SEX AND GENDER DIFFERENCES IN OPIOID ADDICTION TREATMENT

SEX AND GENDER DIFFERENCES IN THE MANAGEMENT AND TREATMENT OF OPIOID ADDICTION

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Abstract

Background and Objectives: Opioid addiction is a major contributor to the global burden of disease and carries a significant risk of morbidity and mortality. Individuals with opioid addiction are subject to numerous adverse consequences including infectious diseases, medical complications, psychiatric disorders, and social disintegration. Women especially experience a heightened vulnerability to the adverse medical and social consequences of opioid addiction as a result of biological sex characteristics and socially-defined gender roles, which increases their risk for poor treatment outcomes. The general objective of this thesis if to investigate sex and gender differences in the management and treatment of opioid addiction with a focus on hormonal influences, genetic variation, and sociobehavioral characteristics including substance use behavior, health status, and social functioning.

Methods: Using various methodologies, we compared the biological and social characteristics of men and women with opioid addiction in the context of methadone treatment. We assessed sex and gender differences in methadone treatment outcomes using a systematic review of the literature and a meta-analysis, which was developed based on published protocol. Next, we used data from the multi-centre GENOA cross-sectional study including 250 patients with opioid addiction recruited from Ontario methadone clinics to measure testosterone levels among men and women compared to non-opioid using controls; total serum testosterone was assayed using ELISA and RIA

techniques. Following this study, we conducted a systematic review and meta-analysis to test the effect of opioid use on testosterone levels, performing subgroup analyses by sex and type of opioid used. Using the previous GENOA sample, we then completed genotype analysis on variants of *BDNF* and *DRD2* genes to test the genetic effect on continued opioid use, measured through urine drug screening. Finally, we recruited an additional 503 participants meeting criteria for opioid use disorder who were receiving treatment with methadone, from which we obtained information on drug use patterns and addiction severity using the Maudsley Addiction Profile (MAP) tool to evaluate sex and gender differences.

Results: In our initial systematic review, we found 20 studies collectively showing that women were less likely than men to report alcohol use, employment, or legal involvement, but were more likely to misuse amphetamines. Using the GENOA dataset of methadone patients, we found a significant reduction in testosterone level among men but not women, which was associated with methadone dose. We also determined that testosterone did not fluctuate significantly between menstrual cycle phases. In line with these findings, our systematic review showed a significant suppression in mean testosterone level among men that use opioids compared to controls, but not in women. Our results also showed that methadone did not affect testosterone differently than other opioids. Among GENOA participants, *BDNF rs6265* and *DRD2 rs1799978* genetic variants were not significantly associated with continued opioid use while in methadone maintenance treatment. Our final study identified sex and gender differences in substance use, health status, and social functioning. Women were younger, had children, were

current smokers, had higher rates of benzodiazepine use, more frequent physical and psychological health problems, family history of psychiatric disorders, more partner conflict, and began regular use of opioids through a physician prescription. In comparison, men were more likely to be employed and to report cannabis and amphetamine use.

Conclusions: This thesis has demonstrated that men and women are differentially affected by opioid addiction and experience sex- and gender-specific challenges throughout the course of methadone treatment that are likely to impact treatment outcomes. The identification of clinically-relevant sex and gender differences is important to our understanding of the addiction profile, and can therefore be used to promote strategies for effective treatment and management of opioid addiction among men and women incorporating both biological and social perspectives.

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List of Abbreviations

OST	opioid substitution therapy
MMT	methadone maintenance treatment
DSM	Diagnostic and Statistical Manual of Mental Disorders
RCT	randomized controlled trial
MeSH	medical subject heading
NOS	Newcastle-Ottawa scale
GRADE	Grading of Recommendations Assessment, Development and Evaluation
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
CINAHL	Cumulative Index to Nursing and Allied Heath Literature
OR	odds ratio
CI	confidence interval
SMD	standardized mean difference
GnRH	gonadotropin-releasing hormone
LH	luteinizing hormone
FSH	follicle-stimulating hormone
GENOA	Genetics of Opioid Addiction
OATC	Ontario Addiction Treatment Centres

PGP	Population Genomics Program
HIREB	Hamilton Integrated Research Ethics Board
HSO	health services organization
MINI	Mini International Neuropsychiatric Interview
ELISA	enzyme-linked immunosorbent assay
RIA	radioimmunoassay
SD	standard deviation
STROBE	Strengthening of Reporting Standards in Observational Studies
HPG	hypothalamic-pituitary-gonadal
MD	mean difference
NSAIDS	non-steroidal anti-inflammatory drugs
CONSORT	Consolidated Standards of Reporting Trials
BDNF	brain-derived neurotrophic factor
DRD2	dopamine receptor D2
SNP	single nucleotide polymorphism
CATC	Canadian Addiction Treatment Centres
MAF	minor allele frequency
MAP	Maudsley Addiction Profile
THC	tetrahydrocannabinol
FDR	False Discovery Rate
SAMHSA	Substance Abuse and Mental Health Services Administration

Declaration of Academic Achievement

This sandwich thesis combines six individual projects prepared for publication in peer-reviewed academic journals. I am the primary author of all studies included and have made substantial contributions to each piece of work by developing the research questions, writing study protocols, managing data, performing statistical analysis, and writing the manuscripts. The detailed contributions of all authors are described following each study in the thesis.

CHAPTER 1

1.1 Background

Opioid addiction is a major contributor to the global burden of disease. Recent prevalence estimates suggest that opioid addiction affects over 15.5 million individuals worldwide and carries a significant risk of morbidity and mortality (1). In North America, an estimated 1 million individuals are dependent on opioids (2), which is driven by increasing rates of illicit opioid use. Increases in the availability and utilization of opioids for the management of pain have prompted a shift from heroin use to non-medical prescription opioid use (3, 4). Canada currently ranks first in global opioid analgesic consumption (5), sustaining a significant burden of opioid-related hospital admissions and mortality nationwide (5-8). Further, individuals with opioid addiction are subject to numerous adverse consequences including infectious disease, medical complications, psychiatric illness, and social disintegration (9). Collectively, these factors have evoked higher rates of healthcare utilization leading to considerable increases in social and health expenditure (10, 11).

Treatment for opioid addiction primarily consists of medication-assisted interventions, formally known as opioid substitution therapy (OST). Methadone maintenance treatment (MMT) is the most common form of opioid agonist therapy implicated for the management of opioid use disorders (12, 13) and currently serves over 35,000 patients in Ontario alone (14-16). Methadone, a synthetic opioid agonist, is an effective substitute

medication for opioid addiction because of its comparably long half-life and ability to provide relief of withdrawal symptoms (17). Similar to other long-acting opioid alternatives such as buprenorphine, methadone is dispensed in prescribed doses to patients under clinical supervision. There is evidence supporting the effectiveness of methadone treatment in reducing opioid-related risks when individuals are successfully retained in treatment (13, 18, 19). However, there are a number of patients that do not experience a positive response to MMT; an estimated 25% of patients discontinue treatment within the first two years or have persistent opioid use while receiving treatment (20, 21).

1.2 Sex and gender

A dramatic growth in rates of prescription opioid use and treatment admissions among women is indicative of problematic opioid use also affecting women (4, 22-26), yet available treatment interventions remain targeted towards primarily male heroin users (27). Women experience a heightened vulnerability to the adverse medical and social consequences of opioid addiction (28, 29) as a result of biological sex characteristics and socially-defined gender roles.

Sex and gender research has developed from the increasing recognition of their role as important determinants of health, which offer unique contributions to our knowledge of health and disease (30, 31). Sex is a biological construct that classifies individuals as male or female based on fundamental human characteristics, including anatomy, physiology,

genetics, and hormones. Sex plays a major role in health and disease as individuals experience certain processes differently based on these biological factors. Gender, however, refers to the social construct of ‘maleness’ or ‘femaleness’, and is often based on cultural and historic influences. Socially-determined norms and expectations affect individuals across many dimensions, including their thoughts, actions, and behaviors, which collectively form their gender role.

The concepts of sex and gender have slowly been incorporated into health literature, but the field of addiction medicine is still lacking in this regard. Addiction is grounded in complex biological and sociocultural etiologies, therefore research on both sex and gender is critical to our understanding of addiction. Sex and gender differences in MMT have been previously identified and used to inform clinical practice and current standards of care, however the literature is limited by the scarcity of studies, poor methodological quality, and small samples.

Understanding the issues that men and women experience when it comes to the management and treatment of opioid addiction may uncover specific factors that individually make them more susceptible to opioid-related risks and treatment challenges. A thorough re-evaluation of sex- and gender-related factors for men and women with opioid addiction is needed to strengthen our understanding of addiction and inform the development of appropriate, gender-sensitive prevention and treatment strategies.

1.3 Thesis objectives

The general objective of this thesis is to investigate sex and gender differences in outcomes related to the management and treatment of opioid addiction with a focus on hormonal influences, genetic variation, and sociobehavioral characteristics including substance use behavior, health status, and social functioning.

This thesis is comprised of six individual papers, forming Chapters 2 to 7, which explore the biological and social profiles of men and women receiving treatment for opioid addiction. For the purpose of this thesis, opioid addiction is used interchangeably with alternative definitions, including opioid dependence (DSM-IV) and opioid use disorder (DSM-5).

The first two papers focus on evaluating the state of the literature with respect to sex differences in MMT. The following two published articles investigate the sex-specific effects of opioid use on testosterone levels using both primary research and a systematic review and meta-analysis. The fifth paper explores the genetic contribution to opioid addiction and methadone treatment response, and the final paper provides an extensive description of sex and gender differences in substance use behavior, health status, and social functioning. Collectively, these papers aim to identify specific issues that men and women face in the context of opioid addiction treatment from multiple angles, covering both biological and social perspectives. What do we currently know about how men and women with opioid addiction compare? How are men and women differentially affected by opioid addiction? What sex- and gender-related factors are important indicators of

treatment success? We seek to answer these questions using data from a combination of observational studies and systematic reviews.

In Chapter 2, we created a protocol for a systematic review that assessed the status of literature on sex differences in MMT. The aim of this paper was to develop a rigorous methodological plan that encompasses key components of a systematic review and meta-analysis: search strategy, study screening, data extraction, risk of bias assessment, and statistical analysis. This protocol was published in *Systematic Reviews* (32) and set the groundwork for the full systematic review, which is included in Chapter 3.

The purpose of the systematic review in Chapter 3 was to gain an understanding of what is currently known about sex differences in the context of MMT. We aimed to determine how men and women differ in specific outcomes that are common indicators of treatment success within areas of substance use, health status, and social functioning. Through this, we also evaluated the quality of the available evidence, highlighting areas for future research. This manuscript has been accepted for publication with the *Canadian Medical Association Journal (CMAJ) Open*.

In Chapter 4, we examined the effect of opioid use on hormone levels with a specific focus on testosterone. Considering that sex hormones are known to play a key role in the biological basis of sex differences, we used a case-control study design to analyse testosterone levels in men and women with opioid addiction receiving MMT. We also aimed to identify other methadone-related treatment factors that are associated with testosterone level and examine the variability of testosterone across menstrual cycle

phases in women. This study revealed that hormone imbalances may be an area of concern in the treatment of opioid addiction in the clinical setting. This primary research paper has been published in *Scientific Reports* (33).

Based on what we learned from this study, we developed our next research question for a systematic review and meta-analysis, which is included in Chapter 5. Our objective for this systematic review was to examine the association between opioids and testosterone levels among men and women. Our intention was to build upon current literature and provide quantifiable data on the magnitude of testosterone suppression. Given that much of the literature focused on men, we also sought to determine whether women receiving long-term opioids have low testosterone levels compared to clinical reference ranges. Finally, we were interested in whether testosterone suppression varied by the type of opioid used, specifically methadone versus other opioids. This review was published in *Drug and Alcohol Dependence* (34).

In Chapter 6, we explored the genetic contribution to methadone treatment for opioid addiction with a specific focus on genes that are involved in addictive and reward behaviors; *brain-derived neurotrophic factor* and *dopamine receptor D2*. Although we were unable to perform a separate analysis for men and women due to sample size limitations, we demonstrated that the genetic variation across these particular genes is not linked to methadone treatment response, contrary to what the evidence suggests. This manuscript has been accepted for publication with *Addiction Science & Clinical Practice* and is currently in press.

Chapter 7 includes the final paper of this six-part series that focuses on sex- and gender-specific patterns in areas of substance use behavior, health status, and social functioning. Based on what we learned from our systematic review in Chapter 3, we aimed to highlight changes in the current population of opioid users receiving MMT as well as provide an updated and extensive description of sex and gender differences across multiple domains of functioning. To conclude this chapter, we discussed areas where trends among men and women have changed, with an emphasis on how prevention strategies and treatment interventions can incorporate these sex-and gender-sensitive factors. This manuscript has been submitted to the journal *Biology of Sex Differences.*

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CHAPTER 2

Study 1

Sex differences in outcomes of Methadone Maintenance Treatment (MMT) for opioid addiction: A protocol for a systematic review

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2.1 Abstract

Background: Use of methadone for the treatment of opioid addiction is an effective harm-reduction approach, though variability in treatment outcomes among individuals has been reported. Men and women with opioid addiction have been known to differ in factors such as opioid use patterns and characteristics at treatment entry, however little has been reported about differences in methadone treatment outcomes between men and women. Therefore, we present a protocol for a systematic review which aims to provide a summary of existing literature on sex differences in outcomes of methadone treatment for opioid addiction.

Methods: Electronic search of PubMed/MEDLINE, EMBASE, and PsycINFO databases will be conducted using a priori defined search strategy. Two authors (MB and BD) will independently screen potential articles for eligibility using pre-determined inclusion and exclusion criteria and extract key information using a data extraction form designed for this study. Discrepancies will be resolved using a third party (ZS). The primary outcome will be response to treatment defined as abstinence from illicit opioid use. Secondary outcomes will be assessed on several domains and will include outcomes such as treatment retention/duration, methadone-related adverse events, psychiatric comorbidity, criminal behavior, employment, social relations, and mortality. A meta-analysis will be conducted if possible; risk of bias and overall quality of evidence will be assessed to determine confidence in the estimates.

Discussion: We anticipate that this review will highlight how men and women differ in methadone treatment outcomes and allow us to generate conclusions that can be applied to treatment in a clinical setting.

Systematic Review Registration: PROSPERO CRD42013006549

KEYWORDS Opioid addiction/dependence; methadone maintenance treatment; sex differences; systematic review, protocol

2.2 Background

The use of illicit opioids continues to pose a problem both at the individual and societal levels, even more so with the exponentially increasing rates of prescription opioid use in North America (1-3), increasing the risk of the development of opioid addiction. Infection (4), medical and psychiatric comorbidity (5), polysubstance use (5), and criminal behavior (6) are among a few of the risks associated with opioid addiction, in addition to a rise in opioid-related deaths (2).

Methadone Maintenance Treatment (MMT) is the most widely used harm-reduction approach to treating opioid addiction (7). Methadone is a synthetic analgesic with the ability to inhibit the euphoric effects of opioids and provide relief of withdrawal symptoms due to its longer duration of action (8). MMT began to receive attention shortly after its development in the early 1940s, which led to the opening of methadone clinics across the world, and later in North America (9). Since then, the number of patients entering treatment has grown about fivefold (10). It is estimated that there are >30,000 registered methadone patients in Ontario, Canada alone (11), which represents approximately 25% of Ontario's illicit opioid user population (10). Although progress has been made with MMT, it is evident that it is still not widely used in opioid addiction populations on the larger scale.

Despite the documented effectiveness of methadone as a substitute opioid therapy, methadone has also been reported to produce a large inter-individual variability in response (12), adding an additional layer of complexity to treatment strategies.

Traditionally, the population of individuals suffering from opioid addiction has been primarily men, with most studies at the time focusing on opioid-dependent men (13, 14). In the most recent 30 years, there has been an increase in the number of women with opioid addiction (15), which calls for a re-examination of literature on sex differences in opioid addiction in general and response to MMT specifically.

Sex differences in opioid addiction (10, 16-18) and methadone treatment (16, 19-22) have been reported; significant sex differences in age, ethnicity, marital status, education, and employment (23), as well as patterns of drug use (21), treatment entry (24), and social support (25) have been identified. Women are typically younger, married, unemployed, and have an earlier onset age of heroin use (26). Men often use opioids for recreational purposes (16) and have a slower disease progression than women (24). Additionally, men report earlier treatment entry, more frequent utilization of substance abuse treatment, and fewer psychological and medical problems at treatment admission compared to women (17). It is becoming clear that treatment needs for men and women are not the same, which points to a demand for separate treatment strategies. The available studies on opioid addiction in the literature are often limited to men (27) or specific ethnic groups, focus on clinical profiles prior to or at treatment entry (16, 19-22), or investigate methadone dose as a single outcome of treatment in association with other factors (28-31). Sex differences have also been examined in opioid addiction patients treated with methadone in association with factors including prescription opioid use (32), drug use patterns (20), drug treatment utilization (33), psychiatric comorbidity (5, 34), smoking outcomes (35), and quality of life (36); however, little has been reported about differences

in methadone treatment outcomes between men and women. Few studies have investigated methadone treatment retention, response, remission, adverse events, health status, social relations, criminal activity, and mortality with a specific focus on sex difference, providing inconsistent results and leaving a large gap in the literature with regards to sex differences in response to MMT.

It is also evident that men and women vary in multiple aspects of addiction characteristics and should therefore be provided sex-specific treatment. Implementation of separate treatment approaches for men and women may prove to be a more efficient way to manage this disorder and eventually improve patient-related health outcomes. This review aims to determine whether or not men and women differ in methadone treatment outcomes.

2.3 Objectives

The objective of this review is to summarize the current status of literature regarding sex differences in methadone treatment outcomes by systematically reporting the available research to date. Specifically, we aim to: (1) assess how men and women differ in methadone outcomes related to drug-use behavior, health status, and sociobehavioural functioning; (2) when suitable, combine the statistical outcomes in a summary estimate through meta-analytical approaches; (3) critically appraise the literature and determine areas that require further investigation.

2.4 Methods

2.4.1 Inclusion and exclusion criteria

This systematic review will include completed randomized controlled trials (RCTs) and observational studies of methadone treatment outcomes in men and women. Included studies must have been conducted in the context of methadone treatment for opioid addiction. Studies including patients that are undergoing a substitute opioid therapy (SOT) other than methadone (i.e. buprenorphine/naloxone, naltrexone) or using a substitute opioid for the purpose of detoxification (not maintenance) will be excluded. Studies investigating patient subpopulations such as pregnant women or incarcerated individuals, or patients that are using methadone for the treatment of a condition other than opioid addiction (i.e. chronic pain) will also be excluded. Participants shall include both men and women who present with opioid addiction and are undergoing methadone treatment. No age or ethnicity limitations will be applied. The primary outcome of this review will be the presence of sex differences in methadone treatment response, defined as abstinence from illicit opioid use and measured through self-report and/or urinalysis. Secondary outcomes will be assessed across three life domains: drug-use related behavior, health status, and sociobehavioural functioning. These outcomes include treatment retention/duration, remission status post-treatment, polysubstance abuse, methadone dose, drug-related adverse events, health status, psychological status, mortality, criminal activity, high risk sexual behavior, social support/relations, and

employment. A complete list of how these outcomes are described, defined, and measured in the literature is available in Table 2.1.

2.4.2 Search strategy

We shall identify all studies relevant to this review with no language or time restraints. We will search the PubMed/MEDLINE, EMBASE, and PsycINFO databases for relevant articles. Relevant search terms and their MeSH (medical subject heading) equivalents will be used in varying combinations; refer to Table 2.2 for the complete search strategy. In order to maximize the number of relevant articles retrieved, treatment outcomes will not be included in the search. Articles will be excluded by limiting the searches to humans. We will also manually review reference lists of included studies for studies that may have been missed in the initial search. Grey literature will not be reviewed as we are looking for complete published data only.

2.4.3 Data screening

Two independent raters (MB and BD) will screen all citations and abstracts retrieved using the search strategy and identify all eligible articles. Articles that meet the pre-determined criteria will be included for full-text review. Disagreements at any phase of the review process will be resolved by discussion or in the case where a consensus is not reached, a third independent rater (ZS) will determine eligibility. Ineligible studies will be excluded from the review and reasons for exclusion will be recorded. Inter-rater

agreement will be calculated using the Kappa statistic (37) for each phase of screening. Authors will be contacted directly if further clarification is needed.

2.4.4 Data extraction

The two authors (MB and BD) will independently extract data from the studies using a pilot-tested pre-established data extraction form (see Appendix I). Information obtained will include the author and year of publication, city and country of publication, title of article, journal name, study design, and description of sample population, including total number of men and women study participants, mean age (total and men vs. women), and ethnicity. Primary and secondary outcomes, outcome measures, statistical analyses, results, and conclusions will also be recorded. In the case of missing or incomplete data, authors will be contacted for further details. Data will be combined to produce a summary estimate in a meta-analysis if the extracted data allows it.

2.4.5 Assessment of quality

Two authors (MB and BD) will independently assess the risk of bias of included studies using the Newcastle-Ottawa Scale (NOS) (38) for observational studies and the Cochrane Collaboration's tool (39) for assessing risk of bias in RCTs. For observational studies, two authors (MB and BD) will independently assess the risk of bias of each included study using an adapted version of a modified NOS, specific to the context of this review. This will include seven questions spread across four domains of evaluation; methods for

selecting study participants (i.e. selection bias), methods to control for confounding (i.e. performance bias), statistical methods (i.e. detection bias), and methods for measuring exposure and outcome variables (i.e. information bias). Risk of bias is measured on a scale of 0 (high risk of bias) to 3 (low risk of bias) and a specific description with examples of both high and low bias is provided. Items regarding selection of participants (i.e. representativeness of sample) and ascertainment of outcome (i.e. objective vs. subjective measures) were retained, while other items relating to the comparability of groups and adequate follow-up for cohort and case/control studies were removed as these were not directly applicable to our topic of interest. We also introduced categories that emphasize statistical methods, confounding effects, and reporting of data to ensure that bias in methodology is assessed. These scales will be used to measure the risk of bias on a per study basis or categorized by domain to develop a general conclusion about the sources of bias in the studies included in this review (see Appendix I). Cochrane's tool for assessing risk of bias in RCTs includes 7 domains; random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each of these domains will be evaluated according to high or low risk of bias and will also be assessed on a per study or per domain basis. If a meta-analysis is possible, we will use the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework to rate the quality of evidence through investigation of risk of bias, imprecision (random error), inconsistency, indirectness, and publication bias. We will then summarize the

evidence for individual outcomes in summary of findings tables, which will allow for assessment of our confidence in the estimates.

2.4.6 Statistical analyses and heterogeneity

The results of this systematic review will be reported in a narrative and where possible, a combined statistical manner using meta-analysis. The Kappa statistic will be used to measure level of agreement between independent raters. For dichotomous outcomes, we will compute a pooled odds ratios using the Mantel-Haenszel random effects model, in which the model is able to estimate between study variation through an evaluation of each study's final results and a Mantel-Haenszel fixed effect meta-analysis result.

For the summary estimates, we will employ a random effects model, which assumes variation between studies and their respective effect sizes. The nature of observational studies in this population is highly variable, therefore heterogeneity will be accounted for and will allow us to develop aggregate estimates. We will assess the participants, methods, and results of included studies for heterogeneity, which will allow us to determine whether results can be compared across studies. Possible sources of heterogeneity include age groups, study design, methodology, and definition of outcome. In case of heterogeneity, subgroup analyses according to these different categories will be performed. Included studies will be presented in the form of a forest plot. We will use Review Manager 5.1 software for all statistical analysis and results will be presented using 95% confidence intervals.

2.4.7 Presenting and reporting of results

We will report the systematic review according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (40). A flow diagram will be used to summarize the selection process of studies at each phase and summary tables will be used to report study characteristics and presence of sex differences per methadone outcome. Publication bias will also be examined and assessed using Egger's plot.

2.5 Discussion

Using evidence from this systematic review, we expect to draw conclusions regarding the presence of sex differences in outcomes of MMT for opioid addiction. This review will not only provide us with summary evidence for which we can objectively make inferences about the current status of literature, it will also allow us to critically evaluate the methodological quality and risk of bias present in the available evidence. We aim to acknowledge inconsistencies in the literature and attempt to understand reasons for them. The literature on methadone treatment focuses primarily on men and little is known about women or how the sexes compare. We anticipate that this review will highlight how men and women differ in methadone treatment outcomes and allow us to generate conclusions that can be applied to treatment in a clinical setting. We will encourage healthcare professionals to make use of this information and approach men and women suffering from opioid addiction with different treatment strategies, catered to each sex specifically. We are hopeful that this review will ultimately establish the need for further examination

into sex differences in methadone treatment in an effort to improve treatment prognosis for individuals dealing with this complex disorder**.**

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Author Contributions: All authors contributed to the development of this paper. ZS and MB jointly conceived the paper, BD assisted with development of the methodology. MB wrote the first draft of the protocol and created the data extraction forms, and the remainder of authors (BD, MS, RA, LT, and ZS) reviewed several versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare that they have no competing interests.

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2.8 Figures and tables

Table 2.1 Definition of methadone treatment outcomes for assessment

Outcome	Definition	Measurement of Variable	Statistics	Studies
DRUG USE-RELATED BEHAVIOUR				
Response to treatment	Abstaining from illicit opioid use throughout treatment duration	- Urine screening - Self reported opioid use (daily or weekly) over specified time period	Percentage, mixed model ANOVA, Cochran-Mantel-Haenszel statistic	(41-44)
Treatment retention or duration	Proportion or participants completing treatment; days in treatment from first to last day of therapy	- Number of days patient remains in treatment - Proportion of patients retained in treatment for pre-specified duration of study	Cox proportional hazards model, Kaplan-Meier survival curve,	(41-43)
Remission status post-treatment	Abstinence from use of illicit opioids at follow-up	- Urine screening - Self-reported opioid use (any) after treatment	t-test, 2x2 factorial ANOVA	(42, 44-46)
Polysubstance use	Use of at least two (non-opioid) substances throughout the course of treatment	- Self-reported use of substances daily or weekly or in last 30 days - Net reduction in proportion of drug abuse after specific duration	Percentage, Fischer's Exact Test	(42, 43)
HEALTH AND METHADONE-RELATED OUTCOMES				
Methadone dose	Average daily methadone dose	- Milligrams/day - Mean methadone dose after specific duration in treatment	Difference in means (SD)	(43)
Drug-related adverse events	Reaction to treatment drug	- Interview/physical examination - Number of hospitalizations	Percentage, t-test	(17)
Health status	Change in health status during course of therapy;	- Interview/physical examination - Number of hospitalizations	ANOVA	(17, 45)
Psychological status	Comorbidity of psychiatric disorders	- Self-reported psychiatric problems - Number of reported symptoms - Validated psychiatric assessments	Percentage, relative risk, ANOVA, Chi-square	(17, 47-49)
Mortality	Treatment-related death or illicit drug use mortality	- Mortality causes - Number of deaths - Annual death rate per year of	Standardized mortality ratio (SMR), Kaplan-	(50)

		age	Meier survival curve	
SOCIOBEHAVIORAL FUNCTIONING				
Criminal behavior	Involvement in illegal activities, arrests, or incarcerations throughout treatment or at follow-up	- Interview/self-report - Current legal status	Percentage, t-test, ANOVA	(17, 42, 44)
High-risk sexual behaviour	Involvement in behaviours that put the patient at high risk for HIV and other infections	- Use of injection methods (30 days prior) - Number of sex partners - Incidence of unprotected sex	Weighted least-squares estimation procedure, repeated measures ANOVA	(51, 52)
Social relations/support	Patient's conception of his/her relationship with others	- Self-report - Number of close friends/family - Marital and family status - Ratings of interactions	ANOVA	(17)
Employment	Status of employment and evidence of financial income	- Change in self-reported employment status during treatment - Employment status after treatment	Percentage, difference in means (SD)	(17, 42, 44, 45)

Table 2.2 Search strategy for retrieval of relevant articles from multiple databases

Database	Search Strategy
MEDLINE n=401	1. Opioid-related disorders/dt, rh, th [Drug Therapy, Rehabilitation, Therapy] 2. Opiate substitution treatment/ 3. Methadone/ 4. Sex Characteristics/ 5. sex differences.m_titl. 6. gender differences.m_titl. 7. sex.m_titl. 8. male.m_titl. 9. female.m_titl. 10. men.m_titl. 11. women.m_titl. 12. 1 or 2 or 3 13. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 14. 12 and 13 15. limit 14 to humans
EMBASE n=180	1. Opioid-related disorders/dt, rh, th [Drug Therapy, Rehabilitation, Therapy] 2. Opiate substitution treatment/ 3. Methadone/ 4. Sex Characteristics/ 5. sex differences.m_titl. 6. gender differences.m_titl. 7. sex.m_titl. 8. male.m_titl. 9. female.m_titl. 10. men.m_titl. 11. women.m_titl. 12. 1 or 2 or 3 13. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 14. 12 and 13 15. limit 14 to humans
PsycINFO n=241	1. exp Methadone Maintenance/ or exp Methadone/ 2. exp Human Sex Differences/ 3. sex.m_titl. 4. male.m_titl. 5. female.m_titl. 6. men.m_titl. 7. women.m_titl. 8. 2 or 3 or 4 or 5 or 6 or 7 9. 1 and 8 10. limit 9 to humans

CHAPTER 3

Study 2

Sex differences in outcomes of methadone treatment for opioid use disorder: a systematic review and meta-analysis

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3.1 Abstract

Background: Opioid use disorder is a serious international concern with limited treatment success. Men and women differ in their susceptibility to opioid use disorder and response to methadone treatment and can therefore benefit from sex-specific treatment. We performed a systematic review of the literature on outcomes of methadone maintenance treatment for opioid use disorder in men and women related to drug use, health status and social functioning.

Methods: We searched PubMed, EMBASE, PsycINFO, and CINAHL for observational or randomized controlled studies involving adults 18 years of age or older undergoing methadone treatment for opioid use disorder. Studies were included if they investigated sex differences in methadone treatment outcomes. Two authors independently reviewed and extracted data. Meta-analyses were performed when possible; risk of bias and quality of evidence were also assessed.

Results: Twenty studies with 9732 participants were included, of which 18 were observational and 2 were randomized controlled trials. Men and women differed significantly in alcohol use (odds ratio [OR] 0.52, 95% confidence interval [CI] 0.31 to 0.86), amphetamine use (OR 1.47, 95% CI 1.12 to 1.94), legal involvement (OR 0.63, 95% CI 0.47 to 0.84) and employment during treatment (OR 0.39, 95% CI 0.21 to 0.73). Opioid use patterns were similar among men and women. Risk of bias was moderate, and quality of evidence was generally low.

Interpretation: Sex differences were evident in polysubstance use, legal involvement, and employment status among men and women receiving methadone treatment for opioid use disorders. Although the quality of evidence is low, this review highlights the need for improved implementation of sex-specific treatment strategies.

3.2 Introduction

Canadians have recently surpassed the US citizens to become the highest opioid analgesic consumers in the world (1). In 2012, *CMAJ* published a report showing that about 200,000 Canadians regularly use prescription opioids (2), which are becoming the most commonly used drugs of abuse (3). There has been a surge in opioid prescriptions of 150% over the last decade (4). As a result, the number of hospital admissions and deaths due to opioid use and overdose has grown significantly (5). In addition to the collective healthcare costs, each untreated case of opioid addiction has a social cost of Can$45 000 annually per person (6), a major economic outlay.

Currently, there are about 35 000 patients receiving substitute opioid therapy with methadone at addiction treatment centers in Ontario (7). Several maintenance treatment programs are available, with methadone being the most commonly prescribed treatment for opioid use disorder (8). Effectiveness rates of 20%–70% for methadone maintenance treatment are reported in the literature (9-12). Treatment response in opioid use disorder is difficult to define and has been broadly described in the literature, which makes clinical interpretation of these studies challenging. There are no agreed-on criteria that characterize a treatment as a success or failure; therefore, there is no accurate way to know whether treatment is working or if the healthcare resources invested in treatment are producing any benefit.

There is evidence, however, of high variability in response to methadone treatment between patients (13), which indicates that patients may have different treatment needs.

Men and women especially are known to differ in addiction susceptibility and behaviour including first opioid use, progression to regular use, and treatment entry (14-16). It is also likely that men and women differ in MMT outcomes, although these differences are unclear in the literature. Hence, current treatment standards that offer the same clinical management of opioid use disorder for women and men may not achieve optimum treatment outcomes for both sexes.

3.3 Objectives

We provide a systematic review of the literature on sex differences in methadone treatment outcomes related to drug use behavior, health status, and social functioning using a meta-analysis where possible. We critically evaluated the evidence and highlighted areas for future research. Our aim was to identify possible sex-specific patient needs that can be addressed with an individualized treatment strategy to produce improved treatment outcomes, increased treatment efficacy and decreased risk of adverse events.

3.4 Methods

This review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (23) (completed checklist in Appendix II). The review has been registered with PROSPERO (no. CRD42013006549), and the detailed methods have been previously reported in a protocol (17).

3.4.1 Literature search

We searched PubMed/MEDLINE, EMBASE, PsycINFO, and CINAHL databases from inception to Aug. 11, 2014, for relevant articles. The following search terms and their MeSH subject headings were used: "opioid-related disorders"; "opiate substitution treatment"; "methadone maintenance"; "sex differences/characteristics"; "gender differences"; "sex"; "male"; "female"; "men"; and "women." No language or time constraints were applied; however, the search was limited to people aged 18 years or older. The complete search strategy is found in the published protocol (17). We included completed, published and peer-reviewed studies to ensure that we captured only high-quality evidence; we did not screen the grey literature. Included studies were supported primarily by national funding agencies, and no conflicts of interest were declared.

3.4.2 Study selection

We included observational studies and randomized controlled trials (RCTs) that focused on sex differences among patients receiving methadone treatment for opioid use disorders. In this review, "opioid use disorder" encompasses all classifications of opioid-related disorders set forth by the Diagnostic and Statistical Manual of Mental Disorders that have been used to date (i.e. abuse, dependence, addiction).

We included studies if their primary focus was sex differences in methadone treatment outcomes including treatment response, retention, remission status after treatment,

polysubstance abuse, methadone dosage, drug-related adverse events, health status, psychological status, death, criminal activity, high-risk sexual behavior, social support and employment. Although marital status is not a direct outcome of methadone treatment, it is worthwhile to examine this variable. Having a partner can be indicative of a stable and supportive environment for the patient, which can positively influence the recovery process (18, 19).

We were interested in all types of patients, regardless of ethnicity or geographic location. We excluded studies involving patients receiving an opioid substitution treatment other than methadone or using methadone for the treatment of chronic pain.

3.4.3 Data collection

Two of us (M.B. and A.B.) independently reviewed articles at each screening stage; disagreements were resolved by consensus. We extracted data in duplicate using a pilot-tested data extraction form (17). Studies not meeting our inclusion criteria were excluded and reasons for exclusion were recorded. We obtained information on study characteristics and design, sample population, and methadone treatment outcomes.

3.4.4 Risk of bias assessment

For observational studies, 2 authors (M.B. and A.B.) independently assessed risk of bias using a version of the Newcastle-Ottawa Scale (20) that was modified for this review. We assessed risk of bias using the Cochrane Collaboration's tool for RCTs (21). We applied

the Grading of Recommendations, Assessment, Development, and Evaluation framework (22) to the meta-analyses to evaluate the confidence in the summarized evidence.

3.4.5 Data synthesis

We used a Mantel-Haenszel random effects model for the summary estimate, assuming heterogeneity between studies. We computed a pooled odds ratio (OR) for dichotomous outcomes and standardized mean difference (SMD) for continuous outcomes. Summary measures are presented with corresponding 95% confidence intervals (CIs). We assessed heterogeneity using the I^2 statistic. Publication bias was evaluated using funnel plots. We performed analyses using Review Manager 5.3 (The Cochrane Collaboration).

3.5 Results

3.5.1 Search results and study characteristics

We included 20 studies with 9732 participants in the review (Fig. 3.1) (24-43). The strength of agreement between the 2 independent raters was high for title screens (κ value = 0.823, 95% CI 0.736 to 0.910), abstract screens (κ value = 0.898, 95% CI 0.760 to 1.000) and full-text screens (κ value = 0.834, 95% CI 0.615 to 1.000).

We included 18 cohort studies and 2 RCTs. The studies were conducted in the United States (16), Israel (2), Spain (1), and Sweden (1). The sample size for each study varied from 53 to 2683 participants, and studies included mostly men. The most frequently

reported ethnicities were white, black, and Hispanic. Characteristics of the 20 studies are presented in Table 3.1.

3.5.2 Risk of bias assessment

We evaluated bias (selection, performance, detection, and information) for all of the studies. Generally, risk of bias was moderate to high for the observational studies and low for RCTs (Appendix II).

3.5.3 Sex differences in methadone treatment outcomes

3.5.3.1 Substance use

Eleven studies focused on polysubstance use during treatment (Table 3.1). We performed a separate meta-analysis for each substance reported: alcohol, amphetamines, benzodiazepines, cannabis, and cocaine.

The odds of self-reporting alcohol use while receiving methadone treatment were significantly lower among women than among men (24, 30, 39) (OR 0.52, 95% confidence interval 0.31 to 0.86) (Table 3.2, Fig. 3.2). The odds of amphetamine use while receiving methadone treatment were significantly greater among women than among men (35, 38) (OR 1.47, 95% CI 1.12 to 1.94) (Table 3.2, Fig. 3.3).

No significant differences were seen in the use of opioids or other substances while receiving methadone treatment (see Appendix II for respective forest plots for opioids, benzodiazepine, cannabis and cocaine use), in treatment retention (Appendix II) or in methadone dosage (Appendix II).

3.5.3.2 Health status

As per the protocol (17), we intended to analyze health outcomes, including methadone-related adverse events, health and psychological status; however data on these outcomes were unsuitable for a meta-analysis.

3.5.3.3 Social functioning

Women were less likely to report arrests or legal supervision (including probation or parole) during treatment than were men (24, 30) (OR 0.63, 95% CI 0.47 to 0.84) (Table 3.2; Fig. 3.4). Women were also less likely than men to be employed (24, 25, 28, 30, 39) compared to men (OR 0.39, 95% CI: 0.21 to 0.73) (Table 3.2; Fig. 3.5). Studies that measured high-risk sexual behavior had highly variable outcome definitions, which precluded a meta-analysis. No significant differences were found in marital status (married or common-law) between men and women during methadone treatment (24, 25, 30, 39) (Table 3.2; Appendix II).

3.5.3.4 Long-term prognosis

Six studies assessed outcomes of long-term methadone maintenance treatment. Patients were followed longitudinally or were identified retrospectively, with follow-up periods varying from 1 to 25 years after treatment completion. These studies provided data on several treatment-related outcomes, including illicit opioid use (5 studies), legal involvement (2 studies), employment (2 studies), and death (3 studies). Owing to large differences in follow-up periods, a meta-analysis was not suitable; we therefore provide a brief summary of findings.

