Steady-state methadone pharmacokinetics in opioid agonist treatment: Influencing factors and clinical outcomes

Fatemeh Chalabianloo

Thesis for the degree of Philosophiae Doctor (PhD)
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“ Life is like riding a bicycle. To keep your balance, you must keep moving.”

- Albert Einstein

Abstract in English

Background:

Methadone maintenance treatment (MMT) is recommended for the treatment of opioid addiction. A considerable inter-individual variability in methadone daily doses and serum concentrations has been reported; however, the underlying causes are not fully understood. The aim of this thesis was to investigate the influence of genetic, pathophysiological and pharmacological factors on serum methadone concentration-to-dose ratio (CDR), and to explore the relationship between serum methadone concentration and clinical outcomes in MMT.

Methods:

The thesis has used observational data from three different sources including two retrospective laboratory databases on therapeutic drug monitoring (TDM) (papers I and II), and a prospective cohort data on patients undergoing MMT (papers III and IV). Linear mixed model analyses were used to investigate the impact of CYP genetic polymorphisms (paper I), age, gender and co-medication (paper II), as well as liver fibrosis and BMI (paper III) on methadone CDR. Association between serum methadone concentrations and subjective symptoms of withdrawal and adverse effects were also investigated (paper IV).

Results:

Paper I: The homozygous carriers of *CYP2B6*6* had significantly higher methadone CDR compared with non-carriers ($P < 0.001$). Paper II: Women as compared to men had 9% lower CDR, whereas the ratio was not influenced by age. Concomitant medication with CYP inducers reduced methadone CDR by 36%, whereas CYP3A4

inhibitors increased it by 36%. Paper III: There was no significant relationship between CDR and liver fibrosis (coefficient: 0.70; 95% CI: -2.16, 3.57; *P*: 0.631) or cirrhosis (-0.50; -4.59, 3.59; 0.810) compared to no/limited fibrosis. Participants with a BMI of 25-30 kg/m² had higher CDR (2.34; 0.22, 4.45; 0.031) compared with those who had lower BMI. Paper IV: The total SOWS score ($P < 0.001$); the specific subjective withdrawal symptoms of anxiety ($P = 0.004$), bone and muscle aches ($P = 0.003$), restlessness ($P = 0.017$), and (slightly) shaking ($P = 0.046$), also use of heroin ($P = 0.015$) and alcohol ($P = 0.011$) were associated with lower methadone concentrations. Cannabis use was slightly related to higher methadone concentrations ($P = 0.049$).

Conclusion:

This thesis demonstrates that genetic polymorphisms in CYP2B6, gender, BMI and concurrent medication with CYP inducers and CYP3A4 inhibitors may explain some of the variations in dose-adjusted serum methadone concentrations. Age, degree of liver fibrosis and the other CYP polymorphisms do not seem to be associated with methadone CDR. Additionally; we have shown associations between the subjective opioid withdrawal symptoms as well as substance use, and serum methadone concentrations. Our findings confirm the current clinical and research challenges in MMT regarding a large inter-individual variability in methadone pharmacokinetics and influence of several factors. The thesis supports the importance of an individually tailored dosage based on the possible factors involved and self-perceived opioid withdrawal symptoms to achieve the desired serum methadone concentration. This is crucial to reduce relapse to substance use with associated risks and to optimize the treatment outcomes.

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Sammendrag på norsk (Abstract in Norwegian)

Bakgrunn:

Metadonsubstitusjon er anbefalt for behandling av opioidavhengighet. En betydelig inter-individuell variasjon i metadons daglige doser og oppnådde serumkonsentrasjoner er rapportert. Mulige underliggende årsaker er imidlertid ikke fullt ut forstått. Formålet med denne forskningen var å undersøke om genetiske, pato-fysiologiske og farmakologiske faktorer kan påvirke metadon serumkonsentrasjon-dose-ratio (CDR), og å utforske sammenhengen mellom serumkonsentrasjon og kliniske utfall i metadon substitusjonsbehandling.

Metoder:

Dette prosjektet har brukt observasjonsdata fra tre forskjellige kilder, inkludert to retrospektive laboratoriedatabaser av terapeutisk legemiddelovervåking (artiklene I og II), og en prospektiv kohortdatabase om pasienter under substitusjonsbehandling med metadon (artiklene III og IV). Lineær regresjon analysemetode ble brukt for å undersøke effekten av CYP genetiske polymorfismer (artikkel I), alder, kjønn og samtidig behandling med andre legemidler (artikkel II), samt leverfibrose og BMI (artikkel III) på metadon CDR. Sammenhengen mellom metadon serumkonsentrasjon og subjektive abstinenssymptomer og bivirkninger ble også undersøkt (artikkel IV).

Resultater:

Artikkel I: Homozygote bærere av *CYP2B6*6* hadde signifikant høyere metadon CDR sammenlignet med ikke-bærere ($P < 0.001$). Artikkel II: Kvinner hadde sammenlignet med menn 9 % lavere CDR, mens denne ratioen ikke var påvirket av alder. Samtidig medisinering med CYP induserende legemidler reduserte metadon CDR med 36 %, mens CYP3A4 hemmende legemidler økte denne med 36 %. Artikkel III: Det var ingen signifikant sammenheng mellom CDR og leverfibrose (koeffisient: 0.70; 95% KI: -2.16, 3.57; *P*: 0.631) eller cirrhose (-0.50; -4.59, 3.59; 0.810) sammenlignet med

ingen/begrenset fibrose. Deltakerne med en BMI på 25-30 kg/m² hadde høyere CDR (2.34; 0.22, 4.45; 0.031) sammenlignet med de som hadde lavere BMI. Artikkel IV: Total SOWS-score ($P < 0,001$), de spesifikke subjektive abstinenssymptomene angst ($P = 0.004$), ben- og muskelsmerter ($P = 0.003$), rastløshet ($P = 0.017$) og (i lavere grad) skjelving ($P = 0.046$), også bruk av heroin ($P = 0.015$) og alkohol ($P = 0.011$) var assosiert med lavere metadonkonsentrasjoner i serum. Cannabisbruk var (i lavere gard) relatert til høyere serumkonsentrasjoner av metadon ($P = 0.049$).

Konklusjon:

Våre forskningsfunn viser at genetiske polymorfismer i CYP2B6, kjønn, BMI og samtidig behandling med CYP induserende og/eller CYP3A4 hemmende legemidler kan forklare noen av variasjonene i dosejusterte serumkonsentrasjoner av metadon. Alder, grad av leverfibrose og andre CYP polymorfismer ser ikke ut til å være assosiert med metadon CDR. Vi har også funnet assosiasjoner mellom subjektive opioidabstinenssymptomer samt rusmiddelbruk, og metadonkonsentrasjoner i serum. Våre funn bekrefter dagens kliniske og forskningsmessige utfordringer knyttet til metadonbehandling med tanke på en stor inter-individuell variasjon i legemiddelets farmakokinetikk. Resultatene er også i tråd med tidligere forskning som viser at metabolismen av metadon kan bli påvirket av flere faktorer. Samlet sett støtter vår forskning viktigheten av en individuelt tilpasset dosering basert på mulige involverte faktorer og selvopplevde opioidabstinenssymptomer for å oppnå en tilstrekkelig og ønsket serumkonsentrasjon som kan redusere tilbakefall til rusmiddelbruk med tilhørende risiko og for å optimalisere behandlingen.

Papers included in this thesis

Paper I

Kringen MK, Chalabianloo F, Bernard JP, Bramness JG, Molden E, Høiseth G. Combined effect of *CYP2B6* genotype and other candidate genes on a steady-state serum concentration of methadone in opioid maintenance treatment. Therapeutic Drug Monitoring 2017;39(5):550–5.

Paper II

Chalabianloo F, Westin AA, Skogvoll E, Bramness JG, Spigset O. Methadone serum concentrations and influencing factors: a naturalistic observational study. Psychopharmacology 2019;236(11):3159–67.

Paper III

Chalabianloo F, Høiseth G, Vold JH, Johansson KA, Kringen MK, Dalgard O, Ohldieck C, Druckrey-Fiskaaen KT, Aas C, Løberg E-M, Bramness JG, Fadnes LT. Impact of liver fibrosis and clinical characteristics on dose-adjusted serum methadone concentration. Journal of Addictive Diseases 2022;31(3):1-11.

Paper IV

Chalabianloo F, Fadnes LT, Høiseth G, Ohldieck C, Vold JH, Aas C, Løberg E-M, Johansson KA, Bramness JG. Subjective symptoms and serum methadone concentrations: what should guide dose adjustments in methadone maintenance treatment? A naturalistic cohort study from Norway. Substance Abuse Treatment, Prevention, and Policy 2021;16(1):39.

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List of tables and figures

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Abbreviations

BMI	Body Mass Index
CDR	Concentration-to-Dose Ratio
CI	Confidence Interval
CYP	Cytochrome P-450
DOT	Directly Observed Treatment
EDDP	2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
eGFR	Estimated Glomerular Filtration Rate
HCV	Hepatitis C Virus
HIV	Human Immune-deficiency Virus
LMM	Linear Mixed Model
LSM	Liver Stiffness Measurement
MMT	Methadone Maintenance Treatment
OAT	Opioid Agonist Treatment
OOWS	Objective Opiate Withdrawal Scale
OUD	Opioid Use Disorder
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SOWS	Subjective Opiate Withdrawal Scale
TDM	Therapeutic Drug Monitoring
WHO	World Health Organization

1. Introduction

1.1 Opioid dependence

Opioids are among the world's oldest known psychoactive drugs, with the use of derivatives from the opium poppy recorded for thousands of years. A wide range of opioids are used for medicinal and recreational purposes, and include natural (i.e. plant-based), semi-synthetic, and synthetic opioids. Opioids are WHO-listed essential medicines for acute and cancer pain, palliative care, and treatment of opioid dependence (1).

Opioid dependence - as defined in international classification of diseases (ICD-10, ICD-11, and DSM-IV) - presents a chronic relapsing disorder with a maladaptive pattern of opioid use involving a constellation of behaviors. These include physiological signs of tolerance and withdrawal, loss of control over use, craving and preoccupation with non-therapeutic use, and continued use despite causing harm (2,3) In North America, the term opioid use disorder (OUD) (from the American Psychiatric Association's DSM-5) (4) is often used in preference to opioid dependence (Table1), which is the applied term in ICD-10 and DSM-IV. Presence of 2–3 criteria is considered mild, 4–5 moderate, and 6 or more is severe on the OUD spectrum in DSM-5.

Table 1. DSM-5* criteria for opioid use disorder

A problematic pattern of opioid use leading to clinically significant impairment or distress, as manifested by at least two of the following, occurring within a 12-month period:
1. Opioids are often taken in larger amounts or over a longer period of time than was intended.
2. There is a persistent desire or unsuccessful efforts to cut down or control opioid use.
3. A lot of time is spent in activities to obtain the opioid, use the opioid, or recover from its effects.
4. Craving, or a strong desire or urge to use opioids.
5. Recurrent opioid use resulting in a failure to fulfill major role obligations at work, school, or home.
6. Continued opioid use despite having persistent or recurrent social or interpersonal problems caused by or exacerbated by the effects of opioids.
7. Important social, occupational, or recreational activities are given up or reduced because of opioid use.
8. Recurrent opioid use in situations in which it is physically hazardous.
9. Continued opioid use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.
10. Toleranceª as defined by either of the following:
(a) A need for markedly increased amounts of opioids to achieve intoxication or desired effect.
(b) A markedly diminished effect with continued use of the same amount of an opioid.
11. Withdrawalª

*DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.
Severity: Mild, 2–3 symptoms; moderate, 4–5 symptoms; severe, 6 or more symptoms.
ªThis criterion is not met for individuals taking opioids solely under appropriate medical supervision.

Among all illegal substances, opioids denote the highest disease burden, treatment demand, healthcare costs, social marginalization and mortality (5-7). Worldwide, opioid-use disorder affects more than 16 million people (age-standardized prevalence of 500 per 100,000) with a higher prevalence in the Middle East and east Asia, and highest prevalence of 1347 per 100,000 in the USA in 2017, and is a major contributor to premature death (8,9). In 2019, around 46,000 deaths (5% of all deaths) and 4,700,000 disability-adjusted life years (6% of total disease burden) were caused by opioid-use disorders in the USA among people under the age of 70 years (10). Norway is one of the European countries with a high prevalence of drug-related deaths of which about 90% are caused by opioids (11). The frequency of drug-related deaths was 6.1 per 100,000 people of 15–64 years of age in 2020, and these were mostly associated with the use of heroin, morphine, and other synthetic/semi-synthetic opioids that were either prescribed or illicitly acquired (12). This is a worrying trend and strongly encourages the implementation of effective and safe treatment methods.

1.2 Opioid agonist treatment

Opioid agonist treatment (OAT) refers to the prescribed use of methadone or buprenorphine as long-acting opioid agonists in the treatment of opioid dependence, although other opioids can be used (13). OAT is the most evidence-based and effective treatment for opioid dependence, and methadone and buprenorphine are included in the WHO`s list of the essential medicines (14). The treatment reduces illicit opioid use and the related harms across multiple health outcomes including all cause and overdose mortality, and improves physical and mental wellbeing and quality of life (15-24). A recent large systematic review and meta-analysis (25) has investigated the overall all-cause and cause-specific mortality both by setting and by participant characteristics during OAT (using methadone or buprenorphine) and the time out of OAT. Fifteen RCTs including 3852 participants and 36 primary cohort studies including 749,634 participants were analyzed. The rate of all-cause mortality during OAT was more than half of the rate seen during time out of OAT. This association was consistent regardless of patient sex, age, geographic location, and the status for human immune deficiency virus (HIV), hepatitis C virus (HCV) or injection/non-injection substance use. During OAT, there was significantly lower risk of mortality related to suicide, cancer, cardiovascular diseases, drug and alcohol use, and incarceration including the time after release. All-cause mortality was six times higher in the four weeks after OAT cessation, remaining double the rate for the remainder of time not receiving OAT.

Despite the strong evidences for the efficacy of OAT among people with opioid dependence, and the implementation of the treatment since 1960s, access to OAT remains limited in many settings, and the global coverage is low. Future work to increase access could have important population-level benefits. In Norway, OAT was not implemented until 1998 (26), however, the current coverage rate is estimated around 60% of people with opioid dependence (27). Currently, there are more than 8000 people enrolled in OAT in Norway using methadone (35%) mainly as oral racemic formulation (liquid or tablet) but also as the R-enantiomer (liquid), and different formulations of buprenorphine (62%) either as sublingual tablets (mono

preparation or combined with naloxone) or depot injections (weekly or monthly) as the main substitution medications. The remainder 3% of the patients receive oral morphine depot tablets as an alternative medication due to specific medical conditions (28). Recently, heroin-assisted treatment is also under consideration to reach the most marginalized people with opioid dependence who do not profit from conventional substitution medications in OAT (29).

1.3 Methadone

1.3.1 Historical and treatment aspects

Methadone was first synthesized by the pharmaceutical company Bockmühl and Ehrhart in 1937 as a spasmolytic agent later named amidon. Further work indicated that amidon had 5-10 fold greater analgesic effect than meperidine (30). Existing proprietary concerns over the name Amidone led the Council on Pharmacy and Chemistry of the American Medical Association to declare methadon (without the e) the generic designation for 6-dimethylamino-4, 4-diphenyl-3-heptanone (31,32). The name was formally changed to methadone in 1948 (33). Early clinical work with methadone focused on its analgesic potential. Human laboratory studies found methadone had greater analgesic properties per milligram than morphine and a similar toxicity profile, with induction of lightheadedness, nausea, and decreases in pulse and respiratory rate (34,35). When used in clinical pain management, significant relief of traumatic, post-operative, and malignant pain were noted (36,37).

Methadone's ability to relieve the opiate withdrawal syndrome was noted as early as 1947 and within two years it became the preferred medication for detoxification at the national narcotics hospital in Lexington, Kentucky (38). Upon taking methadone, opioid dependent individuals in withdrawal found their symptoms relieved; those with active addiction did not experience euphoria or request their usual and available doses of injected morphine; and, after chronic administration, sudden cessation of methadone produced withdrawal syndrome lasting longer than following morphine cessation (39).

It was not until 1964 when the scientists and clinicians Vincent P. Dole and Marie Nyswander at the Rockefeller Medical Research University began to evaluate methadone maintenance in a group of 22 heroin-addicted individuals as a means of long-term medication-assisted treatment for opioid dependence (40). This work helped to establish that not only did methadone relieve opiate withdrawal but, when at steady-state, it also blocked the euphoric and sedating effects of superimposed opiates (40,41). Thus, with methadone, the major components of both the positive and negative reinforcing effects of short-acting opiates such as heroin were reduced and craving subdued allowing the individual to concentrate on non-addictive activities. Combined with a comprehensive program of rehabilitation, patients showed marked improvement; they returned to school, obtained jobs, and became reconciled with their families. Medical and psychometric tests disclosed no signs of toxicity, apart from constipation. The scientists underscored that the treatment required careful medical supervision and many social services. These experiences have been the basis for all subsequent substitution treatment for opioid dependence.

Today, methadone is the most widely used treatment of opioid dependence worldwide (42) and its effectiveness is sufficiently documented in numerous clinical studies (43). The treatment reduces opiate withdrawal symptoms and illicit opioid use, increases retention rate in OAT with subsequent reduction in morbidity and mortality, and gives opioid dependent people a chance to undergo social rehabilitation (18,19,43-45).

1.3.2 Physiological and clinical effects

Methadone administration produces an acute dose-dependent physiological effect typical of mu opioid agonists, including pupil constriction (i.e. miosis), decreased gastrointestinal motility, and decreased respiratory rate (46). Chronic administration leads to small but reliable reductions in respiratory rate, blood pressure and heart rate along with increased body temperature (47,48). The main subjective effects of methadone are including euphoria and sedation that last longer time than with the use of short-acting opioids such as heroin and morphine. Relatively low doses of methadone in opiate-naive individuals and sufficiently high doses of methadone in

opioid-tolerant individuals can lead to fatal overdose consequent to respiratory depression and cardiopulmonary failure (46,49).

The early observations on the long-term maintenance treatment with methadone in opioid dependent individuals confirmed its efficacy in suppressing withdrawal as well as preventing the emergence of craving and withdrawal signs and symptoms (40,41). These investigations also showed that tolerance developed to the sedative and euphoric effects with chronic use. The findings and development of cross-tolerance to other opioids have been documented in several studies on long-term methadone treatment; however, the degree of tolerance differs between individuals and is based on the phase of administration, the dose taken, and the various effects of methadone (41,50-52). In addition, the cross-tolerance between methadone and other opioids is incomplete; meaning that a high degree of opioid tolerance does not eliminate the possibility of methadone overdose, iatrogenic or otherwise. A careful dose adjustment is therefore recommended when switching from other opioids to methadone, as equipotent doses may not always be used from one day to the next (53).

Studies have also examined the effect of methadone on a broad array of psychomotor performance and other cognitive function measures. Studies on the acute effects of methadone (as for other opioids) generally have reported slowed response time in the absence of significant performance deficits, however, tolerance is reported to develop to some of these effects with chronic use (54,55). Finally, chronic administration of methadone at high daily doses has been shown to create a dependence profile very similar to that seen with morphine, including development of tolerance with dose escalation requirements, and emergence of an opioid withdrawal syndrome following abrupt cessation (but this emerges later and persists longer than that seen after morphine discontinuation) (56).

Effect of methadone used as an OAT medication for opioid dependence is most frequently measured in terms of retention in treatment and reduction in illicit opioid use, although improvements in psychosocial function and medical condition have also

been documented (57). Such effects appear to be dose related with most patients stabilizing at doses between 60-120 mg daily (58). Mean one-year retention in treatment is approximately 60% and can vary based on adherence to dosing practices and other individual and treatment-related factors (59-62). Finally, there is strong evidence on the significance of treatment retention in reducing all-cause and overdose death (25). Dose optimization is therefore crucial to increase the effectiveness of methadone maintenance treatment (MMT) and reduce mortality.

1.3.3 Adverse effects

During the induction phase of MMT, patients are being withdrawn from heroin and may therefore show typical withdrawal symptoms, which should be differentiated from methadone-induced side effects (53). They may exhibit some or all of the following signs and symptoms associated with acute withdrawal from heroin or other opioids: lacrimation, rhinorrhea, sneezing, yawning, excessive perspiration, goose-flesh, fever, chilliness alternating with flushing, restlessness, irritability, weakness, anxiety, depression, dilated pupils, tremors, tachycardia, abdominal cramps, body aches, involuntary twitching and kicking movements, anorexia, nausea, vomiting, diarrhea, intestinal spasms, and weight loss. Withdrawal symptoms due to discontinuation of methadone among patients undergoing methadone maintenance are similar to withdrawals from other opioids (63-67) such as morphine, however, the onset is slower, the course is more prolonged, and the symptoms are less intensive (53).

The most frequently observed adverse reactions related to methadone treatment include lightheadedness, dizziness, sedation, nausea, vomiting, and sweating. Other and less frequent side effects include: neuro-psychological (asthenia, headache, seizures, agitation, confusion, disorientation, dysphoria, euphoria, insomnia, hallucinations), cardiovascular (arrhythmias, bradycardia, QT-interval prolongation and other ECG abnormalities, cardiomyopathy, flushing, hypotension, palpitations, phlebitis, syncope), respiratory (pulmonary edema, respiratory depression), gastrointestinal (abdominal pain, anorexia, biliary tract spasm, constipation, dry

mouth, glossitis), hematologic (reversible thrombocytopenia), metabolic (hypokalemia, hypomagnesemia, weight gain, edema), skin (pruritus, urticarial and other skin rashes), hormonal (amenorrhea, antidiuretic effect, reduced libido and/or potency), and urological (urinary retention or hesitancy) adverse reactions (53, 68-71).

Despite its adverse effects, methadone safety is sufficiently established in clinical practice (72). Nevertheless, like other opioid agonists, methadone has the potential to induce lethal respiratory suppression when given in doses that exceed an individual's tolerance (46,49). Likewise, when under-dosing, bothersome objective and subjective withdrawal symptoms are among the factors that can adversely affect treatment satisfaction, resulting in relapse to illegal opioid use with the consequent risk of overdose and death. This emphasizes the importance of balancing an efficacious dose to achieve the desired therapeutic effect, against either a too low dose, leading to withdrawal symptoms and relapse to illicit opioid use or a too high dose, causing adverse effects and toxicity.

1.3.3.1 Opiate withdrawal rating scales

Two rating scales (Figure 1) presented by Handelsman and colleagues in 1987 (64), have been widely used for measuring the signs and symptoms of opiate withdrawal. The Subjective Opiate Withdrawal Scale (SOWS) contains 16 symptoms rated by the patient on a scale of 0 (not at all) to 4 (extremely). The Objective Opiate Withdrawal Scale (OOWS) contains 13 physically observable signs, rated present or absent, based on a timed period of observation of the patient by a rater. Opiate users admitted to a detoxification ward had significantly higher scores on the SOWS and OOWS before receiving methadone as compared to after receiving methadone for 2 days. Opiate users seeking treatment were challenged either with placebo or with 0.4 mg naloxone. Post-challenge SOWS and OOWS scores were significantly higher than pre-challenge scores in the naloxone but not the placebo group. The researchers demonstrated good inter-rater reliability for the OOWS and good intra-subject reliability over time for both scales in controls and in patients on MMT. These scales are demonstrated to be

valid and reliable indicators of the severity of the opiate withdrawal syndrome over a wide range of common signs and symptoms.

Figure 1. Subjective and objective opiate withdrawal scales

Subjective Opiate Withdrawal Scale (SOWS)

Instructions: We want to know how you're feeling. In the column below today's date and time, use the scale to write in a number from 0-4 about how you feel about each symptom right now.

Scale: **0 = not at all** **1 = a little** **2 = moderately** **3 = quite a bit** **4 = extremely**

	DATE					
	TIME					
	SYMPTOM	**SCORE**	**SCORE**	**SCORE**	**SCORE**	**SCORE**
1	I feel anxious					
2	I feel like yawning					
3	I am perspiring					
4	My eyes are tearing					
5	My nose is running					
6	I have goosebumps					
7	I am shaking					
8	I have hot flushes					
9	I have cold flushes					
10	My bones and muscles ache					
11	I feel restless					
12	I feel nauseous					
13	I feel like vomiting					
14	My muscles twitch					
15	I have stomach cramps					
16	I feel like using now					
	TOTAL					

Mild Withdrawal = score of 1 – 10
Moderate withdrawal = 11 – 20
Severe withdrawal = 21 – 30

Objective Opioid Withdrawal Scale (OOWS)

Date ..... Time .....

OBSERVE THE PATIENT DURING A 5 MINUTE OBSERVATION PERIOD THEN INDICATE A SCORE FOR EACH OF THE OPIOID WITHDRAWAL SIGNS LISTED BELOW (ITEMS 1-13). ADD THE SCORES FOR EACH ITEM TO OBTAIN THE TOTAL SCORE

	SIGN	MEASURES		SCORE
1	Yawning	0 = no yawns	1 = ≥ 1 yawn	
2	Rhinorrhoea	0 = < 3 sniffs	1 = ≥ 3 sniffs	
3	Piloerection (observe arm)	0 = absent	1 = present	
4	Perspiration	0 = absent	1 = present	
5	Lacrimation	0 = absent	1 = present	
6	Tremor (hands)	0 = absent	1 = present	
7	Mydriasis	0 = absent	1 = ≥ 3 mm	
8	Hot and Cold flushes	0 = absent	1 = shivering / huddling for warmth	
9	Restlessness	0 = absent	1 = frequent shifts of position	
10	Vomiting	0 = absent	1 = present	
11	Muscle twitches	0 = absent	1 = present	
12	Abdominal cramps	0 = absent	1 = Holding stomach	
13	Anxiety	0 = absent	1 = mild - severe	
	TOTAL SCORE			

Range 0-13
Handelsman, L., Cochrane, K. J., Aronson, M. J. et al. (1987) Two New Rating Scales for Opiate Withdrawal, *American Journal of Alcohol Abuse,* 13, 293-308

1.3.4 Pharmacological aspects

1.3.4.1 Specific pharmacokinetic properties

Methadone is a synthetic opioid receptor agonist, usually administered orally as a racemic mixture of R- and S-methadone, even though the R-enantiomer accounts for the opioid effect. It is rapidly absorbed across the intestinal lumen (with an absorption half-life of 15-60 min) and enters the portal circulation and then the liver. The peak plasma concentration is achieved after 2–4 h and the elimination half-life at steady-state is 24-28 h (73-75). Due to the long terminal half-life, methadone can be administered as a single dose a day. The steady-state plasma concentration is not approximated until day 5 of methadone dosing (i.e. after 5 half-lives). Increasing dose before steady-state is achieved will result in an accelerated increase in plasma levels, which can contribute to the risk of overdosing (e.g. sedation or respiratory suppression). Once steady-state is achieved, the ratio of peak- (serum concentration 2-4 h after dose intake) to-trough concentration (serum concentration just before the next dose intake) is approximately 1.6-2.0. Exceeding this ratio may be an indication of increased methadone clearance due to changes in elimination and/or metabolism (74,76).

