Injury, Infection, and Recovery from Opioid Use Disorders in Michigan

by

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A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Epidemiological Science)
in the University of Michigan
2018

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Dedication

For my mom.

Acknowledgements

There are so many people to thank for this work. First and foremost, my sincerest thanks to my advisors and dissertation co-chairs, Drs. Betsy Foxman and Marisa Eisenberg. It has been a privilege to work so closely with two successful and inspiring women in science.

Betsy, thank you for helping me find the type of science that gets me excited. You pushed me to think critically, taught me to be confident about my skillset, and encouraged me at every step. It is because of your support that I could face the challenges I encountered and leave the PhD pursuing a career I truly want. Thank you for all the time you dedicated to making me a scientist – I wouldn't have gotten through this PhD without you.

Marisa, your enthusiasm for science and commitment to your students is something I will aspire to as I move through my own career. Thank you for being so engaged as our project evolved over four years after its humble beginnings in the classroom. Your words of encouragement during the PhD kept me confident in myself and my skills and inspired me to keep moving our research moving forward – thank you for checking in every step of the way.

PhD. To my partner, Eric, thank you for being my biggest cheerleader during the most intense part of my PhD and for helping me finish this journey. You made sure I ran across the finish line with your unending support and I can't wait to see where life takes us next. A special thank you to my grandparents, Maureen, Ken, and Eleanor. Finally, thank you to my best friends, Chrissy, Kristin, Mandy, and Amanda and my family members, including my Aunts Sue, Donna, Joyce, and Kristin, Uncles Kenny, Roman, Doug, Chris, and Rich, and cousins Lindsey, Jay, Katie, Ben, Josh, Trevor, Sophie, Emma, and Grace. You all remind me to laugh loud, hug hard, and smile wide – I love you all very much.

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List of Abbreviations

Ag   Agonist Only

AgAt   Agonist+Antagonist

AIC   Akaike Information Criterion

AIDS   Acquired Immune Deficiency Syndrome

ASSIST   Alcohol, Smoking, and Substance Involvement Screening Test

At   Antagonist Only

BIC   Bayesian Information Criterion

CDC   Centers for Disease Control and Prevention

CI   Confidence Interval

COMM   Current Opioid Misuse Measure

CPR   Cardiopulmonary Resuscitation

DAA   Direct-Acting Antiviral

DMM   Dirichlet Multinomial Mixture

DNA   Deoxyribonucleic acid

FDR   False Discovery Rate

GAD   Generalized Anxiety Disorder

HCV   Hepatitis C Virus

HIV   Human Immunodeficiency Virus

HMP   Human Microbiome Project

IDU   Injection Drug Use

IQR   Interquartile Range

LCA	Latent Class Analysis
LRT	Likelihood Ratio Test
MDHHS	Michigan Department of Health and Human Services
MetaHIT	Metagenomics of the Human Intestinal Tract
MMSE	Mini-Mental State Examination
N	Neither Agonist nor Antagonist
NA	Not applicable
NHANES	National Health and Nutrition Examination Survey
NK	Naloxone Knowledge
NS	Not Sampled
NSDUH	National Survey on Drug Use and Health
OD	Overdose
OEND	Overdose Education and Naloxone Distribution
OUD	Opioid Use Disorder
PAM	Partition Around Medoid
PHQ-9	Patient Health Questionnaire-9
PR	Prevalence Ratio
PWID	People who Inject Drugs
PWUO	People who Use Opioids
rRNA	Ribosomal Ribonucleic Acid
SUD	Substance Use Disorder
SVR	Sustained Virologic Response
US	United States of America

Abstract

The opioid crisis is an ongoing serious public health challenge in the United States. Starting in the late 1990s, increases in prescriptions for opioid pharmaceuticals led to increases in nonmedical prescription opioid use and, in some cases, subsequent heroin use. These changes have had significant impacts on public health. Drug overdose mortality quadrupled between 1999 and 2016 and increases in injection drug use have led to a rise in bloodborne viral infections. Improving substance use disorder treatment outcomes is a major public health priority; 40-60% treated for a substance use disorder relapse within a year of completing treatment.

This dissertation explored three public health priorities related to the opioid crisis: injury, infection, and recovery. In chapter 2, we focused on injury prevention by examining overdose experiences, naloxone knowledge, attitudes towards overdose risk, and justice involvement among a sample of adults who used opioids in a justice diversion addiction treatment program in Michigan. We used latent class analysis to identify two general justice involvement patterns that occurred prior to treatment: the first was characterized by many recent arrests and little incarceration time, and the second involved incarceration prior to diversion. Only 56.2% of participants had heard of naloxone and identified it as an overdose treatment, yet 68.1% had experienced and 79.2% had witnessed an overdose. These results highlighted the universal need for overdose education and naloxone distribution in the justice diversion addiction treatment setting.

In chapter 3, we characterized the potential impact of interventions to combat the growing incidence of hepatitis C virus (HCV) infection among young people who inject drugs (PWID). We developed an age-stratified ordinary differential equation HCV transmission model fit to surveillance data from Michigan. We predicted that treating 10% of PWID per year in Michigan could reduce HCV cases by over half. Coupling HCV treatment with behavioral interventions could further reduce HCV incidence and prevalence.

In chapter 4, we explored a potential new target for adjunctive treatments to promote recovery from opioid use disorders by examining how substance use impacts the gut microbiota. The gut microbiota is the community of living bacteria in the human gut. It is increasingly recognized as an important communicator along the gut-brain axis, the crosstalk pathways between the gut and brain, and may also modify psychopathology. We studied 46 patients receiving outpatient addiction treatment in Michigan who were exposed to opioid agonists (prescription opioids and heroin) and antagonists (naltrexone or naloxone). We found that opioid agonist exposure tended to decrease bacterial community diversity and was associated with lower abundance of *Roseburia*, a butyrate producer, and *Bilophila*, a microbe important in bile acid metabolism. We did not find these changes in participants who were concurrently or singly exposed to opioid antagonists. These findings were consistent with those from murine models of morphine exposure and highlighted directions for future work that further explores whether psycho-adjunctive treatments that promote gut health could improve opioid use disorder treatment outcomes.

In chapter 5, I close by highlighting some of the lessons I learned through my dissertation work, and propose new research avenues within the areas of injury, infection, and recovery, that focus on mitigating the negative impacts of the opioid crisis on public health.

Chapter 1   Introduction

This dissertation explores several public health challenges related to the opioid crisis, including injury prevention, bloodborne viral infections, and recovery. In this chapter, I discuss the changing epidemiology of opioid use and opioid use disorders (OUDs) in the United States (US) over the past two decades. I introduce three public health priorities related to the opioid crisis: reducing accidental overdose fatalities through overdose education and naloxone distribution, minimizing the growing incidence and prevalence of hepatitis C virus (HCV) infections, and developing new strategies to support recovery from OUDs. I explore each of these topics further in the remainder of the dissertation.

1.1   Changing Epidemiology of Opioid Use in the United States

In 2016, at least 2.1 million Americans aged 12 years and older had an OUD; only 21.1% received treatment.[1,2] OUDs, as described further below, are formally defined by the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) as medical illnesses related to opioid use or misuse.[3,4] Opioids are a class of drugs that include both licit prescription pain relievers (e.g. oxycodone, hydrocodone, and morphine) and illicit substances (e.g. heroin).[1] Opioid drugs bind to receptors that control nociception in the brain, spinal cord, and gut; licit opioids are therefore commonly prescribed to treat pain.[5] The 2016 Centers for Disease Control and Prevention (CDC) Guidelines highlight the need for judicious opioid prescribing practices given the potential harms associated with opioids, including risk for developing OUDs.[5] At high doses, both licit and illicit opioids can cause overdose, respiratory depression, or death.[5]

Formal diagnosis with an OUD involves meeting two or more diagnostic criteria in the past 12 months; these criteria capture a spectrum of impairments to a person's social functioning, health, and control over using opioids (Figure 1.1).[3,4] OUDs can include several physiologic symptoms, such as tolerance (the need to increase the dose of opioids used to achieve the same physiologic effect or 'high'), cravings to use opioids, and withdrawal (a syndrome occurring upon the removal of opioids often involving nausea, vomiting, diarrhea, chills, depression, and cravings to use opioids).[3] In addition, several cognitive and behavioral symptoms capture behavioral patterns of continued use despite social, personal, or health consequences.[3]

Over the past two decades, growing morbidity and mortality from OUDs and related health problems in the US led to recognition of the opioid crisis and the declaration of a national emergency in 2017.[6,7] Opioid pharmaceuticals were increasingly prescribed for the treatment of pain beginning in the mid- to late-1990s; these increases coincided with the introduction of OxyContin by Purdue Pharma in 1995 and the adoption of pain as the fifth "vital sign," akin to blood pressure, heart rate, etc.[8] Opioid prescribing rates rose steadily from 61.9 prescriptions per 100 Americans in 2000 to a high of 81.3 prescriptions per 100 Americans in 2012.[8–10] Accordingly, past 30 day use of prescription opioids rose from 5% of Americans aged ≥20 years in 1999 to 6.9% in 2003, and remained stable through 2011.[11] During this period, prescription of high strength opioids also increased.[11] Among adults who used prescription opioids in 2011, 37% used prescription opioids more potent than morphine compared to 17% in 1999.[11] Opioids more potent than morphine include fentanyl, hydromorphone, methadone, oxycodone, and oxymorphone, as a smaller dosage amount (i.e. milligrams) of these opioids would provide the same pain relief that could be obtained with a standard dose of morphine.[12] In 2012, an analysis of US prescribing patterns showed that medical practitioners specializing in family practice

prescribed the highest share of total opioid prescriptions (18.2% of opioid prescription), followed by internal medicine (15.1%), nurse practitioners or physician's assistants (11.2%), and general practice (11.2%).[13] Not surprisingly, opioid prescribing rates were highest in pain medicine, surgery, and physical medicine and rehabilitation specialties.[13]

Changes in opioid prescribing practices were accompanied by a 75% increase in chronic nonmedical prescription opioid use (i.e. nonmedical use for 200 or more days of the year).[14] Nonmedical prescription opioid use is defined as use beyond the prescribed purpose, use of a greater amount of opioids, and/or use without a prescription.[1] In 2010, approximately 5% of Americans aged ≥12 years misused prescription opioids, and 0.4% chronically misused prescription opioids.[14] Long-term opioid use and nonmedical prescription opioid use are risk factors for developing an OUD.[5,15,16] Becker *et al.* found that nearly 13% of adults who used prescription opioids nonmedically met diagnostic criteria for an OUD.[15] Prevalence of OUD is even higher among young adults; 24% of adults aged 26-34 years who used prescription opioids nonmedically in the past year had an OUD in 2014.[16] Risk of developing an OUD is lower for people who use opioids as prescribed, but increases with duration of opioid prescription.[17,18] Edlund *et al.* conducted a longitudinal study of 568,640 individuals to detect incident OUD in the 18-months after an opioid prescription and noted that 0.12% of people prescribed opioids for ≤90 days developed an OUD whereas 6.1% of people prescribed opioids for >90 days at an average daily dose of ≥120 morphine equivalent milligrams (a standardized opioid dosage unit to account for differences in prescription opioid strengths) developed an OUD.[18] By comparison, 0.004% of people not prescribed opioids developed an OUD during the period of study.[18]

Opioid prescribing rates have declined since 2012; there were 66.5 opioid prescriptions per 100 Americans in 2016.[10] These declines may, in part, be attributable to policies that curb

nonmedical prescription opioid use, such as prescription drug monitoring programs, which track physician prescribing patterns and patient prescription dispensing (i.e. the number and type of opioids prescribed) to identify patient diversion (e.g. patients seeking prescriptions from multiple physicians).[19] However, increases in heroin use starting in the late 2000s suggest that the current opioid crisis cannot be curbed with measures solely focused on prescribing practices.[8,16,19–21] Prevalence of past year heroin use more than doubled during 2002-2016; 948,000 US adults used heroin in 2016.[2] Muhuri *et al.* estimated that 3.6% of people who use opioids nonmedically initiate heroin use within 5 years, suggesting that nonmedical prescription opioid use may lead to heroin use.[22] More than three-quarters of people who use heroin report using prescription opioids nonmedically beforehand.[20,22] The higher availability and lower cost of heroin relative to prescription opioids may further influence the transition from prescription opioid to heroin use.[19] A more recent change in the opioid crisis is the introduction of illicitly manufactured fentanyl, a synthetic opioid that is 50-100 times more potent than morphine, into the heroin supply.[23] As I discuss further below, the introduction of this more potent opioid contributed to the recent sharp increases in overdose mortality in the US.[23,24]

In 2013, prescription OUDs and prescription opioid overdose were estimated to cost $78.5 billion in health care, OUD treatment, criminal justice system expenses, and lost productivity.[25] US life expectancy decreased in the US for two consecutive years (i.e. 2015 and 2016) related to increasing rates of opioid overdose.[26–28] Mitigating these negative consequences to health and mortality are major and immediate public health priorities.[7] In the remainder of this chapter, I introduce three public health priorities related to the opioid crisis. Each is further explored chapters 2-5.

1.2 Increases in Overdose Mortality

Opioid overdose deaths in the US have more than quadrupled since 2000.[24,29] In 2016, there were 42,249 opioid overdose deaths.[24] The number of deaths from opioid overdose outnumbered deaths from motor vehicle crashes for the first time in 2016.[24,30] An average of 115 people die from an opioid overdose in the US each day.[31]

The CDC has characterized three distinct periods of the opioid overdose epidemic.[31] First, prescription opioid overdose deaths, such as those from oxycodone, rose steadily during the period 1999-2009.[31] The second period began in 2010, when deaths from heroin overdose increased sharply.[31] By 2015, heroin overdose deaths outnumbered those due to prescription opioids.[31] The third period began in 2013 with the introduction of illicitly manufactured fentanyl and fentanyl analogs into supplies of heroin and other illicit drugs.[31] Deaths due to synthetic opioids, most of which involve fentanyl or fentanyl analogs, now outnumber heroin and prescription opioid overdose deaths and were involved in 45.9% of all opioid overdose deaths in 2016.[23,31]

Continued increases in opioid overdose deaths through 2016 highlight the need for immediate responses to reduce fatal and nonfatal overdose deaths. One response is to distribute naloxone to people who use opioids.[32–34] Naloxone is an opioid antagonist that can reverse life-threatening respiratory depression caused by an opioid overdose. In chapter 2, I describe the potential benefits of overdose education and naloxone distribution in the justice diversion addiction treatment setting.

1.3   Injection Drug Use and Bloodborne Viral Infections

In 2012, 1.9% of people who used prescription opioids nonmedically and 47.8% of people who used heroin injected substances (e.g. heroin and prescription opioids).[35] Sharing syringes while injecting drugs can lead to bloodborne viral infections, including HCV and human immunodeficiency virus (HIV).[36] Injection drug use is the most common risk factor for new HCV infections in the US.[36] The number of new HCV infections more than tripled during 2010-2016, and increases were especially sharp among young adults aged 20-29 years.[36] HCV infection can cause life-threatening liver damage during years of chronic, asymptomatic infection.[37] More than half of people who inject drugs (PWID) in the US are infected with HCV.[38]

Newly approved, highly effective direct-acting antivirals made HCV curable in 2012.[39] However, treatment uptake among PWID is low.[40,41] Modeling studies support that increasing rates of HCV treatment could decrease HCV prevalence and interrupt transmission.[42] In chapter 3, I quantify the potential impact of HCV treatment and behavioral interventions on HCV prevalence and incidence among young PWID in Michigan.

1.4   Recovery from Opioid Use Disorders

Unmet need for the treatment of OUDs is high: only 37.5% of people with a heroin use disorder and 17.5% of people with a prescription OUD received OUD treatment in 2016.[2] Effective treatments for OUDs include medication assisted treatments such as methadone, buprenorphine, and naltrexone, which should be provided alongside behavioral counseling.[43] Relapse rates after SUD treatment are high; 40-60% of people with SUDs relapse within one year of treatment.[44,45] Return to use may be even higher among people who use opioids without

medication assisted treatment.[46,47] These statistics highlight the need for both increasing uptake of effective OUD treatments and the identification of adjunctive treatments that support OUD recovery. In chapter 4, I summarize results from a pilot study of one potential target for future adjunctive treatments that is increasingly recognized for its role in psychopathology: the gut microbiota.[48]

1.5   Dissertation Aims

The overall goal of this dissertation is to examine three inter-related public health priorities connected to the opioid crisis. In chapter 2, we characterize justice involvement characteristics, overdose experiences, naloxone knowledge, and overdose attitudes among a sample of people who use opioids receiving treatment in a justice diversion residential addiction treatment program. Further, we examine whether overdose experiences differ by justice involvement and how overdose experiences and justice involvement are associated with naloxone knowledge, overdose risk reduction attitudes, and confidence in overdose response. We use these results to highlight the need for overdose education and naloxone distribution in the justice diversion addiction treatment setting.

In chapter 3, we review the development of a hepatitis C virus transmission model among young people who inject drugs. We fit the model to hepatitis C virus surveillance data from Michigan for a set of 10,000 scenarios generated through stratified random sampling of parameter ranges that were identified through literature review. We then simulate the potential impact of several interventions, including HCV treatment, decreased injection drug use initiation, and several other interventions. We discuss how these results could inform public health strategies to reduce hepatitis C infections among young people who inject drugs in Michigan.

In chapter 4, we focus on the area of recovery. Increasingly, the gut microbiota is explored as a modifier of health and disease, including psychopathology. We examine how opioids influence the gut microbiota among a sample of 46 patients receiving outpatient substance use disorder treatment. We note how differences in diversity, enterotypes, and genera may reflect the negative impact of opioids on gut health, and discuss how these results could inform future, more comprehensive studies of OUD recovery.

In the final chapter, I reflect on some of the defining events in my PhD, the skills they helped me gain, and the challenges I faced while conducting interdisciplinary research. I close with an outline of future directions for research on injury, infection, and recovery from opioid use disorders.

Figure 1.1 DSM-5* Criteria for Diagnosis with an Opioid Use Disorder (OUD)

Withdrawal	Tolerance	Cravings	Hazardous Use	Much Time Spent Using	Activities Given Up to Use
Repeated Attempts to Quit/Control Use	Using Larger Amounts over Long Periods	Neglected Major Roles to Use	Social/ Interpersonal Problems Related to Use	Physical/ Psychological Problems Related to Use	
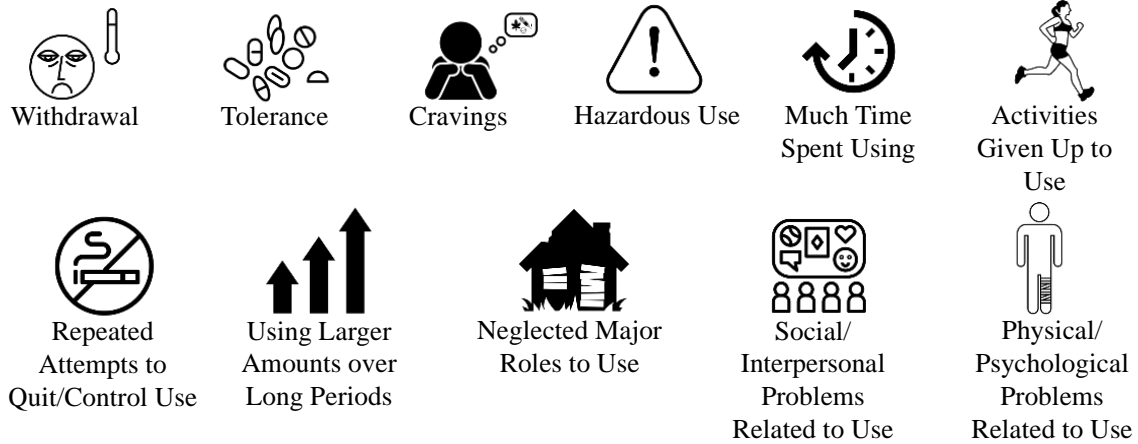
The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) defines OUDs by the presence of two or more of the above criteria in the past 12 months.[3,4] These criteria are meant to encompass the cognitive, physiologic, and behavioral consequences of opioid use.[3,4] The severity of an OUD is defined by the number of criteria endorsed, with 2-3 criteria indicating a mild OUD, 4-5 criteria indicating a moderate OUD, and 6 or more criteria indicating a severe OUD.[3,4]

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Chapter 2   Justice Involvement Patterns, Overdose Experiences, and Naloxone Knowledge among Men and Women in Criminal Justice Diversion Addiction Treatment

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2.1   Author Summary

One response to the four-fold increase in opioid overdose mortality in the US between 1999-2016 has focused on training people who use opioids (PWUO) to reduce their own overdose risk and respond to witnessed overdoses by providing naloxone.[1,2] While community access to naloxone increased in Michigan after state officials approved a 2017 standing order for pharmacy-based distribution, addiction treatment services, the correctional system, and justice diversion programs are an underutilized mechanism for overdose education and naloxone distribution.[3–5] We characterized justice involvement, overdose experiences, naloxone knowledge, and overdose risk reduction and response attitudes among a sample of 514 PWUO in a justice diversion residential addiction treatment program in Michigan. Most participants had experienced (68.1%) or witnessed (79.2%) an overdose. However, only 56.2% had heard of naloxone and correctly identified it as an overdose treatment. History or type of justice involvement was not associated with overdose experience, suggesting a universal need for overdose education and naloxone distribution independent of justice involvement prior to treatment. Participants with more overdose experience were more likely to know about naloxone.

Our results suggest that all PWUO in justice diversion addiction treatment programs should be educated about reducing their overdose risk, responding to an overdose with effective actions, and be provided naloxone before treatment completion.

2.2 Abstract

Criminal justice diversion programs are one major pathway to addiction treatment that could provide overdose education and distribute naloxone to people who use opioids (PWUO). Pre-treatment justice involvement patterns, overdose experiences, naloxone knowledge, and overdose risk reduction and response attitudes may help identify current needs for overdose education and naloxone distribution (OEND) occurring during justice diversion addiction treatment. PWUO are at high risk for overdose post-treatment and post-incarceration and commonly witness overdoses. OEND among PWUO is cost-effective and reduces overdose mortality in community settings.

We characterized the demographic characteristics, overdose experiences, naloxone knowledge, and overdose attitudes among a sample of 514 PWUO in a justice diversion residential addiction treatment program during 2014-2016. We conducted a latent class analysis, stratified by gender, to characterize justice involvement given the complexity of pre-treatment patterns. We used quasi-Poisson regression models with robust standard errors to examine prevalence of naloxone knowledge and overdose attitudes by overdose experiences and justice involvement.

Most participants had personally experienced (68.1%) and/or witnessed another person (79.2%) experience an overdose. Only 56.2% had heard of naloxone and correctly identified it as an opioid overdose treatment. Two justice involvement groups were identified, and these differed

by gender: low involvement (20.3% of men and 46.5% of women), characterized by older age at first arrest, more arrests in the past year, and less time incarcerated; and high involvement (79.7% of men and 53.5% of women), characterized by younger age at first arrest and more lifetime arrests and time incarcerated. Men and women who had personally experienced an overdose were more likely to have heard of naloxone and to correctly identify it as an overdose treatment after adjustment for age and race (Prevalence Ratio, PR [95% Confidence Interval, CI]: Men 2.1 [1.6-2.9], Women 2.0 [1.3-3.1]). Among women without naloxone knowledge, those who had witnessed an overdose felt more ready and confident about responding to an overdose than women who had not witnessed an overdose (PR [95% CI]: 2.1 [1.0-4.3]). This association was attenuated among men (PR [95% CI]: 1.3 [0.98-1.7]).

All PWUO in criminal justice diversion addiction treatment programs would benefit from OEND given the high propensity to experience and witness overdoses and low knowledge of naloxone across justice involvement backgrounds and genders. Among those who did not know about naloxone, discrepancies between perceived ability to respond to an overdose and knowledge may need to be resolved during overdose education programming.

2.3   Introduction

Mortality from opioid overdose increased more than 4-fold from 1999 to 2016 in the United States (US); more than 42,000 people died from an opioid overdose in 2016.[1,2] Training witnesses to identify and respond to an opioid overdose and equipping them with naloxone, an opioid antagonist that reverses the respiratory depression caused by high doses of opioids, reduces opioid overdose mortality.[6] However, a challenge of existing overdose education and naloxone distribution (OEND) programs is to identify appropriate candidates for training, that is,

people who are highly likely to personally experience and/or witness an overdose.[7] Several characteristics increase risk for experiencing or witnessing an overdose. People who have experienced an overdose in the past are more likely to witness or experience a future overdose.[8–11] Overdose risk is heightened in the period immediately following incarceration or addiction treatment.[10,11] Men are more likely to witness overdoses while women and non-Hispanic white individuals are more likely to experience overdoses.[8,10,11] The relationship of age and overdose risk varies by study.[10,11]

PWUO are targeted for OEND programs due to their high propensity to experience and witness overdoses; they are both potential overdose victims and bystanders who could respond.[7–9,12–20] Approximately 50-96% of people who use illicit drugs witness an overdose, and 17-68% personally experience an overdose.[10] Naloxone distribution to PWUO is cost-effective, especially when combined with addiction treatment, and reduces opioid overdose mortality.[6,21–23] Further, PWUO are willing to administer naloxone to other PWUO.[15,24]

There are several key elements of OEND programs. First, participants are trained to recognize the signs and symptoms of an overdose (e.g. loss of consciousness, shallow breathing, pale, blue, or cold skin, small pupils, or a limp body) and educated about behaviors that increase overdose risk.[18,25] Second, they are provided naloxone and trained to effectively respond using naloxone, cardiopulmonary resuscitation (CPR), rescue breathing, or by involving professional medical services.[25] Naloxone is an opioid antagonist that reverses the respiratory depressive effects caused by high doses of opioids.[3] It is currently available in injectable and intranasal formulations.[3] Some OEND programs include other education components. For instance, one program educated PWUO about Good Samaritan Laws, which protect overdose witnesses and victims against legal prosecution for illegal activities discovered when witnesses involve

professional medical help.[25,26] The authors found that correct knowledge of the law increased the odds of 911 calling 3.6-fold in instances of overdose.[26]

To jointly maximize the number of PWUO who are well-positioned to respond to an overdose or benefit from receipt of naloxone and the cost-effectiveness of training, OEND should occur in settings where opioid use disorders (OUDs) are prevalent.[7] In the US, this includes jails, prisons, and addiction treatment services; these settings converge in justice diversion addiction treatment programs, which provide PWUO facing legal prosecution with addiction treatment to avoid criminal charges or to reduce incarceration time.[5,7,27,28] PWUO are referred to justice diversion programs by law enforcement, drug courts, or the correctional system.[5,29–32] Justice diversion addiction treatment programs can be categorized into two types by the time at which people are diverted.[33] Pre-booking programs divert individuals to treatment directly after arrest to avoid criminal charges.[5,33] Individuals in pre-booking programs often avoid incarceration altogether.[5,33] Alternatively, post-booking programs provide treatment to individuals awaiting charges or sentencing or who have already been charged and sentenced to attend treatment; these individuals often spend time in jail while awaiting sentencing.[33] Individuals could also be diverted after violating parole or probationary agreements and may or may not have been incarcerated prior to parole or probation.[33]

The post-incarceration and post-treatment periods are marked by high rates of return to opioid misuse and elevated overdose and overdose mortality rates.[28,34–37] These highlight the need to incorporate OEND and maintenance medications (i.e. methadone, buprenorphine, and naltrexone) in justice diversion and related settings.[5,28,34–43] Binswanger *et al.* showed that risk of drug overdose death was 129-fold higher during the first two weeks following release from incarceration (jail or prison) among a sample of former inmates in Washington relative to the

non-incarcerated population.[34] In the treatment setting, Davoli *et al.* showed that mortality in the post-treatment period was 21-fold higher than mortality in the general population using a sample of >10,000 Italians who used heroin.[44] Most of this elevated risk of death occurred in the first four weeks post-treatment.[44] Similarly, Ravndal and Amundsen suggested that mortality rates were nearly 16-fold higher in the four weeks post-treatment compared to >4 weeks.[45] A loss of physiologic tolerance (i.e. the need to use more of a drug to achieve the same effect) during the period of non-use during incarceration or treatment, in the context of relapse to drug use, is the likely reason that the first month post-treatment is a critical period for overdose risk.[34,44–48]

Providing naloxone before community re-entry after incarceration could reduce overdose risk for both the released individual and their substance use network in this critical period.[49,50] At the population level, Bird *et al.* found that opioid overdose mortality rates were reduced by 36% after implementation of a Scottish policy that provided all released individuals with naloxone.[49] Parmar *et al.* attempted to assess whether providing naloxone prior to community re-entry reduced the risk of opioid overdose death after release by conducting a randomized controlled trial of naloxone on release for >1600 individuals incarcerated in England.[50] However, their trial was unable to compare the risk of post-release overdose death for participants who received and did not receive naloxone because participants who received and used their naloxone most often used it to respond to an overdose they witnessed.[50] While not the intended purpose of this study, these results highlight the potential for naloxone to benefit both PWUO and their network.

PWUO become involved with justice diversion addiction treatment programs at various stages of justice involvement.[5,33] Alongside these variable pathways to treatment, the aspects of justice involvement preceding diversion (e.g. arrest history, age at first arrest, cumulative incarceration time), history of overdose experiences and witnessed overdose, naloxone

knowledge, overdose risk reduction and response attitudes, and demographic characteristics are not thoroughly described. However, high prevalence of witnessed overdose and experienced overdose in related settings (e.g. jail, prison, and community corrections) support investigating the justice diversion setting further. For example, a study of PWUO in community corrections found that 40% experienced an opioid overdose and that 78% of PWUO who experienced an overdose had also witnessed an overdose.[51] Because previous overdose experience is a risk factor for future overdose experience and for witnessing an overdose, identifying correlates of overdose experience among PWUO could help with targeting of OEND.[8,52] In addition, aspects of justice involvement, including multiple incarcerations and property and drug crimes are associated with elevated overdose risk and mortality, suggesting that the way PWUO are referred to justice diversion programs is important in their overdose risk.[30,53,54] Taken together, this research supports describing overlaps in justice involvement, overdose experiences, and naloxone knowledge to inform planning of OEND in the justice diversion context.

Describing attitudes towards overdose risk and responses may also help determine the potential impact of OEND and identify barriers to behavior change. Recent overdose education interventions have incorporated motivational interviewing theory, which identifies discrepancies between beliefs and behaviors to motivate behavior change.[55–57] Attitudes, beliefs, and intentions are believed to be important predictors of behavior change.[55] PWUO in justice diversion addiction treatment may have varying motivations to change overdose risk behaviors given that their primary motivation for enrolling in treatment may have been to avoid legal repercussions (rather than a desire to stop using substances). Studying motivations to change overdose risk behaviors is therefore important for intervention planning. In addition, ineffective responses to overdose (e.g. causing pain to wake an overdose victim or injecting an overdose victim with

something) are highly prevalent among PWUO.[8,16] While OEND increases confidence in responding to an overdose among people with initially low confidence, it is unknown whether uptake of effective overdose responses is modified by false beliefs (i.e. high baseline confidence regarding empirically ineffective overdose responses).[58] Characterizing discrepancies between overdose response knowledge and confidence in responding could determine whether overdose education that stresses the replacement of prior beliefs is required.

Our study aimed to inform OEND planning using a sample of 514 PWUO in a residential justice diversion addiction treatment program in Michigan. We had three objectives. Our first objective was descriptive and sought to add to the literature regarding the characteristics of PWUO in justice diversion addiction treatment programs. Specifically, we described participants' prior involvement in the criminal justice system and history of overdose. We evaluated these separately by gender to account for potential differences in justice involvement and overdose history for men and women. Second, we evaluated whether overdose experiences differed by justice involvement history. Finally, we examined the relationship of overdose experiences and justice involvement with naloxone knowledge, overdose risk reduction attitudes, and confidence in overdose response.

2.4   Methods

2.4.1   Study Description

The analytic sample was drawn from a study of 817 adult (≥18 years) patients in a residential addiction treatment program in Michigan during October 2014 – January 2016. Typical treatment duration for the facility is 60-90 days and treatment populations are separated by gender. This facility's clients included those with no justice involvement and clients who were

diverted to treatment through their involvement with Michigan's criminal justice system (i.e. law enforcement, courts, or corrections, including probation and parole). Clients in justice diversion addiction treatment could be diverted at the pre- or post-booking stage or after violating parole or probationary supervision agreements.

Potential study participants were approached by research assistants and asked to complete a self-administered survey via paper and pencil. To be eligible for the survey, participants had to be ≥18 years, speak English, be able to provide informed consent, and be able to see, speak, and hear. We excluded people who were acutely intoxicated, mentally incompetent, or unable to provide informed consent for any other reason. The survey took approximately 45 minutes to 1 hour to complete. Participants were compensated $5. This study was approved by the Institutional Review Board at the University of Michigan (HUM00078507).

2.4.2   Inclusion Criteria

Because the focus of our analysis was justice diversion addiction treatment, we excluded 36 participants whose treatment was not prompted by the justice system (Figure 2.1). These participants answered no to the question "Was this treatment prompted or suggested by the criminal justice system (judge, probation/parole officer, etc.)?" An additional four participants who skipped this question were also excluded. Participants included in this analysis could be diverted at the pre- or post-booking stage after various criminal justice system interactions (e.g. arrested, charged, sentenced, or under probationary or parole supervision), and may or may not have served time in jail or prison before treatment.

As the main interest of our study was describing opportunities for OEND and naloxone is only relevant to opioid overdose reversal, we restricted the sample to participants who reported

lifetime use of heroin or prescription opioids that were not prescribed by a doctor. This criterion excluded 238 participants. The most commonly used substances among excluded participants were alcohol (n=223), tobacco (n=196), cannabis (n=154), cocaine (n=90), and hallucinogens (n=46). An additional 26 participants were removed due to missing responses for demographic characteristics (age, gender, race, education and living situation), number of overdoses and timing of most recent overdose, number of witnessed overdoses, naloxone and CPR knowledge, and self-efficacy to reduce overdose risk for themselves and to respond to a witnessed overdose. After these exclusions, a total of 514 participants remained for analysis.

2.4.3 Measures

2.4.3.1 Justice Involvement

Five items quantified lifetime and recent justice involvement: age at first arrest (median: 18, range: 9-59 years), number of past-year arrests (median: 1, range: 0-42 arrests), number of lifetime arrests (mode: 6-10, categories: 1-2, 3-5, 6-10, 11-49, 50-99, or 100 or more arrests), number of months during the past year spent in jail or prison (median: 5.3, range: 0-12 months), and lifetime number of years spent in jail or prison (median: 3.5, range: 0-41.3 years). We formed categorical variables using quartile or tertile breaks from distributions in the analytic sample, with modifications when appropriate to enhance interpretability (e.g. juvenile versus adult age at first arrest). Categorical variables included age at first arrest (9-17, 18-20, or 21-59 years), past-year arrests (0, 1-2, 3-42 arrests), lifetime arrests (1-5, 6-10, 11 or more arrests), past-year time spent in jail or prison (0-1.9, 2-5.9, 6-10.9, or 11-12 months), and total time spent in jail or prison (0-0.9, 1-3.4, 3.5-7.4, or 7.5-41.3 years).

2.4.3.2 Overdose Experiences

The survey introduced participants to the definition of an overdose with the following statement: "The following questions are about experiences with taking too much drugs or medications/pills. This is sometimes called 'poisoning,' 'nodding out,' or an 'overdose' or 'OD.'" Participants reported the number of overdoses experienced, timing of their most recent overdose, and substances used during the most recent overdose. We formed binary variables for any lifetime overdose and any past-year overdose. We also assessed the number of lifetime overdoses as a 3-level categorical variable (none, 1-5, or ≥6 overdoses). Finally, we summarized whether the participant's most recent overdose involved heroin or prescription opioids.

2.4.3.3 Witnessed Overdose

Consistent with previous studies, the following statement introduced participants to the definition of a witnessed overdose:

> *The following questions are about times you have seen **someone else** taking too much drugs or medications/pills, and/or drinking too much alcohol. This is sometimes called an "overdose." When someone has an overdose, they might have blue skin color, convulsions, or difficulty breathing, lose consciousness, collapse, cannot be woken up, or have a heart attack or die.*[14]

Participants reported the number of overdoses they witnessed and drugs used by the victim during the most recently witnessed overdose. We formed a binary variable for any witnessed overdose and assessed the number of witnessed overdoses (none, 1-5, or ≥6 witnessed overdoses). Finally, we summarized whether the most recently witnessed overdose involved heroin or prescription opioid use by the victim.

2.4.3.4 Naloxone and Cardiopulmonary Resuscitation (CPR) Knowledge

Participants reported whether they had heard of naloxone and identified its purpose as an overdose treatment, drug treatment for opioid dependence, detox, other, or don't know (multiple responses were allowed). For the analysis, participants who had both heard of naloxone and identified its purpose as an overdose treatment were defined as having naloxone knowledge, while all others had no knowledge.

Similarly, participants reported whether they had heard of CPR and identified one or more purposes (response choices included: used when someone stops breathing, used to help someone when their heart stops, used to wake someone up when they're tired, other, or don't know). Participants were classified as having CPR knowledge if they had heard of CPR and identified its purpose as helping when someone stops breathing or when their heart stops.

2.4.3.5 Motivation to Reduce Overdose Risk

We summarized participant's motivation to reduce their overdose risk on scales of 1 to 10 using two items: the importance ([1] not important to [10] very important) and readiness ([1] not ready to [10] very ready) to reduce their overdose risk. To identify OEND candidates who were ready to change their risk, we created a binary variable for participants with at least neutral (≥ 6) importance and readiness scores.

2.4.3.6 Readiness and Confidence in Responding to a Witnessed Overdose

Participants reported their readiness to respond to an overdose they witnessed ([1] not ready to [10] very ready) and their confidence in responding ([1] not confident to [10] very confident) on scales of 1 to 10. We created one binary variable for participants with neutral or lower (<6) confidence and readiness and a second binary variable for participants with high confidence and readiness (≥8). The high confidence and readiness measure was based on the distribution of scores from the analytic sample. Approximately two-thirds of participants reported readiness and confidence scores ≥8.

2.4.3.7 Demographics and Substance Use

We examined several demographic characteristics of participants, including age (categorized as 18-29, 30-44, 45-67), housing (dichotomized into temporary housing [rooming house/hotel, halfway house/group home, inpatient treatment facility/hospital, jail, shelter, or homeless] versus stable housing [house/apartment or friend/family member's house]), education (college or higher versus high school/GED or less), race (black, white, other, or multiple races), and ethnicity (Hispanic vs. non-Hispanic). In multiple regression models, we collapsed race/ethnicity into non-Hispanic white versus non-white participants (regardless of ethnicity) due to limited numbers of racial/ethnic minorities in the sample. We also summarized lifetime and past-year heroin and illicit prescription opioid use (defined as use not as prescribed by a doctor).