Jimenez-Trevino and colleagues (31) found that, 25 years after completion of treatment, the proportion of men using heroin was significantly greater than that of women (32.5% v. 0%; p=0.04). In the remaining 4 studies, no significant sex differences were found in illicit opioid use at 4 weeks (24) or 1 year follow-up (28, 33, 37).

Both Marsh and Simpson (33) and Savage and Simpson (37) found a greater proportion of men than women reporting lifetime arrest or incarceration (>3 days) at follow-up (30% v. 12%; $p<0.05$ and 27% v. 15%; $p<0.05$, respectively). The proportion of men reporting employment (>6 months) during the first year after treatment or at 1 year of follow-up was also significantly greater than that of women (33, 37) (51% v. 31%; $p<0.05$ and 68% v. 41%; $p<0.05$). Risk of death at 1 year did not differ significantly between men and women (27, 43) (Table 3.2; Appendix II). Jimenez-Trevino and colleagues (31) also found no significant difference in mortality between men and women at 25 years.

3.6 Discussion

3.6.1 Main findings

In this review, we have summarized the results from 20 studies on sex differences in methadone treatment outcomes. We found that women were less likely than men to use alcohol, report arrests or legal supervision, and be employed during treatment. However, women were more likely than men to use amphetamines during treatment.

Our findings are however consistent with the trends observed in past individual studies of patients with opioid use disorder and those in methadone treatment (14-16, 24, 44). They are also in line with traditional sex role expectations; (e.g. higher unemployment among women, more alcohol use among men).

3.6.2 Limitations

The current literature on methadone treatment outcomes lacks common definitions or standard measurements for treatment response, and what constitutes good or poor treatment response remains unclear. We included a comprehensive list of outcomes to account for this variation in definitions. As well, the differences in outcome measurements made it impossible to combine the results of all studies, and several studies were not suitable for meta-analyses (29, 31, 33, 34, 36, 37, 41, 42). As a result, individual meta-analyses in this review contained, at most, 5 studies, thus providing limited generalizability.

Sex differences in this review may actually be a representation of the general population and not specific to patients receiving methadone treatment. For instance, the association between men and criminal behavior is seen in the general population (45) and therefore may not be directly attributable to opioid use disorder or methadone treatment. We were also unable to establish any causality in outcomes because the data were cross-sectional. Thus, we cannot draw conclusions regarding possible significant improvements in outcomes between treatment initiation and completion.

The nature of treatment interventions was poorly described and there were differences in treatment practices among RCTs (32, 40) and among studies conducted in private methadone programs (25, 34). However, most studies had comparable methadone treatment practices, and specific outcomes were consistent across studies.

We assessed risk of bias and overall quality of evidence to evaluate our confidence in our findings. Most studies were at a moderate to high risk for bias, in several cases owing to small or unrepresentative samples, failure to adjust for confounders and lack of objective outcome assessment. The overall quality of evidence was low to moderate, most likely because of differences in outcome measurement between studies, allowing for high variation and heterogeneity.

3.6.3 Implications for practice and research

Using an extensive list of outcomes, this review has shown how men and women differ in their response to methadone treatment. These findings may be useful to inform the design

of existing sex-specific treatment programs or the development of comprehensive sex-specific patient-centred treatment models in areas where they have not yet been introduced. Programs are encouraged to incorporate medical care, other substance-use treatment programs (e.g. alcohol or amphetamine abuse), counseling, mental health services, and employment needs into their treatment plans, since these services are known to be associated with improved treatment outcomes (46).

The current program standards and clinical guidelines for methadone maintenance treatment issued by the College of Physicians and Surgeons of Ontario (47) were developed from individual study findings and are therefore not informed through systematic summaries of the evidence. The findings of our systematic review may be useful in informing development and updates of best-practice guidelines. However, more methodologically sound studies are required to provide a better understanding and characterization of sex differences in the use of methadone treatment in opioid use disorders, for health care providers, health policy-makers and patients.

With additional high-quality studies, a more conclusive record of sex differences in methadone treatment could be developed. Subsequent studies could assess the efficacy of sex-specific treatment and determine which approaches lead to positive outcomes. Moreover, an investigation into potential explanations for sex differences – whether they are biologically or socially driven – is needed so that these factors can also be incorporated into treatment. It would also be of interest to assess knowledge of sex differences within methadone clinics, and ways in which awareness of these differences could be heightened. Furthermore, we do not know which outcomes are important to

patients themselves. Lastly, the cost-effectiveness of integrating specialized treatment for men and women should be assessed, because implementation of these strategies would likely reduce overall costs, treatment-related and otherwise.

3.6.4 Conclusions

Sex differences were evident in polysubstance use, legal involvement and employment status among men and women receiving methadone treatment for opioid use disorders. More studies are needed to better elucidate the presence of sex differences in methadone treatment outcomes. Studies need to be replicated in larger samples, using standardized assessments and measures, and with appropriate statistical testing to improve our understanding of sex differences and draw appropriate conclusions that can be applied in the clinical setting. With further research, it is our hope that these findings can be helpful in improving treatment for patients with opioid use disorders and the overall field of research in opioid addiction.

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Contributors: Monica Bawor and Zainab Samaan developed the research question and interpreted the data. Monica Bawor and Anuja Bhalerao screened articles and extracted data for the review. Monica Bawor and Brittany Dennis performed statistical analyses. Carolyn Plater, Andrew Worster, Michael Varenbut, Jeff Daiter, David Marsh, Dipika Desai and Guillaume Pare were jointly responsible for clinical interpretation and organization of the results. Rebecca Anglin and Meir Steiner were involved in data interpretation. Lehana Thabane assisted with statistical analysis and data interpretation. Monica Bawor, Brittany Dennis and Zainab Samaan drafted the manuscript, and all of the authors critically revised it. All of the authors approved the final version to be published and agreed to be guarantors of the work.

Competing interests: None declared.

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3.9 Figures and tables

Figure 3.1 Flow diagram for included studies

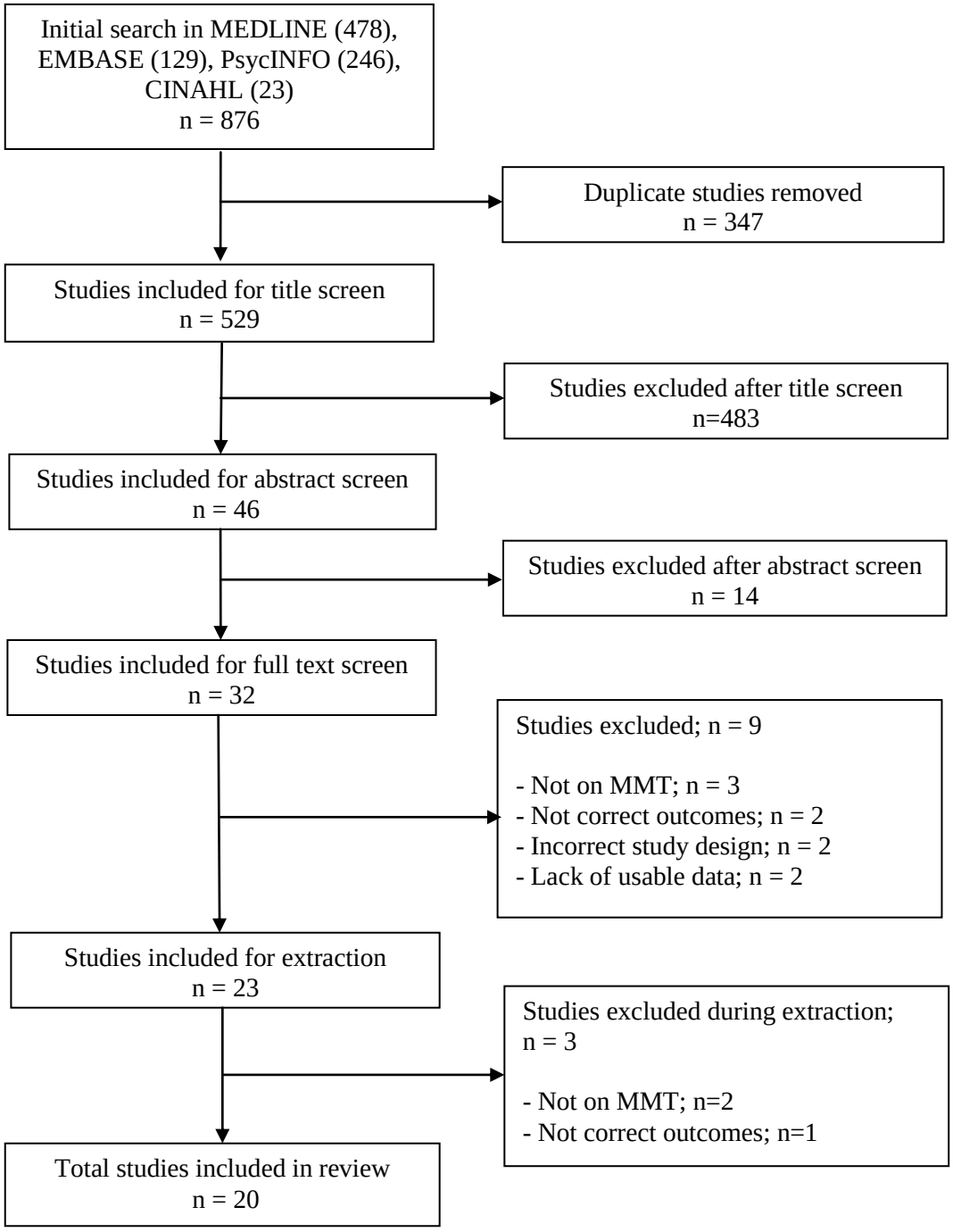


Table 3.1 Study characteristics

Author (Year)	**Study location**	**Study design**	**Total sample size; n**	**Sample size; n (%)**	**Age; mean [SD]**	**Ethnicity (%)**	**Outcomes Measured**
Anglin (1987)[24]	Los Angeles, USA	Cohort	546	M: 282 (51.7) W: 264 (48.4)	M: 33.6 W: 30.4	Anglos (77.7%) Chicanos (22.3%)	• Illicit opioid use • Treatment retention • Polysubstance use • Legal involvement • Marital status • Employment • Long-term prognosis
Brown (1993)[25]	Brooklyn, USA	Cohort	468	M: 291 (62.2) W: 177 (37.8)	M: 37.7 W: 35.8	Black (55.6%) Hispanic (44.4%)	• Illicit opioid use • Polysubstance use • Health status • Psychological status • Legal involvement • Marital status • Employment
Camacho (1996)[26]	Fort Worth, USA	Cohort	326	M: 223 (68.0) W: 103 (32.0)	M: 38.0 W: 34.0	Black (16%) Mexican American (45%) White (36%) Other (4%)	• Methadone dose • Sexual risk behavior
Chatham (1999)[27]	Fort Worth, USA	Cohort	405	M: 279 (64.1) W: 126 (31.1)	M: 37.6 W: 34.4	Mexican American (43%) Caucasian (36%) African American (16%)	• Illicit opioid use • Treatment retention • Polysubstance use • Health status • Psychological status • Legal involvement

							• Sexual risk behavior • Marital status • Employment
Grella (2012)[28]	Los Angeles, USA	Cohort	343	M: 191 (55.7) W: 152 (44.3)	M: 58.3 (4.9) W: 55.0 (4.1)	White (71.1%) Hispanic (26.8%) Other (2.0%)	• Health status • Psychological status • Employment • Long-term prognosis
Haug (2005)[29]	San Francisco, USA	Secondary data analysis	78	M: 42 (53.9) W: 36 (46.2)	M: 42.9 (7.95) W: 45.5 (7.62)	Caucasian (35%) African American (32%) Latino (12%) Other (12%)	• Illicit opioid use • Polysubstance use • Health status • Psychological status
Hser (1990)[30]	Los Angeles, USA	Cohort	720	M: 392 (54.4) W: 328 (45.6)	M: 33.4 W: 30.2	Anglo (74.2%) Chicano (25.8%)	• Illicit opioid use • Treatment retention • Polysubstance use • Legal involvement • Marital status • Employment
Jimenez-Trevino (2011)[31]	Oviedo, Spain	Cohort	53	M: 41 (77.4) W: 12 (22.6)	M: 51.2 (10.1) W: 49.8 (3.8)	NR	• Long-term prognosis
Jones (2005)[32]	Baltimore, USA	RCT	55	M: 36 (65.5) W: 19 (34.5)	M: 37.3 (1.2) W: 35.0 (1.5)	White (46%) Non-white (54%)	• Illicit opioid use • Treatment retention
Marsh (1986)[33]	Fort Worth, USA	Cohort	175	M: 91 (52.0) W: 84 (48.0)	M: 26.8 W: 24.6	Black (52%) White (48%)	• Long-term prognosis

Mulvaney (1999)[34]	Philadelphia, USA	Cohort	548	M: 343 (63.0) W: 205 (37.0)	NR	Black (58%) Hispanics (42%)	• Illicit opioid use • Polysubstance use • Health status • Psychological status • Legal involvement • Marital status • Employment
Peles (2006)[35]	Tel-Aviv, Israel	Cohort	470	M: 339 (72.1) W: 131 (27.9)	M: 37.3 (8.3) W: 34.5 (7.5)	Mainly Isreali	• Illicit opioid use • Treatment retention • Polysubstance use • Methadone dose
Rutherford (1997)[36]	Philadelphia, USA	Cohort	72	M: 44 (61.1) W: 28 (38.9)	M: 39.7 W: 35.2	White (51.4%) Black (45.8%)	• Employment
Savage (1980)[37]	Forth Worth, USA	Cohort	1483	M: 1151 (77.6) W: 332 (22.4)	M: 27.4 W: 25.9	Black (46.2) White (31.6%) Puerto Rican (9.8%) Mexican American (12.4%)	• Long-term prognosis
Schiff (2007)[38]	Jerusalem, Israel	Secondary data analysis	2683	M: 2352 (87.7) W: 331 (12.3)	NR	Mainly Israeli	• Illicit opioid use • Treatment retention • Polysubstance use
Schilling (1991)[39]	New York City, USA	Cohort	244	M: 135 (55.0) W: 109 (45.0)	M: 38.9 (8.8) W: 34.5 (5.8)	White (22%) Black (54%) Hispanic (23%) Other 1%	• Illicit opioid use • Treatment retention • Polysubstance use • Sexual risk behavior • Marital status • Employment

Schottenfeld (1998)[40]	West Haven, USA	RCT	58	M: 39 (67.2) W: 19 (32.8)	M: 33 W: 33.4	White (75.9%) Other (24.1%)	• Illicit opioid use • Treatment retention • Polysubstance use
Steer (1980)[41]	Philadelphia, USA	Cohort	150	M: 107 (71.3) W: 43 (28.7)	NR	Black (70%) White (30%)	• Psychological status
Stenbacka (2003)[42]	Stockholm, Sweden	Cohort	331	M: 233 (70.4) W: 98 (29.6)	NR	Swedish	• Legal involvement
Webber (1999)[43]	Bronx, USA	Cohort	524	M: 302 (58.0) W: 222 (42.0)	Median (Min-Max) M: 37.1 (21.6-66.0) W: 34.7 (19.9-66.1)	Hispanic (63%) Black (23%) White (14%)	• Illicit opioid use

M = men; W = women; NR = Not reported; SD = standard deviation

Table 3.2 Sex differences among included studies: Summary of meta-analysis results

Outcome	No. of studies	Subjects; n		Pooled OR or SMD (95% CI)	I^2 %	Summary of sex differences	GRADE quality of evidence
		M	W				
Illicit opioid use							
Cohort studies[24,30,43]	3	976	814	0.81 (0.50, 1.31) p=0.39	82 p=0.003	--	very low[a,b]
RCTs[32,40a,40b]	3*	75	38	1.39 (0.61, 3.19) p=0.44	0 P=0.72	--	moderate[c]
Treatment retention[27,30,35]	3	1010	585	1.01 (0.62, 1.63) p=0.97	77 p=0.01	--	low
Polysubstance use							
Alcohol use[24,30,39]	3	809	701	0.52 (0.31, 0.86) p=0.01	77 p=0.01	Women less likely to use alcohol	moderate[a,d]
Amphetamine use[35,38]	2	2691	462	1.47 (1.12, 1.94) p=0.006	0 p=0.96	Women more likely to use amphetamines	low
Benzodiazepine use[35,38]	2	2691	462	0.94 (0.70, 1.27) P=0.70	44 P=0.18	--	low
Cannabis use[35,38]	2	2691	462	0.85 (0.67, 1.08) p=0.18	0 p=0.67	--	low
Cocaine use[35,38,39]	3	2826	571	1.07 (0.64, 1.78) p=0.80	76 p=0.01	--	very low[a-d]
Methadone dose (maintenance [26,35]	2	562	234	-2.38 (-5.67, 0.91) p=0.16	0 p=0.82	--	low
Legal involvement[24,30]	2	674	592	0.63 (0.47, 0.84) p=0.002	39 p=0.20	Women less likely to report arrests or legal supervision	moderate[a,b,d]
Employment[24,25,28,30,39]	5	1291	1030	0.39 (0.21, 0.73) p=0.003	91 p<0.0001	Women less likely to be employed	moderate[a,c-e]
Marital status[24,25,30,39]	4	1100	878	0.96 (0.75, 1.21) p=0.71	0 P=0.53	--	low

Mortality[27,43]	2	581	353	1.61 (0.60, 4.33) p=0.35	83 p=0.02	--	low

M = men; W = women; OR = odds ratio; SMD = standardized mean difference; CI = confidence interval; RCT = randomized controlled trial

* This meta-analysis included two studies, but one study (Schottenfeld et al. 1998) had two methadone intervention arms (65mg and 20mg), which were both included separately

[a] Differences in outcome definition and measurement among studies

[b] Studies did not adjust for relevant treatment-related confounders (i.e. methadone dose, opioid use, other medications, etc.)

[c] Small sample sizes and wide confidence intervals across studies

[d] Significant association at $p<0.01$

[e] Inadequate statistical measures and some missing data

Figure 3.2 Alcohol use over the past six months of treatment

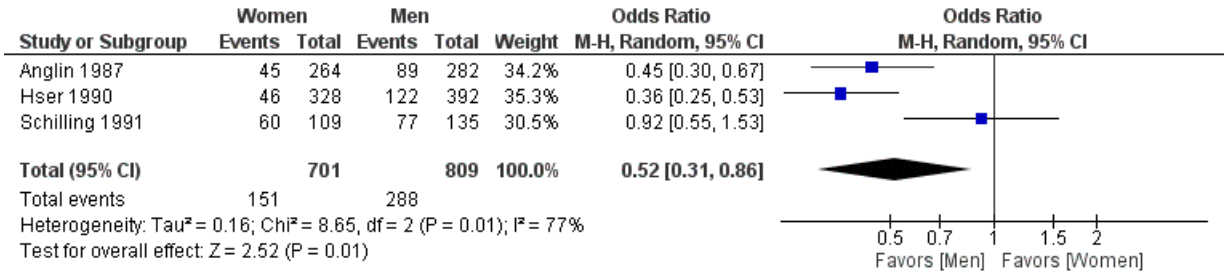


Figure 3.3 Amphetamine use over the last six months of treatment

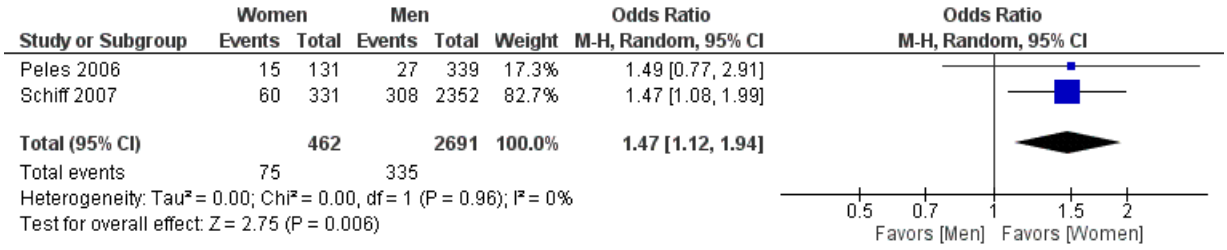


Figure 3.4 Self-reported legal status during treatment

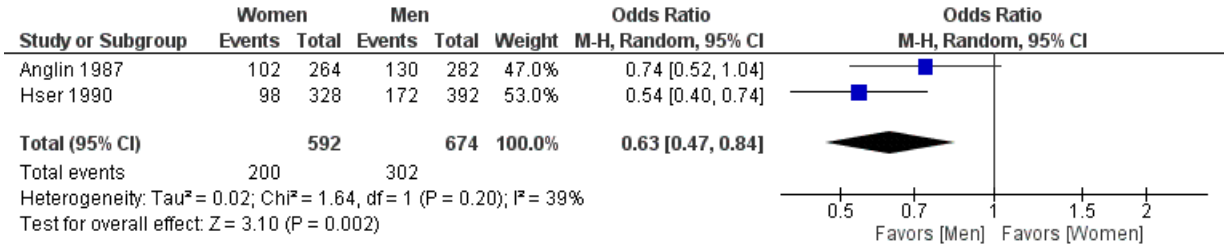
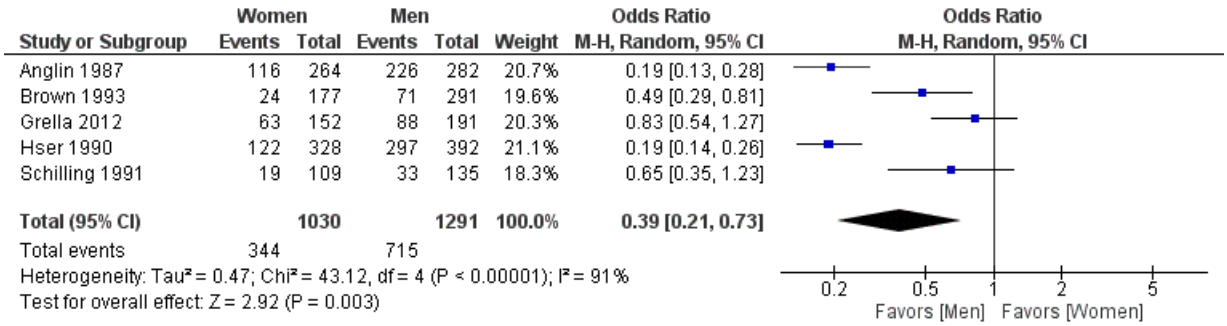


Figure 3.5 Self-reported employment during treatment



CHAPTER 4

Study 3

Methadone induces testosterone suppression in patients with opioid addiction

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Subject Areas

Substance use, opioid dependence, opioids, methadone treatment, sex differences, sex hormones, testosterone, androgen deficiency

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4.1 Abstract

Sex hormones may have a role in the pathophysiology of substance use disorders, as demonstrated by the association between testosterone and addictive behaviour in opioid dependence. Although opioid use has been found to suppress testosterone levels in men and women, the extent of this effect and how it relates to methadone treatment for opioid dependence is unclear. The present multi-centre cross-sectional study consecutively recruited 231 patients with opioid dependence from methadone clinics across Ontario between June and December of 2011. Demographic details, substance use and psychiatric history, and biological samples were obtained. The control group included 783 non-opioid using adults recruited from a primary care setting in Ontario, Canada. Average testosterone level in men receiving methadone treatment was significantly lower than controls. No effect of opioids on testosterone level in women was found and testosterone did not fluctuate significantly between menstrual cycle phases. In methadone patients, testosterone level was significantly associated with methadone dose in men only. We recommend that testosterone levels be checked in men prior and during opioid therapy, so testosterone deficiency caused by opioids may be treated accordingly and lead to successful methadone treatment outcomes.

4.2 Introduction

Opioid dependence has traditionally been observed in men (1, 2), however the increased prevalence of prescription opioid drug abuse has led to an increase in opioid use and dependence in women (3). This trend has sparked interest in the sex-related aspects of the disorder. To date, sex differences have been reported in many aspects of opioid dependence and treatment (4-10), leading to the need for an individual addiction profile for men and women. Sex hormones are often studied as the biological basis for sex differences due to their role in central nervous system regulation, implicating the endocrine system in the pathophysiology of substance use disorders and addictive behaviour (11).

The emerging research on sex hormones in addiction has shed light on the association between testosterone and specific addictive behaviours in men and women, including impulsivity, aggression, risk-taking, and sensation-seeking (12, 13). This provides evidence for the importance of testosterone in substance use disorders including opioid dependence.

Reviews of the literature on testosterone in chronic opioid use report an opioid-induced deficiency in androgen function (14-16). Opioids exert inhibitory effects on the hypothalamus, the area responsible for production of gonadotropin-releasing-hormone (GnRH). GnRH normally acts on the pituitary gland to stimulate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH); when GnRH is inhibited, this leads to low LH and FSH causing suppression of sex hormone secretion from the gonads

(17). Although these findings are supported in samples of men, levels of sex hormones including testosterone in opioid-dependent women have not been extensively studied to date (18). Due to the increase of chronic opioid use in women, a re-examination of the literature is necessary.

4.3 Objectives

The purpose of this study is to examine serum total testosterone level in men and women with opioid dependence receiving methadone treatment. We aim to (1) determine what effect opioids have on testosterone in this sample and if this effect is present in both men and women; (2) identify other methadone-related treatment factors that are associated with testosterone level; and (3) examine the variability of testosterone level across menstrual cycle phases in women.

4.4 Methods

4.4.1 Study design

Cross-sectional data were collected from the Genetics of Opioid Addiction (GENOA) research program, a collaboration between Ontario Addiction Treatment Centres (OATC) and the Population Genomics Program (PGP), Chanchlani Research Centre at McMaster University. Data were collected for the GENOA study from four OATC outpatient methadone clinics specializing in opiate agonist therapy across Southern Ontario, Canada

between June and December of 2011. Recruitment consisted of a structured interview conducted on site by a trained OATC clinical staff member and completion of study-specific case report forms. Demographics, anthropometric measurements, history of past substance use, psychiatric diagnosis, and medical conditions were obtained, in addition to urine and blood samples. This study was carried out in accordance with guidelines and approval by the Hamilton Integrated Research Ethics Board (HIREB) and written informed consent was obtained from each study participant.

4.4.2 Study participants

Men and women aged 18 and older were recruited consecutively from OATC clinics. Inclusion criteria consisted of current enrolment in MMT for opioid dependence and having the ability to provide written informed consent. Exclusion criteria were the use of opioid substitution therapy other than methadone for opioid dependence, inability to communicate in English, and refusal to provide biological samples. Patients received supervised daily methadone doses, addiction counseling (including methods for coping with stress, reacting to environmental stressors, developing constructive social networks, etc.), and regular medical follow up as per usual clinical care.

The control group was a sample of adults aged 18-74 who were screened for DSM-IV dysthymic disorder in a primary care university-affiliated Health Services Organization (HSO) located in Southern Ontario, Canada. This population was an English-speaking,

middle class, suburban family community, which consisted of individuals without opioid dependence (37-39).

4.4.3 Outcome measures

The primary outcome is serum total testosterone level. Covariates include continued opioid use (use of illicit opioids detected by bi-weekly urine screens, measured as the percentage of positive opioid urine screens per total number of urine screens available), methadone treatment duration (length of time in months between the methadone start date and date of most recent methadone dose reported by the patient or obtained from clinic records), methadone dose (current daily dose of methadone at time of interview), polysubstance use (use of a minimum of two substances of abuse in addition to opioids, which include stimulants, hallucinogens, inhalants, cannabis, barbiturates, benzodiazepines, performance-enhancing drugs, or diet pills within the last 12 months; these data were acquired through self-report using the Mini International Neuropsychiatric Interview (M.I.N.I.) Version 6: Drug and Alcohol Modules) (40), and smoking (self-reported average number of cigarettes smoked daily). Age of initial opioid use (self-reported age at which subject began using opioids regularly) was accounted for in the analysis.

4.4.4 Laboratory analysis

Serum total testosterone level in the MMT sample was measured using enzyme-linked immunosorbent assay (ELISA) technique (Enzo Life Sciences, Plymouth Meeting, PA, USA); intra-assay variation is 3.3%, while inter-assay variation is 9.8%, with sensitivity of 2.6%. Serum testosterone in the control sample was measured with the Coat-A-Count total testosterone solid phase radioimmunoassay (RIA) kit (Diagnostic Products Corp., Los Angeles, CA, USA); intra-assay variation is 7.2%, inter-assay variation is 9.4%, and sensitivity is 3.6%. Different assays were used for testosterone measurement between MMT and control groups because the hormone assay method used in the past was RIA, which was used for our control group, whereas ELISA is currently the preferred method of hormone analysis. Standard curves of both assays showed a comparable detectable range and appropriate sensitivities, therefore the methods are unlikely to lead to discrepancies in testosterone levels between samples. Qualitative and seminquantitative urine analysis for opioids was conducted using iMDx^{TM} Prep Assay [NOVX Systems Inc, Richmond Hill, Ontario, Canada] and performed weekly or bi-weekly throughout the study period as part of routine clinical care.

4.4.5 Statistical analysis

All continuous variables are presented as a mean and standard deviation and dichotomous variables are presented as a proportion of the sample population. Data for testosterone level showed a skewed distribution on a normal probability plot in the MMT and control groups; these distributions were transformed with the natural logarithm before inclusion in the multivariable regression analysis. Extreme outliers were removed based on the

maximum and minimum detectable limit of the hormone assays in the laboratory. Multiple imputation methods were used for missing data.

Multivariable linear regression analyses were conducted to determine differences in mean log-transformed testosterone levels between men and women MMT subjects and controls (n=1014) and to determine which methadone-related factors are associated with testosterone level (n=231), with the following covariates included in the model: age, sex, age of initial opioid use, number of cigarettes smoked per day, methadone dose, duration on methadone treatment, polysubstance use, and continued illicit opioid use (based on urine test results). Sub-group analysis by sex was decided *a priori*. A linear regression was also performed to test if there was a significant difference in serum total testosterone level across follicular and luteal phases of the menstrual cycle and the post-menopausal phase in control women (n=419) while accounting for age and smoking status. Patients on hormonal medication including birth control, hormone replacement therapy, and thyroid medications were removed from the sample.

The study is reported in adherence to the Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement for observational studies (41). The results are reported as an estimate of the association expressed as a mean difference or model coefficient, corresponding 95% confidence interval and associated p-value. All statistical analyses were performed using STATA Version 11.

4.5 Results

4.5.1 Sample characteristics

Of the initial 260 subjects undergoing methadone treatment that were recruited, 29 subjects were excluded from the study (duplicate entries = 5, buprenorphine treatment = 3, undetectable or out-of-range testosterone levels = 15, hormone replacement therapy = 2, using prescription opioids for chronic pain = 4). Therefore, 231 subjects in total were included in the analysis (Fig. 4.1). The sample consisted of 56.7% men with mean age 38.3 (SD 11.0) and 43.3% women with mean age 35.2 (SD 9.4). The majority of the sample population (84.4%) was of European ethnicity. Refer to Table 4.1 for additional information on demographics, substance use history, and treatment outcomes.

4.5.2 Effect of opioid use and methadone treatment on testosterone

Control participants included 287 (36.7%) men and 496 (63.3%) women who were not using opioids. Serum testosterone levels were compared between Methadone Maintenance Treatment (MMT) patients and control subjects for men and women separately using linear regression. Men with opioid dependence undergoing methadone treatment had significantly suppressed testosterone levels (mean = 100.10 ng/dL, standard deviation (SD) 72.21) compared to controls (mean = 414.74 ng/dL, SD 141.81 ng/dL) (estimated β= -1.661; 95% confidence interval (CI) -1.793, -1.529; p<0.0001). Testosterone levels for women on MMT did not differ significantly compared to controls

(mean = 36.61 ng/dL, SD 23.19 ng/dL and mean = 25.93 ng/dL, SD 15.20 ng/dL, respectively) (estimated β= 0.063; 95% CI -0.098, 0.224; p=0.441). Table 4.2 presents a statistical summary of testosterone level in both samples by sex.

4.5.3 Factors associated with testosterone level in methadone treatment

A linear regression analysis was employed with log-transformed mean testosterone as the dependent variable to determine factors associated with testosterone levels. Sex was positively associated with testosterone in this model as expected, with men having a higher testosterone level than women (estimated β=1.034; 95% CI 0.857, 1.211; p<0.0001). Testosterone level was found to be inversely associated with methadone dose (estimated β= -0.002; 95% CI -0.003, -0.000; p=0.018) (Table 4.3), indicating that a higher methadone dose is correlated with lowered testosterone levels. In the subgroup analysis by sex, testosterone level was inversely associated with methadone dose (estimated β= -0.003; 95% CI -0.005, -0.001; p=0.003) (Fig. 4.2) and positively associated with the number of cigarettes smoked per day (estimated β= 0.011; 95% CI 0.000, 0.021; p=0.046) in men. This suggests that being a heavier smoker, as measured by the number of cigarettes smoked daily, is correlated with higher testosterone levels. Although no significant correlations were found in the sample of women, polysubstance use showed a positive trend of association with testosterone level (estimated β= 0.244; 95% CI -0.004, 0.493; p=0.054), suggesting that women who use multiple substances in addition to opioids are likely to have higher testosterone levels (Table 4.3).

4.5.4 Testosterone variability across menstrual cycles phases in women

We employed a linear regression to determine whether serum total testosterone level differs between menstrual cycle phases (follicular and luteal) and the menopause phase in our control sample of women (n=419). Results demonstrated no difference in testosterone level between all three phases (estimated β= -0.992; 95% CI -21.263, 19.279; p=0.923) when controlling for age and smoking status (Fig. 4.3). This suggests that testosterone does not fluctuate significantly across phases of the menstrual cycle or during menopause.

4.6 Discussion

The objectives of this study were to examine the overall effect of opioids on serum testosterone level in men and women, determine what factors are associated with testosterone level in this sample, and examine the variability in testosterone level across menstrual cycle phases in women.

Our results have confirmed the suppressive effect of opioids on testosterone in men undergoing methadone treatment, however they also demonstrate that methadone does not suppress testosterone levels in women. There is limited information on testosterone levels in women with opioid dependence who are currently undergoing methadone treatment and this study has aimed to add to the scant literature.

This sex-specific difference in opioid effects on testosterone is indicative of a distinct biological mechanism between men and women. Opioids exert their effects on the gonads

and suppress the release of sex hormones (19). In women, β-estradiol is the primary sex hormone and opioids may act to primarily suppress β-estradiol and target testosterone as a secondary androgen. This is supported by studies looking at the role of opioids in estrogen release. Findings from these studies demonstrate that estradiol was suppressed in opioid-dependent women (20) and after methadone consumption (18). Studies also report the effect of opioids on prolactin release (21, 22). Prolactin is a hormone responsible primarily for milk production during pregnancy, however it also has a role in sexual behaviours. Prolactin may act to mediate this relationship by inhibiting GnRH secretion, causing a decrease of estrogen in women and testosterone in men (23).

The second objective of this study was to investigate the association between testosterone and methadone-related factors. In MMT patients, we found that methadone dose was inversely associated with testosterone level, indicating that the relationship between opioids and testosterone is dose-dependent. Our sub-group analysis by sex showed that this dose-dependent association was present in men only. This is consistent with previous studies in the literature (12, 24, 25), however these studies did not control for other relevant factors such as duration on treatment and continued illicit opioid use. In order to estimate the magnitude of this effect, we used the exponentiated beta coefficient to reverse the logarithmic transformation and multiplied this by 10 so that methadone dose can be quantified in 10mg increments. We found that for each 10mg increase in methadone dose, there is a 0.97 ng/dL decrease in testosterone level (estimated $\exp(\beta)=0.969$; 95% CI 0.950, 0.989; p=0.003), suggesting that men with a higher methadone dose will be more likely to have more suppressed testosterone. In addition, we

observed a positive association between the number of cigarettes smoked daily and serum testosterone level in men. Using the same manipulation, we estimate that for each additional cigarette smoked per day, there is a 1.01 ng/dL increase in testosterone level in men (estimated exp(β)=1.011; 95% CI 1.000, 1.021; p=0.046). This may be explained by the effect of smoking on methadone metabolism, where smoking is an enzyme inducer in the liver accelerating the metabolism of methadone and hence reduces methadone blood level and its inhibition of testosterone (26). This association may also be related to addictive behaviour, where smoking as an addictive behaviour is associated with the risk-taking behavioural profile of testosterone (27). We speculate that this may also be a reason for why men methadone patients have a difficult time with smoking cessation.

Low testosterone in men has been associated with poor quality of life, as well as erectile dysfunction, hypogonadism, symptoms of fatigue, weakness, and mood disturbances (14, 28). Improvement in self-reported quality of life assessed across multiple dimensions has been shown to improve treatment outcomes such as retention and overall health in methadone patients after one year of stabilization on MMT (29). By treating testosterone deficiency, it is suspected that patients will experience improvements in quality of life and therefore demonstrate successful treatment outcomes. Healthcare providers should be aware of the effect of opioids on testosterone and that these symptoms can be actively managed by testosterone therapy. Health care providers should also ensure that patients are being prescribed the lowest possible dose for effective substitution opioid treatment to minimize testosterone suppression.

These findings are applicable to the larger population of methadone patients attending clinics across Ontario and across North America as well. Our patients were recruited from multiple sites of varying geographic locations, all of which follow a standardized treatment regimen, therefore making our sample representative of the overall methadone patient population.

Our final objective was to examine the variability of testosterone levels across menstrual cycle phases in women. In pre-menopausal women, testosterone level does not vary between follicular and luteal phases, and it also does not differ significantly among post-menopausal women. A few studies have found that total testosterone differed between phases, with it being the highest in the luteal (30, 31) or mid-cycle phase (32, 33). However, studies also report no significant differences between cycle phases (34-36). The variability in these findings may be explained by different methods of measuring testosterone (i.e. free, bound, or total; plasma vs. serum; diluted vs. non-diluted, etc.), by the use of different tests (i.e. hormone assays, mass spectrometry), or by differences in defining cycle phases (follicular, mid-cycle, or luteal). Our study has tested this effect in the largest sample of women to date and confirms that testosterone is not sensitive to menstrual cycle changes. Measurement of serum total testosterone level in future investigations does not need to account for menstrual cycle phase, as testosterone levels at any given time are generally representative of the average testosterone level in women.

4.6.1 Limitations

One of the main limitations of the study is the small sample size when the analysis is divided by sex. There is adequate power to support the associations found in men (86%) however the power for the women sample is much lower (43%), which is not enough to detect any significant associations. Although our sample size for women is one of the largest among studies investigating testosterone in methadone treatment among women, it remains inadequate to draw any significant conclusions on the impact of opioids on testosterone in women, although it may provide some information regarding the magnitude and direction of effect. In addition, the cross-sectional design of this study bears its limitations as observational research as no causal interpretations can be established. Also, some data included in this study were based on self-report and therefore may not be entirely accurate or reliable.