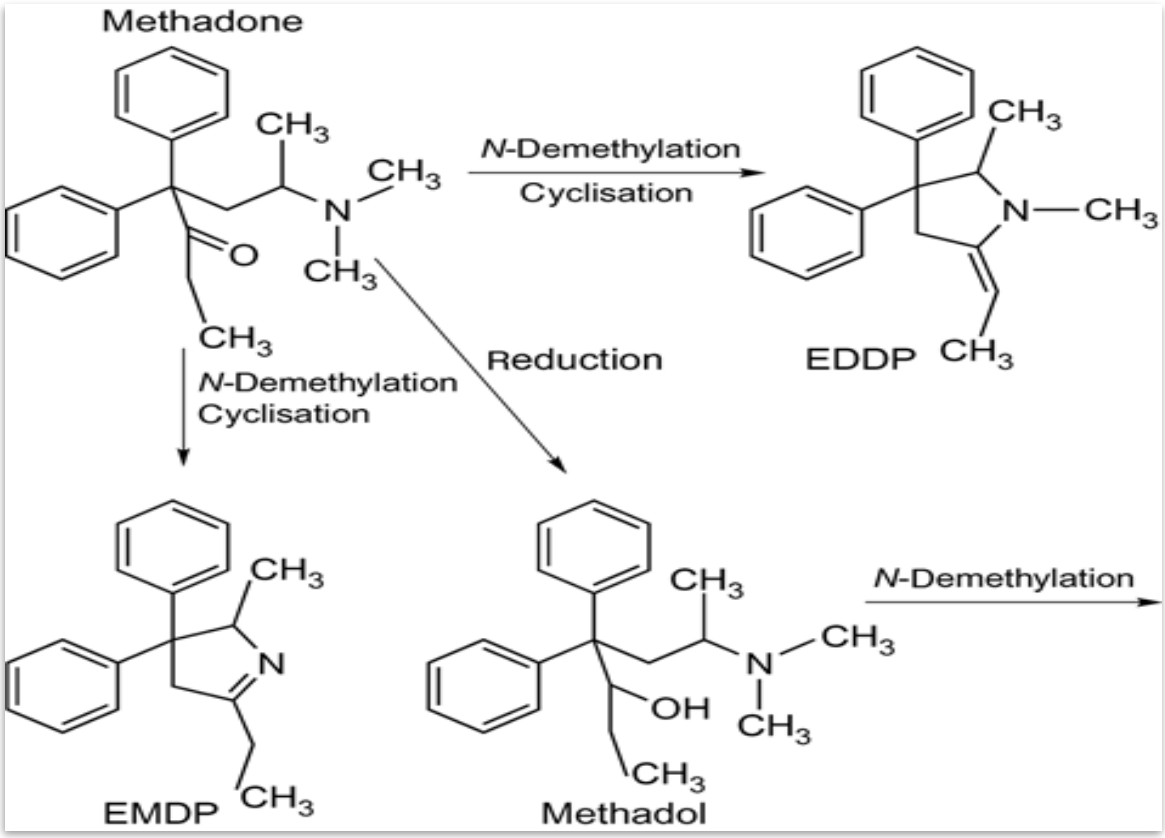
Methadone has a high bioavailability of approximately 70–80% with some variability because of alterations in hepatic first pass metabolism. It is also largely (60–90%) bound to plasma proteins such as albumin and α1-acid-glycoprotein, and 10% is unbound and available for transit across tissue membranes (77,78). Having an apparent volume of distribution of 3.6 L/kg during steady-state, methadone is distributed throughout various tissues such as the liver, intestine, kidneys, muscle, and brain. The rate of distribution into and out of the tissues is different from that of elimination, thus methadone displays bi-exponential (two-compartment) pharmacokinetics (75).

1.3.4.2 Metabolism

Methadone is eliminated mainly by hepatic metabolic clearance, followed by renal and fecal excretion of its metabolites. The drug is extensively metabolized, mainly by the cytochrome P-450 (CYP) enzyme system in the liver, but probably also by intestinal

CYP3A4 and efflux transporters (75,79). Two major processes are involved in the hepatic metabolism of methadone; demethylation and cyclization, resulting in at least ten metabolites, with 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) as the main and inactive metabolic product. Reduction constitutes a third but smaller proportion of methadone metabolism with further demethylation of the produced metabolites (Figure 2). Both methadone and its metabolites are primarily excreted in the feces. Non-metabolized methadone excretion in the urine accounts for less than 11% of the administered dose (70,80,81). The limited amounts of circulating drug that undergo glomerular filtration are partially reabsorbed by the kidney tubules, and this reabsorption is pH-dependent meaning that clearance is inversely related to urine pH (75).

Figure 2. The metabolism pathway of methadone



In vitro and in vivo studies have suggested the involvement of several CYP enzymes in the metabolism of methadone. Although there is no consensus on the relative contributions of each enzyme to the overall disposition of methadone, CYP3A4

appears to play a role (82,83). Hepatic CYP3A4 displays up to 30-fold variability in its activity and its abundance in the gut varies 11-fold (75,82). Measuring the in vitro formation of EDDP from methadone, have confirmed the major involvement of CYP3A4 in this metabolic pathway highly correlated with methadone N-demethylation (75). In addition, CYP3A activity appears to have a modest influence on in vivo methadone disposition, indicating that inhibitors and inducers of this enzyme should be monitored in patients taking methadone (82). Based on these findings and other supporting evidence, CYP3A4 has long been considered to be the main enzyme responsible for the metabolism of methadone (82,83). Although, some new in vivo studies suggest that CYP2B6 accounts for the major part of methadone's metabolism (84,85), at least of the less active enantiomer S-methadone (86). Moreover, some other enzymes including CYP3A5, CYP2D6, CYP2C19, CYP2C9, and CYP1A2 have also to some extent been suggested to be involved in methadone's metabolism (84,87-91). However, the current knowledge on this topic is inconsistent and there is a great uncertainty regarding the clinical relevance of the findings.

Stereo-selectivity toward S- or R-methadone during the various metabolic stages adds to the complexity of the drug's metabolism. For instance, R-methadone has been found to have a lower intrinsic clearance when compared with S-methadone, with a significantly greater fraction of the dose excreted in the urine as S-EDDP and R-methadone than the corresponding enantiomers, suggesting that significantly less R-methadone than S-methadone is metabolized to EDDP. Stereo-selectivity may also involves metabolic stages other than EDDP formation such as binding of methadone to proteins, renal clearance and elimination of EDDP via feces (75).

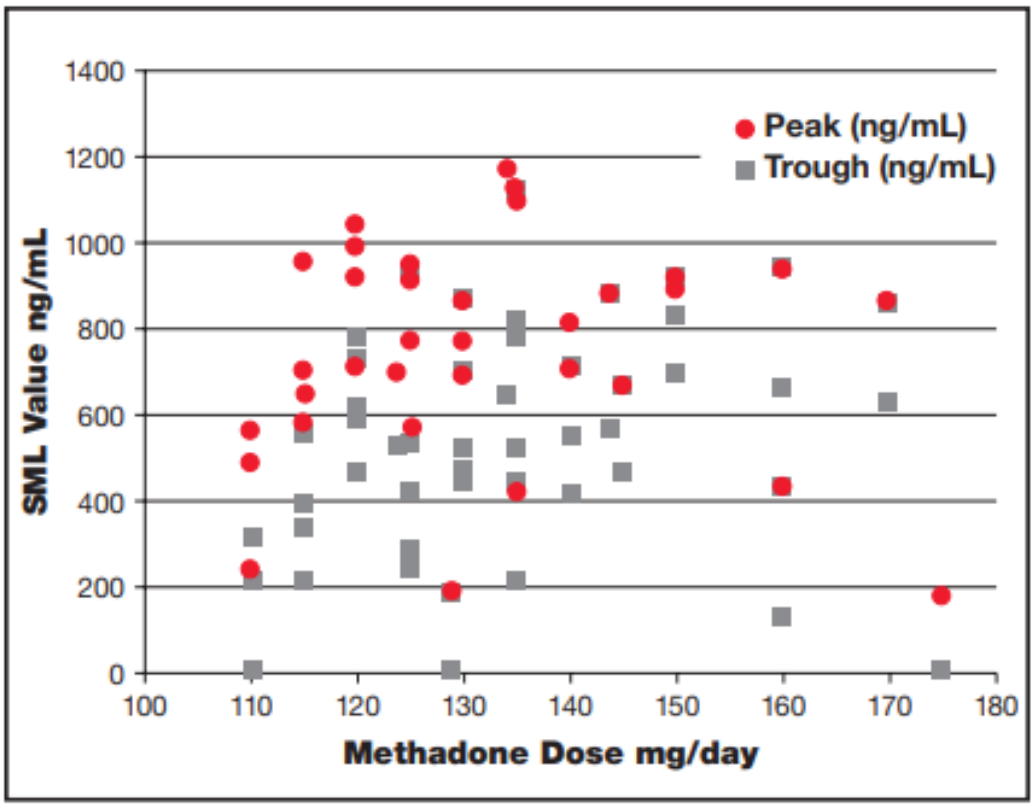
1.3.5 Dose-serum concentration-effect relationship

1.3.5.1 Relationship between the dose and serum concentration

Despite its wide use worldwide among people with opioid dependence, and years of research, our knowledge on methadone pharmacokinetics is still limited. A considerable inter-individual variability in methadone daily doses and serum concentrations has been reported in patients undergoing MMT (43,75,92,93). This has

challenged researchers to demonstrate a clear relationship between a given dose and the concentration achieved in blood (Figure 3). Thus, the optimal dose at steady-state is hard to predict (92,93).

Figure 3. Lack of correlation between dose and peak/trough serum methadone levels (SML) (93)



1.3.5.2 Relationship between the dose and clinical effects

Previous researches have suggested associations between the dose and some of the clinical effects in MMT (52,55-62). As mentioned earlier, reduction in the use of illicit opioids and retention in the treatment are among the most frequently used outcomes to determine the effectiveness of MMT (57,58). Such effects appear to be dose related and numerous non-randomized studies have identified better outcomes for patients on methadone doses ≥60 mg a day compared to lower doses (52,55,58). Most of the patients seem to be stabilized with the daily doses of 60-120 mg having no objective or subjective opioid withdrawal symptoms or craving (58). Higher methadone doses than

120 mg a day have been associated with increased risk of adverse effects such as QT prolongation, and a dose dependent effect on QT prolongation has been shown in some studies (69-73).

1.3.5.3 Relationship between serum concentration and clinical effects

The relationships between serum methadone concentrations and clinical effects are still not fully understood (43). Accordingly, a clinically oriented approach rather than an approach based on serum levels has been suggested for optimizing the methadone daily dosage for individual patients (92-95). Limited studies (96,97), however, have suggested a direct association between the serum methadone concentration and retention in treatment. Others (67,87) have shown that when the serum concentration is too low to inhibit withdrawal symptoms, patients relapse to substance use and drop out of the treatment. Some studies (93,98,99) have indicated that higher concentrations are more likely to reduce opioid craving. Finally, a rapid decline in the trough concentration is related to clinically important responses, notably objective withdrawal symptoms (100).

In a research work (101), the two rating scales (OOWS and SOWS) which were originally developed for measurements of objective and subjective signs of opiate withdrawal, respectively, were used to evaluate potential correlates of methadone effects in relation to serum methadone concentrations. Patients participating in MMT were studied during 24 h after the intake of the daily methadone dose. Methadone concentrations were compared to the subjective (estimated by the patients) and objective (estimated by the investigator) signs of the drug effects before, and 2.5, 5, 9 and 24 h after intake of methadone. Results indicated that, for subjective ratings, the majority of the items investigated corresponded well with the serum methadone concentrations. The most significant associations were found for the following items: low psychomotor speed, alertness, running nose, yawning and anxiety. For objective ratings, only the items rhinorrhea, piloerection and signs of anxiety were significantly associated with the methadone concentrations. The authors concluded that these rating scales might, together with serum methadone determinations, be of considerable value when making dose adjustments for the patients undergoing MMT.

Some researchers have proposed the benefit of a 150 to 600 ng/mL trough racemic methadone serum concentration to suppress opioid craving (75,93,98). This knowledge has to some extent led to the use of therapeutic drug monitoring (TDM) for the surveillance of MMT. Accordingly, methadone steady-state serum concentrations measured around 24 h after dose ingestion (i.e. trough concentrations) have been suggested as a supplementary tool to the clinical assessment for the evaluation of the adequacy of treatment (73,81,102). However, due to the large inter-individual variation in the achieved serum concentrations with the use of given doses, and the sparse knowledge on the relationship with clinical effects in patients on MMT, the role of TDM in dose adjustments is still unclear.

There is a need to increase knowledge on the relationship between dose, serum concentration and clinical outcomes to optimize methadone dosing and accordingly enhance the treatment effect.

1.3.6 Possible factors influencing methadone metabolism

1.3.6.1 Genetic polymorphism in the metabolizing CYP-enzymes

Genetic polymorphisms are important for inter-individual variability in CYP2B6 metabolism, and the most frequent variant affecting the metabolizing phenotype is the 516G>T polymorphism, which exists in several haplotypes. The most studied haplotype is the reduced function variant *CYP2B6*6*, which comprises the 516G>T and 785A>G polymorphisms (103,104). The frequency of *CYP2B6*6* in whites is reported to be 20%–30%, whereas frequencies in Africans/African Americans and Asians are 50% and 15%, respectively (105,106).

The impact of *CYP2B6* genotype on inter-individual variability in pharmacokinetics of methadone has been investigated in several previous studies (84-88,91,92,107-112). CYP2B6 seems to contribute to S-methadone metabolism and, to a lesser extent, to R-methadone metabolism, with the carriers of allele *6 showing higher S- and R,S-methadone plasma levels (87). However, the findings are conflicting. One of the reasons for the inconclusive results may be the fact that enzymes other than CYP2B6

could also be of importance for the metabolism of methadone (82). Other CYP-enzymes such as CYP2D6, CYP2C9, and CYP2C19 have also been linked to the metabolism of methadone, but their relative contribution in vivo is unclear (82-84,87-91). Moreover, some reports have indicated an involvement of CYP3A5 in the in vivo metabolism of methadone (84,86,89). The most frequent polymorphism of CYP3A5 gene has the unusual allele distribution of 90% of the inactive (*3) and 10% of the active (*1) allele. CYP3A5 may represent up to 50% of the total hepatic CYP3A content in subjects expressing it (113,114). CYP3A5 might therefore be an important contributor to the inter-individual variability in methadone metabolism (84), although an in vitro effect has not been demonstrated (88). Finally, genetic variations in ABCB1 gene encoding for the permeability glycoprotein (P-gp) efflux transporter are also shown to influence methadone serum concentrations, consistent with the fact that methadone is a substrate of P-gp (115). However, these findings are inconsistent, and the metabolic pathways of methadone are still not fully understood. Knowledge on the impact of genetic polymorphisms of the CYP-enzymes which are supposed to be involved in methadone metabolism is important regarding dose adjustments and treatment outcomes in MMT.

1.3.6.2 Physiological factors (gender, age, body weight)

Conclusive clinical studies on the influence of gender, age and body weight on methadone metabolism are lacking. A few differences have been reported in terms of treatment approaches, clinical outcomes, and physiological and pharmacological effects between men and women undergoing MMT (70,116). Based on general assumptions, it is conceivable that advanced age may lead to an increased risk of drug accumulation in the body, or patients with higher body mass index (BMI) may need higher doses. The half-life of methadone seems to increase in the elderly; however, this observation is based on limited clinical data suggesting that patients aged 65 years or older have a lower methadone clearance (117). The impact of overweight on methadone metabolism is not sufficiently investigated in previous research; nevertheless, a recent study (118) demonstrated that individuals with overweight had higher methadone serum levels. Possible explanations for this observation could be the changes in body compartment proportions (i.e. the amount of fat tissue that influences

volume of distribution), and an impaired hepatic function due to steatosis (119,120). However, the supporting evidence regarding the influence of gender, age and body weight in MMT is still limited (70,75,118,121). Increased knowledge on the impact of the physiological factors on methadone metabolism is of importance to optimize methadone dose adjustments and clinical outcomes.

1.3.6.3 Pathological factors (hepatic and renal dysfunction)

Liver is the most important organ for the extensive metabolism of methadone (75,79). Metabolism of drugs in the liver depends on hepatic blood flow and liver enzyme activity; both can be affected by liver disease (122,123). Cirrhosis or advanced fibrosis of the liver tissue related to chronic infections or other hepatic diseases, long-term alcohol consumption or even predisposition to specific genotypes of cytochrome P-450 (CYP) enzymes may affect liver function (124,125). Chronic HCV infection is common in patients with injecting substance use (126). Untreated HCV infection can result in liver cirrhosis and even death due to liver failure or hepatocellular carcinoma (127). In a cohort of HCV-infected injecting substance users, a third developed advanced liver disease within three decades (128). The impact of liver cirrhosis and developing portal hypertension and reduced first pass effect on methadone metabolism is not fully understood. Existing studies have not found sufficient evidence to justify and guide methadone dose adjustments due to chronic liver diseases with advanced liver fibrosis (79,118,129-131). Thus, more research on this topic is needed.

Pharmacologically, methadone disposition seems to be relatively unaffected in renal impairment (121). Both methadone and the metabolites are primarily excreted in the feces. Non-metabolized methadone and its major metabolite (EDDP) excretion in the urine accounts for less than 11% of the administered dose (80,81). Nevertheless, the renal elimination of methadone is dependent on the acid-base ratio in urine, and the reabsorption of methadone increases with increasing base in urine (132). It is also supposed that the importance of renal elimination increases at doses above 40-50 mg a day. However most opioid dependent patients with renal failure can be substituted with methadone and dialysis has little effect on the methadone concentration in the blood (133). Apparently, it seems that no dose adjustment is required for mild to

moderate renal impairment, but a dose reduction up to 25-50% may be considered at creatinine clearance of less than 10 ml/min (132,133). However, there is little and conflicting data on the dosage of methadone in renal failure. In order to optimize methadone treatment, there is a need for increased knowledge in this area.

1.3.6.4 Concurrent use of potentially interacting medication

Pharmacokinetic drug interactions are often related to inhibition or induction of the CYP enzymes crucial for the metabolism of the substrate drug with a subsequent increasing or decreasing of the drug's serum concentration, respectively (132). Considering a role of CYP-related metabolism pathways, such interactions will therefore potentially lead to altered serum concentrations of methadone (81,84). Drugs that potentially inhibit methadone metabolism include some antifungals such as fluconazole, antibacterial agents such as ciprofloxacin, antidepressants such as fluoxetine, and proton pump inhibitors such as omeprazole. Other drugs, including the antiepileptic drugs carbamazepine, phenobarbital and phenytoin, have been shown to induce methadone metabolism (134). It may be challenging to predict possible drug interactions, as some compounds may inhibit some CYP enzymes while inducing others. For example, the anti-HIV drug ritonavir has shown to both induce and inhibit the metabolism of methadone. The net impact of ritonavir and other anti-HIV drugs on methadone serum concentration depends, at least in part, on the specific drug combination used in the ingested antiviral regime (136-138).

Despite a large number of patients undergoing MMT worldwide, most studies on drug interactions of methadone are based on in vitro methodology, and very few clinical studies involve serum concentration measurements. Investigating of drug interactions as possible causes of alterations in methadone serum concentration may be considered an important indication for the use of TDM in MMT. Thus, more clinical research by using methadone serum concentrations is needed to increase the knowledge on clinically important drug interactions with methadone. This can help clinicians in decision making for dose adjustments and improving the treatment outcomes.

1.4 Rationale for the research

Methadone has many favorable properties as a substitution medication used in OAT for opioid dependence. Taken orally once a day in sufficient doses, it makes the patients stop using of illicit opioids and retain in the treatment by removing opiate withdrawal symptoms and craving. There is strong evidence for methadone's effectiveness concerning significant reduction of mortality and morbidity and giving the patients a chance to undergo social rehabilitation (15,138). The drug, however, also have some adverse effects like constipation, sweeting, weight gain, decreased sexual drive, and sleepiness (53, 68-71). It is also associated with fatal overdoses (46,49,139). Long-term methadone treatment needs therefore to balance a high enough dose to achieve the wanted effects against the risk of impairment and overdose.

According to the last annual OAT status report from Norwegian Center for Addiction Research (28), above 70% of the patients (30% being women) are aged over 40 with a mean age of 46.5 years, and almost 35% have other medical comorbidities with over 50% being contaminated by HCV. Thus, most of them also use other medications concurrently. Additionally, the frequency of substance use especially those with sedative and respiratory depressant effects such as benzodiazepines and alcohol is high (around 40-50%). These facts underscore the importance of closer follow-up and continuous clinical monitoring in order to reduce the possible adverse effects and risks related to methadone treatment.

Based on theoretical pharmacological considerations, it can be supposed that high serum concentrations of methadone could lead to adverse effects while low concentrations could result in withdrawal symptoms and impaired therapeutic effect. However, due to the large inter-individual variations in obtained serum concentrations by using the recommended daily doses of methadone, the role of TDM in the treatment monitoring and dose adjustment is not fully understood. Each patient presents a unique clinical challenge, and there is no way of prescribing a single best methadone dose to achieve a specific blood level as a "gold standard" for all patients. Clinical signs and patient-reported symptoms of withdrawal syndrome, and continuing illicit opioid use usually remain possible indicators of dose inadequacy.

The major issue is then what should guide dose adjustments in MMT. In other words, what indicates that a given dose is too low, low, enough, high or too high? Adding to the complexity, some researchers have shown that factors other than the dose – such as the patient's expectations and medication preferences, as well as the patient's total physical and mental health condition or improvements in psychosocial functioning – may influence treatment satisfaction (68,111). These findings further challenge clinicians on how to cope with suboptimal treatment outcomes: should the dose be adjusted, or should other problems instead be addressed?

Even if the treatment efficacy is well established, the influence of methadone pharmacokinetics on dose requirements and clinical outcome remains controversial (84). This may be due to lack of knowledge concerning factors that influence methadone metabolism and consequently serum concentration-to-dose ratio (CDR). Variations in methadone's metabolism could potentially explain both deviant serum concentrations and unexpected clinical outcomes. In addition to genetic variations, other inherent clinical characteristics such as gender, age, BMI, and hepatic and renal function, as well as extrinsic factors such as concomitant medication are generally presumed to influence a drug's metabolism. However, the supporting evidence regarding the influence of such factors in MMT is still limited (82,83,90,92).

The underlying mechanisms of methadone metabolism, possible influencing factors, and the relationships between the dose, serum concentration and clinical outcomes should be investigated to provide sufficient evidence base for clinical decision-making. More knowledge in this field is needed in order to improve the therapeutic outcomes and minimize the adverse effects with fatal overdoses as a worst-case scenario.

One of Norway's strengths for clinical research is the stable population and ability to keep track of individuals over time. This provides a minimum loss to follow up. In addition, a rather large group of patients undergoing MMT in Norway are closely followed up, enabling us to access a substantial pool of clinical and laboratory data. Recently, an integrated treatment model for OAT has been established in the Department of Addiction Medicine at Haukeland University Hospital in the city of

Bergen, Norway. The patients are more accessible as they visit the specialized outpatient OAT clinics from one to seven days a week to receive substitution medications under direct observation, and to be closely monitored. These advantages have been used to the full in this research as the shortcomings of the earlier studies to some extent point to such kinds of study designs in achieving more sustainable conclusions.

2. Aims and objectives of the thesis

2.1 Aims

The overall aim of this project was to investigate the influence of genetic, pathophysiological and pharmacological factors on dose-adjusted serum methadone concentration. Further, to explore the relationship between serum methadone concentration and clinical outcomes in patients with opioid dependence undergoing MMT.

2.2 Objectives

To achieve the aims of the thesis, the following objectives were addressed:

1. To clarify the impact on dose-adjusted serum methadone concentration of genetic polymorphisms of some candidate hepatic CYP enzymes (CYP2B6, CYP3A5, CYP2C9, CYP2C19, CYP2D6) among patients undergoing MMT (papers I and III)
2. To investigate the impact on dose-adjusted serum methadone concentration of concurrent use of medications with possible interacting effects through the same CYP enzymes (CYP2B6, CYP3A5, CYP2C9, CYP2C19, CYP2D6) as well as age, gender, and BMI among patients undergoing MMT (papers II and III)
3. To explore the impact on dose-adjusted serum methadone concentration of liver fibrosis and clinical characteristics among patients undergoing MMT (paper III)
4. To investigate whether serum methadone concentration is related to subjective opioid withdrawal symptoms, adverse effects and substance use, and the role that these variables could play in clinical decision on dose adjustment in patients undergoing MMT (paper IV)

3. Material and methods

3.1 Study design

All the four studies have applied naturalistic observational cohort design, either retrospectively (papers I and II) or prospectively (papers III and IV).

3.2 Data sources

This project has used observational data from three different sources; TDM laboratory database at the Center for Psychopharmacology, Diakonhjemmet Hospital (Oslo, Norway) for paper I, TDM laboratory database at the Department of Clinical Pharmacology, St. Olav University Hospital (Trondheim, Norway) for paper II, and cohort data from the INTRO-HCV study (140) conducted at the Department of Addiction Medicine, Haukeland University Hospital (Bergen, Norway) for papers III and IV.

3.3 Samples

Paper I

The TDM database at the Center for Psychopharmacology, Diakonhjemmet Hospital (Oslo, Norway), was reviewed for patients on MMT who had submitted at least one serum sample for the determination of methadone concentration and a blood sample for CYP genotyping as a part of the clinical follow-up. Serum samples were collected at steady-state (≥12 hours after the last intake of methadone). Information about serum concentrations and doses of methadone, age, sex, co-medications and *CYP2D6*, *CYP2C9*, *CYP2C19*, *CYP2B6* and *CYP3A5* genotypes were retrospectively collected from samples submitted in January 2006 through December 2015. Sixty-two patients with 155 serum samples were included in the study.

Paper II

The TDM database at the Department of Clinical Pharmacology, St. Olav University Hospital (Trondheim, Norway) was used. This included measured serum concentrations of methadone from opioid maintenance clinics in Norway since 1999.

The database also contained information obtained from the requisition forms, such as patient data, prescribed methadone dose, time of last dose intake, time of blood sampling, and types and doses of concomitant medications. A total of 11,149 serum concentration analyses of methadone from 3575 subjects were recorded in the laboratory database from October 1999 to July 2017. We excluded records of samples for which the dose and/or the time interval from last intake of medication to blood sampling were lacking or unknown, samples obtained less than 10 h or more than 26 h after last dose, samples where methadone doses were less than 40 mg/d, and samples with non-measurable methadone levels (concentrations less than 7.8 ng/mL). The unique Norwegian 11-digit personal identity number made patient identification consistent. When this number was missing and the subject could not be identified by other means, the sample was excluded. After applying these exclusion procedures, 4425 samples from 1691 subjects were included in the study.

