospective study that recruited patients during their first week on methadone and followed them at 1, 3, 6, and 12 months. The prospective study was closed to enrollment after the first 14 subjects due to slow accrual and these subjects were followed as per protocol but their data were combined with the cross-sectional subjects in creating the population pharmacokinetic model.

As per Federal criteria, all subjects on methadone maintenance were at least 18 years of age and had met Diagnostic and Statistical Manual of Mental Disorders criteria for opiate dependence of at least one year duration prior to initiating methadone. Subjects were

excluded if they were unable or unwilling to provide informed consent, had decompensated liver disease, were in the second or third trimester of pregnancy, or were taking medications known to alter methadone pharmacokinetics (e.g., phenytoin, rifampin, or highly active antiretroviral therapy for HIV). The study was approved by the Human Subjects Research Committee of the Hennepin County Medical Center and conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983). Because data included sensitive psychiatric and drug related information a Certificate of Confidentiality was obtained from the National Institutes of Health National Institute on Drug Abuse. The Hmong speaking population was not literate in English or written Hmong, however, consent forms were translated into Hmong and then read to patients by native Hmong speakers in the presence of a study coordinator who could answer questions about the protocol.

Patient recruitment and enrollment began in October of 2008 and the last patient was enrolled in June, 2011. Recruitment information is included in the study flow diagram below.

Figure 5.1.



While the primary aim of the study was to develop a population pharmacokinetic model of methadone comparing Hmong to non-Hmong patients, various structured and semi-structured psychosocial assessments were completed to help better describe the patient population and for exploratory hypothesis generating purposes. Assessments were conducted during the 3-hour interval between blood draws for trough and peak methadone levels and included:

The Structured Clinical Interview for DSM-IV Axis I Disorders is a structured interview that provides the "gold standard" for current and lifetime DSM-IV diagnoses for axis I disorders.[248]

The Symptom Checklist-90 (SCL-90) is a measure of psychopathology used in clinical and research settings.[249;285] It has been used in methadone patients and in Hmong.[286;287] It is a self-administered assessment that covers nine dimensions of psychopathology (depression, anxiety, obsessive compulsive, somatization, phobic anxiety, paranoid ideation, hostility, interpersonal sensitivity, and psychoticism) and an overall distress dimension.[249] It provides quantitative data that allows for comparisons between and within groups. While the SCL-90 is reliable and has internal consistency, the domains do not have strong predictive validity for Diagnostic and Statistical Manual of Mental Disorders diagnostic categories, thus the SCL-90 may be best suited as a descriptive measure of behavioral symptoms or as a unidimensional measure of symptoms best summarized through the Global Severity Index (GSI), an overall distress dimention.[288-290]

A revised version of the SCL-90 (SCL-90-R) has changed two questions in the anxiety dimension and altered the wording in a few other questions.[288] It has not been as widely validated as the SCL-90 and is copyrighted with requirement of payment for utilization. Despite its limitations, the SCL-90 was chosen for this study because of its public domain status and because it is one of the few psychiatric measures that previously has been used in a Hmong population, thus allowing for some historical comparison. This was its first use in a methadone maintained Hmong population, however.

Original validation studies by Derogatis identified normative values for each dimension in healthy community dwelling adults and compared these to values obtained in

psychiatric (inpatient and outpatient settings) and alcohol dependent males. Normative values were defined as <0.5, borderline scores 0.5-1, and abnormal scores > 1.[249]

The Addiction Severity Index (ASI) is a semi-structured interview that is widely used in clinical and research settings.[247] The ASI covers seven dimensions (e.g., medical, employment, alcohol, drug, legal, family/social, and psychiatric) that may be related to treatment progress and outcome. Past 30 day ratings can be codified using the ASI Composite Index Scores (0-1, with larger scores indicating greater severity).[291] These allow researchers to develop empirically-validated measures of current functioning. While the ASI is non-diagnostic, it is a reliable and valid measure of severity that can be used to plan and track treatment.[247;292] Baseline scores can also predict treatment outcomes for several domains in a methadone maintained population.[293]

All interviews were conducted by a single trained bachelor level research coordinator, thus eliminating inter-rater variability. Interviews were not recorded for later fidelity audits, however. For Hmong subjects, interviews were conducted with the assistance of an interpreter knowledgeable in medical and drug use terminology. The SCL-90 had been previously translated into Hmong[287] and was read aloud by a Hmong interpreter.

We evaluated differences in demographic (age, gender) and psychosocial factors (ASI composite scores, SCID, SCL-90) between Hmong and non-Hmong. Continuous variables were analyzed using t-tests and analysis of variance (ANOVA) whereas

categorical variables were analyzed by Chi-square. Significant p-values were < 0.05 and because of the exploratory nature of this analysis, values were not corrected for multiple testing. Statistical testing was completed using Microsoft Office Excel 2007 (Microsoft Corporation).

Results and Discussion

This study included an initial prospective arm, however, it was abandoned due to recruitment problems. Prior to abandoning this arm, fourteen patients were recruited and data from the thirteen subjects providing blood samples were added to the data-set used to create the pharmacokinetic model only if the data met the study inclusion criteria (e.g., patient had been on stable dose methadone). The data from these thirteen subjects are incorporated into Tables 5.1 and 5.2 and the other descriptive results contained in this chapter.

Table 5.1 Subject demographics

Total Sample Size	206
Male (%)	127 (61.7)
Mean age yrs (SD)	47.2 (13.2)
Hispanic (%)	4 (1.9)
Caucasian (%)	63 (30.6)
African American (%)	44 (21.4)
American Indian (%)	19 (9.2)
Hmong (%)	76 (36.9)

Methadone maintained Hmong were predominantly male and older than their non-Hmong counterparts (Table 5.2). As in our previous retrospective study, the Hmong were on significantly lower doses of methadone than non-Hmong patients.[246]

Table 5.2 Hmong and non-Hmong characteristics

	Hmong (n=76)	**Non-Hmong (n=130)**	**P value**
Male, N (%)	54 (71.1)	72 (55.4)	$< 0.05^{A}$
Age, years (SD)	56.6 (11.6)	41.7(10.7)	$< 0.001^{B}$
Methadone dose, mg (SD)	54.0 (19)	82.4 (31.2)	$< 0.001^{B}$
Time on methadone, yrs (SD)	6.0 (3.9)	1.9 (2.8)	$< 0.001^{B}$

A χ^2 B t-test

Difference in ASI composite scores between Hmong and non-Hmong methadone maintained patients: The Addiction Severity Index (5^{th} Ed) was used to address treatment problems in seven functional domains believed to contribute to treatment outcome (alcohol use, drug use, employment, family and social stability, legal issues, medical problems, and psychiatric problems).

The thirteen subjects recruited for the prospective study whose data were used for pharmacokinetic modeling were excluded from this analysis since the ASI was performed only upon admission to treatment, a time of instability, whereas the all cross-sectional study subjects had been in treatment for at least 2 months. Some subjects had missing data from questions utilized to calculate composite scores. Missing composite scores for

a specific domain were excluded from analysis, thus various degrees of freedom are indicated in Table 5.3.

Because the included participants in this study all had at least 2 months of methadone treatment, it is difficult to compare ASI composite scores to studies in which the ASI was administered upon entry to treatment. We hypothesized that the scores of those in treatment for at least two months would be lower than those entering into treatment because the treatment process will bring improvement across the spectrum of domains. This would be most evident in the alcohol and drug use composite scores, which reflects past 30 day use of alcohol and drugs. Weisner et al. looked at ASI composite scores for 327 HMO members entering treatment for a substance use disorder (not specific to methadone maintenance) and found scores of 0.38 and 0.11 for alcohol and drugs, respectively.[294] Schwartz et al. conducted ASI on 351 opiate addicted subjects entering into methadone maintenance and found alcohol and drug composite scores of 0.09 and 0.32, respectively.[295] Bovasso et al., also examined 310 patients who had been on methadone for 2 to 6 weeks and found alcohol and drug composite scores of 0.12 and 0.36, respectively.[293] The alcohol scores in these studies were higher than our population and those for drugs were higher than we observed in Hmong and non-Hmong other than Weisner's population who was similar to our non-Hmong group. It is not clear what this latter similarity may be related to but it could reflect ongoing non-opiate drug use in the methadone population.

Unexpectedly, medical, family, and psychiatric domains (for non-Hmong only) were worse in our population than those reported by Schwartz; although medical and psychiatric (non-Hmong only) domains were similar to those in Bovasso. Weisner did not report on family domain but our population had worse medical and slightly better psychiatric composite scores. These finding may indicate that our population had worse baseline composite scores in these domains compared to the other studies and our scores represent improvement through time (our hypothesis is correct). Our findings could also indicate that methadone treatment brings improvement in alcohol and drug domains only and the worsening of medical, family, and psychiatric domains are independent of a treatment effect (our hypothesis is false). Without longitudinal repeat measure analysis of ASI composite scores, we cannot reliably make a conclusion on domain improvement during methadone treatment.

Because we have on-site medical and psychiatric services, there is reason for concern about the high medical and psychiatric composite scores. There is little difference in age between our non-Hmong population and those of Schwartz and Weisner, thus aging into illness over the course of treatment cannot explain this difference. It may be that patients entering into methadone treatment have concerns other than medical and psychiatric symptoms (e.g., concern regarding withdrawal), thus biasing the composite downward compared to an in treatment population. Additionally, one element contributing to the psychiatric composite score is whether medications have been taken for psychiatric problems, something that may only occur after entry into treatment and referrals to

psychiatric care are made. The Hmong psychiatric composite scores were similar to Schwartz and lower than Weisner and are not likely due to fewer psychiatric diagnoses than non-Hmong but may be related to differences in the spectrum of diagnoses and perception of problems related to these diagnoses, which is a significant component of the psychiatric composite score. (Differences in diagnoses and symptoms are discussed below in the SCL-90 and SCID sections.)