Investigation into additional sex hormones may provide insight into the biological basis of methadone treatment and potentially decipher the effect that opioids have on women. Larger sample sizes with more sex hormones investigated would be ideal for future study, as well as implementing a prospective follow-up study design to observe whether testosterone levels change throughout the course of treatment with methadone. Future directions may include studying the association between testosterone and risk of relapse, as well as observing testosterone treatment response and retention after treating low testosterone to determine whether these outcomes have improved.

4.6.2 Conclusions

In this study, we provided an investigation of the influence of opioid dependence and methadone treatment on testosterone. We demonstrated that methadone has a dose-dependent suppressive effect on testosterone in men and that testosterone is not sensitive to menstrual cycle changes in women. The results of this study can be used to guide the decision-making process for men and encourage them to seek treatment for opioid dependence. We also recommend that testosterone levels be checked in men prior to undergoing any opioid therapy and at regular intervals thereafter, in order to treat testosterone deficiency caused by opioids.

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M.B. and Z.S. were responsible for the development of the research question, interpretation of data, manuscript writing, and critical revision of the manuscript. M.B. and B.D. performed statistical analyses, and B.D. also contributed to manuscript writing and critical revision. M.C.S. contributed to data interpretation and organization, and critical revision of the manuscript. C.P., A.W., M.V., J.D., D.M., D.D, and G.P. were all jointly responsible for the process of data collection and communication with OATC clinics, as well as clinical interpretation of results and critical revision of the manuscript. R.A. was involved in interpretation of data and critical revision of manuscript. M.S. was responsible for data collection of the control sample and critical revision of the manuscript. M.C. performed all laboratory analyses for testosterone and assisted with

interpretation of data and critical revision of manuscript. L.T. assisted with statistical analysis, interpretation of data, and revision of manuscript. All authors have reviewed and approved the final manuscript.

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4.9 Figures and tables

Figure 4.1 Flow diagram for participants included in study

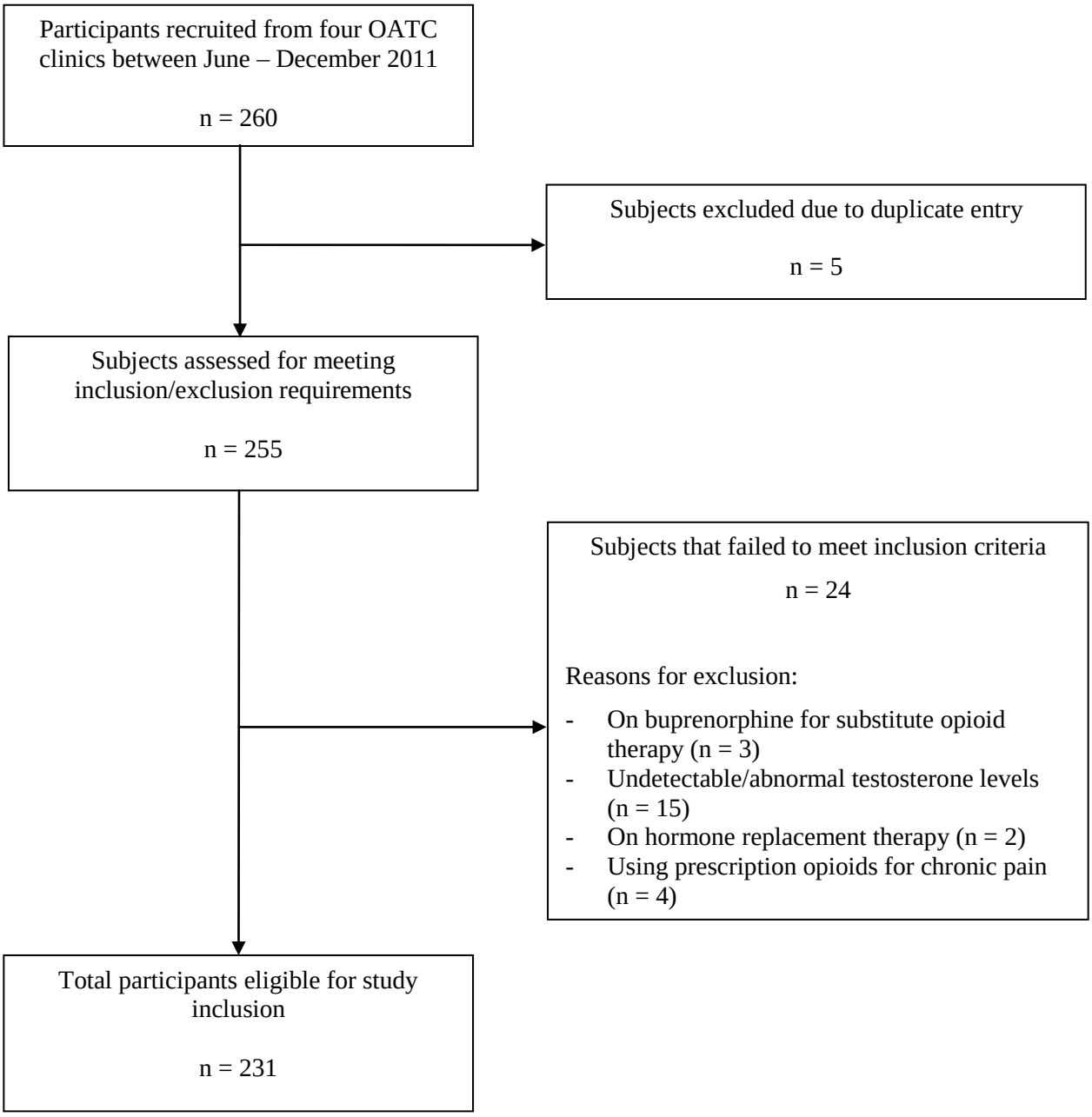


Table 4.1 Demographic characteristics of patients on methadone treatment for opioid addiction

Characteristic	Total (n=231)	Men (n=131)	Women (n=100)
Age in years; mean (SD[a])	36.9 (10.4)	38.3 (11.0)	35.2 (9.4)
BMI[b]; mean (SD)	26.7 (6.4)	26.9 (5.0)	26.6 (8.0)
Married/common law; n (%)	91 (39.4)	53 (40.5)	38 (38.0)
Employed; n (%)	70 (30.3)	45 (34.4)	25 (25.0)
Completed post-secondary education; n (%)	78 (33.8)	33 (25.2)	45 (45.0)
Age of initial opioid use in years; mean (SD)	23.3 (9.3)	23.3 (9.9)	23.3 (8.5)
Current cigarette smokers; n (%)	207 (89.6)	116 (88.5)	91 (91.0)
Number of cigarettes smoked/day; mean (SD)	16.0 (11.1)	17.5 (12.0)	14.1 (9.5)
Polysubstance use; n (%)	102 (44.3)	60 (45.4)	43 (43.0)
Psychiatric comorbidity, self-reported; n (%)	109 (47.2)	55 (42.0)	54 (54.0)
Methadone dose (mg); mean (SD)	87.2 (60.3)	90.2 (65.6)	83.3 (52.8)
Duration on MMT[c] (months); mean (SD)	38.8 (41.8)	40.6 (38.7)	36.4 (45.6)
Illicit opioid use based on urine test results; mean (SD)	18.7 (23.2)	17.0 (21.3)	20.9 (25.6)

[a]SD: standard deviation [b]BMI: Body Mass Index (kg/m^2) [c]MMT: Methadone Maintenance Treatment

Table 4.2 Summary of testosterone levels between men and women on methadone and controls

	MMT[b]					**Controls**				
	n	*Mean Testosterone (ng/dL); [SD[c]]*	*Median*	*Min.*	*Max.*	*n*	*Mean Testosterone (ng/dL); [SD]*	*Median*	*Min.*	*Max.*
Men[a]	131	100.10 ng/dL [72.21] 3.47 nmol/L 2.51]	78.16 ng/dL 2.71 nmol/L	10.53 ng/dL 0.37 nmol/L	347.55 ng/dL 12.06 nmol/L	287	414.74 ng/dL [141.81] 14.39 nmol/L [4.92]	406.34 ng/dL 14.10 nmol/L	109.51 ng/dL 3.80 nmol/L	798.27 ng/dL 27.70 nmol/L
Women	100	36.61 ng/dL [23.19] 1.27 nmol/L [0.81]	28.16 ng/dL 0.98 nmol/L	8.83 ng/dL 0.31 nmol/L	92.22 ng/dL 3.20 nmol/L	496	25.93 ng/dL [15.20] 0.90 nmol/L [0.53]	23.06 ng/dL 0.80 nmol/L	1.44 ng/dL 0.05 nmol/L	106.63 ng/dL 3.70 nmol/L
Total	231					783				

[a]Significant at the $p<0.001$ level [b]MMT: Methadone Maintenance Treatment [c]SD: standard deviation
SI conversion factor: To convert testosterone to nmol/L, multiply values by 0.0347

Table 4.3 Association between serum testosterone level and methadone-related factors

	Total (n=231)				**Men (n=131)**				**Women (n=100)**			
Variable	*Estimated β*	*95% CI*		*p*	*Estimated β*	*95% CI*		*p*	*Estimated β*	*95% CI*		*p*
Age (years)	-0.007	-0.018	0.003	0.16	-0.008	-0.022	0.006	0.27	-0.007	-0.023	0.008	0.34
Sex[e]	1.034	0.857	1.211	<0.001[a]	--	--	--	--	--	--	--	--
Age of initial opioid use (years)	0.001	-0.009	0.011	0.83	-0.001	-0.015	0.014	0.90	0.004	-0.011	0.018	0.64
Number of cigarettes per day	0.003	-0.005	0.011	0.45	0.011	0.000	0.021	0.05[c]	-0.011	-0.024	0.002	0.09
Methadone dose	-0.002	-0.003	-0.000	0.02[c]	-0.003	-0.005	-0.001	0.00[b]	0.001	-0.002	0.003	0.66
Duration on MMT[f] (months)	0.000	-0.002	0.003	0.89	0.002	-0.002	0.006	0.37	-0.002	-0.005	0.002	0.28
Polysubstance use	0.125	-0.064	0.314	0.19	0.101	-0.18	0.382	0.48	0.244	-0.004	0.493	0.05[d]
Illicit opioid use	-0.002	-0.006	0.002	0.32	-0.003	-0.009	0.003	0.36	0.002	-0.007	0.003	0.49

[a]Significant at the p<0.001 level [b]Significant at the p<0.01 level [c]Significant at the p<0.05 level [d]Shows a trend; p=0.050-0.099 [e]Sex values not possible for subgroup analysis by sex [f]MMT: Methadone Maintenance Treatment

Figure 4.2 Methadone dose and serum total testosterone level in men

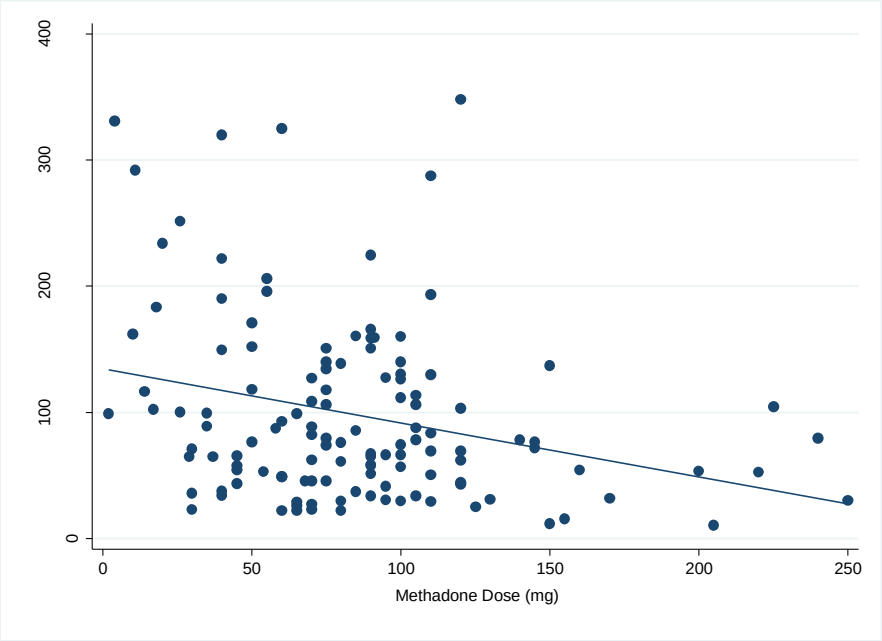
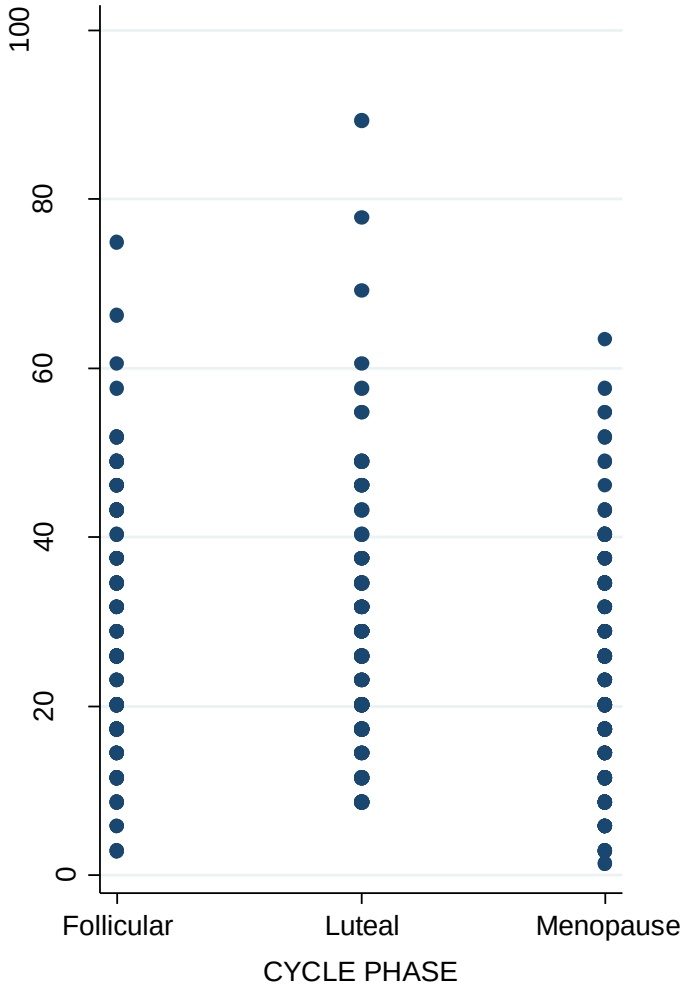


Figure 4.3 Testosterone level across menstrual cycle phases in control women



CHAPTER 5

Study 4

Testosterone suppression in opioid users: A systematic review and meta-analysis

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5.1 Abstract

Background: Whether used for pain management or recreation, opioids have a number of adverse effects including hormonal imbalances. These imbalances have been reported to primarily involve testosterone and affect both males and females to the point of interfering with successful treatment and recovery. We conducted a systematic review and meta-analysis to determine the extent that opioids affect testosterone levels in both men and women, which may be relevant to improved treatment outcomes for opioid dependence and for pain management.

Methods: We searched PubMed, EMBASE, PsycINFO, and CINAHL for relevant articles and included studies that examined testosterone levels in men and women while on opioids. Data collection was completed in duplicate.

Results: Seventeen studies with 2769 participants (800 opioid users and 1969 controls) fulfilled the review inclusion criteria; ten studies were cross-sectional and seven were cohort studies. Results showed a significant difference in mean testosterone level in men with opioid use compared to controls (MD= -164.78; 95% CI: -245.47, -84.08; $p<0.0001$). Methadone did not affect testosterone differently than other opioids. Testosterone levels in women were not affected by opioids. Generalizability of results was limited due to high heterogeneity among studies and overall low quality of evidence.

Conclusions: Our findings demonstrated that testosterone level is suppressed in men with regular opioid use regardless of opioid type. We found that opioids affect testosterone

levels differently in men than women. This suggests that opioids, including methadone, may have different endocrine disruption mechanisms in men and women, which should be considered when treating opioid dependence.

Key words: testosterone, sex hormones, opiates, prescription opioids, methadone

5.2 Introduction

Opioids refer to a class of natural and synthetic drugs that are used for pain management and opioid dependency (1). They exert their analgesic effects by binding to opioid receptors in the brain and spinal cord to inhibit neurotransmitter release (2), causing both a reduction in neurotransmission and an inhibition of sensory neurons responsible for pain sensation. However, opioids also act on the respiratory control centres in the brain to cause a reduction in respiratory function, and they promote a reduction in gastrointestinal motility through their action in the digestive tract (3, 4). When taken appropriately and in recommended dosages, opioids are effective for acute pain relief and management of chronic pain, however they have numerous potential side effects, including sedation, nausea, drowsiness, constipation (5). Other side effects include decreases in sexual function, bone deterioration, hair loss, immunodeficiency, and pain sensitivity (6, 7). Opioids are also known to act on endocrine system function, producing hormonal imbalances that may lead to additional serious adverse effects (8).

Testosterone is a sex steroid that is controlled by the hypothalamic-pituitary-gonadal (HPG) axis and produced through a series of hormonal activations, which include the gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Alterations in testosterone concentration caused by exogenous substances such as opioids can have significant effects on mood, stress reactivity, aggression, and sexual drive (9-11). It is speculated that chronic opioid use leads to suppression of GnRH, which indirectly lowers production of testosterone (8).

In the case of opioid use disorder, testosterone suppression has been documented in opioid-dependent samples (12-16) as well as patients undergoing Methadone Maintenance Treatment (MMT) (17-19). Methadone is a synthetic opioid used to manage opioid use disorder and withdrawal symptoms in substitute opioid therapy (SOT) (20). Treatment with methadone incorporates a harm-reduction approach and involves maintaining patients on a stabilized dose of methadone while slowly tapering off, which can sometimes take years (20). The consequences of testosterone suppression in this particular sample of opioid users may hinder their treatment initiation, maintenance, and recovery.

The incidence of opioid-induced testosterone suppression in women is less commonly examined in the literature. However, a disturbance in female sex hormone levels may also cause the changes that are typically seen in men, and in samples of methadone-treated patients, may lead to poor outcomes and increased risk of relapse.

Based on a review of opioid use and the endocrine system, Katz and Mazer suggest that all opioids suppress testosterone. Studies on individuals with opioid use disorders, methadone-treated patients, and opioid users for chronic pain alike all showed significant suppression of testosterone (8). However, the extent of testosterone suppression was not measured quantitatively. A non-systematic narrative literature review showed similar conclusions (21).

Although previous findings support that all opioids suppress testosterone, direct comparisons of testosterone levels among different opioids have not been performed to

date. It is possible that some opioids affect testosterone more than others, which would be useful in choosing a particular treatment course. Additionally, having information on testosterone level in opioid users compared to the clinically normal ranges would be helpful for healthcare professionals to determine if this reduction in testosterone is clinically significant and when to initiate treatment of its associated symptoms.

These reviews demonstrate that there is a growing interest in this particular topic as a result of increased rates of opioid use and it is likely that additional studies have been conducted since these reviews were published. There is also a lack of quantifiable data to support the effect of opioids on testosterone, which will be appropriately estimated using a summary statistic derived from a meta-analysis of studies that include small samples. Furthermore, examination of the effect of opioids on testosterone levels in women has yet to be completed, and studies that include samples of women are generally small in this particular area of study, therefore a meta-analysis will provide a larger estimate of effect. The quality of the literature also needs to be evaluated to highlight problematic areas for future research and improvement. Hence, the need for a systematic review with updated data that can be combined statistically in a meta-analysis.

5.3 Objectives

The main objective of this systematic review and meta-analysis is to examine the association between opioids and testosterone levels and provide a summary estimate of the magnitude of testosterone suppression. Specifically, we aim to: (1) determine whether

men receiving long-term opioids have low testosterone levels, compared to clinical reference ranges; (2) determine whether women receiving long-term opioids have low testosterone levels, compared to clinical reference ranges; (3) determine if testosterone suppression varies by the type of opioid use, more specifically methadone versus other opioids; and (4) generate clinically relevant evidence through a critical review of the literature.

5.4 Methods

5.4.1 Search Strategy

This review adhered to an *a priori* designed protocol that is available upon inquiry. We systematically searched MEDLINE, EMBASE, PsycINFO, and CINAHL electronic databases from inception to September 19, 2014 for relevant articles. We implemented varying combinations of search terms to reflect differences in indexing among databases. The search was not restricted by language limitations. The complete search strategy can be found in Table 5.1. We manually reviewed reference lists of included studies for relevant citations that may not have been picked up by our search strategy but we did not review the 'grey literature' including dissertations and conference proceedings.

5.4.2 Inclusion and exclusion criteria

The inclusion criteria consisted of observational studies (i.e. cohort, cross-sectional, or case-control) or randomized controlled trials (RCTs) that measured testosterone levels in populations of opioid users, including men and women. We did not limit studies to type or purpose of opioid use (i.e. recreational or therapeutic) or apply any age, ethnicity, or geographic setting limitations. The primary outcome of this review is testosterone level. There were no restrictions based on outcome measurement, such as plasma or serum testosterone, and free, bound, or total testosterone. We excluded studies that included participants on testosterone replacement therapy.

5.4.3 Data screening and extraction

We screened all citations and abstracts retrieved from the search strategy and identified articles for full-text extraction. Two authors (MB and HB) performed the literature search, screening, and data extraction independently; disagreements at any phase of the review process were resolved by discussion or in the case where a consensus was not reached, a third independent rater (ZS) determined eligibility. We recorded the reasons for exclusion and the Kappa statistic for inter-rater agreement of study inclusion at each stage of the screening process. Data were extracted from the studies in duplicate using a pilot-tested data extraction form. In the case where a study previously conducted by the current review authors was included, a third reviewer who was unrelated to either paper verified the data extraction of that particular study. We collected data on the following variables: study characteristics (author, journal, year and place of publication), study design, sample size, age, sex, opioid dose, testosterone level (free and total), time of

blood draw, and statistical analyses performed. We used the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework (22) to rate the quality of evidence of studies included in this review.

5.4.4 Statistical analysis

For the meta-analysis, we employed a random effects model, which assumes variation between studies and their respective effect sizes. We used mean difference (MD) to establish the overall effect size of the difference in mean testosterone levels between opioid users and controls in each of the studies reviewed and have presented these in a forest plot. The analysis was performed separately by sex due to the large variance in testosterone levels between men and women. We planned subgroup analyses by opioid type (methadone for opioid dependence versus opioids for other conditions). We reported the results using 95% confidence intervals (CI) and performed all statistical analyses using STATA (23) and meta-analyses using Review Manager 5.2 (The Cochrane Collaboration, London, UK). This systematic review is reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (24).

5.5 Results

Of the 50 articles retrieved from the initial search and a thorough screen of the reference lists, 17 studies were included in the review (Fig. 5.1); seven cohort and ten cross-

sectional studies. Studies were excluded at each stage of screening for reasons pertaining to incorrect outcome of interest, failure to include appropriate comparison group, and lack of primary research; four studies excluded after title search, 25 studies excluded in the abstract screen, and three studies excluded after full-text screen. Inter-rater agreement was 0.7, 0.4, and 0.4 for the title, abstract, and full-text screen, respectively. Initial disagreements at the abstract and full-text screen stages were later resolved by consensus, whereby the majority of studies were included to undergo screening at the next stage.

5.5.1 Study characteristics

A detailed description of study characteristics is presented in Table 5.2. A total of 800 opioid users were included in the studies, the majority of whom were men (n=646) and 1969 controls (referring to the comparator group in the context of this review). Age of the individuals included in these studies varied from 17 to 58 years with a mean age >30 years for most studies. Study samples reported general opioid dependence (n=3), heroin dependence (n=5), methadone maintenance (n=7), buprenorphine maintenance (n=1), heroin maintenance (n=1), levoacetylmethadol maintenance (n=1), and opioid use for chronic pain (n=4). Daily opioid dose was highly variable among studies; 0.5-40 mg morphine equivalent daily dose and 40-15 mg methadone. Testosterone levels varied from 100 to 700 ng/dL (3.5 to 24.3 nmol/L) in men and from 26 to 55 ng/dL (0.9 to 1.9 nmol/L) in women. Duration of opioid use ranged from months to years, with a minimum of three months and maximum of eleven years; this was referred to as ‘long-term’. Study

publication years varied from 1973 to 2014, however only five of the included studies were published in the last 10 years.

5.5.2 Effect of opioid use on testosterone level in men

We were able to utilize data from 12 studies (including 17 individual samples) to compare the difference in testosterone level in men between opioid users and controls (13, 15-17, 19, 25-31). We found a significantly reduced level of testosterone by a difference of 165 ng/dL (5.7 nmol/L) in men using opioids (n=607) compared to controls (n=1417) (MD= -164.78; 95% CI: -245.47, -84.08; $p<0.0001$) (Table 5.3, Fig. 5.2).

Three other studies were not included in the meta-analysis because they did not use a control group for comparison, rather they drew comparisons to clinical reference ranges of testosterone (32) or utilized a within-subjects cohort design in which the participants had their testosterone levels measured and compared at different time points (14, 33). These individual findings, however, did show significant reductions in testosterone in opioid users.

5.5.3 Effect of opioid use on testosterone level in women

We were able to combine the results of two studies assessing testosterone level in women opioid users in a meta-analysis. There was no significant effect of opioids on testosterone level in opioid-using women (n=121) compared to controls (n=512) (MD= -6.17; 95% CI: -39.87, 27.54; p=0.72) (Table 5.3, Fig. 5.3).

5.5.4 Effect of opioid type on testosterone level in men

We were interested in determining whether certain opioids reduce testosterone levels differently than others, especially those used for substitute opioid therapy. We performed a sub-group analysis using groups of methadone maintenance samples and all other opioids. Although testosterone levels were lower among the methadone-treated group (MD= -181.12; 95% CI: -300.20, -62.05; p=0.003) compared to the non-methadone group (MD= -154.95; 95% CI: -243.45, -66.45; p=0.0006), this difference was not significant.

5.5.5 GRADE quality of evidence

We evaluated our confidence in these findings using the GRADE framework (22). We found that the overall quality of evidence was low, mainly due to a serious risk of bias and inconsistency within the literature, despite the presence of strong associations among outcomes (see Appendix IV for detailed ratings).

5.6 Discussion

This systematic review and meta-analysis sought to evaluate the literature on the effect of opioid use on testosterone levels in men and women. We found that patients using opioids for therapeutic purposes, medication-assisted addiction treatment, or as a drug of abuse and dependence have significantly suppressed testosterone levels compared to non-opioid users, and there was no difference between methadone, a commonly used substitute

opioid therapy, and other types of opioids. This indicates that all opioids suppress testosterone, regardless of drug type or indication of use.

This fact has significant implications for all patients who are prescribed long-term opioids. It is likely that men prescribed methadone treatment for opioid use disorders already have low testosterone levels. Based on clinical observations, one of the concerns that men with opioid dependence have regarding entry to methadone treatment is that their testosterone will be suppressed. Testosterone deficiency is accompanied by symptoms of fatigue, weakness, mood disturbances, and decrease in libido and sexual function, as well as other conditions including erectile dysfunction, and hypogonadism (9, 10). Therefore, it is a common concern for men starting treatment with methadone that they may experience testosterone suppression effects due to methadone. It is important therefore that clinicians treating opioid addiction disorders provide health education to patients to inform them about potential hormonal side effects. It is also recommended that clinicians measure testosterone level before starting SOT and if they find that testosterone is already suppressed. Testosterone replacement therapy should be considered carefully and provided when appropriate. It is expected that treating testosterone deficiency symptoms will improve the overall quality of life of patients (34-36) and potentially opioid addiction treatment outcomes. Based on the current study findings, it is recommended that testosterone levels be monitored in patients prescribed opioids prior to treatment initiation and periodically throughout the course of treatment to allow for appropriate management of testosterone deficiency in men.

Buprenorphine, a synthetic opioid similar to methadone, is also used in substitute opioid therapy, and was explored in a study by Bliesener et al. (2005). Although testosterone levels were greater in the buprenorphine-treated group compared to methadone-treated patients and when compared to non-opioid users, these differences were not significant (19). This finding does potentially highlight the need for further studies with patients undergoing addiction treatment with buprenorphine that include larger sample sizes, in order to clarify this effect.

Our review has confirmed that all opioids suppress testosterone, which is consistent with previous reviews in the literature (8, 10). However, this review is the first to synthesize this information in a meta-analysis and provided a statistical estimate of the magnitude of testosterone deficiency. Our findings demonstrate that testosterone is suppressed by almost 50% in some men and is far below the average clinical reference ranges. We did not observe the same effect in women, which suggests that men and women have different mechanisms of hormonal disturbance caused by opioids. This review can potentially inform both healthcare providers and individuals prescribed opioids about the endocrine disrupting effects of opioid use to make informed decisions about treatment options and other alternatives to be considered. Non-steroidal anti-inflammatory drugs (NSAIDs) and chiropractic care are additional options for pain relief that can prevent the testosterone deficiency caused by opioids. Future studies are required to address whether supplementation with testosterone is an effective approach to the improvement of quality of life and addiction treatment in men receiving opioids.

5.6.1 GRADE quality of evidence

We used the GRADE framework (22) to evaluate our confidence in the estimates derived from the meta-analysis and determined that the studies reported in the literature were of low quality (see Appendix IV for ratings).

The most prominent concerns among the studies were risk of bias and confounding. Some studies had adjusted for potential confounders by considering certain factors in their analyses such as age (31) and methadone dose (29); however the remaining studies either did not take into consideration such factors or failed to report them. Data on duration of opioid use, smoking, concurrent medications, or polysubstance use were collected in some studies but not accounted for when measuring testosterone level. Also, there was often no mention of whether or not participants were undergoing any testosterone replacement therapy at the time of study, or whether other hormonal medications were being used. This also raises the issue of lack of reporting standards among studies in this field. The majority of studies were performed between 1980 and 2000, when standards for reporting studies were different than today. After the introduction of the Consolidated Standards of Reporting Trials (CONSORT) statement and later its multiple extensions in 1996, as well as the gradual uptake of these statements by journals, reporting standards have improved (37). However, important pieces of information related to methodology, statistics, or outcome measurements remain unknown in older studies included in this review, thus impacting the quality assessment.

We noticed a high level of heterogeneity and variability among studies which may be attributed to differences in outcome measurements (i.e. free vs. total vs. bound; plasma vs. serum, different assay methods). This was especially prominent in the studies with women opioid users, where the two studies had an opposite direction of effect (25, 38). This may be due to small sample sizes of individual studies, suggesting that they may not have the power to accurately detect this difference in the outcome or it could be explained by some unknown factors that influenced the control groups. Additionally, in this review, we included samples where participants were dependent on opioids, had serious health concerns, and suffered from lack of adequate healthcare. We also had samples of patients with chronic pain consuming prescription opioids whose doses were regulated and carefully monitored, and taken under safe conditions. Characteristics of these populations may differ, and although the results were consistent among both types of samples, they may have limited comparability in terms of how generalizable the overall summary finding is.

Our overall confidence in the estimates is therefore quite low, however our meta-analysis generally showed a consistent large effect size across multiple studies, which does provide evidence for testosterone deficiency associated with opioid use.

5.6.2 Strengths and limitations

This was a systematically conducted review with rigorous statistics and a large meta-analysis that provided a quantifiable estimate of the effect of opioid use on testosterone

levels. We observed this relationship among men as well as women, which is not commonly reported. We also compared testosterone levels among opioid users being treated with methadone for addiction and opioid use (including prescription opioids) for conditions other than addiction. A thorough evaluation of the status of literature was also performed.

As evaluated by the GRADE framework, the quality of evidence in this field of study is poor, and therefore the results summarized in this review should be interpreted with this consideration in mind. However, this review has brought to light the need for more up to date research using current hormone assay methods, appropriate reporting, and rigorous methodology. It has also been successful in identifying the need for future examination into the effect of opioids on testosterone levels in women. The lack of studies among women opioid users included in this review, as well as the small sample sizes of these studies, poses a challenge in drawing adequate conclusions of this association.

5.6.3 Conclusions

Our systematic review and meta-analysis demonstrated that testosterone levels are suppressed in men receiving opioids, regardless of opioid type or indication of use. These findings may have important potential implications for treatment. Testosterone levels are likely to already be significantly lowered in patients with a history of opioid use. The results of this study can be used by healthcare professionals and patients themselves when choosing to enter substitute opioid treatment for opioid dependence. It is recommended

that testosterone levels are monitored at treatment entry as well as throughout the course of treatment, so that low testosterone and related symptoms may be adequately treated.

5.7 Acknowledgements and author contributions

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Contributors: MB and ZS were responsible for the development of the research question, interpretation of data, manuscript writing, and critical revision of the manuscript. MB and HB were responsible for screening articles and data extraction for the review. MB and BD performed statistical analyses, and BD also contributed to manuscript writing and critical revision. CP, AW, MV, JD, DM, and GP were all jointly responsible for clinical interpretation and organization of results, as well as critical revision of the manuscript. RA and MS were involved in interpretation of data and critical revision of manuscript. MC was responsible for scientific interpretation of hormone values and critical revision of manuscript. LT assisted with statistical analysis, interpretation of data, and revision of manuscript. All authors have reviewed and approved the final manuscript.

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5.9 Figures and tables

Table 5.1 Search strategy

Electronic database	Search terms
MEDLINE n=15	1. "analgesics, opioid" [Title/Abstract] OR 2. "methadone" [MeSH Major Topic] AND 3. "testosterone" [MeSH Major Topic] 4. "human" [MeSH Term]
EMBASE n=27	1. *opiate/ 2. *opiate agonist/ 3. methadone/ 4. testosterone/ 5. 1 or 2 6. 3 and 4 and 5 7. limit 6 to human
PsycINFO n=6	1. *opiates/ 2. *narcotic agonists/ 3. *methadone/ 4. exp Testosterone/ 5. 1 or 2 or 3 6. 4 or 5 7. limit 6 to human
CINAHL n=2	1. MM "analgesics, opioid" AND 2. MM "testosterone"
TOTAL; n=50	

Figure 5.1 Studies selected for inclusion

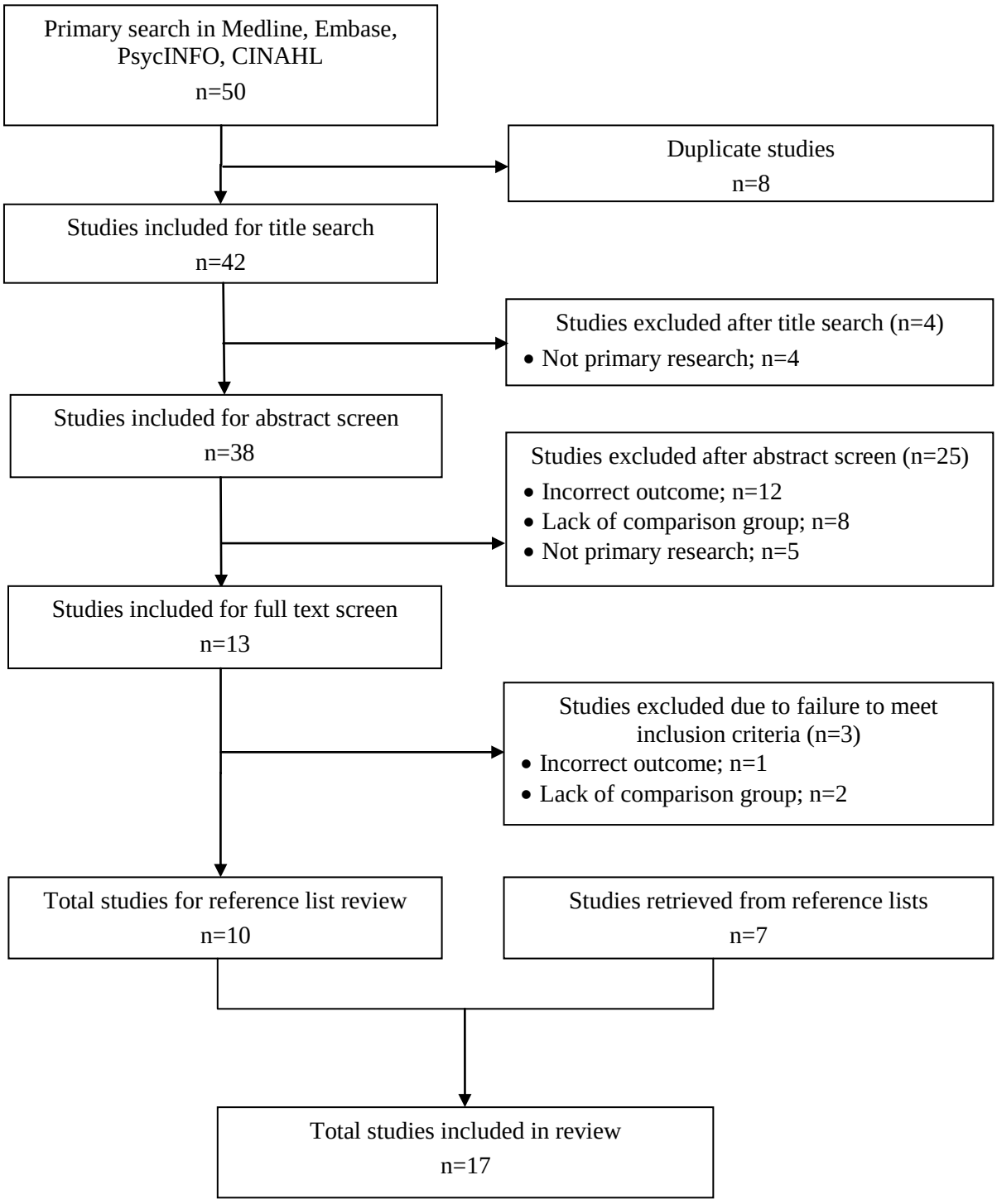


Table 5.2 Study characteristics

Author (Year)	Study Design	Participants (n)	Sex	Age (Yrs); Mean (SD/SE) or Range (Min-Max)	Daily Opioid Dose	Route of Administration	Testosterone (ng/dL); Mean (SD)	P-value (difference between groups)
Abs (2000)	Cohort	CP (29) Control (11)	M	48.4 (SD 11.0) 54.2 (SD 14.0)	4.8 mg ME (SD 3.2)[a] ---	Intrathecal ---	198.9 (149.9) 443.8 (126.8)	<0.001
Azizi (1973)	Cross-sectional	MMT (6) HD (16) Control (25)	M	17-58 17-58 17-58	60-140 mg 60-140 mg ---	Oral NR ---	340 (110) 440 (320) 700 (290)	<0.001[b] <0.02[b]
Bawor (2014)	Cross-sectional	MMT (231) Control (783)	M (131) F (100) M (287) F (496)	38.3 (SD 11.0) 35.2 (SD 9.4) 46.2 (SD 13.1) 44.6 (SD 12.6)	90.2 mg (SD 65.6) 83.3 mg (SD 52.8) --- ---	Oral Oral --- ---	M: 100.1 (72.2) F: 36.6 (23.2) M: 414.7 (141.8) F: 25.9 (15.2)	<0.001[b] NS[b]
Blick (2012)	Cohort	OD (90) Control (759)	M	48.3 (SD 12.0) 52.6 (SD 12.2)	NR ---	NR ---	280 (170) 287 (149)	NS
Bliesener (2005)	Cross-sectional	MMT (37) BUP (17) Control (51)	M	37.5 (6.9) 34.7 (7.4) 35.2 (4.5)	88.4 mg (SD 16.0) 11.2 mg (SD 4.3) ---	Oral Sublingual ---	280 (120) 510 (120) 490 (130)	<0.000[b] NS
Cofrancesco (2006)	Cohort	MMT (33) Control (163)	F	36.3	NR ---	Oral ---	29.7 36.0	0.030
Cushman (1973)	Cohort	MMT (54) HD (23) Control (16)	M	35.0 (7.0) 33.0 (7.0) 31.0 (8.0)	91 mg (SD 25) NR ---	Oral NR ---	577 (284) 523 (279) 589 (246)	NS[b] NS[b]
Daniell (2002)	Cross-sectional	OD (23) Control (27)	M	49.4 (30-78) 57.4 (40-67)	70-120 mg (avg range)	Oral ---	188.5 (193.4) 449.1 (181.1)	<0.001
Daniell (2008)	Cross-sectional	OD (21) Control (16)	F	39.3 (4.9) 42.7 (3.5)	20-30 mg ---	Oral ---	30.7 (21.5) 54.5 (10.3)	<0.001
Finch (2000)	Cross-	CP (11)	M	46.5 (SE 3.5)	0.5-40 mg ME	Intrathecal	141.2 (105.1)	0.0032

	sectional	Control (9)		49.0 (SE 6.0)	(avg range)	---	351. 6 (138.3)	
Malik (1992)	Cross-sectional	HD (33) Control (35)	M	18-50 29.8 (SE 3.3)	37.8 ng/ml ME (SE 5.2) ---	Smoking or vapor inhalation	376.3 (215.4) 630.4 (137.9)	<0.005
Mendelson (1975)	Cohort	MMT (14) HM (12) Control (16)	M	22-47 22-47 22-47	80-150 mg 40-100 mg ---	Oral NR ---	409.1 (181.9) 227.5 (116.6) 622.7 (166.9)	<0.01[b] <0.01[b]
Mendelson (1983)	Cohort	LAAM (9)	M	19-36	50-65 mg	Oral ---	683 (162)	<0.05[c]
Ragni (1988)	Cross-sectional	MMT (42) HD (15) Control (15)	M	25.0 (SE 5.0) 23.0 (SE 6.0) 30.0 (SE 6.0)	40-60 mg Unknown ---	Oral NR ---	520 (190) 550 (120) 490 (110)	NS[b] NS[b]
Rajagopal (2003)	Cross-sectional	CP (20)	M	50.1 (34-77)	>200 mg ME ---	Oral ---	Median: 140 (range 21-381)	NR
Roberts (2002)	Cohort	CP (10)	M	52.4 (SE 4.0)	3.3 mg ME (SD 0.6)	Oral or intrathecal ---	115.3 (81.9)	<0.0001[c]
Wang (1978)	Cross-sectional	HD (54) Control (43)	M	34.6 (SE 1.5) 34.6 (SE 1.5)	>40 ng/ml ---	Smoking, vapor inhalation, or intravenously	521.6 (211.6) 657.1 (207.9)	<0.005

CP=chronic pain; HD=heroin dependence; OD=opioid dependence (general); BUP=buprenorphine maintenance; MMT=methadone maintenance; HM=heroin maintenance; LAAM=levoacetylmethadol maintenance; M=male; F=female; [a]Specified dose is combined for sample of men and women; it is not male-only like the other variables for that study; [b]Compared to control; [c]Within subjects cohort: compared to when subjects were not using opioids
Note: Some studies reported standard error (SE); these values have been transformed to standard deviation for consistency.