Papers III and IV

The studies were conducted at the Department of Addiction Medicine, Haukeland University Hospital (Bergen, Norway) from May 2016 to January 2020. All clinical measurements and laboratory data were recorded prospectively in the hospital journal system, as well as in a recently established health registry database linked to the INTRO-HCV study (140) for patients undergoing OAT in Bergen. In addition to incorporating individual health data, the database included relevant information based on clinical surveys and research records.

Paper III: We included information on age, gender, BMI (kg/m^2), genotypes of *CYP2B6* and *CYP3A5*, methadone daily dose (mg/day) and steady-state trough serum concentrations (nmol/L), concurrent medications, self-reported use of illicit substances and alcohol, liver function parameters including the degree of liver stiffness estimated by transient elastography (kPa), renal function parameters including estimated glomerular filtration rate (eGFR), status on HCV infection (presence of antibody and RNA), and HIV infection. In total, 155 patients were included in the study.

Paper IV: We included information on age, gender, daily methadone doses, serum methadone concentrations, subjective opioid withdrawal symptoms, some common subjective opioid adverse effects, and self-reported illicit drug use. We also included information about the time since last dose intake, time of blood sampling, time when symptoms were recorded, numbers of days with directly observed intake per week, and duration of OAT. One hundred and ninety-nine patients consented to participate in the study and started the primary surveys through in-person clinical interviews by a research nurse. At the end of the study, 83 patients had completed the surveys according to the study protocol, with the time difference between the clinical assessments and laboratory measurements being < 14 days (Mean =2, SD =3), and were included in the study.

3.4 Main laboratory analyses

3.4.1 Serum concentration analysis of methadone (papers I, II, III, IV)

For all the four studies the analysis of racemic methadone in serum samples was performed by comparable validated and certified ultraperformance liquid chromatography with tandem mass spectrometry (LC-MS/MS) method, respectively developed at Center for Psychopharmacology, Diakonhjemmet Hospital (paper I), at the Department of Clinical Pharmacology, St. Olav University Hospital (paper II) and at the Department of Medical Biochemistry and Clinical Pharmacology, Haukeland University Hospital (papers III and IV).

During the development phase of the method as well as in routine use, methadone concentrations were measured in nmol/L. The conversion factor from nmol/L to ng/mL for methadone is 0.310.

Paper I: Samples were purified by protein precipitation mixing 500 mL of serum aliquot with 1000 mL of acetonitrile–methanol (90/10 vol/vol), which included the internal standard (protryptyline), followed by centrifugation for 10 minutes (1800 g at -2ºC). Of approximately 5 mL of purified sample was then injected into an Acquity

UPLC system (Waters, Milford, MA) with a Micromass Quattro micro MS detector (Waters) operated in electrospray ionization-positive mode. Chromatographic separation was performed on an Acquity UPLC BEH Shield RP18 column (1.7 mm, 1.0 x 100 mm; Waters). The mobile-phase gradient was based on a mixture of acetonitrile and ammonium acetate buffer (pH 4.8). The retention time of methadone was 2.4 minutes, and the total run time was 5 minutes. Methadone was detected by the m/z transition 310.2 > 265.2. The calibration curve of methadone ranged 500–3000 nmol/L and fitted to a quadratic polynomial function ($r2 > 0.995$). At 1000 nmol/L, the interday imprecision and inaccuracy showed values less than 3% and 2%, respectively ($n = 5$).

Paper II: Methadone was extracted from 0.5 mL serum with 1 mL ammonium carbonate (pH 9.0) after addition of the internal standard methadone-d3. After solid-phase extraction on a Bond-Elut-C18 100 mg column (Agilent, Palo Alto, CA, USA), the extract was evaporated to dryness and the residue was reconstituted in 50 μL methanol, transferred to vials, and injected on an Agilent MSD 1100 LC-MS system (Agilent). Separation was performed on a Supelco Discovery C18 (20 × 4 mm, 5 μm) column (Merck, Darmstadt, Germany) with a mobile phase consisting of methanol:ammonium acetate 60:40. Total run time was 1.5 min. Methadone was monitored after positive chemical ionization at m/z 310.3 > 265.1 and the internal standard methadone-d3 at m/z 313.3 > 268.1. The limit of quantification was 25 nmol/L (7.8 ng/mL) and the method was linear at least to 3000 nmol/L (930 ng/mL). Recoveries were 77% and 90% and inter-day coefficients of variation were 4.2% and 2.5% at low and high concentrations, respectively.

Paper III and IV: Blood samples were drawn from the participants at the OAT clinics according to the study protocol and at trough concentration with a mean time of 21 ± 8 (standard deviation) hours since the last dose intake and no changes in the methadone dose during the last 1-2 weeks (steady-state). Analysis of methadone in all the collected serum samples was performed by the same analytical method using the same laboratory instruments. MS-MS analysis was performed with electrospray ionization

(ESI) in positive ion mode (Agilent Technologies 6410AA triple quadrupole LC-M-MS, CA, USA). The limit of quantification was 20 nmol/L and the method was linear at least to 4000 nmol/L. Recoveries were 100% and 91% and inter-day coefficients of variation were 2.7% and 5.1% at low and high concentrations, respectively.

3.4.2 Concentration-to-dose ratio (papers I, II, III)

The primary outcome variable for papers I, II and III was the dose adjusted serum concentration of methadone expressed as concentration-to-dose ratio (CDR). CDRs were reported instead of actual concentrations to take into account the large variations in methadone daily doses used when the samples were obtained. CDR was calculated by dividing the measured serum concentration (in ng/mL or nmol/L) by the daily dose (in mg) used by the patient at the time of sampling, i.e. (ng/mL or nmol/L)/(mg/d). By using this measure, the estimated values could be compared within as well as between subjects without taking variations in the dosage into consideration. The unit for CDR is not repeated when using CDR in the thesis.

3.4.3 Genotyping (papers I and III)

Genotyping of the blood samples was performed using TaqMan-based realtime polymerase chain reaction assays routine analysis at the Center for Psychopharmacology for papers I and III. The determination of *CYP2B6*6* haplotype was based on genotyping of 516G.T (rs3745274) and 785A.G (rs2279343) variants. The presence of both 516TT and 785GG was interpreted as *CYP2B6*6/*6*, whereas the presence of 516GT and 785AG or 785GG was interpreted as *CYP2B6*1/*6*. The combinations of 516GG and 785AA or 785AG were interpreted as *CYP2B6*1/*1*. The determination of *CYP3A5*3* haplotype was based on genotyping of 219-237A>G (rs776746). The presence of 219-237GG was interpreted as *3/*3, whereas the presence of 219-237AG was interpreted as *1/*3. Absence of any of the analyzed variants was determined as either *CYP3A5*1* or *CYP2B6*1*, i.e. the wild-type alleles. The patients who presented 2 of any of the reduced function alleles were defined as poor metabolizers, those who presented 1 reduced function allele were defined as

intermediate metabolizers, whereas the remaining patients were classified as extensive metabolizers.

3.5 Assessments

The clinical assessments included self-reported use of substances, subjective symptoms of withdrawal and adverse effects, and measurements of liver stiffness. Information on age (based on date of birth) and gender were recorded in the clinical or laboratory data basis for each participant. BMIs were calculated digitally by research nurses based on the participants' height and weight measurements at the outpatient clinics (papers III and IV).

3.5.1 Substance use (papers III and IV)

Self-reported use of illicit drugs – including heroin or other opioids, amphetamines (amphetamine and/or methamphetamine), benzodiazepines, and cannabis – as well as alcohol, and frequencies of use (categorized as no use at all, or frequent use including either several times a month, weekly, or daily use) during the last month were recorded for the participants.

3.5.2 Assessment of liver stiffness (paper III)

Liver Stiffness Measurements (LSM) were assessed by vibration controlled transient elastography (VCTE) using FibroScan (Model 430 Mini). LSM is correlated to liver fibrosis stage (141). Exclusion criteria were pregnancy, presence of an implantable medical device, and BMI≥30 kg/m² (to avoid erroneous measurements using standard probes that were not adapted to obese individuals). The cutoff values for fibrosis stage (hereby fibrosis measures) for all the participants were as following: LSM≤7 kPa for no/limited fibrosis, LSM 7< kPa<12 for fibrosis, and LSM ≥12 kPa for cirrhosis (141) – those with LSM≥20 kPa in the last category represented cirrhosis state with significant portal hypertension (142).

3.5.3 Assessment of subjective symptoms (paper IV)

Participants who confirmed the presence of withdrawal symptoms were interviewed by a research nurse using the validated standard questionnaire (SOWS) (64), which

covers 16 self-perceived symptoms: anxiety, yawning, perspiring, tearing, runny nose, goosebumps, shaking, hot flushes, cold flushes, bone and muscle aches, restlessness, nausea, vomiting, muscle twitches, stomach cramps, and feeling like using. Respondents rated the intensity of symptoms on a five-point scale from 0 (not at all) to 4 (extreme); possible scores range from 0 to 64 (1–10 = mild withdrawal, 11–20 = moderate withdrawal, 21–30 = severe withdrawal). In addition, all participants were asked six questions on some of the most common subjective adverse effects attributed to methadone treatment, including euphoria, perspiring, nausea, concentration difficulties, feeling "brain fog," and reduced sexual desire; these symptoms were rated in the same way as for the withdrawal symptoms. Perspiring and nausea were defined as adverse effects or withdrawal symptoms based on how each participant perceived them. In addition, one open-ended question asked about other possible symptoms when these were self-perceived to be related to methadone.

Table 2. An overview on the applied methods and materials for this thesis

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Design	Retrospective observational cohort study	Retrospective observational cohort study	Prospective observational cohort study	Prospective observational cohort study
Sources	Laboratory TDM database, Diakonhjemmet hospital, Oslo	Laboratory TDM database, St. Olav university hospital, Trondheim	Clinical and laboratory data, Haukeland university hospital, Bergen	Clinical and laboratory data, Haukeland university hospital, Bergen
Patients with available data	64	3575	199	199
Patients eligible / included	62	1691	155	83
Main laboratory analyses	Serum methadone concentration and CYP genotypes	Serum methadone concentration	Serum methadone concentration and CYP genotypes	Serum methadone concentration
Assessments			Degree of liver stiffness/fibrosis, substance use	Subjective sym. of withdrawal and adverse effects, substance use
Measures included	Serum methadone concentration, dose, age, sex, last dose time, co-medications, CYP genotypes 2B6, 3A5, 2D6, 2C9, 2C19	Serum methadone concentration and dose, age, sex, last dose time, co-medications	Serum methadone concentration and dose, age, sex, last dose time, co-medication, BMI, eGFR, substance use, fibrosis stage, CYP genotypes 2B6, 3A5	Serum methadone concentration and dose, age, sex, last dose- time, time to records, observed intake, OAT duration, Subjective sym. of withdrawal and adverse effects, substance use

BMI: body mass index, CYP: cytochrome P-450 liver enzymes, eGFR: estimated glomerular filtration rate, OAT: opioid agonist treatment, TDM: therapeutic drug monitoring.

3.6 Statistical analysis

The quantitative analyses for this thesis have been performed using IBM SPSS version 21.0 for paper I, STATA/SE 15.1 for paper II, and STATA/SE 16.0 for papers III and IV. Statistical significance was set at the $P < 0.05$ level.

Paper I

Mean and 95% confidence interval (CI) or median and interquartile range was calculated for continuous descriptive variables, and 1-way analysis of variance was used to test the differences between groups. For categorical descriptive variables, contingency table and Pearson x2 or Fisher exact tests were used. Hardy–Weinberg equilibrium was tested for each individual single-nucleotide polymorphism (SNP) using a web tool. Linear mixed model (LMM) analyses were used to study the effect of gene variants in CYP enzymes on the CDR of methadone. To investigate the effects of CYP genes (CYP2B6, CYP3A5, CYP2C9, CYP2C19, and CYP2D6), these variables were inserted as covariates in the analysis together with age, gender, and time between last dose administration and serum sampling. The model allows for adjustment between repeated measurements of methadone serum concentration within each subject by enabling the inclusion of multiple serum concentration measurements per patient in the analyses, which strengthen the inter-individual comparisons. Because the dependent variable (methadone CDR) was not normally distributed, this variable was logarithmically transformed for all measurements before statistical analyses. All the criteria for mixed model analysis were achieved. Mean group estimates from mixed model analyses were then back transformed before presentation.

Paper II

Data were presented as means with 95% confidence intervals and range for continuous variables, as appropriate. The mean methadone dose, serum concentration, and CDR, as well as patient age and time between last dose and sampling (the difference between the recorded interval and 24 h, reported as Time- 24) in each patient were compared with respect to gender using Student's t test. The distribution of the CDR was found to be right-skewed and to achieve near normality the natural logarithm (loge) of methadone CDR was employed as the outcome variable in the statistical model.

Multiple samples were often available from the same patient. Thus, a LMM was used assuming that each individual patient possesses a random intercept (i.e. an individual "offset") in addition to being affected by fixed factors. The fictitious reference group illustrated 40-year-old men, not taking any of the interacting drugs and with a blood sample obtained 24 h after the last dose intake. The variables included in the model were firstly gender, age, time interval from last intake of medication to blood sampling, and concomitant treatment by a total of 46 drugs most frequently recorded to be used in combination with methadone. LMM analyses were applied to study the effect on methadone CDR of concomitant use of other drugs including some inhibitors and inducers of the CYP enzymes (143) supposed to have an impact in methadone metabolism and possibly lead to pharmacokinetic drug interactions. We included CYP3A4, CYP2D6, and CYP2C19 inhibitors in the analyses, whereas CYP2B6, CYP2C9, and CYP1A2 inhibitors were excluded from the main analyses due to too few observations (3, 3, and 5 samples, respectively). In addition, CYP inducers were included (143). Other drugs were included in the model when the drug was recorded in at least 20 samples. Model estimation proceeded forwards, i.e. all these drugs as well as age, gender, and time difference from 24 h between last dose administration, and serum sampling were included one by one as covariates in the unadjusted statistical analysis. Then, adjusted LMM analyses for specific covariates were undertaken based on existing theoretical and empirical knowledge about methadone metabolism and the number of samples included in each group. These covariates were age, gender, sampling time, and co-medication with CYP inducers and CYP3A4, CYP2D6 and CYP2C19 inhibitors. Parameter estimates from the LMM analyses were back-transformed by exponentiation to yield expected CDRs.

Paper III

Basic descriptive data were presented as means (SD) for continuous variables, and numbers with percentages for categorical variables. LMM analyses were applied to investigate possible associations between the explanatory variable of liver fibrosis stage and the outcome variable namely dose-adjusted steady-state serum methadone concentration presented as CDR. In total, 192 observations from 155 patients were

included in the LMM analyses as 37 participants had two sets of records for CDR and fibrosis measures. Interaction analyses ruled out any interacting factor between liver fibrosis and the *CYP* genotypes regarding CDR. Confounding variables were age, gender, BMI, renal function, use of interacting co-medications and the different genotypes of *CYP2B6* and *CYP3A5*. The relevant variables were included one by one as categorical variables in the unadjusted statistical analyses. Renal function measures were not included in the regression analyses due to no recorded severe renal failure (eGFR<30 *ml/min/1.73m*^{2}) (144). There was not recorded any highly suspected interacting medications (145) such as anti-HIV agents and other strong CYP3A4 inhibitors or CYP inducers (143) in our data. The remaining recorded medications without a known interaction potential with methadone were therefore not included in the regression model. Then, the confounding effects of the variables on CDR were investigated using adjusted multivariate LMM analyses. Participants with BMI>30 kg/m² (n=46) were excluded from the adjusted analysis as the measurements of liver stiffness were not possible or - if so - reliable in this group. For participants with two sets of measures, possible changes in CDR and fibrosis measures between the two recording times (pre-treatment and post-treatment) were assessed by adding the time factor in the analysis, and no effects of time were found. We also conducted some sensitivity analyses using LMM to reveal other possible associations or interacting factors when indicated. The intercept presented a woman younger than 50 years old, with BMI<25 kg/m², liver fibrosis measure≤7 kPa, *CYP2B6* genotype *1/*1 and *CYP3A5* genotype *3/*3 who's CDR was 10. Student's t test was used to compare the differences in serum concentrations and CDR between the groups with different combinations of CYP2B6 and CYP3A5 polymorphisms.

Paper IV

Continuous variables were presented as means with standard deviations (SD), as well as ranges when needed. Comparisons of study variables between the participants with and without reported subjective opioid withdrawal symptoms were performed using Mann–Whitney tests for continuous variables and chi-square tests for categorical variables. To avoid Type II statistical errors by overlooking important variables due to

the study's naturalistic design, we also included variables with a *P*-value < 0.10 in the adjusted regression analyses. Linear mixed model analysis was applied to investigate possible associations according to the aim of the study. We included in the main analyses all 16 SOWS items, the 6 subjective adverse effects, and self-reported substance use during the month prior to interviews as dependent variables. The responses to the open-ended question were excluded from the statistical analyses due to scant applicable data. All these variables were included one by one in the unadjusted statistical analyses. Then, adjusted LMM analyses for the specific variables showing statistically significant associations with the serum methadone concentration were undertaken. The results obtained in the main analyses were adjusted for age, gender, and the absolute time difference between blood sampling and the recording of the symptoms.

Table 3. Statistical analyses and outcome measures used in the studies of this thesis

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Software	IBM SPSS 21.0	STATA/SE 15.1	STATA/SE 16.0	STATA/SE 16.0
Statistical analysis				
Frequency tables	X	X	X	X
Pearson chi-square	X			X
Student's t test		X	X	
Mann–Whitney test				X
Interaction analysis		X	X	
Sensitivity analysis			X	X
Linear mixed model	X	X	X	X
Outcome measures				
Dependent variable	Serum methadone concentration-to-dose ratio (CDR)	Serum methadone concentration-to-dose ratio (CDR)	Serum methadone concentration-to-dose ratio (CDR)	Subjective opioid withdrawal symptoms
Independent variables	CYP2B6 genotypes	Interacting Co-medications	Liver fibrosis degree	Serum methadone concentration
Other co-variables	Age, sex, last dose time, CYP3A5, CYP2C9, CYP2C19, CYP2D6 genotypes	Age, sex, last dose time	Age, sex, body mass index, CYP2B6, CYP3A5 genotypes	Age, sex, absolute time diff. between sampling and record of symptoms

3.7 Ethical considerations

All the studies were approved by the respective Regional Committee for Medical Research Ethics. *Paper I:* Regional Committee for Medical Research Ethics and the Hospital Investigational Review Board (reference number 2012/1149). *Paper II:* Regional Committee for Medical and Health Research Ethics in Mid Norway (approval No. 2017/316/REK midt). For papers I and II, the committees had made exceptions from obtaining consent from patients whose serum methadone concentrations were used in the study due to serum samples were taken at past time,

difficulties in re-contacting the patients, and because use of the previously measured serum concentrations did not cause any inconvenience to the patients.

Papers III and IV: The studies were approved by the Regional Committee for Medical and Health Research Ethics in Vest Norway (approval No. 2017/297/REK vest), and all the participants had signed a written informed consent on agreement about using of routine and research data for this purpose and for taking part in the study. Except for expending a couple of hours, there were no other disadvantages or increased risks related to the study participation. It would not have any consequences for the patient's treatment if the patient did not consent to participate. Even if the patient agreed to participate, he or she could later withdraw the consent without affecting the treatment. All information was treated confidentially and with respect for privacy and in accordance with laws and regulations. The information was stored electronically in a database approved by the Norwegian Data Protection Authority (146). The database was secured against access by unauthorized persons. Directly identifiable information (name and birth date) was stored separately from the other information in the database. The study data was indirectly identifiable with a link code only available to authorized persons, and the results of the studies were recorded anonymously. The study participants could request access to the information that was registered about them and had the right to demand correction of any errors. Participants could at any time demand collected biological samples and information about these deleted from the database, without having to state any reason. Deletion of biobank material would not involve deletion from anonymised research files that had already been used in the research. All data will be deleted in accordance with current lows and regulations when the purpose of the studies is completed.

4. Results

4.1 Objective 1 (papers I and III)

To clarify the impact on dose-adjusted serum methadone concentration of genetic polymorphisms of candidate hepatic CYP enzymes (CYP2B6, CYP3A5, CYP2C9, CYP2C19, CYP2D6) among patients undergoing MMT

4.1.1 Basic characteristics

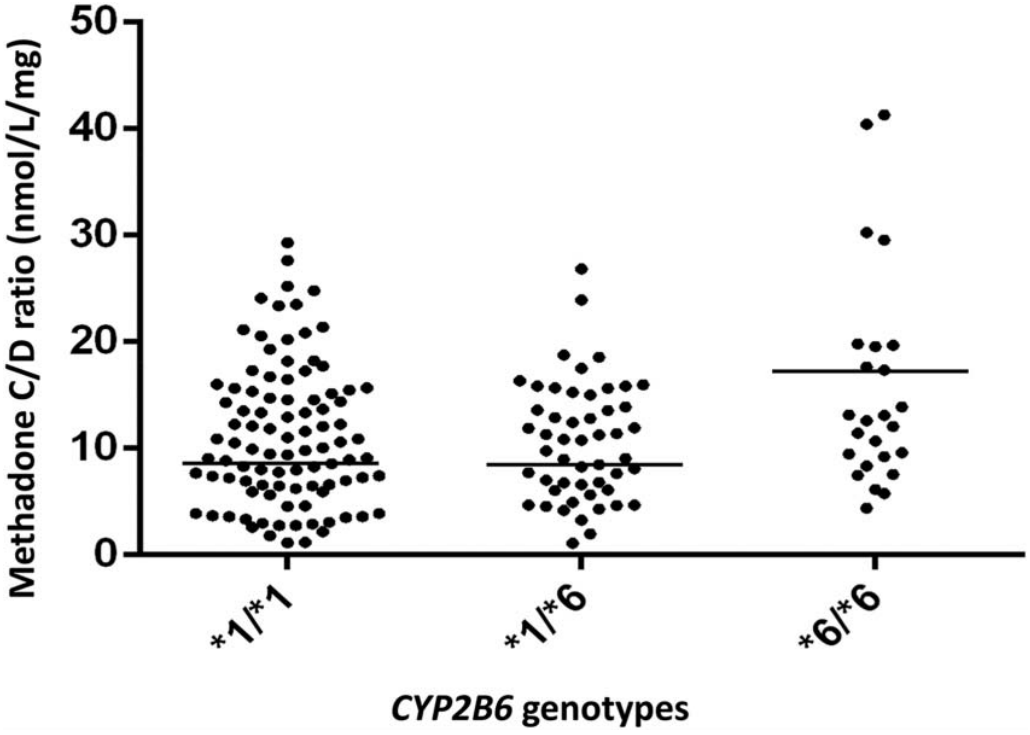
Paper I: Overall, 155 serum samples from 62 patients were included from TDM laboratory database at the Center for Psychopharmacology, Diakonhjemmet Hospital (Oslo, Norway) in this study. Twenty-eight patients (45%) were females and 34 (55%) were male. The mean age at the first serum measurement was 40.9 (95% CI: 38.4, 43.4) years. The mean methadone dose at the first serum measurement was 109.5 mg/d (100.4, 118.7), and the median dose was 100 mg/d (interquartile range 80–130). The mean time between sample collection and time since last methadone intake was 23.8 hours (22.7, 24.9). The frequencies of the *CYP2B6*6* and *CYP3A5*3* alleles were 0.24 and 0.09, respectively and all the studied gene variants were in Hardy–Weinberg equilibrium.

Paper III: Among 155 study participants from the Department of Addiction Medicine, Haukeland University Hospital (Bergen, Norway), a third (33%) were women, and mean (SD) age was 45 (10) years with OAT duration of 9 (5) years. The mean methadone dose and serum concentration in all 192 observations were 99 (25) mg/day and 1248 (559) nmol/L, respectively, giving a mean CDR of 13 (6) with a wide range of 3-38. For the *CYP2B6* genotypes 96 (62%) constituted the wild-type genotype (*1/*1), and the respective 54 (35%) and 5 (3%) were heterozygote (*1/*6) or homozygous (*6/*6) carriers of the reduced function genotype. For the *CYP3A5* genotypes 119 (77%) constituted the homozygote form of the reduced function genotype (*3/*3), while the 36 (23%) remaining were heterozygote carriers (*1/*3) except for one participant having the unusual wild-type genotype (*1/*1); both were categorized in the same group (*1/*1&*1/*3).

4.1.2 Main findings

Paper I: In this study we found that homozygous carriers of *CYP2B6*6* had significantly higher CDR of methadone compared with non-carriers ($P < 0.001$), whereas heterozygous carriers of *6 were similar to non-carriers ($P = 0.925$). The respective estimated mean CDRs were 17.8 and 9.1 for homozygous and heterozygous carriers of *CYP2B6*6*, and 9.2 for non-carriers. The methadone CDRs from all the 155 serum samples are presented in Figure 4 according to *CYP2B6* genotype. Homozygous carriers of *CYP3A5*3* had significantly higher CDR than the other group, consisting of both non-carriers and heterozygous carriers ($P = 0.009$). In addition, heterozygous carriers of *CYP2C9*2* or **3* and *CYP2C19*2* or **3* were associated with significantly higher methadone CDR ($P = 0.038$ and $P = 0.023$, respectively). For CYP2D6, there were no significant differences in CDR between the different genotype groups.

Figure 4. Individual serum concentration-to-dose ratios (CDR) in different *CYP2B6* genotype groups. Lines represent estimated mean values for CDR calculated in the mixed model analyses (paper I)



Paper III: In this study, the associations between CDR and the *CYP2B6**6/*6 genotype compared to *1/*1, or between the heterozygote and homozygote genotypes of *CYP2B6*

did not reach the statistical significance level. Participants with *1/*6&*6/*6 genotypes - presented as one group - had a higher mean CDR compared with the wild-type group (15 vs. 13; *P*: 0.035). Although not statistically significant, participants with homozygote genotype of *CYP2B6*6* variant allele had a higher CDR than those with wild-type; 17 (3) vs. 13 (6), or heterozygote genotypes; 14 (7). Regarding CYP3A5, participants composing the minority group with *CYP3A5**1/*1&*1/*3 genotypes (n=44) had a higher CDR of 16 (7) compared to the dominating group (n=148) with the homozygote genotype *3/*3 having a mean CDR of 13 (6).