The worse family/social status composite score in our population may be attributed to a lowered tolerance for adversity after the treatment process has commenced. In other words, prior to treatment family and social instability may be considered as normal and dissatisfaction with these situations is not present. During the treatment process the family or social situation may not change, but the patient's dissatisfaction with these situations may increase as they enter into more stable drug and alcohol free lives. Thus we may see higher severity scores in patients in treatment compared to those just entering treatment.

There was no difference in employment composite scores between Hmong and non-Hmong and our population and that of Schwartz or Bovasso. Because this composite score queries driver's license status and availability of an automobile, as well as days of paid work and salary amount, lack of transportation despite employment may bias the answer. More likely, our results are representative of ongoing well documented difficulties in gainful employment for patients in methadone maintenance.[296-298] Bovasso,

however, found that this domain has poor specificity in predicting ongoing employment problems two year into treatment.[293] For the Hmong there may be an additional component of educational and language barriers that further contribute to difficulties with employment.

Table 5.3 ASI composite scores Hmong and non-Hmong

Composite Domain	Hmong (n=76)	Non-Hmong (n=129)[A]	df	t-statistic	P value
Medical	.28(.39)	.4 (.38)	153	2.15	< 0.05
Employment	.76 (.26)	.82 (.23)	141	1.59	>0.1
Alcohol Use	.00(.00)	.05 (.09)	132	5.76	<0.001
Drug Use	.02 (.04)	.1 (.1)	186	8.79	<0.001
Legal[B]	.00 (.00)	.09 (.18)	--	--	--
Family/Social	.17 (.07)	.27 (.18)	180	5.24	<0.001
Psychiatric	.07 (.13)	.28 (.23)	203	8.67	< 0.001

A One non-Hmong patient did not complete the ASI; B no Hmong had legal problems so comparison could not be made

Brown et al. evaluated ethnic differences in ASI composite scores in methadone maintained patients who had been in treatment for 2-6 weeks.[299] This comparison included gender as a co-factor and only African American and Hispanic patients. Results showed African Americans had higher composite scores in alcohol only. There were, however, gender differences with women having lower employment. There were no significant ethnicity x gender interactions in composite scores. Because Brown did not include a Caucasian reference group we cannot determine how these groups compare to a Caucasian population.

In a separate analysis of ASI composite scores using analysis of variance comparing Caucasian, African American, and Hmong composite scores (American Indian and Hispanic sample size was too small to include), we found that there was no difference in medical composite score but that all other identified differences remained (Table 5.4). Separate comparisons of the Caucasian and African American groups did not show differences in these areas indicating that identified ethnic differences were driven by the Hmong and the acceptability of comparing Hmong to the aggregate group of non-Hmong. Given the large standard deviations, it may be that these negative findings are due to underpowered sample size and interethnic differences would have been identified with a larger sample size. Future studies with larger sample sizes are needed for verification.

Table 5.4

Composite Domain	Caucasian	African American	Hmong	$F_{2,179}$	P
Medical	0.39 (.37)	0.41 (.38)	.28(.39)	2.25	> 0.1
Employment	0.79 (.25)	0.85 (.19)	.76 (.26)	1.88	> 0.1
Alcohol Use	0.04 (.07)	0.04 (.08)	.00(.00)	11.3	< 0.001
Drug Use	0.11 (.10)	0.10 (.10)	.02 (.04)	28.0	< 0.001
Legal[A]	0.07 (.15)	0.08 (.17)	.00 (.00)	--	> 0.1
Family/Social	0.27 (.19)	0.24 (.18)	.17 (.07)	8.11	< 0.001
Psychiatric	0.31 (.22)	0.25 (.26)	0.07 (.13)	27.2	< 0.001

A No Hmong had legal issues so Caucasian and African Americans were compared with t-test

Symptom Checklist-90 Dimension Differences between Hmong and non-Hmong:

Of the nine dimensions assessed in the SCL-90, the only normative value found in this study was for the Hostility dimension in the Hmong population (Table 5.5). The Hmong

had abnormal scores for the Somatization dimension only whereas non-Hmong had abnormal scores in the Obsessive-Compulsive and Depression dimension. All other dimensions in both Hmong and non-Hmong were in the borderline range.

The Hmong and non-Hmong significantly differed in Somatization, Interpersonal Sensitivity, Depression, Hostility, and Paranoid Ideation dimensions with the Hmong having a higher score in the Somatization dimension only. Because of concerns over the discriminant validity of the various dimensions of the SCL-90, it has been recommended that the GSI be utilized as a unidimensional measure of psychiatric symptom severity.[290] We found no difference in GSI scores between Hmong and non-Hmong.

Table 5.5 SCL-90 Hmong and non-Hmong

Composite Area	Hmong (n=76)	Non-Hmong (n=130)	df	t-statistic	P value
Somatization	1.17 (.78)	.91 (.56)	119	2.49	< 0.05
Obsessive-Compulsive	1.00 (1.02)	1.03 (.82)	134	0.21	>0.1
Interpersonal Sensitivity	.51 (.68)	.78 (.77)	174	2.54	< 0.01
Depression	.70 (.72)	1.05 (.79)	170	3.16	< 0.005
Anxiety	.63 (.73)	.81 (.73)	160	1.71	0.09
Hostility	.46 (.62)	.59 (.74)	179	1.29	> 0.1
Phobic Anxiety	.50 (.63)	.52 (.71)	173	0.29	> 0.1
Paranoid Ideation	.52 (.72)	.73 (.75)	161	1.98	0.05
Psychoticism	.73 (.93)	.70 (.81)	142	0.26	> 0.1
Global Severity	.73 (.69)	.81 (.61)	143	0.75	> 0.1

Comparing our SCL-90 scores to those of other methadone maintained populations has proved difficult because most studies utilize SCL-90 early in treatment rather than in an already stabilized population. Rounsaville et al., evaluated 72 methadone patients with comorbid psychiatric illness who had been on methadone for at least 6 weeks.[300] He reported only the GSI results, which had a mean score of 1.8, more than twice the level we found. Woody et al., reported an SCL-90 GSI score of 1.5 approximately 6 months after one of three psychotherapeutic interventions in 110 methadone patients.[219] Platt et al., evaluated ethnic differences in SCL-90 scores for each of the nine dimensions (no GSI scores reported) in a population of 900 white and black methadone patients.[301] Here they found that only Obsessive-Compulsive and Depression dimensions differed by ethnicity with blacks scoring lower in each. They, however, conclude that SCL-90 scores do not need ethnic or gender adjustments. While we did find dimension differences between Hmong and non-Hmong, determining whether these differences are due to behavioral symptoms or are an indicator that the scores for Hmong need to be weighted differently is beyond the scope of the current study.

A report of SCL-90 scores in patients evaluated at methadone treatment intake and 6 months later found significant improvement in all dimensions at 6 months.[302] While direct comparisons between this report and our population are not possible, it should be noted that our SCL-90 scores are higher in the somatization, phobic anxiety, and psychoticism dimensions in both Hmong and non-Hmong. Both Hmong and non-Hmong scored lower in the interpersonal sensitivity, hostility, and paranoid ideation dimensions.

The non-Hmong but not Hmong had higher scores in the obsessive-compulsive, depression, anxiety, and GSI dimensions. These findings indicate that despite general improvements in drug use through time, there continues to be ongoing behavioral symptoms in methadone patients. The specifics of these problems vary between Hmong and non-Hmong yet the overall level of difficulty as assessed by the GSI is similar. Rounsaville did not find that these symptoms predicted treatment retention at 6 months and we do not have longitudinal data to evaluate whether these symptoms predict retention in the current study.[302]

We dichotomized GSI scores to normal (< 0.5) and borderline plus abnormal (≥ 0.5) and evaluated whether there was a relationship between GSI and urine drug screen results being positive for any drug other than methadone (i.e., amphetamine, benzodiazepines, cocaine, and opiates). The findings were not significant, of the 119 subjects with a GSI ≥ 0.5, 34.5% had positive screens and 31.7% were positive in the 82 subjects with a GSI < 0.5 ($\chi^2_{df=1} = 0.165$, $p = 0.68$). Thus, in a cross-sectional assessment of drug use in a methadone population, there was no relationship between a dichotomized GSI score and drug use. Likewise, there was no difference in methadone dose based on dichotomized GSI score with mean (SD) dose of 71.5 (34.2) mg and 66.5 (28.6) mg for GSI ≥ 0.5 and < 0.5, respectively ($t_{df=191} = 1.11$, $p = 0.27$).

SCL-90 in Hmong: Westermeyer et al. have measured SCL-90 scores in both psychiatrically ill and non-psychiatrically ill Hmong.[303] The GSI in the non-ill Hmong

was 0.51, lower than our population, however the psychiatrically ill Hmong had GSI scores between 0.97 and 1.09 (for adjustment disorder diagnoses and other psychiatric diagnoses, respectively) both higher than in our population. For the nine dimensions, there were mixed differences between our population and that of Westermeyer: our population had higher scores than Westermeyer's non-ill for Somatization, Obsessive-Compulsive, Anxiety, Hostility, Phobic Anxiety, and Psychoticism dimensions; lower scores for Interpersonal Sensitivity, Depression, and Paranoia dimensions. Compared to those with adjustment disorder diagnoses, our population had lower scores for all dimensions except Somatization and Psychoticism. The lower scores in our Hmong population may be related to a cohort effect in that as a refugee population, the Hmong have undergone stressors that may have decreased through time as they adjust to their new setting.[304] Indeed, Westermeyer also found significantly improved dimension sores for GSI, Somatization, Hostility, and Phobic Anxiety over two years in non-psychiatrically ill Hmong.[287] The high Somatization score in the methadone maintained Hmong may be explained by an older age than in Westermeyer or by the large number of methadone maintained Hmong with war-related injuries, which contributed to their opium exposure and subsequent development of addiction. There is no clear explanation for the higher psychoticism score in our population versus that of Westermeyer and why both our Hmong and non-Hmong psychoticism scores are greater than other methadone populations, especially in light of the paucity of psychotic disorder diagnoses from formal interview (see section on SCID below).