Table 5.3 Summary of meta-analysis results

Group	No. of studies	Subjects; n		Pooled MD (95% CI)	I^2 %	Summary of differences	GRADE quality of evidence
		Opioid users	Controls				
Men							
Methadone treatment	6	284	410	-181.12 (-300.20, -62.05) p=0.003	95 p<0.0001	Testosterone is significantly lower in all opioid users compared to controls	Low[a,b]
Opioids (excluding methadone)	11	323	1007	-154.95 (-243.45, -66.45) p=0.0006	92 p<0.0001		Low[a,b]
All opioids	17	607	1417	-164.78 (-245.47, -84.08) p<0.0001	96 p<0.0001		Low[a,c,d]
Women							
All opioids	2	121	512	-6.17 (-39.87, 27.54) p=0.72	97 p<0.0001	No significant difference in testosterone between opioid users and controls	Very low[c,e]

[a]Some studies did not adjust or control for potential confounders (age, BMI, duration of opioid use, opioid dose, smoking, etc.)
[b]Large mean difference in testosterone levels between opioid users vs. control (p<0.01)
[c]Significant differences in patient populations and outcome measurements may limit generalizibility
[d]Large mean difference of testosterone (159.08ng/dL) in opioid users vs. control (p=0.0002)
[e]High variability in direction and magnitude of effect between studies, potentially attributed to differences in characteristics and sample size of patient vs. control groups

Figure 5.2 Effect of opioid use on testosterone level in men

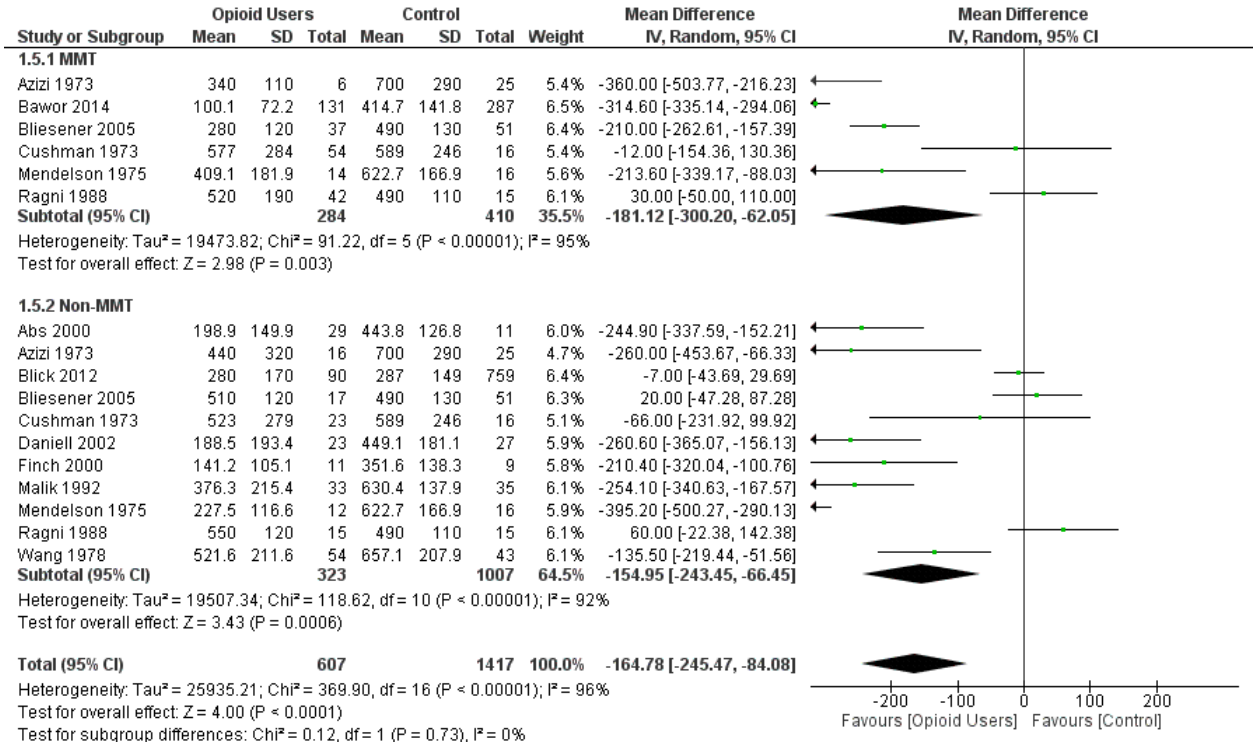
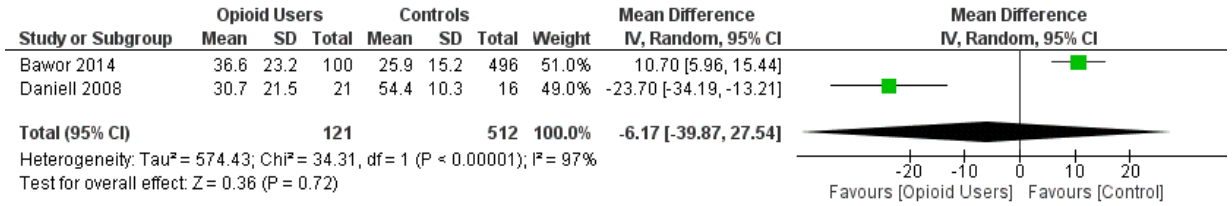


Figure 5.3 Effect of opioid use on testosterone level in women



CHAPTER 6

Study 5

Contribution of *BDNF* and *DRD2* genetic polymorphisms to continued opioid use in patients receiving methadone treatment for opioid use disorder: an observational study

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6.1 Abstract

Background: The heritability of opioid use disorder has been widely investigated, however the influence of specific genes on methadone treatment outcomes is not well understood. The association between response to methadone treatment and genes that are involved in substance use behaviors and reward mechanisms is poorly understood, despite evidence suggesting their contribution to opioid use disorder. The aim of this study was to investigate the effect of brain-derived neurotrophic factor (*BDNF*) and dopamine D2 receptor (*DRD2*) polymorphisms on continued opioid use among patients on methadone treatment for opioid use disorder.

Methods: *BDNF* 196G>A (*rs6265*) and *DRD2* -241A>G (*rs1799978*) genetic variants were examined in patients with opioid use disorder recruited from methadone treatment clinics across Southern Ontario, Canada. We collected demographic information, substance use history, blood for genetic analysis, and urine to measure opioid use. We used regression analysis to examine the association between continued opioid use and genetic variants adjusting for age, sex, ethnicity, age of initial opioid use, methadone dose, duration of treatment, and number of urine screens.

Results: Among 240 patients treated with methadone for opioid use disorder, 36.3 percent (n=87) and 11.3 percent (n=27) had at least one risk allele for *rs6265* and *rs1799978*, respectively. These genetic variants were not significantly associated with continued opioid use while on methadone maintenance treatment (*rs6265*: odds ratio

[OR] = 1.37, 95% confidence interval [CI] = 0.792, 2.371, p = 0.264; *rs1799978*: OR = 1.27, 95% CI = 0.511, 3.182, p = 0.603).

Conclusions: Despite an association of *BDNF rs6265* and *DRD2 rs1799978* with addictive behaviors, these variants were not associated with continued illicit opioid use in patients treated with methadone. Problematic use of opioids throughout treatment with methadone may be attributed to non-genetic factors or a polygenic effect requiring further exploration. Additional research should focus on investigating these findings in larger samples and different populations.

Keywords: opioid use disorder, methadone maintenance treatment, treatment response, BDNF, Val66Met, DRD2

6.2 Introduction

Rates of illicit opioid use are continuing to rise on a global scale, with North America being among the regions with most problematic levels of opioid use (1, 2). Now identified as a growing public health problem, the use of illicit opioids is putting individuals at risk for opioid-related problems, including psychological and physical dependence. The development of opioid use disorder is influenced by a combination of environmental, behavioral, and biological factors, which contribute to the chronic and relapsing nature of the illness. Treatment for opioid use disorder with methadone, a synthetic opioid agonist, has been shown to be effective in reducing rates of relapse (3, 4), however there are a number of patients that continue to abuse opioids while in treatment with little to no progress in their recovery.

The success of opioid agonist treatments is likely to be influenced by individual differences in gene profiles (5). Evidence for the heritability of opioid use disorders has long been established (6-14), from which an interest in the specific genetic variability of opioid use disorder and methadone maintenance treatment (MMT) has evolved (5, 15). Existing genetic studies have explored the therapeutic response to MMT with a focus on opioid use relapse and methadone dosing. Opioid receptor genes, specifically *OPRM1*, and methadone metabolism genes, including *ABCB1* and *CYP450* are among the most commonly studied genes to date (16-22). However, the association between methadone treatment response and other genes such as those involved in substance use behaviors and

reward mechanisms remains unknown, despite evidence suggesting their contribution to opioid use disorder (23, 24).

The brain-derived neurotrophic factor (*BDNF*) gene encodes the neurotrophic protein, BDNF, which modulates neuron survival and neurotransmission (25). Located on chromosome 11p13-15, *BDNF* has been identified as a strong candidate gene in multiple psychiatric and substance use disorders (26-29) including opioid use disorder (30-32), as well as for certain addictive behaviors such as drug-seeking, impulsivity, polysubstance use, and cigarette smoking (33-35). The *BDNF* 196G>A single nucleotide polymorphism (SNP) (*rs6265*, also known as Val66Met) is found in the pro-*BDNF* region of the gene and inhibits secretion of the BDNF protein. Val66Met has been linked with deficits in neurotrophin and neurotransmitter release in specific areas that are responsible for behavior, learning, and memory (36, 37). In the context of methadone treatment, *BDNF* has been explored in relation to BDNF plasma levels (30) and methadone dose (38), with only one study examining methadone treatment response to date (39). In their study of 91 patients enrolled in an MMT program, de Cid and colleagues found that a haplotype block in the BDNF genomic region (GenBank accession number NC_000011; including 21 polymorphisms in a 63.8kb region of coding sequence, and 3′ and 5′ untranslated regions) containing this specific SNP was more frequent in non-responders compared to responders. However, the generalizability of these findings is limited by small sample size, large confidence intervals (CIs), and short period of urinalysis testing (previous four urine screens) (39).

Similarly, the dopamine receptor D2 (*DRD2*) gene plays a major role in opioid use disorders because of its involvement in the reward-dependence pathway (40). The *DRD2* gene is localized to chromosome 11q23 and is responsible for the synthesis of dopamine D2 receptors, which are involved in many signaling and neurotransmission processes underlying addiction, including motivation, pleasure, and reward. A reduction in dopamine receptor signaling has been linked to reward-deficiency syndrome, whereby continuous use of opioids acts to compensate for this inhibited dopamine release or "low reward" state (41). The dopaminergic system mediates withdrawal and drug-related learning (42) and is therefore an important candidate gene for studying opioid use and methadone treatment response. To date, most of the addiction literature involving *DRD2* focuses on the *Taq1A* (*rs1800497*) polymorphism (43-46). There is also widespread evidence for an effect of *Taq1A* on methadone dose, metabolism, and response, which is most often associated with poor outcomes (24, 40, 43, 47, 48). However as the *DRD2* gene is heavily involved in the activation of dopamine-reward circuitry, it is likely that other SNPs that have not been investigated as extensively as *Taq1A* are associated with methadone treatment outcomes. A promising target polymorphism, *DRD2* -241A>G (*rs1799978*), is of particular interest as it has shown preliminary evidence for an association with opioid use disorder and methadone dose in a sample of 85 German drug users admitted to an outpatient methadone treatment center (43).

Despite evidence for a strong association with addictive and reward behaviors, few studies of *BDNF rs6265* and *DRD2 rs1799978* in the context of opioid dependence and response to methadone treatment are available, and those are often limited by small

samples or variation in the definitions of methadone treatment response. Based on existing literature there is high potential for these SNPs to demonstrate an effect on methadone treatment response, which may have important implications for treatment prognosis.

6.3 Objectives

The current study aims to examine the genetic contribution to methadone treatment response (continued opioid use) in individuals with opioid use disorder with specific focus on addiction-related genes, *BDNF* and *DRD2*. We hypothesize that carriers of the minor alleles of both *rs6265* and *rs1799978* will be more likely to engage in continued illicit opioid use during methadone treatment, indicating poor treatment response.

6.4 Methods

We have reported detailed methods of this study sample previously (49). Data used in this study were collected as part of the Genetics of Opioid Addiction (GENOA) research program, in collaboration with Canadian Addiction Treatment Centres (CATC; formerly known as Ontario Addiction Treatment Centres, or OATC) and the Population Genomics Program at McMaster University. This study is a cross-sectional analysis of men and women with a DSM-IV opioid dependence disorder recruited consecutively from four outpatient methadone clinics across Southern Ontario between June and December of

2011. This study was approved by the Hamilton Integrated Research Ethics Board (HIREB) and written informed consent was obtained from each participant.

Participants were included in the study if they were ≥ 18 years of age, enrolled in a methadone treatment program at the CATC clinics, were on a stabilized dose for the past 3 months, and able to provide consent and blood samples. We utilized the genetic information from 240 participant blood samples from the GENOA study in this investigation, in addition to substance use and medical history obtained through structured clinical interviews.

Illicit opioid use (referring to the use of illegal opioids, such as heroin, or using prescription painkillers that were not prescribed for the given individual/condition) was detected by weekly/bi-weekly urine screens and measured as the percentage of positive urine screens per total number of urine screens available. Participants with < 80 percent negative opioid urine screens were classified as using illicit opioids during treatment, or treatment non-responders. We also collected information on demographics, methadone treatment duration, methadone dose, age of initial opioid use, and psychiatric history.

6.4.1 SNP selection and genotyping

We selected the *BDNF* and *DRD2* genes on the basis of evidence supporting their involvement in opioid dependence and addictive behavior. The *rs6265* and *rs1799978* SNPs were the preferred choices for the purpose of this investigation because of their association with substance use and psychiatric disorders among various clinical

populations (33-35, 50-52). We isolated DNA from whole blood and performed genotyping using the Applied Biosystems® ViiA™ 7 Real Time PCR System (Life Technologies Corp, Carlsbad, CA, USA) with Applied Biosystems TaqMan Genotyping Master Mix (Life Technologies Corp) as described previously (49). The genotype call rates were 97.7 percent and 99.2 percent for *BDNF rs6265* and *DRD2 rs1799978*, respectively.

6.4.2 Urine toxicology

All participants underwent qualitative and semiquantitative urine analysis weekly/bi-weekly using the iMDx™ Analyzer and Prep Assay (NOVX Systems Inc, Richmond Hill, ON, Canada). The urine toxicology assays are implemented as part of the treatment model to monitor methadone adherence and to identify use of opioids. The iMDx™ test is able to differentiate between natural and synthetic opioids, allowing for easier identification of specific opioid use. Urine samples were collected and assayed at the respective methadone clinic sites.

6.4.3 Statistical analysis

Sample demographics are summarized using descriptive summary measures expressed as mean (standard deviation, SD) for continuous variables and number (percent) for categorical variables. Genotype and allele frequencies were computed and tested for Hardy-Weinberg equilibrium.

We performed univariate analyses on sample characteristics to evaluate differences between responders and non-responders; Student's *t*-test was used for mean differences and Chi Square was used for categorical variables. We chose to use the results from these comparisons and include significant variables as covariates in our logistic regression model. We performed multivariable logistic regression analysis with opioid use as the binary dependent variable and the two genetic variants, *BDNF rs6265* (A/A vs. A/G vs. G/G) and *DRD2 rs1799978* (G/A vs. A/A) as independent categorical variables adjusting for age, sex, ethnicity, methadone dose (mg), and duration in treatment (months). We also adjusted for the total number of urine screens to eliminate any effect of more frequent urine sampling, suggesting problematic behavior throughout treatment (change in outcome per opioid screen). We classified continued opioid use as having < 80 percent negative opioid urine screens (treatment non-responders). This classification was based on data from our current sample and previous literature demonstrating that 30 to 80 percent of opioid urine screens generally test negative throughout the course of methadone treatment (53-55). Given the maximum value of this range, individuals with greater than 80 percent negative screens (or alternatively, less than 20 percent positive screens) are considered to be in good standing and therefore responding well to treatment. Given that 85 percent of the participants were of self-reported European origin, we did not perform sub-group analyses based on ethnicity due to small sample size of other ethnic groups in our study; this ethnic distribution is in keeping with our region population mix. In our regression, participants of European origin were compared to non-European origin, and men were compared to women.

Regression results including model coefficients (odds ratio, OR), corresponding confidence intervals, and associated p-values are reported. The criterion for statistical significance was set at alpha = 0.05. There were no missing data in our analysis. We performed all statistics using STATA Version 12 (StataCorp LP, College Station, USA). The study is reported in adherence to the Strengthening the Reporting of Observational Studies in Epidemiology statement (56).

We confirmed the statistical power of this investigation *post-hoc* using Quanto Version 1.2.4 (Morrison & Gauderman, 2009, California, USA) with treatment response (continued opioid use) as the outcome variable. Using an additive gene-only unmatched case-control (1:2) model including 240 methadone patients and a two-sided test with p=0.05 level of significance, we have 85 percent power to evaluate the effect of *BDNF rs6265* (minor allele frequency, MAF: 0.20) on methadone treatment response with an odds ratio of 1.5, and 70 percent power to examine the effect of *DRD2 rs1799978* (MAF: 0.05) on methadone treatment response with an odds ratio of 1.8.

6.5 Results

6.5.1 Sample demographics

Of the initial 260 participants recruited from methadone clinics, 20 participants were excluded from the study (duplicate entries = 5, buprenorphine treatment = 3, missing blood sample or urine data = 8, being prescribed opioids for chronic pain condition = 4).

Therefore, 240 participants in total were included in the analysis (Fig. 6.1). The sample consisted of 144 (60.0%) men and 96 (40.0%) women with a total mean age 37.1 (SD =10.4). Participants of European ethnicity made up 85 percent of the sample. A majority of participants (81.3%; n=195) reported having a family history of mental illness or addiction. Responders and non-responders were comparable across the majority of factors. Additional details of sample characteristics are shown in Table 6.1.

6.5.2 Genotypic profile

Genotype frequencies for *rs6265* and *rs1799978* are presented in Table 6.1. They did not deviate significantly from Hardy-Weinberg equilibrium (p=0.21 for *rs6265*; p=0.36 for *rs1799978*) and minor allele frequencies were consistent with previous literature; 0.20 for *rs6265* and 0.05 for *rs1799978*. Among our sample of 240 methadone patients, 36.3 percent (n=87) had at least one *rs6265* risk allele and 11.3 percent (n=27) had at least one *rs1799978* risk allele.

6.5.3 Genetic effect on opioid use during treatment

The continued use of opioids during methadone treatment is an indication of treatment non-response and can be measured objectively across samples. This allows us to examine whether there is a genetic component to outcomes of methadone treatment. On average, 18.9% (SD 24.1) of total urine screens throughout the duration of treatment with methadone were positive for opioids in the total sample. Similar patterns were observed

among genotype frequencies of responders and non-responders (Table 6.1). Our logistic regression analysis showed that the minor alleles of *BDNF rs6265* and *DRD2 rs1799978* were not associated with continued opioid use during methadone treatment, while adjusting for age, sex, ethnicity, age of initial opioid use, methadone dose, duration in treatment, and total number of opioid urine screens (*rs6265*: OR=1.37, 95% CI=0.792, 2.371, p=0.260; *rs1799978*: OR=1.28, 95% CI=0.511, 3.182, p=0.603) (Table 6.2).

6.6 Discussion

Genetic association studies in addiction research aim to characterize genetic differences and variation in the processes that underly addiction and response to treatment. Patients with opioid use disorder have significant interindividual variability in their clinical response to treatment, which may be in part attributed to genetic factors. Variation in addiction-related genes, such as *BDNF* and *DRD2*, due to polymorphisms in the genetic sequence may confer susceptibility to continued opioid use while on methadone treatment for opioid use disorder.

6.6.1 Summary of findings

In this study, we explored the effect of *BDNF rs6265* and *DRD2 rs1799978* polymorphisms on an important methadone treatment outcome, continued opioid use, which represents an objective measurement of response to treatment. In our sample of

240 methadone patients of primarily European origin, we were unable to confirm a role for these specific SNPs in continued opioid use during treatment.

Our findings are in line with a study by de Cid and colleagues (2008), the only other study to examine the influence of *BDNF rs6265* in methadone treatment response (39). They performed a haplotype analysis of 30 SNPs in the *BDNF* coding region, including *rs6265*, in a sample of 91 Caucasian individuals receiving methadone treatment for opioid use disorder. Grouping their sample into responders and non-responders, they were unable to establish an effect of *rs6265* on response to methadone treatment (39), but found that a haplotype block containing this specific SNP appeared more frequently in non-responders compared with responders. However, the generalizability of these findings is limited by small sample size, large confidence intervals, and short period of urinalysis testing (previous four urine screens, or approx. one month) (39). With respect to other methadone outcomes, another study demonstrated no effect of *rs6265* on methadone dose in a sample of 227 former heroin-dependent individuals in methadone treatment (38).

The *rs1799978* SNP of *DRD2* has only been examined in association with opioid dependence or methadone dose in two single-SNP and haplotype analyses. In both studies by Hung et al. (2011) and Doehring et al. (2009), the minor allele of *rs1799978* was more common in opioid users (40, 43). However, Hung et al. (2011) demonstrated that carriers of the minor allele required also higher methadone doses in their sample of 321 methadone patients (40), which was not a consistent finding in the study by Doehring and

colleagues for a sample of 85 German drug users (43), thus suggesting a potential ancestral influence of *rs1799978* in methadone dose.

Although methadone dose was a significant predictor of continued opioid use in our regression analysis, this was an expected finding given the available evidence on methadone dosing in treatment (16, 57). We included this variable to ensure that any effect found between the SNPs and continued opioid use was not explained by the relationship between methadone dose and continued opioid use.

6.6.2 Implications

Contrary to what the current literature suggests, although there may be a potential role for both *BDNF rs6265* and *DRD2 rs1799978* in susceptibility to opioid use disorder, the present study shows that these variants do not appear to exert large effects on continued illicit opioid use during treatment with methadone. Treatment response may however be influenced by the collective genetic risk conferred through multiple SNPs across several different genes (a polygenic effect). It is also possible that the continued illicit use of opioids during methadone treatment may be a result of other clinically relevant factors (i.e. medical or psychiatric comorbidity, social circumstances, life stressors, etc.).

6.6.3 Future directions

Given that there is little conclusive evidence to support a genetic impact in methadone treatment, there is a need for well-designed powerful genome-wide association studies to

identify specific SNPs that are relevant to methadone treatment response. Perhaps with this information, we will be able to simultaneously examine multiple candidate genes to understand the genetic composition of polygenic psychiatric disorders. Implementing the use of a gene score to assess individuals' genetic load may prove to be a promising approach to predicting and identifying those patients who require closer monitoring or alternate treatment strategies to overcome their continued opioid use.

Future research should focus on investigating these questions in larger samples and various populations to ensure validity. Furthermore, a thorough examination of other non-genetic determinants of continued opioid use may prove useful to identify problematic areas that require modifications in treatment delivery. Additionally, the genetic effects of withdrawal symptoms and adverse methadone events may be a promising area of study.

6.6.4 Strengths and limitations

To our knowledge, this is one of few studies to investigate the potential for an allelic effect of *BDNF rs6265* and *DRD2 rs1799978* on continued illicit opioid use among a large sample of methadone patients. These factors have not been thoroughly investigated in the context of methadone treatment response (as measured objectively through urine toxicology screens), highlighting a novel direction of research in the treatment of opioid use disorder with opioid agonist treatments. Through this analysis, we aim to stimulate further research into potential polygenic influences in methadone treatment outcomes, as well as confirm our findings in a larger sample of methadone patients. The uniformity of

our cohort, attributed to consistency in delivery of treatment and standard of care across CATC clinics, ensures representativeness of the entire methadone patient population across Ontario, and likely throughout all of Canada. Our objective selection and definition of outcome measurements—specifically, continued opioid use—is also a noteable strength in this study.

Despite our negative findings, this study should be replicated in a larger sample using multiple genes in order to confirm a lack of association between *BDNF rs6265* or *DRD2 rs1799978* and methadone treatment response. Because the frequencies of the minor alleles were relatively low in our sample, a larger sample size may be required to be able to estimate with confidence the effect of these variants on continued illicit opioid use.

6.6.5 Conclusions

In summary, the present study has demonstrated a lack of association between the two genetic variants (*BDNF rs6265* and *DRD2 rs1799978)* and MMT response, contrary to what previously had been believed about the role of these variants in psychiatric disorders and addictive behavior. Further research with larger samples is needed to re-evaluate this question, as well as to investigate multiple genes simultaneously to assess polygenic effects on susceptibility to poor treatment response. Nevertheless, this study elucidates the potential for other non-genetic determinants that may contribute to continued opioid use during methadone treatment and brings attention to further questions regarding the role of genetics in addiction research.

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Author's Contributions: M.B. and Z.S. were responsible for the development of the research question, interpretation of data, manuscript writing, and critical revision of the manuscript. B.D. also contributed to manuscript writing and critical revision. C.P., A.W., M.V., J.D., D.M., D.D, and G.P. were all jointly responsible for the process of data collection and communication with OATC clinics, as well as clinical interpretation of results and critical revision of the manuscript. R.A. and M.S. were involved in

interpretation of data and critical revision of manuscript. C.T. and G.P. performed genetic testing and assisted with interpretation of data and critical revision of manuscript. L.T. assisted with statistical analysis, interpretation of data, and revision of manuscript. All authors have reviewed and approved the final manuscript

Competing Interests: The authors declare that they have no competing interests.

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6.9 Figures and tables

Figure 6.1 Flow diagram for participants included in study

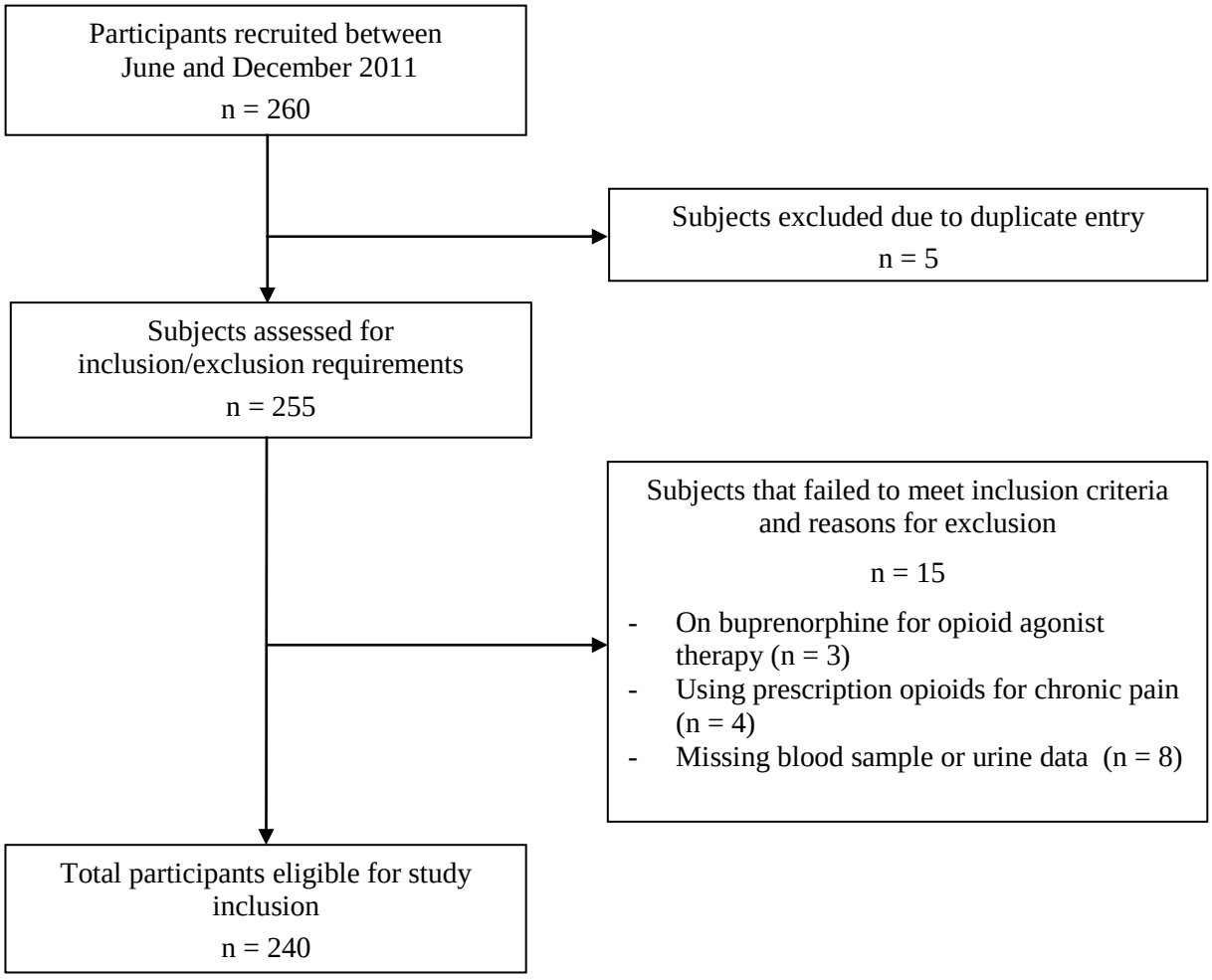

Table 6.1 Characteristics of patients on methadone treatment for opioid use disorder

Characteristic	**Total (n=240)**	**Responders (n=167)**	**Non-responders (n=73)**	***p*-value**
Age in years; mean (SD)	37.1 (10.4)	37.1 (10.7)	36.9 (9.6)	0.868
Male; n (%)	144 (60.0)	105 (62.9)	39 (53.4)	0.169
Married/common law; n (%)	93 (38.8)	66 (39.5)	27 (37.0)	0.711
Employed; n (%)	72 (30.0)	53 (31.7)	19 (26.0)	0.375
Completed post-secondary education; n (%)	81 (33.8)	52 (31.1)	29 (39.7)	0.196
Ethnicity				
European; n (%)	203 (84.6)	139 (83.2)	64 (87.7)	0.381
Native North/South American; n (%)	19 (7.9)	14 (8.4)	5 (6.8)	0.686
Asian; n (%)	2 (0.8)	2 (1.2)	0 (0)	0.348
Persian; n (%)	1 (0.4)	1 (0.6)	0 (0)	0.508
Age of initial opioid use in years; mean (SD)	23.1 (9.2)	22.3 (8.8)	25.0 (9.8)	0.037
Current cigarette smokers; n (%)	214 (89.2)	145 (86.8)	69 (94.5)	0.210
Number of cigarettes smoked/day; mean (SD)	18.0 (10.1)	18.6 (10.5)	16.6 (9.2)	0.158
Psychiatric comorbidity, self-reported; n (%)	116 (48.3)	81 (48.5)	35 (47.9)	0.937
Family psychiatric history; n (%)	195 (81.3)	133 (79.6)	62 (84.9)	0.334
Alcohol use disorder; n (%)	42 (17.5)	29 (17.4)	13 (17.8)	0.934
Methadone dose (mg); mean (SD)	89.5 (60.8)	97.5 (67.5)	71.2 (35.9)	0.002
Duration of MMT (months); mean (SD)	40.5 (42.6)	44.2 (44.1)	31.8 (37.8)	0.042
Total number of opioid urine screens; mean (SD)	65.7 (23.7)	64.9 (20.7)	67.5 (29.6)	0.278
Opioid use (% positive urine screens); mean (SD)	18.9 (24.1)	5.4 (5.6)	49.8 (21.4)	<0.001
BDNF rs6265 genotype frequencies; n (%)				
G/G	153 (63.8)	110 (65.9)	43 (58.9)	0.302
A/G	81 (33.8)	52 (31.1)	29 (39.7)	0.196
A/A	6 (2.5)	5 (3.0)	1 (1.4)	0.458
DRD2 rs1799978 genotype frequencies; n (%)				
A/A	213 (88.8)	150 (89.8)	63 (86.3)	0.427
A/G	27 (11.3)	17 (10.2)	10 (13.7)	0.427

BDNF: *brain-derived neurotrophic factor*; *DRD2*: *dopamine receptor D2*; SD: standard deviation; MMT: methadone maintenance treatment

Table 6.2 Summary of multivariable regression results

Continued opioid use	**OR**	**95% CI**	***p*-value**
Age: year	1.00	0.966, 1.038	0.928
Sex: male	0.64	0.349, 1.178	0.152
Ethnicity: European	1.83	0.715, 4.697	0.207
Age of initial opioid use: year	1.02	0.986, 1.060	0.235
Methadone dose: milligram	0.99	0.983, 0.998	0.016
Duration on treatment: month	1.00	0.987, 1.005	0.364
Total number of opioid screens	1.01	0.997, 1.024	0.148
BDNF rs6265: allele (A)	1.37	0.792, 2.371	0.260
DRD2 rs1799978: allele (G)	1.28	0.511, 3.182	0.603

BDNF: *brain-derived neurotrophic factor*; *DRD2*: *dopamine receptor D2*; OR: odds ratio; CI: confidence interval
LR $\chi^2(7) = 20.23$, Prob> $\chi^2 = 0.0165$, Psuedo $R^2 = 0.0728$, Log likelihood = -128.822

CHAPTER 7

Study 6

Sex differences in substance use, health, and social functioning among opioid users receiving methadone treatment: a multicentre cohort study

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7.1 Abstract

Background: Despite the growing numbers of men and women with opioid use disorder in Canada, sex-specific issues in treatment have not been re-examined in the current population of patients with opioid addiction. We aimed to evaluate sex differences in substance use, health, and social functioning among men and women currently receiving methadone treatment for opioid use disorder in Ontario, Canada.

Methods: We recruited 503 participants with opioid dependence disorder receiving methadone maintenance treatment. We collected data on demographics, treatment characteristics, psychiatric history, addiction severity, and drug use patterns through urinalysis. We performed adjusted univariate analyses and logistic regression to identify distinct factors affecting men and women.

Results: Among our sample of 54% (*n*=266) men and 46% women (*n*=226) with mean age 38.3 years, less than half of participants were employed (35.6%), married (31.8%), and had completed a high school education (27.9%). Compared to men, women had frequent physical and psychological health problems, family history of psychiatric illness, childcare responsibilities, and began using opioids through a physician prescription. Men had higher rates of employment, cigarette smoking, and cannabis use compared to women.