2.4.4 Latent Class Analysis

2.4.4.1 Latent Class Measurement Model

Latent class analysis (LCA) is a statistical technique used to uncover unobserved (i.e. latent) subgroups from patterns (i.e. covariance) of observed variables.[59] It is helpful for empirically-identifying clusters (subgroups) of individuals who share patterns of characteristics in what may otherwise seem to be a homogeneous sample.[59] Lorvick *et al.* previously described three classes of justice involvement (low, medium, and high) among women who used drugs in California based on their incarceration and community corrections involvement.[60]

In this study, we used LCA to identify distinct subgroups defined by involvement in the criminal justice system. Based on prior literature, we hypothesized that criminal justice involvement history could be a useful means of identifying subgroups that would be more likely to benefit from targeted OEND.[17,30,53,54,61] To do so, we used five observed justice involvement variables: age at first arrest (9-17, 18-20, or 21-59 years), past-year arrests (0, 1-2, 3-42 arrests), lifetime arrests (1-5, 6-10, 11 or more), past-year time spent in jail or prison (0-1.9, 2-5.9, 6-10.9, or 11-12 months), and total time spent in jail or prison (0-0.9, 1-3.4, 3.5-7.4, or 7.5-41.3 years).

LCA measurement models provided two types of results. First, we obtained the prevalence of each latent justice involvement class in the sample. Second, we obtained item response probabilities, which reflect the proportion of participants who endorse each observed justice involvement variable within each justice involvement class. Our interpretation of item response patterns by class provided the basis for the investigator-assigned class labels that summarized the overarching identity of each latent class.

We selected the number of latent classes using a combination of interpretability and model fit indices (Akaike Information Criterion [AIC], Bayesian Information Criterion [BIC],

adjusted BIC, and entropy) for latent class measurement models with two to six classes. Smaller values of the AIC and BIC, and larger values of entropy indicate better relative model fit. As discussed further below, model entropy can also help determine whether latent class assignment can be formulated as a categorical (rather than a latent) variable in subsequent analyses.[62] After selecting the number of classes for the initial model, we ensured convergence to a globally optimal solution using 1,000 random start values. We completed LCA analyses in SAS version 9.4 using PROC LCA.[59]

2.4.4.2   Justice Involvement by Gender

Because men and women are treated separately in many residential addiction treatment programs, including the facility where these data were collected, we assessed whether the justice involvement measurement model operated similarly in groups defined by gender (men versus women). We fit the LCA measurement model with and without constraining item response probabilities to be equal across genders, testing the null hypothesis that item response patterns were the same for men and women.[59] We used a likelihood ratio test (LRT) to identify group-level non-invariance in measurement; rejecting the LRT ($p<0.05$) implied that the underlying measurement model differed by gender and that gender stratified models should be used for the remainder of analyses.

2.4.4.3   Assignment of Justice Involvement Class

Before examining the relationship of justice involvement with outcomes, we formed a categorical variable that represented each participant's most likely latent class membership. Posterior probabilities of class assignment were estimated for each participant as part of fitting

the measurement model; these reflected the probability that each participant belonged to each class and described the uncertainty in their class assignment. We assigned participants to their most likely latent justice involvement class using their maximum posterior probability (i.e. the modal class assignment approach) and examined pseudo-class draws in sensitivity analyses (see details below).[63] Both approaches can bias associations towards the null as they fail to incorporate uncertainty in class assignment after individuals are classified into a single class.[62,64–66] However, it is generally considered acceptable to use class assignment as a categorical variable in further analyses for measurement models with entropy values of >0.8.[62]

2.4.4.4 Regression Analyses

After assigning justice involvement class, we examined the prevalence of overdose experiences, naloxone knowledge, motivation to reduce overdose risk, and readiness and confidence in responding to an overdose by the latent classes of justice involvement. Further, we summarized the relationship of overdose experiences (lifetime and past-year), witnessed overdose, and justice involvement with naloxone knowledge and self-efficacy outcomes using prevalence ratios from quasi-Poisson regression models with robust standard errors.[67] Motivation to reduce overdose risk and confidence in responding to an overdose were only examined among participants without naloxone knowledge to focus the analysis on a population with clear need for naloxone training. We summarized results using prevalence rather than odds ratios because the high prevalence of many outcomes of interest in this study prevented the odds ratio from approximating the prevalence ratio.[67,68] We adjusted bivariate prevalence ratios reaching statistical significance ($p<0.05$) for age (18-29, 30-44, and 45-67 years) and race (non-Hispanic white vs. other race/ethnicities).

2.4.5 Sensitivity Analyses

To assess whether the relationships between justice involvement and overdose experiences and knowledge were robust to the modal class assignment LCA approach, we used a second class assignment method, pseudo-class draws.[63] We conducted 20 imputations based on the posterior probabilities of class assignment (i.e. the pseudo-class approach) using the final LCA measurement model to assign class.[63] We repeated Poisson regressions for each imputed dataset for all associations between justice involvement and overdose outcomes that reached statistical significance using the modal class assignment approach. After calculating prevalence ratios for each imputed dataset, we pooled results using imputation procedures.[69]

2.5 Results

2.5.1 Demographic Characteristics, Overdose Experiences, and Naloxone Knowledge

The majority of participants were white (74.7%), non-Hispanic (95.3%) and aged 30-44 years (Table 2.1). Nearly half were arrested for the first time as juveniles (47.9%). Most were arrested once or twice in the year before treatment or jail (41.6%); nearly one-third had no arrests in the year before treatment. Participants spent a median of 3.5 years in their lifetime and 5.3 months of the past year incarcerated.

Most participants had experienced (68.1%) and/or witnessed (79.2%) an overdose, and 42.7% had an overdose in the past year. Among those who experienced or witnessed an overdose, the most recent event often involved opioids (72.6% of most recently experienced overdoses, 83.3% of most recently witnessed overdoses). Only 62.1% had heard of naloxone, but 90.6% of those who had heard of it correctly identified it as an overdose treatment. Only 4 of the

195 participants who had not heard of naloxone correctly identified it as an overdose treatment, so we conceptualized naloxone knowledge as the combination of hearing of naloxone and correctly identifying it as an overdose treatment for the remainder of the analysis. CPR recognition was nearly universal (99.4%); 84.3% recognized that it was used to resuscitate someone who stops breathing and 56.8% recognized that it was used to help someone whose heart stops.

2.5.2   Overdose Risk Reduction and Response Attitudes by Naloxone Knowledge

Just over half of participants (56.2%) had heard of naloxone and correctly identified it as an overdose treatment; knowledge was higher among women than men (66.9% women vs. 51.8% of men, Table 2.2). Over 90% of women reported neutral or higher importance and readiness to reduce their own overdose risk regardless of naloxone knowledge. Women without naloxone knowledge had lower confidence and readiness to respond to an overdose they witnessed than women who knew about naloxone (p=0.04), though few women (16/151, 10.6%) had low confidence and readiness. Conversely, most women (72.2%) reported high confidence and readiness (scores ≥8) to respond to an overdose, and this did not differ by naloxone knowledge (p=0.11).

Overall, 81.0% of men reported neutral or higher scores for readiness and importance in reducing their overdose risk. Only 12.7% of men reported neutral or lower confidence and readiness in responding to an overdose they witnessed while high confidence and readiness (scores ≥8) were common (65.0%). No risk reduction or response attitudes differed by naloxone knowledge among men.

2.5.3   Justice Involvement LCA Measurement Model Fit

Fit indices for models with two to six latent justice involvement classes are shown in Table 2.3. While the BIC and adjusted BIC indicated optimal fit for the three-class model, the two-class model had significantly higher entropy, had larger and thus more stable classes, and was more interpretable than the models with larger numbers of classes. We therefore chose the two-class model for the remaining analysis.[62]

2.5.4   Justice Involvement by Gender

Descriptive analysis showed that justice involvement characteristics differed by gender (Table 2.1), therefore, we evaluated justice involvement by gender using a multiple group LCA. Men had more cumulative justice involvement (i.e., lifetime arrests and time spent in jail or prison) than women, although women had more arrests in the year before treatment. Men were more commonly arrested for the first time as a juvenile than women.

We rejected the null hypothesis of measurement invariance using the LRT (χ^2=72.0, degrees of freedom: 24, p-value<0.05). This result implied that item response probabilities and latent class interpretations differed by gender. To assess whether the solution was sensitive to the model's start values, we repeated the analysis using 1,000 randomly drawn starting values and recovered the optimal solution for all values. We used the gender non-invariant model for the remainder of analyses.

The gender non-invariant model recovered two justice involvement classes for each gender that we termed "high" and "low" involvement (Figure 2.2). Men with low justice involvement (20.3% of men) were characterized by older age at first arrest (median: 19, mean: 22.2 years), few lifetime arrests (80.6% had 1-5 arrests), and less incarceration time (median

past-year: 4.0 months, mean past-year: 4.3 months, median lifetime: 0.8 years, mean lifetime: 1.2 years); 72.2% had 1-2 arrests in the year before treatment. Men with high justice involvement (79.7% of men) were more commonly arrested for the first time as a juvenile (65.3%), had more past-year (median: 8, mean: 7.2 months) and lifetime incarceration time (median: 6, mean: 8.3 years), and had more lifetime arrests (81.4% had 6 or more lifetime arrests).

Although the classes among women shared similarities with men, the defining features and item response probabilities differed. Women with low justice involvement (46.5% of women) were more likely to be arrested at an older age at first arrest (84.2% aged ≥18 years), had few lifetime arrests (75.7% with 1-5 arrests) and spent less time incarcerated (lifetime median: 0.3, mean: 0.5 years; past-year median: 2.6, mean: 2.8 months). Women with high justice involvement (53.5% of women) were younger at their first arrest (70.3% <21 years), had more lifetime arrests (50.6% had ≥11 arrests), and spent more time incarcerated (lifetime median: 4.4 years, mean: 2.9 years; past-year median: 4 months, mean: 5.4 months).

2.5.5 Overdose Knowledge, Experience, and Attitudes by Justice Involvement

Men with high justice involvement had lower prevalence of past-year overdose in bivariate associations (34.4% high involvement vs. 50.0% low involvement, Figure 2.3). However, prevalence did not differ after adjustment for age and race (Figure 2.4). Past-year overdose prevalence was higher among women than men (48.1% high involvement vs. 62.9% low involvement, Figure 2.3). Prevalence among women also did not statistically differ by justice involvement (Figure 2.4). Men with high involvement were older (76.6% high involvement vs. 48.6% low involvement aged ≥30 years) and less commonly white (69.1% high involvement vs. 81.9% low involvement). These patterns were similar among women.

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2.5.6   Overdose Experience and Naloxone Knowledge

Across genders, naloxone knowledge was highest among participants who had lifetime overdose experience, past-year overdose experience, or who had witnessed an overdose (Figure 2.5). Only 26.0% of men who had never experienced an overdose were knowledgeable of naloxone. Conversely, 65.7% of men with lifetime overdose experience and 73.5% of men who overdosed in the past year had naloxone knowledge. Women had similar patterns but higher overall prevalence of naloxone knowledge. There was little difference in prevalence of naloxone knowledge between women with past year overdose experience (79.5%) and those with lifetime overdose experience (77.2%).

2.5.7   Associations of Overdose Experience and Justice Involvement with Naloxone Knowledge and Overdose Attitudes

Experiencing an overdose (lifetime or past year) or witnessing an overdose was associated with increased prevalence of naloxone knowledge; these associations remained after adjustment for age and race (Figure 2.6). Associations of naloxone knowledge with lifetime overdose experience were higher in magnitude than associations with past-year and witnessed overdose. Men who experienced an overdose in their lifetime were 2.1-fold more likely to have naloxone knowledge than men who had not experienced an overdose after adjusting for age and race (95% CI: 1.6-2.9). Similarly, women who experienced an overdose in their lifetime were twice as likely to be knowledgeable of naloxone compared to women who had not after adjusting for age and race (PR [95% CI]: 2.0 [1.3-3.1]).

Next, we compared overdose attitudes among participants without naloxone knowledge. In this subset of participants, women who witnessed an overdose were more likely to report high readiness and confidence in responding relative to women who had not witnessed an overdose after adjustment for age and race (PR [95% CI]: 2.1 [1.0-4.3], p=0.045). The association of witnessed overdose with confidence and readiness to respond was attenuated among men without naloxone knowledge (PR [95% CI]: 1.3 [0.98-1.7], p=0.07). We found no statistically significant differences in prevalence of naloxone knowledge or overdose attitudes by justice involvement after adjustment for age and race among men or women.

2.5.8   Sensitivity Analysis: Multiple Imputation of Justice Involvement

Assigning justice involvement classes with multiple imputation (i.e. the pseudo-class approach) yielded similar results to modal class assignment. The lower bivariate prevalence of past-year overdose among men with high justice involvement relative to low involvement remained statistically significant (PR [95% CI]: 0.69 [0.50-0.95]). The bivariate association of justice involvement with naloxone knowledge among men no longer reached statistical significance after multiple imputation (PR [95% CI]: 0.79 [0.61-1.0], p=0.08). The attenuation of these estimates compared to the modal class assignment approach was consistent with the results from a simulation study that compared both approaches.[64]

2.6   Conclusions

Using a large sample of adults in a residential justice diversion addiction treatment program, this study examined how history of justice involvement related to overdose risk and naloxone knowledge. Our primary finding was that nearly all participants had experienced and/or

witnessed an overdose, whereas just over half had heard of naloxone and correctly identified it as an overdose treatment. In contrast with our expectations, we found no differences in overdose experiences, naloxone knowledge, or overdose attitudes by justice involvement. These findings suggest that all clients, regardless of their path to treatment, are candidates for diversion-based OEND. These elements are described further below.

Our study identified two subgroups of justice involvement among a sample of PWUO that reflect two general pathways to justice diversion addiction treatment. Men with low involvement became involved with the justice system at an older age and entered treatment after many recent arrests, likely to avoid incarceration. Men with high involvement had more cumulative justice involvement and spent much of the year before treatment incarcerated. These individuals likely entered treatment after awaiting trial and sentencing in jail or through diversion from a jail or prison case manager. Women had similar pathways to treatment, but overall had more past year arrests and spent less time incarcerated than men. The sample of women was nearly half high involvement and half low involvement, whereas nearly 80% of men had high involvement. Patterns of justice involvement in this study reflect both justice involvement patterns in the general population and the selection process for diversion programs, such as the one where this study was conducted.

The prevalence of overdose experience history and witnessed overdose experience history in our study approached the maximum estimates reported in a 2015 systematic review.[10] The fact that just over half of participants had heard of naloxone and identified it as an overdose treatment highlights the need for overdose and naloxone education as part of OEND conducted in this setting. Naloxone knowledge was particularly low among participants who had never personally experienced an overdose. While we cannot comment on whether these individuals experienced

an overdose post-treatment, the fact that they were in treatment for an OUD and have no knowledge of naloxone implies that they would benefit from OEND during treatment. We found no differences in overdose experiences, naloxone knowledge, or overdose attitudes by justice involvement, supporting that OEND should be provided to all PWUO in justice diversion addiction treatment.

More than 80% of PWUO in this study reported neutral or higher importance and readiness to reduce their overdose risk. Clients in justice diversion programs could therefore be engaged participants in overdose risk reduction programming. Few (12.1%) participants reported low confidence in their overdose response and readiness to respond to an overdose they witnessed. While the absence of naloxone knowledge overlapped with low confidence to respond among women, we did not find that naloxone knowledge informed response confidence among men. Among participants without naloxone knowledge, witnessing an overdose increased confidence and readiness to respond, although the estimate among men was only marginally statistically significant. Taken together, these results suggest that justice diversion clients in our sample felt confident and ready to respond to an overdose even without knowledge of naloxone. OEND programs should address discrepancies in knowledge and attitudes by discouraging ineffective overdose responses while encouraging uptake of effective responses, such as naloxone, CPR, rescue breathing, and involvement of professional medical services. Motivational interviewing, a counseling style that addresses discrepancies between beliefs and behaviors, could be applied for this purpose.[55]

2.6.1.1 Strengths and Limitations

This study had several strengths. First, we used LCA to describe overarching patterns of justice involvement history. This approach leveraged the covariance of observed justice involvement indicators to capture more information than was contained in each single justice involvement indicator. Further, we used a large sample of PWUO diverted to addiction treatment in both the pre-booking and post-booking stages. This sample therefore included the spectrum of potential clients eligible for addiction treatment following their justice involvement. Another benefit of the large sample size was our ability to completely stratify our analysis by gender to allow for differences in justice involvement, overdose experience history, witnessed overdose history, and naloxone knowledge between men and women. Previous studies support that justice involvement and overdose risk differ by gender.[8,10,11,61]

Our findings are not without limitation. We studied participants from a single addiction treatment facility in the mid-Western US. The prevalence of naloxone knowledge, overdose experience, and witnessed overdose may reflect levels of OEND implementation specific to the Midwest and may not be generalizable to justice diversion addiction treatment programs in other regions. Further, our study participants attended treatment because of their involvement with the criminal justice system. The patterns of pre-treatment justice involvement we described may differ from justice involvement patterns among PWUO seeking treatment for other reasons. In addition, we were unable to determine when clients were diverted relative to the time they committed the crimes preempting treatment. We therefore cannot comment on specific differences between those diverted at the pre-booking, post-booking, or parole/probation stage beyond those supported by the LCA results.[33] The extent to which the patterns in our study reflect characteristics of justice involvement in the general population is unknown and our ability

to evaluate this is complicated by the lack of systematic published criteria for diversion program eligibility. This lack of objective evaluation criteria further limited our ability to untangle the sources of gender and other disparities (e.g. by race) in diversion. In our study population, most diversion clients entered treatment after incarceration. Given the variability in diversion programs, it is difficult to determine whether the patterns of justice involvement observed here would extend to other states.[5]

Our study relied on self-reported characteristics from the pre-treatment period to draw conclusions and therefore all findings are subject to recall bias. The cross-sectional design limited our ability to define the temporal sequence of events (e.g. whether individuals experienced or witnessed overdoses before or after their involvement with the justice system and when they learned about naloxone relative to their overdose experiences). We also cannot comment on post-treatment experienced or witnessed overdoses. Further, we relied on self-reported scales of motivation to reduce overdose risk and readiness and confidence in responding to an overdose. These scales are not validated and cut points for high readiness and confidence to witness an overdose were based on distributions from the analytic sample. We therefore cannot comment on the sensitivity of these measures to capture motivation, confidence, or actions (i.e. overdose risk behaviors or overdose responses).

Our analytic approach also had several limitations. First, the use of modal class assignment and pseudo-class draws to assign participants to a single justice involvement class likely resulted in underestimates of the magnitude of associations between justice involvement and overdose-related outcomes.[62,64–66] While it is generally acceptable to assign class for models with entropy values of >0.8, we may have detected a stronger effect if we had used another approach.[62] The disadvantages of implementing other approaches for this descriptive analysis did

not outweigh the benefits of the simpler approaches applied given that our purpose was to describe patterns in justice involvement and examine the prevalence of other variables within these classes. For example, we could have added our outcomes of interest (e.g. naloxone knowledge) into the LCA measurement model as covariates, but this would have changed the measurement model's classes, resulting in different measurement models for each outcome. Further, the current implementation of LCA with covariates in SAS estimates odds ratios. The high prevalence of outcomes examined in this study presents a challenge where odds ratios do not estimate relative changes in prevalence, limiting the interpretability of odds ratios.[67,68] Other approaches considered (e.g. the inclusive classify-analyze approach) would have also changed the formation of latent classes and is recommended for use with only one outcome.[64]

2.6.1.2 Conclusions

The low prevalence of naloxone knowledge and high prevalence of overdose experience and of witnessing an overdose in our sample of PWUO in a justice diversion addiction treatment program in Michigan suggests that OEND should be incorporated into justice diversion addiction treatment. Given the low levels of naloxone knowledge, OEND should be provided to all clients, regardless of pre-treatment overdose experience or justice involvement. OEND may need to address discrepancies between overdose response knowledge and confidence in responding to an overdose to minimize ineffective responses to a witnessed overdose.

Figure 2.1 Inclusion Criteria for a Sample of 514 People who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016
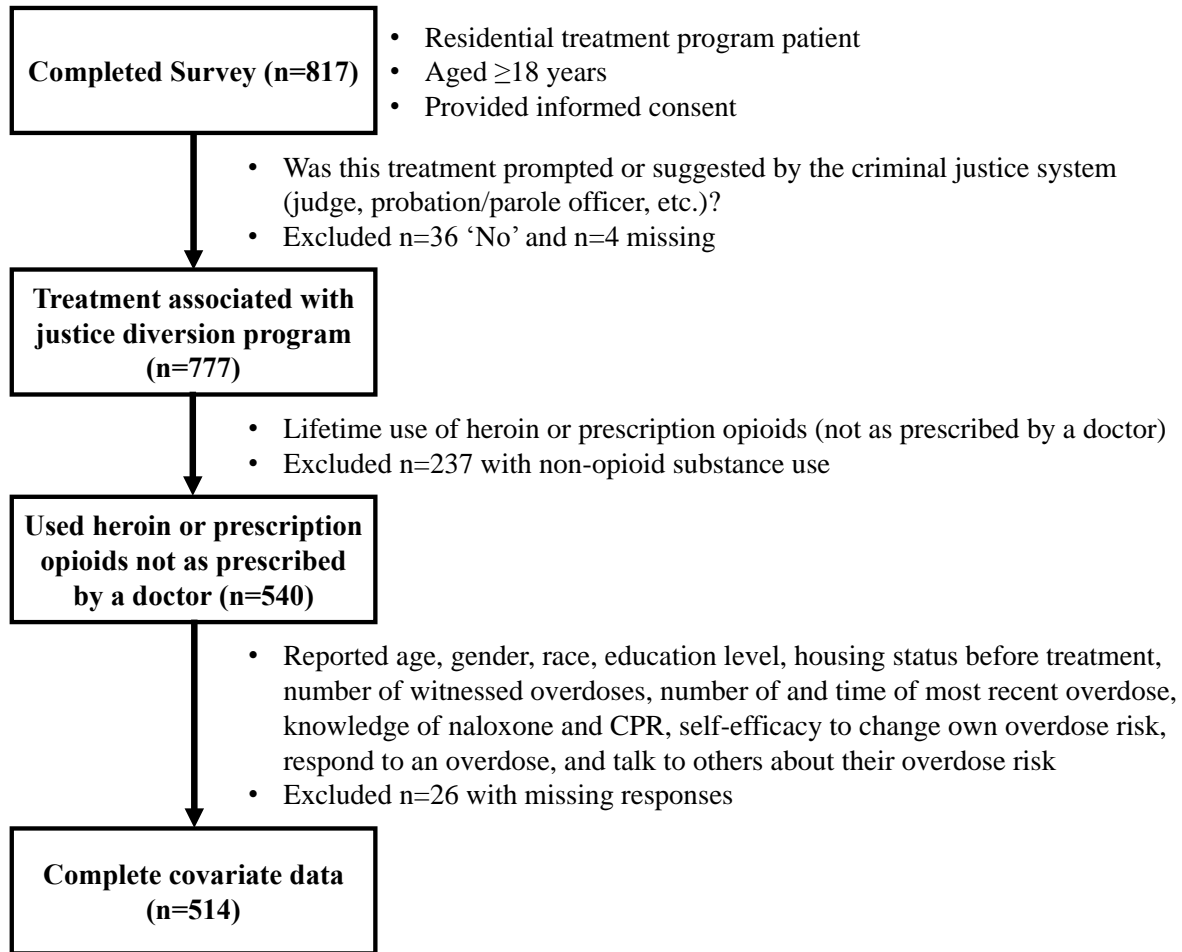
Our study included a sample of 514 PWUO in residential treatment who enrolled in our study, affirmed their involvement in justice diversion, used heroin or prescription opioids (not as prescribed by a doctor) in their lifetime, and had complete data on covariates of interest.

Table 2.1 Sample Description of 514 People who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 by Gender

	Total	Women	Men
	n (%)	n (%)	n (%)
Total	514 (100)	151 (100)	363 (100)
Justice Involvement[a]			
Age at 1st arrest (years)			
Missing	2 (0.4)	0 (0)	2 (0.6)
9-17	246 (47.9)	46 (30.5)	200 (55.1)
18-20	138 (26.9)	48 (31.8)	90 (24.8)
21-59	128 (24.9)	57 (37.8)	71 (19.6)
Median (IQR)	18 (16-20.5)	19 (17-22)	17 (15-19)
Lifetime arrests			
Missing	0 (0)	0 (0)	0 (0)
1-5	173 (33.7)	61 (40.4)	112 (30.9)
6-10	171 (33.3)	49 (32.5)	122 (33.6)
11 or more	170 (32.1)	41 (27.2)	129 (35.5)
Arrests in year before treatment or jail			
Missing	3 (0.6)	0 (0)	0 (0)
0	167 (32.5)	24 (15.9)	143 (39.4)
1-2	214 (41.6)	77 (51.0)	137 (37.7)
3-42	130 (25.3)	50 (33.1)	80 (22.0)
Median (IQR)	1 (0-3)	2 (1-3)	1 (0-2)
Time spent in jail or prison in lifetime (years)			
Missing	7 (1.4)	4 (2.7)	3 (0.8)
0-0.9	107 (20.8)	66 (43.7)	41 (11.3)
1-3.4	135 (26.3)	47 (31.1)	88 (24.2)
3.5-7.4	134 (26.1)	23 (15.2)	111 (30.6)
7.5-41.3	131 (25.5)	11 (7.3)	120 (33.1)

Median (IQR)	3.5 (1-7.5)	1.1 (0.3-3)	5 (2.3-9.8)
Time spent in jail or prison in past year (months)			
Missing	17 (3.3)	8 (5.3)	9 (2.5)
0-1.9	119 (23.2)	43 (28.5)	76 (20.9)
2-5.9	134 (26.1)	59 (39.1)	75 (20.7)
6-10.9	125 (24.3)	28 (18.5)	97 (26.7)
11-12	119 (23.2)	13 (8.6)	106 (29.2)
Median (IQR)	5.3 (2-10)	3.1 (1.4-6)	6.5 (2.9-12)
Overdose Experience			
Experienced an overdose	350 (68.1)	114 (75.5)	236 (65.0)
Most recent overdose involved heroin and/or prescription opioids[b]	254 (72.6)	87 (76.3)	167 (70.8)
Experienced an overdose in the year before treatment	219 (42.7)	83 (55.0)	136 (37.5)
Number of experienced overdoses in lifetime			
0	164 (31.9)	37 (24.5)	127 (35.0)
1-5	225 (43.8)	63 (41.7)	162 (44.6)
6 or more	125 (24.3)	51 (33.8)	74 (20.4)
Witnessed Overdose			
Witnessed any overdose	407 (79.2)	127 (84.1)	280 (77.1)
Most recently witnessed overdose involved heroin and/or prescription opioids[c]	339 (83.3)	117 (92.1)	222 (79.3)
Number of witnessed overdoses in lifetime			
0	107 (20.8)	24 (15.9)	83 (22.9)
1-5	269 (52.3)	84 (55.6)	185 (51.0)
6 or more	138 (26.9)	43 (28.5)	95 (26.2)
Knowledge of Overdose Responses			
Heard of naloxone	319 (62.1)	109 (66.9)	210 (57.9)
Identified purpose of naloxone[d]	289 (90.6)	101 (92.7)	188 (89.5)
Heard of CPR	511 (99.4)	150 (99.3)	361 (99.5)
Identified purpose of CPR (when someone stops breathing and/or heart stops)[e]	497 (97.3)	147 (98.0)	350 (97.0)

Demographic and Social Characteristics			
Age (years)			
18-29	174 (33.9)	69 (45.7)	105 (28.9)
30-44	200 (38.9)	57 (37.8)	143 (39.4)
45-67	140 (27.2)	25 (16.6)	115 (31.7)
Median (IQR)	34 (27-46)	31 (26-40)	36 (28-48)
Race			
Black	83 (16.2)	18 (11.9)	65 (17.9)
White	384 (74.7)	116 (76.8)	268 (73.8)
Other	13 (2.5)	3 (2.0)	10 (2.8)
Multiple races	34 (6.6)	14 (9.3)	20 (5.5)
Hispanic Ethnicity	24 (4.7)	5 (3.3)	19 (5.2)
College or higher education	184 (35.8)	66 (43.7)	118 (32.5)
Temporary housing in past 3 months[f]	290 (56.4)	77 (51.0)	213 (58.7)
Substance Use			
Lifetime heroin use	347 (67.5)	117 (77.5)	230 (63.3)
Heroin use in the past year[g]	249 (71.9)	93 (79.5)	156 (67.8)
Lifetime prescription opioid use (not as prescribed a doctor)	485 (94.4)	144 (95.4)	341 (93.9)
Used prescription opioids in the past year (not as prescribed by a doctor)[h]	271 (55.9)	96 (66.7)	175 (51.3)

[a]Latent class analysis allows for missing values in indicators and uses information on available indicators to create classes for participants with missing data. Therefore, totals for justice involvement may not add to the full sample size.

[b]Among those who experienced an overdose. Includes most recent experienced overdose events where the participant reported they used heroin and/or prescription opioids. An additional 7 participants (5 men, 2 women) did not report substances used.

[c]Among those who witnessed an overdose. Includes most recently witnessed overdose events where the participant reported that the victim used heroin and/or prescription opioids. An additional 9 participants (6 men, 3 women) did not know or did not report substances used by the victim.

[d]Among those who had heard of naloxone.

[e]Among those who had heard of CPR.

[f]Includes living in a halfway house or group home, inpatient facility, jail, shelter, or homeless.

[g]Among those who used heroin in their lifetime. An additional 16 participants (10 men, 6 women) reported lifetime heroin use but declined to answer questions about past year heroin use.

[h]Among those who used prescription opioids in their lifetime. An additional 19 participants (14 men, 5 women) reported lifetime prescription opioid use but declined to answer questions about past year prescription opioid use.

Table 2.2 Overdose (OD) Attitudes by Naloxone Knowledge (NK) among Men and Women who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=514)

OD Attitudes	Total		Women		Men	
	NK[a]	No NK	NK[a]	No NK	NK[a]	No NK
Total: n (%)	289 (56.2)	225 (43.8)	101 (66.9)	50 (33.1)	188 (51.8)	175 (48.2)
Importance of reducing own OD risk: Median (IQR)	10 (9-10)	10 (9-10)	10 (10-10)	10 (10-10)	10 (8-10)	10 (9-10)
Readiness to reduce own OD risk: Median (IQR)	10 (9-10)	10 (9-10)	10 (10-10)	10 (10-10)	10 (9-10)	10 (9-10)
Higher than neutral (≥6) importance and readiness in reducing own OD risk: n (%)	246 (85.1)	186 (82.7)	93 (92.1)	45 (90.0)	153 (81.4)	141 (80.6)
Readiness to respond to an OD: Median (IQR)	10 (7-10)	10 (7-10)	10 (8-10)	10 (7-10)	10 (7-10)	10 (7-10)
Confidence in responding to an OD: Median (IQR)	10 (7-10)	10 (7-10)	10 (8-10)	9 (7-10)	10 (7-10)	10 (7-10)
Lower than neutral (≤5) confidence and readiness in responding to an OD: n (%)	31 (10.7)	31 (13.8)	7 (6.9)*	9 (18.0)*	24 (12.8)	22 (12.6)
High (≥8) confidence and readiness to respond to an OD: n (%)	198 (68.5)	147 (65.3)	77 (76.2)	32 (64.0)	121 (64.4)	115 (65.7)

[a]Naloxone knowledge was defined as having heard of naloxone and recognizing its purpose as an overdose reversal agent.
*Chi-squared test supported statistically significant differences between women with and without NK (p=0.04).
Abbreviations: NK: naloxone knowledge, OD: Overdose.

Table 2.3 Fit of a Latent Class Justice Involvement Measurement Model by Number of Classes among a Sample of People who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=514)

Classes	Log Likelihood	AIC	BIC	Adjusted BIC	Entropy
2	-2897.1	595.7	701.7	622.4	**0.82**
3	-2852.7	532.9	**694.1**	**573.5**	0.69
4	-2841.6	536.7	753.0	591.1	0.71
5	-2822.0	**523.5**	795.0	591.8	0.73
6	-2809.3	524.1	850.8	606.4	0.73

Bold font indicates optimal fit index value of the tested solutions.

Figure 2.2 Patterns of Justice Involvement among Men and Women who use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=363 men and 151 women)
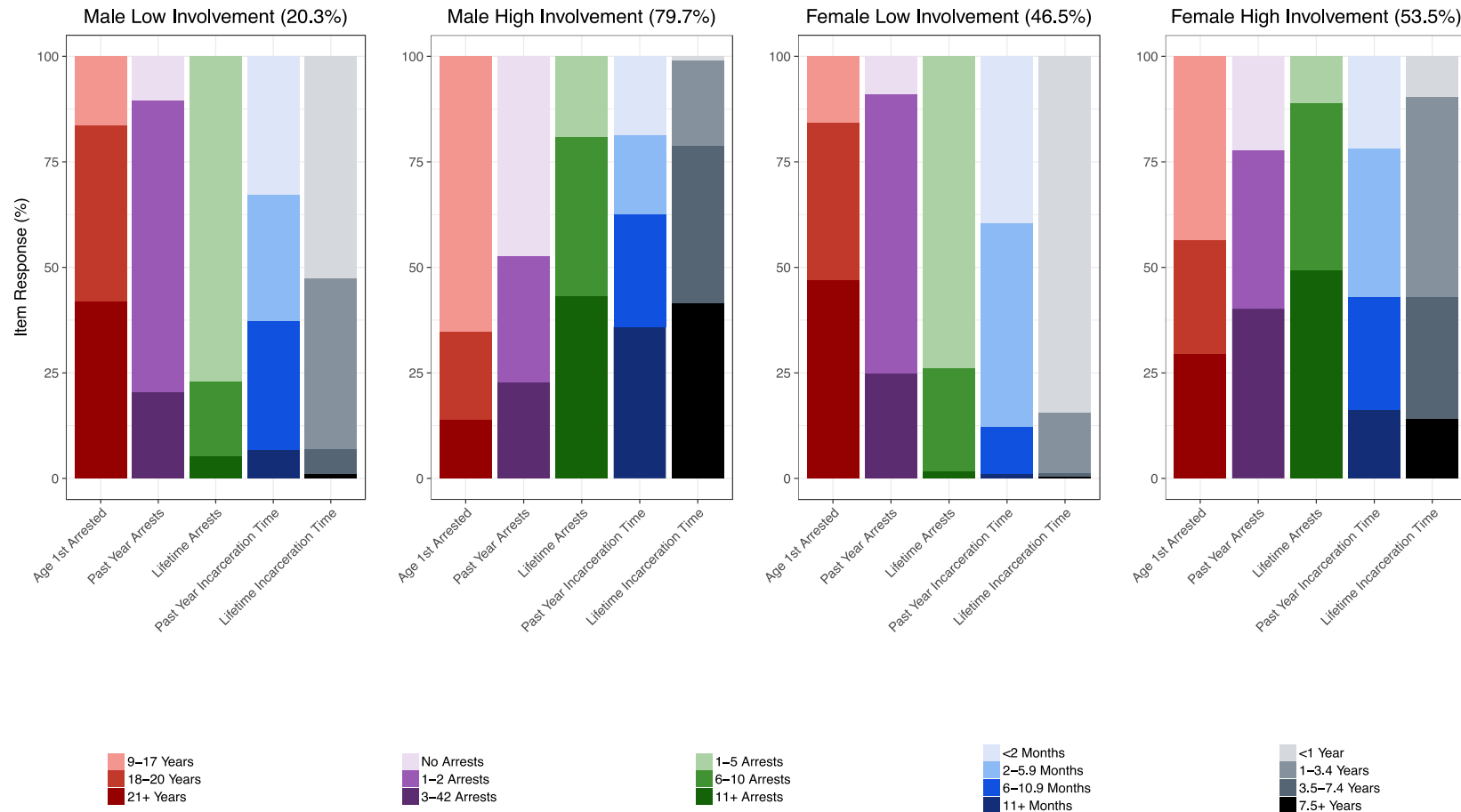
Two justice involvement classes per gender were identified among a sample of 514 PWUO in justice diversion addiction treatment. Men with low involvement (20.3% of men) were arrested for the first time at an older age and arrested more often in the past year. Men with high involvement (79.7%) had more arrests and incarceration time. Similar classes emerged among women, but women had more past year arrests and spent less time incarcerated than men. Women with low involvement comprised 46.5% of the sample and high involvement was slightly more common (53.5% of women).