Finally, in an earlier report from our clinic of Hmong initiating methadone maintenance, Azeem et al., noted SCL-90 scores in abnormal ranges in all domains (all scores were > 1.75).[227] This would indicate that the opiate addicted Hmong have quite abnormal behavioral symptoms at time of admission and that these symptoms improve considerably through time.

SCID diagnoses Hmong versus non-Hmong: The Structured Clinical Interview for DSM-IV (SCID) is the "gold-standard" semi-structured interview for diagnosing psychiatric illness.[248] This diagnostic tool assesses for the presence of both current and life-time diagnoses. In this study, only the section on Axis I disorders was used, thus we did not assess for Axis II, or personality, disorders.

Results of current SCID Axis I diagnoses are presented in Table 5.6. Some subjects had multiple diagnoses, thus the total number of diagnoses exceeds that of subjects. Analysis was performed using chi-square for each diagnostic domain comparing the number of diagnoses within that domain to total number of diagnoses between Hmong and non-Hmong. Overall, the non-Hmong have more SCID diagnoses than Hmong. Interestingly there was no difference in the number of current substance related diagnoses between groups. While there was not statistical power to evaluate specific diagnoses within domains, it should be noted that although the non-Hmong had more anxiety disorders, the Hmong are disproportionately affected by post-traumatic stress disorder (71% versus

18% of anxiety diagnoses in non-Hmong), a likely consequence of their combat exposure and refugee status.

Table 5.6 Number of total current SCID diagnoses

SCID Axis I Diagnosis	Hmong (n=76 patients, 85 diagnoses)	Non-Hmong (n=125 patients, 230 diagnoses)*	Significance[A]
No current diagnosis	**51**	**36**	**P < 0.001**
Schizophrenia/Psychotic Disorders	**2**	**3**	**Insufficient sample size**
Schizophrenia	1	1	--
Delusional	1		--
Brief psychotic	0	1	--
Psychotic NOS	0	1	--
Anxiety Disorders	**17**	**127**	**P < 0.001**
Generalized Anxiety	1	32	--
Panic Disorder	1	24	--
Agoraphobia	0	4	--
Specific phobia	0	9	--
Social Phobia	2	10	--
Obsessive Compulsive	1	23	--
PTSD	12	23	--
Anxiety due to general medical condition	0	1	--
Anxiety NOS		1	--
Mood Disorders	**4**	**38**	**P < 0.05[B]**
Dysthymic Disorder	1	11	--
Major Depression	3	13	--
Bipolar Disorder		12	--
Mood disorder due to general medical condition	0	2	--
Substance Related Disorders	**11**	**23**	**P > 0.1**
Alcohol Dependence	0	1	--

Amphetamine Dependence	0	1	--
Cannabis Abuse	0	2	--
Cannabis Dependence	0	4	--
Opioid Abuse	1	0	--
Opioid Dependence	7	4	--
Cocaine Dependence	1	2	--
Sedative-hypnotic Dependence	0	1	--
Substance induced anxiety	1	2	--
Substance induced mood	1	5	--
Substance induced psychotic	0	1	--
Eating disorders	**0**	**3**	**Insufficient sample size**
Binge eating disorder	0	2	--
Bulimia nervosa	0	1	--
Total number of diagnoses	**85**	**230**	**$P < 0.05^{C}$**

*Five non-Hmong did not complete SCID interview.
A χ^2 for number of diagnoses observed in a domain against total number of diagnoses across all domains
B Yates' χ^2
C χ^2 total number of diagnoses against number of subjects
Note: Some subjects had more than one current diagnosis, thus total number of diagnoses exceeds total number of subjects

Westermeyer conducted diagnostic interviews based on earlier DSM-III criteria in ninety seven community-dwelling Hmong and found that 54% were without an Axis I diagnosis.[303] Seventeen patients (18%) had clear DSM diagnoses with most being in the depressive domain. The remainder was affected by adjustment disorders related to their recent immigration. In this study, we found that 67% of Hmong were without a current DSM-IV diagnosis, despite coming from a population expected to be at higher risk due their history of opiate addiction compared to Westermeyer's non-addicted population.

This may be related to our population having been in the United States considerably longer and further out from the effects of war than Westermeyer's relatively new refugee population assessed in 1977, just as Hmong were arriving as refugees. However, as Westermeyer's population acculturated to the United States, adjustment disorder diagnoses decreased and as a result the number of patients with no DSM diagnoses likely increased.[287] But this increase may also relate to a treatment effect in that these new immigrants with diagnoses on initial assessment could have received treatment and thus some of these diagnoses may have resolved. Our population was receiving treatment for opiate dependence and had ready access and referral to psychiatric care. We do not have data on the number of subjects receiving psychiatric care at the time of the study to determine number of those receiving psychiatric treatment versus those outside of psychiatric treatment.

Conclusion

We found distinct differences in addiction severity, behavior, and psychiatric diagnoses between Hmong and non-Hmong. These differences may affect measures of addiction treatment outcome with methadone. For example, the lower methadone dose requirements and better retention of Hmong in treatment described in Chapter 3 could potentially be explained by less symptom severity as seen in ASI composite scores and a lower burden of DSM-IV psychiatric diagnoses. This is less likely as reports of non-medication based treatment in Hmong show similar outcomes to reports of these

treatments provided to non-Hmong; although direct comparison has yet to be made.[65;210;212]

Ongoing drug use during methadone maintenance is associated with higher methadone doses.[305] This reflects attempts to curb drug use by subsequently increasing the methadone dose. In our study there was no difference in DSM-IV substance related diagnoses between Hmong and non-Hmong that could explain the difference in dose between these populations. Additionally, psychiatric comorbidity has provided mixed information regarding methadone treatment outcome with studies of Axis I diagnoses showing no influence on retention and Axis II diagnoses negatively affecting retention.[91;306;307] We did not track patients longitudinally to test the influence of psychiatric comorbidity on methadone dose or treatment outcome. Nor did we control for time in treatment in our comparisons; thus the difference between Hmong and non-Hmong may be influenced by a treatment effect.

Chapter 6

Recapitulation

This dissertation evaluated the population pharmacokinetics of methadone in an ethnically diverse population using non-linear mixed effects models. This project represents the largest known study of methadone population pharmacokinetics and the only one performed in an ethnically diverse population.

Chapter 1 provided a general review of the three Food and Drug Administration approved pharmacotherapeutic agents for the treatment of opiate dependence. Chapter 2 reviewed the clinical pharmacology of methadone as used in the treatment of opiate dependence. In these chapters it was clear that treatment outcome as measured by retention in treatment and reduction in illicit opiate use is best achieved with methadone maintenance. Despite more than forty-five years of research on methadone maintenance, there are few factors that can be used to predict treatment outcome. While methadone dose is generally associated with treatment outcome, large interstudy and interindividual variability in plasma concentrations has made it difficult to link dose response to pharmacokinetic parameters.

Chapter 3 introduced us to the Hmong and their paradoxically exceptional treatment outcome in methadone maintenance on lower doses of methadone than their non-Hmong counterparts. This retrospective study helped to form the hypothesis that their better treatment outcome is related to higher methadone exposure as determined through population pharmacokinetics.

The results of the population pharmacokinetic study were presented in Chapter 4. We found that the lower methadone dose requirement is explained by higher apparent bioavailability of methadone in Hmong. Other influences on methadone pharmacokinetics, more specifically clearance, include age, body mass index, and single nucleotide polymorphisms in the *ABCB1* and *CYP2B6* genes. The pharmacogenetic results are consistent with previous preclinical and clinical studies of these polymorphisms; although, this is the first report of their influence on pharmacokinetic parameters rather than on surrogates such as plasma level or dose requirement.

Psychosocial differences between Hmong and non-Hmong were presented in Chapter 5. Although the Hmong were generally older, have lower addiction related morbidity, fewer legal complications, and a different spectrum of psychiatric illness than non-Hmong, there remained significant comorbidity when compared to general or other methadone maintained populations. While this dissertation was not focused on elucidating cultural contributors to treatment outcome or methadone response, it should be noted that the Hmong do just as poorly as non-Hmong when their opiate dependence is treated without

pharmacotherapy. Thus, while the potential for culture to influence outcome is acknowledged, there remain sufficient grounds to hypothesize a biological (i.e., pharmacokinetic and/or pharmacodynamic) influence as well.

The results of this dissertation point to several avenues of further research:

- Population pharmacokinetic studies are feasible in methadone maintained populations and can be used to study other populations with unique treatment outcomes or dosing requirements.
- Direct oral versus intravenous methadone studies in Hmong to confirm their increased bioavailability compared to non-Hmong.
- Prospective study of a population upon entry into methadone maintenance to determine whether pharmacokinetic parameters could be used to predict treatment retention.
- Drug interaction studies of methadone and medications used in the treatment of HIV, hepatitis C, and tuberculosis. Currently, traditional pharmacokinetic studies are available but there is limited understanding of the wide interindividual difference in the development of symptoms due to shifts in methadone pharmacokinetics. Population pharmacokinetics would allow a larger population and combined modeling of pharmacokinetic and pharmacodynamic (e.g., opiate withdrawal scale scores or pupilometry) parameters.
- Replication in a buprenorphine (another long-acting opioid medication used in the treatment of opiate dependence) maintained population.