Conclusion: Our results have revealed different patterns of substance use, health, and social functioning among men and women currently receiving methadone treatment for

opioid addiction in Ontario, Canada. This information can be used to develop an integrative treatment regimen that caters to the individual needs of men and women, as well as to inform methadone treatment protocols to include specialized services (including vocational counselling, childcare and parenting assistance, medical assistance, relationship or domestic violence counselling, etc.) and increase their availability and accessibility on a larger scale.

Keywords: substance use disorders, opioid addiction, methadone maintenance treatment, sex differences, women's health

7.2 Introduction

The last decade has witnessed significant changes in patterns of illicit opioid use in Canada (1). Increases in the availability and utilization of opioids for the management of pain conditions in primary care settings (2) have resulted in the shift from heroin use to non-medical prescription opioid use (3). The number of opioid prescriptions has more than doubled over the last two decades (4, 5), and has been associated with a significant burden of opioid-related mortality nationwide with highest rates reported in Ontario (6-8). Currently ranking first in global opioid analgesic consumption (9), Canadians are at a heightened risk for opioid abuse and dependence, giving rise to a major public health crisis (10).

Higher rates of prescription opioid use among women have been consistently documented across studies in Canada and the United States (3, 11-14). Patterns of opioid prescribing are higher among women (15), who are more likely than men to suffer from poor health including pain conditions (16), making them especially vulnerable to misuse prescription narcotics. Indeed, the number of women seeking treatment for opioid-related disorders has markedly increased since the 1960s, reaching current levels that are comparable to men (17).

With the rising number of women seeking treatment for opioid-related problems, there is growing need for a re-evaluation of sex and gender differences in opioid dependence and treatment. Methadone maintenance treatment (MMT) is the most common form of opioid agonist therapy implicated for the management of opioid use disorders and currently

serves over 35,000 patients in Ontario alone, a pronounced increase compared to 7,800 in 2001 (18-20). However, most of what we currently know about methadone treatment is based primarily on studies that included small proportions of women, if at all (21, 22). Existing treatment options remain targeted towards opioid users of the past; primarily young, inner-city, heroin-injecting men. Despite the demographic transformation of this population, available prevention strategies and treatment options have not been revised to accommodate these developments.

The identification of these sex- and gender-specific patterns has been imperative for informing standards of care and clinical practice thus far, however many of these studies were completed in the 1990s and are not representative of today's population of opioid users, nor have they accounted for advancements in assessment tools and research methodology. There is a critical need for a thorough re-evaluation of sex- and gender-related factors for men and women with opioid use disorder.

7.3. Objectives

Our objectives in this study are to (1) provide an updated and extensive description of the current population of opioid users in methadone treatment in Ontario, Canada; and (2) evaluate sex differences in substance use, health status, and social functioning among men and women currently receiving methadone treatment for opioid use disorder.

7.4 Methods

7.4.1 Study design and participant recruitment

We collected data for this study as part of the Genetics of Opioid Addiction (GENOA) research program, in collaboration with Canadian Addiction Treatment Centres (CATC; formerly known as Ontario Addiction Treatment Centres, or OATC) and the Population Genomics Program (PGP) at McMaster University. Details of study methods have been reported previously (23-25). We have since expanded our recruitment setting to include 13 opioid agonist treatment clinics.

We screened all eligible candidates for study inclusion. Participants were included in the study if they were ≥ 18 years of age, meeting criteria for DSM-IV opioid dependence disorder, attending CATC clinics for methadone treatment, and able to provide written consent and blood samples. Participants attending the clinics for opioid agonist treatment other than methadone were not eligible for this study. We chose to include only patients who are receiving methadone treatment as this is the most common opioid agonist treatment in Canada and is covered by most provincial health insurance plans, which allows us to recruit the largest sample possible. Other opioid maintenance treatments (e.g. buprenorphine, naltrexone) are less commonly used and also have distinct biochemical and physiological properties, which would increase the heterogeneity among the sample and render our findings less applicable to the opioid patient population as a whole.

Upon agreeing to participate in the study, participants provided informed consent and underwent baseline assessment, which consisted of a structured clinical interview administered by trained research staff. We collected self-reported data on demographics,

treatment characteristics, age of initial opioid use, and psychiatric history. We also collected information on drug use patterns, measured through urinalysis, and addiction severity across multiple domains using the Maudsley Addiction Profile (MAP) tool. This study was approved by the Hamilton Integrated Research Ethics Board (HIREB; Study ID 11-056).

7.4.2 Maudsley Addiction Profile (MAP)

We used the MAP instrument (26) to measure functioning across several life domains related to addiction; substance use, physical and psychological health symptoms, health risk behavior, and social functioning. The MAP evaluates numerous outcomes, which are common indicators of treatment performance in substance use disorders. Outcomes are evaluated based on the previous month. Originally developed in 1998 for patients with substance use disorders in the UK, it is now widely used and has demonstrated internal reliability and validity (26).

Data on substance use (including alcohol, heroin, illicit methadone, illicit benzodiazepines, cocaine/crack, amphetamines, and cannabis) including the number of days of use, amount, and route of administration was collected for the previous 30-day period. The health risk behavior domain assessed injection drug use, including number of days and frequency of sharing injecting equipment, as well as sexual behavior, including frequency of unprotected sex and number of sexual partners in the previous 30-day period. Frequency of physical and psychological health symptoms were assessed on a

scale ranging from “Never” to “Always”; these responses were tabulated into a single score out of total score of 40, with higher values indicating more frequent health problems. The social functioning domain consisted of interpersonal conflict (days of contact and conflict with partner, family, and friends; represented as a proportion of days of conflict over days of contact in the analysis), employment (days employed and days missed from work), and criminal activity (number of days committed crime and number of times daily). Crime included selling drugs, fraud/forgery, shoplifting, theft from property or vehicle. For analysis purposes these were combined into a single variable representing *any* crime.

7.4.3 Substance use

We collected data on weekly/bi-weekly qualitative and semiquantitative urine analysis using the iMDx™ Analyzer and Prep Assay (NOVX Systems Inc, Richmond Hill, ON, Canada). Urine drug screens are used as part of the clinical care model to monitor methadone adherence, as well as to identify use of illicit opioids and other substances of abuse (including cocaine, cannabis, and benzodiazepines). The cut-off concentrations for detection by urinalysis were: 300 ng/ml for opiates, benzodiazepines, benzoylecgonine (cocaine metabolite), 100 ng/ml for oxycodone, and 50 ng/ml for tetrahydrocannabinol (THC). The iMDx™ assay is designed to distinguish between opioid classifications, including naturally-occurring and synthetic opioids (27). Urine samples were obtained and analysed by trained clinic staff at the methadone clinic sites.

In this study, substance use behavior (including opioids, amphetamines, benzodiazepines, cannabis, and cocaine) was measured as the percent of positive urine screens per total number of available urine screens for each respective drug of interest over the previous three-month period. Alcohol abuse and dependence were measured according to the Mini International Neuropsychiatric Interview (M.I.N.I.) Version 6.0 (28). Self-reported drug use in the past 30 days was collected using the MAP.

7.4.4 Statistical analysis

We summarized descriptive sample characteristics using mean (standard deviation, SD) for continuous measures and number (percentage) for categorical variables. For variables with non-normal distributions, we reported median and interquartile range (Q_1 and Q_3). We performed adjusted univariate analyses on substance use behavior, health symptoms, and social functioning to test differences between men and women (defined as their biological sex) using multivariable linear regression for continuous variables and logistic regression for binary variables, while controlling for age, methadone dose, and duration of methadone treatment. Variables with non-normal distributions were log-transformed prior to the analysis and differences were reported on the log scale. The primary outcome was opioid use measured through urine drug screening; all other outcomes were secondary. We used the False Discovery Rate (FDR) (29) method to control type 1 error rate when performing multiple comparison and adjusted p-values accordingly. A sensitivity analysis was completed using the self-reported MAP assessment to measure substance use compared to urine drug screening. Regression model estimates including

odds ratio (OR) for binary variables, mean difference (MD) for continuous variables, 95% confidence intervals (CI), and p-values are reported.

We did not employ imputations for missing data in our analysis as the proportion of missing data was negligible (4.1%) (30). Our sample size was adequately powered to perform multivariable logistic regression with 10 events per variable and 16 covariates in a sample of 226 women (31). We used STATA Version 12 (StataCorp LP, College Station, USA) for all statistical analyses and we reported this study in adherence to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (32).

7.5 Results

We recruited a total of 503 participants receiving opioid agonist treatment from 13 CATC clinics. Among them, three participants were excluded because they had switched to treatment with buprenorphine rather than methadone. Further, eight participants were excluded as a result of failure to obtain blood and urine samples. A total of 492 participants were included in subsequent analyses (Figure 7.1).

7.5.1 Demographic and clinical characteristics

Our sample consisted of 54% (*n*=266) men and 46% women (*n*=226), with a mean age of 38.3 years and mean methadone dose of 77.6 mg (SD=44.1). Less than half of

participants were employed (35.6%) and had completed a high school education (27.9%). Age of initial regular opioid use was 25 years and age of first entry into methadone treatment was 32.2 years among the total sample. Almost half (44.2%) reported first contact with opioids through a doctor's prescription for a medical illness (Table 7.1).

Women were younger than men (36.9 years, vs. 39.5 years) and receiving a lower methadone dose (73.3 mg vs. 81.3 mg). Women also more commonly reported having had their first contact with opioids through a physician prescription (Table 7.1). Men and women were similar in their age of first regular opioid use, duration of treatment, and number of previous treatments for opioid use disorder.

7.5.2 Substance use behavior

We collected data on participants' substance use using urine toxicology screens and self-reported assessment with the MAP. Apart from cigarette smoking, which was prevalent in the majority of our sample (84.1%), cannabis and alcohol were the most commonly reported substances of use within the past month (47% and 46%, respectively), followed by cocaine (18%) according to the MAP (Table 7.2). Alcohol abuse and dependence were diagnosed using the M.I.N.I. in 9.5% of the entire sample. In the previous three months, the percentage of participants with substance use measured by urine toxicology was highest for opioids (48.5%), followed by benzodiazepines (39.6%), cocaine (34.7%), and cannabis (23.1%) (Table 7.2).

Men and women were similar in their rates of opioid use measured through urine drug

screening within the last three months (48.5% for both). Cannabis use in the past three months was less likely among women compared to men (17.6% vs. 27.8%), and women had significantly fewer positive cannabis urine screens (MD= -16.55; 95% CI= -26.90, -6.19; *p*=0.011) (Table 7.2). These results were consistent when assessed using the MAP (Figure 7.2). Women also reported more frequent use of benzodiazepines compared to men (44.1% vs. 35.7%; OR=1.60; 95% CI=1.10, 2.33; *p*=0.055).

Although alcohol use was reported more frequently among men within the past month (48.5% vs. 42.9%), no differences in alcohol-related disorders among men and women were observed when assessed by the M.I.N.I. (Table 7.2). In total, 84% of the participants were current smokers, and women reported smoking significantly fewer cigarettes daily compared to men (15.4 vs. 18.3; MD= -2.81; 95% CI= -4.79, -0.84; *p*=0.024) (Table 7.2). No sex differences among other substance use were observed.

7.5.3 Health status

Patterns of physical health symptoms demonstrate that over a third of the sample reported chronic pain (35.0%) and a quarter of participants self-reported presence of hepatitis C virus (24.7%); 10.4% of the sample reported both. In contrast, human immunodeficiency virus (HIV) rates were very low (0.8%, *n*=4) (Table 3). Scores for physical and psychological symptoms, measured by the MAP, were also low (15.8 and 13.3 out of 40, respectively). Apart from reporting unprotected sex (42.9%), health risk behavior was not frequently reported.

Women endorsed symptoms of physical and psychological illness more frequently than men, observed by significantly greater scores on the MAP health domains; 17.4 vs. 14.5 for physical health (MD=3.18; 95% CI=1.83, 4.53; $p<0.001$), and 14.7 vs. 12.0 for psychological health (MD=2.77; 95% CI=1.20, 4.34; $p=0.007$) (Table 7.3). Women were also significantly more likely to report a family psychiatric history compared to men (OR=2.35; 95% CI= 1.53, 3.62; $p<0.001$). A greater proportion of men reported positive HIV status, but rates of hepatitis C (27.4% in men vs. 21.7% in women) and chronic pain (35.3% for men vs. 34.5% for women) were equally prevalent among men and women (Table 7.3).

7.5.4 Social functioning

Among our participants, 35.6% reported current employment; the median number of days worked in the past month was 8 for men and 0 for women (Table 7.3). Criminal activity within the past month was rare (5.3%). Less than half of participants were married (31.8%) and a majority reported having children (62.9%).

Women were less likely to report currently employment compared to men (27.1% vs. 42.9%; OR=0.46; 95% CI=0.31, 0.68; $p<0.001$), but were more likely to report having children to care for (73.2% vs. 54.1%; OR=2.88; 95% CI= 1.90, 4.36; $p<0.001$) (Table 7.3).

7.6 Discussion

The results of this study confirm that trends in illicit opioid use in Canada are undergoing dynamic changes, giving rise to a new sociodemographic profile of opioid users. Compared to past literature, the mean age of current opioid-dependent patients enrolled in MMT has increased from 25 to 38 years (21, 33, 34), starting regular use of opioids later (25 years of age now compared to 21 years in the 1990s) and entering treatment at a later age than before (currently 32 years compared to 27 years of age) (17, 21, 34-36). There has been a 30% increase in the proportion of patients who began using opioids after receiving a prescription from a doctor (20% in the 1960s to 50%) (17), usually for the management of chronic pain, which was present in a third of patients. We also observed an approximate 60% decrease in injection drug use (36, 37) and 50% reduction in rates of HIV (38). We have witnessed a gradual deviation from alcohol use to cannabis (35, 36), and greater rates of benzodiazepine use (39). Criminal activity has also declined significantly compared to earlier studies (34% to 5% among current opioid users) (36, 39).

Women, who are close to half of the opioid user population, experience a higher burden of disease related to opioid use disorders, with respect to physical and psychological disorders and related symptoms. Women are more likely to have initiated their substance dependence through prescription opioids, presumably because of their higher rates of chronic pain (13). Indeed, women are known to experience heightened pain perception and sensitivity, and to have lower levels of opioid analgesia compared to men (40). This disparity in opioid prescribing may also be attributed to the utilization of healthcare

services, as women tend to seek medical care for pain-related conditions more often than men (41). Heroin use is also decreasing as a result of this dependence on prescription opioids among women, and therefore we are witnessing lower rates of HIV that normally would have been caused by unsafe heroin injection practices.

Cannabis is now the most prevalent drug of abuse in North America, even though it remains illegal across Canada and most of the United States (9). Given the considerable rate of chronic pain among participants, it is possible that cannabis is being used as an adjunctive therapy to manage pain. Women are less likely to use cannabis than men, consistent with earlier investigations (42, 43), which may be attributed to the social stigma associated with substance use among women. Alternatively, women may be deterred from using cannabis because of the potential legal implications of this behavior.

Although both alcohol and cocaine use continue to be problematic among opioid users in MMT, the disparity that has been seen in the past, with men more likely to abuse alcohol (34, 35) and women more likely to abuse cocaine (42-44), is less apparent. Alcohol use has become a concern for women as well, perhaps because of changes in social roles and attitudes regarding its use (45, 46). Also, it is expected that cocaine use is decreasing as it caters primarily to a younger inner-city group of users, which is characteristic of the former opioid user population (47).

In comparing self-reported substance use measured by the MAP to urine toxicology screening in our sensitivity analysis, we observed considerable under-reporting of benzodiazepine, cocaine, and opioid use. This finding is likely a result of social

desirability bias, or in the case of benzodiazepine use, it may also come from a prescription for an anxiety-related disorder, although we do not have the data to confirm this. Overall, objective measures, such as urine screening, are more reliable than self-report in identifying drug use in men and women alike and should be used regularly across all methadone programs and in future research studies, if possible.

Women experience a heightened vulnerability to the adverse medical and social consequences of opioid dependence (36, 48) as a result of biological sex characteristics and socially-defined gender roles. Although sex and gender differences in MMT have been previously investigated, the literature is limited by the scarcity of studies, poor methodological quality, and small samples. A recent systematic review and meta-analysis found 20 studies, many of which were completed over a decade ago, specifically evaluating methadone treatment outcomes among men and women (in press, Bawor et al.). Men were more likely to be employed, and to report a history of legal involvement and alcohol-related problems, and women were more likely to have used illicit amphetamines throughout the course of treatment (in press, Bawor et al.). Sex differences in physical health (36), co-morbid psychiatric conditions (35, 49, 50), and substance use behavior (34, 35, 42, 43, 51) have also been documented, however findings appear to be conflicting and generally based on subjective self-reported measures.

7.6.1 Implications and future directions

Based on the documented changes in the illicit use of opioids, prevention strategies and

modifications to available opioid addiction treatment programs are needed. Current treatments were initially developed using research from the 1990s targeting heroin users (52), and thus their applicability to the growing population of prescription opioid users is questioned. Guidelines for the treatment of opioid addiction with methadone (53) require a thorough re-evaluation to incorporate this transition and the implementation of new intervention strategies that address the evolving trends in substance use, health, and social functioning is strongly encouraged.

Women also experience a greater burden of disease from opioid dependence with respect to medical problems, health outcomes, and social impairment, elucidating the need for interventions that address these core areas of functioning for women (54). Currently available best practice guidelines for methadone maintenance treatment in Canada outline barriers to treatment and highlight areas for improvement, however these recommendations rely largely on a small and weak body of evidence comprised of outdated literature reviews. Similarly, the U.S. federal guidelines for opioid treatment programs and medication-assisted treatment developed by Department of Health & Human Services, Substance Abuse and Mental Health Services Administration (SAMHSA) (55) acknowledge that women require specialized treatment services, however they are not sufficiently comprehensive as the focus is primarily on pregnancy, physical or sexual abuse, and complex medical problems in women. Furthermore, guidelines for pharmacologically assisted treatment of opioid dependence set forth by the World Health Organization (WHO) in 2009 (56) acknowledge areas where women

experience particular difficulty and they emphasize the need for gender-sensitive treatment services, but admit that data on such programs are sparingly available. First, it is necessary to implement appropriate prevention strategies in general, but especially for women. As our results have shown, women are more likely to be exposed to opioids mainly through prescriptions for pain and other medical conditions. This information can be used to inform both patients and physicians, and in the assessment of individual benefit or risk of opioid-related harms. Alternative treatment and therapeutic options should be considered in the management of pain conditions that require the use of opioid analgesics.

Behavioral therapy and social services can supplement current pharmacological treatment programs in order to develop an integrated patient-centered model of care. Emphasizing the need for fundamental services, such as vocational counselling, childcare and parenting assistance, medical assistance, relationship or domestic violence counselling, and smoking cessation among women is likely to significantly improve the treatment and management of opioid use disorder (57). Similar strategies should be implemented for men in treatment, who experience distinct sex- and gender-specific characteristics of addiction (i.e. HIV, cannabis and amphetamine use). This field of research would benefit from future studies that evaluate the efficacy of these programs compared to standard care and assess patient-important outcomes that can be incorporated into a personalized treatment approach.

7.6.2 Strengths and limitations

This study is limited by its cross-sectional design, whereby sociobehavioral determinants of opioid use disorder were assessed at a single time-point that captured a period of 30 days (or three months in the case of urine screening). A longer time frame would be more appropriate considering the chronicity of the illness and long treatment duration (58). In addition, some of the trends we observed in this study may be attributed to general population differences rather than the specific context of opioid users in methadone treatment. Nevertheless, such factors are still an important consideration for treatment among men and women.

Despite these limitations, our study had numerous strengths. We offer a comprehensive update of factors characterizing a large sample of opioid users receiving methadone treatment within the Canadian context. Our study also provides a descriptive profile of sex differences in methadone treatment, clarifying previous gaps in the literature. We used an objective measure of urine toxicology and performed a sensitivity analysis using self-reported substance use in order to strengthen credibility in our findings. Based on our results, the response rate for MMT in this sample is generally comparable to other studies in the literature (30-80% of opioid urine screens generally test negative (39, 59, 60)), confirming the representativeness of this sample. Finally, our data were derived from a multisite study, whereby standardized treatment procedures are implemented across all 13 clinic sites, yielding a large representative and geographically diverse sample.

7.6.3 Conclusions

The results of this study have revealed new patterns in substance use, health, and social factors among men and women currently receiving MMT for opioid use disorder in Ontario, Canada. We have uncovered clinically-relevant sex differences that can be used to advance our understanding of addiction and promote strategies for effective treatment and management of opioid use disorder among men and women.

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Author Contributions: MB and ZS conceived the ideas for this project and developed the research questions. C.P., A.W., M.V., J.D., D.M., D.D, and G.P. were all jointly responsible for the process of data collection and communication with CATC clinics. L.T. assisted with statistical analysis, interpretation of data, and revision of manuscript. All authors contributed to the interpretation of data and critical revision of the manuscript. The final version has been approved by all authors.

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7.9 Figures and tables

Figure 7.1 Eligibility and screening of candidates for inclusion in the GENOA study

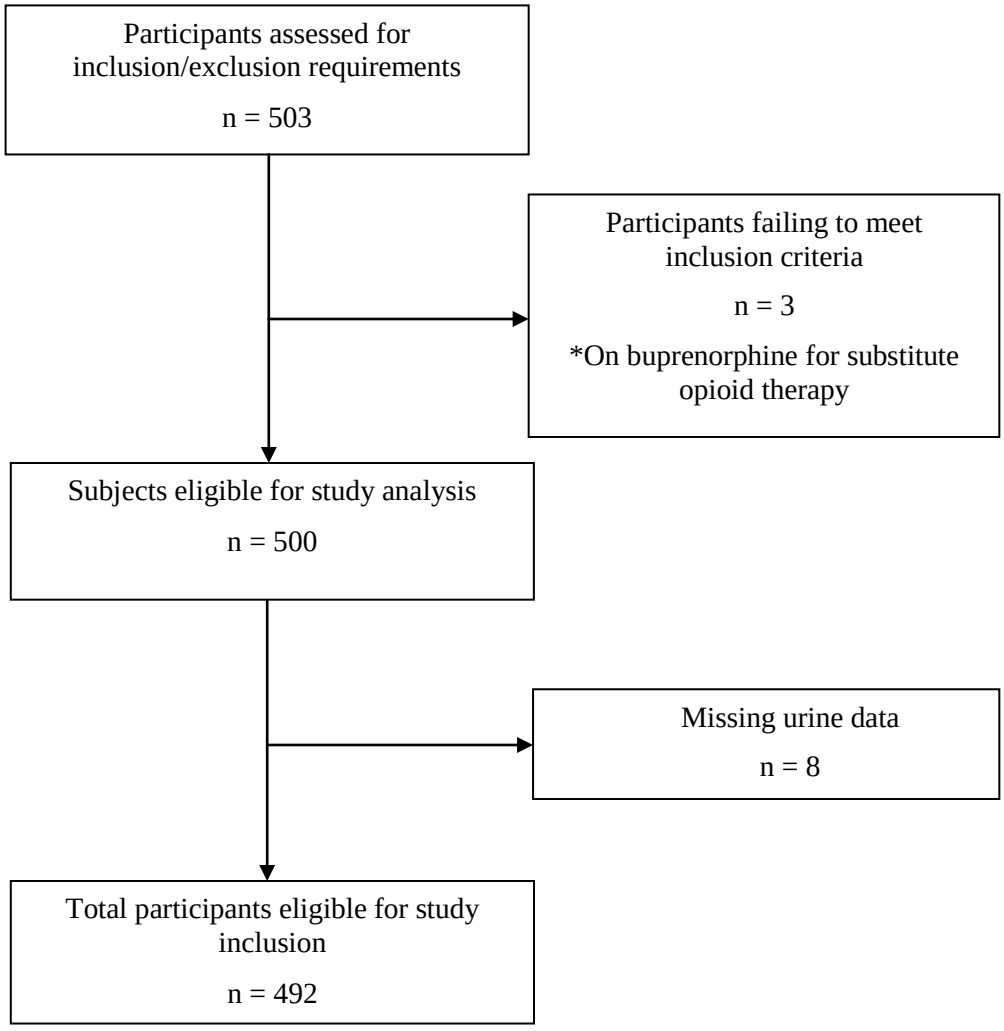


Table 7.1 Demographic and clinical characteristics of opioid-dependent men and women receiving methadone treatment

		Sex	
Characteristic	Total *n=492*	Men *n=266*	Women *n=226*
Age, years; mean (SD)	38.3 (11.0)	39.5 (11.7)	36.9 (9.9)
European ethnicity; n (%)	393 (80.9)	219 (83.0)	174 (78.4)
Completed high school education; n (%)	136 (27.9)	65 (24.6)	71 (32.0)
Substance use and treatment history			
Age of initial opioid use, years; mean (SD)	25.0 (8.7)	24.9 (9.2)	25.1 (8.0)
Physician prescribed first opioid use; n (%)	217 (44.2)	100 (37.7)	116 (51.6)
Daily methadone dose, mg; mean (SD)	77.6 (44.1)	81.3 (48.3)	73.3 (38.3)
Age of first MMT, years; mean (SD)	32.2 (9.6)	32.7 (10.1)	31.5 (9.0)
Duration of MMT (months); mean (SD)	51.6 (49.3)	52.9 (50.7)	49.9 (47.7)
Previous treatment, any; n (%)	149 (30.7)	90 (34.2)	59 (26.6)
Previous MMT treatments, number; mean (SD)	1.5 (1.1)	1.6 (1.1)	1.4 (0.9)

SD: standard deviation; MMT: methadone maintenance treatment

Table 7.2 Substance use behavior among men and women

		Sex		Adjusted analyses, men vs. women		
Outcome	Total *n=492*	Men *n=266*	Women *n=226*	OR/MD	95% CI	Adj. *p*
Primary						
Opioid use in prior three months, urine screening; n (%)	239 (48.5)	129 (48.5)	110 (48.5)	1.03	0.71, 1.50	0.911
Secondary						
Proportion of use in prior three months, urine screening; n (%)						
Amphetamines	23 (4.7)	15 (5.6)	8 (3.5)	0.68	0.28, 1.66	0.616
Benzodiazepines	195 (39.6)	95 (35.7)	100 (44.1)	1.60	1.10, 2.33	0.055
Cannabis	114 (23.1)	74 (27.8)	40 (17.6)	0.57	0.37, 0.89	0.056
Cocaine	171 (34.7)	97 (36.5)	74 (32.6)	0.80	0.54, 1.17	0.417
Ecstasy	23 (4.7)	13 (4.9)	10 (4.4)	1.00	0.69, 1.44	0.985
Positive urine screens in prior three months, percent; median (Q_1, Q_3)						
Amphetamines	0 (0, 0)	0 (0, 0)	0 (0, 0)	-0.56	-1.11, -0.01	0.141
Benzodiazepines	0 (0, 27.3)	0 (0, 20.0)	0 (0, 30.8)	-0.00	-0.27, 0.27	1.008
Cannabis	0 (0, 100.0)	0 (0, 100.0)	0 (0, 50.0)	-16.55	-26.90, -6.19	0.011
Cocaine	0 (0, 12.5)	0 (0, 17.7)	0 (0, 7.7)	-0.20	-0.52, 0.12	0.397
Opioids	0 (0, 25.0)	0 (0, 27.8)	0 (0, 20.0)	-0.21	-0.45, 0.06	0.273
Alcohol use disorder, M.I.N.I.; n (%)						
Alcohol dependence	26 (6.3)	14 (6.5)	11 (5.7)	0.81	0.34, 1.90	0.809
Alcohol abuse	13 (3.2)	7 (3.2)	6 (3.1)	0.84	0.27, 2.58	0.844
Smoking behavior, self-report						
Current smokers; n (%)	412 (84.1)	212 (80.0)	199 (88.8)	1.93	1.14, 3.27	0.053
Cigarettes smoked daily; mean (SD)	16.9 (10.4)	18.3 (11.7)	15.4 (8.6)	-2.81	-4.79, -0.84	0.024
Age of first smoking, years; mean (SD)	15.5 (5.4)	15.5 (5.5)	15.4 (5.3)	0.11	-0.91, 1.12	0.909

Sensitivity Analysis						
Proportion of use in prior month, MAP; n (%)						
Alcohol	227 (46.0)	129 (48.5)	97 (42.9)	0.73	0.50, 1.05	0.213
Heroin	57 (11.5)	35 (13.2)	21 (9.3)	0.54	0.29, 1.00	0.142
Illicit methadone	26 (5.3)	11 (4.1)	14 (6.2)	1.43	0.60, 3.41	0.635
Illicit benzodiazepines	53 (10.7)	29 (10.9)	23 (10.2)	0.90	0.49, 1.66	0.879
Cocaine	89 (18.0)	42 (15.8)	46 (20.4)	1.53	0.91, 2.59	0.241
Crack	57 (11.5)	31 (11.7)	25 (11.1)	0.86	0.42, 1.77	0.839
Amphetamines	32 (6.5)	16 (6.0)	15 (6.6)	0.39	0.10, 1.50	0.330
Cannabis	241 (46.9)	143 (53.8)	88 (41.2)	0.49	0.34, 0.72	<0.001

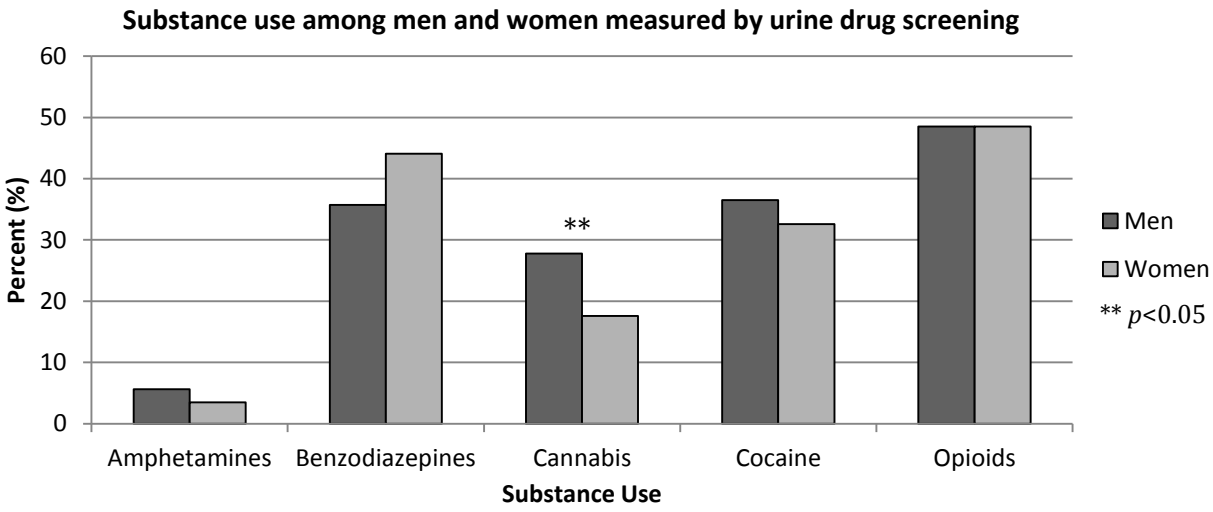
Q_1: quartile 1; Q_3: quartile 3; MINI: Mini International Neuropsychiatric Interview; SD: standard deviation; MAP: Maudsley Addiction Profile: OR: odds ratio; MD: mean difference; CI: confidence interval

All analyses have been adjusted for age, methadone dose, and duration of treatment using multivariable regression and for multiple testing error using False Discovery Rate; results for binary variables reported as OR and results for continuous variables reported as MD.

Variables with non-normal distribution (positive drug urine screens) have been log-transformed for analysis; differences are reported on the log scale.

Note: Data for alcohol use disorder measured by the MINI was only available for 409 participants.

Figure 7.2 Comparison of substance use behavior among men and women measured by urine drug screening and self-report



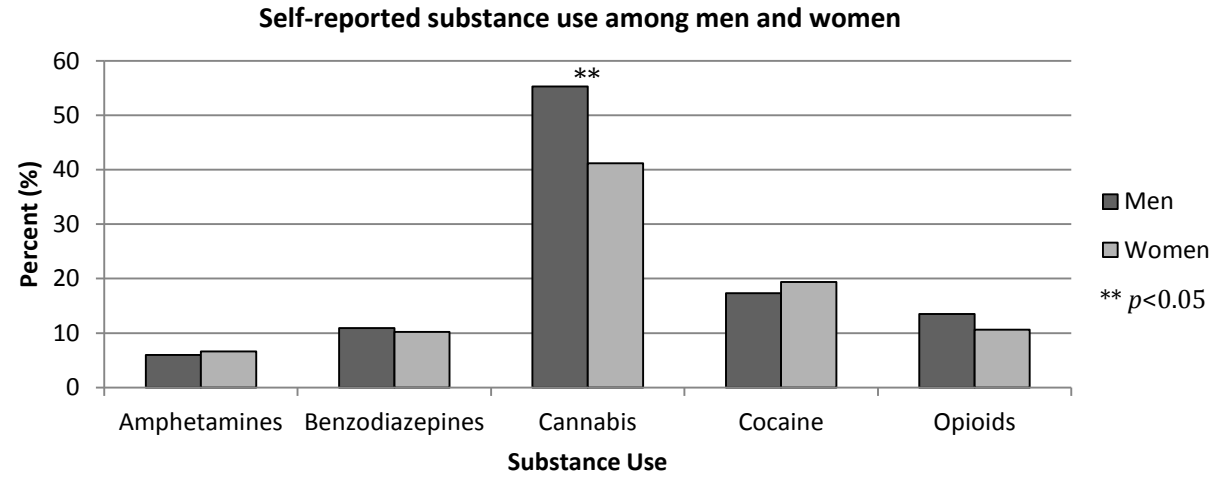


Table 7.3 Health and social functioning among men and women

		Sex		Adjusted analyses, men vs. women		
Outcome	Total *n=492*	Men *n=266*	Women *n=226*	OR/MD	95% CI	Adj. *p*
Physical health symptoms						
MAP physical symptoms score; mean (SD)	15.8 (7.7)	14.5 (7.8)	17.4 (7.3)	3.18	1.83, 4.53	<0.001
HIV+ status; n (%)	4 (0.8)	4 (1.5)	0 (0)	--	--	--
HCV+ status; n (%)	122 (24.7)	73 (27.4)	49 (21.7)	0.88	0.57, 1.38	0.815
Presence of chronic pain; n (%)	173 (35.0)	94 (35.3)	78 (34.5)	1.31	0.87, 1.97	0.368
Mental health symptoms						
MAP Psychological symptoms score; mean (SD)	13.3 (8.8)	12.0 (8.4)	14.7 (9.1)	2.77	1.20, 4.34	0.007
Family psychiatric history; n (%)	350 (70.9)	167 (62.8)	182 (80.5)	2.36	1.53, 3.62	<0.001
Health risk behavior in the prior month						
Injected drugs; n (%)	53 (10.8)	34 (12.8)	19 (8.4)	0.56	0.30, 1.02	0.146
Unprotected sex; n (%)	212 (42.9)	117 (44.0)	95 (42.0)	0.80	0.55, 1.18	0.426
Employment						
Currently employed; n (%)	175 (35.6)	114 (42.9)	61 (27.1)	0.46	0.31, 0.68	<0.001
Paid work in the past month, days; median (Q_1, Q_3)	0 (0, 16)	8 (0, 20)	0 (0, 4)	-0.04	-0.21, 0.13	0.825
Unemployed in the past month, days; median (Q_1, Q_3)	30 (0, 30)	30 (0, 30)	30 (0, 30)	0.02	-0.04, 0.07	0.828
Criminal activity						
Committed crime; n (%)	26 (5.3)	18 (6.8)	8 (3.5)	-0.04	-0.08, 0.00	0.148
Interpersonal relations						
Married/common-law; n (%)	156 (31.8)	85 (32.1)	70 (31.2)	0.94	0.63, 1.39	0.855
Have children; n (%)	309 (62.9)	144 (54.1)	164 (73.2)	2.88	1.90, 4.36	<0.001
Conflict with partner in the past month, percent; median (Q_1, Q_3)	0 (0, 7)	0 (0, 3)	0 (0, 10)	0.11	-0.29, 0.50	0.078
Conflict with family in the past month, percent; median (Q_1, Q_3)	0 (0, 7)	0 (0, 3)	0 (0, 13)	0.42	0.05, 0.80	0.800

MAP: Maudsley Addiction Profile; SD: standard deviation; HIV: human immunodeficiency virus; HCV: Hepatitis C virus; Q_1: quartile 1; Q_3: quartile 3; OR: odds ratio; MD: mean difference; CI: confidence interval
All analyses have been adjusted for age, methadone dose, and duration of treatment using multivariable regression and for multiple testing error using False Discovery Rate; results for binary variables reported as OR and results for continuous variables reported as MD. *Note:* Regression model estimates for HIV+ status were undeterminable.

CHAPTER 8

Conclusion

8.1 Overview

In this thesis, we examined sex and gender differences in the management and treatment of opioid addiction with a focus on hormonal influences, genetic variation, and sociobehavioral characteristics including substance use behavior, health status, and social functioning.We have demonstrated that men and women are differentially affected by opioid addiction and experience sex- and gender-specific challenges throughout the course of methadone treatment that are likely to impact treatment outcomes.

This section summarizes the findings and conclusions of each individual piece of work included in the thesis. We also discuss implications of this work in its entirety, highlighting clinical applications and contributions to the field of addiction medicine. We also comment on areas that warrant future research.

8.2 Sex and gender differences in opioid addiction treatment

Our initial study (Chapter 3) aimed to identify sex differences in the context of methadone treatment through a systematic review of the literature, developed on the basis of the published protocol (1) (Chapter 2). Using common indicators of treatment success within the areas of substance use, health status, and social functioning, we determined that women were less likely than men to use alcohol, report arrests or legal supervision, and be employed during treatment. However, women were more likely to use amphetamines during treatment compared to men.

The conclusions gathered from this study substantiate the presence of sex differences in methadone treatment outcomes, specifically in relation to substance use, employment, and criminal activity. The applicability of these findings is however limited. The literature is fraught with inconsistency in patterns of sex differences and variation in definitions of treatment response. Many studies in this area of research also date back as far as the 1980s and are subject to high risk of bias and poor methodological quality (4-16).

Moreover, increasing rates of illicit opioid use (15-19) and opioid-related treatment admissions (20) among women illustrate the fact that opioid-related problems are affecting a growing number of women. Thus, our study affirms a need for the re-evaluation of sex and gender differences in current opioid users. This systematic review has established a framework for the remainder of the thesis, in which we searched for explanations to support these sex differences through biological and sociobehavioral avenues. We revisited these findings in the final study of the thesis (Chapter 7).