Figure 2.3 Prevalence of Overdose Experiences, Response Knowledge, and Attitudes by Justice Involvement among People who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=363 men and 151 women)
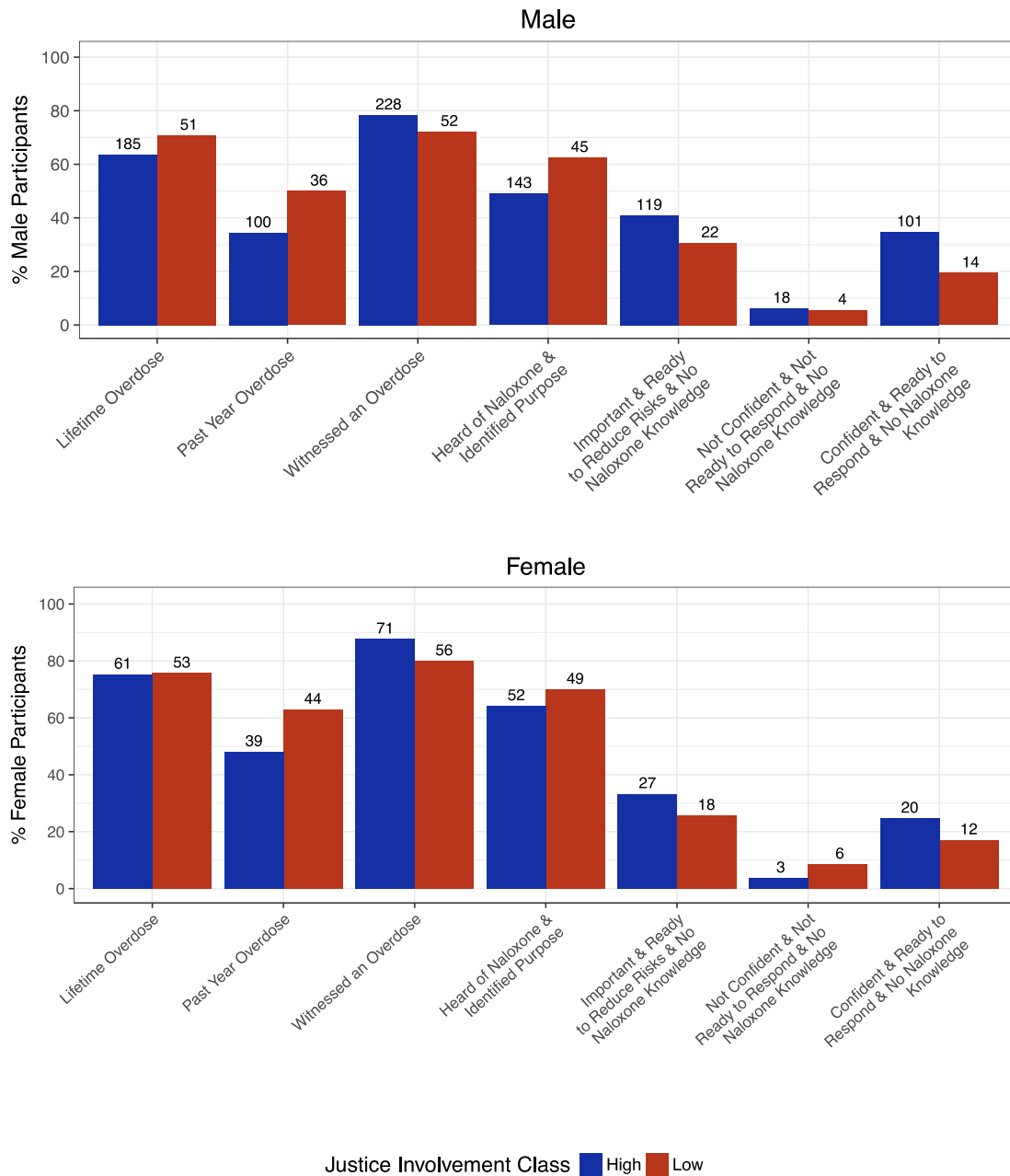
Prevalence of overdose experiences, naloxone knowledge, and overdose attitudes (bars) are annotated with the number of participants with each outcome. Prevalence of experiencing an overdose and witnessing an overdose was high across justice involvement groups and was slightly higher among women than men. Naloxone knowledge was also higher among women compared to men and did not differ by justice involvement. In both genders, few participants reported low confidence and readiness to respond to an overdose and did not have naloxone knowledge, while other overdose self-efficacy outcomes were more common.

Figure 2.4 Associations of Justice Involvement with Overdose Experiences among Men and Women who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=363 men and 151 women)
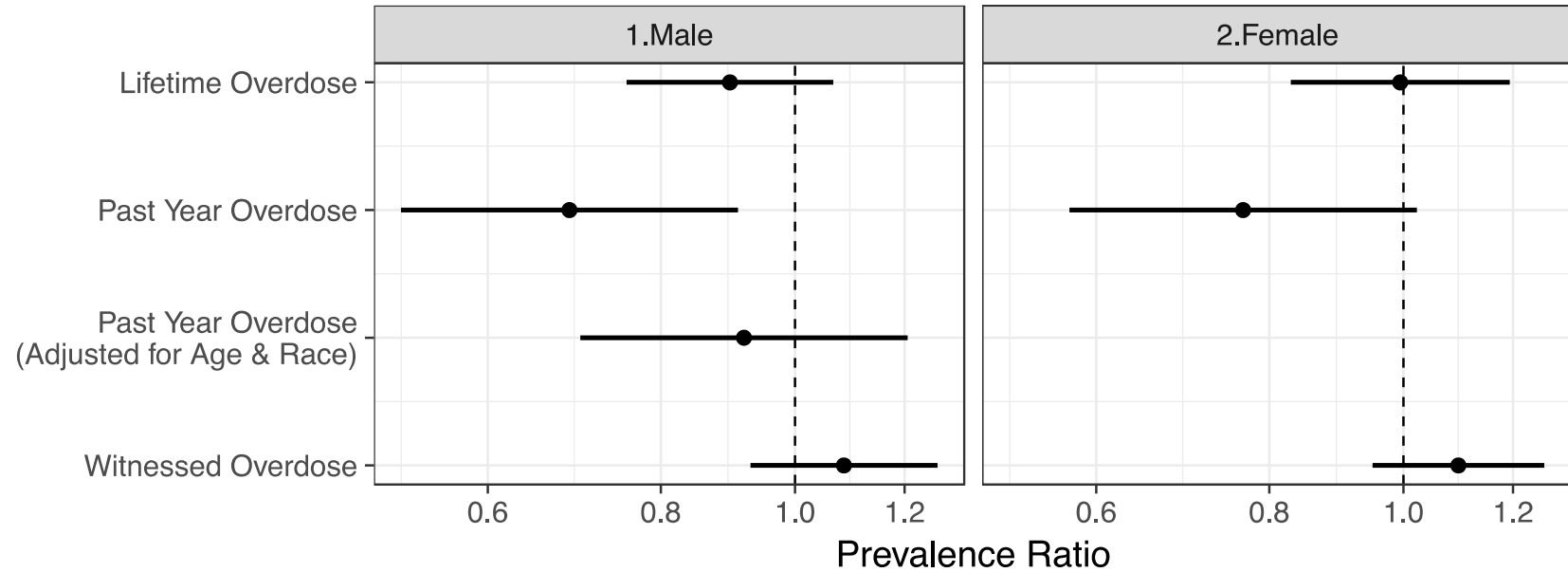
We summarized the association of justice involvement (referent: low involvement) with overdose experiences using prevalence ratios. Overdose experiences were not associated with justice involvement among men or women. Past year overdose was slightly less common among men in bivariate analyses but this association did not remain after adjustment for age and race.

Figure 2.5 Prevalence of Naloxone Knowledge (NK) by Overdose Experiences among People who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=363 men and 151 women)
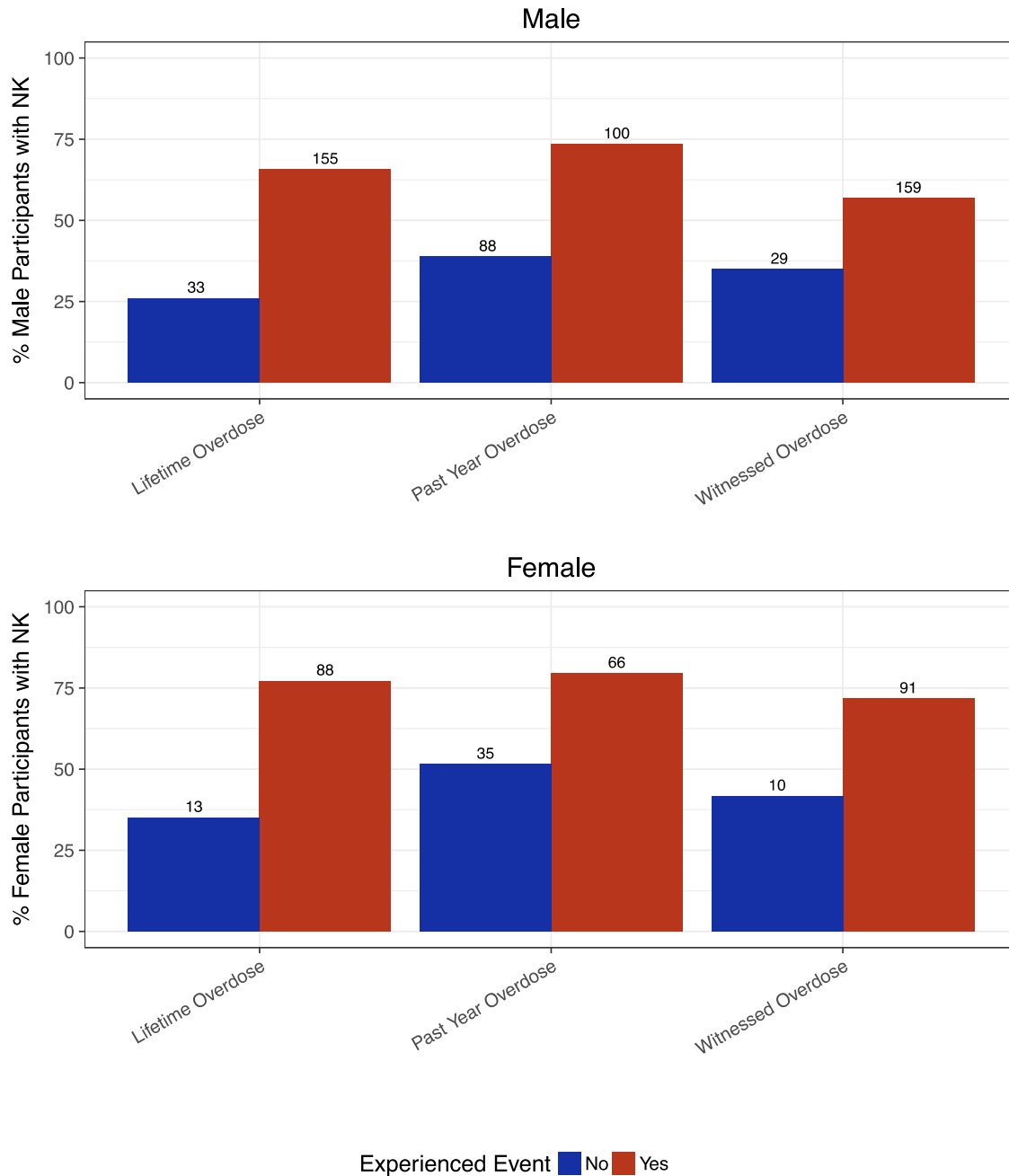
Prevalence of naloxone knowledge (bars) for participants with and without overdose experiences are annotated with the number of participants with naloxone knowledge in each category. Prevalence of naloxone knowledge was higher among men and women who experienced overdoses or witnessed an overdose. Naloxone knowledge prevalence differences were largest between participants with and without lifetime overdose experience for both men and women. Abbreviations: NK: naloxone knowledge.

Figure 2.6 Associations of Overdose Experiences and Justice Involvement with Naloxone Knowledge and Overdose Attitudes among Men and Women who Use Opioids in Justice Diversion Addiction Treatment during 2014-2016 (n=363 men and 151 women)
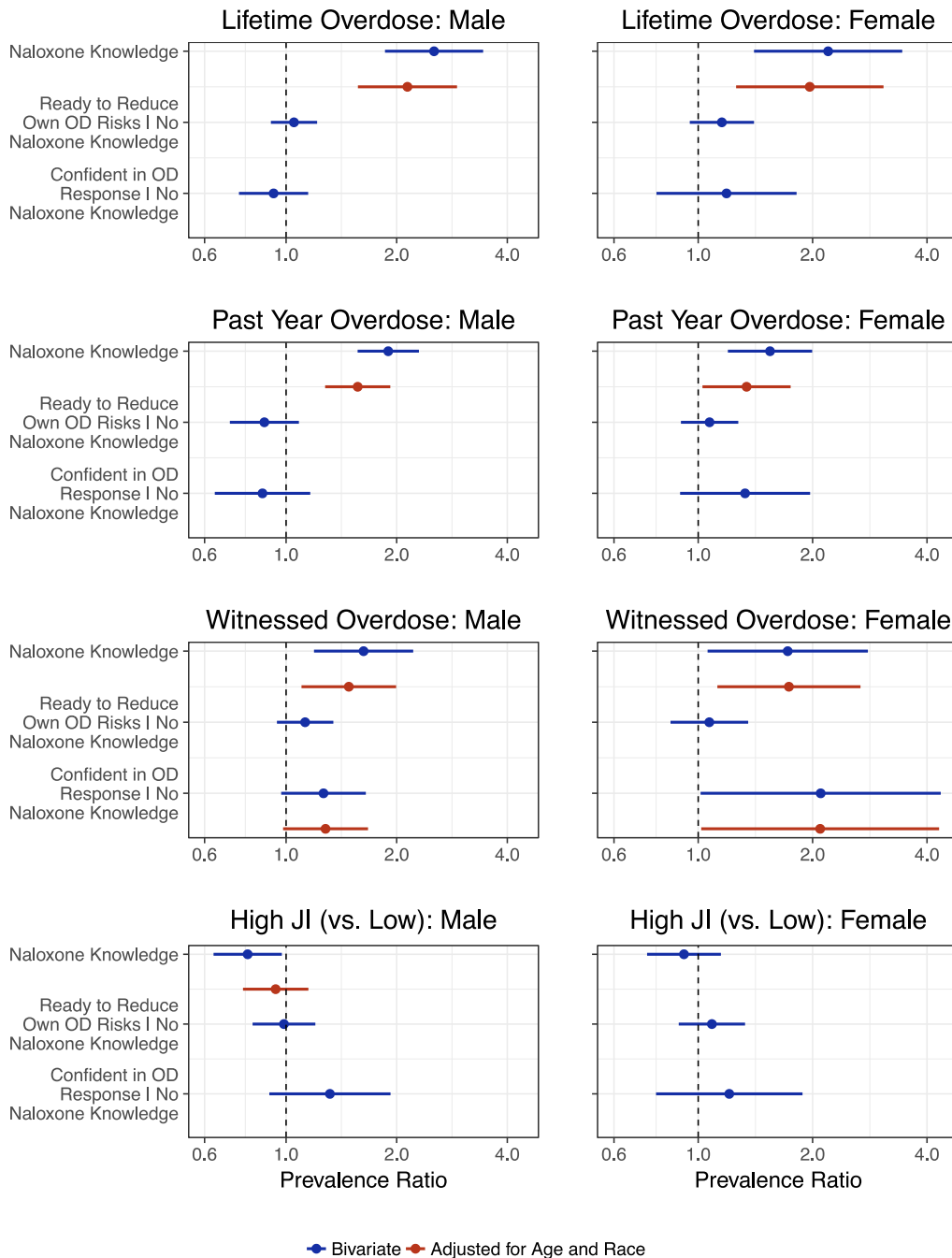
Naloxone knowledge prevalence was higher among men and women who experienced or witnessed an overdose. Readiness and confidence outcomes were only assessed among participants without naloxone knowledge. Among women without naloxone knowledge, witnessing an overdose increased confidence and readiness to respond. This association was attenuated among men (p=0.07). Abbreviations: JI: Justice Involvement.

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Chapter 3   Hepatitis C Transmission in Young People who Inject Drugs: Insights Using a Dynamic Model Informed by State Public Health Surveillance

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3.1   Author Summary

Increasing injection drug use arising from the opioid crisis has led to a concurrent increase in the incidence of hepatitis C virus (HCV) infections. How best to interrupt HCV transmission among young people who inject drugs (PWID) is unknown. We assessed this question in a simulation framework informed by HCV trends among young PWID in Michigan. Concurrent treatment of currently injecting and formerly injecting PWID could reduce prevalence and incidence; however, the predicted case reductions from treatment interventions varied widely. High cure rates from HCV treatment could be achieved by integrating HCV treatment into addiction treatment or syringe services programs.

3.2   Abstract

Increasing use of heroin and non-medical use of prescription opioids are major contributors to the observed increases in the incidence of hepatitis C Virus (HCV) infections in US young adults since the late 1990s. How best to interrupt transmission and decrease HCV prevalence in young people who inject drugs (PWID) is uncertain.

We developed an age-stratified transmission model of PWID aged 15-64, which we fit to Michigan HCV surveillance data among young PWID aged 15-29. We sampled ranges of model parameters using 10,000 Latin hypercube samples. We used the best-fitting 10% of simulations to predict the potential impact of primary (reducing injection initiation), secondary (increasing cessation, reducing injection partners, or reducing injection drug use relapse), and tertiary (HCV treatment) interventions on incident and prevalent HCV cases. We summarized these results by the predicted median percent reduction in acute and chronic HCV cases and range of predicted reductions across the best fitting 10% of simulations.

Treating both current and former PWID led to the greatest predicted reductions in HCV prevalence and incidence. Treating 10% of current and former PWID per year could reduce HCV prevalence by 69.8% (range: 47.9-74.0%) and acute cases by 65.8% (range: 24.2-73.2%) among PWID aged 15-29 when 90% were cured (i.e. achieved sustained virologic response [SVR] to treatment). Treatment results were somewhat sensitive to the proportion cured. Treating 10% of current and former PWID with an SVR of 60% predicted a reduction of chronic HCV cases by 55.0% (range: 36.2-59.5%). Restricting treatment to 10% of former PWID per year with a 90% SVR reduced the potential impact of treatment compared to treating both current and former PWID (median predicted prevalence decrease: 47.8%, range: 20.5-62.4%, median predicted decrease in acute cases: 44.4%, range: 4.1-62.3%).

Primary and secondary interventions also reduced predicted HCV prevalence and incidence. For instance, reducing the number of syringe sharing partners per year by 10% predicted a 16.6% (range: 12.7-27.3%) reduction in chronic cases and a 19.7% (range: 13.0-32.4%) reduction in acute cases. In simulations of combinations of interventions, reducing injection initiation, syringe sharing, and relapse rates each by 10% while increasing cessation

rates by 10% predicted a 49.1% (range: 40.1-67.3%) reduction in chronic HCV and a 57.7% (range: 47.1-77.3%) reduction in acute HCV.

While these results are specific to Michigan, our approach could be applied in other states conducting HCV surveillance to identify local-level intervention opportunities. Our results highlight the need for HCV treatment among both current and former PWID. High SVR rates could be achieved by integrating HCV treatment into addiction treatment or syringe services programs. Further, these results support scaling up both primary and secondary interventions to further reduce HCV prevalence and incidence in Michigan.

3.3   Introduction

The epidemiology of hepatitis C virus (HCV) infections in the United States (US) has changed dramatically over the last decade, with notable increases in HCV incidence among young people aged approximately 15-29 years.[1–5] These changes in incidence have been associated with increases in opioid and injection drug use (IDU).[1–5] In the US, up to 2.6% of adults have injected drugs in their lifetime and more than half of US people who inject drugs (PWID) have HCV infection.[6–11] IDU is the primary risk factor for new HCV infections in the US.[5] After decades of asymptomatic chronic infection, HCV leads to liver-associated morbidity and mortality (e.g. cirrhosis and hepatocellular carcinoma).[12,13]

In the US, transmission modeling studies have shaped HCV screening, treatment, and prevention policies by increasing our understanding of HCV transmission dynamics, forecasting prevalence of HCV-related liver diseases, and simulating the impact, costs, and benefits of highly effective direct-acting antivirals (DAAs) among PWID and other groups disparately

burdened by HCV.[12,14–19] Multiple studies support the cost-effectiveness of treating PWID with DAAs to interrupt HCV transmission, a strategy known as 'treatment as prevention.'[15,16,18,20–43]

To our knowledge, only one modeling study has evaluated the potential impacts of HCV treatment among young PWID. In this study, Echevarria *et al.* suggested that treating just 5 per 1,000 young PWID (<30 years) in Chicago could halve HCV prevalence in this age group over 10 years (from 10% to 5%).[15] This large predicted reduction from a modest intervention stemmed in part from the low baseline HCV prevalence among young PWID in Chicago compared with their older counterparts.[15,44,45] HCV seroprevalence studies in several US cities (Baltimore, Chicago, Los Angeles, New York City, San Diego, and Seattle) suggest that HCV prevalence among young US PWID varies widely across the US, with estimates ranging 10-53%; however, estimates are unavailable for a majority of states and cities.[15,44–49] This absence of local HCV seroprevalence estimates limits our ability to evaluate prevalence trends and the potential impact of interventions. We demonstrate here that HCV public health surveillance data collected as part of nationally notifiable and state-reportable condition surveillance might be used to evaluate the potential impact of interventions among young PWID in locations without a systematic characterization of HCV prevalence.[50]

HCV incidence increases in young adults and adolescents were first identified using HCV public health surveillance data.[2,51,52] As part of HCV surveillance, laboratories and physicians report positive HCV lab results to state health departments, who apply standard case definitions to stage HCV as acute or chronic.[53–56] Underreporting of HCV cases limits use of surveillance data for purposes other than description and outbreak monitoring.[2] Klevens *et al.* estimated the magnitude of acute HCV under-reporting at approximately 12.3-16.8 cases per case reported in a nationwide study.[57] However, Onofrey *et al.* suggested that the magnitude of under-detection

may be even higher: up to 138 acute cases per reported acute case.[58] In addition to under-reporting, there is high variability in capacity to collect risk factor or demographic information, trace contacts, and connect people with HCV infection to further testing and treatment.[2,5] These limitations have discouraged use of public health surveillance data for HCV transmission modeling.

We developed an HCV transmission model fit to HCV surveillance data among 15-29 year olds in Michigan that adjusts for case under-reporting. We reviewed the literature to identify ranges for model parameters and simulated the model across 10,000 plausible scenarios using Latin hypercube sampling, a form of stratified random sampling.[50,59] We then evaluated the potential impact of several interventions, including primary prevention (reduced injection initiation), secondary prevention (behavioral initiatives), and tertiary initiatives (HCV treatment) in a counterfactual framework by summarizing the predicted reduction in HCV prevalence (chronic cases) and incidence (acute cases). This modeling framework could be applied to HCV surveillance data from other states and/or adapted for use in other nationally or state-notifiable conditions.

3.4   Methods

A detailed discussion of the model structure, parameters, initial conditions, surveillance data, and parameter estimation process is available in Appendix 3.1. Matlab (The MathWorks Inc, Natick, MA) code for model simulation and R (R Foundation for Statistical Computing, Vienna, Austria) code for figures is freely available at https://github.com/epimath/Hepatitis-C-in-Young-PWID.

3.4.1 Model Structure

An HCV ordinary differential equation (ODE) transmission model of PWID with preferential age mixing was developed and implemented in Matlab R2017b. The model consists of 11 states per age group, with age groups of 15-19 years, 20-25 years, 26-29 years, and 30-64 years (Figure 3.1). Individuals age through groups and transition through a series of compartments within age classes: uninfected, non-PWID with substance dependence or abuse (Z_i), uninfected current or former PWID (S_i or S_{Ni}, respectively), acutely infected current or former PWID (A_i or A_{Ni}, respectively), chronically infected current or former PWID (C_i or C_{Ni}, respectively), immune current or former PWID (I_i or I_{Ni}, respectively), and treated current or former PWID (T_i or T_{Ni}, respectively).

Individuals in our model acquire HCV by injecting drugs; other transmission modes (e.g. perinatal acquisition, unregulated tattoos, sexual transmission) are not considered. IDU is the primary risk factor for new HCV infections in the US, therefore we focus on this risk factor.[5] Non-PWID initiate IDU at an estimated injection initiation rate, θ_i, calibrated to fit HCV surveillance data. Susceptible current PWID acquire new infections at an estimated rate β through effective contact (i.e. syringe sharing) with an infected (acute [A_j] or chronic [C_j]) or treated (T_j) current PWID in any age class. Contact rates between current PWID in each age class are parametrized by partitioning each susceptible individual's (S_i) total contacts (σ_i) into the number of contacts per each age class. This partitioning of contacts is parametrized by a contact matrix, Π, which is adapted from social contact patterns in eight European countries and informed by a study of IDU syringe sharing networks (Appendix 3.1).[60,61] Individuals from any of the current PWID classes can stop injecting drugs and move to their adjacent former PWID class at a cessation rate γ_i while former PWID can begin injecting again after a period of

injection abstinence and enter the current PWID class at a relapse rate κ_i. To maintain a realistic ratio of current to former PWID during model fitting to data, we calculated age-specific relapse rates for each simulation using the sampled cessation rates and prevalence of current and former IDU in the US based on national survey data (Appendix 3.1).[1,6,62]

3.4.2 Surveillance Data, Parameter Estimation, and Parameter Sampling

The Michigan Department of Health and Human Services (MDHHS) receives reports of HCV diagnoses from healthcare providers and laboratories and stages cases as acute or chronic using standardized national case definitions.[53,54] We obtained the number of newly identified acute and chronic HCV cases per year during 2000-2016 (Table 3.1).

We made three adjustments to the acute case series to facilitate model fitting to data (Appendix 3.1). First, we adjusted the number of 2016 cases to the number that would have been detected by the 2012 case definition.[53,55] We assumed that only 74% of 2016 cases would have met the 2012 definition in accordance with an unpublished case series review conducted by MDHHS. Second, we substituted the number of acute cases among 15-19 year olds for the number of new chronic cases reported to MDHHS during the same period adjusted for under-reporting to facilitate model fitting (Figure 3.2). Finally, we adjusted for under-detection of the acute cases by MDHHS surveillance using a mid-point estimate between the correction factor developed by Klevens *et al.* and an estimate from Onofrey *et al.* of 1 case detected per 50 acute infections.[57,58]

To incorporate parameter uncertainty, we drew a stratified random sample of 10,000 parameter sets across plausible ranges gathered through literature review using Latin hypercube sampling (Table 3.2). We used an initial (year 2000) HCV prevalence of 10% among 15-19 year

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olds, 20% among 20-25 year olds, 30% among 26-29 year olds, and 55.2% among 30-64 year

olds in alignment with prevalence estimates from several US locations; no data were available

for Michigan.[10,15,44–49] To optimize model fit to data, we estimated four unknown parameters (the

transmission rate [β] and three age-specific injection initiation rates [θ_i]) in each simulation using

unweighted least squares assuming normally distributed measurement error with equal variances

for each data point. The rate of injection initiation for 30-64 year olds was not estimated as we

had no data to fit to for this age group and because the focus of our analysis was on young

PWID. Instead, we sampled this parameter.

Residual sum of squares values after parameter estimation were used to select the best-

fitting 10% of parameter sets to data (i.e. 1,000 simulations). To determine if a certain range

appeared more consistent with data, we plotted histograms by quartile of fit to visualize

differences in fit along uniformly sampled parameter ranges (Figure 3.3). Parameter estimation

and simulations were run using `fminsearchbnd` and the `ODE15S` solver in Matlab (due to

stiffness in some simulation runs).[63] Appendix 3.1 outlines details for the selection of bounds for

estimated parameters used in `fminsearchbnd` and a discussion of our reasons for expanding

injection initiation rate bounds from values found in the literature.[64–74]

3.4.3   Intervention Simulations

We selected the best-fitting 10% of parameter sets (1,000 simulations) to simulate the

potential impact of interventions on HCV incidence (acute cases) and prevalence (chronic cases)

among 15-29 year olds in Michigan. The model was re-simulated after scaling one or more

parameters, which provided counterfactual estimates of the expected percent reduction in acute

or chronic HCV cases in the presence of interventions compared to baseline model estimates from the model fitting phase (i.e. no intervention).

We simulated primary prevention interventions (reduced injection initiation [θ_i]), secondary prevention interventions (decreased syringe sharing [σ_i], decreased IDU relapse [κ_i], and increased IDU cessation [γ_i]), and tertiary prevention interventions (treatment of former [φ_N] and current PWID [φ_P]).[75] Although parameters cannot be singly classified into primary, secondary, and tertiary (e.g. current PWID treatment is tertiary [treatment] and secondary [reduces transmission]), these terms classify interventions by their most immediate roles. Parameters were scaled at 10%, 20%, and 40%. For treatment, these can be interpreted as treating 10%, 20%, or 40% of PWID per year. We first simulated the expected impact of each intervention alone. Next, we simulated the expected impact of combinations of interventions, specifically to compare the added benefit of secondary interventions on top of primary versus tertiary approaches. Intervention results are summarized by comparing the expected percent case reduction for acute and chronic HCV cases at the end of the 17-year simulation period (year 2016) compared to no intervention.

We conducted three sensitivity analyses for HCV treatment interventions. We first examined the impact of sustained virologic response (SVR), a measure of the percentage cured by HCV treatment.[76] We assumed that approximately 90% of individuals achieved SVR in main intervention simulations in alignment with a recent meta-analysis of DAAs by Ferreira *et al*., and examined values of 60-100% in sensitivity analyses to examine the potential impact of treatment incompletion among PWID.[76–82] We next examined expected case counts when a proportion of current PWID in treatment transmitted HCV. We assumed that 50% of treated current PWID contributed to transmission in the main results, and compared values of 0-100% in sensitivity

analyses. Finally, we simulated a treatment duration of 12 weeks for the main analysis, and examined 8 and 16 week durations in sensitivity analyses; all are typical treatment durations for currently approved DAAs.[13]

3.5   Results

3.5.1   Model Fit to Acute HCV Surveillance Data

The model fit data well for the 1,000 parameter sets used to simulate interventions (Figure 3.4, top). However, under some simulated conditions, case counts were overestimated (Figure 3.4, bottom). Chronic HCV prevalence generally increased throughout the period, especially among 15-29 year olds; however, there was a wide range in predicted prevalence due to the variability of conditions assessed (Figure 3.5). A variety of scenarios across sampled parameter ranges fit the data well (Figure 3.3). Better-fitting parameter sets had a slight tendency towards a lower transmission rate, injection initiation rates, and total contacts among 26-29 year olds, and towards a higher number of total contacts among 20-25 year olds and former PWID prevalence among 26-29 year olds.

3.5.2   Intervention Simulations

We simulated the potential impact of primary (reducing injection initiation), secondary (increasing cessation, reducing injection partners, or reducing injection drug use relapse), and tertiary (HCV treatment) interventions on incident and prevalent chronic HCV cases among young (aged 15-29 years) PWID. Among the best-fitting 1,000 simulations, treating former PWID predicted the largest reduction in chronic HCV prevalence (chronic HCV cases) and incidence (acute HCV cases) among 15-29 year olds at year 17 of simulation (Figure 3.6). While

this represents an 'on average' finding (over the 1,000 tested parameter sets), there were some scenarios wherein high current PWID prevalence made treating current PWID equally as effective as treating former PWID. In general, the predicted percent case reductions from treatment were highly dependent on parameter values, and therefore the predicted effectiveness of treatment spanned a large range, especially among former PWID.

Primary and secondary interventions also reduced prevalence and incidence, but less so than treatment (Figure 3.6). However, predicted impacts of these interventions were less variable than treatment interventions. Reductions in syringe sharing and injection initiation were predicted to be more effective than decreasing relapse. For instance, reducing the number of syringe sharing partners per year by 10% reduced HCV prevalence by 16.6% (range: 12.7-27.3%) and acute cases by 19.7% (range: 13.0-32.4%).

When considered in combination, adding secondary interventions enhanced the predicted reductions from primary prevention initiatives (reduced injection initiation) and HCV treatment when provided at lower levels (Figure 3.7). Treating former and current PWID together also reduced the uncertainty in treatment effect compared to treatment of former or current PWID alone. Treating 10% of both current and former PWID per year predicted a reduction in HCV prevalence by 69.8% (range: 47.9-74.0%) and incidence by 65.8% (range: 24.2-73.2%) among PWID aged 15-29 when we assumed that 90% were cured (i.e. achieved SVR). Reducing injection initiation, syringe sharing, and relapse rates each by 10% while increasing cessation rates by 10% reduced HCV prevalence by 49.1% (range: 40.1-67.3%) and acute cases by 57.7% (range: 47.1-77.3%). These magnitudes of predicted reductions were similar to those predicted for treatment of 10% of former PWID per year, but with much less variability.

3.5.3 Sensitivity Analyses

We assessed the impact of three aspects of treatment on predicted reduction of HCV incidence and prevalence. Reducing the proportion of treated current PWID who achieved SVR (i.e. were cured by treatment) made treatment less effective (Figure 3.8). The magnitude of these effects was similar for former PWID (data not shown). Treatment duration and the proportion of treated current PWID who shared syringes (and contributed to transmission during treatment) did not impact predicted treatment effectiveness.

3.6 Conclusions

We developed and implemented an HCV transmission model among young PWID informed by Michigan HCV surveillance data, and leveraged our model to evaluate the relative benefits of primary, secondary, and tertiary interventions for reducing HCV prevalence and incidence. The incorporation of state-level surveillance data allowed us to evaluate interventions in a framework consistent with the HCV incidence trends in Michigan. Simulation results suggested that HCV treatment was the most effective strategy, especially when both former and current PWID received treatment. Both singly and in combination with secondary prevention measures, such as decreasing relapse, increasing cessation, and decreasing syringe sharing, treatment predicted substantial reduction of chronic HCV infections. In line with the concept of 'treatment as prevention,' treatment also yielded reductions to acute HCV. We found no decrease in predicted reductions from HCV treatment when PWID continued to share syringes and contributed to transmission during treatment, supporting current recommendations that all PWID be provided treatment, regardless of their IDU behaviors.[13]

There are several important caveats to our findings about HCV treatment. First, the predicted impact of treatment was highly uncertain across scenarios tested, all of which represent reasonable approximations to the current HCV epidemic and PWID prevalence, given their parametrization using findings from empirical studies. In addition, compartmental HCV models that do not account for the dynamic network structure of syringe sharing are known to overestimate treatment effects, especially their treatment as prevention potential.[83] Incorporating network structure was beyond the scope of this model; however, we did incorporate some heterogeneity in IDU contact patterns based on age, and modeled contact patterns under a variety of scenarios. Further, our model was optimized at low estimates of the transmission rate, β, given its range of values in the literature.[84–86] This may reflect and account for some of the exaggeration of contact rates that contribute to the overestimation of HCV treatment's effectiveness in non-network models. Nonetheless, the predicted impacts of treatment should be taken as an upper bound of the potential treatment effect and the integration of a network model within our current framework is an area for future model development. Finally, treatment results were somewhat sensitive to cure rates. Taken together, these results emphasize the importance of engaging PWID in treatment until cure, and highlight the need to incorporate behavioral interventions that compliment treatment, given the uncertainty about its effects.

Primary prevention interventions that reduce injection initiation and secondary prevention interventions, which reduce syringe sharing, promote injection cessation, and prevent relapse, consistently predicted reductions in HCV prevalence and incidence. These results were robust across tested scenarios (i.e. parameter sets). Thus, while HCV treatment is a major public health priority, primary and secondary interventions should be implemented concurrently. These simulated primary and secondary interventions are meant to represent real-world initiatives, such

as expanded access to addiction treatment services and medication assisted treatments, which help PWID stop using and prevent relapse, or the "Staying Safe," "Break the Cycle," or "Change the Cycle" interventions, which promote safe drug injection and prevent people who use drugs from initiating injection.[87,88] With respect to treatment, several studies have found that providing HCV treatment within existing addiction treatment or syringe services programs is feasible and produces cure rates similar to those seen in randomized controlled trials of DAAs among non-PWID, even when patients continue to use drugs.[76,79,81,82] In addition to reducing the public health impacts of HCV directly, interventions that include behavioral risk reduction focused on IDU could concurrently reduce the economic costs of HCV, which increase with earlier age of infection, and the economic and societal costs of heroin and other opioid use disorders.[89,90] Our results therefore reinforce the need for an integrated care model. For example, harm reduction programs should provide co-located services that include provision of sterile injecting equipment, bystander overdose response training, bloodborne virus testing and referral to (or coordination of) treatment for HCV and Human Immunodeficiency Virus, and other services, such as mental health counseling, addiction treatment, or opioid maintenance therapy.[91]

3.6.1.1 Limitations

Like all modeling exercises, we made several simplifying assumptions, and we were limited by existing data. Our model only considers HCV acquisition through IDU and we focus results on PWID aged 15-29 given available data. Our results may not be generalizable to other age or risk groups. Cases with missing risk factor data were assumed to be PWID, consistent with PWID being the most common risk factor for HCV.[3,5,92] Because surveillance data suffers from under-reporting and missing data, we applied a reporting rate to adjust for under-detection

of cases by surveillance during the time period under study, although the true number of undetected cases is unknown.[57,58] We used a scaled version of newly reported chronic cases instead of the acute case series among 15-19 year olds due to the high variability in reported acute cases in this age group. To address uncertainty in model parameter values, we sampled nearly all parameters and used values most consistent with surveillance data to simulate interventions.

Our model assumed homogeneous mixing beyond age and used a contact matrix from Mossong *et al.*, a European study of social contacts, that may not reflect the age-related patterns underlying syringe sharing.[60] We therefore expanded the ranges presented by Mossong *et al.* in an effort to capture many plausible syringe sharing scenarios. However, treatment effects may still be overestimated and should be interpreted as the maximum possible impact of treatment.[83] Treatment interventions also assumed a treatment-naïve population in model fitting, however this is likely realistic given the historically low treatment rates among PWID during the time period under study.[93–96] Finally, in some simulations, our model's estimated injection initiation rates exceeded values found in the literature.[64–74] This discrepancy in injection initiation rates could be for several reasons, including that injection initiation rates were not available for Michigan, that available studies were conducted among specific populations (e.g. Canadian street youth), or because high estimated values of injection initiation rates were artefacts of poorly fitting simulations or ill-suited initial conditions to data. Intervention simulations were conducted on the best fitting 10% of simulations, and therefore reflect the potential impact of interventions for values consistent with empirical studies and these higher values.

3.6.1.2   Conclusions

HCV surveillance data is a valuable source of information for understanding HCV transmission and identifying local intervention opportunities among young PWID. In Michigan, HCV treatment could reduce prevalence and incidence. The impact of treatment is more certain when both former and current PWID are treated and when treatment is combined with behavioral interventions that reduce injection drug use initiation or syringe sharing, increase injection cessation, and reduce relapse. PWID at all stages of use or recovery should be connected to HCV treatment alongside primary and secondary prevention interventions.