- Evaluation of other commonly used medications in Hmong to determine if this increase in bioavailability extends to medications other than methadone.

A limitation to the overall approach of this dissertation is that pharmacokinetic and pharmacodynamic parameters were not integrated into a single pk-pd model. Methadone dose requirements are influenced by both pharmacokinetic and pharmacodynamic factors. As demonstrated in chapter 4, methadone pharmacokinetics shows wide inter-individual variability and is influenced by ethnicity, age, weight, and genetics. We did not, however, assess methadone pharmacodynamics. Common pharmacodynamic assessment of methadone, and opiates in general, includes measures of pupil size, pain tolerance, respiratory suppression, and neuroendocrine response. Other measures more specific to patients receiving methadone for treatment of opiate addiction (as opposed to methadone for analgesia) are relief of opiate withdrawal symptoms and cross-tolerance to superimposed opiates. Evaluating how genetic variants in pharmacodynamic related genes (e.g., mu-opioid receptor gene) influence these responses may be additionally informative.

Many of the assessed pharmacodynamic end-points of methadone are influenced by medical and psychiatric comorbidities, psychosocial factors, and the use of other drugs such as alcohol, benzodiazepines, and cocaine. Therefore, future studies developing a pharmacokinetic-pharmacodynamic model of methadone will require integration of data presented in both Chapters 4 and 5. While some basic outcome data (e.g., GSI score of

the SCL-90 and urine drug screen results) were incorporated into the POPPK model and analyzed using general additive models none were influential on pharmacokinetic parameters. Modeling pharmacodynamic parameters was not performed, however.

Studies that model both methadone pharmacokinetics and pharmacodynamics may help improve treatment outcome for a disease known to increase morbidity and mortality, disrupt family networks and social function, and significantly contribute to the global burden of disease.

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Appendix A

Research Design and Methods

Based on retrospective data presented in Chapter 3, we hypothesized that pharmacokinetic differences contribute to the overall superior outcome in methadone maintained Hmong compared to non-Hmong. This overall hypothesis was tested by comparing the population pharmacokinetics of methadone in Hmong and non-Hmong (Specific Aim).

Specific Aim: To compare the pharmacokinetics of methadone (clearance, volume of distribution, and half-life) in Hmong to non-Hmong using population pharmacokinetics.

Hypothesis: In Hmong compared to non-Hmong, methadone clearance is lower.

Methods and Procedures:

Recruitment: Hmong and non-Hmong methadone maintained patients were recruited from a cross-sectional sample of patients in treatment at the Hennepin County Medical Center (HCMC) Addiction Medicine Program in Minneapolis, Minnesota. The Hmong attending the clinic have their origin in the mountains of Laos. The non-Hmong in the clinic originate mostly from the Upper Midwest and are 50% non-Hispanic Caucasian,

38% non-Hispanic African American, 4% American Indian, and 8% Other (including Hispanic Caucasian, Hispanic African American, non-Hmong Asian, and mixed). This non-Hmong population served as a comparison group and, while Hispanics were underrepresented compared to national statistics of methadone admissions (20-30% Hispanic)[17;308], their number was representative of other methadone maintained populations within Minnesota.

In order to avoid recruiting patients during the initial dose titration phase following admission, eligible subjects were required to be on methadone for at least 8-weeks prior to study. Subjects were not matched based on methadone dose or length of time in treatment. The rationale for this was that a population pharmacokinetic approach can adapt to and is even strengthened when employed in a population with a diversity of medication exposure.[241;242] Furthermore, it allowed us to expand the pool of eligible participants and simplify the recruitment process.

Inclusion/Exclusion Criteria: 1) at least 18 years of age (Federally determined lower age limit for methadone maintenance); 2) enrolled in the HCMC Addiction Medicine Program and, therefore, met Federal criteria for methadone maintenance (i.e., DSM-IV opiate dependence of at least 1-year duration); 3) subjects were taking methadone for at least 8-weeks prior to study day; 4) medical record indicated no end-stage liver disease as this would adversely affect methadone kinetics[152;202]; 5) end-stage renal disease does not appear to influence methadone kinetics and was not an exclusion criterion[201]; 6) no use of medications that significantly alter methadone metabolism, specifically phenytoin[309],

rifampin[310], and HIV medication[311] (review of the HCMC Addiction Medicine Program clinical population indicated that fewer than 5% of patients were on these medications and, therefore, excluding them did not impact the generalizability of our recruitment population); 7) medical record indicated patient was not in the second or third trimester of pregnancy as this could alter methadone kinetics[153]; 8) subjects were not enrolled if they were unable to provide informed consent (e.g., severely psychotic).

Procedure: Following consent, a blood specimen (10 ml) was drawn for methadone trough levels approximately 24-hours following a directly observed methadone dose (time of dosing data was stored in Methasoft, a computerized methadone dispensing system). Patients then received their usual methadone dose and remained in clinic for three to four hours until a second blood specimen (10 ml) was drawn for measurement of methadone peak levels. An additional blood specimen (20 ml) was collected for genetic studies. Plasma was stored at -80^{o}C until analyzed (see method below). Previous studies have confirmed that freezing and storage of specimens does not result in sample degradation.[312] A urine specimen was also collected for routine urine toxicology (EMIT). This standard test detects opiates, cocaine, amphetamines, benzodiazepines, alcohol, and the major methadone metabolite EDDP.

Patient medications were reviewed and documented. Patients had their weight and height measured for calculation of body mass index (kg/m^2). Length of time in methadone

treatment as well as length of time on current methadone dose was documented. These data were tracked within our automated dispensing system.

Analysis of methadone: Blood samples were placed on ice and, within 45 minutes of being drawn, were centrifuged at 2000 g for fifteen minutes at 4^{o} centigrade for plasma separation. Plasma was immediately stored in 2.0 mL Nunc Cryotubes (Thermo Fisher Scientific, Rochester, NY) at -80^{o} centigrade until analyzed. Plasma levels of each methadone enantiomer were determined using an LC-MS/MS protocol adapted from Foltz et al.[250] Briefly, 100 ul of human plasma was extracted with 400ul of hexane at pH 11.0 (200 ul of 500 mM sodium bicarbonate). After 10 minutes of vigorous shaking the samples were centrifuged at 12500 rpm for 5 minutes. A 250 ul aliquot of the supernatant was collected. Supernatant was dried at ambient temperature under a stream of nitrogen for 5 minutes. Dried sample were reconstituted with 100 ul of mobile phase for LC-MS/MS.

LC-MS/MS was performed using a TSQ Quantum Classic LC-MS/MS (Thermo Scientific, Waltham, MA) with Agilent 1200 HPLC (Agilent, Santa Clara, CA) and a Chiral-AGP column (5 cm x 2.0 mm, 5 um particle size, Regis Technologies, Morton Grove, IL). Calibration and quality control using R and S methadone and their tritiated counterparts (Cerilliant, Round Rock, TX) revealed an assay lower level of quantitation of 2.75 ng/ml for R-methadone and 2.25 ng/ml for S-methadone and a linear range of detection measured between 2.75-687 ng/ml and 2.25-565 ng/ml, respectively. Although

not tested beyond these ranges, Liang et al. found this methodology to be linear up to 1000 ng/ml for each enantiomer.[250] Between and within assay variability percent coefficient of variation were below 6% for both enantiomers. Assay variability and calibration curve data are presented in the tables below.

Table A.1 Between and within assay variability (QCs – ng/ml)

(R)-Methadone	**Low QC (11.01)**	**Med QC (220.11)**	**High QC (440.22)**
Mean (n=5)	10	199	407
%Bias	-9.0%	-9.4%	-7.5%
Between run %CV	5.7%	2.1%	3.4%
Within Run %CV	5.5%	2.0%	3.7%

(S)-Methadone	**Low QC (8.99)**	**Med QC (179.89)**	**High QC (359.78)**
Mean (n=5)	8	165	337
%Bias	-7.9%	-8.4%	-6.2%
Between run %CV	5.3%	2.4%	3.8%
Within Run %CV	4.7%	2.3%	3.9%

Table A.2 Calibration Curve

(R)-Methadone	**Slope**	**Intercept**	**R-Squared**
Mean (n=5)	0.01016874	0.002459638	0.9994
S.D	9.06928E-05	0.001216237	0.00043589
%CV	0.9%	49.4%	0.04%

(S)-Methadone	**Slope**	**Intercept**	**R-Squared**
Mean (n=5)	0.01236746	0.002920947	0.99942
S.D	0.00021938	0.001567568	0.000535724
%CV	1.8%	53.7%	0.05%

Table A.3 Back calculated values (units ng/ml)

(R)-Methadone	TRUE	Mean (n=5)	%CV	%Bias
	2.75	2.78	6.6%	0.9%
	5.5	5.5	6.1%	0.3%
	27.5	27.3	4.3%	-0.6%
	137.6	139.2	3.2%	1.2%
	275.1	274.9	2.0%	-0.1%
	412.7	403.9	2.3%	-2.1%
	550.3	561.9	1.7%	2.1%
	687.8	685.5	2.3%	-0.3%

(S)-Methadone	TRUE	Mean (n=5)	%CV	%Bias
	2.25	2.28	4.2%	1.2%
	4.5	4.5	4.6%	0.0%
	22.5	22.3	4.2%	-0.9%
	112.4	114.7	4.6%	2.0%
	224.9	225.6	1.7%	0.3%
	337.3	329.2	1.3%	-2.4%
	449.7	458.9	2.1%	2.0%
	562.2	560.4	2.1%	-0.3%

Urine drug screening: Urine specimens collected on day of study were analyzed for amphetamine, benzodiazepine, barbiturates, cocaine, and opiates using a commercial immunoassay (EMIT, Beckman, Brea, CA). The presence of methadone metabolite (EDDP) in urine was also determined (CEIDA, Microgenics, Fremont, CA). All urine drug screening was performed on site in a CLIA and College of American Pathologists certified laboratory at the Hennepin Faculty Associates (Minneapolis, MN).