Sex hormones are often studied as the biological basis for sex differences due to their involvement in central nervous system regulation, implicating a role for the endocrine system in the substance use disorders and addictive behavior (21). In Chapter 4, we explored the association between methadone used in the treatment of opioid addiction and testosterone levels among men and women. Using data from the GENOA investigation, we found a significant dose-dependent reduction in testosterone level among men but not women, which is in agreement with previous literature (22-24). Hormonal imbalances attributed to long-term opioid use including methadone may have negative implications on quality of life, sexual function, and mood (25, 26), which are likely to influence how patients perform in treatment (27). We have confirmed that opioid effects on testosterone have distinct biological mechanisms between men and women, which may highlight a potential area of concern in the treatment of men with opioid addiction in the clinical setting. Hormone imbalance especially with regards to testosterone should be evaluated upon initiating treatment with methadone.

In agreement with our findings from Chapter 4, testosterone suppression in men receiving MMT has been documented across multiple studies (24, 28-31). However these studies suffer from a limited sample size, outdated analytic approaches, and general lack of methodological quality (32). Furthermore, the literature does not offer a credible account of the effect of opioids on testosterone among women (33). We were also interested in whether the testosterone deficiency is attributed to methadone specifically or to chronic opioid use in general. We performed a systematic review and meta-analysis (Chapter 5) to

address these concerns with the intention of building upon current literature and providing quantifiable data on the magnitude of testosterone suppression among men and women. Including our data from the previous study (Chapter 4), our systematic review demonstrated that testosterone is suppressed substantially in men and falls far below the average clinical reference ranges. We did not observe the same effect in women, which further confirms that men and women have different mechanisms of hormonal disturbance caused by opioids. We also showed that methadone did not affect testosterone differently than other opioids, suggesting that the observed testosterone suppression is not attributed to methadone alone, but to opioids in general. Despite the high levels of confounding and bias across included studies, this review has important clinical implications. It can potentially be used to inform both healthcare providers and individuals prescribed opioids about the endocrine disrupting effects of opioid use to make informed decisions about treatment options and other alternatives to be considered.

Shifting the focus to other biological mechanisms involved in opioid addiction, Chapter 6 explored the genetic contribution to methadone treatment for opioid addiction with a specific focus on genes that are involved in addictive and reward behaviors, namely *BDNF* and *DRD*2. Our initial intent was to examine whether there is an existing sex-specific genetic effect on methadone treatment response, however we were unable to answer this question due to insufficient power for subgroup analyses among men and women. Therefore we tested the association between the *BDNF rs6265* and *DRD2 rs1799978* genetic variants and continued opioid use in our total sample of GENOA participants. We demonstrated that the genetic variation across these particular genes is

not linked to methadone treatment response, contrary to what the evidence suggests (34, 35). Despite the inability to assess sex differences in this study, we have nevertheless highlighted the importance of investigating the collective polygenic effect on opioid use in an adequately powered sample, while also exploring other non-genetic factors that may contribute to continued opioid use (i.e. medical or psychiatric comorbidity, social circumstances, life stressors, etc.), some of which are likely sex-related.

Considering the limitations outlined by the initial systematic review in Chapter 3 and in subsequent chapters, we chose to examine sex differences in a large, updated sample of 503 current opioid users receiving MMT. In Chapter 7, we included the final paper of this six-part series that focused on sex- and gender-specific patterns across clinically relevant domains including substance use behavior, health status, and social functioning. In our sample, women were younger, had children, were current smokers, had higher rates of benzodiazepine use, more frequent physical and psychological health problems, family history of psychiatric disorders, more partner conflict, and began regular use of opioids through a physician prescription. In comparison, men were more likely to be employed, and to use cannabis and amphetamines.

Compared to previous findings from the systematic review that was outlined in Chapter 3, patterns in employment and criminal activity are consistently more common among men. However we observed important changes in substance use. Women are more likely to have initiated their substance dependence through prescription opioids, presumably because of their higher rates of chronic pain (18), yet they are less likely to use cannabis than men, which is in agreement with earlier investigations (36, 37). Although both

alcohol and cocaine use continue to be problematic among opioid users in MMT, the disparity that has been seen in the past, with men more likely to abuse alcohol (2, 6) and women more likely to abuse cocaine (36-38), is now less apparent.

8.3 Implications and future directions

8.3.1 Implications for research

Sex and gender are important factors that contribute to the comprehensive understanding of addiction, yet research in this area remains largely inadequate and underdeveloped. First and foremost, we encourage future research in addiction to incorporate sex and gender into their study design and analysis to elucidate the role of these important health determinants in addiction and treatment on a broader level. We also recommend that sex and gender differences are examined in current populations of opioid users employing appropriate methodologies and objective measures to ensure that the available evidence is accurate and up-to-date. Furthermore, we suggest investigating sex and gender differences using interdisciplinary perspectives in order to promote insight into novel therapeutic targets.

Specific research goals may include examination of domestic or intimate partner violence among men and women, as well as how child custody may be involved in the motivation to enter treatment or remain abstinent from opioids. Based on our findings, it would be important for future research to also assess whether testosterone returns to baseline levels

after opioid lowering or detoxification, and also if testosterone replacement is likely to be chosen by patients over opioid lowering or detoxification. Additionally, an investigation of female sex hormones is warranted to evaluate specifically how they are influenced by opioids and what effect this may have on treatment outcomes.

Future studies should focus on evaluating the efficacy of sex- and gender-sensitive treatment programs in comparison to standard gender-neutral interventions in adequately designed randomized trials to determine whether these specific approaches lead to improved treatment outcomes. On the same note, conducting cost-effectiveness studies of specialized treatment for men and women would likely elucidate the financial benefits of such programs with respect to treatment-related and societal costs.

Patient-important treatment outcomes among men and women are also a point of interest that is often overlooked, yet would provide valuable information for the development of personalized patient-centered treatment strategies. Evaluating knowledge of sex and gender differences among clinical staff and incorporating knowledge translation to improve awareness and education of these concepts may also provide benefit to all individuals that are involved in the treatment of opioid addiction including clinicians, allied health professionals, and patients themselves.

8.3.2 Implications for practice

First, it is necessary to implement appropriate education and prevention strategies for all opioid users, but also tailored to men and women individually. We have shown that

women are more likely to be exposed to prescription opioids than men, mainly due to presenting to care providers for treatment for pain and other medical conditions. Given this knowledge, caution should be exercised in prescribing opioids to women especially, who are more susceptible to opioid use disorders and related risks. Alternative treatment and therapeutic options should be considered in the management of pain conditions that require the use of opioid analgesics.

Women also experience a greater burden of disease from opioid dependence with respect to medical problems, health outcomes, and social impairment, elucidating the need for interventions that address these core areas of functioning for women. Behavioral therapy and social services can supplement current pharmacological treatment programs in order to develop an integrated patient-centered model of care. Emphasizing the need for fundamental services, such as vocational counselling, childcare and parenting assistance, domestic violence counselling, medical assistance, and smoking cessation among women is likely to significantly improve the treatment and management of opioid use disorder and related harms. Efficacy of women-sensitive substance abuse treatment programs has been evidenced in a number of studies (40, 41) and these programs should be made accessible across various geographic jurisdictions on both a provincial and national scale. Similar strategies should be implemented for men in treatment, who experience distinct sex- and gender-specific characteristics of addiction (i.e. testosterone suppression, cannabis use, etc.). Programs to help decrease recidivism and incarceration may also be helpful.

8.3.3 Implications for policy

Based on the documented changes in the illicit use of opioids among men and women, modifications to available prevention and treatment programs are needed. Given the dramatic increase in non-medical prescription opioid use, it is important to re-evaluate current policies surrounding prescribing practices. There are a number of solutions that can be implemented to manage this growing public health issue. Research on the role of pain as a predictor of treatment response using standardized measurement tools when possible is required to inform clinical practice. We also encourage the utilization of currently available recommendations and the implementation of alternative non-opioid therapies for managing pain conditions. Furthermore, effective strategies to reduce the availability of opioids or modify prescribing patterns are essential, in addition to allocating resources for monitoring prescription opioid misuse.

Approaches and strategies for opioid substitute treatment also require adjustments, given the changing trends in illicit opioid use. Current treatments were initially developed based off research from the 1990s targeting male heroin users, and thus their applicability to the current population of opioid users, half of which are now women, is questionable. Furthermore, current treatment guidelines have been developed from selective study findings and are therefore not informed through systematic summaries of the evidence. Guidelines for the treatment of opioid addiction with methadone require a thorough re-evaluation to incorporate these sex and gender influences using appropriate evidence-based methods.

8.4 Concluding remarks

The identification of sex and gender differences in the management and treatment of opioid addiction is critical to our understanding of the addiction profile of men and women. In this thesis, we have demonstrated that men and women are differentially affected by opioid addiction and experience sex- and gender-specific challenges throughout the course of methadone treatment.

Specifically, we showed that (1) women were less likely than men to report alcohol use, employment, or legal involvement, but were more likely to misuse amphetamines; (2) men, but not women, experience a significant dose-dependent reduction in testosterone level in response to methadone; (3) men that use opioids, but not women, had significant suppression in testosterone level compared to controls, and methadone did not affect testosterone differently than other opioids; (4) *BDNF rs6265* and *DRD2 rs1799978* genetic variants were not significantly associated with continued opioid use while on methadone maintenance treatment; and (5) women were younger, had children, were current smokers, had higher rates of benzodiazepine use, more frequent physical and psychological health problems, family history of psychiatric disorders, more partner conflict, and began regular use of opioids through a physician prescription. In comparison, men were more likely to be employed and to use cannabis and amphetamines.

We have drawn attention to issues within the addiction literature and highlighted problems that pervade our currently established treatment regimens. Furthermore, we

presented a comprehensive update and descriptive profile of sex- and gender-related factors characterizing opioid users receiving methadone treatment in Canada, clarifying previous gaps in the literature. Using various research methodologies, we have made substantial contributions to the literature on opioid addiction treatment among men and women from a multidisciplinary standpoint, and we have shed light on a promising area of research for the advancement of clinical practice and health policy for men and women living with opioid addiction.

8.5 References

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Appendix I

SYSTEMATIC REVIEWS

PROTOCOL Open Access

Sex differences in outcomes of methadone maintenance treatment for opioid addiction: a systematic review protocol

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Abstract

Background: Use of methadone for the treatment of opioid addiction is an effective harm-reduction approach, although variability in treatment outcomes among individuals has been reported. Men and women with opioid addiction have been known to differ in factors such as opioid use patterns and characteristics at treatment entry; however, little has been reported about differences in methadone treatment outcomes between men and women. Therefore, we present a protocol for a systematic review which aims to provide a summary of existing literature on sex differences in outcomes of methadone treatment for opioid addiction.

Methods/Design: Electronic search of PubMed/MEDLINE, EMBASE, PsycINFO, and CINAHL databases will be conducted using *a priori* defined search strategy. Two authors (MB and BBD) will independently screen potential articles for eligibility using pre-determined inclusion and exclusion criteria and extract key information using a data extraction form designed for this study. Discrepancies will be resolved using a third party (ZS). The primary outcome will be sex differences in response to treatment defined as abstinence from illicit opioid use. We will also assess sex differences in treatment outcomes including treatment retention, remission status post-treatment, polysubstance abuse, methadone dose, drug-related adverse events, health status, psychological status, mortality, criminal activity, high risk sexual behavior, social support/relations, and employment. A meta-analysis will be conducted if possible; risk of bias and overall quality of evidence will be assessed to determine confidence in the estimates.

Discussion: We anticipate that this review will highlight how men and women differ in methadone treatment outcomes and allow us to generate conclusions that can be applied to treatment in a clinical setting.

Systematic review registration: PROSPERO CRD42013006549

Keywords: Opioid addiction/dependence, Methadone maintenance treatment, Sex differences, Systematic review, Protocol

Background

The use of illicit opioids continues to pose a problem both at the individual and societal levels, even more so with the exponentially increasing rates of prescription opioid use in North America [1-3], increasing the risk of development of opioid addiction. Infection [4], medical and psychiatric comorbidity [5], polysubstance use [5], and criminal behavior [6] are among a few of the risks associated with opioid addiction, in addition to a rise in opioid-related deaths [2].

Methadone maintenance treatment (MMT) is the most widely used harm-reduction approach to treating opioid addiction [7]. Methadone is a synthetic analgesic with the ability to inhibit the euphoric effects of opioids and provide relief of withdrawal symptoms due to its longer duration of action [8]. MMT began to receive attention shortly after its development in the early 1940s, which led to the opening of methadone clinics across the world, and later in North America [9]. Since then, the number of patients entering treatment has grown about fivefold [10].

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It is estimated that there are >30,000 registered methadone patients in Ontario, Canada, alone [11], which represents approximately 25% of Ontario's illicit opioid user population [10]. Although progress has been made with MMT, it is evident that it is still not widely used in opioid addiction populations on the larger scale.

Despite the documented effectiveness of methadone as a substitute opioid therapy, methadone has also been reported to produce a large inter-individual variability in response [12], adding an additional layer of complexity to treatment strategies. Traditionally, the population of individuals suffering from opioid addiction has been primarily men, with most studies at the time focusing on opioid-dependent men [13,14]. In the most recent 30 years, there has been an increase in the number of women with opioid addiction [15], which calls for a re-examination of literature on sex differences in opioid addiction in general, and response to MMT specifically.

Sex differences in opioid addiction [10,16-18] and methadone treatment [16,19-22] have been reported; significant sex differences in age, ethnicity, marital status, education, and employment [23], as well as patterns of drug use [21], treatment entry [24], and social support [25] have been identified. Women are typically younger, married, unemployed, and have an earlier onset age of heroin use [23]. Men often use opioids for recreational purposes [16] and have a slower disease progression than women [24]. Additionally, men report earlier treatment entry, more frequent utilization of substance abuse treatment, and fewer psychological and medical problems at treatment admission compared to women [17]. It is becoming clear that treatment needs for men and women are not the same, which points to a demand for separate treatment strategies. The available studies on opioid addiction in the literature are often limited to men [26] or specific ethnic groups, focus on clinical profiles prior to or at treatment entry [16,19-22], or investigate methadone dose as a single outcome of treatment in association with other factors [27-30]. Sex differences have also been examined in opioid addiction patients treated with methadone in association with factors including prescription opioid use [31], drug use patterns [20], drug treatment utilization [32], psychiatric comorbidity [5,33], smoking outcomes [34], and quality of life [35]; however, little has been reported about differences in methadone treatment outcomes between men and women. Few studies have investigated methadone treatment retention, response, remission, adverse events, health status, social relations, criminal activity, and mortality with a specific focus on sex difference, providing inconsistent results and leaving a large gap in the literature with regards to sex differences in response to MMT.

It is also evident that men and women vary in multiple aspects of addiction characteristics and should therefore be provided sex-specific treatment. Implementation of separate treatment approaches for men and women may prove to be a more efficient way to manage this disorder and eventually improve patient-related health outcomes. This review aims to determine whether or not men and women differ in methadone treatment outcomes.

Objectives

The objective of this review is to summarize the current status of literature regarding sex differences in methadone treatment outcomes by systematically reporting the available research to date. Specifically, we aim to:

1. Assess how men and women differ in methadone outcomes related to drug-use behavior, health status, and sociobehavioral functioning.
2. When suitable, combine the statistical outcomes in a summary estimate through meta-analytical approaches.
3. Critically appraise the literature and determine areas that require further investigation.

Methods/Design

Inclusion and exclusion criteria

This systematic review will include completed randomized controlled trials (RCTs) and observational studies of methadone treatment outcomes in men and women. Included studies will focus primarily on sex differences, as opposed to studies on separate populations of men or women. Included studies must also have been conducted in the context of methadone treatment for opioid addiction. Studies including patients that are undergoing a substitute opioid therapy other than methadone (that is, buprenorphine/naloxone, naltrexone) or using methadone for the purpose of detoxification (not maintenance) will be excluded. Studies investigating patient subpopulations such as pregnant women or incarcerated individuals will be excluded as they are too specific to represent the overall population of opioid-dependent individuals and may not allow for the application and generalizability of our findings to community samples. Sex differences in these populations may also be influenced by their environment, leading to a high potential for confounding and bias in the outcomes studied. Patients that are using methadone for the treatment of a condition other than opioid addiction (that is, chronic pain) will also be excluded. Participants shall include both men and women who are receiving methadone treatment for a diagnosis of opioid dependence. No other limitations will be applied (including age or ethnicity) as our intent is to retrieve all articles on sex differences in methadone treatment without restrictions based on population characteristics. The primary outcome of this review will be the presence of sex differences in methadone treatment response, defined as abstinence

from illicit opioid use and measured through self-report and/or urinalysis. Sex differences in treatment outcomes will also be assessed with respect to three life domains: drug use-related behavior, health status, and sociobehavioral functioning. These outcomes include treatment retention/duration, remission status post-treatment, polysubstance abuse, methadone dose, drug-related adverse events, health status, psychological status, mortality, criminal activity, high risk sexual behavior, social support/relations, and employment. A complete list of how these outcomes are described, defined, and measured in the literature is available in Table 1.

Search strategy

We shall identify all studies relevant to this review with no language or time restraints. We will search the PubMed/MEDLINE, EMBASE, PsycINFO, and CINAHL databases for relevant articles. Relevant search terms and their medical subject heading (MeSH) equivalents will be used in varying combinations; refer to Table 2 for the complete search strategy. In order to maximize the number of relevant articles retrieved, treatment outcomes will not be included in the search. We will use a wide search to include titles, abstracts, and keyword fields to avoid missing important articles whose title may not reflect the content of the article. Articles will be excluded by limiting the search to humans. We will also manually review reference lists of included studies for studies that may have been missed in the initial search. Grey literature will not be reviewed as we are looking for complete published data only.

Data screening

Two independent raters (MB and BBD) will screen all citations and abstracts retrieved using the search strategy and identify all eligible articles. Articles that meet the predetermined criteria will be included for full-text review. Disagreements at any phase of the review process will be resolved by discussion or, in the case where a consensus is not reached, a third independent rater (ZS) will determine eligibility. Ineligible studies will be excluded from the review and reasons for exclusion will be recorded. Inter-rater agreement will be calculated using the Kappa statistic [48] for each phase of screening. Authors will be contacted directly if further data clarification is needed.

Data extraction

The two authors (MB and BBD) will independently extract data from the studies using a pre-established pilot-tested data extraction form (see Additional file 1). Information obtained will include the author and year of publication, city and country of publication, title of article, journal name, study design, and description of sample population, including total number of men and women study participants, mean age (total and men versus women), and ethnicity. Primary and secondary outcomes, outcome measures, statistical analyses, results, and conclusions will also be recorded. In the case of missing or incomplete data, authors will be contacted for further details. Data will be combined to produce a summary estimate in a meta-analysis if the extracted data allows it.

Assessment of quality

Two authors (MB and BBD) will independently assess the risk of bias of included studies using the Newcastle-Ottawa Scale (NOS) [49] for observational studies and the Cochrane Collaboration's tool [50] for assessing risk of bias in RCTs. For observational studies, two authors (MB and BBD) will independently assess the risk of bias of each included study using an adapted version of a modified NOS, specific to the context of this review. This will include seven questions spread across four domains of evaluation; methods for selecting study participants (selection bias), methods to control for confounding (performance bias), statistical methods (detection bias), and methods for measuring exposure and outcome variables (information bias). Risk of bias is measured on a scale of 0 (high risk of bias) to 3 (low risk of bias) and a specific description with examples of both high and low bias is provided. Items regarding selection of participants (representativeness of sample) and ascertainment of outcome (objective versus subjective measures) were retained, while other items relating to the comparability of groups and adequate follow-up for cohort and case-control studies were removed as these were not directly applicable to our topic of interest. We also introduced categories that emphasize statistical methods, confounding effects, and reporting of data to ensure that bias in methodology is assessed. These scales will be used to measure the risk of bias on a per study basis or categorized by domain to develop a general conclusion about the sources of bias in the studies included in this review (see Additional file 2). Cochrane's tool for assessing risk of bias in RCTs includes seven domains; random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias. Each of these domains will be evaluated according to high or low risk of bias and will also be assessed on a per study or per domain basis. If a meta-analysis is possible, we will use the Grading of Recommendations, Assessment, Development, and Evaluation framework to rate the quality of evidence through investigation of risk of bias, imprecision (random error), inconsistency, indirectness, and publication bias. We will then summarize the evidence for individual outcomes in summary of findings tables, which will allow for assessment of our confidence in the estimates.

Table 1 Definition of methadone treatment outcomes for assessment

Outcome	Definition	Measurement of variable	Statistics	Studies
Drug use-related behavior				
Response to treatment	Abstaining from illicit opioid use throughout treatment duration	Urine screening	Percentage	[36-39]
		Self reported opioid use (daily or weekly) over specified time period	Mixed model ANOVA Cochran-Mantel-Haenszel statistic	
Treatment retention or duration	Proportion of participants completing treatment; days in treatment from first to last day of therapy	Number of days patient remains in treatment	Cox proportional hazards model	[36-38]
		Proportion of patients retained in treatment for pre-specified duration of study	Kaplan-Meier survival curve	
Remission status post-treatment	Abstinence from use of illicit opioids at follow-up	Urine screening	t-test	[37,39-41]
		Self-reported opioid use (any) after treatment	2x2 factorial ANOVA	
Polysubstance use	Use of at least two (non-opioid) substances throughout the course of treatment	Self-reported use of substances daily or weekly or in last 30 days	Percentage Fischer's Exact Test	[37,38]
		Net reduction in proportion of drug abuse after specific duration		
Health and methadone-related outcomes				
Methadone dose	Average daily methadone dose	Milligrams/day	Difference in means (SD)	[38]
		Mean methadone dose after specific duration in treatment		
Drug-related adverse events	Reaction to treatment drug	Interview/physical examination	Percentage	[17]
		Number of hospitalizations	t-test	
Health status	Change in health status during course of therapy	Interview/physical examination	ANOVA	[17,40]
		Number of hospitalizations		
Psychological status	Comorbidity of psychiatric disorders	Self-reported psychiatric problems	Percentage	[17,42-44]
		Number of reported symptoms	Relative risk	
		Validated psychiatric assessments	ANOVA	
			Chi-square	
Mortality	Treatment-related death or illicit drug use mortality	Mortality causes	Standardized mortality ratio (SMR) Kaplan-Meier survival curve	[45]
		Number of deaths		
		Annual death rate per year of age		
Sociobehavioral functioning				
Criminal behavior	Involvement in illegal activities, arrests, or incarcerations throughout treatment or at follow-up	Interview/self-report	Percentage	[17,37,39]
		Current legal status	t-test	
			ANOVA	
High-risk sexual behavior	Involvement in behaviors that put the patient at high risk for HIV and other infections	Use of injection methods (30 days prior)	Weighted least-squares estimation procedure	[46,47]
		Number of sex partners	Repeated measures ANOVA	
		Incidence of unprotected sex		
Social relations/support	Patient's relationship status and conception of his/her relationship with others	Self-report	ANOVA	[17]
		Number of close friends/family		
		Marital and family status		
		Ratings of interactions		
Employment	Status of employment and evidence of financial income	Change in self-reported employment status during treatment	Percentage Difference in means (SD)	[17,37,39,40]
		Employment status after treatment		

ANOVA, analysis of variance.

Table 2 Search strategy for retrieval of relevant articles from multiple databases

Database	Search strategy
MEDLINE n = 401	1. Opioid-related disorders/dt, rh, th [Drug Therapy, Rehabilitation, Therapy]
	2. Opiate substitution treatment/
	3. Methadone/
	4. Sex Characteristics/
	5. sex differences.m_titl.
	6. gender differences.m_titl.
	7. sex.m_titl.
	8. male.m_titl.
	9. female.m_titl.
	10. men.m_titl.
	11. women.m_titl.
	12. 1 or 2 or 3
	13. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11
	14. 12 and 13
	15. limit 14 to humans
EMBASE n = 180	1. Opioid-related disorders/dt, rh, th [Drug Therapy, Rehabilitation, Therapy]
	2. Opiate substitution treatment/
	3. Methadone/
	4. Sex Characteristics/
	5. sex differences.m_titl.
	6. gender differences.m_titl.
	7. sex.m_titl.
	8. male.m_titl.
	9. female.m_titl.
	10. men.m_titl.
	11. women.m_titl.
	12. 1 or 2 or 3
	13. 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11
	14. 12 and 13
	15. limit 14 to humans
PsycINFO n = 241	1. exp Methadone Maintenance/ or exp Methadone/
	2. exp Human Sex Differences/
	3. sex.m_titl.
	4. male.m_titl.
	5. female.m_titl.
	6. men.m_titl.
	7. women.m_titl.
	8. 2 or 3 or 4 or 5 or 6 or 7
	9. 1 and 8
	10. limit 9 to humans
CINAHL n = 23	1. Opioid abuse (TX All Text)
	2. Methadone (TX All Text)
	3. Methadone treatment programs (MJ Word in Major Subject Heading)
	4. Gender differences (TX All Text)
	5. Sex differences
	6. 1 or 2 or 3 and 4 or 5
	7. limit 6 to human

Statistical analyses and heterogeneity

The results of this systematic review will be reported in a narrative and informative manner; we will discuss issues of study design and statistical analysis methods of the included studies to determine which studies are most informative and reliable. Where possible, we will assess the studies in a combined statistical manner using meta-analysis. The Kappa statistic will be used to measure level of agreement between independent raters. For dichotomous outcomes, we will compute pooled odds ratios using the Mantel-Haenszel random effects model, in which the model is able to estimate between study variation through an evaluation of each study's final results and a Mantel-Haenszel fixed effect meta-analysis result.

For the summary estimates, we will employ a random effects model, which assumes variation between studies and their respective effect sizes. The nature of observational studies in this population is highly variable, therefore heterogeneity will be accounted for and will allow us to develop aggregate estimates. We will assess the participants, methods, and results of included studies for heterogeneity, which will allow us to determine whether results can be compared across studies. Possible sources of heterogeneity include age groups, study design, methodology, and definition of outcome. In case of heterogeneity, subgroup analyses according to these different categories will be performed. Included studies will be presented in the form of a forest plot. We will use Review Manager 5.1 software (The Cochrane Collaboration, London, UK) for all statistical analysis and results will be presented using 95% confidence intervals.

Presenting and reporting of results

We will report the systematic review according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines [51]. A flow diagram will be used to summarize the selection process of studies at each phase and summary tables will be used to report study characteristics and presence of sex differences per methadone outcome. Publication bias will also be examined and assessed using Egger's plot.

Discussion

Using evidence from this systematic review, we expect to draw conclusions regarding the presence of sex differences in outcomes of MMT for opioid addiction. This review will not only provide us with summary evidence for which we can objectively make inferences about the current status of literature, it will also allow us to critically evaluate the methodological quality and risk of bias present in the available evidence. The literature on methadone treatment focuses primarily on men and little is known about women or how the sexes compare. We anticipate that this review will highlight how men and women differ in methadone treatment outcomes and allow us to generate conclusions that can be applied to treatment in a clinical setting. We will encourage healthcare professionals to make use of this information and approach men and women dealing with opioid addiction using different treatment strategies, catered to each sex specifically. We are hopeful that this review will ultimately establish the need for further examination into sex differences in methadone treatment in an effort to improve treatment prognosis for individuals dealing with this complex disorder.

Additional files

Additional file 1: Data extraction form for included studies. This form contains the information which we intend to extract from included studies during the data extraction process. It includes general study information, methods and description of sample, outcomes, and results.

Additional file 2: Adapted version of a modified Newcastle-Ottawa Scale (NOS) for single use in specific context. This form demonstrates the modified version of the NOS categorized by domain of evaluation and supported with examples of levels of bias. Observational studies will be assessed on risk of bias based on this form.

Abbreviations

MMT: methadone maintenance treatment; NOS: Newcastle-Ottawa Scale; RCT: randomized controlled trial.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MB: conception and design, manuscript writing, critical revision, development of data extraction forms and quality assessment tools, and final approval of manuscript. BBD: interpretation and methodology, help with development of quality assessment tool, critical revision, and final approval of manuscript. RA: consultations for search strategy and quality assessment, critical revision, and final approval of manuscript. MS: interpretation of literature, critical revision, and final approval of manuscript. LT: methodology, critical revision, and final approval of manuscript. ZS: conception and design, critical revision, methodology, and final approval of manuscript. All authors read and approved the final manuscript.

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SYSTEMATIC REVIEW FULL-TEXT EXTRACTION FORM **Initials:**

A. STUDY INFORMATION **Study ID:**

Last Name of First Author, Initial: **Year of Publication:**

Title of Article:

Journal Name: **City, Country:**

B. METHODS

Study Design: **# of Participants:**

Description of Sample Population (Age, Ethnicity):

C. RESULTS

MMT-related outcome(s):

Statistical Analysis:

Multiple Testing Error Accounted For (If so, indicate how):

How did the study handle missing data?

Results:

Sex Differences Reported:

Inclusion [must check all to be included]

- ☐ The study is looking at MMT patient populations
- ☐ Study participants on MMT for the treatment of opioid addiction
- ☐ The study participants are human
- ☐ The study has been completed
- ☐ The study is in English

Exclusion [exclude study if any of the following is checked]:

- ☐ Incomplete studies
- ☐ Abstract only
- ☐ The primary focus of the study is not MMT related
- ☐ The study focuses on a different SOT (i.e. suboxone)
- ☐ The study is on animal populations

Comments:

Adapted version of a modified Newcastle-Ottawa Scale for single use in specific context

Modified Newcastle-Ottawa Scale (NOS)

Legend

0 = Definitely no (high risk of bias)
1 = Mostly no
2 = Mostly yes
3 = Definitely yes (low risk of bias)

Domain of evaluation: Methods for selecting study participants (*i.e. Selection bias)*

Is the source population (cases, controls, cohorts) appropriate and representative of the population of interest?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: A consecutive sample or random selection from a population that is representative of the condition under study.

Example of moderate risk of bias: A consecutive sample or random selection from a population that is not highly representative of the condition under study.

Example of high risk of bias: The source population cannot be defined or enumerated (i.e. volunteering or self-recruitment).

Domain of evaluation: Methods to control confounding (*i.e. Performance bias)*

Is the sample size adequate and is there sufficient power to detect a meaningful difference in the outcome of interest?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: Sample size was adequate and there was sufficient power to detect a difference in the outcome.

Example of high risk of bias: Sample size was small and there was not enough power to test outcome of interest.

Did the study identify and adjust for any variables or confounders that may influence the outcome?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study identified and adjusted for all possible confounders that may influence estimates of association between exposure and outcome (*i.e. Was the patient being treated for a medical condition such as chronic pain and was being prescribed opioids while on methadone treatment?*)

Example of moderate risk of bias: The study identified and reported possible variables that may influence the outcome but did not explore the interaction.

Example of high risk of bias: The study either did not report any variables of influence or acknowledge variables of influence when it was clear they were present.

Domain of evaluation: Statistical methods (*i.e. Detection bias)*

Did the study use appropriate statistical analysis methods relative to the outcome of interest?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study reported use of appropriate statistical analysis as required (*i.e. adjusting for an unbalanced distribution of a specific covariate among sexes, or correcting for multiple testing error*)

Example of moderate risk of bias: The study either used correct statistical methods but did not report them well, or used the incorrect methods but reported them in detail.

Example of high risk of bias: The study did not use appropriate statistical analysis as required (*i.e. did not adjust for an unbalanced distribution of a specific covariate among sexes, or correct for multiple testing error when necessary*) or did not report them adequately.

Is there little missing data and did the study handle it accordingly?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study acknowledged missing data to be less than 10% and specified the method of handling it.

Example of moderate risk of bias: The study either had greater than 15% but they specified the method they used to handle it.

Example of high risk of bias: The study had greater than 15% missing data and did not handle it at all.

Domain of evaluation: Methods for measuring outcome variables (*i.e. Information bias)*

Is the methodology of the outcome measurement explicitly stated and is it appropriate?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study provides a detailed description of the outcome measure(s) which are appropriate for the outcome of interest.

Example of moderate risk of bias: The study provides a somewhat complete description of outcome measurements and they are justified.

Example of high risk of bias: The study provides limited information on the methods of measuring the outcome and the measure is not appropriate considering the outcome.

Is there an objective assessment of the outcome of interest?

0	1	2	3
(high risk of bias)			(low risk of bias)

Example of low risk of bias: The study used objective methods to discern the outcome status of participants (*i.e. laboratory measurements, medical records*).

Example of moderate risk of bias: The study relied on subjective data as the primary method to discern outcome status of participants (*i.e. self-report*).

Example of high risk of bias: The study had limited reporting about assessment of outcomes.

Appendix II

Supplementary Information (online only)

Table S1. Completed PRISMA checklist for adherence to reporting standards

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6 + In protocol
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	In protocol
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7 + In protocol

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7 + In protocol
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	In protocol
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7 + In protocol
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7 + In protocol
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7 + In protocol
Section/topic	**#**	**Checklist item**	**Reported on page #**
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7 + In protocol
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9 + Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9 + Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	9 + Tables S2 and S3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9-11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9-11 + Table 2 + Figs. 2-5
Risk of bias across	22	Present results of any assessment of risk of bias across studies (see Item 15).	9 + Tables

studies			S2 and S3
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	12
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	12-13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

Table S2. Risk of bias assessment for observational studies

		SELECTION BIAS	PERFORMANCE BIAS		DETECTION BIAS		INFORMATION BIAS		
Author, Last name	Year	Is the **source population** representative?	Is the **sample size** adequate and is there sufficient **power**?	Did the study adjust for **confounders**?	Did the study use appropriate **statistics** for outcome of interest?	Is there little **missing data** and was it handled appropriately?	Are the **methods or outcome measurements** explicitly stated and is it appropriate?	Is there an **objective assessment** of outcomes?	Total (out of 21)
Anglin	1987	2	1	1	2	1	1	1	9
Brown	1993	1	1	1	2	2	2	1	10
Camacho	1996	2	2	1	2	2	3	1	13
Chatham	1999	2	2	1	2	2	3	3	15
Grella	2012	1	2	1	1	1	2	1	9
Haug	2005	1	1	1	2	2	3	3	13
Hser	1990	2	2	1	2	2	1	0	10
Jimenez-Trevino	2011	1	1	1	2	0	2	1	8
Marsh	1986	1	1	1	1	2	1	0	7
Mulvaney	1999	2	2	1	2	2	2	2	13
Peles	2006	2	1	1	2	1	3	2	12
Rutherford	1997	1	1	1	2	1	2	0	8
Savage	1980	2	2	1	1	2	1	0	9
Schiff	2007	2	2	1	2	1	1	3	12
Schilling	1991	1	1	1	2	2	2	0	9
Steer	1980	2	1	2	3	2	2	0	12
Stenbacka	2003	2	2	1	2	1	3	3	14
Webber	1999	1	2	2	2	2	2	2	13

0 = Definitely no; 1 = Mostly no; 2 = Mostly yes; 3 = Definitely yes

Table S3. Risk of bias assessment for RCTs

Author, Last name	Year	1. Was the **allocation sequence generated** adequately?	2. Was allocation **concealed** adequately?	3. Was **knowledge of intervention** adequately prevented?	4. Were **incomplete data** adequately addressed?	5. Are reports of the study free of **selective outcome reporting**?	6. Was the study free of other problems that could put it at **high risk of bias**?
Jones	2005	1	1	1	1	1	1
Schottenfeld	1998	1	1	1	1	1	1

1 = Low risk of bias

Figure S1. Cohort and randomized controlled studies measuring illicit opioid use during treatment

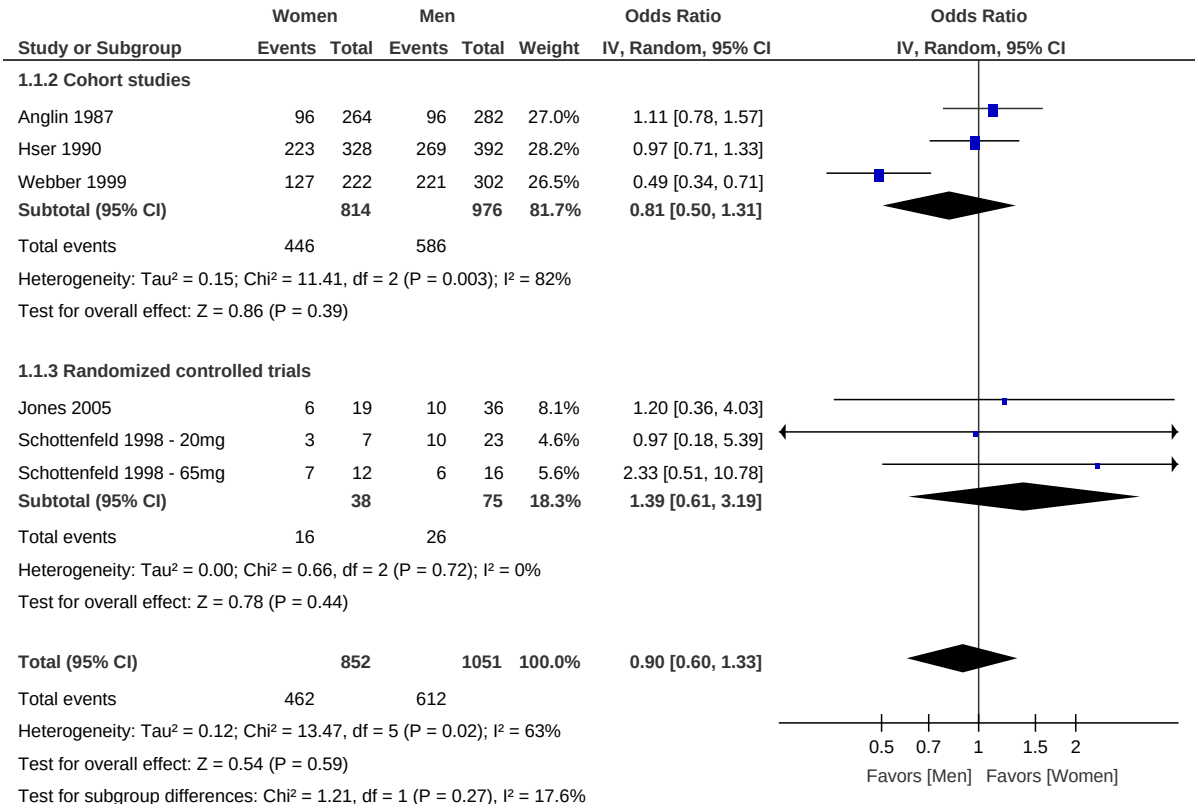


Figure S2. Number of subjects with 12-20 months of treatment retention

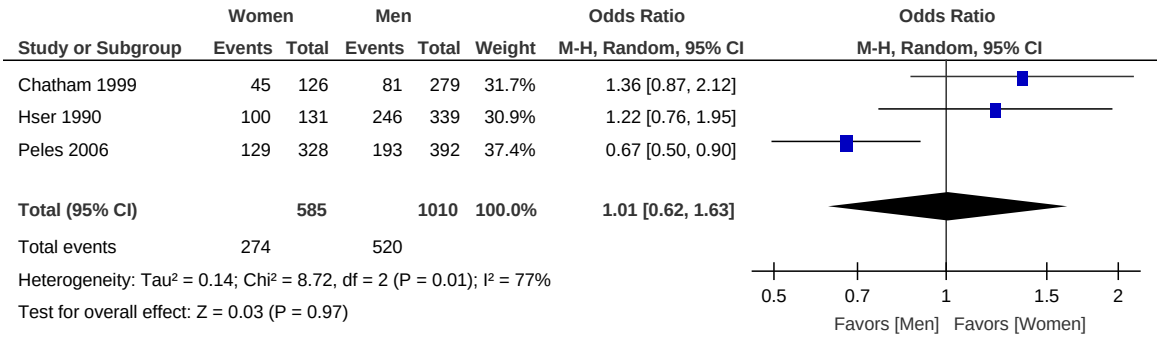


Figure S3. Benzodiazepine use over the last six months measured using urine toxicology