3.6.1.3   Acknowledgments

Figure 3.1 Hepatitis C Transmission Model among People who Inject Drugs: Model States and Parameters

Non-PWID Past Year Abuse or Dependence (Z_i) $\eta_Z\mu_i$

θ_i $\eta_P\mu_i$

$\alpha\omega$

$\delta\varepsilon(1-\xi)$ $\eta_P\mu_i$ $(1-\alpha)\omega$ $\eta_P\mu_i$

Susceptible Current PWID (S_i) $\beta\pi_{i,j}\sigma_i$ Acute HCV, Current PWID (A_i) $(1-\delta)\varepsilon$ Chronic HCV, Current PWID (C_i) φ_P Treated Current PWID (T_i)

$\eta_P\mu_i$

Immune Current PWID (I_i)

$\delta\varepsilon\xi$

κ_i γ_i κ_i γ_i κ_i γ_i κ_i γ_i κ_i γ_i

$\delta\varepsilon\xi$ Immune Former PWID (I_{Ni})

$\eta_N\eta_P\mu_i$

Susceptible Former PWID (S_{Ni}) Acute HCV, Former PWID (A_{Ni}) $(1-\delta)\varepsilon$ Chronic HCV, Former PWID (C_{Ni}) φ_N Treated Former PWID (T_{Ni})

$\eta_N\eta_P\mu_i$ $\delta\varepsilon(1-\xi)$ $\eta_N\eta_P\mu_i$ $\eta_N\eta_P\mu_i$ $(1-\alpha)\omega$ $\eta_N\eta_P\mu_i$

$\alpha\omega$
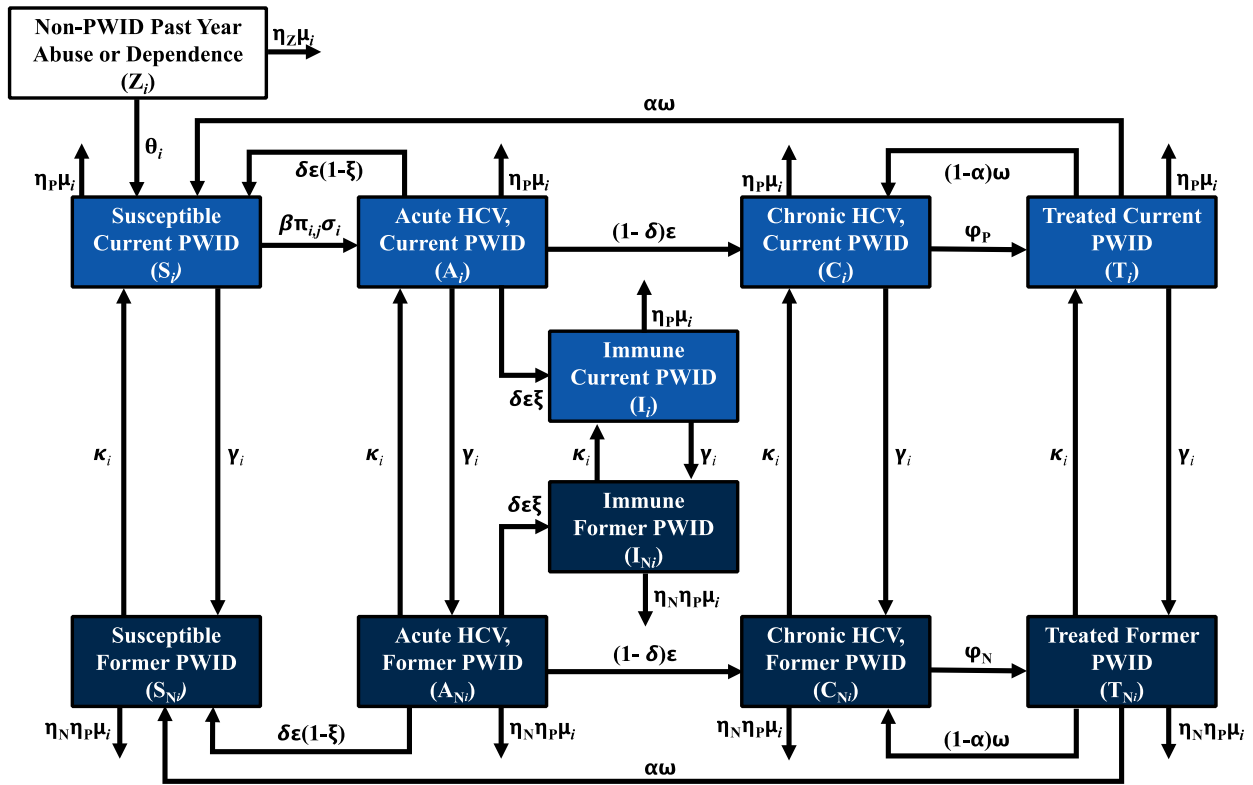
This diagram outlines model states and parameters controlling flows between states of an HCV transmission model among PWID in Michigan. Non-PWID with substance abuse or dependence (Z_i) begin injecting drugs (S_i) at a rate θ_i and acquire acute infection (A_i) through effective contact with an HCV-infected PWID (A_j or C_j or T_j) of the same or discordant age ($\Pi_{j,i}$) at a transmission rate (β). Chronic infection (C_i) develops at a rate ε among a proportion of acute cases, $(1-\delta)$, and resolves in the remaining δ acute cases, of whom ξ develop sterilizing immunity and the remaining $(1-\xi)$ become susceptible to reinfection. Chronic infection can be treated (T_i) at a rate φ_P, for a duration of ω^{-1} years, after which point susceptibility to reinfection ensues. PWID stop injecting drugs at a rate γ_i and transition to former PWID states (denoted by State$_{Ni}$). Former PWID can begin injecting drugs after a period of abstinence at a rate κ_i. Death occurs at a rate μ_i, which is elevated among current PWID by a factor η_P and among non-PWID by a factor η_Z. Former PWID mortality rates are closer to mortality rates from the general Michigan population (μ_i) by multiplying the current PWID mortality increase factor by a protective factor η_N. Subscript i denotes parameter or state age class (1: 15-19 years, 2: 20-25 years, 3: 26-29, 4: 30-64 years) and subscript j denotes the age group of contacts from whom susceptible current PWID (S_i) can acquire infection. Individuals move through age groups based on the duration predicted by the age range captured in each group (ν_i, not depicted for simplicity) and new 15 year-olds are added to the non-PWID compartment each year at a rate $\nu_0\psi_N Z_0$ (not depicted for simplicity).

Table 3.1 Acute HCV Cases among 15-29 Year Olds Detected by Viral Hepatitis C Surveillance—Michigan, 2000-2016

Characteristic	N (%)
Total	343 (100.0)
Female[a]	178 (51.9)
Age	
15-19[b]	48
20-25	180
26-29	118
Race	
Black or African American	10 (2.9)
White	286 (83.4)
Other or Multiple Races	21 (6.1)
Unknown	25 (7.3)
Ethnicity	
Hispanic or Latino	11 (3.2)
Not Hispanic or Latino	195 (56.9)
Unknown	137 (39.9)
Injected Drugs in the 2 Weeks to 6 Months Before Symptom Onset[c]	
Yes	226 (65.9)
Unknown or Missing	117 (34.1)
Case Status	
Confirmed	333 (97.1)
Probable	10 (2.9)

[a]All remaining cases were male. No cases were unknown or other gender.
[b]For simulations, we used a case series of newly reported chronic infections to MDHHS among 15-19 year olds, which was scaled for under-reporting (Appendix 3.1).
[c]67 cases who reported no IDU in the 2 weeks to 6 months before symptom onset were excluded from the present analysis.

Figure 3.2 Acute and Newly Reported Chronic HCV Cases Detected by Viral Hepatitis Surveillance among PWID Aged 15-19—Michigan, 2000-2016
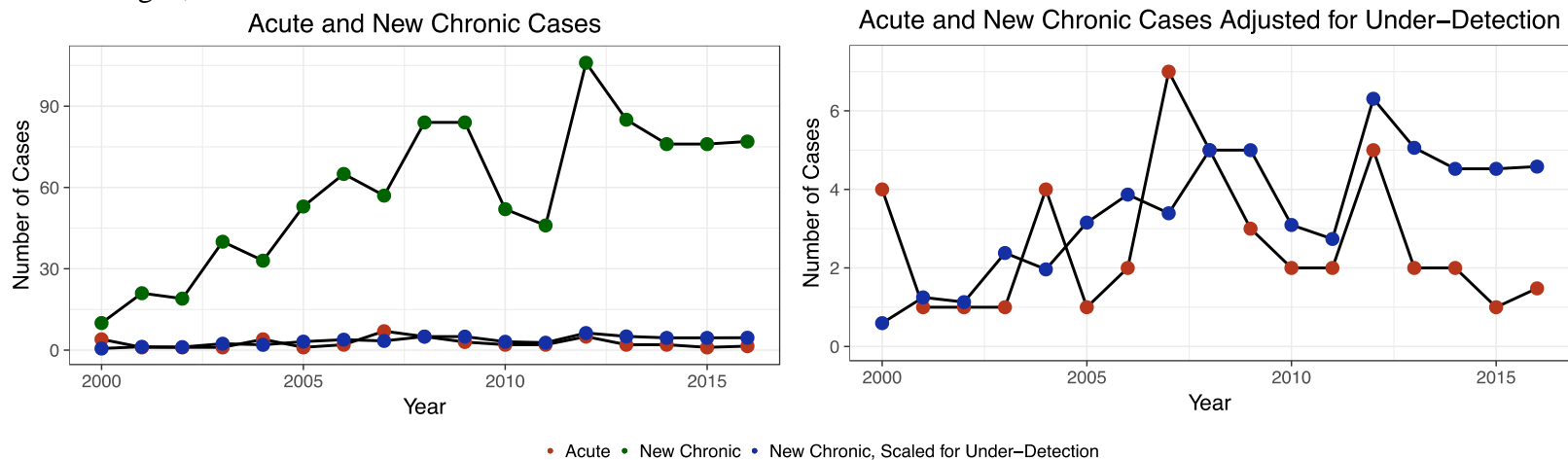
The acute HCV case series among 15-19 year olds (red) was characterized by few cases with high variability, presenting a challenge for model fitting to data, whereas newly reported chronic cases, another case series used to summarize HCV incidence, showed a more consistent trend (green). We used the 15-19 new chronic case series (blue) scaled by the under-reporting rate from Klevens *et al.* during model fitting.[57]

Table 3.2 Parameters and Sampling Ranges Incorporated through Latin Hypercube Sampling for an HCV Transmission Model among PWID in Michigan

Parameter	Best Fit Value[a] (Sampled Range or Estimate Bounds)	Units	Definition	Source
α	NA (Intervention)	%	Percent treated who are cured (achieve sustained virologic response)	Intervention sensitivity analysis, tested scenarios from Aspinall et al.,[78] Butner et al.,[79] Eckhardt et al.,[81] Ferreira et al.[76], Hellard et al.,[77] Saeed et al.,[80] Trabut et al.[82]
β	0.000019 (0-10.0)[b]	%	Probability of infection given contact with HCV	Fit to data from CDC,[84] Hasan et al.,[85] Mitsui et al.[86]
γ_1	0.42 (0.07-1.2)	Years^{-1}	Injection cessation rate (15-19 years)	Chang et al.,[97] Fazito et al.,[98] Genberg et al.[99]
γ_2	0.18 (0.07-1.2)	Years^{-1}	Injection cessation rate (20-25 years)	Chang et al.,[97] Fazito et al.,[98] Genberg et al.[99]
γ_3	1.0 (0.07-1.2)	Years^{-1}	Injection cessation rate (26-29 years)	Chang et al.,[97] Fazito et al.,[98] Genberg et al.[99]
γ_4	0.08 (0.07-1.2)	Years^{-1}	Injection cessation rate (30-64 years)	Chang et al.,[97] Fazito et al.,[98] Genberg et al.[99]
δ	0.19 (0.15-0.5)	Years^{-1}	Probability of spontaneously resolving acute HCV	Chung,[100] Heller & Rehermann,[101] Thomas & Seeff,[102] Westbook & Dusheiko[103]
ε^{-1}	0.5 (NS)	Years^{-1}	Duration of acute infection	Westbook & Dusheiko[103]
ζ_1	0.52 (0.15-1.0)	%	Prevalence of past year injection drug use (ages 15-19, year 2000)	NSDUH (ICPSR)[62] & Tempalski et al.[1]
ζ_2	0.30 (0.26-1.0)	%	Prevalence of past year injection drug use (ages 20-25, year 2000	NSDUH (ICPSR)[62] & Tempalski et al.[1]
ζ_3	0.56 (0.19-1.0)	%	Prevalence of past year injection drug use (ages 26-29, year 2000	NSDUH (ICPSR)[62] & Tempalski et al.[1]

ζ_4	0.59 (0.16-1.1)	%	Prevalence of past year injection drug use (ages 30-64, year 2000	NSDUH (ICPSR)[62] & Tempalski et al.[1]
η_N	0.37 (0.18-0.54)	Unitless	Standardized mortality ratio: former vs. current PWID	Mathers & Degenhardt[104]
η_P	11.7 (2.5-15.3)	Unitless	Standardized mortality ratio: current PWID vs. general population	Degenhardt et al.,[105] Evans et al.,[106] Vlahov et al.[107]
η_Z	4.3 (4.3-4.4)	Unitless	Standardized mortality ratio: non-PWID who use drugs vs. general population	Veldhuizen & Callaghan[108]
θ_1	4.2 (0.05-5.0)[b]	Years^{-1}	Injection initiation rate (ages 15-19 years)	Fit to data from Arreola et al.,[71] Bluthenthal et al.,[73] Chami et al.,[70] Ompad et al.,[66] Parriott et al.,[64] Reddon et al.,[74] Roy et al.,[65] Roy et al.,[67] Young & Havens[69]
θ_2	1.9 (0.034-5.0)[c]	Years^{-1}	Injection initiation rate (ages 20-25 years)	Fit to data from Arreola et al.,[71] Bluthenthal et al.,[73] Chami et al.,[70] Lake et al.,[72] Ompad et al.,[66] Parriott et al.,[64] Reddon et al.,[74] Roy et al.,[65] Roy et al.,[67] Young & Havens[69]
θ_3	10.0 (0.034-10)[b]	Years^{-1}	Injection initiation rate (ages 26-29 years)	Fit to data from Arreola et al.,[71] Bluthenthal et al.,[73] Lake et al.,[72] Ompad et al.,[66] Stein et al.,[68] Young & Havens[69]
θ_4	0.046 (0.034-1.0)[b]	Years^{-1}	Injection initiation rate (ages 30-64 years)	Fit to data from Arreola et al.,[71] Lake et al.,[72] Stein et al.,[68] Young & Havens[69]
κ_1	0.030 (0.0000012-2.5)[c]	Years^{-1}	Injection drug use relapse rate (ages 15-19 years)	Calculated and checked for consistency with previous work (Evans et al.,[109] HHS,[91] Hubbard et al.,[110] McLellan et al.[111])
κ_2	0.062 (0.00077-1.7)[c]	Years^{-1}	Injection drug use relapse rate (ages 20-25 years)	Calculated and checked for consistency with previous work

				(Evans *et al.*,[109] HHS,[91] Hubbard *et al.*,[110] McLellan *et al.*[111])
κ_3	0.16 (0.000046-1.4)[c]	Years[-1]	Injection drug use relapse rate (ages 26-29 years)	Calculated and checked for consistency with previous work (Evans *et al.*,[109] HHS,[91] Hubbard *et al.*,[110] McLellan *et al.*,[111] Shah *et al.*[112])
κ_4	0.0027 (0.000062-0.78)[c]	Years[-1]	Injection drug use relapse rate (ages 30-64 years)	Calculated and checked for consistency with previous work (Evans *et al.*,[109] HHS,[91] Hubbard *et al.*,[110] McLellan *et al.*,[111] Shah *et al.*[112])
λ_1	10 (NS)	%	Initial HCV prevalence (ages 15-19 years, year 2000)	Amon *et al.*,[46] Boodram *et al.*,[44] Echevarria *et al.*,[15] Garfein *et al.*,[47] Garfein *et al.*,[45] Garfein *et al.*,[48] Jordan *et al.*[49]
λ_2	20 (NS)	%	Initial HCV prevalence (ages 20-25 years, year 2000)	Amon *et al.*,[46] Boodram *et al.*,[44] Echevarria *et al.*,[15] Garfein *et al.*,[47] Garfein *et al.*,[45] Garfein *et al.*,[48] Jordan *et al.*[49]
λ_3	30 (NS)	%	Initial HCV prevalence (ages 26-29 years, year 2000)	Amon *et al.*,[46] Boodram *et al.*,[44] Echevarria *et al.*,[15] Garfein *et al.*,[47] Garfein *et al.*,[45] Garfein *et al.*,[48] Jordan *et al.*[49]
λ_4	55.2 (NS)	%	Initial HCV prevalence (ages 30-64 years, year 2000)	Degenhardt *et al.*[10]
μ_1	55.3 (NS)	Persons*100,000 Persons[-1]*Years[-1]	Mortality rate (ages 15-19 years)	CDC Wonder[113] & Freide *et al.*[114]
μ_2	91.5 (NS)	Persons*100,000 Persons[-1]*Years[-1]	Mortality rate (ages 20-25 years)	CDC Wonder[113] & Freide *et al.*[114]
μ_3	119.0 (NS)	Persons*100,000 Persons[-1]*Years[-1]	Mortality rate (ages 26-29 years)	CDC Wonder[113] & Freide *et al.*[114]

μ_4	493.0 (NS)	Persons*100,000 Persons^{-1}*Years^{-1}	Mortality rate (ages 30-64 years)	CDC Wonder[113] & Freide *et al.*[114]
ν_0^{-1}	1 (NS)	Years	Rate of entry of new 15-year olds per year	Not applicable
ν_1^{-1}	5 (NS)	Years	Duration in 15-19 year old age class	Not applicable
ν_2^{-1}	6 (NS)	Years	Duration in 20-25 year old age class	Not applicable
ν_3^{-1}	4 (NS)	Years	Duration in 26-29 year old age class	Not applicable
ν_4^{-1}	35 (NS)	Years	Duration in 30-64 year old age class	Not applicable
ξ	42 (0-45)	%	Probability of developing immunity after spontaneous acute HCV clearance	Little data, informed by Mehta *et al.*[115]
o_1	0.93 (0.43-1.0)	%	Prevalence of formerly injecting drugs (ages 15-19, year 2000)	Lansky *et al.*[6] & NSDUH (ICPSR)[62]
o_2	0.94 (0.71-1.0)	%	Prevalence of formerly injecting drugs (ages 20-25, year 2000)	Lansky *et al.*[6] & NSDUH (ICPSR)[62]
o_3	2.1 (0.81-2.1)	%	Prevalence of formerly injecting drugs (ages 26-29, year 2000)	Lansky *et al.*[6] & NSDUH (ICPSR)[62]
o_4	1.9 (1.6-2.7)	%	Prevalence of formerly injecting drugs (ages 30-64, year 2000)	Lansky *et al.*[6] & NSDUH (ICPSR)[62]
$\pi_{1,1}$	3.0 (1.6-9.3)	Contacts*Persons^{-1} * Days^{-1}	Sampled number of 15-19 year old contacts aged 15-19 years	Mossong *et al.*[60] & Williams *et al.*[61]
$\pi_{1,2}$	0.32 (0.25-1.6)	Contacts*Persons^{-1} * Days^{-1}	Sampled number of 20-25 year old contacts aged 15-19 years	Mossong *et al.*[60] & Williams *et al.*[61]
$\pi_{1,3}$	0.16 (0.12-0.47)	Contacts*Persons^{-1} * Days^{-1}	Sampled number of 26-29 year old contacts aged 15-19 years	Mossong *et al.*[60] & Williams *et al.*[61]
$\pi_{1,4}$	0.42 (0-3.1)	Contacts*Persons^{-1} * Days^{-1}	Sampled number of 30-64 year old contacts aged 15-19 years	Mossong *et al.*[60] & Williams *et al.*[61]
$\pi_{2,1}$	0.84 (0.3-1.2)	Contacts*Persons^{-1} *Days^{-1}	Sampled number of 15-19 year old contacts aged 20-25 years	Mossong *et al.*[60] & Williams *et al.*[61]

$\pi_{2,2}$	2.5 (0.92-3.7)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 20-25 year old contacts aged 20-25 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{2,3}$	0.64 (0.35-2.3)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 26-29 year old contacts aged 20-25 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{2,4}$	4.3 (0-4.5)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 30-64 year old contacts aged 20-25 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{3,1}$	0.33 (0.16-0.44)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 15-19 year old contacts aged 26-29 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{3,2}$	0.63 (0.47-1.8)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 20-25 year old contacts aged 26-29 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{3,3}$	1.1 (0.64-2.3)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 26-29 year old contacts aged 26-29 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{3,4}$	3.3 (0-6.4)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 30-64 year old contacts aged 26-29 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{4,1}$	1.0 (0-2.8)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 15-19 year old contacts aged 30-64 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{4,2}$	3.0 (0-4.7)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 20-25 year old contacts aged 30-64 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{4,3}$	1.7 (0-5.0)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 26-29 year old contacts aged 30-64 years	Mossong et al.[60] & Williams et al.[61]
$\pi_{4,4}$	33 (13-34)	Contacts*Persons^{-1}*Days^{-1}	Sampled number of 30-64 year old contacts aged 30-64 years	Mossong et al.[60] & Williams et al.[61]
ρ^{-1}	50 (NS)	True Cases per Surveillance-Detected Case	Reporting rate	Informed by Klevens et al.[57] & Onofrey et al.[58]
σ_1	14.3 (5.0-20)	Contacts*Years^{-1}	Syringe sharing partners among PWID aged 15-19 years	Wide range sampled (e.g. Conrad et al.,[116] Eckhardt et al.,[117] Kim et al.,[118] Mackesey-Amiti et al.,[119] Williams et al.[61])
σ_2	10.1 (5.0-20)	Contacts*Years^{-1}	Syringe sharing partners among PWID aged 20-25 years	Wide range sampled (e.g. Conrad et al.,[116] Eckhardt et al.,[117] Kim et al.,[118] Mackesey-Amiti et al.,[119] Williams et al.[61])

σ_3	9.9 (5.0-20)	Contacts*Years^{-1}	Total number of syringe sharing partners among PWID aged 26-29 years	Wide range sampled (e.g. Conrad *et al.*,[116] Eckhardt *et al.*,[117] Kim *et al.*,[118] Mackesey-Amiti *et al.*,[119] Williams *et al.*[61])
σ_4	16.2 (5.0-20)	Contacts*Years^{-1}	Syringe sharing partners among PWID aged 30-64 years	Wide range sampled (e.g. Conrad *et al.*,[116] Eckhardt *et al.*,[117] Kim *et al.*,[118] Mackesey-Amiti *et al.*,[119] Williams *et al.*[61])
τ	NA (Intervention)	%	Proportion of currently injecting PWID who transmit during treatment	Intervention sensitivity analysis, tested scenarios across full range (0-100%)
φ_P	NA (Intervention)	Years^{-1}	Treatment rate, current PWID	Intervention, tested various scenarios including Mehta *et al.*[93] & Saeed *et al.*[80]
φ_N	NA (Intervention)	Years^{-1}	Treatment rate, former PWID	Intervention, tested various scenarios including Mehta *et al.*[93] & Saeed *et al.*[80]
ψ_1	1.9 (1.6-2.1)	%	Prevalence of past year dependence or abuse without IDU (ages 15-19, year 2000)	NSDUH (ICPSR)[62]
ψ_2	1.4 (1.1-1.5)	%	Prevalence of past year dependence or abuse without IDU (ages 20-25 year 2000)	NSDUH (ICPSR)[62]
ψ_3	0.79 (0.37-1.0)	%	Prevalence of past year dependence or abuse without IDU (ages 26-29, year 2000)	NSDUH (ICPSR)[62]
ψ_4	0.46 (0.24-0.48)	%	Prevalence of past year dependence or abuse without IDU (ages 30-64, year 2000)	NSDUH (ICPSR)[62]
ψ_N	2.2 (0.81-3.0)	%	Prevalence of past year dependence or abuse without IDU (ages 15-19, years 2000-2016)	NSDUH (ICPSR)[62]

ω^{-1}	NA (Intervention)	Years	Treatment duration	Sensitivity analysis, tested scenarios from AASLD[13]
P_1	723,319 (NS)	Persons	Average population of 15-19 year olds in Michigan, 2000-2016	CDC Wonder[120] & Freide et al.[114]
P_2	815,922 (NS)	Persons	Average population of 20-25 year olds in Michigan, 2000-2016	CDC Wonder[120] & Freide et al.[114]
P_3	483,070 (NS)	Persons	Average population of 26-29 year olds in Michigan, 2000-2016	CDC Wonder[120] & Freide et al.[114]
P_4	4,605,115 (NS)	Persons	Average population of 30-64 year olds in Michigan, 2000-2016	CDC Wonder[120] & Freide et al.[114]
Z_0	144,097 (NS)	Persons	Number of 15 year olds	CDC Wonder[120] & Freide et al.[114]

[a]Best fit value is the parameter's value for the best fitting single simulation to acute HCV data of 10,000 total simulations.

[b]β estimates were obtained from an initial value 0.00035 for all 10,000 simulations.

[c]Initial values for each injection initiation rate were sampled from the following ranges from the literature prior to parameter estimation: θ_1 (0.05-1.5), θ_2 (0.034-1.5), and θ_3 (0.034-1.5) and were bounded by the following ranges: θ_1 (0.05-5.0), θ_2 (0.034-5.0), and θ_3 (0.034-5.0) during parameter estimation and fit to acute HCV data.

[d]Presented as the best fitting parameter set's value and range of calculations for 10,000 simulations based on fit to acute HCV data. Values of κ_i were not sampled, but rather were calculated from γ, ζ, η_N, η_P, μ, ν, o, and P, for each parameter set (Appendix 3.1).

Figure 3.3 Residual Sum of Squares Distribution and Latin Hypercube Sampling Parameter Ranges by Fit to HCV Surveillance Data
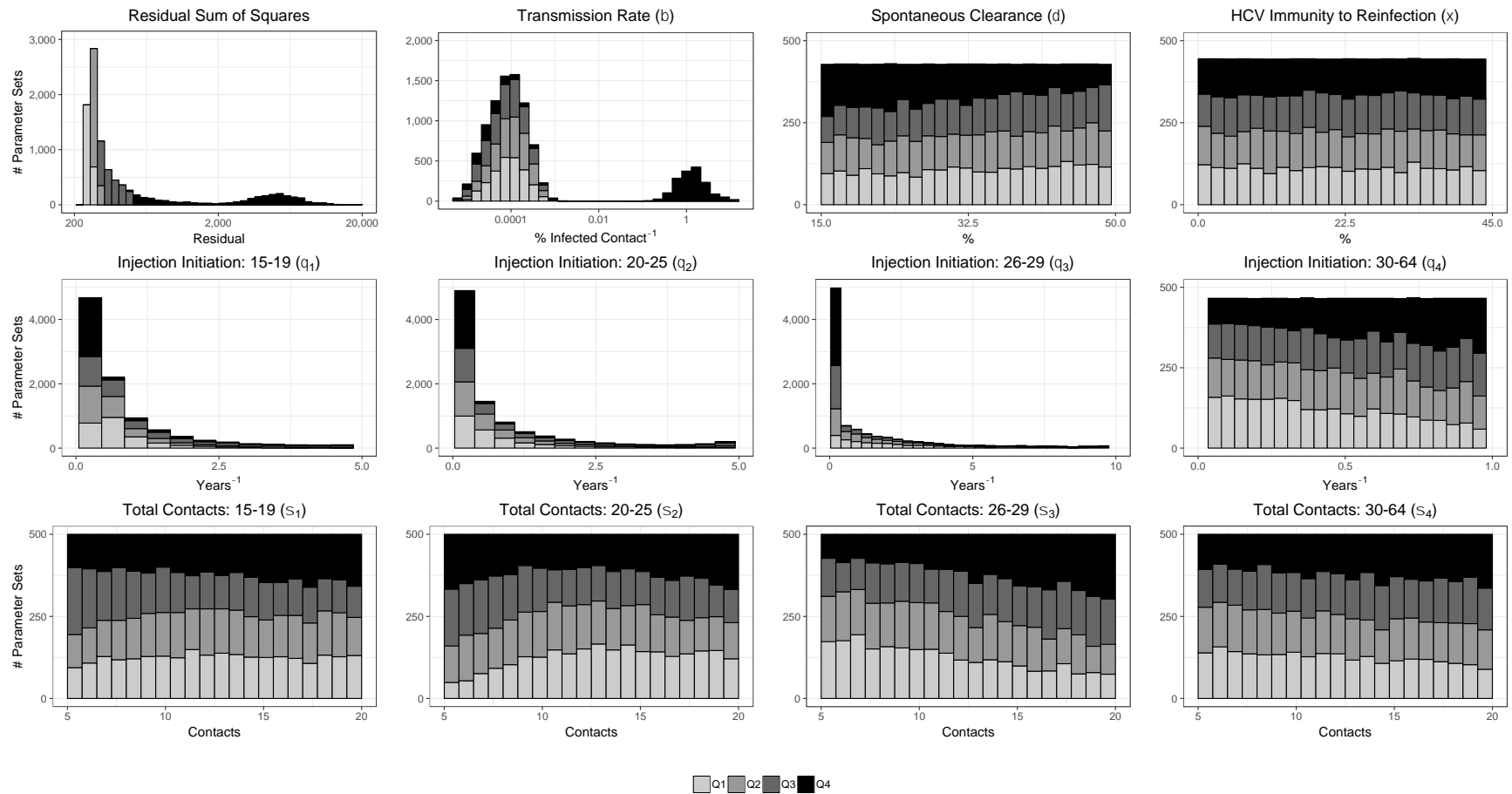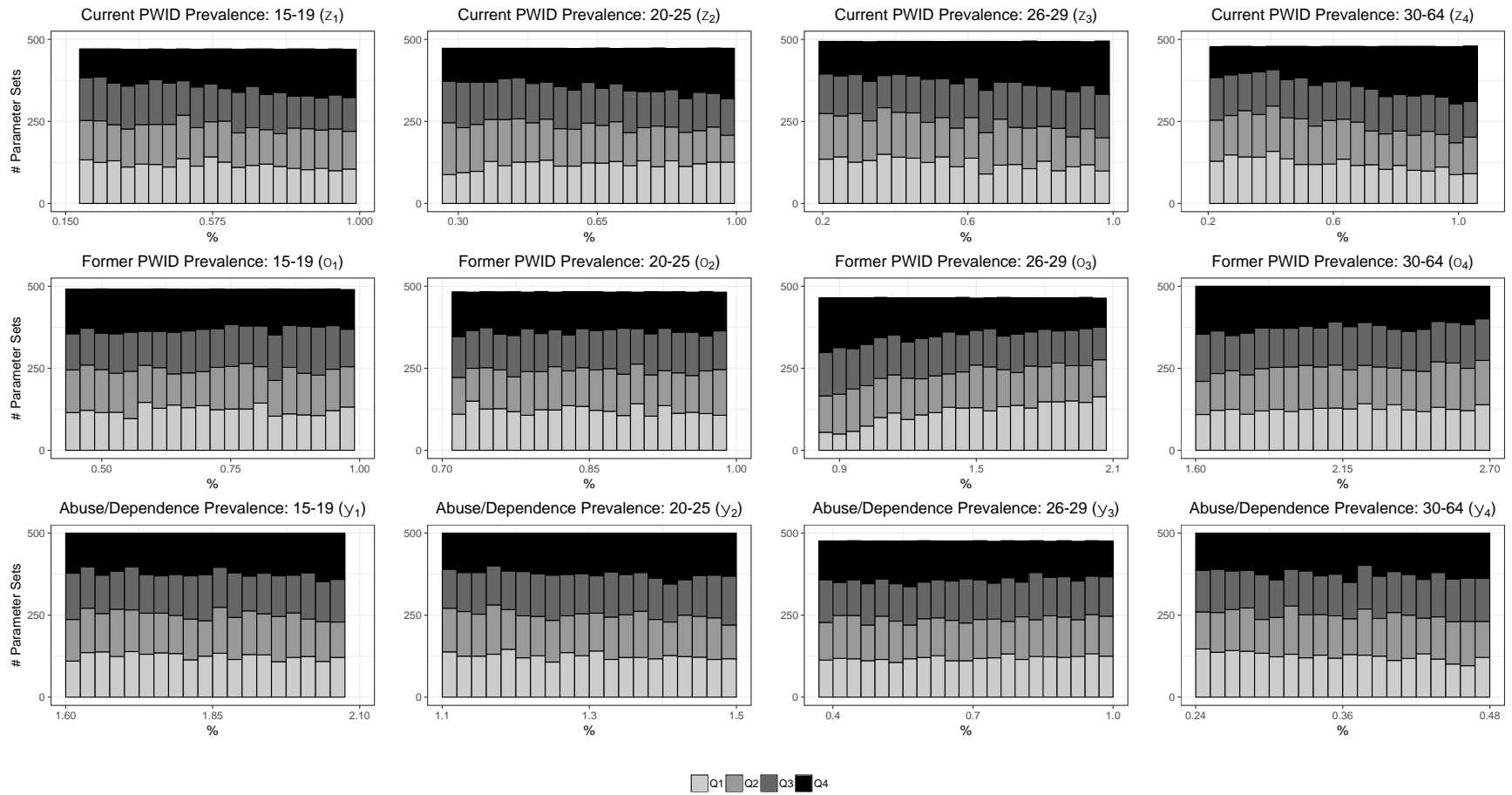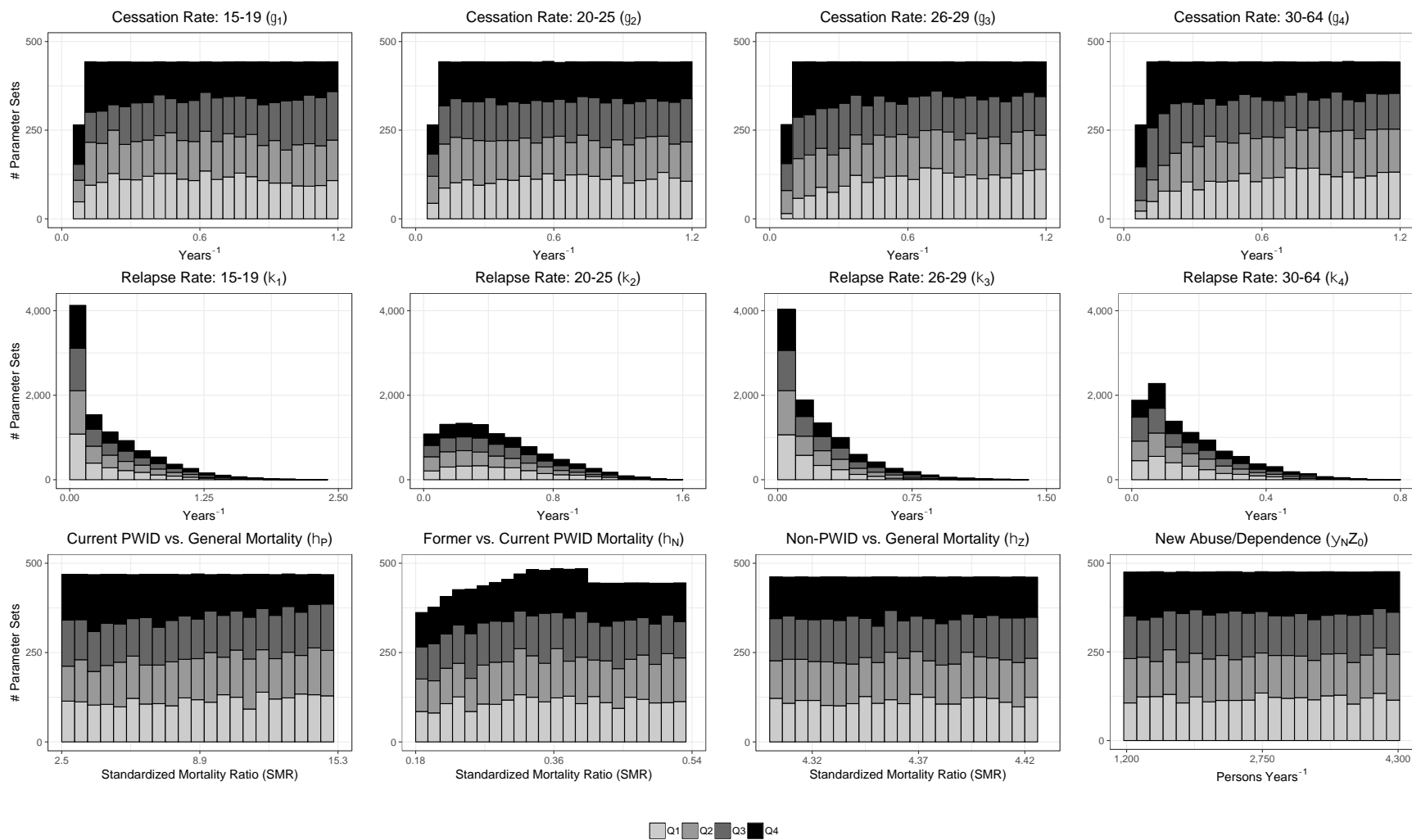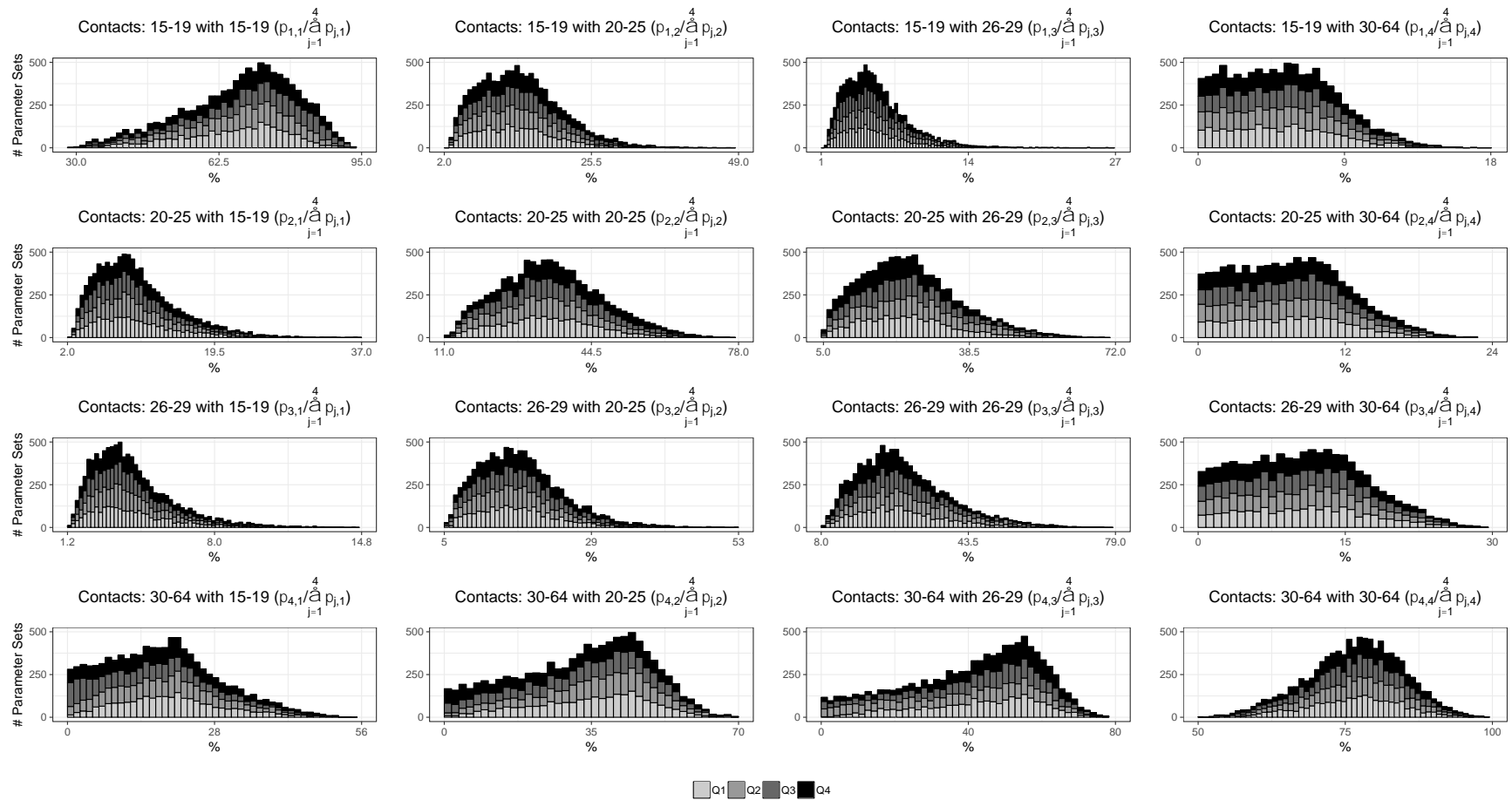
Latin hypercube sampling was used to sample 10,000 parameter sets determining model state flows and initial conditions across plausible ranges and we summarized each simulation by its quartile of fit (based on the residual sum of squares) to acute HCV data, where Q1 (light grey) represents the best fitting 25% of models and Q4 (black) shows the worst fit (residual sum of squares in the highest quartile) to data. Parameters fit to data (transmission rate [β] and injection initiation rates [θ_i]) or parameters adjusted (η_N) or calculated from other parameters (relapse rate [κ_i], proportion of contacts that occur with each age group [$\pi_{j,i}/\Sigma\pi_{j,i}$ for $j=1$ to 4]) are not expected to be sampled uniformly. Most sampled parameters fit well throughout their sampled range. Here we transform the contact parameters, $\pi_{j,i}$, to depict the sampled percent of total syringe sharing contacts that occur with PWID from each age group.