Population Pharmacokinetic Analysis For determination of population pharmacokinetics, a nonlinear mixed-effects modeling (NONMEM) approach was used with a sparse sampling design. All methadone concentrations from all subjects were simultaneously analyzed, and the experimental unit was the population; this contrasts

with conventional PK analyses in which the individual is the unit of experimentation. One advantage of this approach was that it allowed for sparse sampling within individuals. Another advantage was that the kinetic modeling was strengthened by having subjects with a wide range of medication exposure making it ideal for cross-sectional studies containing subjects who have been in treatment for differing lengths of time. The trade-off was that a sufficient number of individuals needed to be included to characterize the population. It has been shown that bias and precision are acceptable as sample size increases above 50 subjects.[313-316] This approach has been validated for kinetics of total methadone and each of its enantiomers in a methadone maintained population that underwent both dense and sparse sampling strategies.[239]

The software package NONMEM 7.2.0 and PDx-Pop 5.0 (both Icon Development Solutions, Ellicott City, MD) is tailored specifically for population pharmacokinetic applications and was used for these analyses. The mean, noted as a "typical value" (TV) in NONMEM terminology, of each pharmacokinetic parameter, and the variance of that parameter, frequently expressed as a coefficient of variation (CV), were the population parameters of interest. For example, the typical value of clearance (TVCL) in the population, and its variance, were estimated. In addition, relationships between a parameter and subject-specific covariates (age, weight, sex, ethnicity, concomitant drug use, etc.) were identified. Although there is flexibility in modeling, continuous covariates such as age and weight entered the model in an additive way. For example, when

examining the relationship between weight (WT) and clearance, the following model might be used, where the THETAs represent regression parameters.

```
TVCL = THETA(1) + THETA(2)*WT
```

Categorical covariates typically enter the model in a multiplicative way. For example, if sex is being examined, the following model may be used, where THETA(2) is the fractional change in clearance due to SEX.

```
TVCL = THETA(1)
IF (SEX.EQ.1) TVCL = TVCL*THETA(2)
```

In a population model, variance parameters usually enter the model in an exponential way, reflecting a typical underlying log-normal distribution of physiological parameters. So the population model for clearance might be

```
TVCL = THETA(1)
CL = TVCL * EXP(ETA(1))
```

Where THETA(1) is the regression parameter for the population average clearance, and the ETA is the regression parameter characterizing the variability (CV) of clearance in the population.

In this study, we hypothesized that methadone clearance is decreased in Hmong compared to non-Hmong. The examples above were extended to specifically test this hypothesis as follows.

```
TVCL = THETA(1)
IF (ETHNICITY.EQ.HMONG) TVCL = TVCL*THETA(2)
```

This model provided an estimate of the fractional difference in clearance (THETA(2)) between Hmong and non-Hmong.

Hypothesis testing of competing models was based on the Likelihood Ratio Test which examines the difference in the objective function values (OFV; similar to a sum-of-squares metric) between two nested models. The difference in OFV is approximately Chi-Square distributed with degrees of freedom equal to the difference in the number of parameters in two nested models. For alpha=0.05 and 1 degree of freedom, the OFV needs to decrease by 3.8 units to declare the larger model is significantly better than the smaller nested model. In this example, the model that doesn't include ethnicity [TVCL = THETA(1)] is fully nested within the larger model with ethnicity. In this model, if adding the covariate (ETHNICITY.EQ.HMONG) decreases the OFV by more than 3.8 units, we would conclude ethnicity was a significant determinant of clearance. Relationships with other covariates were similarly tested.

All analyses in NONMEM were carried out with the First Order Conditional Estimation method with Interaction (FOCE-I). Maximum *a posteriori* Bayesian *post hoc* estimates of each individual's pharmacokinetic parameters were obtained. These empirical Bayes parameter estimates were used to calculate, for each individual, clearance and volume of distribution.

Methadone is a racemic mixture so measurement of methadone exposure is insufficient to detect potential pharmacokinetic differences between enantiomers. In order to take this into account, we quantified both the R-methadone and S-methadone enantiomers. For the POPPK analyses then, the kinetics of R-methadone and S-methadone were separately determined.

Descriptive Measures For descriptive and exploratory hypothesis generating purposes, patients underwent the following assessments during the interval between blood draws for trough and peak methadone levels:

The Structured Clinical Interview for DSM-IV Axis I Disorders provides the "gold standard" for current and lifetime DSM-IV diagnoses for axis I disorders.[248]

The Symptom Checklist-90 (SCL-90) is a measure of psychopathology used in clinical and research settings.[249;285] It has been used in methadone patients and in Hmong.[286;287] It

is a self-administered assessment that covers a range of psychopathology (e.g., depression, anxiety, obsessive compulsive, somatization, phobic anxiety, paranoid ideation, hostility, interpersonal sensitivity, and psychoticism). It provides quantitative data that allows for comparisons between and within groups.

The Addiction Severity Index (ASI) is a semi-structured interview that is widely used in clinical and research settings.[247] The ASI covers seven dimensions (e.g., medical, employment, alcohol, drug, legal, family/social, and psychiatric) that may be related to treatment progress and outcome. Past 30 days ratings can be codified using the ASI Composite Index Scores.[291] These allow researchers to develop empirically-validated measures of current functioning.

Hmong/American Acculturation Scale is a structured two-component scale that measures Hmong cultural affiliation and acculturation to American society. Previous work in Hmong has shown that difficulties in acculturation contribute to mental health related illness[304], which may affect methadone maintenance treatment outcome.

Descriptive Statistics We evaluated differences in demographic (age, gender) and psychosocial factors (ASI composite scores, SCID, SCL-90) between Hmong and non-Hmong.

Limitations in Ethnic Comparisons A limitation of this study was the comparison of a homogenous Hmong population to a heterogeneous non-Hmong population. Microsatellite data indicate that the Hmong are a distinctly homogenous population.[317;318] While haplotype analyses show separation between Caucasians and Africans there remains a fair amount of heterogeneity within each group.[319] It was, however, not clinically feasible to find a homogenous comparison population for the Hmong. Instead, the non-Hmong were used as the main comparison group although ethnicity (e.g., Caucasian, African-American, etc.) was evaluated as a covariate in the population pharmacokinetic (POPPK) analyses.

The methods used in this study were appropriate for population estimates of methadone kinetics but were not as strong as direct estimates that can obtained from individuals through dense sampling. The intensity of dense sampling, however, means that often only a small number of individuals can be studied. Most previous methadone pharmacokinetic studies, therefore, have included only 5-25 subjects making analysis of population based variability difficult.[320-322] The strengths of a POPPK approach, however, offset this limitation because, through it, we were able to access a larger population (an outpatient setting) and to study a group (the Hmong) that would otherwise not participate in dense sampling protocols. Furthermore, population pharmacokinetics is not dependent upon utilizing each sample and accommodates missing data into its modeling by fitting data that are present into Bayesian estimates to establish expected values.

Estimation of clearance was expressed as clearance over the fraction of methadone absorbed following oral dosing (CL/F). This is standard convention for pharmacokinetic studies of orally administered medications. Since methadone has >90% oral bioavailability[323], absorption was expected to be uniform with little variability.

There are no specific *a priori* power calculations for nonlinear mixed-effects models. However, methodology from simulation studies indicate that with fifty subjects per group, a 30% difference in a parameter (such as CL or AUC) with 30% interindividual variability can be generally detected with an alpha of 0.05 and a power of 0.80.[324] The sample size of this study was considerably larger than fifty making it likely that the risk for Type I and II error has been minimized.

This study was performed in an outpatient setting. While, compliance with methadone dosing in the days prior to testing could not be guaranteed it was unlikely as Federal regulations for methadone treatment require directly observed dosing 6 days/week during the first 3 months in treatment, 5 days/week during the second 3 months, 4 days/week during the third 3 months, and 1 day/week for months 9-12. After 1-year in treatment, directly observed dosing must occur 2 days/month and after 2 years in treatment directly observed dosing must occur on at least 1 day/month. We, therefore, had extensive computerized dispensing records of directly observed methadone dosing for most subjects. These records document time and amount of all methadone doses an individual

received. We could not, however, determine if some subjects also used illicitly obtained supplemental methadone.

Not all of the proposed descriptive measures have been validated in the Hmong population. The SCL-90 has been validated in the Hmong.[287;304] Neither the ASI nor SCID (although point prevalence of DSM diagnoses in general Hmong population has been assessed) have been validated in Hmong.[303] Since these measures were used in an exploratory manner, this was not a major limitation to the assessment of outcomes measures.

Appendix B

Genetic influence on methadone pharmacokinetics: Methods and genotyping data

Whole blood drawn for genetic analysis was immediately shipped to the Rutgers Cell and DNA Repository for DNA extraction on an AutoPure LS automated DNA extractor using the Puragene Reagent System (GENTRA Systems, Qiagen, Valencia, CA). In short, RNase was added to the WBC lysis stage with isopropanol precipitation of the DNA and resuspension in 1X TE buffer (pH 8.0). This methodology routinely provides high molecular weight DNA of 20 kb or greater with an average yield of 30 μg/ml whole blood.