4.1.3 Other findings

Paper I: We did not find statistically significant difference in age (*P* = 0.086), sex (*P* = 0.236), dose of methadone (*P* = 0.134), or time between the last dose intake and blood sampling (*P* = 0.853) between patients in any of the *CYP2B6* genotype groups. The estimated mean values from the mixed model analyses for absolute methadone serum levels were 1169 nmol/L for homozygous carriers of *CYP2B6*6*, 669 nmol/L for heterozygous carriers of *CYP2B6*6*, and 751 nmol/L in non-carriers. The absolute concentrations in homozygous carriers were significantly higher than in non-carriers (*P* = 0.003). The heterozygous carriers were not significantly different from the non-carriers (*P* = 0.286). In the *CYP2B6 *6/*6* group, the mean dose administered was within the recommended range of 60–120 mg.

Paper III: We could show that serum methadone concentrations were significantly higher among the participants with *CYP2B6*6* allele variants in comparison to the wild-type genotype; 1705 (276) vs. 1143 (444) nmol/L, *P* = 0.006. Among 43% of the participants with *CYP3A5**1/*1&*1/*3 genotypes, the combined *CYP2B6* genotype was either heterozygote or homozygote *6 variant allele and these patients had higher serum methadone concentrations; 1728 (931) and 1632 (156) nmol/L respectively, compared with the participants with combined wild-type *CYP2B6* genotype group having a mean serum concentration of 1292 (448) nmol/L.

4.2 Objective 2 (papers II and III)

To investigate the impact on dose-adjusted serum methadone concentration of concurrent use of medications with possible interacting effects through the same CYP enzymes (CYP2B6, CYP3A5, CYP2C9, CYP2C19, CYP2D6) as well as age, gender, and body weight among patients undergoing MMT

4.2.1 Basic characteristics

Paper II: The 4425 samples from 1691 patients used in this study included 3013 samples (70%) from 1187 men, and 1412 samples (30%) from 504 women obtained from TDM laboratory database at the Department of Clinical Pharmacology, St. Olav University Hospital (Trondheim, Norway). Mean age of the patients at the time of sampling was 38.4 (7.2) years. Mean daily dose of methadone was 111 (39) mg, of serum concentration was 344 (181) ng/mL, and of CDR was 332 (184) (ng/mL)/(100 mg/d). More than 170 drugs were recorded as concomitant medications, with at least one co-medication recorded in 1148 samples (26%). Eighteen CYP inhibitors and four CYP inducers were recorded in 79 and 37 samples, respectively. Twenty-six other drugs (including esomeprazole and carbamazepine which were also included in the former group) were recorded in at least 20 samples. Valproate (valproic acid) (n = 123), oxazepam (n = 122), mirtazapine (n = 105), alimemazine (trimeprazine) (n = 100), chlorprothixene (n = 90), and olanzapine (n = 85) were the most frequently recorded co-medications.

Paper III: Since the information on BMI was not recorded in TDM database used for paper II, we included this variable prospectively obtained in paper III. The mean BMI for the 155 participants in this study was 27 (6) kg/m², with 45% having a BMI <25, 25% having a BMI of 25-30 and 30% with a BMI >30. The last group were, however, excluded from the adjusted regression analysis as the measurements of liver stiffness were not possible or - if so - reliable in this group. Other relevant demographic and clinical characteristics of the participants are provided under the objective 3.

4.2.2 Main findings

Paper II: In this study, we could show that concomitant medication with CYP inducers reduced methadone CDR by 36%, whereas CYP3A4 inhibitors as a group increased it by 36%. Of the four inducers in the former group, carbamazepine (n = 30) showed to have the most significant effect on CDR ($P < 0.001$). Nevirapine (n = 1) had also a significant effect ($P = 0.034$), whereas phenobarbital (n = 4) and efavirenz (n = 2) was not found to influence CDR significantly ($P = 0.243$ and $P = 0.102$, respectively). The CYP3A4 inhibitors recorded in this study (n = 15) were as following; atazanavir (n = 6), diltiazem (n = 1), erythromycin (n = 2), fluconazole (n = 3), indinavir (n = 1), nelfinavir (n = 1) and saquinavir (n = 1).

We also found that women as compared to men had 9% lower CDR, whereas the ratio was not influenced by age, at least in the age group included in this study. Women used on average 8 mg higher daily methadone doses than men, but had about 26 ng/mL lower serum concentrations, resulting in correspondingly lower CDR. The combined effects of the most relevant variables (age, gender, time from last dose intake to sampling, CYP inducers, and inhibitors of CYP3A4, CYP2D6, and CYP2C19) on the loge-transformed methadone CDR in the adjusted analysis are shown in Table 4.

Table 4. The effects of different variables in adjusted linear mixed model on the log_e-transformed and expected methadone serum concentration-to-dose ratio (CDR) (paper II)

	Log_e (methadone CDR)			Expected methadone CDR (ng/mL) / (100 mg/d)	
Variable	Estimate	95% CI	*p*-value	Mean (95% CI)	Change (%) (95% CI)
Intercept[a]	1.128	1.017, 1.239	<0.001	309 (276, 345)	
Age (per year)	0.002	-0.001, 0.005	0.176	310 (276, 343)	+ 0 (- 0, + 1)
Gender (women)	-0.092	-0.144, -0.040	**0.001**	282 (239, 332)	**- 9 (- 13, - 4)**
Sampling time[b]	0.007	-0.017, 0.030	0.560	311 (272, 335)	+ 1 (- 2, + 3)
CYP inducer	-0.452	-0.588, -0.324	**<0.001**	197 (154, 250)	- **36 (- 44, - 28)**
CYP3A4 inhibitor	0.304	0.094, 0.515	**0.005**	419 (304, 578)	**+36 (+ 10, + 68)**
CYP2D6 inhibitor	-0.071	-0.222, 0.080	0.360	288 (221, 374)	- 7 (- 20, + 8)
CYP2C19 inhibitor	0.003	-0.153, 0.146	0.965	310 (237, 399)	+ 0 (- 14, + 16)

[a]The intercept represents a 40-year-old man not using any of the interacting drugs, having a blood sample obtained 24 hours after the last methadone intake, [b]Per hour (the difference between recorded time and 24 hours from the last dose intake), Abbreviation: CI= Confidence interval.

Paper III: We showed that participants with a BMI of 25-30 kg/m² had higher CDR (coefficient: 2.34; 95% CI: 0.22, 4.45; *P*: 0.031) compared with those who had BMI<25 kg/m².

4.2.3 Other findings

Paper II: Neither age, time interval from last drug intake to sampling, nor CYP2D6, CYP2C19, CYP2B6, CYP2C9, or CYP1A2 inhibitors had statistically significant effects on CDR. Men were slightly older than women, and the recorded mean time from last dose intake to sampling was slightly shorter in women than in men. Out of the 26 frequently recorded concomitant medications (Table 5) carbamazepine (as also mentioned under CYP inducers), was found to reduce the CDR by 38%.

Table 5. The most frequently recorded concomitant medications (n) (paper II)

Alimemazine (100)	Esomeprazol (21)	Mirtazapine (105)	Sertralin (26)
Carbamazepine (30)	Ibuprofen (26)	Olanzapine (85)	Trimetoprim (65)
Chlorpromazine (23)	Lamotrigine (32)	Oxazepam (122)	Valproate (123)
Chlorprothixene (90)	Levomepromazine (56)	Paracetamol (35)	Venlafaxine (35)
Citalopram (33)	Melatonine (34)	Pregabalin (60)	Zopiclone (49)
Diazepam (22)	Methylphenidate (32)	Quetiapine (33)	
Escitalopram (51)	Mianserin (39)	Risperidone (24)	

Further, we observed that in 65% of the samples the recorded daily dose of methadone was 80–120 mg. Doses higher than 120 mg/d and lower than 80 mg/d were recorded in about 25% and 10% of the samples, respectively. In about 50% of the samples, the serum concentrations were within the recommended reference range at our laboratory (200–400 ng/ mL). Almost 30% and 20% of the records presented higher or lower concentrations than this range, respectively. The intra-class correlation with regard to the random effects was high (65% correlation between different intra-individual samples), illustrating that every patient was much like himself/herself regarding repeated measurements, whereas the inter-individual variations were large.

Paper III: Other findings in this paper are presented under the objectives 1 and 3 (Paper III).

4.3 Objective 3 (paper III)

To explore the impact on dose-adjusted serum methadone concentration of liver fibrosis and clinical characteristics among patients undergoing MMT

4.3.1 Basic characteristics

Paper III: 145 out of 155 (94%) participants in this study had positive HCV antibodies, of those 56 (36%) had HCV RNA (regardless of completing the treatment with direct acting antiviral medications). None of the participants had HIV antibodies/antigens, and no one was recorded with severe renal failure or was under

the treatment by co-medications that could interact with methadone. The mean BMI was 27 (6) kg/m². Almost 90% of the patients frequently (weekly to daily during the last month) used at least one illicit substance, of which more than half also had used alcohol.

Mean liver fibrosis measure was 7 kPa, where 82% had no/limited fibrosis with a mean value of ≤7 kPa and 11% had fibrosis measures between 8 and 11 kPa. For the remaining 9 (7%) participants the mean liver fibrosis measure was ≥12 kPa indicating a possible cirrhosis state, probably complicated with portal hypertension in 3 patients with measures ≥20 kPa. Mean methadone dose, serum concentration and CDR for each of the categories are illustrated in table 6.

Table 6. Methadone dose, serum concentrations, and serum concentration-to-dose ratio (CDR) in patients with different stages of liver fibrosis[1] (paper III)

	N	Dose (mg) Mean (SD)	Serum concentration (nmol/L) Mean (SD)	Concentration-to-dose ratio (nmol/L)/mg) Mean (SD)
Liver fibrosis measure				
No/limited fibrosis, ≤7 kPa (Ref)	107	98 (23)	1290 (609)	14 (6)
Fibrosis, 7<kPa<12	14	91 (29)	1189 (463)	14 (8)
Cirrhosis, ≥12 kPa	9	100 (17)	1239 (485)	12 (5)
Portal hypertension, ≥20 kPa	3	110 (20)	1379 (391)	12 (3)

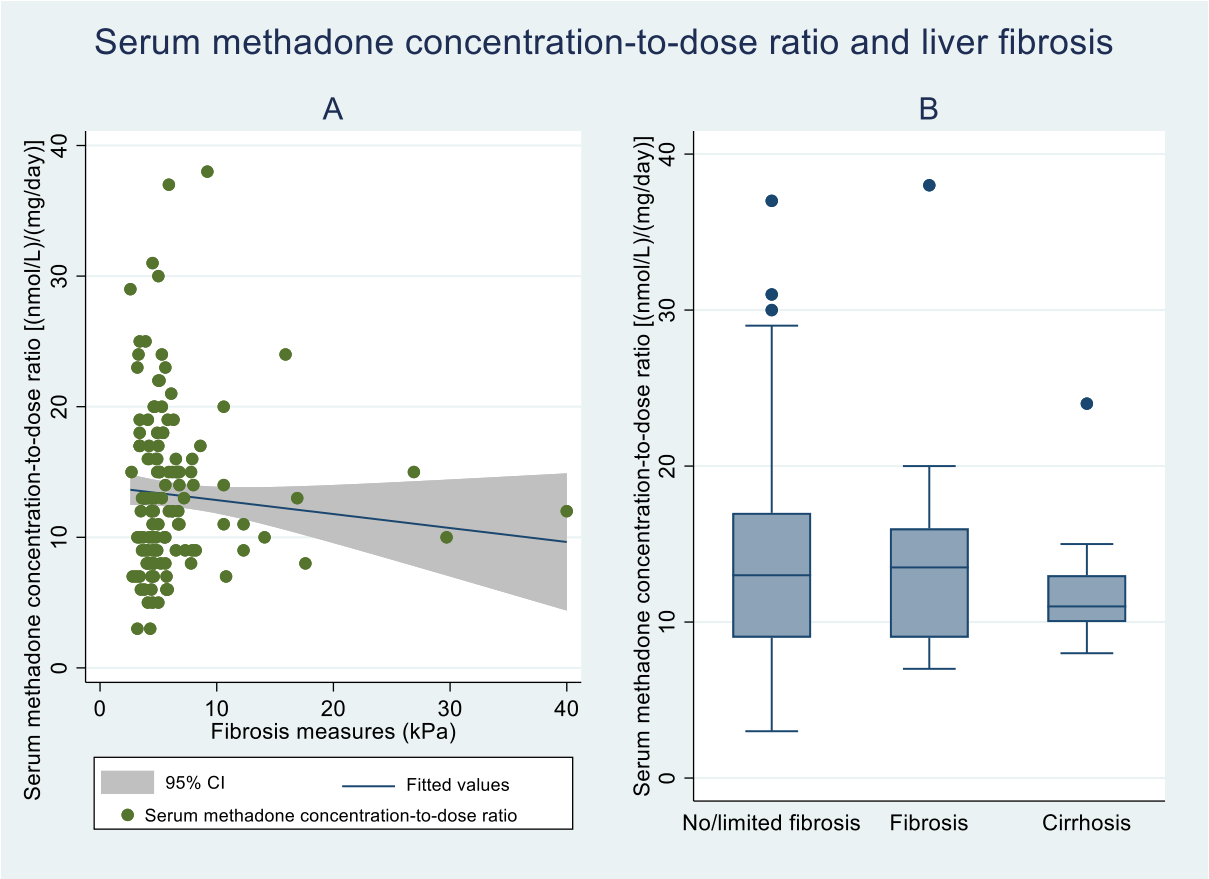
[1]Patients with BMI>30 were excluded, Ref: the reference group.

4.3.2 Main findings

Paper III: Although we observed some differences in the serum methadone concentrations, CDR did not change considerably among those with higher degrees of fibrosis measures or between the different categories of liver fibrosis (Figure 5). The LMM was adjusted for the different genotypes of *CYP2B6* and *CYP3A5* as well as age groups, gender, and BMI categories, and there was no significant relationship between

CDR and liver fibrosis (coefficient: 0.70; 95% CI: -2.16, 3.57; *P*: 0.631) or cirrhosis (-0.50; -4.59, 3.59; 0.810) compared to no/limited fibrosis.

Figure 5. Serum methadone concentration-to-dose ratio (CDR), and liver fibrosis measures and stages in study participants* on methadone maintenance treatment (paper III)



Liver stiffness measures: Limited fibrosis: ≤7 kPa; Fibrosis: 7<kPa<12; Cirrhosis: ≥12 kPa.

*For 192 observations including two sets of measures in 37 participants. Fibrosis measures illustrate 130 observations from 107 participants due to excluding of the individuals with BMI>30 kg/m² (n=46) and missing data (n=2).

4.3.3 Other findings

Other findings in paper III are presented under the objective 1 (regarding *CYP2B6* and *CYP3A5* genotypes) and objective 2 (regarding BMI).

4.4 Objective 4 (paper IV)

To investigate whether serum methadone concentration is related to subjective opioid withdrawal symptoms, adverse effects and substance use, and the role that these variables could play in clinical decision on dose adjustment in patients undergoing MMT

4.4.1 Basic characteristics

Paper IV: For all the 83 participants in this study, the mean age was 45 (9) years; 33% were women, and 54% reported mild to moderate subjective opioid withdrawal symptoms with a mean total SOWS score of 9 (12) at the time of the interviews. The mean time from last dose intake to blood sampling was 21 (8) hours, and the patients had 4 (2) days per week with directly observed treatment (DOT). The mean methadone daily dose and serum concentration were 97 (24) mg and 374 (188) ng/mL, respectively. All had experienced one or more subjective adverse effects, and 73 (88%) reported frequent use (from daily to several times a month) of at least one substance during the month prior to the surveys.

4.4.2 Main findings

Paper IV: In the unadjusted LMM analysis, we found statistically significant inverse associations, although weak to moderate correlations, between serum methadone concentrations and total SOWS scores ($P = 0.011$), and for the specific symptoms of anxiety ($P = 0.009$), bone and muscle aches ($P = 0.007$), and restlessness ($P = 0.021$) out of the 16 subjective opioid withdrawal symptoms based on the SOWS questionnaire, as well as for use of heroin ($P = 0.028$) and alcohol ($P = 0.008$). Except for a significant direct association with nausea reported as an adverse effect of methadone ($P = 0.040$), no associations were found between the other subjective adverse medication effects and methadone serum concentrations.

When adjusting in the LMM analyses for age, gender, and the absolute time difference between blood sampling and the recording of the symptoms (Table 7), we found that the associations between serum methadone concentrations and total SOWS scores; the

specific withdrawal symptoms of anxiety, bone and muscle aches, and restlessness; and use of heroin and alcohol still remained highly significant. There was a tendency toward higher serum concentrations among those who reported nausea as an adverse effect ($P = 0.057$). Obtaining P-values of <0.10 by analyzing the withdrawal symptom of shaking as well as use of cannabis in the unadjusted LMM, we also added these variables to the adjusted model and found slight associations with serum methadone concentrations ($P = 0.046$ and $P = 0.049$, respectively).

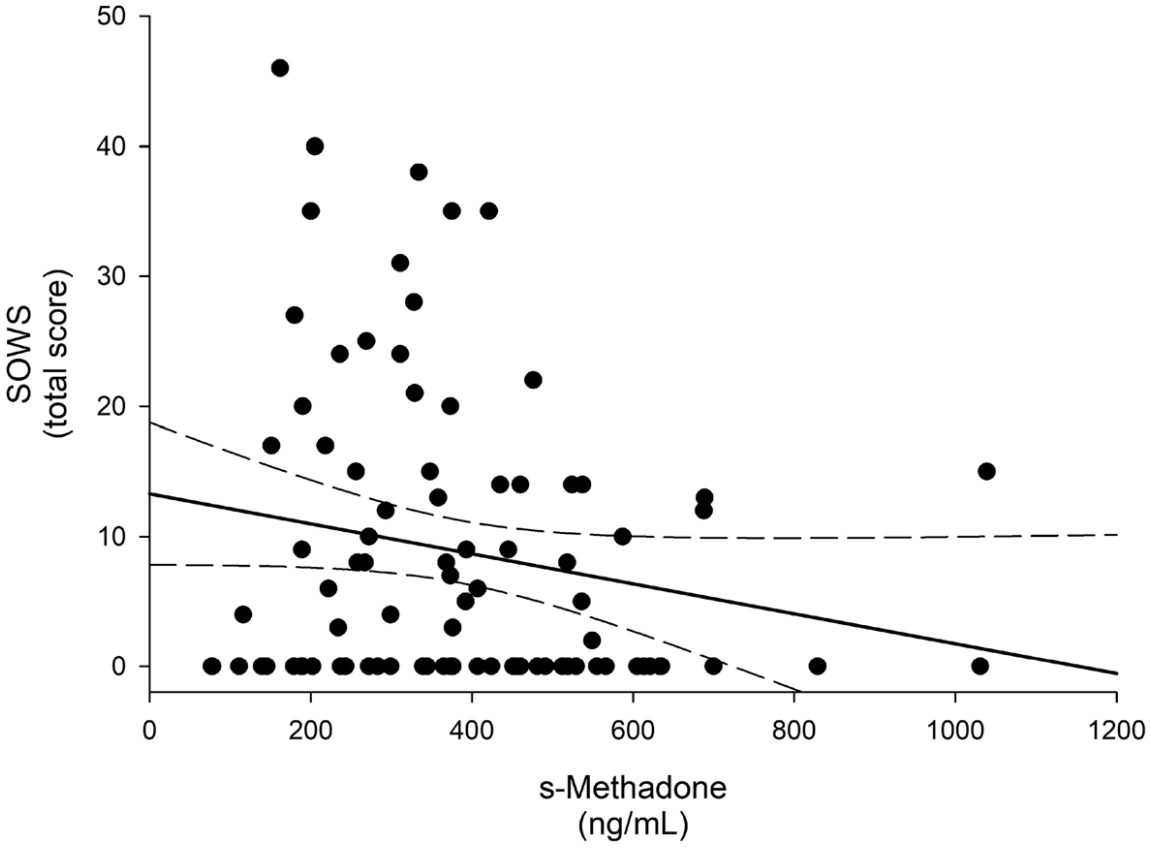
Table 7. The adjusted* associations between serum methadone concentrations and the selected study variables in linear mixed model (paper IV)

	Coefficient	95% CI [a]	*P*-value
Total SOWS[b] Score	-4.3	-5.6, -2.9	<0.001
Anxiety	-0.5	-0.8, -0.2	0.004
Bone- and muscle ache	-0.5	-0.9, -0.2	0.003
Restlessness	-0.5	-9.7, -0.9	0.017
Shaking	-0.3	-0.6, -0	0.046
Nausea (as adverse effect)	0.3	-0.1, 0.6	0.057
Heroin use	-0.2	-0.4, -0	0.015
Alcohol use	-0.4	-0.7, -0.1	0.011
Cannabis use	0.5	0, 10.4	0.049

*Adjusted for age, gender and absolute time difference between blood sampling and record of the symptoms; [a] Confidence interval; [b] Subjective opioid withdrawal symptoms.

Figure 6 from this paper shows the relationship between the recorded total SOWS scores and the measured serum methadone concentrations, illustrating a weak correlation with wider confidence intervals at lower and higher concentrations.

Figure 6. Scatter plot with regression line by recorded total SOWS[a] scores and measured serum methadone concentrations in 83 participants. The solid and dashed lines represent the regression line and 95% confidence intervals (paper IV)



[a]Subjective opioid withdrawal symptoms

4.4.3 Other findings

Participants who had reported subjective opioid withdrawal symptoms had lower serum concentration-to-dose ratios ($P = 0.039$), and more frequently received DOT ($P = 0.026$) compared to those in the other group. There were no differences between the groups with regard to age, gender, or self-reported use of illicit substances and alcohol.

5. Discussion

This thesis explores associations between methadone dose, serum concentration, and clinical outcomes, as well as the conceivable influencing elements including genetic, physiological, pathological and pharmacological factors in MMT for opioid dependence. Despite years of clinical experiences and research, the impact of such factors in methadone metabolism is not fully understood, and the current clinical practice in this area varies considerably. These uncertainties face clinicians with obvious challenges when it comes to methadone dose adjustments, and assessments of treatment efficacy and plausible risks.

The discussion section starts by a short summary of the research's key findings and will be followed by discussing the results as well as the most relevant clinical implications of the findings. Finally, the main methodological aspects of the thesis will be discussed.

5.1 Summary of the main findings

In paper I, we investigated the impact of genetic polymorphisms of CYP2B6 and other candidate CYP genotypes on methadone CDR. We could demonstrate a significant increase (90%) in methadone CDR among the homozygous carriers of *CYP2B6*6* compared with non-carriers, indicating a slow metabolizer phenotype. This finding suggests that polymorphisms in *CYP2B6* genotypes may explain some of the variations in dose-adjusted serum concentrations of methadone.

In paper II, we explored possible effects of gender, age, and various co-medications on methadone CDR. We found that women used on average 8 mg higher daily methadone doses than men, but had about 26 ng/mL (87 nmol/L) lower serum concentrations, resulting in a correspondingly 9% lower CDR. The ratio was not influenced by age. The study also showed that concomitant medication with CYP inducers reduced the methadone CDR by 36%, while CYP3A4 inhibitors increased it correspondingly.

In paper III, we studied the impact of liver fibrosis and clinical characteristics on methadone CDR. We showed that the dose-adjusted serum concentration of methadone did not increase among participants with higher degrees of liver fibrosis, even among those with possible advanced cirrhosis. We also found a direct association between BMI and CDR. Nevertheless, no statistically significant relationships with *CYP2B6* genotypes were found in this study.

In paper IIIV, we investigated the association between serum methadone concentration and subjective opioid withdrawal symptoms. Although the correlations were not very strong, the total SOWS score, and the specific subjective withdrawal symptoms of anxiety, bone and muscle aches, restlessness, and (slightly) shaking, as well as use of heroin and alcohol were associated with lower methadone concentrations. Cannabis use was slightly related to higher methadone concentrations.

5.2 Discussion of the results and clinical implications of the thesis

5.2.1 Clinical challenges in MMT: dose adjustments and use of TDM

As described in the introductory section, a considerable inter-individual variation in methadone daily doses and serum concentrations has been reported in patients undergoing MMT (43,75). This poses a major challenge for clinicians regarding the daily dose assessments i.e. balancing an effective dose to achieve the desired therapeutic effect against an inappropriate dose, which causes withdrawal symptoms or adverse effects.

Although TDM generally provides clinicians with a tool to better monitor patients' treatment, its role in MMT is not entirely clear. The reason is an insufficient evidence on correlations between methadone dose, serum concentrations and clinical outcomes. Despite this uncertainty, TDM has been used by clinicians to monitor methadone treatment along with greater emphasis on closer clinical assessments (147).
In this research work, we have used TDM as well as clinical data obtained from patients undergoing MMT to explore various factors that may contribute to the observed variations in methadone metabolism, and clarify the role of TDM in

treatment monitoring and dose adjustments. To achieve these purposes, we have conducted the studies presented in this thesis with a special focus on investigating the issues and research questions that are discussed below.

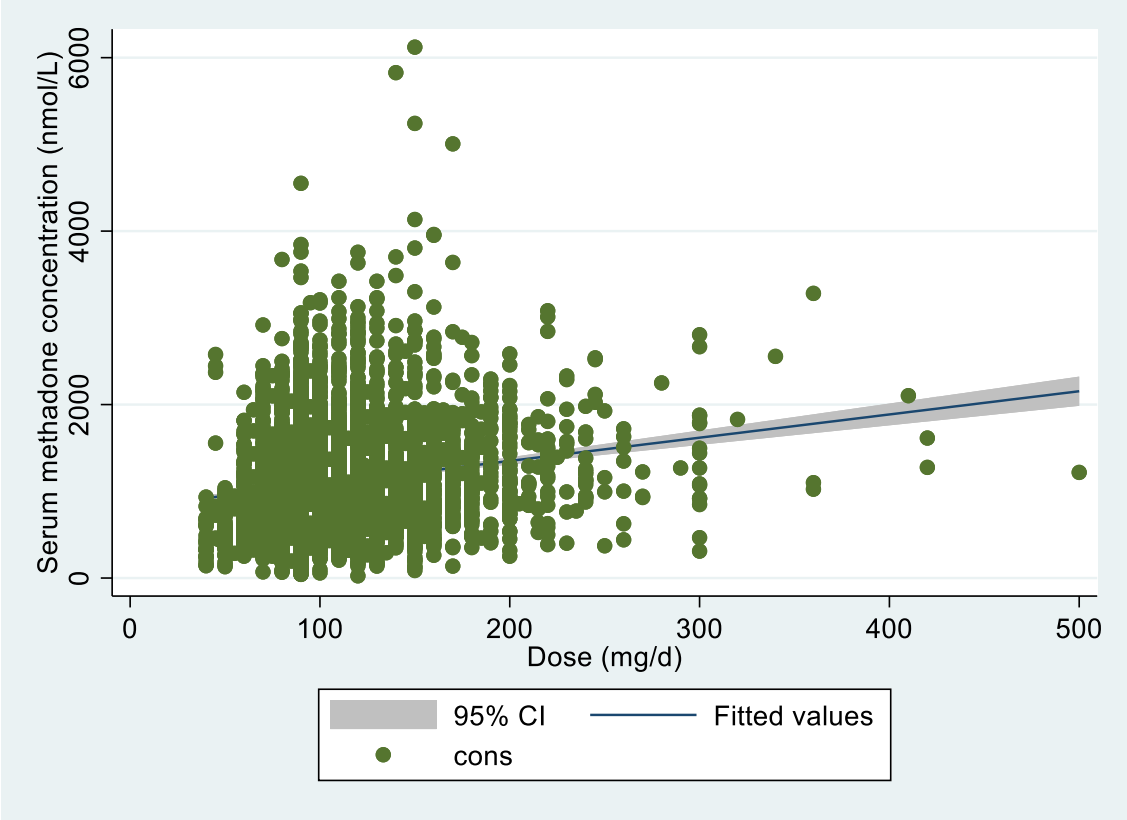
5.2.2 The relationship between methadone dose and serum concentration

Using the large TDM database in paper II we could show a considerable variation (adjusted R^2=0.032) in the relationship between methadone doses and serum concentrations measured at steady-state (Figure 7). The mean daily dose of methadone was 111 (39) mg, and the recorded daily dose was within the recommended range of 80–120 mg in 65% of the samples. Overall mean serum methadone concentration was 344 ng/mL (1147 nmol/L), with a SD of 181 ng/mL (603 nmol/L). In only about half the samples, the serum concentrations were within the recommended reference range at our laboratory (180-360 ng/mL or 600-1200 nmol/L). Mean CDR across all samples was 11 (SD: 6).