Figure 3.4 Model Fit to Acute HCV Cases Detected by Public Health Surveillance in Michigan among PWID, 2000-2016
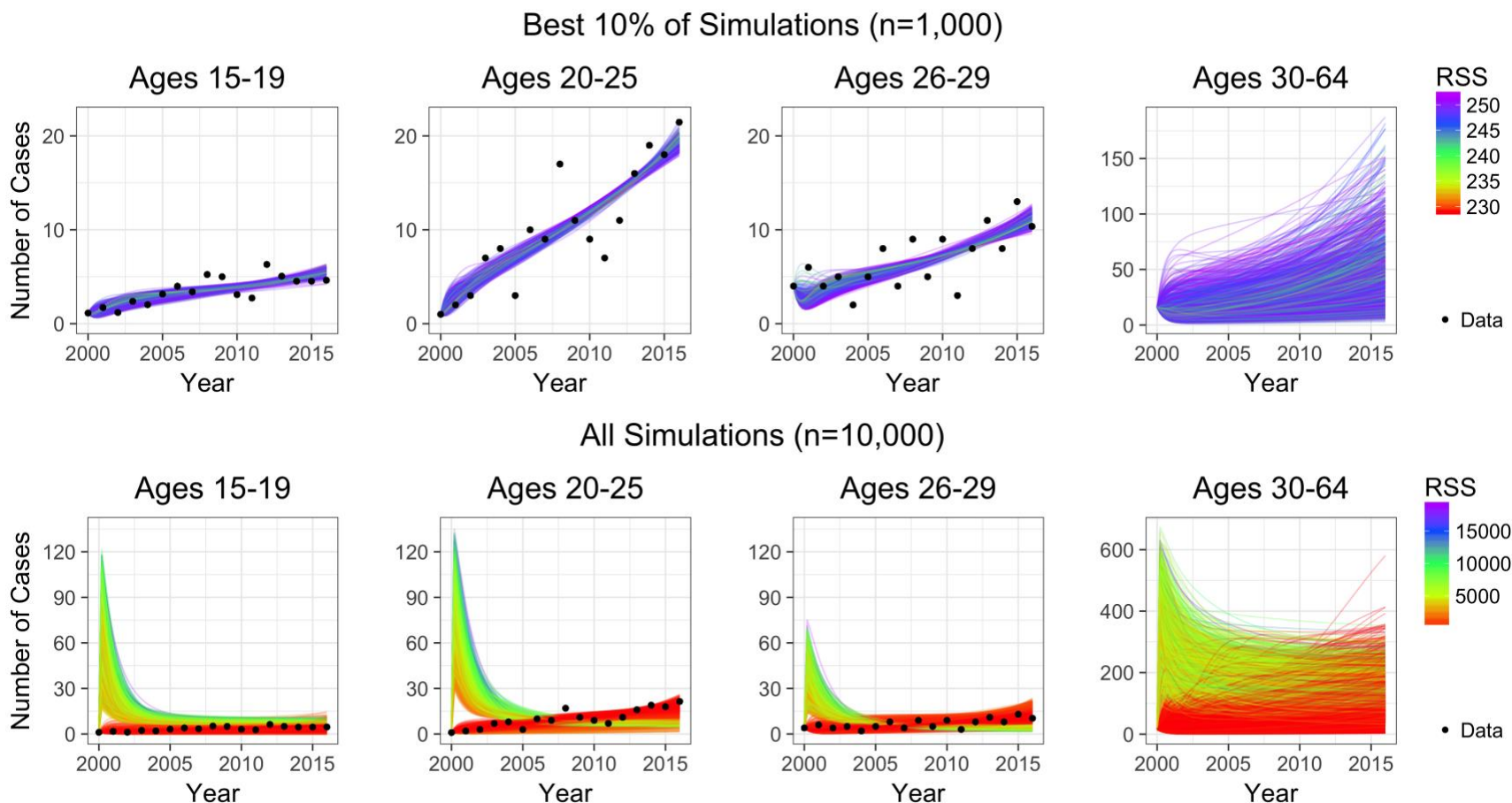
An ordinary differential equation HCV transmission model among PWID was fit to HCV surveillance-detected acute HCV cases aged 15-29 years reported to the Michigan Department of Health and Human Services during the years 2000-2016. Parameters were sampled across plausible ranges using 10,000 Latin hypercube samples. Model fit (colored lines) to data (black points) is shown by the residual sum of squares values. Results are shown for the best fitting 10% of simulations (top) and for all 10,000 simulations (bottom) for each age group. We did not fit to data for PWID aged 30-64 and instead show predicted acute HCV cases.

Figure 3.5 Predicted Chronic Hepatitis C Cases among PWID in Michigan from a HCV Model Fit to HCV Surveillance Data in Michigan, 2000-2016
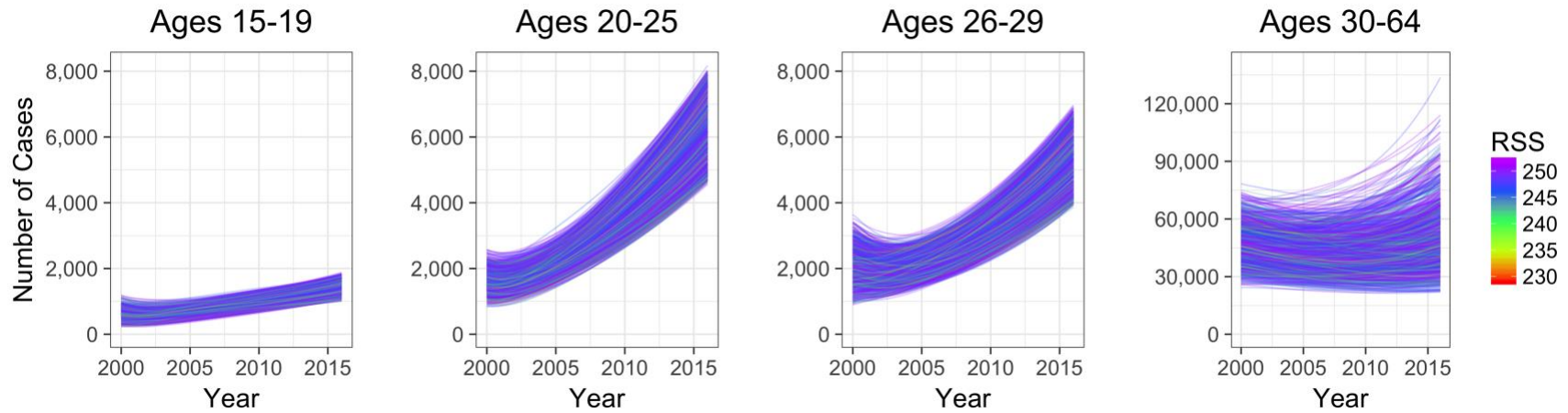
An ordinary differential equation HCV transmission model among PWID was fit to HCV surveillance-detected acute HCV cases aged 15-29 years reported to the Michigan Department of Health and Human Services during the years 2000-2016. Each graph summarizes the predicted number of chronic HCV cases in Michigan among the best fitting 10% to data (n=1,000 simulations) by age and fit to data (color of lines).

Figure 3.6 Counterfactual Simulations of Primary, Secondary, and Tertiary Interventions to Reduce HCV Prevalence and Incidence among Young PWID
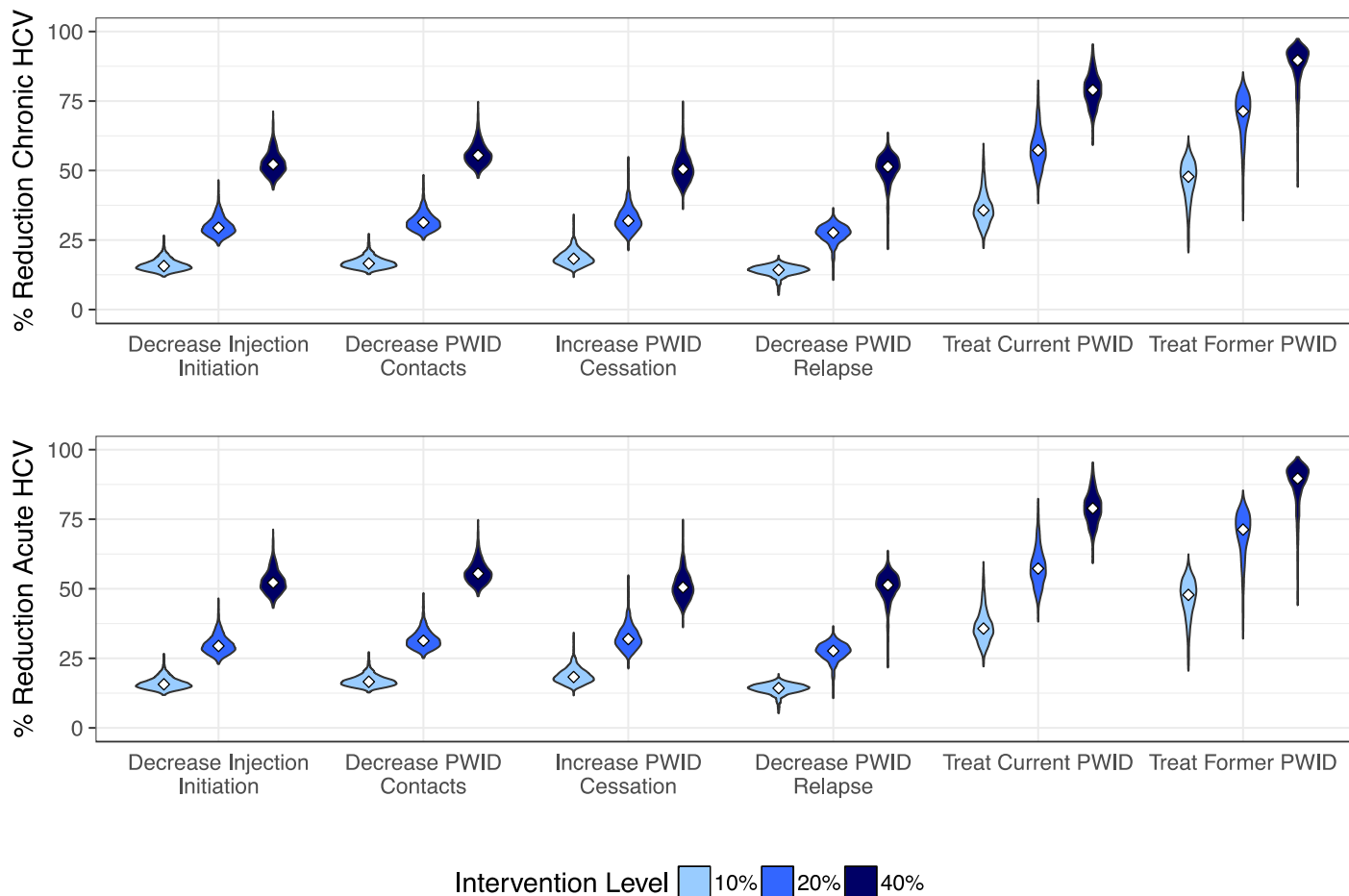
The distribution of predicted percent reduction in chronic HCV (top) and acute HCV (bottom) for each of 6 interventions among the best-fitting 10% of parameter sets to data are depicted as violin plots. Diamonds denote the median percent reduction. Treating former PWID and current PWID were associated with the largest predicted reductions to HCV prevalence. Other measures also reduced HCV, and generally had less variability across scenarios than treatment interventions.

Figure 3.7 Counterfactual Simulation of Combined Interventions to Reduce HCV Prevalence and Incidence among Young PWID
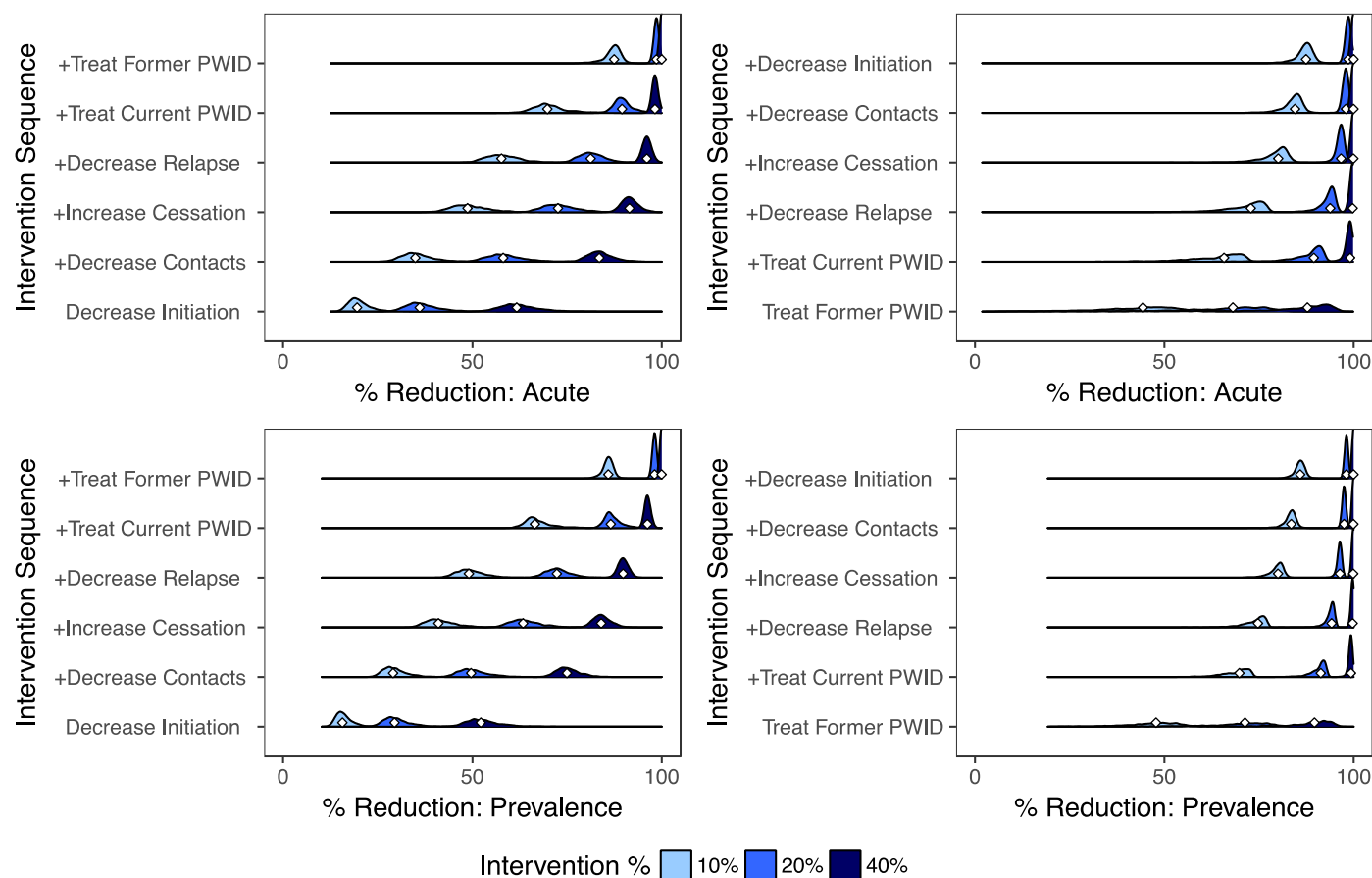
Histograms of the predicted percent reduction in acute (top) and chronic (bottom) among 15-29 year olds after 17-years simulation are plotted using the best fitting 1,000 parameter sets. All percent reductions are calculated compared to no intervention and interventions are sequentially added from a base of primary (left) versus tertiary (right) interventions. Diamonds denote the median percent reduction. Both strategies reduced HCV prevalence and incidence. Combined former and current PWID treatment had the largest impact on prevalence and incidence. The precision of predicted reductions grew with higher intensity interventions or when secondary interventions were combined with treatment.

Figure 3.8 Sensitivity of Predicted Effects of HCV Treatment among Current PWID to Cure Rate, Continued Syringe Sharing During Treatment, and Treatment Duration
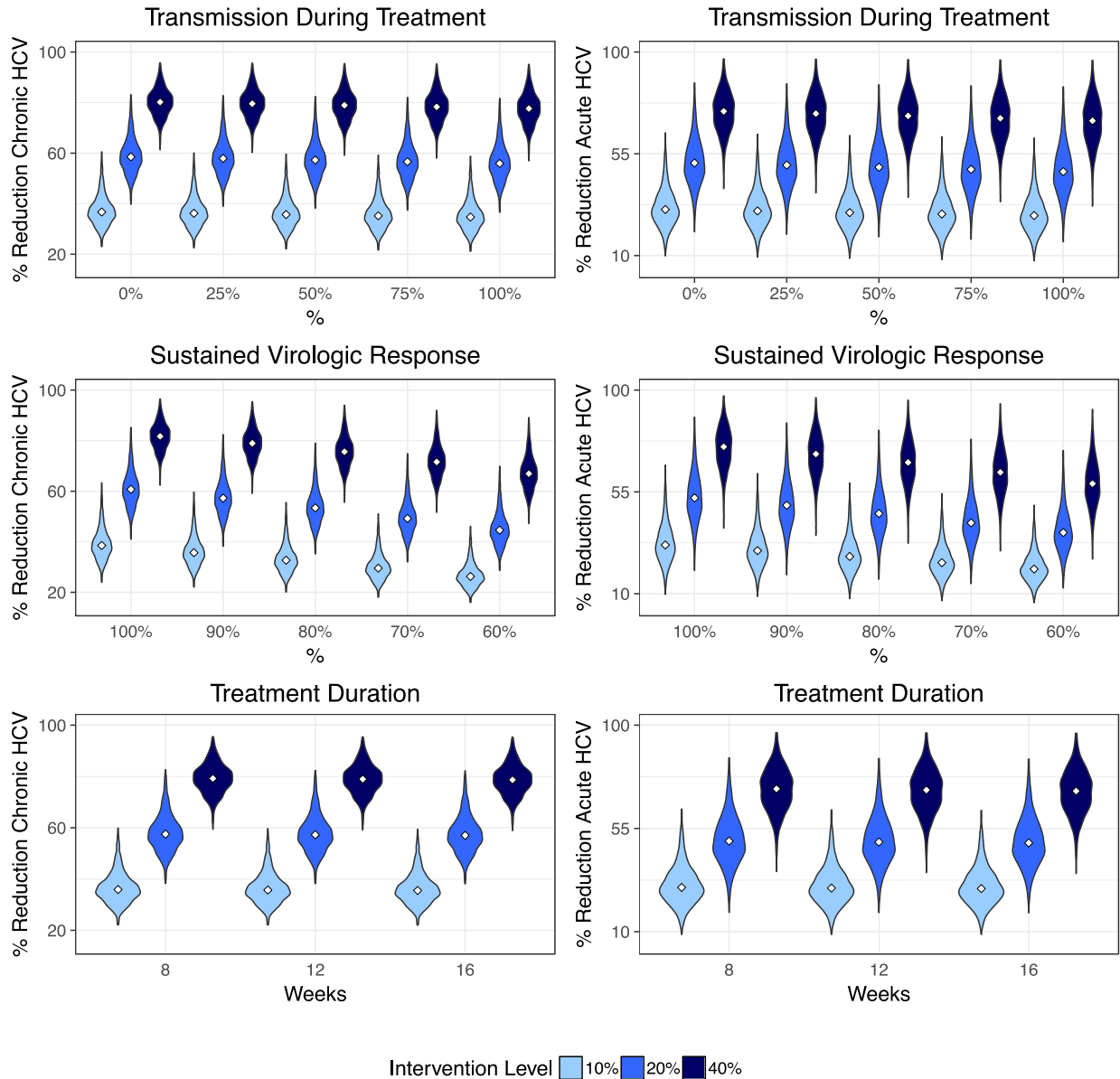
The distribution of predicted % reduction in HCV prevalence (left) and acute HCV (right) among 15-29 year olds for several treatment scenarios implemented among current PWID for the best 10% of parameter sets fitting to data are presented as violin plots. Diamonds denote the median percent reduction. We show the potential impact of ongoing syringe sharing during treatment (top), cure rates (middle), and treatment duration (bottom). The cure rate (% of treated current PWID achieving sustained virologic response) moderately impacted treatment effectiveness, whereby lower cure rates decreased the predicted impact of treatment.

Appendix 3.1 Detailed Methods for Model Fitting and Parameter Estimation for an HCV

Transmission Model among PWID in Michigan

Model Structure

An HCV ordinary differential equation (ODE) transmission model of PWID with preferential age mixing was developed in Matlab R2017b (The MathWorks Inc, Natick, MA). The model consists of 11 states per age group, with age groups of 15-19, 20-25, 26-29, and 30-64 years (Figure 3.1). We obtained the number of people with substance dependence or abuse (non-PWID, Z_i), former PWID, and current PWID by multiplying the Michigan population size for each age group (P_i) by several US national prevalence estimates described in further detail in Table 3.2.[1,6,62] Individuals age through groups and transition through a series of compartments within age classes: uninfected, non-PWID with substance abuse or dependence (Z_i), uninfected current or former PWID (S_i or S_{Ni}, respectively), acutely infected current or former PWID (A_i or A_{Ni}, respectively), chronically infected current or former PWID (C_i or C_{Ni}, respectively), and treated current or former PWID (T_i or T_{Ni}, respectively).

Movement through compartments is governed by a set of 44 differential equations shown in (1), where subscript i denotes the age class (1: 15-19 years, 2: 20-25 years, 3: 26-29 years, 4: 30-64 years) moving through compartments and subscript j denotes the age class of effective contacts from whom infection is acquired (i.e. by sharing injecting equipment). In the youngest age class, non-PWID without HCV infection are added each year to the Z_1 compartment based on the prevalence of substance abuse or dependence among 15-19 year olds from the National Survey on Drug Use and Health (NSDUH) and the average population size of 15 year-olds during 2000-2016 in Michigan.[62,114,120] These are represented with the term $\nu_0 \psi_N Z_0$ (omitted

from the equations below for simplicity). In other age groups, individuals age to the subsequent

age class at a rate ν_i. Mortality occurs at a rate μ_i, which is elevated for current PWID and

moderately elevated for former PWID and non-PWID with substance abuse or dependence (Z_i)

compared to age-specific mortality rates for the general population in Michigan through

standardized mortality ratios η_P, η_N, and η_Z, respectively. [104–108,113,114] The value of η_N is scaled

during model simulation so that the mortality among former PWID cannot fall below general

mortality rates, μ_i.

$$\frac{dZ_i}{dt} = -\theta_i Z_i - \eta_Z \mu_i Z_i + \nu_{i-1} Z_{i-1}$$

$$\frac{dS_i}{dt} = \theta_i Z_i - \beta \sigma_i S_i \sum_{j=1}^{4} \pi_{j,i}(A_j + C_j + \tau T_j) + \alpha \omega T_i + \delta \epsilon (1-\xi) A_i$$

$$+ \kappa_i S_{N_i} - \gamma_i S_i + \nu_{i-1} S_{i-1} - \nu_i S_i - \eta_P \mu_i S_i$$

$$\frac{dA_i}{dt} = \beta \sigma_i S_i \sum_{j=1}^{4} \pi_{j,i}(A_j + C_j + \tau T_j) - \epsilon A_i + \kappa_i A_{N_i} - \gamma_i A_i$$

$$+ \nu_{i-1} A_{i-1} - \nu_i A_i - \eta_P \mu_i A_i$$

$$\frac{dC_i}{dt} = (1-\delta)\epsilon A_i + (1-\alpha)\omega T_i - \phi_P C_i + \kappa_i C_{N_i} - \gamma_i C_i$$

$$+ \nu_{i-1} C_{i-1} - \nu_i C_i - \eta_P \mu_i C_i$$

$$\frac{dI_i}{dt} = \delta \epsilon \xi A_i + \kappa_i I_{N_i} - \gamma_i I_i + \nu_{i-1} I_{i-1} - \nu_i I_i - \eta_P \mu_i I_i$$

$$\frac{dT_i}{dt} = \phi_P C_i - \omega T_i + \kappa_i T_i - \gamma_i T_i + \nu_{i-1} T_{i-1} - \nu_i T_i - \eta_P \mu_i T_i$$

$$\frac{dS_{N_i}}{dt} = \delta \epsilon (1-\xi) A_{N_i} + \alpha \omega T_{N_i} + \gamma_i S_i - \kappa_i S_{N_i} + \nu_{i-1} S_{N_{i-1}} - \nu_i S_{N_i} - \eta_N \eta_P \mu_i S_{N_i}$$

$$\frac{dA_{N_i}}{dt} = -\epsilon A_{N_i} + \gamma_i A_i - \kappa_i A_{N_i} + \nu_{i-1} A_{N_{i-1}} - \nu_i A_{N_i} - \eta_N \eta_P \mu_i A_{N_i}$$

$$\frac{dC_{N_i}}{dt} = (1-\delta)\epsilon A_{N_i} + (1-\alpha)\omega T_{N_i} - \phi_N C_{N_i} + \gamma_i C_i - \kappa_i C_{N_i}$$

$$+ \nu_{i-1} C_{N_{i-1}} - \nu_i C_{N_i} - \eta_N \eta_P \mu_i C_{N_i}$$

$$\frac{dI_{N_i}}{dt} = \delta \epsilon (1-\xi) A_{N_i} + \gamma_i I_i - \kappa_i I_{N_i} + \nu_{i-1} I_{N_{i-1}} - \nu_i I_{N_i} - \eta_N \eta_P \mu_i I_{N_i}$$

$$\frac{dT_{N_i}}{dt} = \phi_N C_{N_i} - \omega T_{N_i} + \gamma_i T_i - \kappa_i T_{N_i} + \nu_{i-1} I_{N_{i-1}} - \nu_i T_{N_i} - \eta_N \eta_P \mu_i T_{N_i} \tag{1}$$

Individuals acquire HCV by injecting drugs; other transmission modes (e.g. perinatal acquisition, unregulated tattoos, sexual transmission) are not considered. Non-PWID flow into the susceptible PWID compartment (S_i) at an estimated injection initiation rate, θ_i, calibrated to fit acute case data from initial values based on empirical studies of injection initiation.[64–74] Susceptible PWID acquire new infections at an estimated rate β through effective contact between a susceptible PWID (S_i) and an acutely (A_j) or chronically (C_j) infected individual in any age class. A proportion (τ) of treated current PWID (T_j) also contribute to transmission during intervention simulations, as described further below. We set upper and lower bounds for estimated values of β from values in the literature.[84–86] Initial model fitting suggested that values were optimized at approximately 0.0000035 and we therefore used this value as the initial condition of β for each model simulation.

Contact rates between individuals in each age class are parametrized by a contact matrix, Π, and the total number of contacts per susceptible PWID (S_i), σ_i, described in further detail below. Newly infected PWID (A_i) have acute HCV infection for an average duration of 6 months (ε^{-1}), at which point 50-85% ($1-\delta$) develop chronic infection (C_i).[100–103] A fraction (ξ, 0-45%) of acutely infected individuals who spontaneously clear their HCV infection have sterilizing immunity and move to the immune class (I_i) while the remaining individuals ($1-\xi$) move back to the susceptible class (S_i) where they can be re-infected at the same rate as infection-naive individuals. While there is little data to inform this parameter, we used findings by Mehta *et al.*[115] to inform the sampling range for ξ.

Chronically infected individuals are treated (T_i and T_{Ni}) at rates φ_P and φ_N for a duration of ω^{-1} and become susceptible to HCV re-infection after treatment in the current PWID state by returning to the susceptible class (S_i). A proportion (τ) of current PWID receiving treatment (T_i)

transmit HCV (i.e. share syringes during treatment). Like all former PWID, treated former PWID (T_{Ni}) do not transmit HCV. Individuals from any of the current PWID classes can stop injecting drugs and move to their adjacent former PWID class at a cessation rate γ_i. Former PWID can begin injecting again after a period of injection abstinence and enter the current PWID class at a relapse rate κ_i.

Surveillance Data

The Michigan Department of Health and Human Services (MDHHS) receives reports of HCV diagnoses from healthcare providers and laboratories. Case status (acute or chronic) is determined using standardized national case definitions and cases are recorded in a centralized surveillance system.[53–56] We obtained the number of newly identified acute HCV cases per year during 2000-2016 (Table 3.1). Risk factors (e.g. recent injection drug use) are collected by surveillance systems, but not for all cases; however, injection drug use (IDU) is the most common mode of acquisition for new HCV cases nationally and in Michigan.[3,92] For the purposes of the model, we assumed that cases with no known risk factors injected drugs during the 2 weeks to 6 months before they were reported to MDHHS, and therefore were infected with HCV by IDU.

Acute and chronic HCV are under-reported in the US for several reasons, including that 20-30% of cases are asymptomatic, not all cases see a healthcare provider, not all cases who see a provider are diagnosed, and some of those diagnosed are never reported to state health departments.[57,121] CDC developed a correction factor representing the estimated number of acute cases occurring for each surveillance-detected acute HCV case (1 surveillance detected case per 16.8 true cases).[57] However, work by Onofrey *et al*. suggested that case under-detection by

surveillance systems was more severe given the stringency of the 2012 acute case definition (1 surveillance detected case per 138 true cases).[57,58] We use the inverse of an approximate middle point from these studies (1 surveillance detected case per 50 true cases) as a reporting rate, ρ, to scale the number of reported acute cases detected by surveillance during parameter estimation.

The high variability in the acute case series among 15-19 year olds presented a challenge during model fitting (Figure 3.2). However, the series of newly reported chronic cases to MDHHS among 15-19 year olds more consistently increased during the time period of interest and had significant overlap with the acute case series when scaled by the Klevens *et al.* estimate for under-reporting.[57] Therefore, we use the scaled newly reported chronic case series for 15-19 year olds as a proxy for acute cases and note the high variability in the acute case series as a limitation preventing model fitting to the observed acute data. State health departments also use newly reported chronic case reports among young PWID as indicators of HCV trends beyond those available from the sparse acute HCV surveillance data given their limited funding to detect and follow-up on acute cases and the stringency of the case definition before 2016.[50,52] This assumes that, given their young age, these individuals acquired infection relatively, consistent with patterns of substance use and injection drug use in the US.[62]

We made a final modification to the number of 2016 cases for all age groups based on a change in the case definition.[53,55] This change increased the number of acute cases detected by surveillance in 2016 by removing the requirement that confirmed cases be free from acute hepatitis A or B and introducing a probable class classification with less stringent laboratory requirements.[55] To maintain consistency throughout the time period of study, we therefore assumed that only 74% of 2016 cases (for all age groups) would have met the 2012 definition in

accordance with an unpublished case series review conducted by MDHHS and used this scaled version of 2016 cases for model fitting.

Initial Conditions

The initial number of acute HCV cases was set to the number of acute cases detected by MDHHS during 2000 who endorsed IDU in the HCV incubation period (2 weeks to 6 months before symptom onset) or who had unknown or missing responses regarding IDU multiplied by the inverse of the reporting rate (ρ^{-1}). As noted above, this included a scaled version of newly reported chronic cases for 15-19 year olds. Because the HCV prevalence in 15-64 year olds in Michigan is unknown, we used several HCV prevalence estimates from US empirical studies conducted outside of Michigan to inform the initial HCV prevalence.[10,15,44–49] We set initial HCV prevalence to 10% for 15-19 year olds, 20% for 20-25 year olds, 30% for 26-29 year olds, and 55.2% for 30-64 year olds.[10,15,44–49]

Individuals were designated current or former PWID in all relevant states based on published and unpublished estimates of past year and lifetime PWID prevalence.[1,6] Upper sampling bounds for the prevalence of current PWID came from a meta-analysis of past year injection drug use by Tempalski *et al.* while former PWID prevalence upper sampling bounds came from a meta-analysis of lifetime PWID prevalence by Lanksy *et al.* (Table 3.2).[1,6] Lower bounds for the sampling ranges of current and former PWID prevalence as well as the entire sampling range for the prevalence of non-PWID with substance abuse or dependence were based on an unpublished analysis of data from NSDUH (contact first author for more information).[62] We calculated the year 2000 US national prevalence of substance abuse or dependence by age group for any of the following substances: heroin, prescription opioids, stimulants and/or cocaine

without lifetime IDU, and set the 95% confidence interval bounds as sampling ranges (Table 3.2).[62] The lower bounds for former PWID prevalence and current PWID prevalence were set to the point estimates for prevalence of lifetime without past year (former PWID) or past year (current PWID) injection of any of the following substances: heroin, prescription opioids, stimulants and/or cocaine using NSDUH data from 2000 (Table 3.2).[62] These substances were chosen because they are commonly injected, meaning that people with abuse or dependence and no lifetime IDU history may be at risk for initiating IDU and thus could be at risk for HCV if they share syringes.[122]

PWID prevalence estimates were applied to the Michigan population and, after subtracting the total number of HCV cases, were used as the initial number of uninfected current and former PWID (S_i and S_{Ni}). We assumed that there were no treated current or former PWID during parameter estimation, which is reasonable given that changes in treatment availability and targeting of treatment to PWID occurred only recently.[93–96]

Contact Rates between Infected and Susceptible Current PWID

We sampled the total number of contacts per susceptible individual from 5-20 contacts per person; this parameter is known to vary widely in empirical studies.[61,116–119] We then adapted the contact matrix, Π, from a study of age-specific physical contacts per day in eight European countries, so that it reflected the proportion of total contacts per susceptible PWID that occurred with PWID in each age group.[60] First, we summed the number of contacts among persons aged 30-64 years from the original 5-year age groups for each country-specific matrix (both contacts by 30-64 year olds to other age groups and contacts by other age groups to 30-64 year olds). We used the maximum contacts from the eight matrices as the upper bounds for Latin hypercube

sampling. We used the minimum as the lower bound for sampling, with one modification. Because Mossong *et al.* included parent-child contacts in their study, we sampled to a lower bound of 0, regardless of the observed minimum value, in all sampling ranges including 30-64 year-old contacts with other age groups.[60] In our study, this allowed us to include the possibility of a very low syringe sharing rate of younger individuals with this large and oldest age group, which likely reflected parent-child contacts in the original study. We should also note that our study did not have perfect overlap with the age groups used in Mossong *et al.*; however, they were approximately similar (e.g. 20-24 in Mossong *et al.* vs. 20-25 in our study).[60] We formed age groups for the model that were consistent with the age reporting structure in NSDUH, which more directly influenced PWID prevalence than contact rates.[62]

After values for the number of contacts were sampled with Latin hypercube sampling, we transformed the contact matrix to column-wise proportions, which represent the proportion of each age group's total contacts that occurred with people from each age group. We multiplied these proportions by the total number of contacts per year, σ_i, to obtain the number of syringe sharing contacts in each age group per susceptible PWID per year. These proportions included those reported in a study of PWID age-specific syringe sharing rates.[61]

Solving Steady-State Equations for PWID Relapse Rates

During initial model design and fit to data, we noticed that model simulations generally produced population-level trends where current PWID outnumbered former PWID. These initial findings were inconsistent with empirical studies, which suggest that there are more former PWID than people currently injecting drugs.[1,6,62] Therefore, to maintain a realistic ratio of current to former PWID during model fitting to data throughout the simulation period, we

calculated age-specific relapse rates (κ_i) for each simulation using the sampled parameters for each simulation. As described in detail below, this procedure rearranged the system of ordinary differential equations to solve for κ_i, relying on the assumption that the system of differential equations was at steady-state (all derivatives set to equal 0). The assumption of steady-state is analogous to the principle that prevalence approximately equals the product of incidence and duration when the prevalence, incidence rate, and mortality rate are stable, there is no net migration of individuals with the condition, and the condition is rare.[123]

We rearranged the ODEs to solve for the four relapse rate parameters (κ_i). The system of 11 equations per age-group was rewritten as three ODEs per age-group (non-PWID [Z_i], current PWID [C_i], and former PWID [F_i]) as each state within the three groups had the same net inflows and outflows. Each equation was set to 0 and we obtained:

$$\frac{dZ_1}{dt} = \nu_0 \psi_N Z_0 - (\theta_1 + \eta_Z \mu_1 + \nu_1) Z_1 = 0$$

$$\frac{dC_1}{dt} = \theta_1 Z_1 + \kappa_1 F_1 - (\gamma_1 + \eta_P \mu_1 + \nu_1) C_1 = 0$$

$$\frac{dF_1}{dt} = \gamma_1 C_1 - (\gamma_1 + \eta_N \eta_P \mu_1 + \nu_1) F_1 = 0$$

$$\frac{dZ_2}{dt} = \nu_1 Z_1 - (\theta_2 + \eta_Z \mu_2 + \nu_2) Z_2 = 0$$

$$\frac{dC_2}{dt} = \theta_2 Z_2 + \kappa_2 F_2 + \nu_1 C_1 - (\gamma_2 + \eta_P \mu_2 + \nu_2) C_2 = 0$$

$$\frac{dF_2}{dt} = \gamma_2 C_2 + \nu_1 F_1 - (\kappa_2 + \eta_N \eta_P \mu_2 + \nu_2) F_2 = 0$$

$$\frac{dZ_3}{dt} = \nu_2 Z_2 - (\theta_3 + \eta_Z \mu_3 + \nu_3) Z_3 = 0$$

$$\frac{dC_3}{dt} = \theta_3 Z_3 + \kappa_3 F_3 + \nu_2 C_2 - (\gamma_3 + \eta_P \mu_3 + \nu_3) C_3 = 0$$

$$\frac{dF_3}{dt} = \gamma_3 C_3 + \nu_2 F_2 - (\kappa_3 + \eta_N \eta_P \mu_3 + \nu_3) F_3 = 0$$

$$\frac{dZ_4}{dt} = \nu_3 Z_3 - (\theta_4 + \eta_Z \mu_4 + \nu_4) Z_4 = 0$$

$$\frac{dC_4}{dt} = \theta_4 Z_4 + \kappa_4 F_4 + \nu_3 C_3 - (\gamma_4 + \eta_P \mu_4 + \nu_4) C_4 = 0$$

$$\frac{dF_4}{dt} = \gamma_4 C_4 + \nu_3 F_3 - (\kappa_4 + \eta_N \eta_P \mu_4 + \nu_4) F_4 = 0 \qquad (2)$$

Rearranging the equations in (2) as a function of the parameter of interest, the relapse rate (κ_i), from the equations for dC_i/dt gave (3), which are shown directly from above and in terms of the additional parameters required to calculate compartment sizes.

$$\kappa_1 = \frac{(\gamma_1 + \eta_P\mu_1 + \nu_1)C_1 - \theta_1 Z_1}{F_1} = \frac{(\gamma_1 + \eta_P\mu_1 + \nu_1)\zeta_1 P_1 - \theta_1\psi_1 P_1}{o_1 P_1}$$

$$\kappa_2 = \frac{(\gamma_2 + \eta_P\mu_2 + \nu_2)C_2 - \theta_2 Z_2 - \nu_1 C_1}{F_2} = \frac{(\gamma_2 + \eta_P\mu_2 + \nu_2)\zeta_2 P_2 - \theta_2\psi_2 P_2 - \nu_1\zeta_1 P_1}{o_2 P_2}$$

$$\kappa_3 = \frac{(\gamma_3 + \eta_P\mu_3 + \nu_3)C_3 - \theta_3 Z_3 - \nu_2 C_2}{F_3} = \frac{(\gamma_3 + \eta_P\mu_3 + \nu_3)\zeta_3 P_3 - \theta_3\psi_3 P_3 - \nu_2\zeta_2 P_2}{o_3 P_3}$$

$$\kappa_4 = \frac{(\gamma_4 + \eta_P\mu_4 + \nu_4)C_4 - \theta_4 Z_4 - \nu_3 C_3}{F_4} = \frac{(\gamma_4 + \eta_P\mu_4 + \nu_4)\zeta_4 P_4 - \theta_4\psi_4 P_4 - \nu_3\zeta_3 P_3}{o_4 P_4} \quad (3)$$

Rearranging from the equations for dF_i/dt gave:

$$\kappa_1 = \frac{\gamma_1 C_1}{F_1} - \eta_N\eta_P\mu_1 - \nu_1 = \frac{\gamma_1\zeta_1 P_1}{o_1 P_1} - \eta_N\eta_P\mu_1 - \nu_1$$

$$\kappa_2 = \frac{\gamma_2 C_2 + \nu_1 F_1}{F_2} - \eta_N\eta_P\mu_2 - \nu_2 = \frac{\gamma_2\zeta_2 P_2 + \nu_1 o_1 P_1}{o_2 P_2} - \eta_N\eta_P\mu_2 - \nu_2$$

$$\kappa_3 = \frac{\gamma_3 C_3 + \nu_2 F_2}{F_3} - \eta_N\eta_P\mu_3 - \nu_3 = \frac{\gamma_3\zeta_3 P_3 + \nu_2 o_2 P_2}{o_3 P_3} - \eta_N\eta_P\mu_3 - \nu_3$$

$$\kappa_4 = \frac{\gamma_4 C_4 + \nu_3 F_3}{F_4} - \eta_N\eta_P\mu_4 - \nu_4 = \frac{\gamma_4\zeta_4 P_4 + \nu_3 o_3 P_3}{o_4 P_4} - \eta_N\eta_P\mu_4 - \nu_4 \quad (4)$$

During simulations, we used the sampled values for all parameters shown in (4) to calculate the value of κ_i. Negative values from both (3) and (4) were possible given sampling ranges. Because more of the κ_i values calculated from (4) were positive, we used the dF_i/dt (4) instead of (3). When a negative value was calculated given the sampled parameters, we reset the value for κ_i to the minimum value for relapse rates found in the literature (0.1).[91,109–112]

Least Squares Estimation Methods

We chose an unweighted least squares method, which assumes that all points have normally distributed errors of the same magnitude (maximum likelihood with equal variances for each data point). Compared with other least squares weights considered, this strategy fit both the

smaller and larger case counts, whereas other methods (e.g. weighted least squares with variances proportional to the size of each data point) were unable to fit the larger data points that occurred later in the case series.