Purified DNA (mean concentration 116 ng/μl) was shipped to the University of Minnesota for sequencing of twenty-one single nucleotide polymorphisms across a number of genes whose products are implicated in methadone pharmacokinetics (see table B.1). Primer design (table B.2) and DNA sequencing was performed using Sequnom's (San Diego, CA) iPLEX Gold reaction and MassARRAY System for MALDI-TOF (Matrix-assisted laser desorption ionization - time of flight) mass spectrometry based sequencing.

Table B.1 Selected Polymorphisms for genetic analysis

Gene	SNP	Functional Effect	Nucleotide Change	Amino Acid Change	Citation(s)
CYP3A4	rs2740574	↓ activity	-392A>G	NA	251‡
	rs28371759	↑ activity	20070T>C	L293P	252
	rs4986909	↓ activity	22026C>T	P416L	253
CYP2B6	rs3211371	↑ activity	25505C>T	R487C	254‡
	rs3745274	↓ activity	516G>T	Q172H	255‡
	rs2279343	↑ activity	785A>G	K262R	256
	rs8192709	unknown	64C>T	C22R	257
CYP2D6	rs1065852	splice defect	100C>T	P34S	258
	rs5030656	↓ activity	2615-2617delAAG	K281del	259†
CYP2C19	rs3758581	↑ activity	80161A>G	I331V	260
ABCB1	rs1045642	↑expression	3435C>T	NA	251‡
	rs6949448	unknown	86979750A>G	NA	261‡
	rs2235067	unknown	86987858G>A	NA	261‡
	rs2032582	↓ activity	2677G>A/T	S893A/T	261‡
	rs1922242	unknown	87011603A>T	NA	261‡
	rs1128503	↑ activity	1236C>T	NA	261
	rs2520464	unknown	87039022 A>G	NA	261‡
	rs3789243	unknown	87058822C>T	NA	261‡
	rs9282564	↑ activity	61A>G	N21D	251‡
CYP1A2	rs762551	↑ inducibility	-163C>A	NA	263

‡implicated in methadone dose, level, or effect

Table B.2

Gene	rs number: primer sequence
CYP3A4	
	rs2740574: aatttcaagtattttggaatgaggacagccatagagacaagggca**[**A/G**]**gagagaggcgatTTAATAGATT TTATGCCAATGGCTCCACTTGAG
	rs28371759: GATTTACCTAAAATGTCTTTCCTCTCCTTTCAGCTCTGTCCGATC**[**C/T**]**GG AGCTCGTGGCCCAATCAATTATCTTTATTTTTGCTGGCTATGA
	rs4986909: TTCACCGTGACCCAAAGTACTGGACAGAGCCTGAGAAGTTCCTCC**[**C/T**]**T GAAAGGTACAAGG**Y**CCCTGGGAAGGGAGCCCTCCCTGAACCAGC
CYP2B6	
	rs3211371: CCCCAGGAGTGTGGTGTGGGCAAAATACCCCCAACATACCAGATC**[**C/T**]** GCTTCCTGCCCCGCTGAAGGGGCTGAGGGAAGGGGGTCAAAGGAT
	rs3745274: CTGCTTCTTCCTAGGGGCCCTCATGGACCCCACCTTCCTCTTCCA**[**G/T**]**TC CATTACCGCCAACATCATCTGCTCCATCGTCTTTGGAAAACGA
	rs2279343: ACAGTGTGGAGAAGCACCGTGAAACCCTGGACCCCAGCGCCCCCA**[**A/G**]** GGACCTCATCGACACCTACCTGCTCCACATGGAAAAAGTGGGGTC
	rs8192709: CTCTTCCTTGCACTCCTCACAGGACTCTTGCTACTCCTGGTTCAG**[**C/T**]**GC CACCCTAACACCCATGACCGCCTCCCACCAGGGCCCCGCCCTC
CYP2D6	
	rs1065852: CTGGTGGACCTGATGCACCGGCGCCAACGCTGGGCTGCACGCTAC**[**C/T**]**C ACCAGGCCCCCTGCCACTGCCCGGGCTGGGCAACCTGCTGCATG
	rs5030656: CAGCCCCCCCGAGACCTGACTGAGGCCTTCCTGGCAGAGATGGAG**[**- /AAG**]**GTGAGAGTGGCTGCCACGGTGGGGGGCAAGGGTGGTGGGTTGA

CYP2C19	
	rs3758581: ACTTGTGTCTTGTCAGCTAAAGTCCAGGAAGAGATTGAACGTGTC**[**A/G**]**T TGGCAGAAACCGGAGCCCCTGCATGCAGGACAGGGGCCACATGC
ABCB1	
	rs1922242: GTTAAAAACTTTTATATGTACAATTCTTACATACGCACAAAAATT**[**A/T**]**G TAAAGGAATATATCCTATCCTTATTCCTTATCAATGAATAAGTG
	rs6949448: gctcttgaggccattctaatattcaccgaagctttagaaccacCA**[**C/T**]**TTCAGGTcaatgtttttcaacatggga ttcaaggtccactgatgg
	rs2235067: TTGAGAAACAGTTGTAATTATGCAGGAGAGAAAGTACAAGACCCT**[**A/G**]** AACTAAGGCAGGGACATCTCTGAGGTAGAACCTGTAAGAATGGGT
	rs2032582: ATGTTGTCTGGACAAGCACTGAAAGATAAGAAAGAACTAGAAGGT**[**A/G/ T**]**CTGGGAAGGTGAGTCAAACTAAATATGATTGATTAATTAAGTA
	rs1045642: CATTGCCTATGGAGACAACAGCCGGGTGGTGTCACAGGAAGAGAT**[**A/C/ T**]**GTGAGGGCAGCAAAGGAGGCCAACATACATGCCTTCATCGAGT
	rs1128503: TGCCTTGAAGTTTTTTTCTCACTCGTCCTGGTAGATCTTGAAGGG**[**C/T**]**CT GAACCTGAAGGTGCAGAGTGGGCAGACGGTGGCCCTGGTTGGA
	rs2520464: tcctaaatctttagatctgaactgtctctaaaactggaaagttac**[**A/G**]**gtctgaatacttattggacatcttcaattgaatgt gccaggcact
	rs3789243: ATAAGTCTCAACATTCTCTGACTGCTTCAGTTCCAACAACGACGC**[**C/T**]**C CATAAATTACATGAGTACCTTAGTAAATTGCACGTGTGGACAGG
	rs9282564: GACCGCAATGGAGGAGCAAAGAAGAAGAACTTTTTTAAACTGAAC**[**A/G**]** ATAAAAGGTAACTAGCTTGTTTCATTTTCATAGTTTACATAGTTG
CYP1A2	
	rs762551: ccagctctcagattctgtgatgctCAAAGGGTGAGCTCTGTGGGC**[**A/C**]**CAGGACGCAT GGTAGATGGAGCTTAGTCTTTCTGGTATCCAGCTG

Rationale for this limited selection of genes and SNPs is presented below. Tables of population variation in selected SNPs include a population reference mostly from the HapMap Project followed by genotype frequencies from the current study.

ABCB1 (ATP-binding cassette, subfamily B, member 1) gene is located on chromosome 7 (7q21.12) and consists of 28 exons extending over 200KB. It encodes the P-glycoprotein efflux transporter which is widely expressed in the intestinal lumen, renal proximal tubule, and blood-brain barrier.[325] Since methadone is a substrate for this transporter, polymorphisms effecting its expression and function could influence methadone exposure through alterations in oral bioavailability or in transfer across the blood-brain barrier. For example, the PGP inhibitors verapamil and quinidine can increase methadone transport across intestinal membrane in an *in vitro* everted gut sac model; and in an *ABCB1* mouse knock out model brain levels of methadone are increased more than 15-fold.[266;326]

We evaluated the effect of nine single nucleotide polymorphisms in *ABCB1* on methadone pharmacokinetic parameters. These SNPs were chosen because prior literature had linked them to functional effects on PGP efflux or methadone transport in particular.

rs1045642: This synonymous (Ile1145Ile) coding region SNP at position 3435C>T of exon 26 has been associated with decreased protein expression and function.[327-329] Thus homozygotes for this variant have increased plasma levels of PGP substrates such as digoxin. Levran et al. evaluated methadone dose requirements, dichotomized as high dose (> 150 mg) and low dose (< 150 mg) and found a trend ($p = 0.054$) towards low dose for TT genotypes but following correction for multiple testing, significance was lost ($p = 0.1977$).[261]

Data from the 1000 Genomes dataset indicate allele distribution of 3435C>T

	African	Asian HCB	European
C*	.883	.600	.466
T	.117	.400	.534
CC	.783	.40	.15
CT	.20	.40	.63
TT	.017	.20	.22

* Reference allele; HCB: Han Chinese

Data from the current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
CC	11	32	9	30	2
CT	37	9	10	30	1
TT	13	2	2	11	0

rs1128503: This is a synonymous (Gly412Gly) coding region SNP at position 1236C>T of exon 13 of the *ABCB1* gene. There is no clear consensus that this SNP has a functional effect as some have reported decreased PGP expression in homozygotes for the C allele with corollary improved glioblastoma survival following temozolomide therapy while others have reported increased irinotecan exposure in those homozygous for the T allele.[330;331]

Data from HapMap Project:

	African	Asian HCB	European
C*	.876	.291	.549
T	.124	.709	.451
CC	.752	.093	.265
CT	.248	.395	.566
TT	0	.512	.168

Data from current study:

	Caucasian*	African American	American Indian	Hmong	Hispanic
CC	19	32	3	5	1
CT	37	10	13	32	2
TT	5	1	5	35	0