Although some limited studies with small sample sizes were able to demonstrate significant correlations between methadone dose and serum concentration (99,148-150), when considering a large number of patients the dosage is supposed to explain less than 50% of the variability of the concentrations of R-methadone, even in patients without co-medications (99). When measuring methadone steady-state concentrations in a study on 18 patients undergoing MMT, there was a 4- to 6-fold inter-individual variability after the values were adjusted to a 70 mg daily dose of racemic methadone (148). Another study with controlled administration of methadone during 3 weeks among 18 patients showed up to a 5- fold inter-individual variability in the trough serum concentrations of methadone for the same dosage (151), and a poor correlation was found between methadone dosage and serum concentration in a study with 32 patients (152). In a larger study conducted on 211 MMT patients, for a given dosage, R-methadone trough serum concentrations corrected for bodyweight varied up to 17- and 41-fold in patients without and with concomitant use of other medications, respectively (99). It has been stated that serum concentrations and methadone dosages are highly correlated when the compliance is good (153,154). However, studies

performed in conditions where compliance problems were excluded also showed large inter-individual variabilities in methadone concentrations up to 3-10-fold (155,156). Additionally, similar variabilities of methadone pharmacokinetics have been demonstrated in single-dose studies involving methadone-free subjects without the issue of poor compliance (74,157). Despite this large inter-individual variability, there seems to be a closer relationship between methadone dose and serum concentration within an individual (158,159) provided that no inducing or inhibiting co-medications are introduced or removed.

Figure 7. Relationship between daily doses and serum concentrations of methadone (paper II)



Because of the large variability of methadone concentrations, several studies have aimed to find the optimum serum concentration for effective maintenance treatment (98,156,160-169). In some studies, such a threshold could not be found (160,165-

167,169), whereas various values ranging from 50 to 600 μg/L (170-2000 nmol/L) of racemic methadone have been proposed by other investigators (98,156,161,163-168). A concentration of 400 μg/L (1300 nmol/L) is often considered as necessary to provide stabilized maintenance and is used as a reference value when performing TDM of methadone (169,170). However, to our knowledge, studies to validate such a threshold are lacking. As the opioid effect of racemic methadone exhibits mainly by the R-enantiomer and considering the wide inter-individual variability of the R-/S-methadone ratio measured in blood (171,177), it could be more reliable to measure the concentration of R-methadone than racemic methadone to correlate serum concentrations with therapeutic outcome. This hypothesis is supported by a study which showed that R-, but not S-methadone trough concentrations were significantly correlated with several items of the SOWS in a group of 25 patients who complained of low dosages (177). In another study using a mean daily dose of 100 mg methadone in MMT, R-methadone at 250 μg/L (830 nmol/L) and racemic methadone at 400 μg/L (1300 nmol/L), but not S-methadone concentrations were associated with therapeutic response i.e. the absence of illicit opioid in urinary tests (99). However, beyond pharmacokinetic factors, pharmacodynamic parameters such as variability in receptors (178) and psychological or social factors (179,180) are of importance for the success of MMT. Altogether, these results suggest an unclear role of TDM in MMT.

In summary, our finding confirms previous reports on considerable variations in methadone pharmacokinetics (75,81,92,181) emphasizing the importance of clinical assessments in decision making on dose adjustments in MMT. Accordingly, the use of TDM for methadone dose adjustment in daily clinical practice is not supported by this thesis. Thus, understanding possible factors that may influence methadone metabolism and serum concentration-to-dose ratio is crucial in order to determine the more specific indications for the use of TDM in MMT, and make reasonable clinical decisions to optimize treatment outcome and reduce harm in this high-risk population.

In the subsections below, we have discussed the impact of the most relevant factors on methadone pharmacokinetics based on our findings in papers I-III.

5.2.3 Genetic factors

Should genetic polymorphisms in metabolizing CYP-enzymes be considered in methadone dose adjustments?

Although the impact of *CYP2B6* genotype on inter-individual variability in pharmacokinetics of methadone, particularly S-methadone, has been investigated in several previous studies (84-88,91,92,107-112), the findings are still not conclusive. In paper I, we could show that the homozygous carriers of *CYP2B6*6* had the largest increase in CDR (>90%) compared with non-carriers, which is in line with the predicted slow metabolizer phenotype (104) as well as in agreement with the results of Crettol et al (88). In an observational study on 245 MMT patients, they found that *CYP2B6*6/*6* carriers had significantly higher steady-state trough S-methadone serum concentrations and a trend toward higher R-methadone serum levels, presenting a higher total steady-state trough R,S-methadone serum concentration. Other previous studies failed to find significant effects of the *CYP2B6* genotype on methadone serum concentration (84,106) although a trend supporting the findings was observed. We found no increase of methadone CDR among heterozygous carriers of *CYP2B6*6* compared with non-carriers that could possibly indicate a recessive genetic effect for the *CYP2B6*6* variant (182) or even other gene effects not considered in this study. In addition, the influence of other non-genetic factors involving this population cannot be ruled out. Yet, *CYP2B6* poor metabolism genotype was associated with serum concentrations above the highest reference values of 600–1200 nmol/L (183), whereas the given dose was within the recommended range of 60–120 mg/d (184). We observed that although not statistically significant, homozygous carriers of *CYP2B6*6* were treated with a 30% lower methadone dose than non-carriers. This could indicate that the patients had undergone some dose-adjustment regimens presumably because of higher serum concentrations. However, we did not have access to the concurrent clinical data for the patients to explore other possible causes. The most expected clinical consequences related to the increased serum concentration are a higher risk of undesirable effects. Some studies have shown that prolongation of QT interval and potentially dangerous cardiac arrhythmias is presumably due to a higher S-methadone

serum concentration (185,186). In our study, we did not measure the concentrations of methadone isomers nor record the results of ECG or other clinical measurements and could therefore not interpret the possible clinical outcomes of an increased total methadone serum concentration among the patients. Measuring the total serum concentration of racemic methadone rather than specific isomers is a limitation in our study. However, the assessment of clinical effect and safety of MMT is most often based on measuring of the total methadone because most TDM laboratories do not perform isomer-specific analysis. Furthermore, we have limited knowledge on the influence of the reduced function *CYP2B*6* variant allele on the different isomers of methadone compared with total serum concentration and possible clinical outcomes. A report examining methadone-related deaths concluded that the risk of methadone fatality may be related, in part, with the *CYP2B6*6* allele (187), supporting the assumption of a possible clinical relevance of this genotype. However, as the influence of methadone pharmacokinetics on clinical outcome is not fully investigated (84), the presence of higher methadone CDR in patients homozygous for *CYP2B6*6* should be interpreted cautiously, taking into account all the possible concurrent medical and sociodemographic factors. In paper III, although serum methadone concentrations were significantly higher among patients with the homozygote and heterozygote genotypes of the *CYP2B6*6* variant allele compared with the wild-type, the differences in CDR did not reach statistical significance in the multivariate regression model. We supposed that including only five participants (3%) with this genotype in this study did not provide enough statistical power to obtain a similar result. The overall conclusion would be that clinical impact of *CYP2B6* genetic polymorphism in methadone metabolism and dose adjustments is still not completely clear. Accordingly, an individually assessed clinical decision-making should be considered based on possible risks and benefits.

In paper II but not in paper III, we could also show a smaller effect (>40%) on methadone CDR in CYP3A5 slow metabolizers compared with non-carriers. There are few clinical data on the role of CYP3A5 polymorphisms on methadone metabolism, however, this is not surprising as the expression of the active allele among whites is

rare (113,114). A possible in vivo impact of CYP3A5 on methadone metabolism is anticipated in few clinical studies; nevertheless, the results are conflicting (84,88,89). The limited and conflicting results are unlikely to support a clinical relevance of CYP3A5 in methadone metabolism, which is in line with our findings. CYP2C9 and CYP2C19 have previously shown to impact methadone metabolism to a lesser extent (87). Although our results in paper I indicated such an effect of the *2 and *3 variant alleles, these findings should be interpreted with caution as there were few patients in each group. The unremarkable increase in CDR seen in heterozygous carriers is considered to have limited clinical relevance, and the effect in homozygous CYP2C9 and CYP2C19 carriers did not reach statistical significance (only 2 and 4 patients had these genotypes, respectively). Finally, although CYP2D6 has been related to methadone metabolism (84), our study showed no impact on methadone CDR by its various genetic polymorphisms. Larger clinical studies are needed to explore the impact of the various CYP polymorphism on MMT.

5.2.4 Physiological factors

Should gender, age and BMI be considered in methadone dose adjustments?

Currently, there are limited clinical studies on the influence of gender and age on methadone metabolism, and the findings are not conclusive (70,116,117). In paper II, we showed that women had 9% lower CDR compared to men, whereas the ratio was not influenced by age. Women used on average 8 mg higher daily methadone doses than men, but had about 26 ng/mL (84 nmol/L) lower serum concentrations, resulting in correspondingly lower CDR. This may indicate that dose increase was considered but did not result in corresponding increase in serum concentration. Some authors have also commented on the need for higher methadone doses among women (81). Methadone metabolism is significantly accelerated in the third trimester of pregnancy (70). At that time, the daily dose often needs to be increased in order to prevent withdrawal symptoms and drug-seeking behavior in the mother. This knowledge together with some studies indicate an inducing effect of estradiol on methadone metabolism related to CYP2B6 (188) and CYP3A4 (189) suggesting a possible role of estradiol as an explanation for the gender difference. However, no significant gender

difference in methadone elimination was found in a smaller study (190). Nor in paper III, we could demonstrate a significant association between CDR and gender, which is probably due to the small sample size of the studies. Despite these discrepancies, we recommend that a possible gender effect on methadone metabolism should be taken into consideration in clinical practice to avoid under-dosing of women. The lack of an age effect on methadone metabolism in papers II and III may be due to the inclusion of only a few serum samples obtained from patients over 60 years of age or a small sample size, respectively. Others have suggested that age may explain some of the inter-individual variations in steady-state methadone levels (191), however, supporting studies are lacking. Larger clinical studies by recruiting older patients are needed to answer the question of the impact of advanced age on methadone metabolism. Thus, there is to date no evidence to recommend methadone dose reduction with increasing age, and any dose adjustment should still be made based on the individual clinical condition and an overall risk-benefit assessment.

In paper III, we found a direct association between overweight (BMI 25–30 Kg/m2) and CDR in the adjusted regression analysis. The impact of body weight on methadone metabolism has not been sufficiently investigated in previous studies. Nevertheless, a recent study (118) demonstrated that individuals with overweight had higher dose-adjusted serum methadone concentrations, which is in line with our findings. Possible explanations for this observation could be the changes in body compartment proportions (i.e., the amount of fat tissue that influences volume of distribution) and liver steatosis (120,192). Conversely, MMT has been related to weight gain (193). If this condition is considered a dose-dependent adverse effect of methadone, higher serum concentrations can be expected, at least in some patients. Accordingly, it is challenging to verify the direction of a potential causal relationship. The clinical implication of the current knowledge may be that overweight does not necessitate higher methadone dosages; in fact, some patients may need dose reduction to avoid weight gain. Further, other influencing factors may warrant individualized dose requirements.

5.2.5 Pathological factors

Should liver fibrosis be considered in methadone dose adjustments?

In paper III, we showed that the dose-adjusted serum concentration of methadone did not increase among participants with higher degrees of liver fibrosis, even among those with possible advanced cirrhosis. Although the study did not find an association between liver fibrosis and methadone concentrations, it does not appear that available research can definitively conclude on this topic (118,129-131). Reduced metabolism of methadone in HCV-infected patients with opioid use disorder was demonstrated in a study (129), but no association between methadone serum levels and liver fibrosis was found. Another study (130) reported a higher concentration of total methadone and the active R-enantiomer in HCV-seropositive patients compared to seronegative patients. Both studies suggest consideration of dose adjustments in methadone-maintained patients with a history of HCV infection. However, the clearance of drugs in general is not considerably altered in patients with chronic active hepatitis without cirrhosis (122,194). In a study on patients undergoing MMT, the researchers could not demonstrate changes in the total body amount of methadone in individuals with mild to moderate chronic liver disease (131). They proposed that dose adjustment was not needed. However, a higher methadone dose requirement has been suggested due to CYP3A4 induction in patients with HCV infection (119). In line with our results, a recent study (118) could not show a significant effect of liver stiffness in patients with ongoing HCV infection on methadone metabolism rates. Our findings may thus indicate that an increased liver fibrosis probably caused by ongoing HCV infection does not immediately warrant methadone dose adjustment without further clinical evaluation. In very severe liver diseases, however, a decreased metabolic capacity is expected, and together with an impaired production of drug-binding proteins, it can result in an increased fraction of free drug (120,192). Nevertheless, the measured protein-bound drug concentration may seem normal; leading to the conclusion that drug metabolism is unaffected. Indeed, the drug clearance is reduced due to increased tissue distribution of the unbound fraction, especially in the presence of edema and ascites (120,192). Drugs with intermediate or high hepatic extraction rates—such as methadone—may have increased oral bioavailability due to portal hypertension and

development of cirrhotic porto-systemic shunts, leading to a reduced first-pass metabolism (195). Increased bioavailability combined with decreased hepatic clearance can cause a considerable accumulation of the drug in the body per time unit (123). Further, a strong relationship between the activity of hepatic CYP enzymes and the severity of cirrhosis has been demonstrated, in which the content and activity of some CYP enzymes, such as 3A, appear to be particularly vulnerable to the effect of liver disease (196). Although we did not find any interacting factor between liver stiffness and the CYP genotypes regarding methadone CDR in paper III, the pattern of CYP enzymes alterations also differs according to the etiology of liver disease (196). Due to the large bioavailability and protein binding capacity, and a long half-life, as well as the considerable inter- and intra-individual variability in the pharmacokinetics of methadone, a close clinical monitoring has been recommended in patients with severe hepatic impairment, although no dose adjustment is suggested in mild and moderate liver diseases (77). We considered fibrosis measures ≥20 kPa to be the indicator of significant portal hypertension, as we did not directly measure hepatic venous pressure. Three participants were found in this category apparently without an impaired metabolic rate of methadone, having a mean CDR of 12. However, the regression model was unable to analyze the data, possibly due to too few individuals in this category. Although the study could not indicate a significant increase in dose-adjusted serum methadone concentration among patients with severe cirrhosis, a close clinical monitoring and observation of overdosing symptoms such as increased sedation, could support a possible accumulation of methadone in the central nervous system. Continuous clinical evaluations should therefore be recommended as the most important tool in the management of severe hepatic impairment among patients undergoing MMT. In some cases, measurement of serum concentration may also reveal intra-individual variations during the treatment course.

5.2.6 Pharmacological factors

Should concurrent use of other medication be considered in methadone dose adjustments?

In paper II we observed that almost one-quarter of the patients used other medication in addition to methadone, some of which were potential inducers or inhibitors of the

hepatic CYP enzymes. CYP inducers as a group were found to reduce methadone CDR by 36%. Among these drugs, the effects of carbamazepine and nevirapine were most significant. A previous study (117) showed that plasma levels of methadone were significantly reduced days after co-administration of carbamazepine with subsequent clinical opioid withdrawal symptoms. The authors proposed methadone dose reduction after discontinuation of carbamazepine to avoid methadone-induced respiratory depression. Others have described withdrawal symptoms days after starting nevirapine among patients on MMT (197,198). This may indicate an inducing effect of the drug on methadone metabolism; however, predicting a net effect is more complicated and depends on possible influences of other antivirals in the recommended combination regimes. Although phenobarbital is a powerful CYP inducer (117,199), we could not demonstrate a reducing effect on methadone CDR, which could possibly be due to too few samples included. Additionally, we showed that the group of CYP3A4 inhibitors increased CDR by 36%. This finding confirms previous research data using in vitro, in vivo and clinical studies on the impact of CYP3A4 in methadone metabolism (82,83,88,200). However, a possible influence of some of the drugs in this group on CYP2B6-related methadone metabolism cannot be ruled out, as the effect of many drugs on CYP2B6 enzyme activity is unknown. Only three patients in our study were recorded with concurrent use of clopidogrel that is a selective CYP2B6 inhibitor (201). It is therefore challenging to conclusively predict the role of CYP2B6 inhibitors on methadone CDR. In addition, CYP2B6 involves stereo-selective methadone metabolism preferentially metabolizing the inactive S-methadone, which was not measured in our study; whereas CYP3A4 exhibits no enantiomer preference (79,90,202), suggesting that stereo-selective inhibition might play a role in varied serum concentrations of the R- and S-methadone (202). Several studies have confirmed the primary roles of CYP3A4 and CYP2B6 in methadone's metabolism with minimal roles of CYP2C9, CYP2C19, CYP3A5, CYP2D6 and other enzymes (75,82,83,87,88,91,108,110,202-204). Our results in paper II showing no associations between methadone CDR and concomitant use of medications influencing certain hepatic CYP enzymes support these findings, together with emphasis on the role of CYP3A4 in methadone metabolism. Summarized, this large observational cohort study

demonstrating significant alterations in methadone serum concentration by concomitant use of hepatic CYP enzyme inducers or CYP3A4 inhibitors is in line with previous reports. Although the clinical implications of our findings require further research, our results call for close clinical monitoring of patients undergoing MMT and concurrent medication. This knowledge along with measurements of methadone serum concentrations can help clinicians in managing the potential drug interactions and accordingly reducing the risk of undesirable effects of the treatment among patients with multiple comorbid conditions.

Methadone is more likely to be co-administered with drugs to treat HIV and HCV infections (126-128), and potential drug-drug interactions can be complicated. Since multiple CYP enzymes are probably involved in its metabolism, inhibitors or inducers of these enzymes can affect the pharmacokinetics of methadone. In turn, methadone can also influence the metabolism and clinical effects of other drugs (205-209). For certain antiviral medicines which are dual inhibitors and inducers for CYP enzymes, their effect on methadone pharmacokinetics can change with time since the effect of induction is usually delayed compared to the effect of inhibition (200). This issue can become even more challenging when using combination antiviral regimens. As new drugs such as direct-acting antiviral agents approved for the treatment of HCV (210-213) may be administered concomitantly with methadone (214-216), the agents that are known to induce or inhibit CYP3A4 or other hepatic enzymes may require close clinical monitoring regarding possible influences on methadone efficacy and toxicity. These may be including opiate withdrawal symptoms or overdosing symptoms. Clinical and laboratory based assessments including measurements of serum methadone concentration are crucial in decision making on the need for methadone dose adjustments. Further clinical interaction studies are needed especially for the use of combination antiviral regimens or other pharmacological interventions in the patients undergoing MMT.

Whereas papers I-III discuss the impact of various factors on MMT and concentration-to-dose ratio, paper IV focuses on the influence of serum methadone concentration on the treatment outcomes. In the following subsection, we discuss the clinical aspects regarding the association between serum methadone concentration and self-perceived effects of the treatment based on the findings in paper IV.

5.2.7 The association between serum methadone concentration and clinical effect

As discussed in the subsections above, clinical practice varies with respect to how the need for methadone dose adjustment is assessed. In the light of the observed limitations regarding the use of TDM as a reliable tool to confirm the indication for dose adjustment, patient-reported symptoms may usually be a valid guide for this purpose. However, there is limited knowledge on this topic involving patients undergoing MMT. Previous research has reported an inverse relationship between serum methadone concentration and common objective withdrawal symptoms (67,87,100). To our knowledge, only a few studies (101,177) have investigated such relationships with regard to subjective withdrawal symptoms. In paper IV, we aimed to explore this matter and could demonstrate similar results; subjective opioid withdrawal symptoms – particularly anxiety, bodily pain, restlessness, and shaking (slightly) – were associated with lower serum methadone concentrations. Although the correlations with the serum methadone concentration were not very strong for these symptoms, the findings are in line with existing theoretical expectations and support that dose adjustments should be based on patient-reported symptoms in clinical practice. A lower dose-adjusted serum concentration among more than half the study participants who experienced withdrawal symptoms is a remarkable finding in this study, and may be partially explained by the fact that increasing the dose was not met by a corresponding increase in serum methadone concentration. Another reason may be that further dose escalation was not considered when the serum concentration measured was close the upper laboratory reference limit. As there is apparently not a clear relationship between methadone dose and serum concentration, this is obviously an unfortunate practice leading to underestimation of the dose that actually is needed. However, considering possible aberrant methadone metabolisms or the influence of

other disturbing factors, an individualized dose optimization based on appropriate risk–benefit assessments might be emphasized as an approach capable of achieving treatment effects and simultaneously avoiding the risk of intoxication (80,92,99,100,217-219). We have also discussed in paper IV other approaches such as dividing the daily dose or converting to another opioid e.g. long-action morphine among those not experiencing the optimal effect despite increasing the dose (93,100,220). When none of these measures can help, other causes such as pharmacodynamical factors and genetic variations affecting opioid receptor activity might be excluded (75,221). Finally, diversion of prescribed methadone take-home doses may be considered as an explanation for lower serum concentrations despite patients' receiving appropriate doses and frequently being observed while taking their medications. A closer clinical follow-up together with assessing of intra-individual alterations in serum methadone concentrations may be considered in such aberrant cases as a support to clinical decisions.

As regard to adverse effects, none of the reported symptoms were significantly related to serum methadone concentrations, except for a slight association with nausea. Studies on such associations are lacking. Nevertheless, it is important to keep in mind other possible physical and psychosocial conditions surrounding the patient, which may influence the total subjective experience and satisfaction with the treatment (68).

We also could show an inverse relationship between the use of heroin and alcohol, and serum methadone concentrations, which is in line with findings in earlier studies (67,87,99). Higher methadone doses are shown to be more effective in reducing heroin use and improving treatment retention (52,55,222). Nevertheless, it is not clear whether the lower serum concentration causes the heroin use or whether some patients intentionally do not use the full dose prescribed to allow the heroin to be felt. It is challenging to answer these questions considering the naturalistic design of the study. Our finding of higher alcohol use among those with lower serum methadone concentrations could be explained by self-experienced replacement to alleviate opioid withdrawal symptoms. Research has also demonstrated an overlapping effect of alcohol on mu-opioid receptors in the central nervous system (223). In addition,

regular low-dose alcohol intake (< 4 alcoholic drink/day) may induce P450 enzymes and thus decrease serum methadone concentrations (70). Considering the increased risk of adverse effects and overdose with the concurrent use of opioids and alcohol, a balanced dosage strategy is important to increase treatment retention and avoid not only relapse but also toxicity. Further, a trend to more use of cannabis was shown among the participants with higher serum methadone concentrations. A possible mechanism may be a central-acting effect to counterbalance undesirable side effects related to methadone treatment, for instance, an antiemetic effect of cannabis (224). Some researchers have also suggested an opioid-saving effect of cannabis in patients with opioid dependence (223). However, use of cannabis seems not to be associated with treatment retention or outcomes such as relapse to heroin use or psychosocial functioning (225). To our knowledge, clinical studies have not yet examined possible associations between cannabis use and the methadone dose or serum concentration. Summarized, there is not sufficient evidence to follow self-perceived adverse effects of the treatment or substance use based on serum methadone concentrations in MMT.

5.2.8 The implications of TDM in MMT

Based on the findings in our thesis supported by the current evidence on the enormous inter-individual variability in methadone pharmacokinetics and the absence of a clear relationship between methadone dose and serum concentrations, TDM does not seem to represent a reliable tool in daily dose adjustments in MMT (75,98,218). Accordingly, a careful clinical follow-up of objective signs and subjective symptoms is considered sufficient for dosage titration in clinical practice (75). Nevertheless, the clinicians might find it useful in some selected situations to could manage the intra-individual alterations in serum methadone concentrations (158,159). Trough serum samples (just prior to intake of the next dose) should be drawn at steady-state i.e. a stabilized dose intake during the last 4–7 consecutive days, for the measurements of serum methadone concentrations (73,81,102). As we have showed through the studies included in this thesis, such assessments can be considered in the situations where a possible influence of some of the described factors on serum concentration is expected. Such conditions may include use of interacting co-medications or suspected

aberrant pathways of metabolism such as CYP genetic polymorphisms. Other investigators have also suggested the use of TDM as support to clinical assessments in similar situations where serum concentrations are expected to change markedly; such as upon induction or cessation of an interacting co-medication (117,134,200), during pregnancy (70,226), among patients with liver cirrhosis or severe renal dysfunction (77,132,133), or increased risk of serious adverse effects especially cardiotoxicity (75,185,186). The latter may be related to the use of higher methadone doses in the presence of CYP inhibition, which may be induced by co-medications or possible genetic polymorphisms. In daily clinical assessments of dose requirements, other factors such as gender, age and BMI should also be considered in MMT, with or without measuring of serum methadone concentrations based on individual medical conditions. It has also been suggested that TDM could be helpful to reveal low compliance, treatment failure or methadone diversion (75,154,227). However, this may be challenging, as a low serum concentration do not necessarily indicate that the methadone dose taken is lower than prescribed. In addition, other non-pharmacological factors such as psychosocial conditions may influence the treatment outcome. Nevertheless, a close clinical monitoring along with measurements of serum concentrations may be considered for the individual follow-up of the patients who complain on insufficient dosage and opioid withdrawal symptoms despite dose increase. Some researchers have also suggested measurements of peak-to-trough-, or methadone/EDDP ratios to assess the rate of methadone metabolism and differences in dosage needs (87,228,229). Higher peak-to-trough ratios, suggesting a shorter elimination half-life, are in agreement with the usual clinical measures taken for such patients, which are to increase methadone dosages and to split the daily dose into several intakes (87). Finally, stereo-selective measurements can be considered in some cases as the concentration of R-methadone rather than racemic methadone has been shown to be correlated with therapeutic outcome in MMT (75,99,177).