Although we chose to use one unweighted least squares scheme, the Matlab code provided for least squares estimation includes four total least squares methods, each with different weighting schemes to minimize the residual sum of squares after fitting to surveillance data. These include weights by the data point size, square root of the data point size, maximum data point size, and unweighted least squares.

Model Fitting with Latin Hypercube Sampling

To incorporate parameter uncertainty, Latin hypercube sampling with 10,000 simulations was used to draw a stratified random sample of parameter sets across plausible ranges (Table 3.2). Ranges for the majority of parameters were based on published estimates except when described here or used in intervention analyses. To optimize model fit to data, we estimated four unknown parameters (the transmission rate [β] and three age-specific injection initiation rates [θ_i]) in each simulation. Parameters were estimated for each simulation by unweighted least squares (equivalent to maximum likelihood assuming normally distributed measurement error with equal variances for each data point). Parameter estimation and simulations were run using `fminsearchbnd` and the `ODE15S` solver in Matlab.[63]

We used `fminsearchbnd` instead of `fminsearch` so that we could set upper and lower limits for the estimated parameters.[63] We set upper and lower bounds for estimated values of β from values in the literature.[84–86] Initial model fitting suggested that values of this parameter were optimized at approximately 0.0000035 (by visual inspection of fit to data) and we therefore

used this value as the initial condition of β for each model simulation. We also fit three injection

initiation rates, θ_i, to fit acute case data from 15-29 year olds from initial values based on

empirical studies of injection initiation.[64–74] While we did set upper and lower bounds for

injection initiation rates to ensure the model provided realistic estimates, we allowed the model

to fit higher injection initiation rates than were found in empirical studies to improve model fit

and accommodate the possibility that Michigan's initiation rates were higher than studies

conducted in other locations. Initial simulations using bounds from empirical studies produced

bimodal distributions, where >20% of parameter sets were assigned the maximum value (the

upper limit from empirical studies). We therefore set upper bounds for parameter estimation that

prevented the formation of bimodal distributions during simulations across our parameter value

ranges to allow maximum flexibility during this process. This resulted in upper bounds for θ_i that

were 3.3 (θ_1, θ_2) or 6.7 (θ_3) fold higher than the upper bound found in the literature, which was

approximately 1.5 per year.[66]

Residual sum of squares values after parameter estimation identified the best-fitting 10%

of parameter sets to data (i.e. 1,000 simulations, Figure 3.3). To determine if a certain range

appeared more consistent with data, we plotted histograms by quartile of fit and examined

distributions alongside their uniformly sampled ranges. Matlab code for model simulation and R

(R Foundation for Statistical Computing, Vienna, Austria) code for figures is available at

https://github.com/epimath/Hepatitis-C-in-Young-PWID.

3.7   References

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Chapter 4   The Association of Opioid Agonist and Antagonist Use with Gut Microbiota Diversity, Genera, and Enterotypes among People in Addiction Treatment

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4.1   Author Summary

The gut microbiota, the living bacterial community in the gut, is increasingly recognized for its influence on health and disease. We explored the relationship between opioid agonist (e.g. heroin and prescription opioids) and antagonist use with the gut microbiota of people in addiction treatment. We identified several attributes of the gut microbiota that differed between people exposed to opioid agonists compared to those with no agonist or antagonist exposure. These included lower microbial diversity and lower abundance of bacteria involved in butyrate production and bile acid metabolism in people exposed to opioid agonists. These differences were not seen in people exposed jointly to opioid agonists and antagonists, suggesting that the antagonizing effects of naltrexone and naloxone may offset some of the impacts of agonist exposure. This work informs future studies that examine the effects of opioid use on gut health, and the relationship of gut-brain-microbiota communication in addiction treatment outcomes.

4.2   Abstract

The gut microbiota is increasingly recognized as a potential determinant of psychopathology and treatment outcomes through its interactions with the brain along the gut-

brain-microbiota axis. We conducted a pilot study to examine human gut microbiota changes associated with use of opioid agonists and antagonists as a first step in understanding how gut health might relate to psychological functioning during addiction treatment.

We recruited 46 patients receiving outpatient addiction treatment at a private addiction treatment facility; consenting individuals reported their substance use in the past month and provided stool specimens. Analysis of self-reported substance use data and medical record review identified opioid agonist (heroin, prescription opioids, and methadone), antagonist (naltrexone), or agonist-antagonist combinations (buprenorphine+naloxone or other opioid+naltrexone). We sequenced participant stool samples using Illumina MiSeq and examined bacterial diversity (Shannon diversity and Chao1 indices), enterotypes (via dirichlet multinomial mixture modeling), and differential relative abundance (using ALDEx2) by opioid agonist-antagonist defined groups.

Among the 46 participants, 5 used opioid agonists only, 4 used agonist-antagonist combinations, 6 used antagonists alone, and 31 used neither. Participants who used only opioid agonists had lower bacterial diversity, *Bacteroidetes* enterotypes, and lower relative abundance of *Roseburia* and *Bilophila.* These differences were not observed between any other groups, including participants who used agonist-antagonist combinations versus participants exposed to neither agonists nor antagonists.

Addiction treatment patients with opioid agonist use had several alterations in the gut microbiota relative to other addiction treatment patients. These were consistent with those observed in murine models of morphine dependence. These changes were not evident in individuals who used both opioid agonists and antagonists. These findings support further characterizing the relationships between opioid agonist and antagonist exposure, gut

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permeability, inflammation, and relapse predictors to inform whether psycho-adjunctive treatments for opioid use disorders could improve addiction treatment outcomes.

4.3   Introduction

Approximately 40-60% of people who receive treatment for substance use disorders (SUDs) relapse in the first year following treatment.[1] SUD-related morbidity and mortality have increased dramatically during the past decade alongside changes in opioid prescribing patterns and nonmedical prescription opioid heroin use and overdose.[2,3] There is an urgent need to improve treatment outcomes to mitigate growing morbidity and mortality from opioid and other SUDs.[1]

The gut microbiota, the bacterial community living in the intestinal tract, is increasingly explored as a determinant of health and disease, and more recently, of psychopathology.[4,5] The gut microbiota may play a role or be a marker for the development of and/or recovery from several psychiatric disorders, including depression, autism spectrum disorders, and schizophrenia.[4,5] Following this recognition, the microbiota was incorporated into the communication pathway that describes the crosstalk between the gut and brain, termed the gut-brain-microbiota axis.[6]

The gut microbiota, and gut barrier function in general, may also influence SUD recovery and treatment outcomes.[7,8] Previous research supports that stress, anxiety, depression, and cravings influence vulnerability to relapse after periods of abstinence, and the microbiota may modulate these relapse precursors.[7–11] For example, Leclercq *et al.* found that chronic alcohol use alters gut barrier function and promotes gut leakiness compared to healthy controls.[12–14] Further, intestinal permeability, which was associated with altered levels of *Lactobacillus* and

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Bifidobacterium, modulated inflammatory markers and relief from depressive symptoms during a three week detoxification.[14] Collectively, the authors surmise that alterations to gut leakiness from chronic drinking and the release of bacterial products and metabolites into the bloodstream promote a neuro-inflammatory state that influences mood and drinking behaviors.[15] These results highlight the potential role of gut barrier function and the microbiota in alcohol addiction treatment outcomes, but less is known about other SUDs.

Wang *et al.* used a murine morphine dependence model to characterize the impact of opioid exposure on the gut microbiota.[16] Mice exposed to morphine exhibited major shifts in their gut microbiota within one day of exposure, and by day three had decreased gut microbiota richness (i.e. number of species), increased abundance of *Enterococcus faecalis,* and decreased abundance of *Flavobacterium, Fusobacteriuam, Suterella,* and *Clostridium* compared to placebo-treated mice.[16] Further research by this group and others has demonstrated that opioid exposure increases intestinal permeability, bacterial translocation to mesenteric lymph nodes and the liver, and risk of enteric and septic infections, dysregulates immune responses, disrupts bile acid metabolism, promotes inflammation, and may even induce the expression of virulence factors in pathogenic bacteria, such as *Pseudomonas aeruginosa*.[16–19]

Interestingly, Wang *et al.* also showed that the observed expansion of *E. faecalis* and changes to bile acid metabolism in morphine treated mice were not found among mice treated with both morphine, an opioid agonist, and naltrexone, an opioid antagonist.[16] This and other work by Banerjee *et al.* suggest that observed microbiota alterations relate to the binding of opioids to μ-opioid receptors and that the gut microbiota of mice treated with morphine and naltrexone differs from that seen in mice treated with morphine alone.[16,18]

Currently approved medication-based treatments for opioid use disorders (OUDs) involve two types of opioid antagonists, which are medications that block the μ-opioid receptor.[20] Naltrexone is an opioid antagonist used to manage cravings for both alcohol and OUDs. Its formulation as methylnaltrexone relieves opioid induced constipation.[21–23] A low dose formulation of naltrexone is currently debated as a potential treatment for the gut mucosal injury and inflammation characteristic of Crohn's Disease and for other inflammatory conditions.[24–26] These applications suggest that the formulation of naltrexone used for OUDs and alcohol use disorders might similarly modulate gut barrier function, which was previously correlated with gut microbiota composition and relapse risk among people with alcohol use disorders.[14]

A second opioid antagonist used in products with buprenorphine, a partial opioid agonist, is naloxone.[20] In combination, buprenorphine-naloxone is one of the most commonly used medication assisted treatments for OUDs.[27] Alone, naloxone is an opioid antagonist that reverses the respiratory depressive effects of an opioid overdose. Naloxone's mechanism of action is similar to naltrexone (i.e. blocks the μ-opioid receptor); it is included in buprenorphine formulations to reduce the risk of abuse. Collectively, the impacts of joint administration of opioid agonists and antagonists (e.g. buprenorphine+naloxone or opioid agonists and naltrexone) and the sole administration of opioid antagonists (e.g. naltrexone) on the human gut microbiota are unknown.

We identified two human studies of people with OUDs that characterized changes to the gut microbiota associated with opioid agonists.[28,29] Barengolts *et al.* studied 99 male veterans to explore how diabetes, metformin use, and OUDs modulated the gut microbiota.[28] They observed a positive association between *Bifidobacterium* and OUDs among men with type 2 diabetes and no metformin use.[28] Xu *et al.* compared the gut microbiota's diversity and composition among

45 Chinese men with SUDs to 48 controls without SUD; 26 participants in the SUD group used heroin and all used alcohol and smoked daily.[29] They observed increased prevalence of *Thauera, Paracoccus,* and *Prevotella* and a trend towards higher diversity among the SUD group.[29]

There are several opportunities to extend the important findings from these studies. First, previous studies commented on two measures of the microbiota (diversity and individual taxa); however, no studies have evaluated enterotypes, a representation of the entire gut community structure, among people or animal models of opioid or other SUDs. A recent review by Costea *et al.* identified three gut microbiota enterotypes found across a variety of settings and populations and discussed the overlap of enterotypes with diseases and diets.[30] Enterotype characterization among people who use opioids could help identify mechanisms underlying dysbioses related to opioid use through their overlap with enterotype patterns observed in other conditions. Second, the joint and single effects of opioid agonist and antagonist exposure on the human gut microbiota remain unknown. These findings could be important for several groups, including people using opioid agonists as prescribed, people with OUDs, and people prescribed medication assisted treatments for an OUD (buprenorphine+naloxone, methadone, and naltrexone) and/or alcohol use disorder (naltrexone).

In summary, opioid agonist exposure disrupts the gut microbiota in mouse models and these changes are incompletely described in humans who use opioids.[16,18,19,28,29] While these effects are antagonized by naltrexone in a murine model, to our knowledge, there are no gut microbiota studies of opioid antagonists among humans.[16,18] The ongoing opioid crisis highlights the urgent need to identify and improve treatment outcomes for OUDs, and improvements in treatment outcomes may be possible through adjunctive treatments that modulate gut microbiota dysbioses and improve gut barrier function.[1,2,15] A first step in achieving these goals is to

describe the gut microbiota's associations with opioid agonist and antagonist exposure in humans. Therefore, we examined the relationship between opioid agonist and antagonist exposures with the gut microbiota of 46 patients receiving outpatient SUD treatment. We describe differences in microbiome diversity, enterotypes, and bacterial genera abundance by opioid agonist exposure and evaluate whether these effects are modified by opioid antagonists.

4.4   Methods

4.4.1   Overview

We consented 46 patients aged 18-60 years who were receiving treatment in an outpatient addiction treatment facility to participate in a gut microbiota and substance use study. We surveyed participants about the substances they used in the 30 days prior and reviewed participant medical records to characterize their opioid agonist and antagonist use. Participants submitted a stool sample and we sequenced the V4 hypervariable region of the 16S ribosomal ribonucleic acid (rRNA) gene to characterize the gut microbiota. Each participant's opioid agonist and antagonist use patterns from survey and medical record data were summarized as agonist only (Ag), agonist+antagonist (AgAt), antagonist only (At), or neither agonist nor antagonist (N). We compared gut microbiota diversity, enterotypes, and genera relative abundance between participants in these four opioid agonist and antagonist groups. Details about each step of participant recruitment and data analysis are summarized below.

4.4.2 Participant Recruitment

4.4.2.1 Study Site and Substance Use Survey

The study population was drawn from a sample of adult (≥18 years) patients from a private, outpatient addiction treatment facility in Michigan during July 2016 – September 2017. The facility provided SUD treatment in both individual and group settings and treatment options included psychotherapy and pharmacotherapy for alcohol, opioid, and other SUDs. The facility offered medication assisted treatments, including buprenorphine+naloxone and naltrexone, but did not offer methadone. Study research assistants approached patients before group and individual appointments about their interest in completing a survey about substance use. To be eligible to participate, patients had to be ≥18 years, speak English, be able to provide informed consent, and able to see, speak, and hear. We excluded people who were acutely intoxicated, mentally incompetent, or unable to provide informed consent for any other reason. The study was approved by the Institutional Review Board at the University of Michigan (HUM00113964).

Interested and eligible participants provided informed consent and completed a 45-minute survey, which could be completed electronically using Qualtrics (Qualtrics, Provo, Utah) or as paper and pencil. The screening survey assessed substance use and demographic characteristics. A total of 124 participants provided informed consent and 92 (74.2%) completed the initial survey within 4 weeks (Figure 4.1). Participants were compensated $5 for their survey.

4.4.2.2 Microbiota Study Eligibility Criteria

Research assistants reviewed results of the substance use survey and invited 65 participants who met eligibility criteria to enroll in a microbiota study. Before enrollment, research assistants administered the Mini-Mental State Exam (MMSE) to check cognitive

function and ability to consent; a total score ≥21 was required to enroll.[31] Further eligibility criteria included age 18-60 years and self-reported use of at least 1 substance in the past 30 days or misuse of prescription opioids during the month before beginning treatment.

We used an abbreviated version of the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) to assess past 30 day substance use. ASSIST is a 12-item questionnaire that asks about use of street opioids, opioid pain medications (with or without a prescription), methadone or buprenorphine+naloxone (with or without a prescription), tobacco, alcohol, cannabis, cocaine (including crack), amphetamines, inhalants, sedatives or sleeping pills, and hallucinogens.[32,33] Participants who only endorsed tobacco were not eligible without use of another substance. Participants reporting past 30-day use (nonmedical or as prescribed) of medication assisted treatments (buprenorphine+naloxone or methadone) were eligible, regardless of past 30 day use of other substances. We used the Current Opioid Misuse Measure (COMM) to capture self-reported misuse of prescribed opioids.[34] The COMM is an eight-item scale that assesses self-reported opioid misuse not captured by ASSIST.[34] Participants with a score >0 for questions 4 and 6-8 were eligible.[34]

4.4.2.3 *Microbiota Study Participants and Stool Sample Collection*

A total of 51 out of 65 eligible participants provided informed consent for the microbiota study; the remaining 14 were not enrolled for reasons listed in Figure 4.1. At enrollment in the microbiota study, participants completed an additional survey about their dietary habits and were compensated $20. Weekly thereafter for three weeks, participants submitted a stool sample (described further below) and completed an additional survey reporting their depression, anxiety, cravings to use drugs or alcohol, dietary habits, and antibiotic use. Participants were

compensated $10 per appointment. All follow-up appointments were completed within one month of enrollment in the microbiota study. We received at least one stool sample from 46 of the 51 participants who provided informed consent for the microbiota study.

4.4.3   Medical Record Review

As part of their written informed consent, participants granted the research team access to their University of Michigan Health System medical record. We reviewed medical encounters at the University of Michigan during the 30 days before completion of the first survey through the day of sample collection. We documented the following items to supplement study survey data: 1) prescription for naltrexone, and 2) prescription for buprenorphine+naloxone.

4.4.4   Measures: Participant Characteristics

4.4.4.1   Opioid Agonist and Antagonist Use

We used a combination of the ASSIST and medical record review to identify any opioid agonist use. We defined opioid agonist use as a binary indicator for either of the following: 1) self-reported opioid use (heroin, methadone, buprenorphine, or prescription opioids used as prescribed or not as prescribed) in the 30 days before study enrollment on the modified ASSIST, or 2) buprenorphine+naloxone use during the day of sample collection in the medical record. We supplemented the ASSIST data on buprenorphine use with data from the medical record because survey questions about medication assisted treatments were added to the modified ASSIST during January 2017 and were not available prior. Of note, one participant who was prescribed opioids to manage pain was included in the group of participants who used opioid agonists.

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We also assessed opioid antagonist use. For the purpose of this study, we defined opioid antagonist use as either of the following according to the medical record: 1) buprenorphine+naloxone use during the day of sample collection, or 2) naltrexone use during the day of sample collection. We included any formulation of either opioid antagonist. Finally, we created a categorical variable describing the overlap in opioid agonist and antagonist use (i.e. agonist only, combined agonist-antagonist, antagonist only, and neither agonist nor antagonist).

4.4.4.2 Depression, Anxiety, and Cravings to Use

We summarized scores from the Patient Health Questionnaire-9 (PHQ-9), a reliable and valid depression severity screening tool (Cronbach's α: 0.86-0.89, Test-retest correlation: 0.84, range: 0-27).[35] We also summarized scores from the Generalized Anxiety Disorder 7-Item Scale (Cronbach's α: 0.92, Test-retest correlation: 0.83, range: 0-21).[36] For reference, a sum $\geq$10 indicated possible GAD.[36] Finally, cravings to use drugs or alcohol were reported using summed scores from a modified version of the Penn Alcohol Craving Scale adapted to capture drug cravings (range: 0-30).[37–39]

4.4.4.3 Microbiota Study Population Characteristics

We summarized average age, self-identified gender (female, male, and other), race (black, white, other, or multiple races), ethnicity (Hispanic vs. non-Hispanic), and self-reported days in SUD treatment at the study site. We calculated predicted past month dietary fiber intake (grams per day) from food frequency questions completed at enrollment in the microbiota study using validated predictive models developed from the National Health and Nutrition Examination Survey (Appendix 4.1).[40] Dietary fiber intake has previously been associated with

the gut microbiota, transit time, and stool water content.[30,41–43] We used the ASSIST to describe alcohol use during the 30 days before completing the substance use survey.[32] Finally, we summarized self-reported antibiotic use during the week of sample collection.

4.4.5   Stool Sample Collection and Sequencing

4.4.5.1   Stool Sample Collection

We based our stool collection protocols on those from Feigelson *et al.*, Flores *et al.*, and Fu *et al.*[44–46] Participants self-collected stool samples by placing two dime-sized scoops of stool into a sterile Sarstedt tube with a spoon lid (Sarstedt, Nümbrecht, Germany) containing a cryopreservant, RNA*later*™ (Ambion, Austin, TX), and 5-10 glass beads (Walter Stern, Washington, NY). Participants then secured the lid, homogenized the sample in RNA*later*™ by shaking, and stored the sample at room temperature for up to two days before returning the sample to a research assistant. Previous research supported that RNA*later*™ preserved the composition of the stool bacterial community at room temperature for up to three days after collection.[45,46] Stool samples were then frozen in 1 mL aliquots at -80C.

4.4.5.2   Illumina Sequencing and Sequence Processing

After all participants were enrolled, we thawed a 1 mL aliquot of each stool sample, centrifuged at 10,000xg, and resuspended in 1 mL 1X phosphate buffered saline (Gibco, Thermo Fisher Scientific, Waltham, MA, pH 7.4). We added 250 uL resuspended stool to each of two wells of a bead beating plate so that each sample would be sequenced in duplicate. We provided plates to the Microbial Systems Molecular Biology Laboratory at the University of Michigan.

They used previously described protocols for deoxyribonucleic acid (DNA) extraction and Illumina MiSeq sequencing of the V4 hypervariable region of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene (Appendix 4.1).[47–49]

We processed sequencing reads using mothur (v1.39.5) and the MiSeq standard operating procedure (https://www.mothur.org/wiki/MiSeq_SOP, accessed November 8, 2017) to perform quality filtering and align sequences to the V4 region of the 16S rRNA gene.[49] We converted sequences to the format required for oligotyping and clustered samples into oligotypes using the procedures and default parameters described by Eren *et al.* (Appendix 4.1).[50–52]

Two samples with fewer than 1,000 reads were removed from further analysis; however, we were able to retain these two participants in the analytic sample using their duplicate sequenced sample. We verified that all mock communities resembled their known compositions (data not shown) and summed duplicate sequenced samples. For this analysis, we examined the first sample submitted per participant, amounting to 46 samples with 2,207,827 sequence reads (21,796 – 77,013 reads per participant) and 354 oligotypes. We assigned oligotype taxonomy using the Ribosomal Database Project (release 11, update 5).[53] We focused this analysis on the first sample per participant after observing little change to taxa diversity metrics (described further below) during the month-long period of study (Appendix 4.1).

4.4.6   Microbiota Measures

4.4.6.1   Genus Relative Abundance and Diversity Metrics

We calculated the relative abundance of genera in each sample (i.e. the number of sequencing reads from each genus divided by the total number of sequencing reads per sample). We used oligotype counts to calculate Shannon diversity and the Chao1 Index, which

summarized within-sample oligotype alpha diversity (number and evenness of oligotypes) and richness (number of oligotypes), respectively. Further, we visualized between-sample (beta diversity) differences using principal coordinate analysis of the Bray-Curtis dissimilarity index to provide a comparable index with other microbiome analyses and of the Morisita index, given its adequate performance with low sample sizes.[54]

Microbiota sequencing data are compositional; the total number of sequences from each sample is bound by an arbitrary depth of sequencing coverage that does not reflect the true abundance of bacteria in the participant's gut.[55] Thus, taxa comparisons between samples must be analyzed on the relative (not absolute) scale.[55] Failure to analyze these data as compositional results in spurious correlations between taxa and biases associations of taxa abundance with other taxa and covariates.[55] We therefore applied two analytic approaches to compare taxa distributions by covariates of interest that accounted for the compositional nature of the data: Dirichlet multinomial mixture modeling and ALDEx2.[56–59]

4.4.6.2   De Novo and Reference-Based Enterotyping

Enterotyping (broadly: community typing methods) distill highly dimensional microbiota taxa data into clusters (i.e. groups of samples exhibiting similar taxa distributions) by leveraging the information from the covariance structure of taxa.[30,57,60] The high dimensionality of microbiome data (number of taxa) poses a challenge during analysis, as testing for associations of single taxa with metadata requires many tests and results can be difficult to interpret without acknowledging co-occurring changes in other taxa. Enterotyping addresses these challenges through data reduction and summary by clustering samples with similar bacterial profiles into groups based on the distribution of taxa in samples.[30]

We applied two clustering approaches that classified each stool sample's genus-level read counts into enterotypes.[30,57] First, we used Dirichlet multinomial mixture (DMM) models, an extension of latent profile analysis adapted for microbiota data by Holmes *et al*.[57] Like traditional latent profile analysis, this technique recovers unobserved (i.e. latent) subgroups based on joint distributions of bacterial genera. DMM-based enterotypes were assigned *de novo* (i.e. based on the data and participants included in our study) using data from all 129 samples collected during the study to standardize DMM assignments across all samples. We compared model fit using the Laplace approximation of negative log models for DMM models with one to five enterotypes and chose the number of enterotypes that optimized model fit (i.e. minimized the Laplace approximation).[57] We then assigned each sample to its most likely enterotype based on posterior probabilities of enterotype assignment. All samples used in this analysis had posterior probabilities ≥90% and were therefore all assigned to an enterotype (minimum posterior probability: 95.3%). We examined the average relative abundance of all genera and the top 20 taxa that were most influential in distinguishing between enterotypes to summarize taxa distributions representative of each enterotype.

Costea *et al*. recently reviewed the enterotyping literature and suggested the existence of three enterotypes in healthy human populations based on their dominant bacterial taxa: *Bacteroides*, Firmicutes, and *Prevotella*.[30] They also created an online reference-based enterotyping tool that assigns uploaded samples to one of these three enterotypes.[30] This tool compares genera relative abundance in uploaded samples to relative abundance from two studies of the healthy human gut microbiota, the Human Microbiome Project (HMP) and the Metagenomics of the Human Intestinal Tract (MetaHIT) study. It also applies enterotyping

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methods described by Arumugam *et al.* (i.e. partitioning around medoid clustering) to assign an enterotype based those observed in HMP and MetaHIT.[30,60]

We uploaded genus relative abundance data from our study to http://enterotypes.org and obtained enterotype assignments and a binary variable indicating whether each sample had similar genera to the observed patterns in reference samples from HMP and MetaHIT. Two of 46 samples were not comparable to reference samples based on this indicator and were therefore assigned as "missing" for their assignment.

4.4.7   Analysis: Association of Microbiota with Opioid Use

4.4.7.1   *Diversity and Enterotypes by Opioid Agonist and Antagonist Use*

We compared all microbiota metrics described below by two indicators of opioid use: opioid agonist use (binary, yes/no) and combined opioid agonist/antagonist use in four categories (agonist only [Ag], agonist+antagonist [AgAt], antagonist only [At], or neither opioid agonist nor antagonist [N]). Using appropriate statistical tests for each exposure and outcome, we compared 1) opioid agonist use versus no use, 2) Ag vs. N, 3) AgAt vs. N, and 4) At vs. N and other pairwise comparisons where relevant.

We compared alpha diversity metrics (Shannon and Chao1) by opioid use and tested for differences using Wilcoxon rank sum tests. We visualized beta diversity metrics as principal coordinate analyses. Next, we compared the distribution of *de novo* enterotypes by opioid use variables with Fisher's exact test. We also described taxa that were influential in assigning enterotypes by their average relative abundance patterns and alpha diversity in *de novo* assigned enterotypes We examined the overlap of *de novo* and reference-based enterotype assignments.

4.4.7.2 *Genus Differential Abundance by Opioid Agonist and Antagonist Use*

We compared abundance of specific genera by opioid use status and other covariates of interest using ALDEx2, an analysis of variance-like tool for compositional data.[58] For each genus, ALDEx2 inferred absolute abundance given the observed abundance matrix over 1,000 Monte-Carlo simulations from a Dirichlet distribution. This simulation-based framework was meant to recognize each sample as a single realization from the gut bacterial communities of participants. To account for the compositional structure of the data, genera abundances for each sample and simulation were transformed to centered log ratios (i.e. log of the ratio of taxa$_i$ abundance for sample j divided by the geometric mean for the abundance of all taxa [$i=1:K$] in sample j). ALDEx2 then calculated each genera's median centered log ratio by group (e.g. opioid agonist use vs. no use) for each simulation. Within-group variability in each taxa's log ratios reflected sampling variation whereas between-group differences represented the biological variation of interest (e.g. by opioid use status). These were used to calculate an effect size for each genus as the median difference in centered log ratios between groups across all simulations divided by the median of the largest detected difference within groups for each condition (e.g. the median of two items: 1) the largest difference in centered log ratios for taxa$_i$ among opioid agonist use group across 1,000 simulations and 2) the largest difference in centered log ratios for taxa$_i$ among people not using opioid agonists across 1,000 simulations). Statistical significance for each effect size was summarized as a p-value corrected for the false discovery rate (FDR) using the Benjamini-Hochberg procedure. We summarized genera relative abundance for all samples as bar charts for taxa with FDR corrected p-values<0.05 identified from the ALDEx2 Wilcoxon rank sum test.

4.4.7.3   *Alcohol and Dietary Fiber*

We examined how dietary fiber intake and past 30-day alcohol use varied by both microbiota characteristics (diversity, enterotypes, and genera differential abundance) and opioid use. These variables are explored as potential alternative explanations for our findings around opioid use given previously documented associations of these characteristics with the microbiota.[14,41–43]

4.5   Results

4.5.1   Study Sample Characteristics

The 46 participants were aged a median of 33.5 years; most were male (56.5%), white (84.7%), and non-Hispanic (89.1%, Table 4.1). Nine (19.6%) used opioid agonists (Figure 4.1). Five used opioid agonists only (heroin or prescription opioids, Ag), four used agonists and antagonists (AgAt, three buprenorphine+naloxone and one prescription opioids and naltrexone). Among the 37 who were unexposed to an opioid agonist, 6 used naltrexone, an opioid antagonist (At), and 31 were exposed to neither opioid agonists nor antagonists (N).

Participants using both opioid agonists and antagonists reported receiving treatment at this facility for longer periods of time compared with other participants. Depression, anxiety, and craving scores were higher in the Ag group compared to others. Antibiotic use was uncommon but one participant was exposed to antibiotics during the week of stool sampling. Fiber intake and alcohol use are further discussed below.

4.5.2 Opioid Agonists Were Associated with Decreased Gut Microbiota Alpha Diversity

We used the Wilcoxon Rank Sum test to compare species diversity (Shannon Index) and richness (Chao1 Index) between opioid agonist vs. no agonist groups and by agonist/antagonist combination groups (Figure 4.2). Diversity metrics were, on average, lower in participants who used any opioid agonist vs. those who did not use opioid agonists but differences did not reach statistical significance (Shannon diversity p=0.055, Chao1 p=0.059). However, diversity (p=0.04) and richness (p=0.02) were significantly lower among Ag vs. N individuals. While we noted similar patterns between the Ag vs. At groups, differences in Shannon diversity (p=0.08) and richness (p=0.13) did not reach statistical significance. We found no statistically different differences in diversity or richness between any other groups (all p>0.19). Plots of principal coordinates from calculation of two beta diversity metrics (Bray-Curtis and Morisita) showed some evidence for clustering in the Ag group (Figure 4.3). However, we avoided further statistical testing for differences given the lack of strong clustering by group and small sample size.

4.5.3 *De Novo* Assigned Enterotypes Differed by Opioid Agonist Use

Fit indices clearly favored a three enterotype model using *de novo* clustering with DMM models (Figure 4.4). Mean relative abundance distributions from samples clustered into each enterotype identified two *Bacteroides* dominated groups and a third dominated by *Prevotella* (23.9% of participants, n=11, Figure 4.5). *Prevotella* and *Bacteroides* were also the two most influential taxa in assigning enterotypes. Among the 35 participants with *Bacteroides* dominated groups, 24 were assigned to an enterotype with elevated relative abundance of *Faecalibacterium*

(*Bacteroides: Faec.*) and 11 were assigned to an enterotype with elevated *Clostridium* cluster XIVa (*Bacteroides: Clost.*).

No participants exposed to opioid agonists had the *Prevotella* enterotype, regardless of antagonist use (Figure 4.6). The distribution of enterotypes differed for the Ag and N groups, likely reflecting that the *Bacteroides: Clost.* enterotype was common in the Ag group. The *Bacteroides: Clost.* group had reduced alpha diversity (Figure 4.7, Kruskal Wallis test for Shannon diversity p-value<0.00001, Chao1 p-value<0.0001). We did not detect any further statistically significant differences in the distribution of enterotypes by other groups (e.g. AgAt vs. N and At vs. N).

4.5.4   Enterotypes Were Largely Consistent with those from Healthy Human Populations

We submitted genera relative abundance for each sample to http://enterotypes.org to determine whether our samples had overlapping genera distributions with two studies of healthy populations (HMP and MetaHIT) and to obtain reference-based enterotype assignments to one of three previously described enterotypes: *Bacteroides, Prevotella,* or Firmicutes.[30] The majority (95.7%, 44 of 46 samples) were similar to the genera distributions observed in HMP and MetaHIT and therefore able to be assigned to reference-based enterotypes (Table 4.2). Among these, all 11 *Prevotella* samples from the DMM method were assigned the *Prevotella* reference enterotype. Nearly all (91.7%, 22 of 24 samples) of the samples assigned to the *Bacteroides: Faec.* enterotype from DMM were assigned to the *Bacteroides* reference enterotype. The remaining were assigned *Prevotella*. Fewer of the *Bacteroides: Clost.* samples were assigned to the *Bacteroides* reference enterotype (81.8%, 9 of 11 samples) and the remaining two samples were unable to be assigned as the algorithm determined that they were outside of the sample

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space of enterotypes observed in HMP and MetaHIT. The two methods agreed that the majority

of samples were *Bacteroides* enterotype(s); 11 or 13 of 46 samples were assigned *Prevotella*

depending on the method used. Regardless of method, the *Prevotella* enterotype never included

participants who used opioid agonists, even with concurrent antagonist use.

4.5.5 Opioid Agonist Use was Related to Specific Genera

We used ALDEx2 to compare genera abundance by opioid agonist (vs. no agonist) and

between agonist/antagonist combination groups while accounting for the compositional data

structure and adjusting for multiple comparisons (i.e. 77 genera tested) using the FDR. *Roseburia*

abundance was reduced (FDR p-value: 0.017) and *Clostridium* cluster XIVa (FDR p-value:

0.045) abundance was increased in samples from participants who used opioid agonists (vs. no

agonist use). We identified nine differentially abundant genera between Ag and N participants

(Figure 4.8). Unclassified Enterobacteriaceae (FDR p-value: 0.026), *Lactobacillus* (FDR p-

value: 0.031), *Clostridium* cluster XIVa (FDR p-value: 0.033), *Faecalicoccus* (FDR p-value:

0.037), *Anaerostipes* (FDR p-value: 0.040), and *Streptococcus* (FDR p-value: 0.045) abundances

were higher in Ag vs. N participants while unclassified Firmicutes (FDR p-value: 0.031),

Bilophila (FDR p-value: 0.037), and *Roseburia* (FDR p-value: 0.043) were less abundant in Ag

vs. N participants. We found no statistically significant differences between other opioid

agonist/antagonist groups.