* Not in Hardy-Weinberg equilibrium (HWE), $\chi^2 = 4.8$

rs2032582: Is a triallelic non-synonymous (Ala893Thr/Ser) coding region SNP at position 2677G>A/T of exon 22 of the *ABCB1* gene. The A allele is relatively uncommon and HapMap database populations with this allele have not been identified. The G allele encodes alanine, the A allele encodes threonine, whereas the T allele encodes serine. There are mixed reports on the effect of the T allele with increased, decreased, and no change in drug exposure or effect.[332]

Data from the HapMap Project:

	African (ASW)	Asian HCB	European
G*	.929	.384	.531
T	.071	.646	.469
A	0	0	0
GG	.857	.093	.257
GT	.143	.581	.549
TT	0	.326	.195

ASW: American Southwest

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AA, AT, TT	7	0	2	21	1
AG, GT	34	7	11	32	0
GG	21	37	8	19	2

The three abovementioned SNPs (1236C>T, 2677G>T, and 3435C>T) are in linkage disequilibrium and comprise two major haplotypes, TTT and CGC. The TTT haplotype significantly decrease PGP substrate transcellular transport in an *in vitro* model.[262] Levran et al. found that methadone maintained patients with the TTT haplotype were five times more likely to require doses greater than 150 mg daily than those with other haplotypes.[261]

rs9282564: Is a non-synonymous (Asn21Asp) coding region SNP at position 61A>G of exon of the *ABCB1* gene. Crettol et al. found that the variant allele had a modest effect in lowering trough levels of methadone but had no effect on peak levels.[251]

Data from HapMap Project:

	African	Asian HCB	European
A*	1	1	.90
G	0	0	.10
AA	1	1	.817
AG	0	0	.167
GG	0	0	.017

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AA	48	43	19	71	3
AG	12	1	2	1	0
GG	1	0	0	0	0

rs1922242: Is an intronic SNP at position 87011603A>T in intron 16 of *ABCB1* gene. While there is no known functional effect of this SNP, it has been associated with more

severe depression scores but not treatment outcome for depressed patients receiving escitalopram.[333]

Data from HapMap Project:

	African	Asian HCB	European
A*	.567	.711	.567
T	.433	.289	.433
AA	.30	.489	.317
AT	.533	.444	.50
TT	.167	.067	.183

Data from current study:

	Caucasian	African American*	American Indian	Hmong	Hispanic
AA	18	15	7	45	0
AT	31	15	13	23	2
TT	12	14	1	3	1

* Not in Hardy-Weinberg equilibrium (HWE), $\chi^2 = 4.44$

rs2235067: Is an intronic SNP at position 86987858G>A in intron 22 of the *ABCB1* gene. Levran et al. found no association between this SNP and methadone dose requirement.[261]

Data from HapMap Project:

	African	Asian HCB	European
G*	.748	.854	.571
A	. 252	. 146	. 429
GG	. 566	.93	.717
GA	.363	.07	.274
AA	.071	0	.009

Data from current project:

	Caucasian	African American	American Indian	Hmong	Hispanic
GG	2	32	16	63	3
GA	14	11	4	8	0
AA	45	0	0	0	0

rs2520464: Is an intronic SNP at position 87039022A>G in intron 4 of the *ABCB1* gene.

Data from HapMap Project:

	African	Asian HCB	European
A*	.173	.721	.447
G	.827	.279	.553
AA	.044	.512	.168
AG	.257	.419	.558
GG	.699	.174	.274

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AA	6	3	5	36	0
AG	35	10	12	35	2
GG	19	30	4	5	1

rs3789243: Is an intronic SNP at position 87058822C>T in intron 3 of the *ABCB1* gene. This SNP has been associated with drug resistant epilepsy in a Han Chinese population. It is unclear whether there is direct influence of this SNP on PGP function or whether this SNP is in linkage disequilibrium with other functional variants.[334] Levran et al. found no association between this SNP and methadone dose requirement.[261]

Data from HapMap Project:

	African	Asian HCB	European
C*	.394	.721	.491
T	.606	.279	.509
CC	.159	.488	.196
CT	.469	.465	.589
TT	.372	.047	.214

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
CC	13	12	3	21	1
CT	32	23	11	41	0
TT	16	8	7	9	2

rs6949448: Is an intronic SNP at position 86979750C>T in intron 25 of the *ABCB1* gene.

Levran et al. found no association between this SNP and methadone dose requirement.[261]

Data from HapMap Project:

	African	Asian HCB	European
C*	.881	.547	.544
T	.119	.453	.456
CC	.788	.326	.274
CT	.186	.442	.540
TT	.027	.233	.186

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
CC	23	30	10	27	2
CT	32	12	9	31	1
TT	7	2	2	14	0

Cytochrome P450 2B6 is one of the major isoforms involved in methadone metabolism. Recent work has identified possible stereoselectivity in methadone metabolism for CYP2B6. In an *in vitro* human liver microsomal assay, CYP2B6 selectively metabolized S-methadone whereas CPY3A4 had no such stereoselectivity.[272] CYP2B6 is generally expressed at a lower level than CYP3A4; however, after controlling for protein content, stereoselective metabolism remained, although to a lesser extent.[271;273] Clopidogrel, a selective inhibitor of CYP2B6, eliminated stereoselective methadone metabolism whereas troleandomycin, a selective CYP3A4 inhibitor, had no effect on stereoselective methadone metabolism.[273] In clinical studies, troleandomycin had no effect on stereoselective methadone disposition, whereas the non-selective CYP3A4 and CYP2B6 inducer rifampicin, increased the ratio of R- to S- methadone.[273] Given these findings, genetic polymorphisms that alter levels of CYP2B6 protein expression or function could result in clinically apparent stereoselective methadone metabolism. We evaluated the effect of select SNPs in CYP2B6 on methadone pharmacokinetics and hypothesized that change in CYP2B6 function would selectively influence S-methadone pharmacokinetics with no effect on R-methadone pharmacokinetics.

rs2279343: is a non-synonymous (Lys262Arg) SNP at position 792A>G in exon 5 of the *CYP2B6* gene and is used primarily to identify the *CYP2B6*4* variants but is also present in *CYP2B6*6* and *CYP2B6*7* variants. A human liver microsome assay with CYP2B6 expressed by this variant did not show alterations in stereoselective methadone metabolism.[273]

Data from European Genome Project:

	African American	Asian	European
A*	.538	.812	.786
G	.462	.188	.214
AA	.385	.708	.619
AG	.308	.208	.333
GG	.308	.083	.048

Data from current study:

	Caucasian	African American	American Indian	Hmong*	Hispanic
AA	31	24	13	28	1
AG	24	16	7	40	2
GG	4	4	1	3	0

* Not in Hardy-Weinberg equilibrium (HWE), $\chi^2 = 5.8$

rs3211371: is a non-synonymous (Arg487Cys) SNP at position 1459C>T in exon 9 of the *CYP2B6* gene. The variant is used to identify *CYP2B6*5* and *CYP2B6*7* variants. The variant allele has been associated with reduced protein expression and mephenytoin metabolism in a human liver microsomal assay.[257]

Data from European Genome Project:

	African American	Asian	European
C*	1	.958	.909
T	0	.042	.091
CC	1	.917	.818
CT	0	.083	.182
TT	0	0	0

Data from current study:

	Caucasian*	African American*	American Indian*	Hmong*	Hispanic
CC	0	0	0	0	0
CT	48	41	18	72	2
TT	14	3	3	0	1

*Not in HWE for any ethnicity

rs3745274: is a non-synonymous (Gln172His) SNP at position 516G>T in exon 4 of the *CYP2B6* gene. The variant is present in the *CYP2B6*6* variant. The variant allele is associated with increased efavirenz exposure and neuropsychiatric effects. A methadone maintained Chinese Han population with the variant required lower methadone doses than those without the variant.[270] Haplotypes containing this variant were also associated with higher trough S-methadone and a greater increase in S-methadone peak trough ratio compared to R-methadone peak trough ratio.[26;251]

Data from HapMap Project:

	African	Asian HCB	European
G*	.58	.849	.73
T	.42	.151	.27
GG	.339	.721	.531
GT	.482	.256	.398
TT	.179	.023	.071

Data from current study:

	Caucasian	African American	American Indian	Hmong*	Hispanic
GG	36	24	12	29	1
GT	21	16	6	40	2
TT	5	4	3	3	0

* Not in Hardy-Weinberg equilibrium (HWE), $\chi^2 = 5.6$

rs8192709: is a non-synonymous (Arg22Cys) SNP at position 64C>T in exon 1 of the *CYP2B6* gene. It is used to identify the *CYP2B6*2A* variant. There have been no direct reports of this SNP on methadone pharmacokinetics.