5.3 Methodological considerations

Validity, which describes the accuracy of a research, is normally related to some internal as well as external components.

5.3.1 Internal validity

The internal validity, i.e. the characteristic of a clinical study to produce valid results, can be affected by random and systematic (bias) errors. Random error is due to chance and can be reduced by increasing the sample size or by decreasing the variation in measurements (reducing measurement error). Bias is any error resulting from methods used by the investigator to recruit individuals for the study, from factors affecting the study participation (selection bias) or from systematic distortions when collecting information about exposures and diseases (information bias). More generally, bias is any deviation in the design, collection, analysis, interpretation, and publication of data leading to conclusions that systematically underestimate or overestimate the true relationship between a given exposure and a specific disease or any other outcome. Bias cannot be minimized by increasing the sample size. Most violations of internal validity can be attributed to selection bias, information bias or confounding (230,231). Understanding bias in the context of research methodology is crucial to improve the quality of work and heighten the reliability of findings. In the following subsections, the main aspects of the internal validity of this research work have been discussed.

5.3.2 Research design

For all the papers included in this research work, a naturalistic observational design has been used. Accordingly, no controlled interventions were conducted. When planning the research, emphasis was placed on the hypothesis and the purposes of the thesis. With the research goals in mind, we believed that the use of observational cohort design could be sufficient and appropriate to enable us to answer the research questions. Initially, the intention was to look at whether there was a relationship between methadone dose and serum concentration, and then explore possible factors that may influence this relationship. During the past decades, the use of TDM data in Norway has provided us with a large pool of laboratory data that gave an opportunity

for this type of research. Papers I and II made therefore use of laboratory TDM data collected from Norwegian patients undergoing MMT, retrospectively. By providing blood samples for CYP genotyping in addition to serum concentration measurements of methadone, the TDM database at the Center for Psychopharmacology, Diakonhjemmet Hospital in Oslo, Norway was quite suitable for carrying out paper I. Similarly, accessing enormous information on concomitant medications together with demographic data on age and gender, the TDM database at the Department of Clinical Pharmacology at St. Olav University Hospital in Trondheim, Norway could provide retrospective data for investigating the impact of interacting co-medications as well as the gender and age on methadone metabolism in paper II. Although the retrospective nature of the data, entailed some limitations especially in terms of access to necessary and desired information, and assurance that the samples were taken correctly and at the right time, it had the advantage of saving decades to collect such a large volume of laboratory data. Nevertheless, a prospective design would be able to give us the opportunity to ensure a broader access to information and optimize the precision of sample collections. The retrospective design probably did not significantly affect our results, as all the uncertain and incomplete data were deleted before the final analyzes were performed. In addition, the results were adjusted for the time between taking the last methadone dose and blood sampling.

For papers III and IV, a prospective cohort design was used. The establishment of an integrated treatment model for OAT in the Department of Addiction Medicine at Haukeland University Hospital in the city of Bergen, Norway, has provided us with access to a large group of patients undergoing MMT. The patients visit the specialized outpatient OAT clinics from one to seven days a week to receive substitution medications (mainly buprenorphine or methadone) under direct observation and close clinical monitoring with a low risk of loss to follow-up. This treatment model has enabled us to access a substantial pool of clinical and laboratory data, which are recorded in the hospital journal system, as well as in a health registry database for clinical and research purposes nested in the INTRO-HCV study (140).

During the planning phase of the research, we were convinced that a prospective cohort design was appropriate to conduct papers III and IV, having opportunity to perform the substantial clinical assessment of subjective opioid withdrawal symptoms and adverse effects, as well as the measurements of liver stiffness and other clinical and laboratory assessments related to the research objectives. The naturalistic observational setting of the studies allowed us to reflect the real clinical life situation through appropriate research methods. In addition, since the purpose of the thesis was to investigate possible associations, but not to prove any causalities, it seemed that the adequacy and suitability of an observation cohort design was convincing.

5.3.3 Selection bias

Both the methods used to select the participants or subjects to be included in a study, and the factors that influence loss to follow-up may result in selection bias (232). There are challenges associated with access to and follow-up of patients with substance use disorders, especially those that are difficult to reach regularly. This fact may have contributed to the selection of individuals with higher stability and more frequent contact with treatment services. The consequences for all papers may be that data is skewed and the results are thus not unambiguously presented. For the two retrospective laboratory-based data materials used for papers I and II, it was not possible to influence the choice of participants and the individuals with greater medication challenges may have been over-represented. Additionally, by defining specific selection criteria for including the measured data in the studies, the probability of selection bias was present. These criteria included methadone dose, time since last dose intake until sampling, concomitant medications, quality of the analysis, and incomplete data sets among others. At the same time, it was important to narrow down the inclusion criteria to increase the likelihood of more robust and complete data, and in order to achieve more reliable results. This bias may possibly have influenced our results to some extent, especially in paper I having smaller sample size than paper II.

For the prospective clinical-based data material used in papers III and IV, the risk of selection bias may include coverage of all patient categories, i.e. group

representativeness, and the risk of loss to follow-up. Specific criteria for including the most correctly measured data in the studies could also have increased the probability of selection bias. These criteria were mainly related the time difference between measurements of serum methadone concentrations and the assessments of subjective opioid withdrawal symptoms and adverse effects, as well as measurements of liver stiffness. Incomplete data sets for some measurements resulted in the exclusion of some data. This was mainly due to loss to follow-up among the most vulnerable participants who were not able to cooperate on the research protocols. All these weaknesses may have disrupted the effect estimates of interest, although this was determined as missing at random. Reasonably, the prospective design of the studies allowed us to optimize data collection and quality of the measurements, and any systematic differences were taken into account by measured covariates and sensitivity analyses. Multiple imputation is an alternative method to deal with missing data, which accounts for the uncertainty associated with missing data. Multiple imputation is implemented in most statistical software under the assumption of missing at random and provides unbiased and valid estimates of associations based on information from the available data. The method affects not only the coefficient estimates for variables with missing data but also the estimates for other variables with no missing data (233). The results may therefore not have been significantly affected in Articles III and IV.

5.3.4 Information and assessment bias

Information bias refers to any systematic differences from the truth that arises in the collection, recall, recording and handling of information in a study, including how missing data is dealt with. Major types of information bias are misclassification bias, observer bias, recall bias and reporting bias. Observational studies may be at greater risk, particularly those relying on self-reports and retrospective data collection. Missing data can be a major cause of information bias, when certain groups of people are more likely to have missing data. Non-differential (random) misclassification of measures that occurs equally in all comparison groups tends to result in underestimation of effect. Whereas differential information bias where there are different levels of inaccuracy between comparison groups, could work in either

direction, resulting in an over- or underestimate of the true effect. Some of the main techniques suggested to minimize the risk of information bias are prospective design with awareness of planning strategies, using standardized methods, and measuring tools, developing well-standardized data collection protocols, neutrality in the formulation of the questions asked by interviewers, and ensuring consistent recording methods to ascertain information or to verify self-reported measures. Multiple imputations may be used if data is thought to be missing at random (232-235).

The risk of information bias might have been greater for papers I and II, having retrospective design in comparison with papers III and IV that were designed prospectively. The data were collected in the respective laboratory databases based on the recorded information on the requisition form used for clinical purposes. Bias may therefore have been occurred in every stage of data collection including obtaining and registration at requisition (especially relevant clinical data such as co-morbid diseases, concurrent medications etc.), as well as transferring the information to the research database. We dealt with this issue by choosing the subjects who had complete dataset, and by assessing the quality of the information. The dataset with essential missing affecting the main study variables or with highly uncertain values were deleted in the final database. The unique Norwegian 11-digit personal identity number made patient identification consistent. When this number was missing and the subject could not be identified by other means, the sample was excluded. An important concern is, however, about the accuracy of the recorded methadone doses at the time of taking blood samples. In addition to the risk of recall bias, the possibility of secondary non-compliance (whether the patients had taken the medication as prescribed) cannot be ruled out (236). Although a prospective study design could reduce this risk, it would not completely resolve the challenge. The participants could have greater incentive, due to their health concerns, to over- or under-report the real dose they had used; for instance excess illicit use of methadone in addition to the prescribed dose to get high, or less use than prescribed due to diversion. There is a possibility that the results in all the papers are influenced due to this limitation, however, the risk is lower for papers III and IV as the doses were mainly taken supervised. Exposed individuals in a cohort

study may be concerned about the exposure and may over-report or more accurately report the occurrence of non-existent symptoms or health outcomes. This may be the case in paper IV regarding self-reporting of subjective opioid withdrawal symptoms to obtain higher methadone doses. Regardless of design, this is an inevitable challenge, especially among patients with substance use disorders with multiple psychological and socioeconomic problems that affect their self-perceived expectations and other incentives. It can therefore not be ruled out that our results may have been over- or under-estimated.

In addition, there is a risk of measurement and assessment bias, which includes both objective and self-reported data, especially for papers III and IV. Errors may have occurred at all stages of blood sampling due to various reasons such as deviations from protocol with respect to time since last dose intake, problems with sampling technique and equipment, errors in recording personal data or during laboratory analyzing, and reporting of serum methadone concentrations. Deviations in objective measurements may also have affected the measures of liver stiffness and BMI. However, we have reduced this risk by standardizing the research protocols and striving to follow the current medical procedures for this purpose. Daily access to the patients in OAT clinics has also made it possible to assess the feasibility of the research activities from one day to the next in order to optimize the situation in relation to the required qualifications. There was also possibility to repeat the measurements when needed.

The main outcome variable in Paper IV was the self-reported symptoms of opioid withdrawal and adverse effects. Obtaining self-perceived information through interviews challenges several aspects of the information assessment process that may include recall, the way questions are asked, interviewer-patient relationship and how the answers are understood and interpreted (237). The patient's current clinical condition, possible use of substances and life events prior to interviews may also have an impact on how the questions are answered. We have tried to minimize this bias by using standardized structural and semi-structural interview forms conducted by experienced and non-independent research nurses. There have also been frequently

meetings between research nurses and research managers to ensure the quality of the data collection and make the implementation as coordinated as possible. The use of these measures has probably reduced the risk of assessment bias and the results are therefore not significantly affected.

For papers III and IV, synchronizing the time for blood sampling and the relevant measurements and assessments such as conducting the interviews with respect to self-reported symptoms or measuring liver stiffness were also some of the challenges. Because some participants were unable to have the necessary examinations conducted on the same day as the blood samples were taken, the sample sizes were reduced to include only those who had a maximum 14-day time interval between laboratory and clinical measurements. Nevertheless, this was considered an important measure to reduce the risk of erroneous findings and conclusions, even though it was at the expense of reduced sample size, which may have increased the risk of Type I and Type II statistical errors.

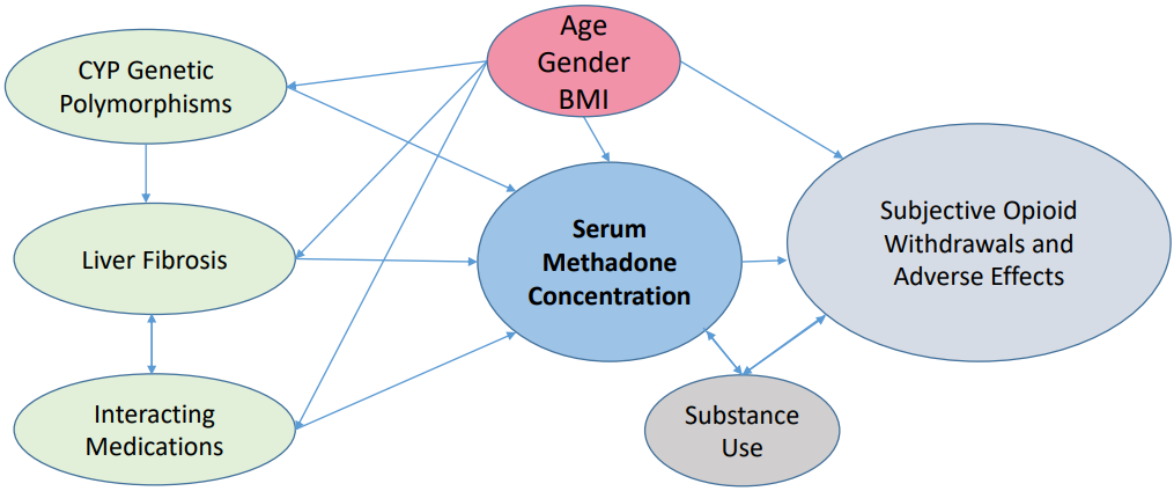
Another concern for the cohort studies is the introduction of social desirability bias among people with substance use disorders. The core of this bias is under-reporting socially undesirable attitudes and behaviors such as substance use, while over-reporting more socially desirable attributes (238). For instance, patients may under-report the use of illicit substances to avoid sanctions such as being reduced in methadone dose or deprived of doses taken home. The risk of this type of imbalance is lower when using independent research nurses who are not involved in patients' clinical treatment as this have been the case in our research. However, the risk of under- or over-estimating the results cannot be fully ruled out in our research.

5.3.5 Confounding

A confounding factor is a variable that correlates (positively or negatively) with both the exposure and outcome. Confounding can be a major problem with any observational (nonrandomized) study. Ignoring confounding in an observational study will often result in a distorted or incorrect estimate of the association or treatment

effect. When defining confounding, it is important to consider the temporal relationship between the purported confounding variable and the exposure variable. A confounding variable must occur or be measured before the exposure variable or the exposure period (239-243). In order to control for confounding, researchers can routinely implement study design procedures (e.g. randomization, study eligibility restriction, and/or a priori participant matching), but most confounding is removed by statistical procedures in the subsequent data analysis (e.g. multivariable regression models or propensity score methods) for both clinical trials and more typically observational studies (244-246). The assumed model of inference and confounding in our research is shown in Figure 8, and illustrated using DAGitty diagram in Figure 9.

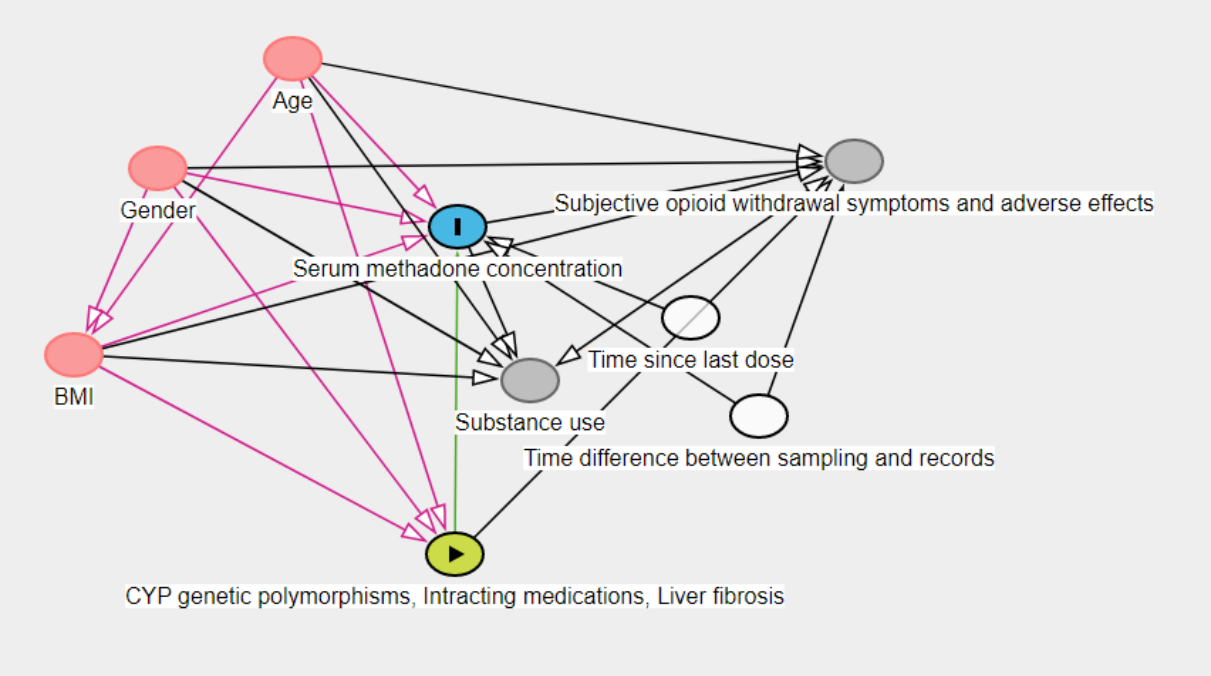
Figure 8. Assumption for inference and confounding model based on the research hypothesis of the possible influencing factors on serum methadone concentration and its impact on the clinical outcomes



The research hypothesis has been that factors such as genetic polymorphisms in hepatic CYP enzymes, liver fibrosis and interacting medications may affect serum methadone concentration, which in turn may influence the self-perceived treatment outcomes such as subjective opioid withdrawal symptoms and adverse effects. When showing an association between an exposure and an outcome in observational studies, a number of possible explanations need to be addressed before making a conclusion on a true cause-effect relationship. As discussed under the previous subsections, an

observed association may be caused by systematic errors (biases), or simply be produced by chance (random error). A third probability is the influence of confounding that distorts the true association between the exposure and the outcome; either by masking associations when they truly exist or by indicating spurious associations when in fact there are no causal relationships (232).

Figure 9. DAGitty diagram illustrating inference and confounding model based on the research hypothesis of the possible influencing factors on serum methadone concentration and its impact on the clinical outcomes



By having serum methadone concentration as the outcome variable in papers I, II and III, the possible factors that could have distorted the results were assumed gender, age and BMI. These variables were included in the regression model as co-variates. In addition, the results were adjusted with regard to the time between the last dose intake and blood sampling to reduce the risk of false findings. During the data collection using the retrospective laboratory databases, the samples where the time was shorter than 10 h or longer than 26 h were excluded from the study material. In paper IV, the outcome variables were subjective symptoms of opioid withdrawal and adverse effects, while serum methadone concentration was the exposure variable. Several

factors such as the use of various substances, or behaviors related to it e.g. non-compliance due to diversion or excess use of illicit methadone, could have affected both the self-experienced symptoms and serum methadone concentrations. It was, however, not possible to detect or avoid such deviations due to the naturalistic method of the research. In addition, a possible impact of substance use on serum methadone concentration is not previously studied. We have therefore constructed a priori theory for association with the factor in our research as shown in Figure 8. In paper IV, the results were adjusted with regard to the time difference between measurement of serum methadone concentrations and recording of the clinical assessments, and the data with time differences longer than 14 days were excluded from the regression analyses. The risk of affecting the results by confounding bias is therefore supposed to be reduced in our research; however, it cannot be fully eliminated.

5.3.6 Statistical considerations

We used the regression model of analysis to adjust for possible confounders and accordingly to reduce the risk of systematic biases. Yet, the cohort studies in this thesis are at risk for confounding as the effect of other unknown confounders only can be fully adjusted by true randomization (247). As we have benefited longitudinal data with repeated measurements for all the papers included in this thesis, the linear mixed model was considered as an appropriate method for analyzing of the data. Linear mixed model is a statistical model containing both fixed effects and random effects, and are widely used to analyze linear regression relationships involving dependent data when the dependencies have a known structure. Because of their advantage in dealing with missing values, mixed effects models are often preferred rather than more traditional approaches such as repeated measures analysis of variance (248). Nevertheless, an observed statistical association between an exposure and outcome in this research dose not necessitate a causal relationship. Likewise, a lack of association does not imply the absence of such relationship. Additionally, another indicator of importance when evaluating an observed association involves an assessment of the strength of the association where a stronger association is more likely to be causal compared to a weaker one. Temporality (cause precedes outcome), consistency

(consistent with others findings), dose-response relationship (biological gradient), specificity (one to one relationship), plausibility and coherence (alteration in exposure alters outcome) are among the criteria indicating the strength of an association (232,249). Considering the naturalistic observational nature of our research, we acknowledge that not all the described criteria have been met in the included papers e.g. temporality criteria in the papers I and II, using retrospective design. However, the findings in all the four studies are consistent with the current knowledge for the specific topics, and to a certain extent support a dose-response relationship especially for the papers I-III.

An association does not necessarily imply a strong correlation between an exposure and an outcome. For instance, although paper IV found associations between some subjective symptoms and serum methadone concentrations, the results must be interpreted in light of the relatively small effect sizes. The observed weak to moderate correlations may reflect possible influences of other factors such as concurrent use of illicit drugs or abstinence from these substances, comorbid somatic and psychiatric conditions, or even manipulation of symptoms to receive higher methadone doses. Furthermore, the probability of Type I and Type II statistical errors due to a small sample size and the naturalistic observational nature of the research should be taken into account for all the papers. Although the scope of our research was not to conclude strictly on a causal relationship, exploring potential associations between the various exposure variables and the outcome variables in a causal inference framework may contribute to reduce the possible risks and support reasonable interpretations of the findings (250).

5.3.7 External validity

External validity refers to the extent to which results from a study can be applied (generalized) to other situations, groups or events, especially for the population that the sample is thought to represent. In the modern research perspective, external validity includes scientific and statistical generalization. Scientific generalization is the characteristic of a study whereby it may generate a coherent, potentially causal,

biological hypothesis applicable to a more general set of clinical circumstances than the specific population under investigation. Statistical generalization is fundamental in survey sampling in which the resulting sample must be statistically representative of the source (or target) population. The key difference between the two features of external validity is that scientific generalization rests on biological rather than on statistical representativeness of the sample (251,252). This can be an important point to be aware of when researching populations with substance use disorders. The higher risk of loss of follow-up and selection bias in studies among this group of people may reduce the external validity of the research (253). In fact, all threats to internal validity are also threats to external validity in research. Consequently, the external validity of our research may also have been affected by the same factors that may have influenced its internal validity. In clinical and cohort studies, there is a need to balance the eligibility criteria to protect participants' safety and to ensure internal validity, as well as to include the study sample of interest in preserving good external validity (253). By using broader inclusion criteria in our research, we have intended to improve the generalizability of the findings. Nevertheless, the importance of access to complete laboratory and clinical data sets made it necessary to limit the sample size in order to improve the robustness of the data. This may have led to the exclusion of the most vulnerable groups of the research population. This is one of the challenges when it comes to research on hard-to-reach populations (254).

5.4 Limitations and strengths

5.4.1 Limitations

In addition to the limitations discussed in the subsections above, the use of retrospective TDM data for research purpose implies also some methodological limitations such as compliance, uncertainty about the exact dose and time of dose taken. As mentioned previously, we therefore included the samples where information about the time of last dose ingestion was available. Administration of daily methadone doses under health staff supervision may also have reduced the risk of non-compliance and diversion. Furthermore, the use of TDM data is based on the assumption that

blood samples are collected at steady-state. Although it was not possible to confirm steady-state conditions for all the concentration measurements included in papers I and II, it is considered unlikely to have significant effect on the findings –at least regarding comparing between the groups- as this was the case for all the samples. Due to the retrospective naturalistic design, we neither could confirm that the information stated on the requisition forms was correct, nor had access to information on variables such as ethnic background, concurrent diagnoses, smoking habits, and concomitant use of herbals, alcohol or illicit substances. This might all affect the results as well as assessing the clinical relevance of the observed CDR variations. Another limitation of this research (for all the papers) was that methadone serum concentrations were measured as the racemate and not as the specific enantiomers of R- and S-methadone. This may complicate the interpretation of the results, as some of the observed effects may have been caused by a disproportional change in the concentrations of the inactive isomer S-methadone. However, the clinical importance of such effects is not known.

Additionally, the low number of observations in papers I, III and IV, and the few numbers of the recorded potentially interacting drugs in paper II may have influenced our findings. This may have resulted in Type I and Type II errors. The small sample size does not allow for drawing certain conclusions about the possible influences of the important clinical and genetic confounding factors. For instance, the sample used for paper III did not include sufficient data on severe renal failure or concomitant medication with a potential interacting effect on methadone metabolism. Ideally, a larger population scale is needed to explore possible influences of genetic factors, which may have influenced the findings in papers I and III. Delayed follow-up was one of the limitations for papers III and IV, as the participants were not able to complete all the clinical interviews, examinations, and blood samplings at the same time. Only approximately half of those who had completed the primary surveys were thus eligible to be included in the research. Furthermore, although paper IV found associations between some subjective symptoms and serum methadone concentrations, the results should be interpreted with caution due to the observed weak to moderate

correlations, possibly because of other interfering factors. The naturalistic nature of the studies in papers III and IV could also have contributed to some limitations by allowing the clinicians to adjust the methadone dose based on their clinical judgment. This may for example have led to inappropriate dose reductions in people with liver impairment, which may affect the findings in paper III. Finally, other factors that are beyond the scope of this research, such as other patient-related factors, may have influenced our results.

5.4.2 Strengths

The major strength of this thesis is the use of several laboratory and clinical databases obtained from Norwegian patients undergoing MMT enabling us to access a large pool of information needed to conduct the research. The available TDM databases provided us with information on genetic and interacting drugs as well as serum methadone concentrations, which were spot on with regard to the main objectives of papers I and II. Access to more than 4000 longitudinal data including repeated measurements of methadone serum concentrations collected from approximately 1700 subjects in paper II and the use of an appropriate analysis method, i.e. linear mixed regression model to deal with the described limitations, can be considered as one of the strengths of this research. In addition, the prospective nature of the studies in papers III and IV, and the treatment platform established in the OAT clinics, allowed us to easily access and continuously meet patients who are otherwise difficult to reach for research purposes. We were thus able to manage data collection more closely and reduce information bias, which in turn can increase the quality of research. Furthermore, the naturalistic observation setting of the studies that reflects real life and actual clinical challenges in the daily practice of MMT may constitute another strength of this work.

6. Concluding remarks and future perspectives

By conducting the studies included in this thesis, we could demonstrate that genetic polymorphisms in CYP2B6, gender, BMI and concurrent medication with CYP enzyme inducers and CYP3A4 inhibitors may explain some of the variations in dose-adjusted serum methadone concentration. We also observed that age, the degree of liver fibrosis and the other CYP polymorphisms were not associated with methadone CDR. Additionally, we found associations between the subjective withdrawal symptoms (the total SOWS score, and the specific symptoms of anxiety, bone and muscle aches, restlessness, and shaking) as well as substance use (heroin, alcohol and cannabis), and serum concentrations of methadone.