4.5.6 Fiber Intake and Alcohol

Nearly all participants used alcohol in the 30 days before the substance use survey (Table

4.1). Alcohol use prevalence was lower among Ag participants (60%) but there were no

statistically significant differences between opioid agonist exposure (Fisher exact p-value=0.68) or Ag and N groups (p=0.60). Shannon diversity was marginally lower among participants with no alcohol use (Wilcoxon rank sum p=0.052, Figure 4.9). Visually, the *Bacteroides: Faec* enterotype was more common in people who used alcohol in the past 30 days (Fisher exact p-value=0.11, Figure 4.10). Using ALDEx2, we were unable to identify any taxa that were statistically significantly associated with alcohol use in this sample of participants (all FDR Wilcoxon rank sum p>0.12).

Average fiber intake was lowest among the Ag group but there were no statistically significant differences by opioid agonist exposure (Wilcoxon rank sum p-value=0.81) or between Ag and N groups (p=0.32, Table 4.1). Gut microbiota richness was positively and linearly associated with fiber intake (Pearson correlation: 0.41, p=0.005, Figure 4.9). Although there were visual differences in fiber consumption by *de novo* assigned enterotypes, they did not reach statistical significance (Kruskal Wallis p-value=0.12, Figure 4.10). We used ALDEx2 with a four-level categorical variable formed from quartiles of fiber intake among participants. No genera were statistically significantly associated with fiber intake; however, we found marginally significant associations with *Collinsella* (FDR Kruskal Wallis FDR p-value=0.051) and *Clostridium* cluster XIVa (FDR Kruskal Wallis FDR p-value=0.075). All other FDR p-values were >0.2.

4.6   Conclusions

In our pilot study of 46 individuals receiving SUD treatment, exposure to opioid agonists was associated with variations in the human gut microbiota. Consistent with results from a murine model by Wang *et al.*, we observed decreased alpha diversity among participants

exposed to opioid agonists only (Ag) vs. neither agonist nor antagonist (N) exposed

participants.[16] Opioid agonist exposure was also associated with *Bacterioides* enterotypes and

variations to several bacterial genera, whose metabolic and functional roles are discussed below.

While decreased alpha diversity was associated with opioid agonist exposure in our study

and in a murine model, Xu *et al.* found that alpha diversity was higher among participants with

SUDs relative to healthy controls.[16,29] These conflicting results may be explained by differences

in study design.[29] We recruited a sample of participants who all had SUDs to help control for

lifestyle and dietary factors associated with being in recovery from addiction and so that all

participants compared had the possibility for an opioid antagonist prescription. However, Xu *et*

al. compared 45 Chinese men with SUDs to and 48 Chinese men without SUDs and did not

evaluate antagonist exposure.[29]

Participants exposed to agonists were more likely to have the *Bacteroides* enterotype with

higher relative abundance of *Clostridium* cluster XIVa. This enterotype also had the lowest

diversity, which was consistent with Costea *et al.*'s comment that *Bacteroides* enterotypes are

generally the least diverse.[30] However, in our study, a second *Bacteroides* enterotype appeared to

have similar diversity to the *Prevotella* enterotype.

No participants exposed to opioid agonists, including those with concurrent antagonist

exposure, had the *Prevotella* enterotype. *Prevotella* has previously been associated with a fiber-

rich diet.[30] As such, this association could reflect the lower fiber consumption among the opioid

agonist only (Ag) group, or could reflect underlying processes common to both low fiber diets

and opioid agonist exposure, including slowed transit time, constipation, and reduced stool water

content.[19,42,43] Given our study design, we were unable to disentangle the effects of fiber and

opioid agonist exposure and our data supported a positive association between fiber and gut

microbiota richness. However, we also observed consistent associations between opioid agonist exposure and three aspects of the gut microbiota (diversity, enterotypes, and genera relative abundance). Murine models suggest that the impacts of low fiber diet and opioid agonist exposure differ.[18] In particular, Banerjee *et al.* showed that the gut barrier compromise and bacterial translocation observed in morphine exposed mice were not observed in mice constipated due to a low fiber diet.[18] Future studies will need to further differentiate the impacts of fiber and opioids on the gut microbiota in humans. We did not detect the Firmicutes enterotype among study participants; however, we cannot determine whether this was a true absence in our population or a reflection of sampling variability and random error. The Firmicutes enterotype has the highest alpha diversity of the three known enterotypes, and is associated with low inflammation, making its complete absence from our study population an interesting finding worth further research.[30,61]

We also observed differences in the relative abundance of several bacterial genera by opioid agonist exposure, including decreased *Roseburia* and *Bilophila* and increased *Clostridium* cluster XIVa, *Lactobacillus, Faecalicoccus, Streptococcus,* and *Anaerostipes*. *Roseburia* abundance was decreased among the opioid agonist group, with or without concurrent antagonist exposure. Species in this genus produce butyrate, a short chain fatty acid associated with colon health.[62] Butyrate's beneficial properties include reduced inflammation, the reduction of colon oxidative stress, and improvement of gut barrier health.[62] Interestingly, this genus is considered part of the *Clostridium* XIVa cluster, a functionally related group of bacteria that spans many genera. Seemingly contrary then, is the association of increased *Clostridium* XIVa cluster abundance with opioid agonist use. A search of the Ribosomal Database Project, the phylogenetic reference assignment tool used in this study, suggested that *Roseburia* and

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Clostridium XIVa were distinct genera for the purposes of taxa assignment and that the *Clostridium* XIVa genera assigned corresponds to three *Clostridium* species of unknown butyrate production status, rather than a reflection of the full breadth of the *Clostridium* XIVa cluster.[53,63,64] Wang *et al.* observed an increase in the relative abundance of *Clostridium* in mice exposed to morphine relative to placebo; however, we are unable to determine whether the species detected in their study are the same as those detected in our study due to limitations of current species identification capabilities from 16S rRNA sequencing.[16]

The association of opioid agonist exposure with *Bilophila* relative abundance may relate to a disruption in bile acid metabolism observed in morphine exposed mice.[18,19] Decreases in *Bilophila*, a bacterial genera known to use bile as a nutrient source, may correlate with the reduced intestinal primary and secondary bile acid levels observed in morphine exposed mice.[18] Reductions in bile acid levels were accompanied by gut microbiota changes, gut barrier disruption and systemic inflammation.[18] The temporal sequence of metabolic and inflammatory changes occurring after morphine exposure and their relationship to the gut microbiota have not yet been determined.[18,65] Interestingly, reductions in *Bilophila* abundance were also observed in inflamed intestinal samples from ulcerative colitis patients and in the gut microbiota of people with autism spectrum disorders vs. healthy controls.[66,67]

The remaining bacteria that were differentially abundant in participants exposed to opioid agonists are less well described. *Faecalicoccus,* a rare taxa in our study (<1.4% abundance across all participants), was elevated in three of five participants in the opioid agonist group. *Faecalicoccus* is largely undescribed in the literature, but is known to produce moderate amounts of butyrate.[68] Graphically, the associations detected among *Anaerostipes, Streptococcus,* and *Lactobacillus* were difficult to interpret and may have been overly influenced by outliers. As

such, we reserve discussion of these genera and point to them as genera that could be explored in future work. Overall, the differential abundances we observed may reflect differences in bacterial metabolism associated with opioid agonist exposure, and could be directions for study in future, larger studies.

We also identified that microbiota diversity, enterotypes, and taxa were similar for participants with combined agonist and antagonist (AgAt) exposure, antagonist only exposure (At), and neither agonist nor antagonist exposure (N). To our knowledge, these similarities have not been previously described in a human study and they align with the observed antagonism of morphine's effects by naltrexone in mice.[16] Taken together, these results suggest that combinations of agonists and antagonists may have benefits beyond their intended application (e.g., naloxone reducing the abuse potential and risk of overdose from the buprenorphine portion of the combination product). These benefits may include those observed in other applications of naltrexone, including its use as a laxative for opioid induced constipation, its potential promotion of gut mucosal healing in Crohn's disease, and its anti-inflammatory applications for treating chronic pain.[21–26] It is important to note that the mechanisms of naltrexone's action in Crohn's disease are the subject of some debate, and that the formulations for use in these applications differ from those used for craving management in alcohol and OUDs.[24,26] However, these applications suggest the possibility that naltrexone improves gut barrier function and this action may have implications for the gut microbiota and naltrexone's effectiveness in mitigating cravings.

This study was limited by the small sample size (n=46), especially the small number of agonist and antagonist exposed individuals. Despite this, we found several biologically plausible associations that deserve follow-up in larger studies. We were not able to adjust for confounding (e.g., by fiber or alcohol use) given our small sample size, and instead presented descriptive statistics that showed the potential extent of confounding by fiber and alcohol use. Larger studies will need to evaluate the potential for confounding further. We did not include healthy controls, but we compared our samples to two large studies to determine whether our samples were in the sample space observed in healthy human gut microbiota studies. While it is encouraging that our samples were within the sample space of HMP and MetaHIT, we must acknowledge that differences in sample collection, processing, and sequencing, and differences in target populations limit inferences that might be made from this comparison.

Nearly all measures were self-reported (except prescriptions for naltrexone or buprenorphine+naloxone that were identified through medical record review) and are therefore subject to recall bias. We had missing data on two important variables, including one dietary measure quantifying cheese intake, which we imputed (Appendix 4.1), and self-reported buprenorphine or methadone use in the past 30 days, which we were able to gather through a medical record review. Nonetheless, this process could have introduced bias into our results.

Several participants were lost to follow-up after consenting for the first substance use survey, and five did not submit stool samples after consenting for the microbiota study. This opens the potential for selection bias if participants who dropped out had more severe substance use disorders or systematically differed from retained participants in some other way. We qualitatively observed that increased hardness of stool samples among participants using opioids

made these samples more difficult to homogenize in RNA*later*™ but did not measure stool consistency or hardness. We expect that participants who used opioid agonists had harder stool and slowed transit time, but are unable to comment on how stool hardness and transit time affect the variation observed in the non-opioid agonist groups. Nonetheless, the strong biological plausibility of the present findings suggest that they are worthwhile to explore further and validate using other more comprehensive methods.

Our study had several offsetting strengths. Methodologically, previous studies did not describe enterotypes or evaluate associations of microbial taxa with opioid exposure using analytic tools appropriate for compositional data, and therefore previous findings may include spurious or biased associations.[55] We were able to evaluate the impact of two factors that could alternatively explain the associations observed between the microbiota and opioids, which were alcohol and dietary fiber. Neither exhibited as strong a relationship as the results for opioids, pointing to opioids as a future direction for further research, yet still highlighting the importance of measuring these potential confounders.

4.6.1.2   Conclusions

Individuals exposed to opioid agonists had differences in gut microbiota diversity, enterotypes, and bacterial genera compared to individuals who used neither an agonist nor antagonist. We observed decreased diversity and richness, an absence of the *Prevotella* enterotype, and decreases in *Roseburia,* a butyrate producer, and *Bilophila*, which may reflect the bile acid dysregulation observed in murine models of morphine dependence.[18,19] Individuals who concurrently used an opioid agonist and antagonist, and individuals who only used naltrexone, an opioid antagonist, did not differ in gut microbiota diversity, richness, or genera

relative abundance compared to individuals who used neither an agonist nor antagonist. These findings suggest that the effects of opioids on the gut microbiota might be antagonized by naltrexone or naloxone. Overall, this study supports conducting future work that further characterizes the relationships between opioid agonist and antagonist exposure, gut permeability, inflammation, and relapse predictors to inform whether psycho-adjunctive treatments for opioid and alcohol use disorders could improve addiction treatment outcomes.

4.6.1.3 Acknowledgments

Figure 4.1 Inclusion Criteria and Opioid Use among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility into a Study of the Gut Microbiota and Opioid Use, 2016-2017
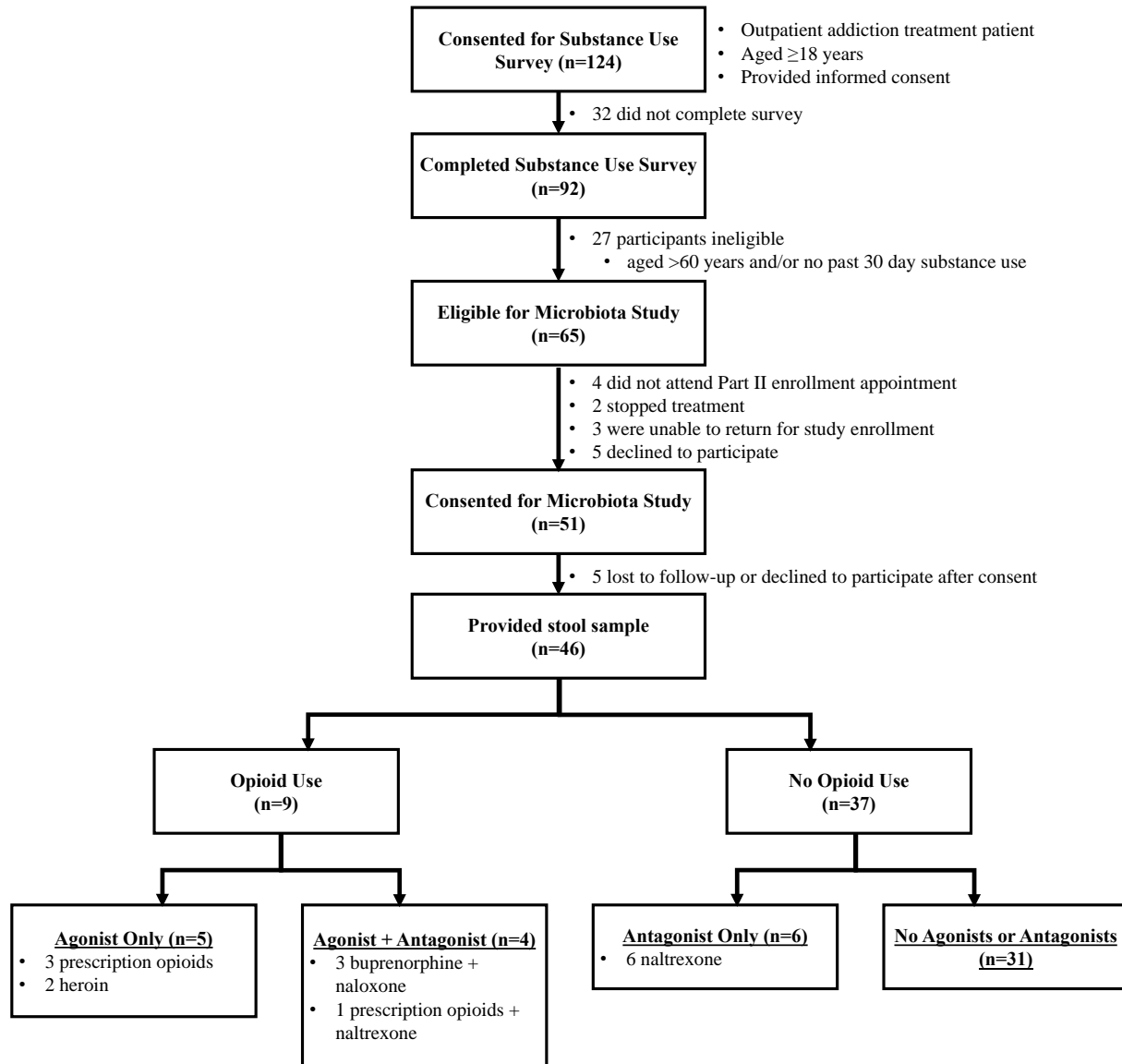
We included 46 participants who were eligible and provided informed consent for both study stages and provided stool samples in the present analysis. Among the 9 using opioids, 5 used only agonists (prescription opioids and heroin) and 4 used an agonist and antagonist combination during the time their sample was provided. Among the 37 not using opioids, 6 used an opioid antagonist and 31 were exposed to neither opioid agonists nor antagonists during the time they provided their stool sample.

Table 4.1 Characteristics of 46 Participants Enrolled from an Outpatient Addiction Treatment Facility into a Study of the Gut Microbiota and Opioid Use, 2016-2017

Characteristic	Total n(%)	Ag n(%)	AgAt n(%)	At n(%)	N n(%)
Total	46 (100)	5 (100)	4 (100)	6 (100)	31 (100)
Age, Median (IQR)	33.5 (26.3-47.5)	38 (31-46)	27.5 (23.5-35.8)	34 (27.5-44.3)	36 (25.5-48)
Gender					
Female	26 (41.3)	1 (20.0)	2 (50.0)	1 (16.7)	15 (48.4)
Male	19 (56.5)	4 (80.0)	2 (50.0)	5 (83.3)	15 (48.4)
Other	1 (2.2)	0 (0)	0 (0)	0 (0)	1 (3.2)
Race					
Black	2 (4.3)	0 (0)	0 (0)	0 (0)	2 (6.5)
White	39 (84.7)	3 (60.0)	4 (100.0)	5 (83.3)	27 (87.1)
Multiple Races	2 (4.3)	1 (20.0)	0 (0)	0 (0)	1 (3.2)
Other	3 (6.5)	1 (20.0)	0 (0)	1 (16.7)	1 (3.2)
Hispanic ethnicity	5 (10.9)	1 (20.0)	0 (0)	1 (16.7)	3 (9.7)
Used alcohol[a]	34 (73.9)	3 (60.0)	3 (75.0)	5 (83.3)	23 (74.2)
Antibiotic use	1 (2.2)	0 (0)	1 (25.0)	0 (0)	0 (0)
Days in Treatment, Median (IQR)	34 (5-74)	12 (3-1,171)	549 (123-949)	23 (6-53)	19 (6-67)
Fiber (grams/day), Median (IQR)[b]	15.7 (14.1-18.4)	13.5 (12.7-17.0)	17.9 (14.9-19.0)	16.9 (13.2-18.3)	15.6 (14.3-17.7)
Depression Score, Median (IQR)[c]	9.5 (6.0-12.8)	13 (12-18)	7 (6.5-8.8)	9 (6.3-11)	9 (5.5-11.5)
Anxiety Score, Median (IQR)[d]	8 (4-10)	13 (9-14)	4.5 (1.5-8)	8 (7-9.8)	7 (3-9.5)
Craving Score, Median (IQR)[e]	9 (5-16)	16 (9-19)	5.5 (6.5-13)	12 (6.8-17.3)	8.5 (5-13.8)

[a]Participants self-reported alcohol use in the 30 days before the substance use survey (before enrolling in the microbiota study).
[b]Fiber intake data were available for 45 of 46 participants (30 of 31 participants who used neither opioid agonists nor antagonists).
[c]Score from the Patient Health Questionnaire (PHQ)-9 (range: 0-27).
[d]Score from the Generalized Anxiety Disorder 7-Item scale (range: 0-21).
[e]Score from the modified Penn Craving Scale (range: 0-30). Data were available for 45 of 46 participants (30 of 31 participants who used neither opioid agonists not antagonists).
Abbreviations: Ag: Opioid agonist only (heroin [n=2] or prescription opioid [n=3]), AgAt: Opioid agonist+antagonist use (buprenorphine+naloxone [n=3] or prescription opioids+naltrexone [n=1]), At: Opioid antagonist use only (naltrexone [n=6]), N: Neither opioid agonist nor antagonist use (n=31), IQR: interquartile range.

Figure 4.2 Gut Microbiota Alpha Diversity among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility by Opioid Agonist and Antagonist Use, 2016-2017
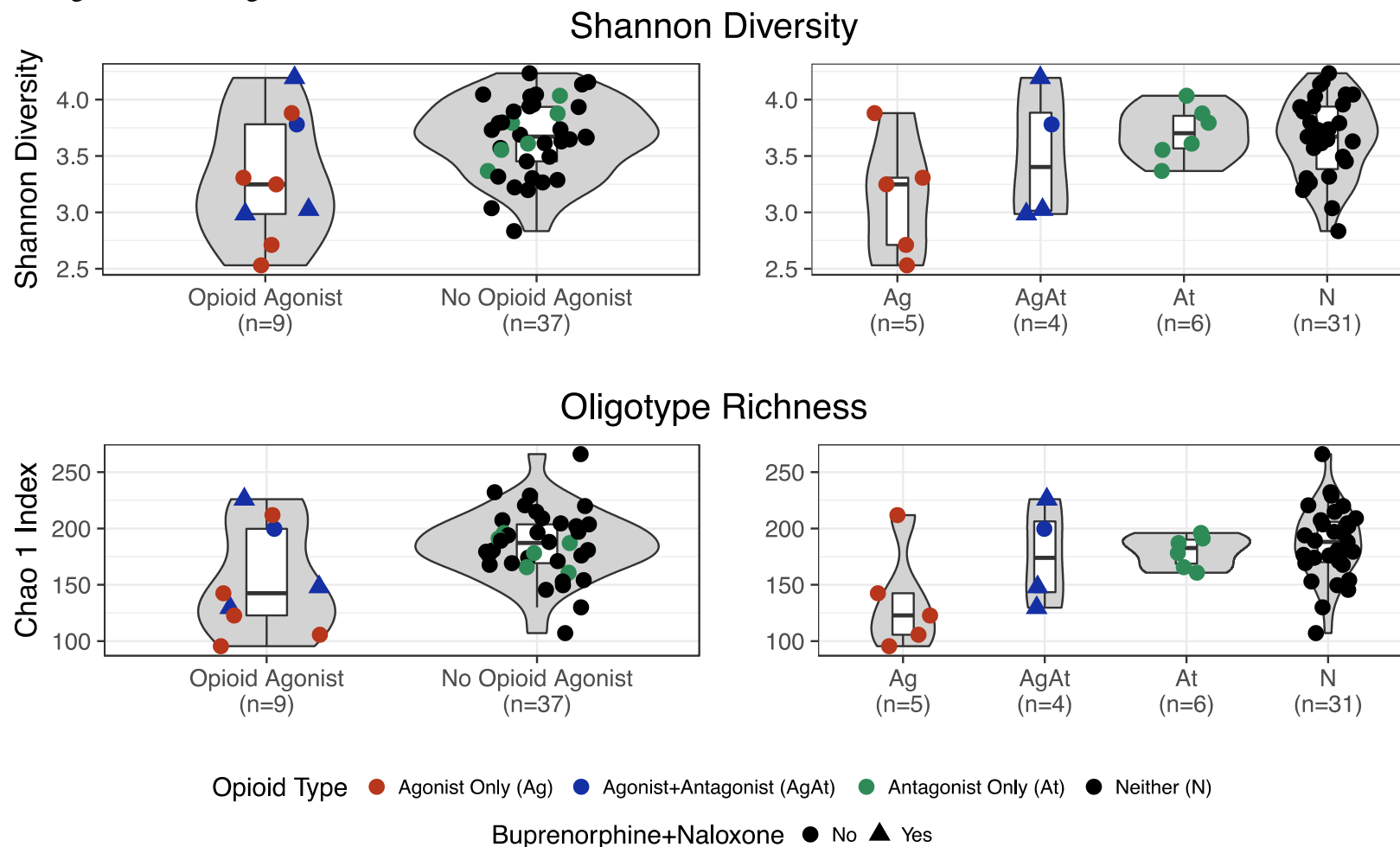
We compared alpha diversity between opioid agonist vs. no agonist groups and between groups using agonists and antagonists versus no use (N) using two metrics. Ag participants had lower diversity compared to N for both Shannon diversity (Wilcoxon rank sum p=0.04) and richness (Chao1 index, p=0.02). No other groups statistically differed, including AgAt vs. N and At vs. N.

Figure 4.3 Gut Microbiota Beta Diversity among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility by Opioid Agonist and Antagonist Use, 2016-2017
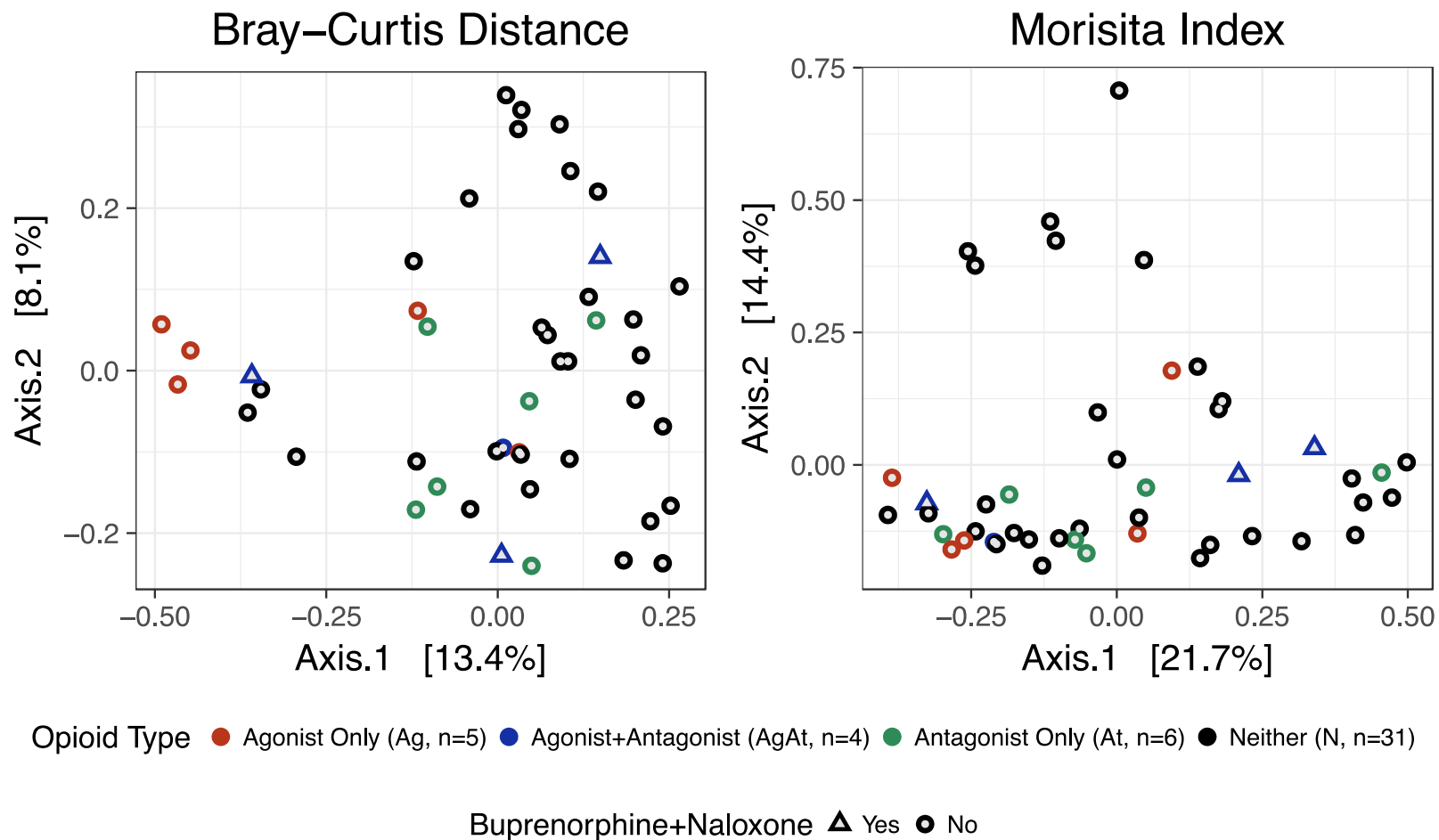
We summarized two beta diversity metrics, Bray-Curtis Distance and the Morisita Index, using a principal components analysis. We observed some clustering of the Ag group using Bray-Curtis. Strong clustering was not observed by opioid agonist-antagonist groups using the Morisita index. For this reason, we avoided further statistical testing.

Figure 4.4 Dirichlet Multinomial Mixture Model Fit (Laplace Approximation) for Models with 1-5 Enterotypes
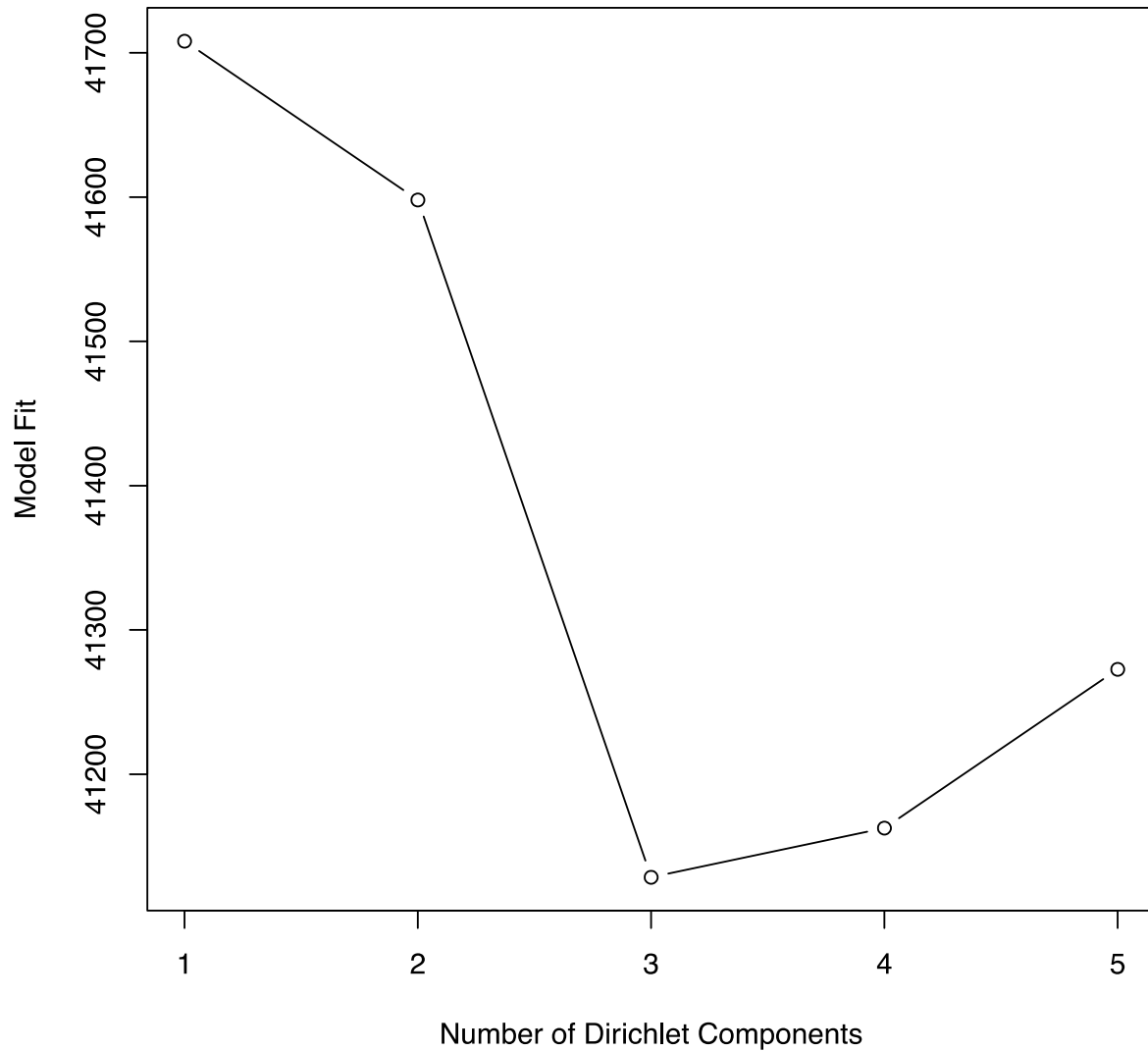
We fit Dirichlet multinomial mixture models with one to five enterotypes. Model fit was optimized by a three enterotype model given the minimization of the Laplace approximation, a measure of model fit.

Figure 4.5 Enterotypes Recovered from *De Novo* Clustering of Gut Microbiota Samples among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility, 2016-2017

The relative abundance of the top 20 genera that were most informative in partitioning samples into three enterotypes accounted for >70% of sequencing reads (top). We identified two *Bacteroides* enterotypes, one with increased *Faecalibacterium* and a second with increased *Clostridium* cluster XIVa. The third enterotype was dominated by *Prevotella.* Twenty-four participants assigned to the *Bacteroides: Faec.* enterotype had higher *Faecalibacterium* (mean *Faecalibacterium* relative abundance of 8.9% *Bacteroides: Faec.*, 2.1% *Bacteroides: Clost.*, 7.2% of *Prevotella*). *Faecalibacterium* was the third most influential genera in assigning enterotypes. Eleven participants assigned to the *Bacteroides: Clost.* enterotype had higher *Clostridium* cluster XIVa (mean *Clostridium* cluster XIVa relative abundance of 4.0% *Bacteroides: Clost.*, 0.86% *Bacteroides: Faec.*, and 0.63% *Prevotella* enterotype). *Clostridium* cluster XIVa was the fifth most influential genera in assigning enterotypes. The fourth most influential genera in assigning enterotypes was *Blautia*, which distinguished the *Prevotella* and two *Bacteroides* enterotypes (mean *Blautia* relative abundance of 8.5% *Bacteroides: Faec.*, 8.6% *Bacteroides: Clost*, and 4.7% *Prevotella*). We also show the relative abundance of all genera (bottom).

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Figure 4.6 *De Novo* Assigned Gut Microbiota Enterotypes among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility by Opioid Agonist and Antagonist Use, 2016-2017
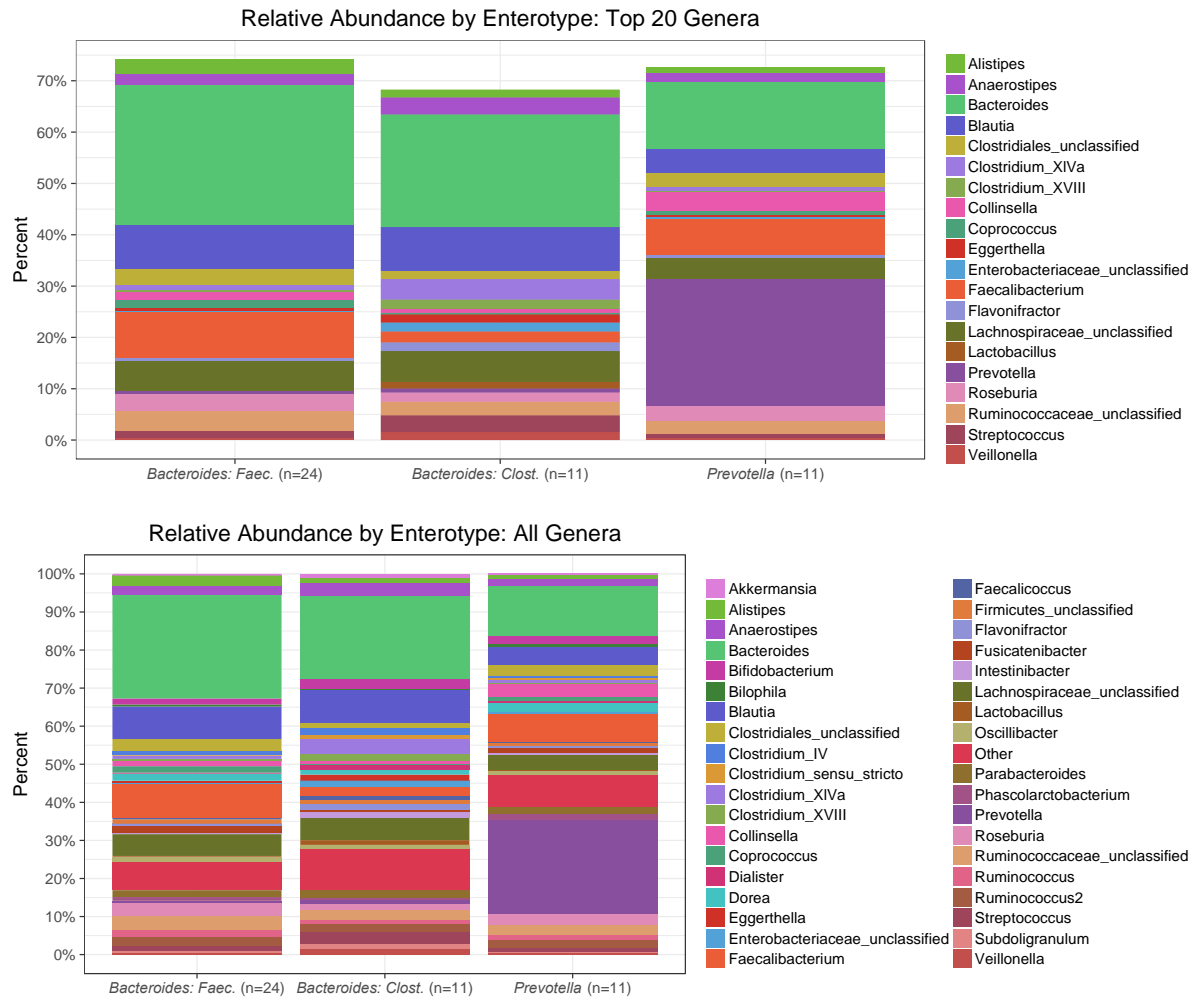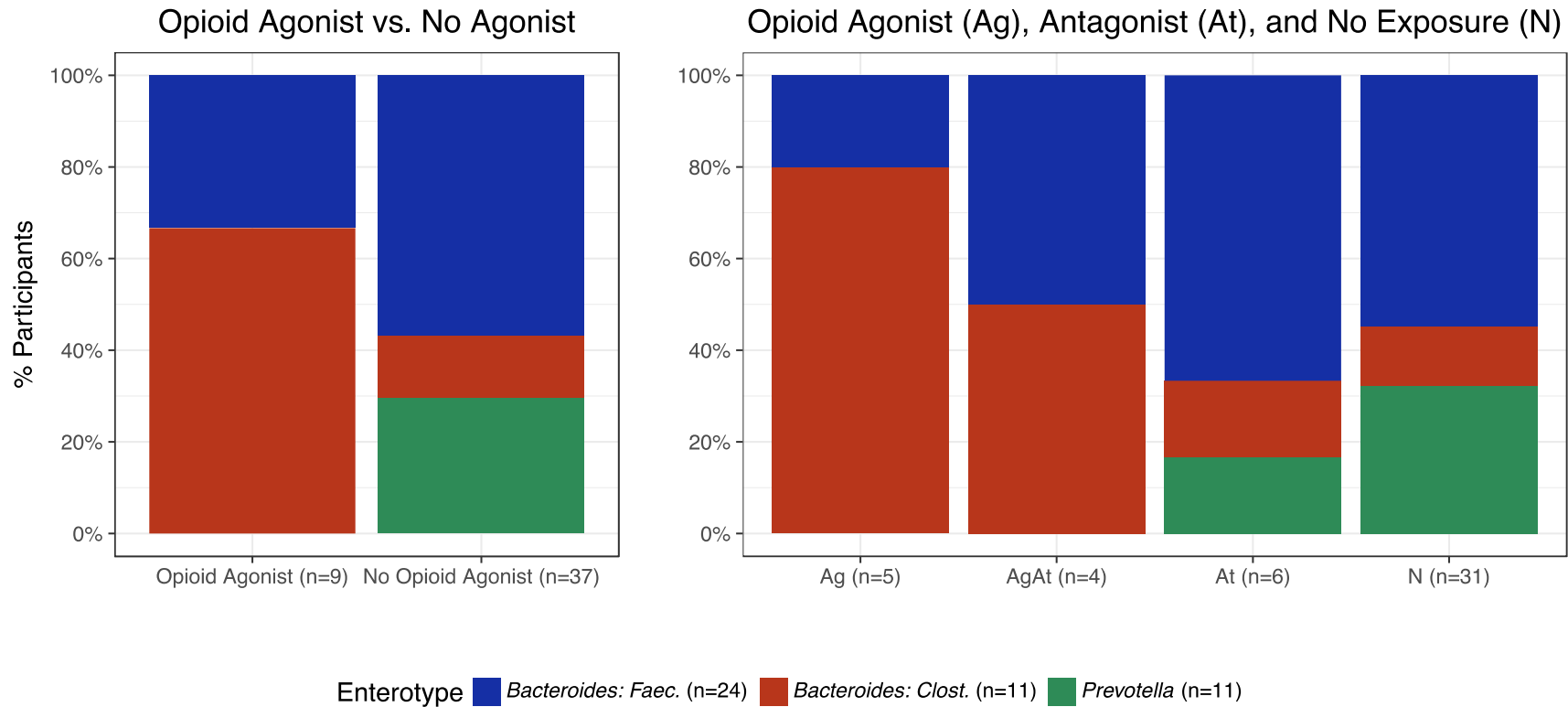

The prevalence of three enterotypes identified through Dirichlet multinomial mixture modeling differed by opioid agonist-antagonist exposure groups. No individuals who used opioid agonists had the *Prevotella* enterotype and 4 of 5 participants who used only opioid agonists (Ag) had a *Bacteroides* enterotype with elevated *Clostridium* cluster XIVa. The distribution of enterotypes differed by opioid agonists vs. no agonists (Fisher exact p-value=0.0036) and between the agonist only group (Ag) vs. the group that used neither opioid agonists nor antagonists (N, Fisher exact p-value=0.0060).