Data from HapMap Project:

	African	Asian HCB	European
C*	.954	.976	.964
T	.046	.024	.036
CC	.907	.952	.928
CT	.093	.048	.072
TT	0	0	0

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
CC	54	43	20	67	3
CT	7	1	1	4	0
TT	0	0	0	0	0

Cytochrome P450 2D6 The CYP2D6 isoform is minimally involved in methadone metabolism.[335] It does, however, have well characterized functional variants that alter metabolism of several drugs including codeine.[336] These variants lead to poor substrate metabolism, extensive substrate metabolism, or ultrarapid substrate metabolism such that poor metabolizers have lower analgesic response to codeine whereas ultrarapid metabolizers are more susceptible to the intoxicating effects of the codeine metabolite morphine.[337;338] There is ethnic variation in these variants with approximately 10% of

Caucasians being poor metabolizers and 25% of Africans being ultrarapid metabolisers.[339]

rs1065852: is a non-synonymous (Pro34Ser) SNP at position 100C>T in exon 1 of the *CYP2D6* gene. It is used to identify the reduced function *CYP2D6*4* variant and, when 1846G>A is absent, it denotes a loss of function, poor metabolizer, *CYP2D6*10* variant. Eap et al., found significantly higher methadone levels in poor metabolizers compared to ultrarapid metabolizers but no difference between the poor and wild type extensive metabolizer groups.[340] De los Cobos, et al., however, did not find concentration or dose requirement differences based on this genotype.[341]

Data from European Genome Project:

	African American	Asian
C*	.90	.543
T	.10	.457
CC	.867	.261
CT	.067	.565
TT	.067	.174

Data from current study:

	Caucasian	African American	American Indian	Hmong*	Hispanic
CC	42	34	18	15	3
CT	20	9	3	56	0
TT	0	0	0	0	0

* Not in Hardy-Weinberg equilibrium (HWE), $\chi^2 = 30.1$

rs5030656: is a deletion (2615-2617del AAG) polymorphism in exon 5 that results in loss of lysine at position 281. It is used to identify the *CYP2D6*9* variant, which results in decreased protein expression and, thus possibly a poor metabolizer phenotype.[342]

No population-based reference data (e.g., HapMap Project) are available for this polymorphism.

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AAG*	58	44	21	71	3
AAGdel	3	0	0	0	0

Cytochrome P450 3A4 Until recently, this isoform has been considered the main enzyme responsible for methadone metabolism.[192;343] *In vitro* models show that it N-demethylates methadone in a non-stereoselective manner.[199;200;343] *In vivo* studies, however, have not shown significant influence of CYP3A4 inhibition on methadone pharmacokinetics. Paradoxically, some medications known to inhibit CYP3A4 appear to increase methadone clearance while others known to induce CYP3A4 have no effect on methadone pharmacokinetics.[192;198;344] This conflicting literature may, in part, be explained by substrate effects on other CYP isoforms (e.g., CYP2B6) as few are CYP3A4 selective.

rs2740574: is a 5'-flanking region SNP (-392A>G) that is used to identify the *CYP3A4*1B* and *CYP3A4*15* variants. Indinavir exposure was reduced by nearly 65% in patients homozygous for this variant.[345] While CYP3A4 does not appear to have stereoselectivity in *in vitro* models, Crettol et al. found that carriers of this variant had higher levels of S-methadone at peak and at trough than non-carriers but that there was no difference in levels of R-methadone.[251]

Data from European Genome Project:

	African American	Asian	European
A*	.321	1	.977
G	.379	0	.023
AA	.071	1	.955
AG	.50	0	.045
GG	.429	0	0

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AA	51	8	19	70	2
AG	11	25	2	2	1
GG	0	11	0	0	0

rs28371759: is a non-synonymous (Leu293Pro) SNP at position 20070T>C in exon 10 of the *CYP3A4* gene and identifies the *CYP3A4*18* variant. This variant has only been identified in Asian population and is thus of particular interest to this study. It is associated with a rapid metabolizer phenotype as measured by testosterone in a cellular construct.[252] In humans, however, the variant is associated with reduced midazolam clearance and metabolite formation.[346]

No population-based reference data (e.g., HapMap Project) are available for this polymorphism.

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
CC	0	0	0	0	0
CT	0	0	0	3	0
TT	61	44	21	68	3

rs4986909: is a non-synonymous (Pro416Leu) SNP at position 22026C>T in exon 11 of the *CYP3A4* gene and identifies the *CYP3A4*13* variant. However, no carriers of this variant were identified in this study so it will not be further discussed.

No population-based reference data (e.g., HapMap Project) are available for this polymorphism.

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
CC	61	43	21	71	3
CT	0	0	0	0	0
TT	0	0	0	0	0

Cytochrome P450 2C19 This isoform metabolizes methadone with stereoselectivity for the R-enantiomer.[199;272] Because expression of CYP2C19 relative to CYP2B6 and

CYP3A4 is low, the clinical role of CYP2C19 in methadone metabolism is minimal. Selective CYP2C19 inhibition with S-mephenytoin did not significantly alter methadone metabolism in a human liver microsomal assay.[199]

rs3758581: is a non-synonymous (Ile331Val) SNP at position 85161A>G in exon 7 of the CYP2C19 gene. Only three carriers of the variant allele were identified in this study so it will not be further discussed.

No population-based reference data (e.g., HapMap Project) are available for this polymorphism.

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AA	0	0	0	0	0
AG	0	0	0	3	0
GG	60	43	18	66	2

Cytochrome P450 1A2 This isoform is primarily involved in metabolism of tricyclic antidepressants and caffeine. It is induced by tobacco and inhibited by ciprofloxacin and fluvoxamine. Case reports of methadone patients experiencing sedation following initiation of ciprofloxacin or fluvoxamine has led to consideration of this isoform in methadone metabolism.[347;348] There is no *in vitro* data to support this isoform as being significantly involved in methadone metabolism, however.[199] *In vivo* assessment of

CYP1A2 activity as measure by salivary caffeine half-life, failed to show an effect of this isoform on steady-state methadone metabolism.[280]

rs762551: is a flanking sequence SNP -163C>A of the *CYP1A2* gene and is used to identify the *CYP1A2*1F* variant. This is a widely studied variant and is associated with increased caffeine metabolism in Caucasian smokers but it is not clear whether this is due to sole effect of the variant, linkage disequilibrium with other variants, and/or gene-environment interactions.[263] Crettol et al. did not find this variant to influence plasma levels of methadone.[251]

Data from HapMap Project:

	African	Asian HCB	European
A*	.566	.667	.721
C	.434	.337	.279
AA	.336	.419	.531
AC	.46	.488	.381
CC	.204	.093	.088

Data from current study:

	Caucasian	African American	American Indian	Hmong	Hispanic
AA	23	17	6	29	3
AC	34	18	12	30	0
CC	4	9	3	12	0

Appendix C

NONMEM control stream for the final model of the population pharmacokinetics of R-methadone

```
$PROB RUN# 224
Output Extracted from file: c:\pdxpop5\rmethrev2\224.res
DataFile:  METHADONEREV2.CSV

MODEL DEFINITION:
 ADVAN2 TRANS2

IF(AGE/40.LE.0) EXIT 1 100
IF(BMI/25.LE.0) EXIT 1 200
TVCL=THETA(1)
TVCL=TVCL*(AGE/40)**THETA(4)
TVCL=TVCL*(BMI/25)**THETA(5)
IF(ABC1.EQ.2) TVCL=TVCL*THETA(6)
  CL=TVCL*EXP(ETA(1))
TVV=THETA(2)
  V=TVV
TVKA=THETA(3)
   KA=TVKA
TVF1=1.0
IF(RACE.EQ.4) TVF1=TVF1*THETA(7)
IF(CYP1.EQ.2)TVF1=TVF1*THETA(8)
F1=TVF1*EXP(ETA(2))
   S2=V/1000
IF (AMT.GT.0) THEN
TDOS=TIME
TPD=0.0
ENDIF
IF (AMT.EQ.0) TPD=TIME-TDOS
SID=ID
DELC=CL-TVCL
DELV=V-TVV
DELF=F1-TVF1

   IPRE=F
```

Y = F + F*ERR(1)

Key to control stream code:

BMI = Body Mass Index

CYP2B615631 = CYP2B6 516 SNP

ABCB1.EQ.2 = *ABCB1* 2677 GG

RACE.EQ.4 = Hmong

CYP1.EQ.2 = *CYP1A2* -163 CC

Appendix D

NONMEM control stream for the final model of the population pharmacokinetics of S-methadone

```
$PROB RUN# 102
$INPUT C ID TIME TAD NTIM EVID AMT SS II
        DV RACE AGE BMI ABCB12677 CYP2B615631
$DATA METHADONEREVS.CSV IGNORE=C
$SUBROUTINES ADVAN2 TRANS2
$PK
        TVCL=THETA(1)*(AGE/40)**THETA(4)*(BMI/25)**THETA(5)
        IF(ABCB12677.EQ.2) TVCL=TVCL*THETA(6)
        IF(CYP2B615631.EQ.1)TVCL=TVCL*THETA(7)
        IF(CYP2B615631.EQ.2)TVCL=TVCL*THETA(8)
          CL=TVCL*EXP(ETA(1))
        TVV=THETA(2)
          V=TVV;;;;;*EXP(ETA(2))
        TVKA=THETA(3)
           KA=TVKA
        TVF1=1.0
        IF(RACE.EQ.4) TVF1=TVF1*THETA(9)
        F1=TVF1*EXP(ETA(2))
           S2=V/1000
        SID=ID
$ERROR
        IPRE=F
        Y = F + F*ERR(1)
$THETA
      (0, 10);[CL]
      (0, 400);[V]
      (1.5 FIX);[KA]
      (-5,-1,0) ;[CL AGE]
      (-5,0.01,5) ;[CL BMI]
      (0,1)  ;[CL ABCB12677 2]
      (0,1)   ;[CL CYP2B615631 1]
      (0,1)   ;[CL CYP2B615631 2]
      (0,1)   ;[F RACE HMONG]
```

```
$OMEGA
  0.1 ;[P]
  0.1 ;[P]
$SIGMA
  0.01 ;[P]
$EST METHOD=1 INTERACTION PRINT=5 MAX=9999 SIG=4 NOABORT
MSFO=102.MSF
$COV
```

Key to control stream code:

BMI = Body Mass Index

CYP2B615631 = CYP2B6 516 SNP

ABCB12677.EQ.2 = *ABCB1* 2677 GG

CYP2B615631.EQ.1 = *CYP2B6* 516 GT

CYP2B615631.EQ.2 = *CYP2B6* 516 TT

RACE.EQ.4 = Hmong