Our findings confirm the current clinical and research challenges in MMT regarding a large inter-individual variability in methadone pharmacokinetics. The results are also in line with previous research demonstrating the influence of several factors, which should be considered during the treatment course. Overall, our research supports the importance of a proper and individually tailored dosage to achieve sufficient serum concentrations of the drug that can alleviate symptoms related to the current condition. In this research, we have presented an inverse relationship between subjective withdrawal symptoms and serum methadone concentrations. Dose adjustments based on self-perceived symptoms could therefore be of importance to achieve the desired effect by the treatment, which mainly is to reduce the risk of relapse to substance use and other associated risks. The major challenge is the observed variations in the relationship between methadone dose and serum concentration, and the diversity of influencing factors including genetic, pathophysiological and pharmacological factors. The use of TDM in MMT should therefore be limited to specific clinical situations such as abnormal metabolism, concomitant use of other medications and severe organ impairment. Increased knowledge in this topic can help clinicians in the daily management of the treatment and clinical decision-making. Being aware of variability in methadone pharmacokinetics, together with taking into account other contributing factors such as the patient's psychosocial situation, could contribute to improved

outcomes and reduced risk in MMT. Thus, further clinical research using other study designs, larger patient samples and including other possible confounding factors are needed to improve the knowledge in MMT. High quality research including clinical trials can obviously reduce the disturbances related to confounding and others biases, especially in subpopulations with substance use disorders. Wider access to and use of laboratory facilities that enable broader methods for the measurement of serum concentrations of racemic methadone, stereo-selective analyses, determination of the metabolites, and other pharmacological and genetic analyses will also contribute to future research opportunities.

7. References

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Appendices

Paper III

Chalabianloo F, Høiseth G, Vold JH, Johansson KA, Kringen MK, Dalgard O, Ohldieck C, Druckrey-Fiskaaen KT, Aas C, Løberg E-M, Bramness JG, Fadnes LT. Impact of liver fibrosis and clinical characteristics on dose-adjusted serum methadone concentration. Journal of Addictive Diseases 2022;31(3):1-11.

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Impact of liver fibrosis and clinical characteristics on dose-adjusted serum methadone concentrations

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ABSTRACT

Background: There is limited knowledge on the causes of large variations in serum methadone concentrations and dose requirements.

Objectives: We investigated the impact of the degree of liver fibrosis on dose-adjusted steady-state serum methadone concentrations.

Methods: We assessed the clinical and laboratory data of 155 Norwegian patients with opioid use disorder undergoing methadone maintenance treatment in outpatient clinics in the period 2016–2020. A possible association between the degree of liver fibrosis and dose-adjusted serum methadone concentration was explored using a linear mixed-model analysis.

Results: When adjusted for age, gender, body mass index, and genotypes of *CYP2B6* and *CYP3A5*, the concentration-to-dose ratio of methadone did not increase among the participants with liver fibrosis (Coefficient: 0.70; 95% CI: −2.16, 3.57; *P*: 0.631), even among those with advanced cirrhosis (−0.50; −4.59, 3.59; 0.810).

Conclusions: Although no correlation was found between the degree of liver stiffness and dose-adjusted serum methadone concentration, close clinical monitoring should be considered, especially among patients with advanced cirrhosis. Still, serum methadone measurements can be considered a supplement to clinical assessments, taking into account intra-individual variations.

KEYWORDS
Opioid agonist treatment; methadone; serum concentrations; liver fibrosis; cirrhosis; CYP genotypes; BMI

1. Introduction

Despite decades of using methadone in opioid agonist therapy (OAT) for opioid use disorder, there is still limited knowledge on the causes of large variations in the drug's metabolism, serum concentration, and dose requirements.[1] Various factors, such as hepatic and renal function, genetic heterogeneity, individual biological characteristics, and concomitant medication, may influence drug metabolism.[2] Understanding possible predictors of

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methadone metabolism and serum concentration is essential for providing optimized doses and person-tailored OAT treatment.

Methadone is metabolized extensively in the liver.[3,4] Metabolism of drugs in the liver depends on hepatic blood flow and liver enzyme activity; both can be affected by liver disease.[5,6] Cirrhosis or advanced fibrosis of the liver tissue related to chronic infections or other hepatic diseases, long-term alcohol consumption, or even predisposition to specific genotypes of cytochrome P-450 (CYP) enzymes may affect liver function.[7,8] Chronic hepatitis C virus (HCV) infection is common in patients with injection substance use.[9] Untreated HCV infection can result in liver cirrhosis and death due to liver failure or hepatocellular carcinoma.[10] In a cohort of HCV-infected injection substance users, a third developed advanced liver disease within three decades.[11] The impact of liver cirrhosis and developing portal hypertension and reduced first pass effect on methadone metabolism is not fully understood. Existing studies have not found sufficient evidence to justify and guide methadone dose adjustments due to chronic liver diseases with advanced liver fibrosis.[4,12–15]

Further, it is unclear whether genetic polymorphisms of the hepatic enzymes involved in methadone metabolism may also be related to the development of liver fibrosis. Polymorphisms in genes encoding for CYP enzymes have been suggested as possible causes of large variations in methadone dose requirements.[16–19] Recently, a possible impact of CYP2B6 has been suggested on methadone metabolism,[17,20,21] with the *6 reduced-function allele demonstrated with higher serum methadone concentrations in some studies.[16,22] Among other CYP enzymes, the CYP3A family appears to play some role.[18] CYP3A5 exhibits genetic polymorphism and the most frequent genotype (90%) has the unusual inactive *3 allele.[23,24] Although some data are available,[18,22,25] the clinical impact of CYP3A5 on methadone metabolism has not been sufficiently investigated. Other inherent clinical characteristics, such as age, gender, body mass index (BMI), and renal function, as well as extrinsic factors, such as concomitant medications, may also be presumed to influence the drug's metabolism.[2,26–28] For instance, based on general assumptions, it is conceivable that impaired renal function with advanced age may lead to an increased risk of drug accumulation in the body, or patients with higher BMI may need higher doses. However, the supporting evidence regarding methadone maintenance treatment is still limited.[4,12,26–28]

In the present study, we aimed to investigate the association between liver stiffness and dose-adjusted steady-state serum methadone concentration, adjusted for age, gender, BMI, renal function, concomitant medication, and genetic polymorphisms in CYP2B6 and CYP3A5.

2. Patients and methods

2.1. Settings and data sources

The Department of Addiction Medicine, Haukeland University Hospital (Bergen, Norway) is responsible for the treatment and follow-up of more than 1000 patients with opioid use disorder receiving OAT, of which almost 40% receive methadone, while the remaining mainly receive buprenorphine. All medical interventions are integrated with psychosocial care provided in multidisciplinary outpatient clinics. Depending on the overall functioning level and decisional capacity, the follow-up of patients ranges from directly observed treatment and consultations to weekly take-home doses. All the clinical measurements and laboratory data are recorded in the hospital journal system as well as in a health registry database for integrated clinical and research purposes nested in the INTRO-HCV study[29] and in connection with a previous study from the same research group.[30]

2.2. Data collection

The research surveys were performed through in-person clinical examinations and blood samplings from May 2016 to January 2020. In the present study, we included information on age, gender, BMI (kg/m^2), genotypes of *CYP2B6* and *CYP3A5*, methadone daily dose (mg/day) and steady-state trough serum concentrations (nmol/L), concurrent medications, self-reported use of illicit substances and alcohol, liver function

parameters (blood levels of alanine aminotransferase (ALT) (IU/L), aspartate aminotransferase (AST) (IU/L), alkaline phosphatase ($ALKPO_4$) (IU/L), and bilirubin (BIL) (μmol/L)), the degree of liver stiffness estimated by transient elastography (kPa), renal function parameters (estimated glomerular filtration rate (eGFR); ml/min/1.73m^2), HCV infection status (presence of antibody and RNA), and human immunodeficiency virus (HIV).

2.3. Participants

A total of 155 patients with opioid use disorder based on the ICD-10 diagnostic criteria undergoing methadone maintenance treatment in OAT Bergen during the study period consented and participated in the study. All participants completed the requested laboratory tests and clinical surveys mentioned above during this period.

2.4. Laboratory analyses of methadone serum concentrations and liver function tests

Blood samples were drawn from the participants at the OAT clinics according to the study protocol and at trough concentration with a mean (standard deviation; SD) time of 21 (8) hours since the last dose intake and no changes in the methadone dose during the last 1–2 weeks (steady state). Analyses of methadone as well as liver function tests (ALT, AST, $ALKPO_4$, and BIL) in all the collected serum samples were performed by the same analytical method using the same laboratory instruments at the Department of Medical Biochemistry and Clinical Pharmacology, Haukeland University Hospital (Bergen, Norway). Serum concentrations of methadone were analyzed using a validated and certified high-pressure liquid chromatography-tandem mass spectrometry (HPLC-MS-MS) method. MS-MS analysis was performed with electrospray ionization (ESI) in positive ion mode (Agilent Technologies 6410AA triple quadrupole LC-M-MS, CA, USA). The limit of quantification was 20 nmol/L, and the method was linear at least to 4000 nmol/L. Recoveries were 100% and 91%, and inter-day coefficients of variation were 2.7% and 5.1% at low and high concentrations, respectively. During the development phase of the method, as well as in routine use, methadone concentrations were measured in nmol/L. The conversion factor from nmol/L to ng/mL for methadone was 0.310.

2.5. Assessing liver fibrosis

Liver stiffness measurements (LSM) were assessed by vibration-controlled transient elastography using FibroScan (Model 430 Mini). The LSM value was correlated to the liver fibrosis stage.[31] Exclusion criteria were pregnancy, the presence of an implantable medical device, and a BMI ≥30 kg/m^2 (to avoid erroneous measurements using standard probes that were not adapted to obese individuals). Participants who consented were requested to fast for 3 h before the procedure. The examination was performed onsite in the OAT clinics according to a standardized procedure.[32] After a minimum of 10 valid measurements were acquired, median LSM values were calculated.[33] Examinations with an interquartile range greater than 30% were classified as unreliable and were excluded from further analyses.[34] The cutoff values for fibrosis stage (hereby fibrosis measures) for all the participants were as follows: LSM ≤7 kPa for no/limited fibrosis, LSM 7 kPa to <12 kPa for fibrosis, and LSM ≥12 kPa for cirrhosis[31]—those with LSM ≥20 kPa in the last category represented cirrhosis state with significant portal hypertension.[35]

2.6. Genotyping

Genotyping of *CYP2B6* and *CYP3A5* was performed using routine analysis TaqMan-based real-time polymerase chain reaction assays at the Center for Psychopharmacology at Diakonhjemmet Hospital (Oslo, Norway). The determination of the *CYP2B6*6* haplotype was based on genotyping of 516 G > T (rs3745274) and 785 A > G (rs2279343) variants. The presence of both 516TT and 785GG was interpreted as *CYP2B6*6/*6*, whereas the presence of 516GT and 785AG or 785GG was interpreted as *CYP2B6*1/*6*. The combinations of 516GG and 785AA or 785AG were interpreted as *CYP2B6*1/*1*. The determination of *CYP3A5*3* haplotype was based on genotyping of 219-237 A > G (rs776746). The presence of

219-237GG was interpreted as *3/*3, whereas the presence of 219-237AG was interpreted as *1/*3. Patients who presented two of any of these alleles were defined as poor metabolisers (PM), those who presented one allele were defined as intermediate metabolisers (IM), and the remaining patients were classified as normal metabolisers (NM).

2.7. Statistical analyses

The statistical analyses were performed using Stata/SE 16.0 (StataCorp, TX, USA). Basic descriptive data were presented as means (SD) for continuous variables, and numbers with percentages for categorical variables. Linear mixed model (LMM) analyses were applied to investigate possible associations between the explanatory variable of liver fibrosis stage and the outcome variable, namely dose-adjusted steady-state serum methadone concentration presented as concentration-to-dose ratio (CDR) in (nmol/L)/(mg/day), although the unit is not repeated when using CDR in the text. In total, 192 observations were included in the LMM analyses, as 37 participants had two sets of records for CDR and fibrosis measures. Interaction analyses ruled out any interacting factor between liver fibrosis and the *CYP* genotypes regarding CDR.

Confounding variables were age, gender, BMI, renal function, use of interacting co-medications, and the different genotypes of *CYP2B6* and *CYP3A5*. The relevant variables were included one by one as categorical variables in the unadjusted statistical analyses. Renal function measures were not included in the regression analyses due to no recorded severe renal failure (eGFR <30 ml/min/1.73 m^2).[36] There were no highly suspected interacting medications,[26] such as anti-HIV agents and other strong CYP-3A4 inhibitors or CYP inducers,[37] in our data. The remaining recorded medications without a known interaction potential with methadone were therefore not included in the regression model. We then investigated the confounding effects of the variables on CDR in an adjusted multivariate LMM model. Participants with BMI >30 kg/m^2 (n = 46) were excluded from the adjusted analysis as the measurements of liver stiffness were not possible or, if so, reliable in this group. For participants with two sets of measures, possible changes in CDR and fibrosis measures between the two recording times (when having HCV infection and post treatment) were assessed by adding the time factor in the analysis, and no effects of time were found. We also conducted some sensitivity analyses using the LMM to reveal other possible associations or interacting factors when indicated. The intercept presented a woman younger than 50 years old, with BMI <25 kg/m^2, liver fibrosis measure ≤7 kPa, *CYP2B6* genotype *1/*1, and *CYP3A5* genotype *3/*3, and whose CDR was 10. The results are presented as coefficients with 95% confidence intervals (95% CI), and *P*-values were considered statistically significant at a level of <0.05.

2.8. Ethical considerations

The study was approved by the Regional Committee for Medical and Health Research Ethics in Vest, Norway (approval No. 2017/297/REK vest). All participants signed a written informed consent agreeing to the use of routine and research data for this purpose and to take part in the study.

3. Results

Table 1 shows the demographic and clinical characteristics of the 155 study participants. A third (33%) were women, and the mean age was 45 (10) years, with a mean OAT duration of 9 (5) years. The mean time from last dose intake to blood sampling was 21 (8) h, and the patients had 4 (2) days per week with directly observed intake of the OAT medications. The mean methadone dose and serum concentration in all 192 observations were 99 (25) mg/day and 1248 (559) nmol/L, respectively, giving a mean CDR of 13 (6) with a wide range of 3–38 (nmol/L)/(mg/day). Out of 145 (94%) participants with positive HCV antibodies, 56 (36%) had HCV RNA (regardless of completing the treatment with direct-acting antiviral medications). None of the participants had HIV antibodies/antigens, and no one was recorded with severe renal failure or was treated with co-medications that could significantly

Table 1. Demographic and clinical characteristics of the study participants undergoing methadone maintenance treatment for opioid use disorder.

Characteristics	All participants (N = 155)
Gender, female, N (%)	53 (33)
Age, years, Mean (SD)	45 (10)
<50, N (%)	102 (66)
≥50, N (%)	53 (34)
OAT duration, years, Mean (SD)	9 (5)
Observed intake of OAT medications, days per week, Mean (SD)	4 (2)
Time from last dose to blood sampling, hours, Mean (SD)	21 (8)
BMI, kg/m^2, Mean (SD)	27 (6)
<25, N (%)	70 (45)
25-30, N (%)	39 (25)
>30, N (%)	46 (30)
Methadone dose, mg/day, Mean (SD)	99* (25)
Serum concentration, nmol/L, Mean (SD)	1248* (559)
Concentration-to-dose ratio, (nmol/L)/(mg/day), Mean (SD)	13* (6)
Liver fibrosis measure, kPa, Mean (SD)	7* (6)
ALT, U/L, Mean (SD)	40 (50)
AST, U/L, Mean (SD)	45 (35)
ALKPO4, U/L, Mean (SD)	96 (20)
Bilirubin, µmol/L, Mean (SD)	7 (4)
Renal function measure (eGFR), ml/min/1.73m^2, Mean (SD)	79 (21)
Anti-HCV positive, N (%)	145 (94)
HCV RNA positive, N (%)	56 (36)
HIV positive, N (%)	0 (0)
Use of co-medication strongly interacting with methadone[1], N (%)	0 (0)
CYP2B6 genotypes	155 (100)
*1/*1	96 (62)
*1/*6	54 (35)
*6/*6	5 (3)
CYP3A5 genotypes	155 (100)
*3/*3	119 (77)
*1/*1 & *1/*3	36 (23)
Use of substances, weekly to daily during the last month, N (%)	140 (90)
Alcohol, N (%)	81 (52)
Heroin, N (%)	17 (12)
Other opioids, N (%)	9 (6)
Cannabis, N (%)	107 (69)
Benzodiazepines, N (%)	99 (64)
Amphetamines, N (%)	45 (29)

ALT: alanine aminotransferase (lab. reference: 10–45 for women, 10–70 for men, IU/L); ALKPO4: alkaline phosphatase (lab. reference: 35–105, IU/L); AST: aspartate aminotransferase (lab. reference: 15–35 for women, 15–45 for men, IU/L); BIL: bilirubin (lab. reference: <19, µmol/L); BMI: Body mass index; eGFR: estimated glomerular filtration rate, *ml/min/1.73m*^{2}; HCV: Hepatitis-C Virus; HIV: Human immune deficiency virus; SD: Standard deviation.

*For 192 observations including two sets of measures in 37 participants.

[1]Some medications were recorded, however, the agents were not in the categories of being moderate or strong inhibitors or inducers of CYP enzyme involving in methadone metabolism.[26,37].

interact with methadone. The mean BMI was 27 (6) kg/m^2. Almost 90% of the patients frequently (weekly to daily during the last month) used at least one illicit substance, of which more than half had also used alcohol.

Regarding the *CYP2B6* genotypes, 96 out of 155 participants (62%) constituted the wild-type genotype (*1/*1), and 54 (35%) and 5 (3%) participants were heterozygote (*1/*6) or homozygous (*6/*6) carriers of the reduced function genotype, respectively. For the *CYP3A5* genotypes, 119 (77%) participants constituted the homozygote form of the reduced function genotype (*3/*3), and the remaining 36 (23%) were heterozygote carriers (*1/*3), except for one participant who had the unusual wild-type genotype (*1/*1) but was categorized into *1/*3 group.

As shown in Table 2, nine participants (7%) had liver fibrosis measures ≥12 kPa, indicating a possible cirrhosis state that probably had developed to portal hypertension in three of those with measures ≥20 kPa. Although some differences in the serum methadone concentrations were observed, CDR did not change considerably among those with higher degrees of fibrosis measures or between the different categories of liver fibrosis (Figure 1A and 1B).

The results of the LMM analyses are presented in Table 3. In the unadjusted analyses, no significant association was observed between methadone CDR and liver fibrosis measures.

Table 2. Methadone dose (mg), serum concentrations (nmol/L) and serum concentration-to-dose ratio [(nmol/L)/(mg/day)] in the study participants[1] on methadone maintenance treatment with different stages of liver fibrosis (kPa).

	N	Dose (mg) Mean (SD)	Serum concentration (nmol/L) Mean (SD)	Concentration-to-dose ratio (nmol/L)/mg) Mean (SD)
Liver fibrosis measure				
No/limited fibrosis, ≤7 kPa	107	98 (23)	1290 (609)	14 (6)
Fibrosis, 7 < kPa < 12	14	91 (29)	1189 (463)	14 (8)
Cirrhosis, ≥12 kPa	9	100 (17)	1239 (485)	12 (5)
• Portal hypertension, ≥20 kPa	3	110 (20)	1379 (391)	12 (3)

SD: Standard deviation.
[1]Patients with body mass index > 30 kg/m² are excluded.

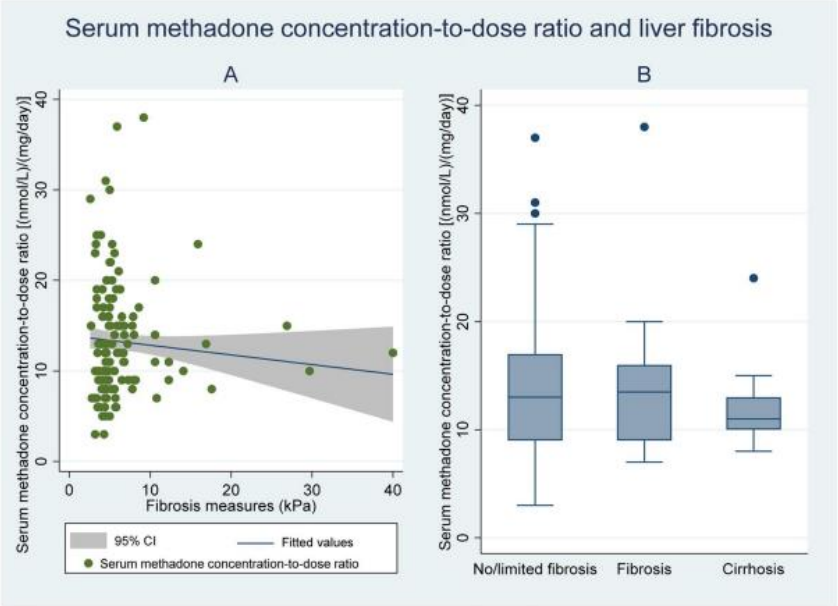

Figure 1. Serum methadone concentration-to-dose ratio [(nmol/L)/(mg/day)], and liver fibrosis measures (kPa) and stages in 155 study participants* on methadone maintenance treatment. Liver stiffness measures: Limited fibrosis: ≤7 kPa; Fibrosis: 7 < kPa < 12; Cirrhosis: ≥12 kPa. *For 192 observations including two sets of measures in 37 participants. Fibrosis measures illustrate 130 observations from 107 participants due to excluding of the individuals with BMI > 30 kg/m² (n = 46) and missing data (n = 2).

When the different stages of liver fibrosis and different genotypes of *CYP2B6* and *CYP3A5,* as well as age groups, gender, and BMI categories, were combined in the adjusted LMM analysis, there was still no significant relationship between CDR and liver fibrosis (coefficient: 0.70; 95% CI: −2.16, 3.57; *P*: 0.631) or cirrhosis −0.50; −4.59, 3.59; 0.810) compared to no/limited fibrosis. Participants with a BMI of 25–30 kg/m² showed higher CDR (2.34; 0.22, 4.45; 0.031) compared with those with BMI <25 kg/m². The associations between CDR and the *CYP2B6**6/*6 genotype compared to *1/*1, or between the heterozygote and homozygote genotypes of *CYP2B6* did not reach the statistical significance level.

4. Discussion

The present study showed that the dose-adjusted serum concentration of methadone did not increase among participants with higher degrees of liver fibrosis, even among those with possible advanced cirrhosis. Although the present study did not find an association between liver fibrosis and methadone concentrations, it does not appear that available research can definitively conclude on this topic.[12–15] Reduced metabolism

Table 3. Associations between methadone serum concentration-to-dose ratio [(nmol/L)/(mg/day)] and liver fibrosis measures (kPa) adjusted for age, gender, BMI (kg/m^2) and *CYP* genotypes, in linear mixed model for 155 participants* on methadone maintenance treatment.

	Unadjusted		Adjusted[†]	
Variables	*P*-value	Coefficient (95% CI)	*P*-value	Coefficient (95% CI)
Intercept			0.000	10.16 (7.87-12.44)
Age (per 10 years)	0.263	−1.11 (-3.10, 0.83)	0.866	0.20 (-2.10, 2.49)
Male gender (compared to female)	0.343	0.97 (-1.04, 2.98)	0.064	2.30 (-0.13, 4.74)
BMI				
<25 kg/m^2		0 (reference)		0 (reference)
25-30 kg/m^2	0.034	2.26 (0.17, 4.34)	0.031	2.34 (0.22, 4.45)
Liver fibrosis measure				
≤7kPa		0 (reference)		0 (reference)
7< kPa < 12	0.730	0.53 (-2.42, 3.47)	0.631	0.70 (-2.16, 3.57)
≥12 kPa	0.680	−0.90 (-5.13, 3.34)	0.810	−0.50 (-4.59, 3.59)

BMI: Body mass index; CI: Confidence interval.
*For 192 observations including two sets of measures in 37 participants. The adjusted model used 130 observations from 107 participants due to excluding of the individuals with BMI > 30 kg/m^2 (n = 46) and missing data (n = 2). There was not found any effect of time (related to the two measurements) on the statistical analyses.
[†]Adjusted for age, gender, BMI and *CYP2B6* and *CYP3A5* genotypes.

of methadone in HCV-infected patients with opioid use disorder was demonstrated in a study,[13] but no association between methadone serum levels and liver fibrosis was found. Another study[14] reported a higher concentration of total methadone and the active *R*-enantiomer in HCV-seropositive patients compared to seronegative patients. Both studies suggest consideration of dose adjustments in methadone-maintained patients with a history of HCV infection. However, the clearance of drugs in general is not considerably altered in patients with chronic active hepatitis without cirrhosis.[5,38] In a study on patients undergoing methadone maintenance treatment, the researchers could not demonstrate changes in the total body amount of methadone in individuals with mild to moderate chronic liver disease.[15] They proposed that dose adjustment was not needed. However, a higher methadone dose requirement has been suggested due to CYP3A4 induction in patients with HCV infection.[39] In line with our results, a recent study[12] could not show a significant effect of liver stiffness in patients with ongoing HCV infection on methadone metabolism rates. Our findings may thus indicate that an increased liver fibrosis probably caused by ongoing HCV infection does not immediately warrant methadone dose adjustment without further clinical evaluation.

In very severe liver diseases, however, a decreased metabolic capacity is expected, and together with an impaired production of drug-binding proteins, it can result in an increased fraction of free drug.[40,41] Nevertheless, the measured protein-bound drug concentration may seem normal, leading to the conclusion that drug metabolism is unaffected. Indeed, the drug clearance is reduced due to increased tissue distribution of the unbound fraction, especially in the presence of edema and ascites.[40,41] Further, drugs with intermediate or high hepatic extraction rates—such as methadone—may have increased oral bioavailability due to portal hypertension and development of cirrhotic porto-systemic shunts, leading to a reduced first-pass metabolism.[42] Increased bioavailability combined with decreased hepatic clearance can cause a considerable accumulation of the drug in the body per time unit.[6] Further, a strong relationship between the activity of hepatic CYP enzymes and the severity of cirrhosis has been demonstrated, in which the content and activity of some CYP isoenzymes, such as 3 A, appear to be particularly vulnerable to the effect of liver disease.[43] Although we ruled out any interacting factor between liver stiffness and the *CYP* genotypes regarding methadone CDR in the present study, the pattern of CYP enzymes alterations also differs according to the etiology of liver disease.[43]

Methadone has a high bioavailability of approximately 70–80%, with a large variability because of alterations in hepatic first-pass metabolism. It is also largely bound to plasma proteins (60–90%).[44] Due to these facts and the considerable inter- and intra-individual variability in the

pharmacokinetics of methadone, as well as its long half-life, close clinical monitoring has been recommended in patients with severe hepatic impairment, although no dose adjustment is suggested in mild and moderate liver diseases.[44] In the present study, we considered fibrosis measures ≥20 kPa to be the indicator of significant portal hypertension, as we did not directly measure hepatic venous pressure. Three participants were found in this category apparently without an impaired metabolic rate of methadone, having a mean CDR of 12 (3). However, the LMM was unable to analyze the data, possibly due to too few individuals in this category. Although the present study could not indicate a significant increase in dose-adjusted serum methadone concentration among patients with severe cirrhosis, close clinical monitoring and observation of overdosing symptoms, such as increased sedation, could support a possible accumulation of methadone in the central nervous system. Continuous clinical evaluations should therefore be recommended as the most important tool in the management of severe hepatic impairment among patients undergoing methadone maintenance treatment. In parallel, measurements of serum concentrations may, in some cases, reveal intra-individual variations during the treatment course.