Figure 4.7 Alpha Diversity in *De Novo* Assigned Gut Microbiota Enterotypes among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility by Opioid Agonist and Antagonist Use, 2016-2017
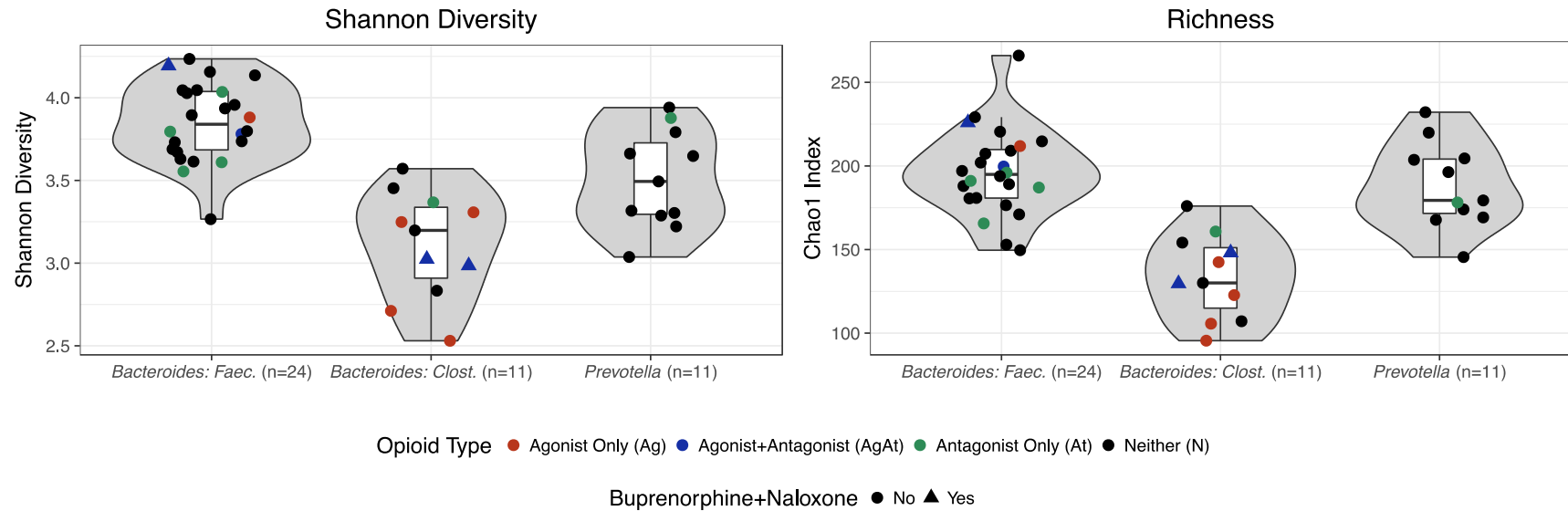
We used Dirichlet multinomial mixture modeling to identify three enterotypes. A hallmark feature of the *Bacteroides: Clost.* group was reduced alpha diversity by both the Shannon diversity (Kruskal Wallis p-value<0.00001) and Chao1 (Kruskal Wallis p-value<0.0001) metrics. We did not detect any statistically significant differences in the distribution of enterotypes by other groups (e.g. AgAt vs. N and At vs. N).

Table 4.2 Reference-Based and *De Novo* Enterotype Assignments among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility, 2016-2017

	De Novo **Assigned Enterotypes** (Method: DMM)			
Reference-Based Enterotypes (Method: PAM)	*Bacteroides: Faec.* n (%)	*Bacteroides: Clost.* n (%)	*Prevotella* n (%)	Total n (%)
Bacteroides	22 (91.7)	9 (81.8)	0 (0)	31 (67.4)
Firmicutes	0 (0)	0 (0)	0 (0)	0 (0)
Prevotella	2 (8.3)	0 (0)	11 (100)	13 (28.3)
Unable to be assigned	0 (0)	2 (18.2)	0 (0)	2 (4.3)
Total	24 (100)	11 (100)	11 (100)	46 (100)

Abbreviations: DMM: Dirichlet multinomial mixture model, PAM: Partitioning around medoid clustering, n: number.

Figure 4.8 Genera Selected by ALDEx2 as Differentially Abundant by Opioid Use among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility, 2016-2017
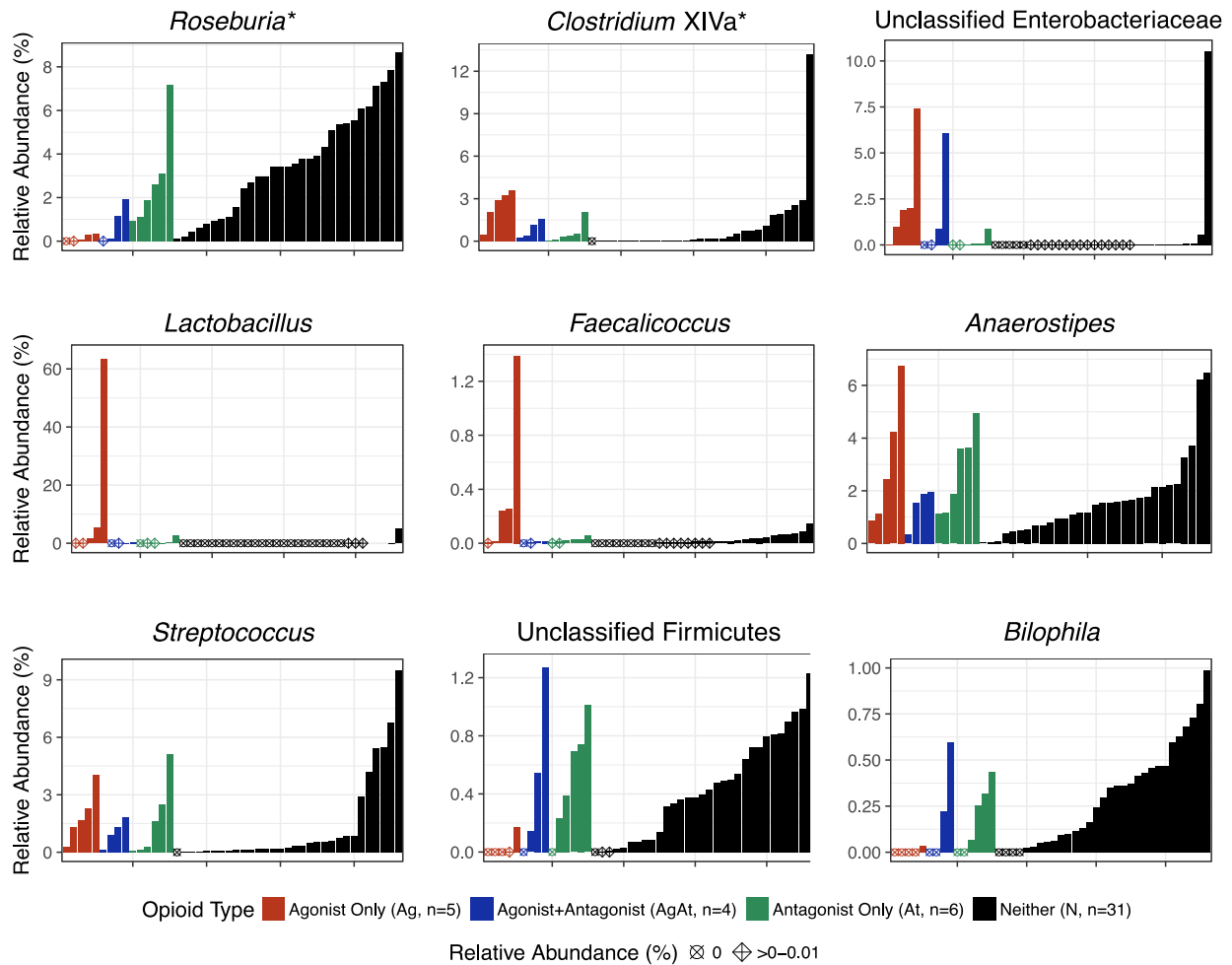
We used ALDEx2 to identify nine genera that were differentially abundant between participants who used opioid agonists (Ag) vs. participants who used neither agonists nor antagonists (N) using a false discovery rate (FDR) corrected p-value<0.05 threshold for the Wilcoxon rank sum test. Unclassified Enterobacteriaceae (FDR p-value: 0.026), *Lactobacillus* (FDR p-value: 0.031), *Clostridium* cluster XIVa (FDR p-value: 0.033), *Faecalicoccus* (FDR p-value: 0.037), *Anaerostipes* (FDR p-value: 0.040), and *Streptococcus* (FDR p-value: 0.045) abundances were higher in Ag vs. N participants while unclassified Firmicutes (FDR p-value: 0.031), *Bilophila* (FDR p-value: 0.037), and *Roseburia* (FDR p-value: 0.043) were less abundant in Ag vs. N participants. We found no statistically significant taxa abundance differences between other opioid agonist/antagonist groups. *Taxa were also differentially abundant in a comparison of opioid agonists (Ag or AgAt) vs. no agonist (At or N) use.

Figure 4.9 Gut Microbiota Alpha Diversity by Alcohol Use and Fiber Intake among 46
Participants Enrolled from an Outpatient Addiction Treatment, 2016-2017
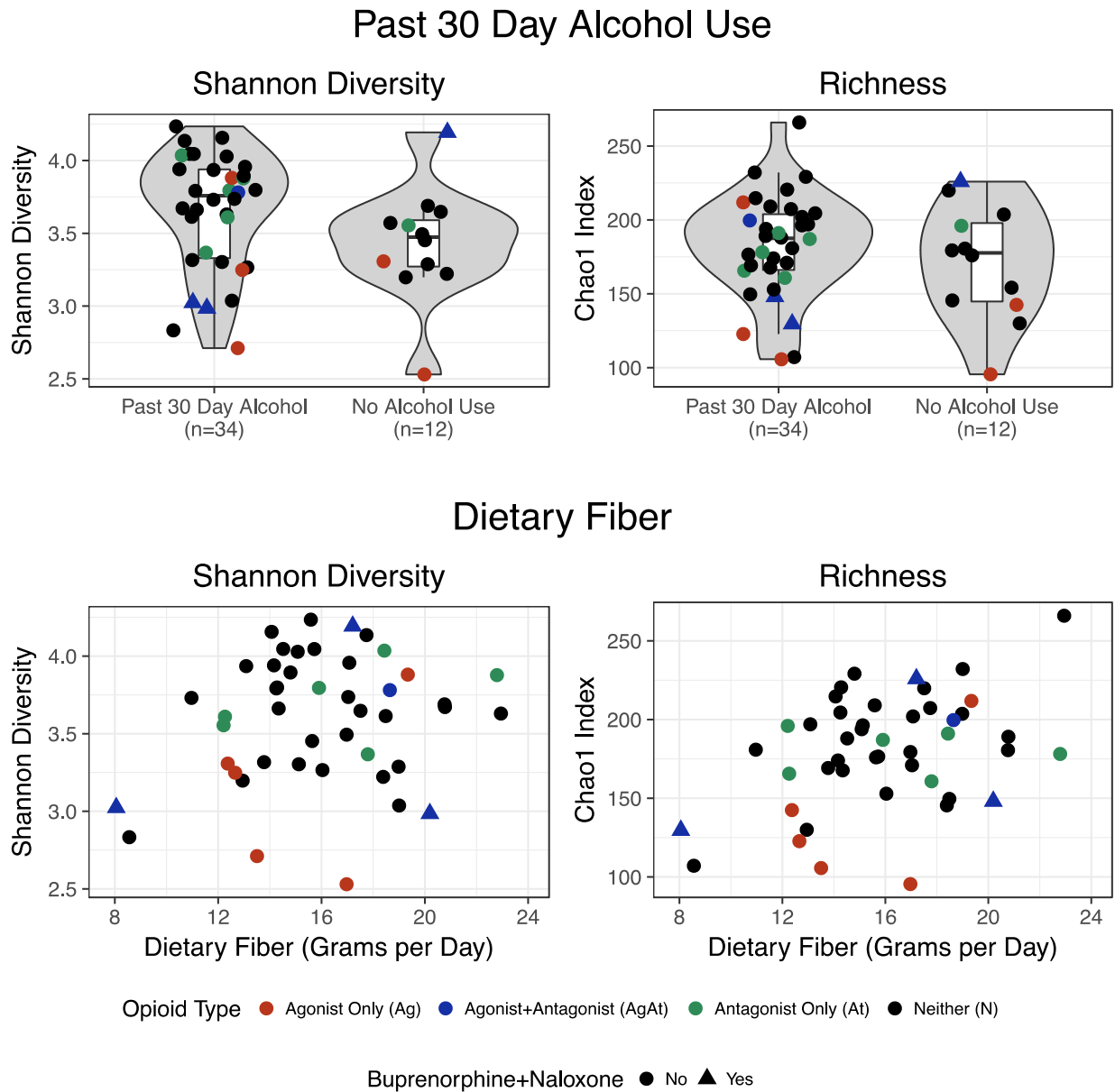
We compared alpha diversity using the Shannon diversity and Chao1 metrics by past 30-day
alcohol use (top) and dietary fiber (bottom). Shannon diversity was marginally lower among
participants who did not use alcohol in the past 30 days (Wilcoxon rank sum p=0.052). Gut
microbiota richness was positively associated with fiber intake and the Pearson correlation (0.41)
between richness and fiber intake reached statistical significance (p=0.005).

Figure 4.10 *De Novo* Assigned Enterotypes by Alcohol Use and Dietary Fiber Intake among 46 Participants Enrolled from an Outpatient Addiction Treatment Facility, 2016-2017
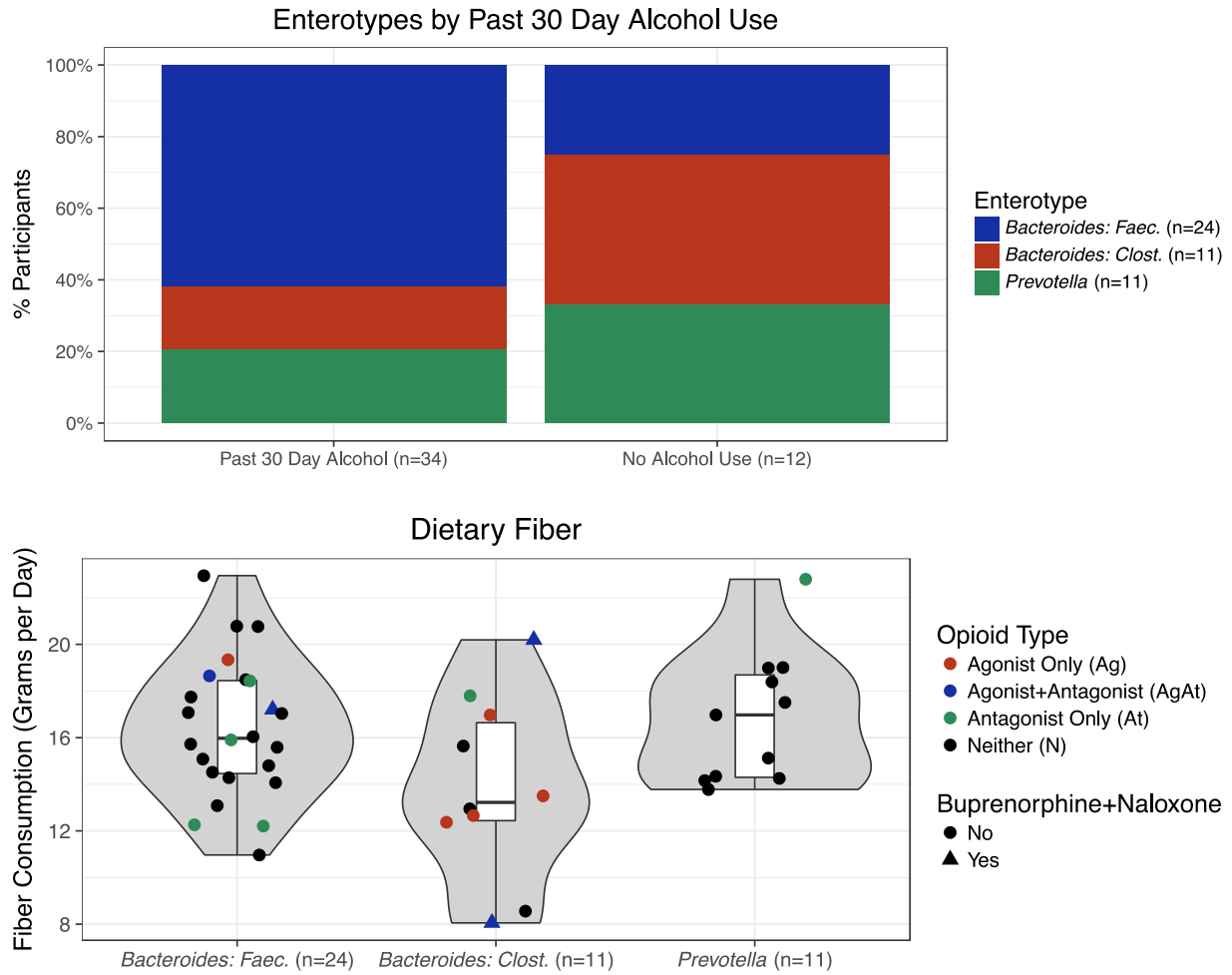
We examined whether enterotype prevalence differed by past 30-day alcohol use (top) or dietary fiber consumption (bottom). The *Bacteroides: Faec* enterotype was more common in people who used alcohol in the past 30 days (Fisher exact p-value=0.11). *Bacteroides: Clost* had the lowest median fiber intake (Kruskal Wallis p-value=0.12).

Appendix 4.1. Supplementary Methods

Dietary Fiber Intake Estimation

Upon enrollment in the microbiota study, participants completed a validated food frequency questionnaire about general past month intake of 25 food and drink items.[69] Due to a skipped question on the survey, participants did not report their cheese intake. We therefore assigned participants the survey weighted mean values of cheese intake using age and race-specific values from the 2009-2010 cycle of the National Health and Nutrition Examination Survey (NHANES), which included the food frequency questionnaire used in our study. We did not use sex-specific values as cheese intake did not differ by sex in NHANES. We calculated predicted past month dietary fiber intake (predicted grams per day) from reported dietary item intake and validated predictive models developed from the National Health and Nutrition Examination Survey.[40]

DNA Extraction and Illumina MiSeq Sequencing

After all participants were enrolled, we thawed a 1 mL aliquot of each stool sample, centrifuged at 10,000xg, and resuspended in 1 mL 1X phosphate buffered saline (Gibco, Thermo Fisher Scientific, Waltham, MA, pH 7.4). We added 250 uL resuspended stool to each of two wells of bead beating plates and provided samples to the Microbial Systems Molecular Biology Laboratory at the University of Michigan. They used standard protocols for deoxyribonucleic acid (DNA) extraction and Illumina MiSeq sequencing of the V4 hypervariable region of the bacterial 16S ribosomal ribonucleic acid (rRNA) gene.[47–49]

First, DNA was extracted using the Qiagen MagAttract PowerMicrobiome kit (Qiagen, Hilden, Germany). DNA libraries for the V4 region of the 16S rRNA gene were generated and

16S DNA was amplified using polymerase chain reaction (PCR) and barcoded dual-index primers for the V4 hypervariable region.[49] Reactions included 5 uL of 4 uM equimolar primer set, 0.15 uL AccuPrime *Taq* DNA High Fidelity Polymerase, 2 uL 10X AccuPrime PCR Buffer II (Thermo Fisher Scientific), 11.85 uL PCR-grade water, and 1 uL DNA and used the following cycling conditions: 2 min at 95°C, 30 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 5 min, and finally 72°C for 10 min. PCR reactions were normalized, pooled, and quantified. The pooled amplicon library was sequenced using Illumina MiSeq with the 500 cycle MiSeq V2 Reagent kit (Illumina, San Diego, CA) with modifications to the primer set (custom read 1/read 2 and index primers were added to the reagent cartridge). Two types of mock communities were included with samples to assess sequencing error rates. Mocks included a community of 10 species (added as bacterial DNA, Zymo Research, Irvine CA, catalog no. D6300) and a mock community of two species added as suspended overnight cell culture in brain heart infusion broth (equal ratios of *Escherichia coli* and *Staphylococcus aureus*). All samples were sequenced in duplicate and mocks were sequenced on each of four DNA extraction plates.

We processed sequencing reads using mothur (v1.39.5) and the MiSeq standard operating procedure (https://www.mothur.org/wiki/MiSeq_SOP, accessed November 8, 2017) to perform quality filtering and align sequences to the V4 region of the 16S rRNA gene.[49] We converted sequences to the format required for oligotyping and clustered samples into oligotypes using the procedures and default parameters described by Eren *et al.*[50,52] Oligotyping uses minimum entropy decomposition methods to identify highly variable nucleotide positions and clusters sequences based on Shannon entropy.[50] Compared with operational taxonomic units, an alternative way to cluster sequences based on distance-based metrics, oligotyping improves identification of taxa at the species or strain level.[50]

Two samples with fewer than 1,000 reads were removed from further analysis; however, we were able to retain these two participants for analysis using their duplicate sequenced sample. We verified that all mock communities resembled their known compositions (data not shown) and summed duplicate sequenced samples. For this analysis, we examined the first sample submitted per participant, amounting to 46 samples with 2,207,827 sequence reads (21,796 – 77,013 reads per participant) and 354 oligotypes. We assigned oligotype taxonomy using the Ribosomal Database Project (release 11, update 5).[53] We chose to focus on the first sequenced sample per participant after observing little change to taxa diversity, relative abundance and other microbiota characteristics during the month-long period of study (further described below).

Gut Microbiota Diversity Among 129 Samples Collected over a 1 Month Follow-Up Period from 46 Participants Enrolled from an Outpatient Addiction Treatment Facility

We examined microbiota diversity among 129 samples collected in our study (up to 3 per participant over a maximum of 1 month study enrollment). As shown in Figure 4.11, we found that sample replicates clustered strongly by participant using the Bray-Curtis distance metric, a measure of beta diversity. We also found little difference in alpha diversity over time (Figure 4.12). Stool sample relative abundance and enterotypes also changed minimally during the study period (data not shown). These findings supported the restriction applied for the present analysis to the first sample received per participant. Below we include plots of microbiota diversity by participant and study visit. Note that study visit indicates the sample number (1-3). Participant identification numbers are withheld to maintain participant confidentiality.

Figure 4.11 Principal Coordinate Analysis of Bray-Curtis Distances by Participant and Study Visit Demonstrate Clustering By Participant
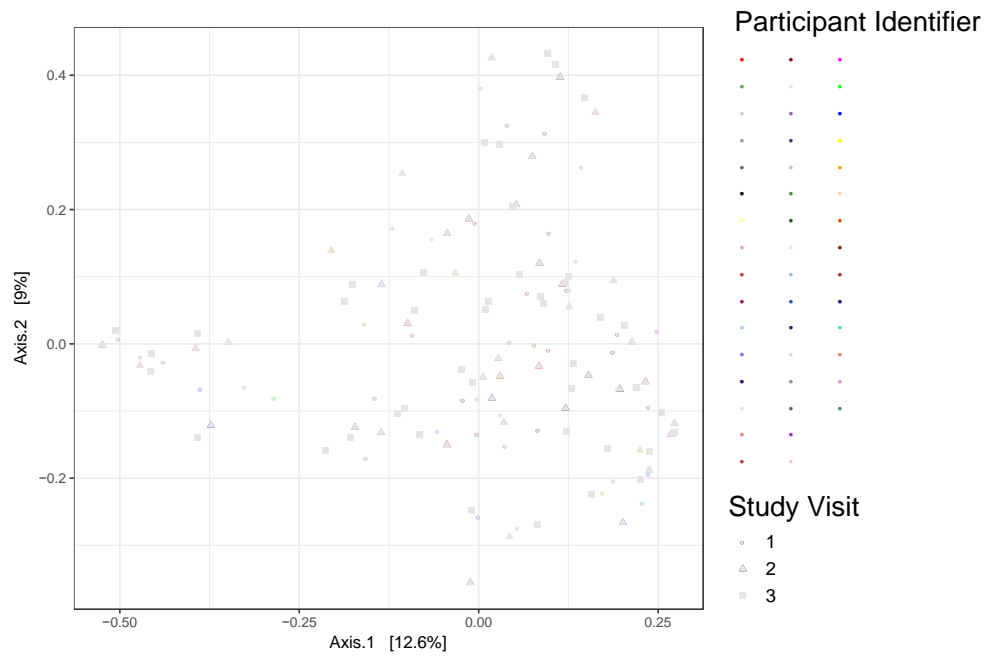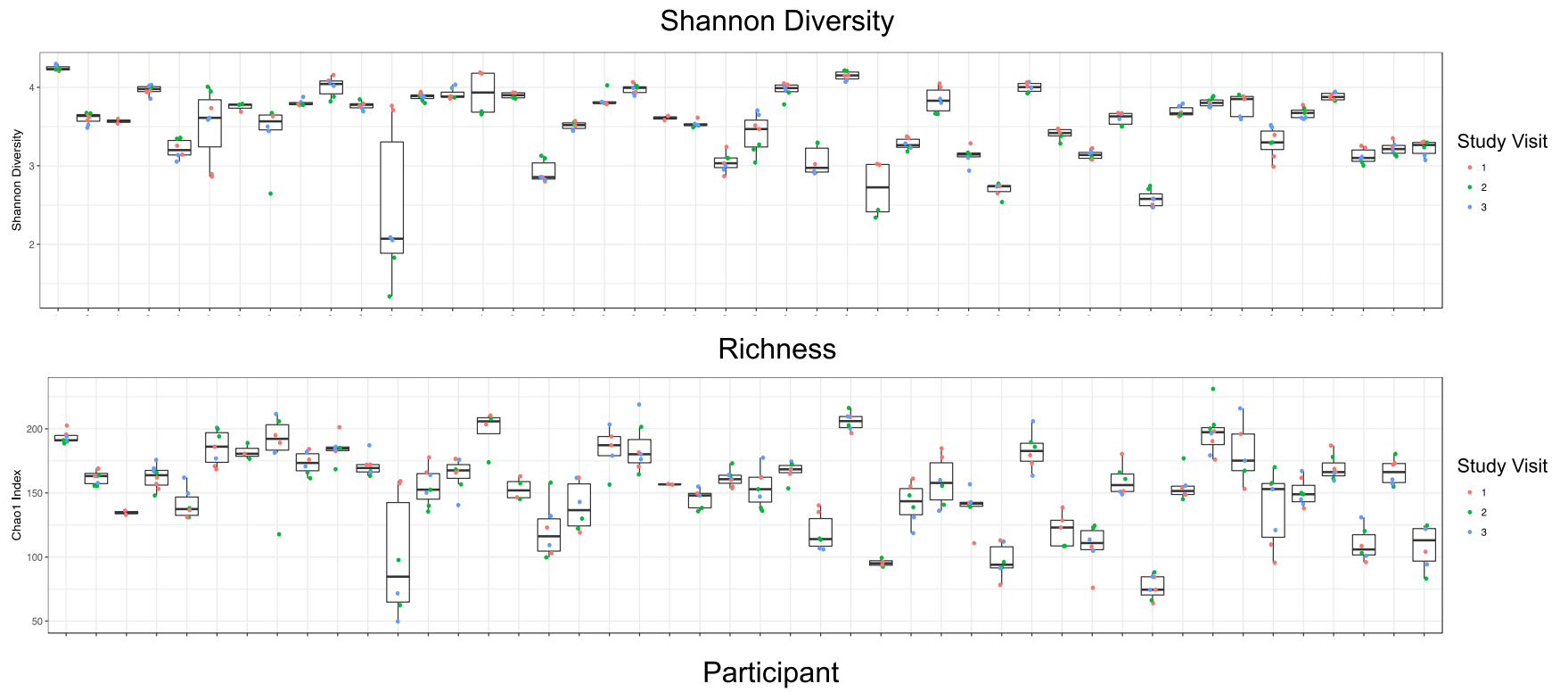
Figure 4.12 Alpha Diversity was Similar Over a One Month Follow-Up Period for Most Participants

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4.7   References

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Chapter 5   Summary and Conclusions

In this chapter, I review some of my experiences during the PhD, and discuss how both my successes and challenges helped me develop independence and embrace interdisciplinary research. I end the chapter with a discussion of several avenues for future research.

5.1   A Reflection on My Successes and Challenges

5.1.1   Learning Methodology Early and Often

When I interviewed for the PhD program, I expressed an interest in becoming more proficient in statistics. To achieve this goal, my advisor suggested that I complete linear algebra and multivariable calculus courses before beginning the PhD and take several advanced biostatistics courses during my first year of the PhD program. At first, I struggled to make sense of this new material and could not see its applications to my research. As I moved through my first year, however, my skills improved, my confidence grew, and I developed a new interest in learning and applying new methods. These new skills were the foundation that let me learn about compositional data analysis and use latent variables across seemingly disparate applications. Over the past four years, I've learned that it's important to push myself through those first few papers or courses and that the process of digging through the details of a new method is something I enjoy.

5.1.2   Starting Over

I completely switched dissertation projects during my second year. It was difficult to admit to myself that I needed to make a change and to have the conversations necessary to switch projects. However, I found my voice after some encouragement from my mentors, and took a few decisive steps to transition my research to an area I had long been interested in: substance use and its intersection with infectious disease epidemiology. Switching focus to this area of research has kept me motivated, passionate, and productive through these past two years in the PhD program. Speaking up and having a few difficult conversations in year two were more than worth the discomfort I felt at the time. In addition, watching my mentors handle and embrace this situation taught me a lot about my own responsibilities as a mentor in the future. I was only able to make a big change because of their support. Mentorship, especially to a PhD student, is not a small responsibility.

5.1.3   Meeting New Mentors

Part of starting over meant that I had to find two new research projects, so I set out to find a third mentor with an interest in substance use epidemiology. I was so fortunate to meet Dr. Amy Bohnert, who graciously integrated me into her research team in Psychiatry, just a few weeks after I had decided to switch gears. My timing in meeting Amy was perfect – she was looking for a research assistant to get a microbiota project running and I was looking for data collection experience, so I was very motivated to take on this challenge. This experience taught me to put myself out there – sending a cold email to a professor I read a newspaper article about gave me a new mentor and colleagues and led to a complete change in my dissertation research.

5.1.4   Data Collection Challenges

I was a research assistant on the microbiota study team and was therefore involved with study setup, participant recruitment, lab work, data entry and cleaning, and analysis. It was incredibly rewarding to see the study grow and I learned so much from interacting with the participants and my colleagues in psychiatry. I'm incredibly thankful to have been this involved in a project for my dissertation.

However, the process of data collection was one of the most laborious tasks during my PhD. Going to the clinic to recruit almost every day, and sometimes walking away with no new participants, left me feeling like I wasn't progressing on some days. An additional challenge was the process of doing risk assessments for participants with thoughts of self-harm or suicide. While I knew our protocols well from the beginning, it took me time to feel comfortable and confident about conducting risk assessments while taking whatever steps necessary to make sure our participants were safe. I was so lucky to be able to rely on the support of my supervisors in Psychiatry, who checked in often and really helped me learn this process.

Although it took time, collecting data taught me so many valuable lessons about research. One of these lessons was about the value of piloting studies. The microbiota study was a pilot, but participant recruitment was slow and we had to reduce the planned size of the study after a few months. We recruited few participants with opioid use disorders, and later learned that many of the clinic's patients who had opioid use disorders only came to the clinic once per month. In addition, patients' late evening appointments often made it challenging for them to stay even later to participate in research. These are exactly the reasons why studies are piloted. Even when a study site seems like a good fit and protocols and surveys are developed beforehand, the pilot phase provides time to really get a feel for the flow of things before doing a larger study.

5.1.5 The Benefits of Interdisciplinary Research

My dissertation research is spread across a couple of topic areas. I have really enjoyed being an interdisciplinary scientist, and often I think about a new way to look at data from one of my projects while working on another.

My best example of this was when I attended a community meeting to learn more about opioid overdose in Washtenaw county, Michigan. I was hoping that attending this meeting would help me plan the overdose research aims I discuss in chapter two. However, the meeting had a speaker, a pharmacologist who outlined the applications of low dose naltrexone for pain management (in combination with opioids). She briefly mentioned naltrexone's potential mucosal healing benefits for Crohn's disease. My mind immediately made a connection to the microbiota project (naltrexone is also used to manage cravings among people with alcohol or opioid use disorders). I took four pages of notes and tried to reason through this idea for months. I was excited when I came across a murine model that supported pursuing this idea further and was able to relate the findings from the murine model to our microbiota study in chapter 4.[1] It was not what I expected from a meeting I attended to learn about overdose but was nonetheless influenced my work in an important way.

5.2 Future Directions for Research

5.2.1 Overdose Education and Naloxone Distribution

As drug overdoses continue to rise, a major research priority must be to determine who is at risk for overdose and who could benefit from overdose education and/or naloxone distribution programs.[2,3] While investigating questions about overdose in chapter 2, we found additional potential avenues for future research with implications for overdose education and naloxone

distribution. A subset of people who use prescription opioids initiate heroin, but we do not yet know how naloxone knowledge differs for individuals with different opioid use initiation patterns.[4] Using the dataset I described in chapter two, we observed lower naloxone knowledge among people who only used prescription opioids compared to participants who transitioned from using prescription opioids to heroin. A deeper exploration of the overdose experiences, healthcare interactions that may have influenced how these individuals initiated their opioid use (e.g. after experiencing pain and receiving a prescription), and criminal justice system interactions could point to missed opportunities for overdose education and/or could support prescribing naloxone in the context of pain treatment or upon release from incarceration.

5.2.2   Substance Use Trends

During chapter 3, I mentioned that we used data from the National Survey on Drug Use and Health (NSDUH) to help parametrize the model. During model development, we examined time trends for substance abuse and dependence and injection drug use but could not explore these trends further for the purposes of model development. An age-period-cohort analysis could provide insight into the drivers of prescription opioid, heroin, and injection drug use changes over time.

5.2.3   Injection Initiation

Injection drug use carries several risks beyond other methods of drug use, including increased overdose risk and the potential for bloodborne viral infections if syringes are shared.[5–7] The initiation period of injection drug use is a particularly risky period for bloodborne viral infections and overdose.[8] Only a handful of interventions have been shown to decrease the

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initiation of injection drug use.[9] Some of these interventions encourage people who are experienced injecting drugs to refuse when asked to help initiate their less experienced peers.[9] In the short term, I would like to conduct a meta-analysis to understand the rates of injection drug use initiation we used in chapter three for the purposes of model fitting. There are only a few studies on this topic and summarizing these would be helpful for future transmission models. In the long-term, I hope to develop novel interventions that prevent injection drug use initiation and reduce the harms associated with injection drug use among people who inject drugs.

5.2.4   Microbiome and Recovery from Opioid Use Disorders

We are only beginning to understand how the interactions between our gut, brain, and gut microbiota impact psychopathology. Expanding our pilot study to consider the gut microbiota, and gut health more generally, as a potential support for recovery from opioid use disorders would be a first step. We need further research into how opioid agonists and antagonists interact with the gut microbiota, gut permeability, and dietary patterns, and how these factors could affect inflammatory markers, neuro-inflammation, mood, cravings, psychopathology, substance use behaviors, and other aspects of mental and physical health. Limited research suggests the potential that opioid antagonists could mitigate some of the negative impacts of opioid agonists on the gut microbiota, but this work needs to be replicated in a larger study of humans and to be studied further in murine models.[1] Nonetheless, the possibilities for this research are exciting, and could have major implications for adjunctive treatments among people who take opioids as prescribed, people in recovery from opioid use disorders, and in the context of medication assisted treatments.

5.3   Conclusions

In conclusion, this dissertation examined three priorities related to the opioid crisis: injury, infection, and recovery. Our results suggest that all participants in justice diversion addiction treatment should receive overdose education and be provided naloxone given the high prevalence of overdose experience and that only half knew about naloxone's application as an overdose treatment. Further, we found that reductions to hepatitis C prevalence and incidence among young people who inject drugs in Michigan could be achieved through HCV treatment, and that these reductions could be strengthened by behavioral interventions. Finally, we found that opioid agonist exposure was associated with several alterations to the gut microbiota, including the promotion of a *Bacteroides* dominated enterotype, lower microbiota diversity, and lower abundance of *Roseburia*, a genus that produces a short chain fatty acid (butyrate) known to promote gut health. Collectively, these findings point to strategies to address three major public health priorities associated with the opioid crisis: injury, infection, and recovery.

5.4   References

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