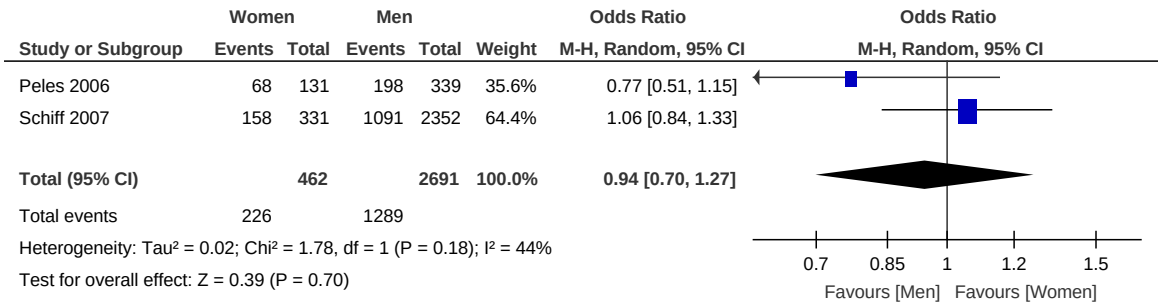


Figure S4. Cannabis use over the last six months measured using urine toxicology

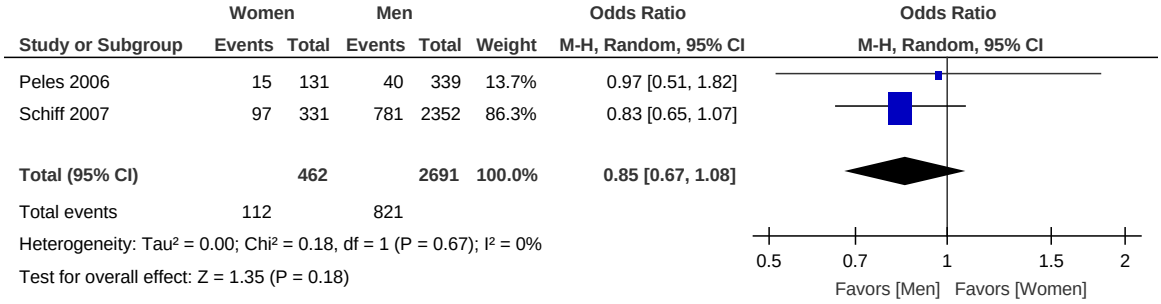


Figure S5. Cocaine use over the last six months measured using urine toxicology

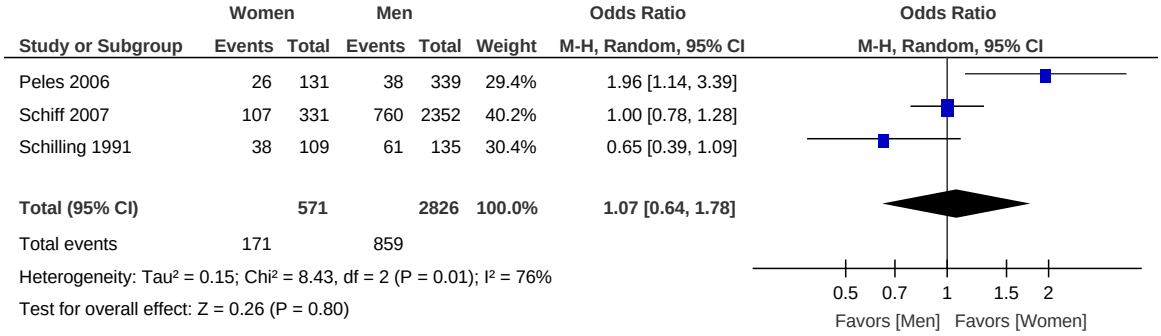


Figure S6. Mean methadone dose after 6-12 months in treatment (mg/day)

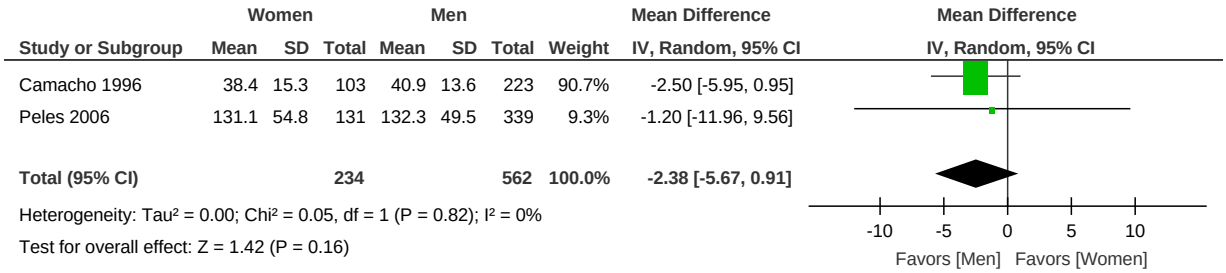


Figure S7. Number of subjects currently married or living with spouse

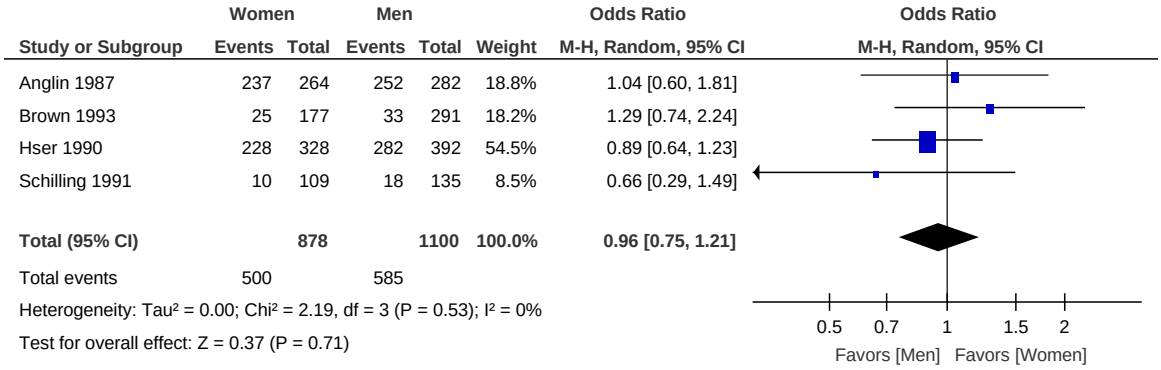
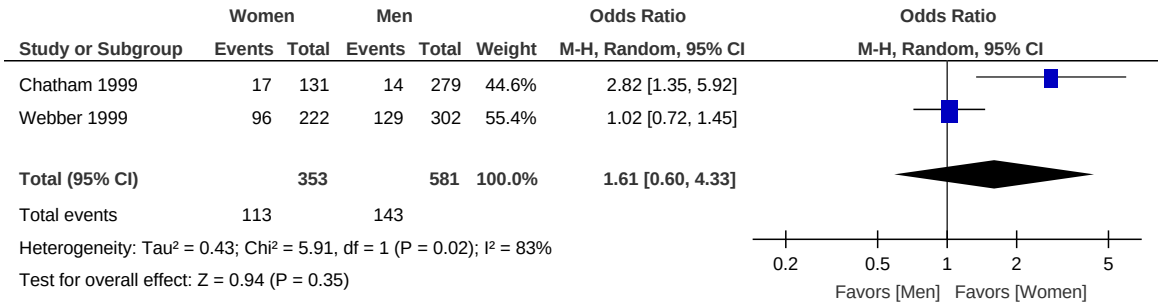


Figure S8. Number of deaths reported at one year after treatment completion



Appendix III

SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
ADDICTION
QUALITY OF LIFE

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Methadone induces testosterone suppression in patients with opioid addiction

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Sex hormones may have a role in the pathophysiology of substance use disorders, as demonstrated by the association between testosterone and addictive behaviour in opioid dependence. Although opioid use has been found to suppress testosterone levels in men and women, the extent of this effect and how it relates to methadone treatment for opioid dependence is unclear. The present multi-centre cross-sectional study consecutively recruited 231 patients with opioid dependence from methadone clinics across Ontario, Canada between June and December of 2011. We obtained demographic details, substance use, psychiatric history, and blood and urine samples from enrolled subjects. The control group included 783 non-opioid using adults recruited from a primary care setting in Ontario, Canada. Average testosterone level in men receiving methadone treatment was significantly lower than controls. No effect of opioids including methadone on testosterone level in women was found and testosterone did not fluctuate significantly between menstrual cycle phases. In methadone patients, testosterone level was significantly associated with methadone dose in men only. We recommend that testosterone levels be checked in men prior and during methadone and other opioid therapy, in order to detect and treat testosterone deficiency associated with opioids and lead to successful methadone treatment outcomes.

Opioid dependence has previously been observed in men[1,2], however the increased prevalence of prescription opioid drug abuse has led to an increase in opioid use and dependence in women[3]. This trend has sparked interest in the sex-related aspects of the disorder. To date, sex differences have been reported in many aspects of opioid dependence and treatment[4–10] leading to the need for separate addiction treatment profiles for men and women.

Sex hormones are often studied as the biological basis for sex differences due to their role in central nervous system regulation, implicating the endocrine system in the pathophysiology of substance use disorders and addictive behaviour[11]. The emerging research on sex hormones in addiction has shed light on the association between testosterone and specific addictive behaviours in men and women, including impulsivity, aggression, risk-taking, and sensation-seeking[12,13]. This provides evidence for the importance of testosterone in substance use disorders including opioid dependence.

Reviews of the literature on testosterone in chronic opioid use report an opioid-induced deficiency in androgen function[14–16], significantly lower than normative levels of testosterone seen in the clinical literature (average range: 300–1000 ng/dL or 10–35 nmol/L for men and 20–85 ng/dL or 0.7–3 nmol/L for women)[17]. Opioids exert inhibitory effects on the hypothalamus, the area responsible for production of gonadotropin-releasing-hormone (GnRH). GnRH normally acts on the pituitary gland to stimulate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH); when GnRH is inhibited, this leads to low LH and FSH causing suppression of sex hormone secretion from the gonads[18]. Although these findings are supported in samples of men, levels of

sex hormones including testosterone in opioid-dependent women have not been extensively studied to date[19]. Due to the increase of chronic opioid use in women, a re-examination of the literature is necessary.

Objectives. The purpose of this study is to examine serum total testosterone level in men and women with opioid dependence receiving methadone treatment. We aim to (1) determine what effect opioids including methadone have on testosterone in this sample and if this effect is present in both men and women; (2) identify other methadone-related treatment factors that are associated with testosterone level; and (3) examine the variability of testosterone level across menstrual cycle phases in women.

Results

Sample characteristics. Of the initial 260 participants undergoing methadone treatment that were recruited, 29 participants were excluded from the study (duplicate entries = 5, buprenorphine treatment = 3, undetectable or out-of-range testosterone levels = 15, hormone replacement therapy = 2, using prescription opioids for chronic pain = 4). Therefore, 231 participants in total were included in the analysis (Figure 1). The sample consisted of 56.7% men with mean age 38.3 (standard deviation [SD] 11.0) and 43.3% women with mean age 35.2 (SD 9.4). The majority of the sample population (84.4%) was of European ethnicity. Refer to Table 1 for additional information on demographics, substance use history, and treatment outcomes. Control participants included 287 (36.7%) men and 496 (63.3%) women who were not using opioids. Mean age was 46.2 (SD 13.1) and 44.6 (SD 12.6) years for men and women, respectively.

Effect of opioid use and methadone treatment on testosterone serum level. Men with opioid dependence undergoing methadone treatment had significantly suppressed testosterone levels (mean = 100.10 ng/dL, SD 72.21, or 3.47 nmol/L, SD 2.51) compared to controls (mean = 414.74 ng/dL, SD 141.81, or 14.39 nmol/L, SD 4.92) (estimated $\beta = -1.661$; 95% confidence interval [CI] -1.793, -1.529; $p < 0.0001$). Testosterone levels for women on MMT did not differ significantly compared to controls (mean = 36.61 ng/dL, SD 23.19, or 1.27 nmol/L, SD 0.81; and mean = 25.93 ng/dL, SD 15.20, or 0.90 nmol/L, SD 0.52, respectively) (estimated $\beta = 0.063$; 95% CI -0.098, 0.224; $p = 0.441$). Table 2 presents a statistical summary of testosterone level in both samples by sex.

Factors associated with testosterone level in methadone treatment. Sex was positively associated with testosterone in this model as expected, with men having a higher testosterone level than women (estimated $\beta = 1.034$; 95% CI 0.857, 1.211; $p < 0.0001$). Testosterone level was found to be inversely associated with methadone dose (estimated $\beta = -0.002$; 95% CI -0.003, -0.000; $p = 0.018$) (Table 3), indicating that a higher methadone dose is correlated with lowered testosterone levels. In the subgroup analysis by sex, testosterone level was inversely associated with methadone dose (estimated $\beta = -0.003$; 95% CI -0.005, -0.001; $p = 0.003$) (Figure 2) and positively associated with the number of cigarettes smoked per day (estimated $\beta = 0.011$; 95% CI 0.000, 0.021; $p = 0.046$) in men. Although no significant correlations were found in the sample of women, polysubstance use showed a positive trend of association with testosterone level (estimated $\beta = 0.244$; 95% CI -0.004, 0.493; $p = 0.054$) (Table 3).

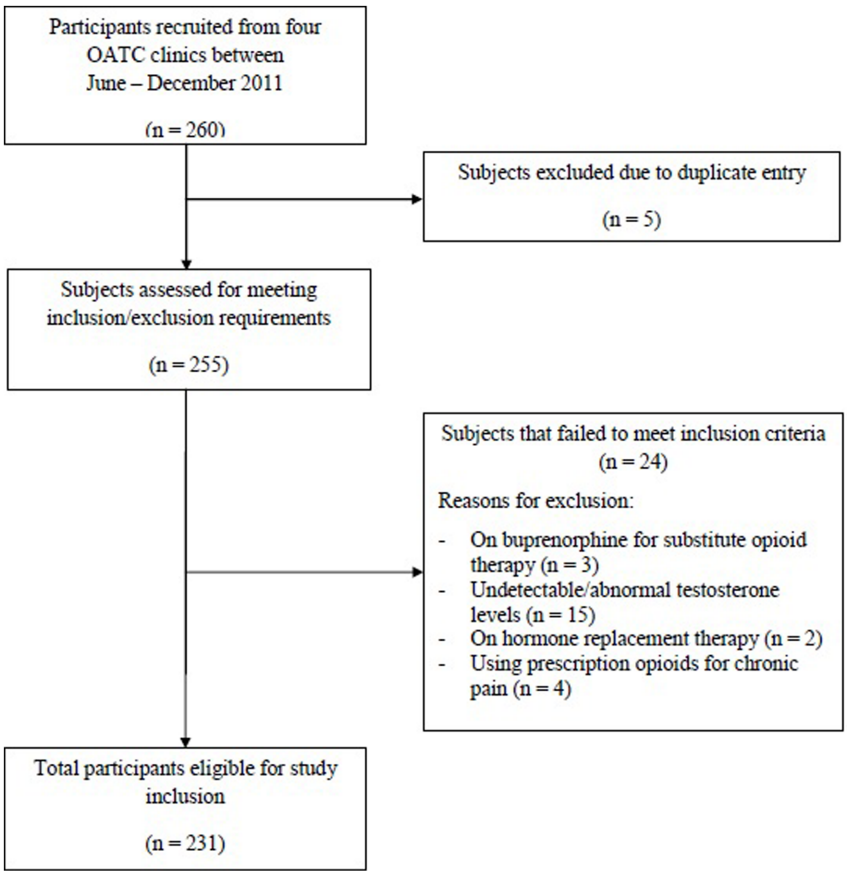

Figure 1 | Flow diagram for participants included in study. Description: Flow of participants throughout each stage of the study and reasons for exclusion.

Table 1 | Demographic characteristics of patients on methadone treatment for opioid addiction

Characteristic	Total (n = 231)	Men (n = 131)	Women (n = 100)
Age in years; mean (SD[a])	36.9 (10.4)	38.3 (11.0)	35.2 (9.4)
BMI[b]; mean (SD)	26.7 (6.4)	26.9 (5.0)	26.6 (8.0)
Married/common law; n (%)	91 (39.4)	53 (40.5)	38 (38.0)
Employed; n (%)	70 (30.3)	45 (34.4)	25 (25.0)
Completed post-secondary education; n (%)	78 (33.8)	33 (25.2)	45 (45.0)
Age of initial opioid use in years; mean (SD)	23.3 (9.3)	23.3 (9.9)	23.3 (8.5)
Current cigarette smokers; n (%)	207 (89.6)	116 (88.5)	91 (91.0)
Number of cigarettes smoked/day; mean (SD)	16.0 (11.1)	17.5 (12.0)	14.1 (9.5)
Polysubstance use; n (%)	102 (44.3)	60 (45.4)	43 (43.0)
Psychiatric comorbidity, self-reported; n (%)	109 (47.2)	55 (42.0)	54 (54.0)
Methadone dose (mg); mean (SD)	87.2 (60.3)	90.2 (65.6)	83.3 (52.8)
Duration on MMT[c] (months); mean (SD)	38.8 (41.8)	40.6 (38.7)	36.4 (45.6)
Illicit opioid use based on urine test results; mean (SD)	18.7 (23.2)	17.0 (21.3)	20.9 (25.6)

[a]SD: standard deviation.
[b]BMI: Body Mass Index (kg/m^2).
[c]MMT: Methadone Maintenance Treatment.

Variability of testosterone level across menstrual cycles phases in women. We employed a linear regression to determine whether serum total testosterone level differs between menstrual cycle phases (follicular and luteal) and the menopause phase in our control sample of women (n = 419). Results demonstrated no difference in testosterone level between all three phases (estimated $\beta = -0.992$; 95% CI −21.263, 19.279; p = 0.923) when controlling for age and smoking status (Figure 3). This suggests that testosterone does not fluctuate significantly across phases of the menstrual cycle or during menopause.

Discussion

The objectives of this study were to examine the overall effect of opioids including methadone on serum testosterone level in men and women, determine what factors are associated with testosterone level in this sample, and examine the variability in testosterone level across menstrual cycle phases in women.

Our results have confirmed the suppressive effect of methadone on testosterone in men undergoing methadone treatment, however they also demonstrate that methadone does not suppress testosterone levels in women. There is limited information on testosterone levels in women with opioid dependence who are currently undergoing methadone treatment and this study has aimed to add to the scant literature.

This sex-specific difference in methadone effects on testosterone is indicative of a distinct biological mechanism between men and women. Opioids including methadone exert their effects on the gonads through the HPG axis and suppress the release of sex hormones[20]. In women, β-estradiol is the primary sex hormone and opioids may act to primarily suppress β-estradiol and target testosterone as a secondary androgen. This is supported by studies looking at the role of opioids in estrogen release. Findings from these studies demonstrate that estradiol was suppressed in opioid-dependent women[21] and after methadone consumption[19]. Studies also report the effect of opioids on prolactin release[22,23]. Prolactin is a hormone responsible primarily for milk production during pregnancy, however it also has a role in sexual behaviours. Prolactin may act to mediate this relationship by inhibiting GnRH secretion, causing a decrease of estrogen in women and testosterone in men[24].

The second objective of this study was to investigate the association between testosterone and methadone-related factors. In MMT patients, we found that methadone dose was inversely associated with testosterone level, indicating that the relationship between methadone and testosterone is dose-dependent. Our sub-group analysis by sex showed that this dose-dependent association was present in men only. This is consistent with previous studies in the literature that looked at the effect of morphine and heroin dosing on testosterone level[12,25,26], however these studies did not control for other relevant factors such as duration on treatment and continued illicit opioid use. Bolelli et al.[25] measured heroin plasma concentration in association with testosterone level in men and also found suppressed testosterone levels, however their study was limited by a very small sample size as well as a lack of an accurate heroin concentration measure; they were unable to control for varying rates of heroin metabolism between participants or by which route the heroin entered the blood. Dev et al.[26] focused on a small sample of cancer patients using morphine for pain management. They found a similar inverse relationship between morphine dose and testosterone level in men. Our study has confirmed this relationship in a large sample of patients specifically using methadone for the treatment of opioid addiction, and we have incorporated an analysis to test this effect in women.

In order to estimate the magnitude of this effect, we used the exponentiated beta coefficient to reverse the logarithmic transformation and multiplied this by 10 so that methadone dose can be quantified in 10 mg increments. We found that for each 10 mg increase in methadone dose, there is a 0.97 ng/dL (0.03 nmol/L) decrease in testosterone level (estimated $\exp(\beta) = 0.969$; 95% CI 0.950, 0.989; p = 0.003), suggesting that men with a higher methadone dose will be more likely to have more suppressed testosterone. In addition, we observed a positive association between the number of cigarettes smoked daily and serum testosterone level in men. Using the same calculation, we estimate that for each additional cigarette smoked per day, there is a 1.01 ng/dL (0.04 nmol/L) increase in testosterone level in men (estimated $\exp(\beta) = 1.011$; 95% CI 1.000, 1.021; p = 0.046). This may be explained by the effect of smoking on methadone metabolism, where smoking is an enzyme inducer in the liver accelerating the metabolism of methadone and hence reduces methadone blood level and its inhibition of testosterone[27]. This association may also be related to addictive behaviour, where smoking as an addictive behaviour is associated with the risk-taking behavioural profile of testosterone[28]. We speculate that this may also be a reason for why male methadone patients have a difficult time with smoking cessation[29].

Low testosterone in men has been associated with poor quality of life, as well as erectile dysfunction, hypogonadism, symptoms of fatigue, weakness, and mood disturbances[14,30]. Improvement in self-reported quality of life assessed across multiple dimensions has been shown to improve treatment outcomes such as retention and overall health in methadone patients after one year of stabilization on MMT[31]. By treating testosterone deficiency, it is suspected that patients will experience improvements in quality of life and therefore

Table 2 | Summary of testosterone levels between men and women on methadone and controls

	MMT[b]					Controls				
	n	Mean Testosterone [SD[c]]	Median	Min.	Max.	n	Mean Testosterone [SD]	Median	Min.	Max.
Men[a]	131	100.10 ng/dL [72.21] 3.47 nmol/L [2.51]	78.16 ng/dL 2.71 nmol/L	10.53 ng/dL 0.37 nmol/L	347.55 ng/dL 12.06 nmol/L	287	414.74 ng/dL [141.81] 14.39 nmol/L [4.92]	406.34 ng/dL 14.10 nmol/L	109.51 ng/dL 3.80 nmol/L	798.27 ng/dL 27.70 nmol/L
Women	100	36.61 ng/dL [23.19] 1.27 nmol/L [0.81]	28.16 ng/dL 0.98 nmol/L	8.83 ng/dL 0.31 nmol/L	92.22 ng/dL 3.20 nmol/L	496	25.93 ng/dL [15.20] 0.90 ng/dL [0.53]	23.06 ng/dL 0.80 nmol/L	1.44 ng/dL 0.05 nmol/L	106.63 ng/dL 3.70 nmol/L
Total	231					783				

[a]Significant at the $p < 0.001$ level.
[b]MMT: Methadone Maintenance Treatment.
[c]SD: standard deviation.
SI conversion factor: To convert testosterone to nmol/L, multiply values by 0.0347.

Table 3 | Association between serum testosterone level and methadone-related factors

	Total (n = 231)				Men (n = 131)				Women (n = 100)			
Variable	Estimated β	95% CI		p	Estimated β	95% CI		p	Estimated β	95% CI		p
Age (years)	−0.007	−0.018	0.003	0.16	−0.008	−0.022	0.006	0.27	−0.007	−0.023	0.008	0.34
Sex[e]	1.034	0.857	1.211	<0.001[a]								
Age of initial opioid use (years)	0.001	−0.009	0.011	0.83	−0.001	−0.015	0.014	0.90	0.004	−0.011	0.018	0.64
Number of cigarettes per day	0.003	−0.005	0.011	0.45	0.011	0.000	0.021	0.05[c]	−0.011	−0.024	0.002	0.09
Methadone dose	−0.002	−0.003	−0.000	0.02[c]	−0.003	−0.005	−0.001	0.00[b]	0.001	−0.002	0.003	0.66
Duration on MMT[f] (months)	0.000	−0.002	0.003	0.89	0.002	−0.002	0.006	0.37	−0.002	−0.005	0.002	0.28
Polysubstance use	0.125	−0.064	0.314	0.19	0.101	−0.18	0.382	0.48	0.244	−0.004	0.493	0.05[d]
Illicit opioid use	−0.002	−0.006	0.002	0.32	−0.003	−0.009	0.003	0.36	0.002	−0.007	0.003	0.49

[a]Significant at the $p < 0.001$ level.
[b]Significant at the $p < 0.01$ level.
[c]Significant at the $p < 0.05$ level.
[d]Shows a trend; $p = 0.050$–0.099.
[e]Sex values not possible for subgroup analysis by sex.
[f]MMT: Methadone Maintenance Treatment.

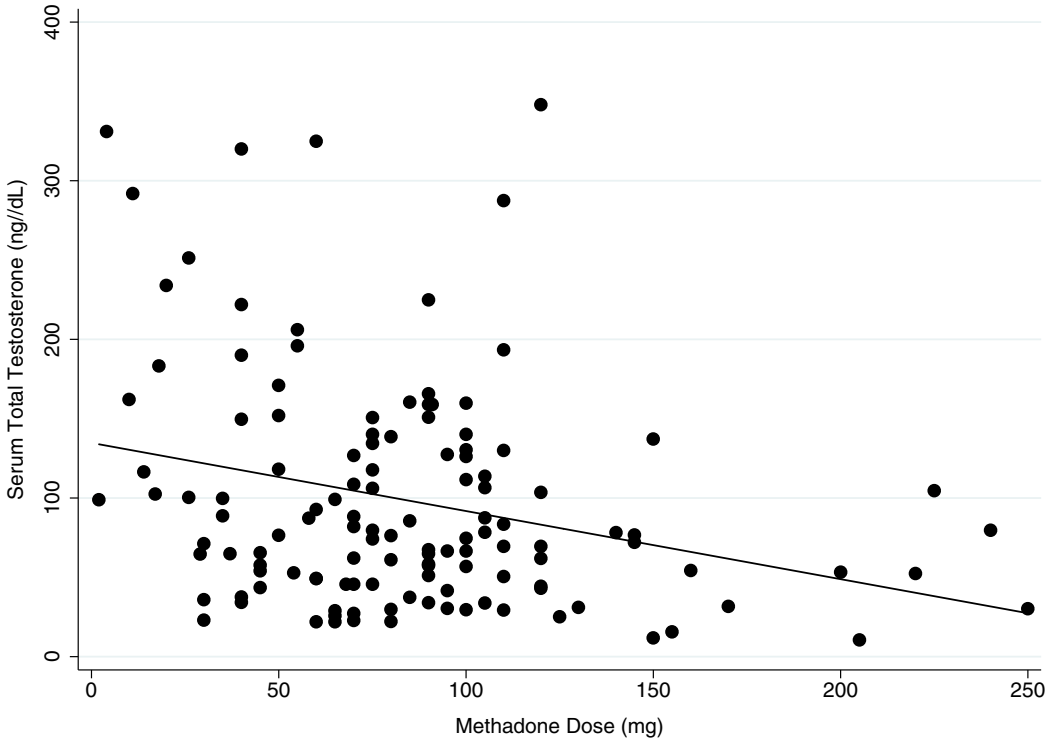

Figure 2 | **Methadone dose and serum total testosterone level in men.** Description: Inverse linear relationship between serum total testosterone level and methadone dose in men on methadone treatment (n = 131).

demonstrate successful treatment outcomes. Healthcare providers should be aware of the effect of opioids including methadone on testosterone and that symptoms related to testosterone deficiency can be actively managed by testosterone therapy. Health care providers should also ensure that patients are being prescribed the lowest possible dose for effective substitution opioid treatment to minimize testosterone suppression.

These findings are applicable to the larger population of methadone patients attending clinics across Ontario and across North America as well. Our patients were recruited from multiple sites of varying geographic locations, all of which follow a standardized treatment regimen, therefore making our sample representative of the overall methadone patient population.

Our final objective was to examine the variability of testosterone levels across menstrual cycle phases in women. In pre-menopausal women, testosterone level does not vary between follicular and luteal phases, and it also does not differ significantly among post-menopausal women. A few studies have found that total testosterone differed between phases, with it being the highest in the luteal[32,33] or mid-cycle phase[34,35]. However, studies also report no significant differences between cycle phases[36–38]. The variability in these findings may be explained by different methods of measuring testosterone (i.e. free, bound, or total; plasma vs. serum; diluted vs. non-diluted, etc.), by the use of different tests (i.e. hormone assays, mass spectrometry), or by differences in defining cycle phases (follicular, mid-cycle, or luteal). Our study has tested this effect in the largest sample of women to date and confirms that testosterone is not sensitive to menstrual cycle changes. Measurement of serum total testosterone level in future investigations does not need to account for menstrual cycle phase, as testosterone levels at any given time are generally representative of the average testosterone level in women.

One of the main limitations of the study is the small sample size of MMT patients when the analysis is divided by sex. There is adequate power to support the associations found in men (86%) however the power for the women sample is much lower (43%), which is not enough to detect any significant associations. Although our sample size for women is one of the largest among studies investigating testosterone in methadone treatment among women, it remains inadequate to draw any significant conclusions on the impact of opioids on testosterone in women, although it may provide some information regarding the magnitude and direction of effect. In addition, some variables included in this study, for example smoking,

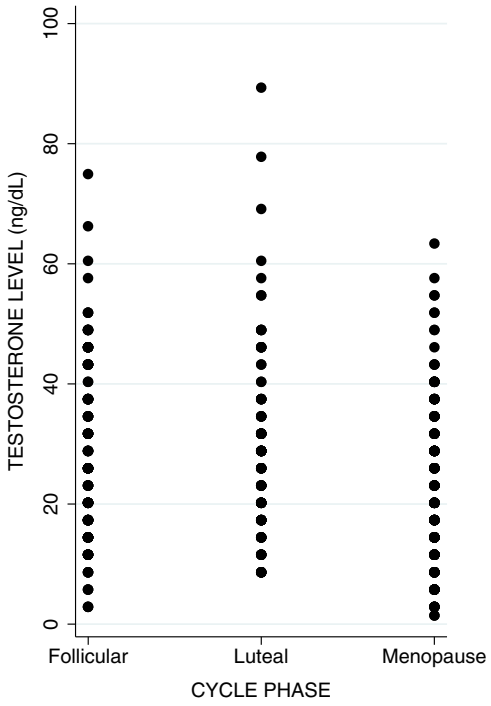

Figure 3 | **Testosterone level across menstrual cycle phases in control women.** Description: Testosterone level shows consistency across follicular and luteal cycle phases, as well as throughout menopause.

were based on self-report and therefore may not be entirely accurate or reliable.

Investigation into additional sex hormones may provide insight into the biological basis of methadone treatment and potentially decipher the effect that opioids have on women. Larger sample sizes with more sex hormones investigated would be ideal for future study, as well as implementing a prospective follow-up study design to observe whether testosterone levels change throughout the course of treatment with methadone. Future directions may include studying the association between testosterone and risk of relapse, as well as observing methadone treatment response and retention after treating low testosterone to determine whether these outcomes have improved.

In this study, we provided an investigation of the influence of opioid dependence and methadone treatment on testosterone. We demonstrated that methadone has a dose-dependent suppressive effect on testosterone in men and that testosterone is not sensitive to menstrual cycle changes in women. The results of this study can be used to guide the decision-making process for men and encourage them to seek treatment for opioid dependence. We also recommend that testosterone levels be checked in men prior to undergoing any opioid therapy and at regular intervals thereafter, in order to treat testosterone deficiency associated with opioids.

Methods

Study design. We collected cross-sectional data from the Genetics of Opioid Addiction (GENOA) research program[39], a collaboration between Ontario Addiction Treatment Centres (OATC) and the Population Genomics Program (PGP), Chanchlani Research Centre at McMaster University. Data were collected for the GENOA study from four OATC outpatient methadone clinics specializing in Opiate Agonist Therapy (OAT) across Southern Ontario, Canada between June and December of 2011. Recruitment consisted of a structured interview conducted on site by a trained OATC clinical staff member and completion of study-specific case report forms. Demographics, anthropometric measurements, and history of current and past substance use, psychiatric diagnoses, and medical conditions were obtained, in addition to urine and blood samples. This study was carried out in accordance with ethical guidelines and approval by the Hamilton Integrated Research Ethics Board (HIREB) and written informed consent was obtained from each study participant.

Study participants. We recruited men and women aged 18 years and older consecutively from OATC clinics. Inclusion criteria consisted of current enrolment in MMT for a diagnosis of opioid dependence according to DSM-IV criteria and having the ability to provide written informed consent. Exclusion criteria were the use of opioid substitution therapy other than methadone for opioid dependence, inability to communicate in English, and refusal to provide biological samples. Patients received supervised daily methadone doses, addiction counseling (including methods for coping with stress, reacting to environmental stressors, developing constructive social networks, etc.), and regular medical follow up as per usual clinical care.

The control group was a sample of adults aged 18–74 years who were screened for DSM-IV dysthymic disorder in a primary care university-affiliated Health Services Organization (HSO) located in Southern Ontario, Canada. This population was an English-speaking, middle class, suburban family community, which consisted of individuals without opioid dependence[40–42].

Outcome measures. The primary outcome is serum total testosterone level. Covariates include continued opioid use (use of illicit opioids detected by weekly or bi-weekly urine screens, measured as the percentage of positive opioid urine screens per total number of urine screens available), methadone treatment duration (length of time in months between the methadone start date and date of most recent methadone dose reported by the patient or obtained from clinic records), methadone dose (current daily dose of methadone at time of interview), polysubstance use (use of a minimum of two substances of abuse in addition to opioids, which include stimulants, hallucinogens, inhalants, cannabis, barbiturates, benzodiazepines, performance-enhancing drugs, or diet pills within the last 12 months; these data were acquired through interviews using the Mini International Neuropsychiatric Interview (M.I.N.I.) Version 6: Drug and Alcohol Modules)[43], and smoking (self-reported average number of cigarettes smoked daily). We also collected age of initial opioid use (self-reported age at which participant began using opioids regularly).

Laboratory analysis. We measured serum total testosterone level in the MMT sample using enzyme-linked immunosorbent assay (ELISA) technique (Enzo Life Sciences, Plymouth Meeting, PA, USA); intra-assay variation is 3.3%, while inter-assay variation is 9.8%, with sensitivity of 2.6%. Serum testosterone in the control sample was measured with the Coat-A-Count total testosterone solid phase radioimmunoassay (RIA) kit (Diagnostic Products Corp., Los Angeles, CA, USA); intra-assay variation is 7.2%, inter-assay variation is 9.4%, and sensitivity is 3.6%. We used different assays for testosterone measurement between MMT and control groups because the hormone assay method used in the past was RIA, which was used for our control group, whereas ELISA is currently the preferred method of hormone analysis. Standard curves of both assays showed a comparable detectable range and appropriate sensitivities, therefore the methods are unlikely to lead to discrepancies in testosterone levels between samples. We conducted qualitative and seminquantitative urine analysis for opioids using iMDx™ Prep Assay [NOVX Systems Inc, Richmond Hill, Ontario, Canada] and performed these weekly or bi-weekly throughout the study period as part of routine clinical care.

Statistical analysis. Continuous variables are presented as a mean and standard deviation and dichotomous variables are presented as a proportion of the sample population. Data for testosterone level showed a skewed distribution on a normal probability plot in the MMT and control groups; these distributions were transformed with the natural logarithm before inclusion in the multivariable regression analysis. Extreme outliers were removed based on the maximum and minimum detectable limit of the hormone assays in the laboratory. We used multiple imputation methods for missing data.

We conducted multivariable linear regression analyses to determine differences in mean log-transformed testosterone levels between men and women MMT participants and controls (n = 1014) and to determine which methadone-related factors are associated with testosterone level (n = 231), with the following covariates included in the model: age, sex, age of initial opioid use, number of cigarettes smoked per day, methadone dose, duration on methadone treatment, polysubstance use, and continued illicit opioid use (based on urine test results). Sub-group analysis by sex was decided *a priori*. We also performed a linear regression to test if there was a significant difference in serum total testosterone level across follicular and luteal phases of the menstrual cycle and the post-menopausal phase in control women (n = 419) while accounting for age and smoking status. Patients on hormonal medication including birth control, hormone replacement therapy, and thyroid medications were removed from the sample.

The study is reported in adherence to the Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement for observational studies[44]. The results are reported as an estimate of the association expressed as a mean difference or model coefficient, corresponding 95% confidence interval and associated p-value. All statistical analyses were performed using STATA Version 11.

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Author contributions

M.B. and Z.S. were responsible for the development of the research question, interpretation of data, manuscript writing, and critical revision of the manuscript. M.B. and B.D. performed statistical analyses, and B.D. also contributed to manuscript writing and critical revision. M.C.S. contributed to data interpretation and organization, and critical revision of the manuscript. C.P., A.W., M.V., J.D., D.M., D.D. and G.P. were all jointly responsible for data collection from OATC clinics, as well as clinical interpretation of results and critical revision of the manuscript. R.A. was involved in interpretation of data and critical revision of manuscript. M.S. was responsible for data collection of the control sample and critical revision of the manuscript. M.C. performed all laboratory analyses for testosterone, and assisted with interpretation of data and critical revision of manuscript. L.T. assisted with statistical analysis, interpretation of data, and revision of manuscript. All authors have reviewed and approved the final manuscript.

Additional information

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Appendix IV

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Review

Testosterone suppression in opioid users: A systematic review and meta-analysis☆

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ABSTRACT

Background: Whether used for pain management or recreation, opioids have a number of adverse effects including hormonal imbalances. These imbalances have been reported to primarily involve testosterone and affect both males and females to the point of interfering with successful treatment and recovery. We conducted a systematic review and meta-analysis to determine the extent that opioids affect testosterone levels in both men and women, which may be relevant to improved treatment outcomes for opioid dependence and for pain management.
Methods: We searched PubMed, EMBASE, PsycINFO, and CINAHL for relevant articles and included studies that examined testosterone levels in men and women while on opioids. Data collection was completed in duplicate.
Results: Seventeen studies with 2769 participants (800 opioid users and 1969 controls) fulfilled the review inclusion criteria; 10 studies were cross-sectional and seven were cohort studies. Results showed a significant difference in mean testosterone level in men with opioid use compared to controls (MD = −164.78; 95% CI: −245.47, −84.08; $p < 0.0001$). Methadone did not affect testosterone differently than other opioids. Testosterone levels in women were not affected by opioids. Generalizability of results was limited due to high heterogeneity among studies and overall low quality of evidence.
Conclusions: Our findings demonstrated that testosterone level is suppressed in men with regular opioid use regardless of opioid type. We found that opioids affect testosterone levels differently in men than women. This suggests that opioids, including methadone, may have different endocrine disruption mechanisms in men and women, which should be considered when treating opioid dependence.

☆ Supplementary materials for this article can be found by accessing the online version of this paper.
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Contents

1. Introduction

Opioids refer to a class of natural and synthetic drugs that are used for pain management and opioid dependency (Fornasari, 2012). They exert their analgesic effects by binding to opioid receptors in the brain and spinal cord to inhibit neurotransmitter release (Mansour et al., 1987), causing both a reduction in neurotransmission and an inhibition of sensory neurons responsible for pain sensation. However, opioids also act on the respiratory control centers in the brain to cause a reduction in respiratory function, and they promote a reduction in gastrointestinal motility through their action in the digestive tract (Narita et al., 2001; Zhang et al., 2008). When taken appropriately and in recommended dosages, opioids are effective for acute pain relief and management of chronic pain, however they have numerous potential side effects, including sedation, nausea, drowsiness, and constipation (Baumann, 2009). Other side effects include decreases in sexual function, bone deterioration, hair loss, immunodeficiency, and pain sensitivity (Benyamin et al., 2008; Hallinan et al., 2008). Opioids are also known to act on endocrine system function, producing hormonal imbalances that may lead to additional serious adverse effects (Katz and Mazer, 2009).

Testosterone is a sex steroid that is controlled by the hypothalamic–pituitary–gonadal (HPG) axis and produced through a series of hormonal activations, which include the gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Alterations in testosterone concentration caused by exogenous substances such as opioids can have significant effects on mood, stress reactivity, aggression, and sexual drive (Borjesson et al., 2011; Kosten and Ambrosio, 2002; Smith and Elliott, 2012). It is speculated that chronic opioid use leads to suppression of GnRH, which indirectly lowers production of testosterone (Katz and Mazer, 2009).

In the case of opioid use disorder, testosterone suppression has been documented in opioid-dependent samples (Azizi et al., 1973; Mendelson et al., 1975a, 1975b, 1984; Wang et al., 1978) as well as patients undergoing methadone maintenance treatment (MMT; Bliesener et al., 2005; Cofrancesco et al., 2006; Cushman, 1973). Methadone is a synthetic opioid used to manage opioid use disorder and withdrawal symptoms in substitute opioid therapy (SOT; Mattick et al., 2009). Treatment with methadone incorporates a harm-reduction approach and involves maintaining patients on a stabilized dose of methadone while slowly tapering off, which can sometimes take years (Mattick et al., 2009). The consequences of testosterone suppression in this particular sample of opioid users may hinder their treatment initiation, maintenance, and recovery.

The incidence of opioid-induced testosterone suppression in women is less commonly examined in the literature. However, a disturbance in female sex hormone levels may also cause the changes that are typically seen in men, and in samples of methadone-treated patients, may lead to poor outcomes and increased risk of relapse.

Based on a review of opioid use and the endocrine system, Katz and Mazer (2009) suggest that all opioids suppress testosterone. Studies on individuals with opioid use disorders, methadone-treated patients, and opioid users for chronic pain alike all showed significant suppression of testosterone. However, the extent of testosterone suppression was not measured quantitatively. A non-systematic narrative literature review showed similar conclusions (Elliott et al., 2011).

Although previous findings support that all opioids suppress testosterone, direct comparisons of testosterone levels among different opioids have not been performed to date. It is possible that some opioids affect testosterone more than others, which would be useful in choosing a particular treatment course. Additionally, having information on testosterone level in opioid users compared to the clinically normal ranges would be helpful for healthcare professionals to determine if this reduction in testosterone is clinically significant and when to initiate treatment of its associated symptoms.

These reviews demonstrate that there is a growing interest in this particular topic as a result of increased rates of opioid use and it is likely that additional studies have been conducted since these reviews were published. There is also a lack of quantifiable data to support the effect of opioids on testosterone, which will be appropriately estimated using a summary statistic derived from a meta-analysis of studies that include small samples. Furthermore, examination of the effect of opioids on testosterone levels in women has yet to be completed, and studies that include samples of women are generally small in this particular area of study, therefore a meta-analysis will provide a larger estimate of effect. The quality of the literature also needs to be evaluated to highlight problematic areas for future research and improvement. Hence, the need for a systematic review with updated data that can be combined statistically in a meta-analysis.

Table 1
Search strategy.

Electronic database	Search terms
MEDLINE *n* = 15	1. "analgesics, opioid" [Title/Abstract] OR 2. "methadone" [MeSH Major Topic] AND 3. "testosterone" [MeSH Major Topic] "human" [MeSH Term]
EMBASE *n* = 27	1. *opiate/ 2. *opiate agonist/ 3. methadone/ 4. testosterone/ 5. 1 or 2 6. 3 and 4 and 5 Limit 6 to human
PsycINFO *n* = 6	1. *opiates/ 2. *narcotic agonists/ 3. *methadone/ 4. exp Testosterone/ 5. 1 or 2 or 3 6. 4 or 5 Limit 6 to human
CINAHL *n* = 2	1. MM "analgesics, opioid" AND MM "testosterone"
Total; *n* = 50	

The main objective of this systematic review and meta-analysis is to examine the association between opioids and testosterone levels and provide a summary estimate of the magnitude of testosterone suppression. Specifically, we aim to: (1) determine whether men receiving long-term opioids have low testosterone levels, compared to clinical reference ranges; (2) determine whether women receiving long-term opioids have low testosterone levels, compared to clinical reference ranges; (3) determine if testosterone suppression varies by the type of opioid use, more specifically methadone versus other opioids; and (4) generate clinically relevant evidence through a critical review of the literature.

2. Methods

2.1. Search strategy

This review adhered to an a priori designed protocol that is available upon inquiry. We systematically searched MEDLINE, EMBASE, PsycINFO, and CINAHL electronic databases from inception to September 19, 2014 for relevant articles. We implemented varying combinations of search terms to reflect differences in indexing among databases. The search was not restricted by language limitations. The complete search strategy can be found in Table 1. We manually reviewed reference lists of included studies for relevant citations that may not have been picked up by our search strategy but we did not review the 'gray literature' including dissertations and conference proceedings.

2.2. Inclusion and exclusion criteria

The inclusion criteria consisted of observational studies (i.e. cohort, cross-sectional, or case–control) or randomized controlled trials (RCTs) that measured testosterone levels in populations of opioid users, including men and women. We did not limit studies to type or purpose of opioid use (i.e. recreational or therapeutic) or apply any age, ethnicity, or geographic setting limitations. The primary outcome of this review is testosterone level. There were no restrictions based on outcome measurement, such as plasma or serum testosterone, and free, bound, or total testosterone. We excluded studies that included participants on testosterone replacement therapy.

2.3. Data screening and extraction

We screened all citations and abstracts retrieved from the search strategy and identified articles for full-text extraction. Two authors (MB and HB) performed the literature search, screening, and data extraction independently; disagreements at any phase of the review process were resolved by discussion or in the case where a consensus was not reached, a third independent rater (ZS) determined eligibility. We recorded the reasons for exclusion and the Kappa statistic for inter-rater agreement of study inclusion at each stage of the screening process. Data were extracted from the studies in duplicate using a pilot-tested data extraction form. In the case where a study previously conducted by the current review authors was included, a third reviewer who was unrelated to either paper verified the data extraction of that particular study. We collected data on the following variables: study characteristics (author, journal, year and place of publication), study design, sample size, age, sex, opioid dose, testosterone level (free and total), time of blood draw, and statistical analyses performed. We used the grading of recommendations, assessment, development, and evaluation (GRADE) framework (Guyatt et al., 2011) to rate the quality of evidence of studies included in this review.

2.4. Statistical analysis

For the meta-analysis, we employed a random effects model, which assumes variation between studies and their respective effect sizes. We used mean difference (MD) to establish the overall effect size of the difference in mean testosterone levels between opioid users and controls in each of the studies reviewed and have presented these in a forest plot. The analysis was performed separately by sex due to the large variance in testosterone levels between men and women. We planned subgroup analyses by opioid type (methadone for opioid dependence versus opioids for other conditions). We reported the results using 95% confidence intervals (CI) and performed all statistical analyses using STATA (StataCorp, 2009) and meta-analyses using Review Manager 5.2 (The Cochrane Collaboration, London, UK). This systematic review is reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

3. Results

Of the 50 articles retrieved from the initial search and a thorough screen of the reference lists, 17 studies were included in the review (Fig. 1); seven cohort and 10 cross-sectional studies. Studies were excluded at each stage of screening for reasons pertaining to incorrect outcome of interest, failure to include appropriate comparison group, and lack of primary research; four studies excluded after title search, 25 studies excluded in the abstract screen, and three studies excluded after full-text screen. Inter-rater agreement was 0.7, 0.4, and 0.4 for the title, abstract, and full-text screen, respectively. Initial disagreements at the abstract and full-text screen stages were later resolved by consensus, whereby the majority of studies were included to undergo screening at the next stage.

3.1. Study characteristics

A detailed description of study characteristics is presented in Table 2. A total of 800 opioid users were included in the studies, the majority of whom were men (*n* = 646) and 1969 controls (referring to the comparator group in the context of this review). Age of the individuals included in these studies varied from 17 to 58 years with a mean age >30 years for most studies. Study samples reported general opioid dependence (*n* = 3), heroin dependence (*n* = 5), methadone maintenance (*n* = 7), buprenorphine maintenance (*n* = 1), heroin maintenance (*n* = 1), levoacetylmethadol maintenance (*n* = 1), and opioid use for chronic pain (*n* = 4). Daily opioid dose was highly variable among studies; 0.5–40 mg morphine equivalent daily dose and 40–15 mg methadone. Testosterone levels varied from 100 to 700 ng/dL (3.5–24.3 nmol/L) in men and from 26 to 55 ng/dL (0.9–1.9 nmol/L) in women. Duration of opioid use ranged from months to years, with a minimum of 3 months and maximum of 11 years; this was referred to as 'long-term'. Study publication years varied from 1973 to 2014, however only five of the included studies were published in the last 10 years.

3.2. Effect of opioid use on testosterone level in men

We were able to utilize data from 12 studies (including 17 individual samples) to compare the difference in testosterone level in men between opioid users and controls (Abs et al., 2000; Azizi et al., 1973; Bawor et al., 2014; Blick et al., 2012; Bliesener et al., 2005; Cushman, 1973; Daniell, 2002; Finch et al., 2000; Malik et al., 1992; Mendelson et al., 1975a; Ragni et al., 1988; Wang et al., 1978).

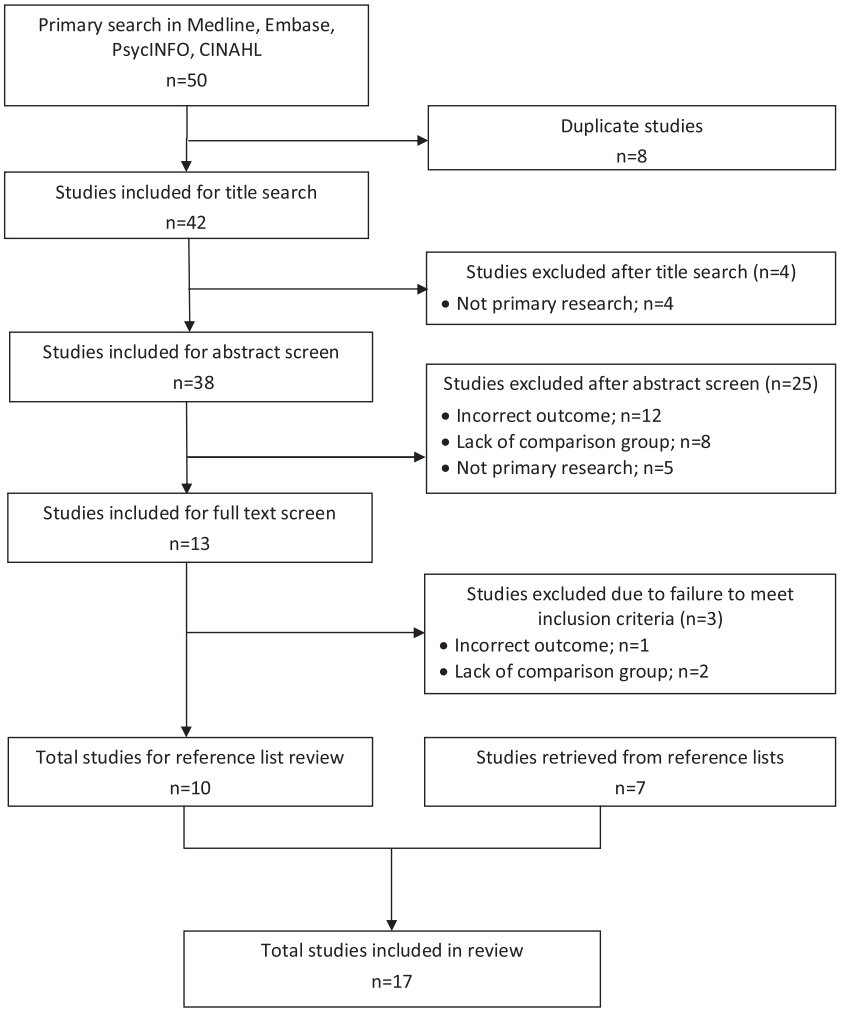

Fig. 1. Studies selected for inclusion with number of studies included after each stage of the screening process.

We found a significantly reduced level of testosterone by a difference of 165 ng/dL (5.7 nmol/L) in men using opioids ($n = 607$) compared to controls ($n = 1417$) (MD = −164.78; 95% CI: −245.47, −84.08; $p < 0.0001$) (Table 3, Fig. 2).

Three other studies were not included in the meta-analysis because they did not use a control group for comparison, rather they drew comparisons to clinical reference ranges of testosterone (Rajagopal et al., 2003) or utilized a within-subjects cohort design in which the participants had their testosterone levels measured and compared at different time points (Mendelson et al., 1984; Roberts et al., 2002). These individual findings, however, did show significant reductions in testosterone in opioid users.

3.3. Effect of opioid use on testosterone level in women

We were able to combine the results of two studies assessing testosterone level in women opioid users in a meta-analysis. There was no significant effect of opioids on testosterone level in opioid-using women ($n = 121$) compared to controls ($n = 512$) (MD = −6.17; 95% CI: −39.87, 27.54; $p = 0.72$) (Table 3, Fig. 3).

3.4. Effect of opioid type on testosterone level in men

We were interested in determining whether certain opioids reduce testosterone levels differently than others, especially those used for substitute opioid therapy. We performed a sub-group analysis using groups of methadone maintenance samples and all other opioids. Although testosterone levels were lower among the methadone-treated group (MD = −181.12; 95% CI: −300.20, −62.05; $p = 0.003$) compared to the non-methadone group (MD = −154.95; 95% CI: −243.45, −66.45; $p = 0.0006$), this difference was not significant.

3.5. GRADE quality of evidence

We evaluated our confidence in these findings using the GRADE framework (Guyatt et al., 2011). We found that the overall quality of evidence was low, mainly due to a serious risk of bias and inconsistency within the literature, despite the presence of strong associations among outcomes. Please refer to the Supplementary Material for detailed GRADE ratings (Table S1[1]) and for funnel plots assessing publication bias (Figs. S1 and S2[2]).

4. Discussion

This systematic review and meta-analysis sought to evaluate the literature on the effect of opioid use on testosterone levels in men

[1] Supplementary material can be found by accessing the online version of this paper.

[2] Supplementary material can be found by accessing the online version of this paper.

Table 2
Study characteristics.

Author (year)	Study design	Participants (*n*)	Sex	Age (yrs); mean (SD/SE) or range (min–max)	Daily opioid dose	Route of administration	Testosterone (ng/dL); mean (SD)	*p*-value (difference between groups)
Abs et al. (2000)	Cohort	CP (29)	M	48.4 (SD 11.0)	4.8 mg ME (SD 3.2)[a]	Intrathecal	198.9 (149.9)	<0.001
		Control (11)		54.2 (SD 14.0)	–	–	443.8 (126.8)	
Azizi et al. (1973)	Cross-sectional	MMT (6)	M	17–58	60–140 mg	Oral	340 (110)	<0.001[b]
		HD (16)		17–58	60–140 mg	NR	440 (320)	<0.02[b]
		Control (25)		17–58	–	–	700 (290)	
Bawor et al. (2014)	Cross-sectional	MMT (231)	M (131)	38.3 (SD 11.0)	90.2 mg (SD 65.6)	Oral	M: 100.1 (72.2)	<0.001[b]
			F (100)	35.2 (SD 9.4)	83.3 mg (SD 52.8)	Oral	F: 36.6 (23.2)	NS[b]
		Control (783)	M (287)	46.2 (SD 13.1)	–	–	M: 414.7 (141.8)	
			F (496)	44.6 (SD 12.6)			F: 25.9 (15.2)	
Blick et al. (2012)	Cohort	OD (90)	M	48.3 (SD 12.0)	NR	NR	280 (170)	NS
		Control (759)		52.6 (SD 12.2)	–	–	287 (149)	
Bliesener et al. (2005)	Cross-sectional	MMT (37)	M	37.5 (6.9)	88.4 mg (SD 16.0)	Oral	280 (120)	<0.000[b]
		BUP (17)		34.7 (7.4)	11.2 mg (SD 4.3)	Sublingual	510 (120)	NS
		Control (51)		35.2 (4.5)	–	–	490 (130)	
Cofrancesco et al. (2006)	Cohort	MMT (33)	F	36.3	NR	Oral	29.7	0.030
		Control (163)			–	–	36.0	
Cushman (1973)	Cohort	MMT (54)	M	35.0 (7.0)	91 mg (SD 25)	Oral	577 (284)	NS[b]
		HD (23)		33.0 (7.0)	NR	NR	523 (279)	NS[b]
		Control (16)		31.0 (8.0)	–	–	589 (246)	
Daniell (2002)	Cross-sectional	OD (23)	M	49.4 (30–78)	70–120 mg (avg range)	Oral	188.5 (193.4)	<0.001
		Control (27)		57.4 (40–67)		–	449.1 (181.1)	
Daniell (2008)	Cross-sectional	OD (21)	F	39.3 (4.9)	20–30 mg	Oral	30.7 (21.5)	<0.001
		Control (16)		42.7 (3.5)	–	–	54.5 (10.3)	
Finch et al. (2000)	Cross-sectional	CP (11)	M	46.5 (SE 3.5)	0.5–40 mg ME (avg range)	Intrathecal	141.2 (105.1)	0.0032
		Control (9)		49.0 (SE 6.0)		–	351. 6 (138.3)	
Malik et al. (1992)	Cross-sectional	HD (33)	M	18–50	37.8 ng/ml ME (SE 5.2)	Smoking or vapor inhalation	376.3 (215.4)	<0.005
		Control (35)		29.8 (SE 3.3)	–		630.4 (137.9)	
Mendelson et al. (1975a, 1975b)	Cohort	MMT (14)	M	22–47	80–150 mg	Oral	409.1 (181.9)	<0.01[b]
		HM (12)		22–47	40–100 mg	NR	227.5 (116.6)	<0.01[b]
		Control (16)		22–47	–	–	622.7 (166.9)	
Mendelson et al. (1984)	Cohort	LAAM (9)	M	19–36	50–65 mg	Oral	683 (162)	<0.05[c]
Ragni et al. (1988)	Cross-sectional	MMT (42)	M	25.0 (SE 5.0)	40–60 mg	– Oral	520 (190)	NS[b]
		HD (15)		23.0 (SE 6.0)	Unknown	NR	550 (120)	NS[b]
		Control (15)		30.0 (SE 6.0)	–	–	490 (110)	
Rajagopal et al. (2003)	Cross-sectional	CP (20)	M	50.1 (34–77)	>200 mg ME	Oral	Median: 140 (range 21–381)	NR
					–	–		
Roberts et al. (2002)	Cohort	CP (10)	M	52.4 (SE 4.0)	3.3 mg ME (SD 0.6)	Oral or intrathecal	115.3 (81.9)	<0.0001[c]
Wang et al. (1978)	Cross-sectional	HD (54)	M	34.6 (SE 1.5)	>40 ng/ml	Smoking, vapor inhalation, or intravenously	521.6 (211.6)	<0.005
		Control (43)		34.6 (SE 1.5)	–		657.1 (207.9)	

Note: Some studies reported standard error (SE); these values have been transformed to standard deviation for consistency.
CP, chronic pain; HD, heroin dependence; OD, opioid dependence (general); BUP, buprenorphine maintenance; MMT, methadone maintenance; HM, heroin maintenance; LAAM, levoacetylmethadol maintenance; M, male; F, female.

[a] Specified dose is combined for sample of men and women; it is not male-only like the other variables for that study.
[b] Compared to control.
[c] Within subjects cohort: compared to when subjects were not using opioids.

and women. We found that patients using opioids for therapeutic purposes, medication-assisted addiction treatment, or as a drug of abuse and dependence have significantly suppressed testosterone levels compared to non-opioid users, and there was no difference between methadone, a commonly used substitute opioid therapy, and other types of opioids. This indicates that all opioids suppress testosterone, regardless of drug type or indication of use.

This fact has significant implications for all patients who are prescribed long-term opioids. It is likely that men prescribed methadone treatment for opioid use disorders already have low

Table 3
Summary of meta-analysis results.

Group	No. of studies	Subjects; *n*		Pooled MD (95% CI)	I^2%	Summary of differences	GRADE quality of evidence
		Opioid users	Controls				
Men							
Methadone treatment	6	284	410	−181.12 (−300.20, −62.05) $p = 0.003$	95 $p < 0.0001$	Testosterone is significantly lower in all opioid users compared to controls	Low[a,b]
Opioids (excluding methadone)	11	323	1007	−154.95 (−243.45, −66.45) $p = 0.0006$	92 $p < 0.0001$		Low[a,b]
All opioids	17	607	1417	−164.78 (−245.47, −84.08) $p < 0.0001$	96 $p < 0.0001$		Low[a,c,d]
Women							
All opioids	2	121	512	−6.17 (−39.87, 27.54) $p = 0.72$	97 $p < 0.0001$	No significant difference in testosterone between opioid users and controls	Very low[c,e]

[a] Some studies did not adjust or control for potential confounders (age, BMI, duration of opioid use, opioid dose, smoking, etc.).
[b] Large mean difference in testosterone levels between opioid users vs. control ($p < 0.01$).
[c] Significant differences in patient populations and outcome measurements may limit generalizability.
[d] Large mean difference of testosterone (159.08 ng/dL) in opioid users vs. control ($p = 0.0002$).
[e] High variability in direction and magnitude of effect between studies, potentially attributed to differences in characteristics and sample size of patient vs. control groups.

testosterone levels. Based on clinical observations, one of the concerns that men with opioid dependence have regarding entry to methadone treatment is that their testosterone will be suppressed. Testosterone deficiency is accompanied by symptoms of fatigue, weakness, mood disturbances, and decrease in libido and sexual function, as well as other conditions including erectile dysfunction, and hypogonadism (Borjesson et al., 2011; Smith and Elliott, 2012). Therefore, it is a common concern for men starting treatment with methadone that they may experience testosterone suppression effects due to methadone. It is important therefore that clinicians treating opioid addiction disorders provide health education to patients to inform them about potential hormonal side effects. It

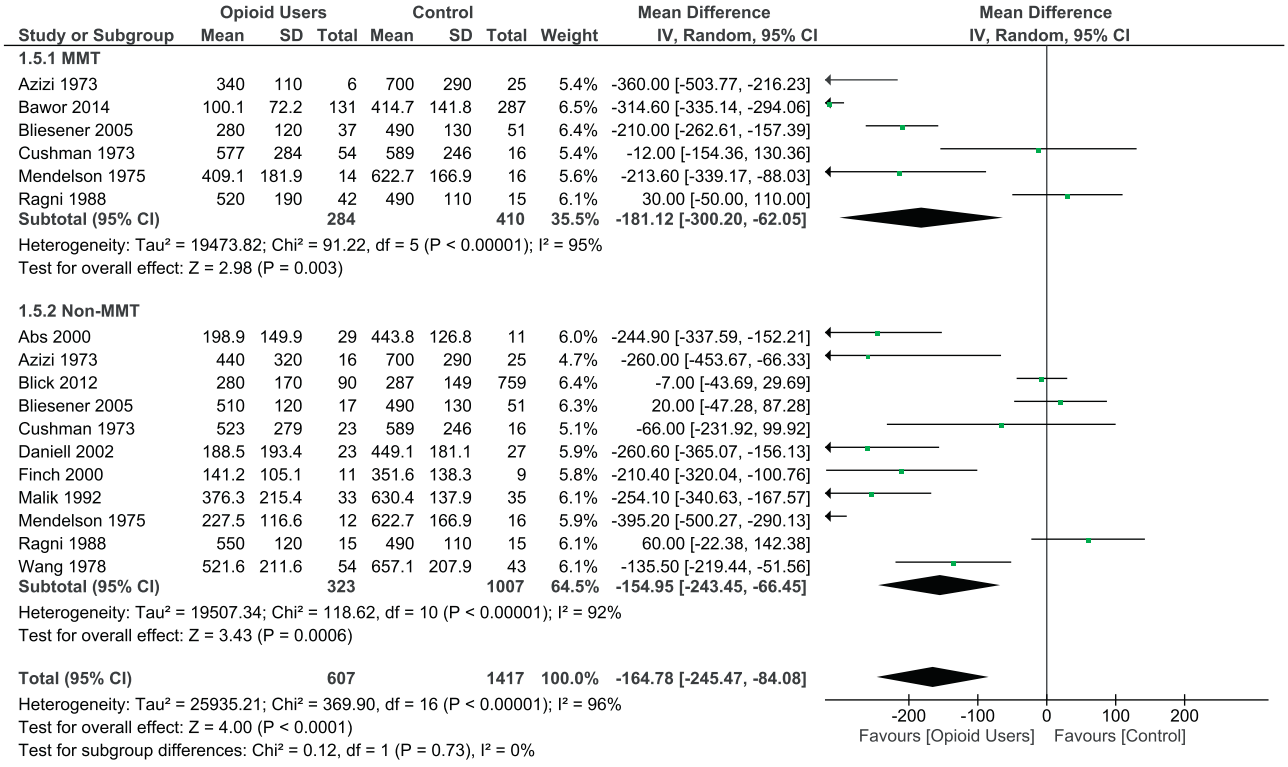

Fig. 2. Effect of opioid use on testosterone level in men. Caption: forest plot representing the relationship between opioid use and testosterone in men using methadone for treatment compared to other opioids.

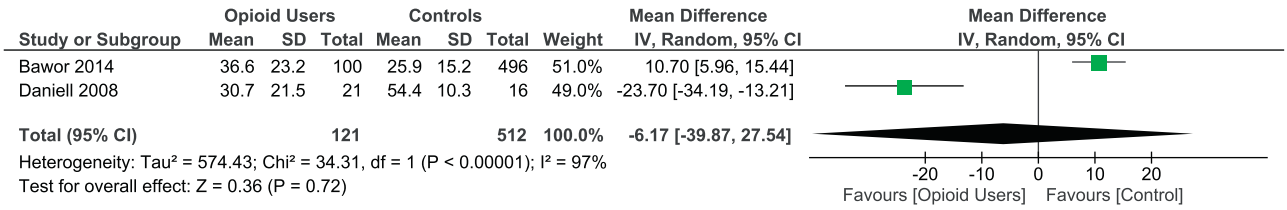

Fig. 3. Effect of opioid use on testosterone level in women. Caption: forest plot representing the difference in testosterone levels between women who use opioids and controls across studies.

is also recommended that clinicians measure testosterone level before starting SOT and if they find that testosterone is already suppressed. Testosterone replacement therapy should be considered carefully and provided when appropriate. It is expected that treating testosterone deficiency symptoms will improve the overall quality of life of patients (Daniell et al., 2006; Dazord et al., 1998; Katznelson et al., 2006) and potentially opioid addiction treatment outcomes. Based on the current study findings, it is recommended that testosterone levels be monitored in patients prescribed opioids prior to treatment initiation and periodically throughout the course of treatment to allow for appropriate management of testosterone deficiency in men.

Buprenorphine, a synthetic opioid similar to methadone, is also used in substitute opioid therapy, and was explored in a study by Bliesener et al. (2005). Although testosterone levels were greater in the buprenorphine-treated group compared to methadone-treated patients and when compared to non-opioid users, these differences were not significant (Bliesener et al., 2005). This finding does potentially highlight the need for further studies with patients undergoing addiction treatment with buprenorphine that include larger sample sizes, in order to clarify this effect.

Our review has confirmed that all opioids suppress testosterone, which is consistent with previous reviews in the literature (Katz and Mazer, 2009; Smith and Elliott, 2012). However, this review is the first to synthesize this information in a meta-analysis and provided a statistical estimate of the magnitude of testosterone deficiency. Our findings demonstrate that testosterone is suppressed by almost 50% in some men and is far below the average clinical reference ranges. We did not observe the same effect in women, which suggests that men and women have different mechanisms of hormonal disturbance caused by opioids. This review can potentially inform both healthcare providers and individuals prescribed opioids about the endocrine disrupting effects of opioid use to make informed decisions about treatment options and other alternatives to be considered. Non-steroidal anti-inflammatory drugs (NSAIDs) and chiropractic care are additional options for pain relief that can prevent the testosterone deficiency caused by opioids. Future studies are required to address whether supplementation with testosterone is an effective approach to the improvement of quality of life and addiction treatment in men receiving opioids.

4.1. GRADE quality of evidence

We used the GRADE framework (Guyatt et al., 2011) to evaluate our confidence in the estimates derived from the meta-analysis and determined that the studies reported in the literature were of low quality (see Table S1[3] of Supplementary Material for ratings).

The most prominent concerns among the studies were risk of bias and confounding. Some studies had adjusted for potential confounders by considering certain factors in their analyses such as age (Malik et al., 1992) and methadone dose (Daniell, 2002); however the remaining studies either did not take into consideration such factors or failed to report them. Data on duration of opioid use, smoking, concurrent medications, or polysubstance use were collected in some studies but not accounted for when measuring testosterone level. Also, there was often no mention of whether or not participants were undergoing any testosterone replacement therapy at the time of study, or whether other hormonal medications were being used. This also raises the issue of lack of reporting standards among studies in this field. The majority of studies were performed between 1980 and 2000, when standards for reporting studies were different than today. After the introduction of the Consolidated Standards of Reporting Trials (CONSORT) statement and later its multiple extensions in 1996, as well as the gradual uptake of these statements by journals, reporting standards have improved (Samaan et al., 2013). However, important pieces of information related to methodology, statistics, or outcome measurements remain unknown in older studies included in this review, thus impacting the quality assessment.

We noticed a high level of heterogeneity and variability among studies which may be attributed to differences in outcome measurements (i.e. free vs. total vs. bound; plasma vs. serum, different assay methods). This was especially prominent in the studies with women opioid users, where the two studies had an opposite direction of effect (Bawor et al., 2014; Daniell, 2008). This may be due to small sample sizes of individual studies, suggesting that they may not have the power to accurately detect this difference in the outcome or it could be explained by some unknown factors that influenced the control groups. Additionally, in this review, we included samples where participants were dependent on opioids, had serious health concerns, and suffered from lack of adequate healthcare. We also had samples of patients with chronic pain consuming prescription opioids whose doses were regulated and carefully monitored, and taken under safe conditions. Characteristics of these populations may differ, and although the results were consistent among both types of samples, they may have limited comparability in terms of how generalizable the overall summary finding is.

Our overall confidence in the estimates is therefore quite low, however our meta-analysis generally showed a consistent large effect size across multiple studies, which does provide evidence for testosterone deficiency associated with opioid use.

4.2. Strengths and limitations

This was a systematically conducted review with rigorous statistics and a large meta-analysis that provided a quantifiable estimate of the effect of opioid use on testosterone levels. We observed this relationship among men as well as women, which is not commonly reported. We also compared testosterone levels among opioid users being treated with methadone for addiction and opioid use (including prescription opioids) for conditions other than addiction. A thorough evaluation of the status of literature was also performed.

As evaluated by the GRADE framework, the quality of evidence in this field of study is poor, and therefore the results summarized in

[3] Supplementary material can be found by accessing the online version of this paper.

this review should be interpreted with this consideration in mind. However, this review has brought to light the need for more up to date research using current hormone assay methods, appropriate reporting, and rigorous methodology. It has also been successful in identifying the need for future examination into the effect of opioids on testosterone levels in women. The lack of studies among women opioid users included in this review, as well as the small sample sizes of these studies, poses a challenge in drawing adequate conclusions of this association.

5. Conclusion

Our systematic review and meta-analysis demonstrated that testosterone levels are suppressed in men receiving opioids, regardless of opioid type or indication of use. These findings may have important potential implications for treatment. Testosterone levels are likely to already be significantly lowered in patients with a history of opioid use. The results of this study can be used by healthcare professionals and patients themselves when choosing to enter substitute opioid treatment for opioid dependence. It is recommended that testosterone levels are monitored at treatment entry as well as throughout the course of treatment, so that low testosterone and related symptoms may be adequately treated.

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Contributors

MB and ZS were responsible for the development of the research question, interpretation of data, manuscript writing, and critical revision of the manuscript. MB and HB were responsible for screening articles and data extraction for the review. MB and BD performed statistical analyses, and BD also contributed to manuscript writing and critical revision. CP, AW, MV, JD, DM, and GP were all jointly responsible for clinical interpretation and organization of results, as well as critical revision of the manuscript. RA and MS were involved in interpretation of data and critical revision of manuscript. MC was responsible for scientific interpretation of hormone values and critical revision of manuscript. LT assisted with statistical analysis, interpretation of data, and revision of manuscript. All authors have reviewed and approved the final manuscript.

Conflict of interest

No conflict declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.drugalcdep.2015.01.038.

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SUPPLEMENTARY MATERIAL

Table S1. Quality of evidence evaluated by GRADE criteria

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Opioid Users	Control	Relative (95% CI)	Absolute		
Women (Better indicated by lower values)												
2	observational studies	no serious risk of bias	serious[a]	serious[b]	no serious imprecision	none	121	512	-	MD 6.17 lower (27.54 to 39.87 lower)	⊕OOO VERY LOW	
Men (Better indicated by lower values)												
12	observational studies	serious[c]	no serious inconsistency	serious[d]	no serious imprecision	very strong association[e]	607	1417	-	MD 164.78 lower (84.08 to 245.47 lower)	⊕⊕OO LOW	
Men - MMT (Better indicated by lower values)												
6	observational studies	serious[c]	no serious inconsistency	no serious indirectness	no serious imprecision	strong association[f]	284	410	-	MD 181.12 lower (62.05 to 300.02 lower)	⊕⊕OO LOW	
Men - Non-MMT (Better indicated by lower values)												
11	observational studies	serious[c]	no serious inconsistency	no serious indirectness	no serious imprecision	strong association[e]	323	1007	-	MD 154.95 lower (66.45 to 243.45 lower)	⊕⊕OO LOW	

[a] High variability in direction and magnitude of effect between studies, potentially attributed to differences in characteristics and sample size of patient vs. control groups
[b] Significant differences in patient populations (methadone maintenance for addiction vs. sustained-action opioids for nonmalignant pain) in duration of opioid use, health, smoking, and age which may reduce generalizability of findings
[c] Some studies did not adjust or control for potential confounders (age, BMI, duration of opioid use, opioid dose, smoking, etc.)
[d] Significant differences in patient populations and outcome measurements may limit generalizability
[e] Large mean difference of testosterone (164.78 ng/dL) in opioid users vs. control ($p<0.0001$)
[f] Large mean difference in testosterone levels between opioids users vs. control ($p<0.0001$)

Figure S1. Funnel plot to assess publication bias in studies including men

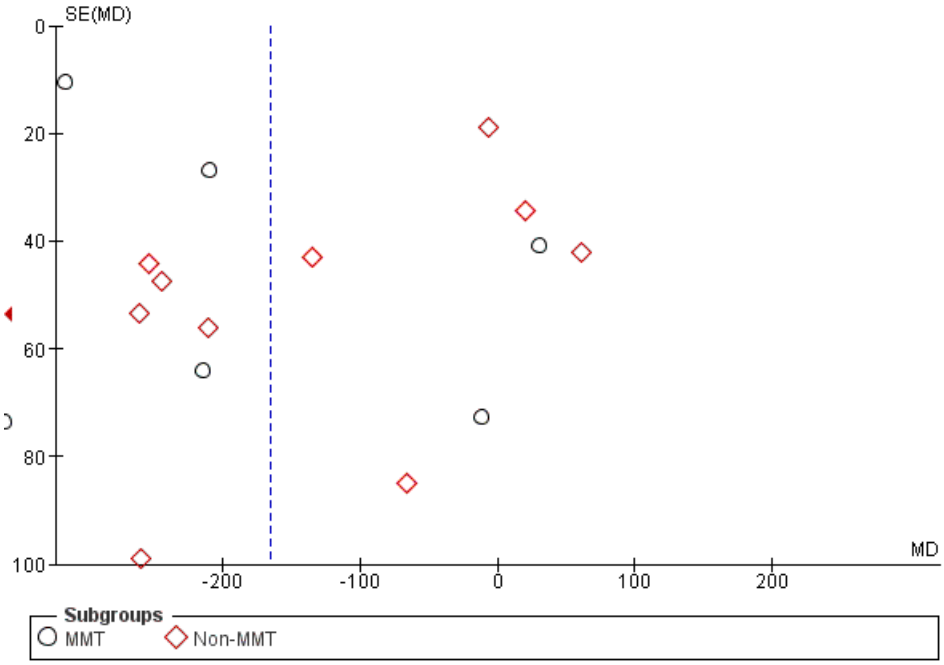

Figure S2. Funnel plot to assess publication bias in studies including women