In the present study, we adjusted the regression model for *CYP2B6* and *CYP3A5* genotypes, age, gender, and BMI as confounding factors that could possibly affect methadone CDR, either directly or indirectly, by influencing the fibrosis degree of the liver. Although serum methadone concentrations were significantly higher among patients with the homozygote and heterozygote genotypes of the *CYP2B6*6* variant allele compared with the wild-type, the differences in CDRs did not reach statistical significance in the multivariate regression model. As we demonstrated such a significant effect of the *CYP2B6*6* variant allele (PM phenotype) on methadone CDR in a previous study,[16] we supposed that including only five participants (3%) with this genotype in the present study did not provide enough statistical power to obtain a similar result. Thus, the genotype differences could not be associated with treatment response or methadone dose requirements. An explanation can be the selectivity toward the non-active enantiomer of *S*-methadone[19,22]. Moreover, limited and conflicting results regarding the possible involvement of CYP3A5 in methadone metabolism[16,18,22,25] are unlikely to support clinical relevance. At the least, our findings do not support such an association.

Among other clinical factors, we found a direct association between overweight (BMI 25–30 Kg/m^2) and CDR in the adjusted LMM analysis. The impact of overweight on methadone metabolism has not been sufficiently investigated in previous research. Nevertheless, a recent study[12] demonstrated that individuals with overweight had higher methadone serum levels, which is in line with our findings. Possible explanations for this observation could be the changes in body compartment proportions (i.e., the amount of fat tissue that influences volume of distribution) and impaired hepatic function due to steatosis.[40,41] Conversely, methadone maintenance treatment has been related to weight gain.[45] If this condition is considered a dose-dependent side effect of methadone, higher serum concentrations can be expected, at least in some patients. It is challenging to verify the direction of a potential causal relationship. The clinical implication of this finding may be that patients who are overweight do not necessarily need higher methadone doses than those who are not overweight, even though some patients may need dose reduction to avoid weight gain as an adverse effect. Further, other influencing factors may warrant individualized dose requirements. Unlike a previous study by some of the current authors,[26] we could not demonstrate a significant effect of gender on methadone CDR, probably due to the smaller sample size of the present study. However, a similar result considering age was found—that is, no impact of age on CDR. A possible impact of considerably reduced renal function could not be investigated in this study, as none of the participants had severe renal failure. Pharmacologically, methadone disposition seems to be relatively unaffected in renal impairment.[28] Further, no concomitant medication with strong interacting effects on methadone was recorded in our data; however, we demonstrated the impact of such

co-medications on methadone CDR in a previous study.[26]

A strength of this study is its naturalistic design and treatment platform, which allowed us to manage data collection more closely and reduce information bias. However, the study has some limitations that must be acknowledged. A small sample size increases the risk of statistical Type II errors. This could have influenced the results regarding patients with severe cirrhosis. Similarly, the small sample size does not allow for drawing certain conclusions about the possible influences of the important clinical and genetic confounding factors. The sample did not have sufficient data on severe renal failure or concomitant medication with a potential interacting effect on methadone metabolism. Moreover, to explore the possible influences of genetic factors, a larger population scale is usually needed. Another limitation is the naturalistic nature of the study, which allowed clinicians to adjust the methadone dose based on their clinical judgment. This may have led to inappropriate dose reductions in people with liver impairment. Further, other factors that are beyond the scope of this research, such as poor compliance with prescribed methadone or other patient-related factors, may have influenced our results. Further clinical research using larger patient samples and including other possible confounding factors is needed to improve the knowledge in this field. Wider access to and use of laboratory facilities that enable the measurement of serum concentrations of various drugs and genetic analyses will also contribute to future research opportunities.

5. Conclusions

This study showed that the dose-adjusted serum concentration of methadone did not correlate with the degree of liver fibrosis. Nevertheless, in patients with liver fibrosis, particularly in the presence of advanced cirrhosis, dose adjustments should mainly be based on close clinical monitoring and individual considerations. Still, measurements of serum methadone levels during treatment can be considered a supplement to clinical assessments, taking into account intra-individual variations.

Disclosure statement

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Paper IV

Chalabianloo F, Fadnes LT, Høiseth G, Ohldieck C, Vold JH, Aas C, Løberg E-M, Johansson KA, Bramness JG. Subjective symptoms and serum methadone concentrations: what should guide dose adjustments in methadone maintenance treatment? A naturalistic cohort study from Norway. Substance Abuse Treatment, Prevention, and Policy 2021;16(1):39.

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Substance Abuse Treatment, Prevention, and Policy

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Subjective symptoms and serum methadone concentrations: what should guide dose adjustments in methadone maintenance treatment? A naturalistic cohort study from Norway

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Abstract

Background: There is little evidence-based guidance on how to optimize methadone dosages among patients with opioid addiction undergoing methadone maintenance treatment (MMT). This study aims to investigate whether self-perceived opioid withdrawal symptoms, adverse effects, and self-reported substance use in patients on MMT are related to serum methadone concentrations and the role that these variables could play in clinical decisions on dose adjustments.

Methods: This naturalistic prospective cohort study included clinical and laboratory measurements from 83 patients undergoing MMT in outpatient clinics in Bergen, Norway, from May 2017 to January 2020. Information on age, gender, methadone daily doses and serum concentrations, subjective opioid withdrawal symptoms using 16 items Subjective Opioid Withdrawal Scale (SOWS) questionnaire, self-reported adverse effects, and substance use was obtained. Linear mixed modelling was used for analyzing the data.

Results: The mean age of the participants was 45 years, and 33% were women. Almost half reported mild to moderate subjective opioid withdrawal symptoms, and all had experienced at least one subjective adverse effect. The use of at least one substance was reported by 88% of the participants. Serum concentration-to-dose ratios were lower among those who had reported subjective opioid withdrawal symptoms (p) = 0.039). The total SOWS score ($p < 0.001$); the specific subjective withdrawal symptoms of anxiety ($p = 0.004$), bone and muscle aches ($p = 0.003$), restlessness ($p = 0.017$), and (slightly) shaking ($p = 0.046$), also use of heroin ($p = 0.015$) and alcohol ($p = 0.011$) were associated with lower methadone concentrations. Cannabis use was slightly related to higher methadone concentrations ($p = 0.049$).

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Conclusions: The findings suggest that the patient's self-perceived symptoms and current clinical condition are related to the serum concentrations of methadone. This interpretation supports dose adjustments based on patient-reported symptoms. In some aberrant cases, measurement of serum concentrations together with other individual assessments may be considered to support the clinical decision.

Keywords: Methadone maintenance treatment, Serum concentrations, Subjective opioid withdrawal symptoms, Adverse effects, Substance use, Opioid agonist treatment

Background

Methadone maintenance treatment (MMT) is an evidence-based medical intervention that reduces illicit opioid use and risk of overdose [1, 2] and mortality [3, 4] among opioid-dependent individuals. Understanding factors that may influence treatment satisfaction and continuity – and accordingly preventing a relapse to illicit opioid use and the subsequent risk of overdose and death – is crucial. Such factors may be opioid withdrawal symptoms and adverse effects related to inappropriate methadone dosages. Thus, balancing an efficacious dose to achieve the desired therapeutic effect against a dose that is either too low, leading to withdrawal symptoms and relapse to illicit opioid use or too high, causing adverse effects and toxicity, is important in clinical practice.

Individualized dose optimization using daily doses of between 60 and 120 mg for most patients appears to be related to increased retention in treatment and reduced illicit opioid use as the most common measures of MMT efficacy [5]. However interindividual variations in methadone dose requirements should be kept in mind [6–8]. Furthermore, some researchers have shown that factors other than the dose – such as the patient's expectations and medication preferences, as well as the patient's total physical and mental health condition or improvements in psychosocial functioning – may influence treatment satisfaction [9, 10]. These findings add to the complexity of the issue challenging clinicians regarding how to cope with suboptimal treatment outcomes: should the dose be adjusted, or should other problems instead be addressed?

One can predict the optimal methadone maintenance dose using various factors based on continuous clinical evaluations [11–13]. Although previous findings suggest an association between the dose and clinical symptoms, the relationships between serum methadone concentrations and treatment effects are still not fully understood [14]. Due to the large interindividual variation in methadone pharmacokinetics, individual serum concentrations after a given dose will vary substantially [12, 14–16]. The optimal dose at steady state thus is hard to predict [12]. Accordingly, a clinically oriented approach rather than an approach based on serum levels has been suggested for optimizing the methadone daily dosage for individual patients [11, 12, 17].

Limited studies [18, 19], however, have suggested a direct association between the serum methadone concentration and retention in treatment. A few clinical studies [20, 21] have shown that when the serum concentration is too low to inhibit objective withdrawal symptoms, patients relapse to substance use and drop out of MMT. Other studies [11, 22, 23] have indicated that higher concentrations are more likely to reduce opioid craving. Finally, a rapid decline in the trough concentration is related to clinically important responses, notably objective withdrawal symptoms [24]. However, there is limited knowledge on the specific subjective symptoms reflecting the serum concentrations of methadone, which clinicians must recognize to guide proper dose adjustments in clinical practice.

The aim of this study is to investigate whether self-perceived opioid withdrawal symptoms, adverse effects, and substance use in patients on MMT are related to serum methadone concentrations and the role that these variables should play in clinical decisions on dose adjustments.

Methods

Design, setting, and data sources

This naturalistic prospective cohort study was conducted at the Department of Addiction Medicine at Haukeland University Hospital in Bergen, Norway, from May 2017 to January 2020. The department is responsible for the treatment and follow-up of more than 1000 patients with opioid dependence receiving opioid agonist treatment (OAT). Almost 40% of these patients receive MMT, while the rest mainly receive buprenorphine-based treatment. All pharmacological interventions are integrated with psychosocial treatment and are provided in multidisciplinary outpatient clinics. The patients are followed-up via directly observed treatment (DOT) and consultations, and take-home doses frequencies are based on the individual treatment courses. All clinical measurements and laboratory data are recorded prospectively in the hospital journal system, as well as in a recently established health registry database for patients undergoing OAT in Bergen. In addition to incorporating individual health data, the

database includes relevant information based on clinical surveys and research records.

In the present study, we included information on age, gender, daily methadone doses, serum methadone concentrations (as the independent variable), subjective opioid withdrawal symptoms (as the primary outcome), some common subjective opioid adverse effects and self-reported illicit drug use (as the secondary outcomes). We also included information about the time since last dose intake, time of blood sampling, time when symptoms were recorded, numbers of days with DOT per week, and duration of OAT.

Participants

One hundred and ninety-nine patients consented to participate in the study and started the primary surveys through in-person clinical interviews by a research nurse. At the end of the study, 83 patients had completed the surveys according to the study protocol, with the time difference between the clinical assessments and laboratory measurements being < 14 days (Mean = 2, SD = 3), and were included in the study.

Assessments of subjective symptoms

As part of the clinical assessments and based on the study protocol, the participants were initially asked whether they were experiencing opioid withdrawal symptoms. Those who confirmed the presence of withdrawal symptoms were interviewed by a research nurse using the validated standard questionnaire, the Subjective Opioid Withdrawal Scale (SOWS) [25], which covers 16 self-perceived symptoms: anxiety, yawning, perspiring, tearing, runny nose, goosebumps, shaking, hot flushes, cold flushes, bone and muscle aches, restlessness, nausea, vomiting, muscle twitches, stomach cramps, and feeling like using. Respondents rated the intensity of symptoms on a five-point scale from 0 (not at all) to 4 (extreme); possible scores range from 0 to 64 (1–10 = mild withdrawal, 11–20 = moderate withdrawal, 21–30 = severe withdrawal).

In addition, all participants were asked six questions on some of the most common subjective adverse effects attributed to MMT, including euphoria, perspiring, nausea, concentration difficulties, feeling "brain fog," and reduced sexual desire; these symptoms were rated in the same way as for the withdrawal symptoms. The selection of the adverse effects was based on the authors clinical experiences, previously published peer-collected data on this population [10], and the most common reported side effects for methadone [26, 27] and opioids in general [28]. Perspiring and nausea were defined as adverse effects or withdrawal symptoms based on how each participant perceived them. In addition, one open-ended question asked about other possible symptoms when these were self-perceived to be related to MMT.

Substance use

Self-reported use of illicit drugs – including heroin or other opioids, amphetamines (amphetamine and/or methamphetamine), benzodiazepines, and cannabis – as well as alcohol, and frequencies of use (categorized as no use at all, or frequent use including either several times a month, weekly, or daily use) during the last month were recorded for the participants.

Measurements of serum methadone concentrations

Blood samples were drawn from the participants at the OAT clinics according to the study protocol on average 21 (SD = 8) hours after the last dose intake, and no changes in dosages were made during the week prior to sampling. Analysis of methadone was performed by the same analytical method using the same laboratory instruments at the Department of Medical Biochemistry and Clinical Pharmacology, Haukeland University Hospital, Bergen. During the development phase of the method and in routine use, methadone concentrations were measured in nmol/L. The conversion factor from nmol/L to ng/mL for methadone is 0.310.

Statistical analyses

Basic descriptive statistical analysis of the data was performed using Stata/SE 16.0 (StataCorp, TX, USA). Continuous variables were presented as means with standard deviations (SD), as well as ranges when needed. Comparisons of study variables between the participants with and without reported subjective opioid withdrawal symptoms were performed using Mann–Whitney tests for continuous variables and chi-square tests for categorical variables. The exact p-values were reported, and values < 0.05 were considered statistically significant. To avoid type-II statistical errors by overlooking important variables due to the study's naturalistic design, we also included variables with a p-value < 0.10 in the adjusted regression analyses.

Linear mixed model (LMM) analysis was applied to investigate possible associations according to the aim of the study. We included in the main analyses all 16 SOWS items, the 6 subjective adverse effects, and self-reported substance use during the month prior to interviews as dependent variables. The responses to the open-ended question were excluded from the statistical analyses due to scant applicable data. All these variables were included one by one in the unadjusted statistical analyses. Then, adjusted LMM analyses for the specific variables showing statistically significant associations with the serum methadone concentration were undertaken. The results obtained in the main analyses were adjusted for age, gender, and the absolute time difference between blood sampling and the recording of the symptoms.

Results

Demographic and clinical data

Table 1 shows the demographic and clinical data of the 83 study participants and comparisons between those with and without reported withdrawal symptoms as the main outcome of the study. For all participants, the mean age was 45 (SD = 9) years; 33% were women, and 54% reported mild to moderate subjective opioid withdrawal symptoms with a mean total SOWS score of 9 (SD = 12) at the time of the interviews. The mean methadone daily dose and serum concentration were 97 (SD = 24) mg and 374 (SD = 188) ng/mL, respectively. Those who had reported subjective opioid withdrawal symptoms had lower serum concentration-to-dose ratios ($p = 0.039$), and more frequently received DOT ($p = 0.026$) compared to those in the other group. All had experienced one or more subjective adverse effects, and 73 (88%) reported frequent use of at least one substance during the month prior to the surveys. There were no differences between the groups with regard to age, gender, or self-reported use of illicit substances and alcohol.

Relationships between subjective opioid withdrawal symptoms and serum methadone concentrations

Figure 1 clarifies the relationship between the recorded total SOWS scores and the measured serum methadone concentrations, illustrating a weak correlation with wider confidence intervals at lower and higher concentrations.

In the unadjusted LMM analysis (Table 2), we found statistically significant inverse associations, although weak to moderate correlations, between serum methadone concentrations and total SOWS scores ($p = 0.011$), and for the specific symptoms of anxiety ($p = 0.009$), bone and muscle aches ($p = 0.007$), and restlessness ($p = 0.021$) out of the 16 subjective opioid withdrawal symptoms based on the SOWS questionnaire, as well as for use of heroin ($p = 0.028$) and alcohol ($p = 0.008$). Except for a significant direct association with nausea reported as an adverse effect of methadone ($p = 0.040$), no associations were found between the other subjective adverse medication effects and methadone serum concentrations.

Adjustment of the results for confounding factors

When adjusting in the LMM analyses for age, gender, and the absolute time difference between blood sampling and the recording of the symptoms (Table 3), we found that the associations between serum methadone concentrations and total SOWS scores; the specific withdrawal symptoms of anxiety, bone and muscle aches, and restlessness; and use of heroin and alcohol still remained highly significant. There was a tendency toward higher serum concentrations among those who reported nausea as an adverse effect ($p = 0.057$). Obtaining p-values of < 0.10 by analyzing the withdrawal symptom of shaking as well as use of cannabis in the unadjusted LMM, we also added these variables to the adjusted model and found

Table 1 Demographic and clinical data in the study participants and comparisons between the groups with and without reported subjective opioid withdrawal symptoms

	All participants	Withdrawals	No withdrawals	
	N = 83	*N* = 45	*N* = 38	*p*-value[a]
Gender, female/male[b]	27/56 (33/67)	15/30 (56/54)	12/26 (44/46)	0.865
Age, years[c]	45 (9, 26–66)	45 (9, 26–62)	44 (10, 26–66)	0.493
Methadone dose, mg/day[c]	97 (24, 20–170)	101 (24, 35–170)	92 (23, 20–150)	0.079
Methadone serum concentration, ng/mL[c]	374 (188, 74–1005)	347 (168, 113–1005)	405 (208, 74–998)	0.145
CDR,[d] (ng/mL)/(mg/day)[c]	4 (2, 1–11)	3 (2, 1–11)	4 (2, 1–9)	0.039
Time since last dose, hours[c]	21 (8, 0–28)	22 (5, 1–28)	19 (10, 0–27)	0.199
Duration of opioid agonist treatment[c]	9 (5, 1–20)	9 (5, 1–18)	9 (5, 1–20)	0.922
Direct observed treatment, day/week[c]	4 (2, 1–7)	4 (2, 1–7)	3 (2, 1–6)	0.026
Self-reported substance use last month[b]	73 (88)	41 (92)	32 (83)	0.187
Heroin[b]	10 (12)	5 (12)	5 (13)	0.971
Other opioids[b]	5 (6)	3 (6)	2 (5)	0.819
Benzodiazepines[b]	50 (62)	29 (64)	21 (55)	0.233
Cannabis[b]	55 (66)	30 (65)	25 (67)	0.828
Amphetamine[b]	26 (31)	15 (33)	11 (30)	0.789
Alcohol[b]	38 (46)	22 (49)	16 (42)	0.542

[a]Significance was tested using Mann-Whitney U-test for continuous and chi-square test for categorical variables
[b]The categorical variables are presented by n (%)
[c]The continuous variables are presented as means with standard deviations (SD) and ranges
[d]Concentration-to-dose ratio

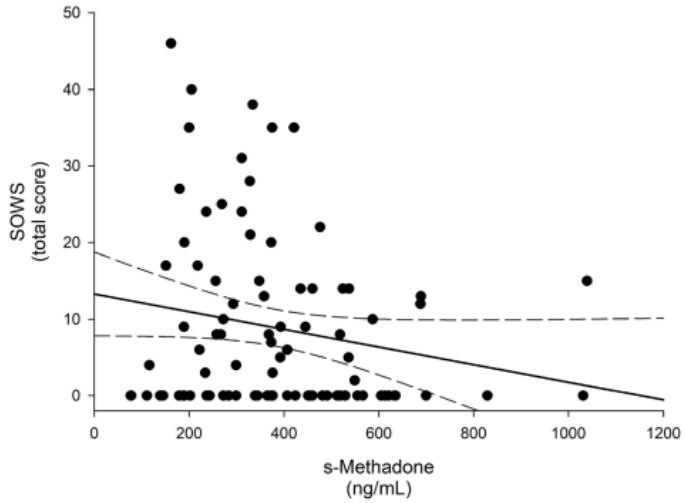

Fig. 1 Scatter plot with regression line by recorded total SOWS[a] scores and measured serum methadone concentrations in 83 participants. The solid and dashed lines represent the regression line and 95% confidence intervals. [a] Subjective opioid withdrawal symptoms

slight associations with serum methadone concentrations ($p = 0.046$ and $p = 0.049$, respectively).

Discussion

In clinical practice, there are different perceptions regarding what methadone dose adjustments should be based on: subjective symptoms or serum concentration measurements, and evidence on this topic is scarce. In this naturalistic cohort study, we have shown associations between serum methadone concentrations and subjective opioid withdrawal symptoms. Previous research has reported an inverse relationship between serum methadone concentrations and general objective withdrawal symptoms [20, 21, 24], but to our knowledge, no studies have investigated such relationships with subjective withdrawal symptoms. Although the correlations with the serum methadone concentration were not very strong for these symptoms, the findings are in line with existing theoretical expectations and support dose adjustments based on patient-reported symptoms in clinical practice.

A lower serum concentration-to-dose ratio among more than half the study participants who experienced withdrawal symptoms is a remarkable finding, meaning that serum concentrations were lower in this group despite these participants' having methadone doses comparable to those of participants who did not report such symptoms. This outcome may be partially explained by the fact that increasing the dose was not met by a corresponding increase in serum methadone concentration. Considering possible aberrant methadone metabolisms or the influence of other disturbing factors, individualized dose optimization based on appropriate risk–benefit assessments might be emphasized as an approach capable of achieving treatment effects [5–8, 12, 29]. Among those not experiencing the optimal effect despite increasing the dose, dividing the daily dose or converting to another opioid such as long-action morphine can be considered [11, 24, 30]. When none of these measures can help, other causes such as pharmacodynamic factors and genetic variations affecting opioid receptors might be excluded [6, 31]. Finally, diversion of prescribed methadone take-home doses may be considered as an explanation for lower serum concentrations in the present study despite patients' receiving appropriate doses and frequently being observed while taking their medications. Measurements of serum concentrations can be considered in such aberrant cases as a support to clinical decisions.

The higher use of heroin among those with lower serum methadone concentrations is also in line with earlier studies [20, 21, 23], indicating that even heroin use may prompt physicians to consider dose escalation. Using higher methadone doses is more effective in reducing heroin use and improving treatment retention [32–34]. However, some patients continue using heroin for its euphoric effects or other reasons, regardless of their serum methadone concentration. In addition, it is not clear whether the lower serum concentration causes the heroin use or whether some patients intentionally do not use the full dose prescribed to allow the heroin to be felt. It is challenging to answer these questions considering the naturalistic design of the study. Our finding of higher alcohol use among those with lower serum methadone concentrations could be explained by self-experienced replacement to alleviate opioid withdrawal

Table 2 The unadjusted associations between serum methadone concentrations and the study variables in 83 participants. All the primary and secondary outcome variables were inserted one by one in linear mixed model

	β-coefficient	95% CI[a]	*p*-value
Total SOWS[b] score	− 3.9	− 7, − 9.1	0.011
Anxiety	− 4.3	− 7.6, − 1.1	0.009
Yawning	− 0.5	− 3.5, 2.5	0.739
Perspiring	− 0.4	− 4.1, 3.3	0.821
Tearing	− 1.5	− 4.1, 1.2	0.283
Nose run	− 1.7	− 4.9, 1.5	0.305
Goose bumps	− 2.1	− 5.5, 1.4	0.244
Shaking	− 2.7	− 5.7, 0.3	0.081
Hot flushes	− 2.6	− 6.2, 8.9	0.143
Cold flushes	− 3.2	− 7.1, 0.8	0.115
Bone- and muscle ache	− 4.9	− 8.4, − 1.4	0.007
Restlessness	− 5.2	− 9.6, − 0.8	0.021
Nausea	− 1.5	− 4.2, 1.2	0.272
Vomiting[d]	–	–	–
Muscle twitches	− 0.9	− 4.2, 2.4	0.590
Stomach cramps	− 1.5	− 4.2, 1.1	0.263
Feel like using[d]	–	–	–
Subjective adverse effects[d]	–	–	–
Euphoria[d]	–	–	–
Perspiring (as adverse effect)	1.4	− 3.0, 5.9	0.526
Nausea (as adverse effect)	3.1	0.1, 6.1	0.040
Concentration difficulties	− 0.7	− 4.7, 3.3	0.731
Feeling "brain fog"	1.5	− 2.2, 5.2	0.427
Decreased sexual desire	2.2	− 2.4, 6.8	0.351
Self-reported substance use [c]	0.2	− 0.9, 1.3	0.754
Heroin	− 2	− 3.8, − 0.2	0.028
Other opioids[d]	–	–	–
Benzodiazepines	0.6	− 3.7, 4.9	0.772
Cannabis	4.7	− 0.5, 9.9	0.077
Amphetamines	− 0	− 3.0, 2.9	0.984
Alcohol	− 4.5	− 7.8, − 1.2	0.008

[a] Confidence interval
[b] Subjective opioid withdrawal symptoms
[c] Frequent use (from daily to several times a month) of illicit drugs and alcohol during the month prior to surveys
[d] The linear mixed model system was not able to analyze these variables due to scant data

Table 3 Associations between serum methadone concentrations and the study variables in 83 participants by using adjusted[a] linear mixed model

	β-coefficient	95% CI [b]	*p*-value
Total SOWS[c] Score	− 4.3	− 5.6, − 2.9	< 0.001
Anxiety	− 0.5	− 0.8, − 0.2	0.004
Bone- and muscle ache	− 0.5	− 0.9, − 0.2	0.003
Restlessness	− 0.5	− 9.7, − 0.9	0.017
Shaking	− 0.3	− 0.6, − 0	0.046
Nausea (as adverse effect)	0.3	− 0.1, 0.6	0.057
Heroin use	− 0.2	− 0.4, − 0	0.015
Alcohol use	− 0.4	− 0.7, − 0.1	0.011
Cannabis use	0.5	0, 10.4	0.049

[a] Adjusted for age, gender and absolute time difference between blood sampling and record of the symptoms
[b] Confidence interval
[c] Subjective opioid withdrawal symptoms

symptoms. Research has also demonstrated an overlapping effect of alcohol on mu-opioid receptors in the central nervous system [35]. In addition, regular low-dose alcohol intake (< 4 alcoholic drink/day) may induce P450 enzymes and thus decrease serum methadone concentrations [26]. Considering the increased risk of adverse effects and overdose with the concurrent use of opioids and alcohol, a balanced dosage strategy is important to increase treatment retention and avoid not only relapse but also toxicity.

Although all participants reported at least one subjective adverse effect related to methadone treatment, none of these symptoms were significantly related to serum concentrations, except for a slight association with nausea. Studies on such associations are lacking. Nevertheless, it is important to keep in mind other possible physical and psychosocial conditions surrounding the patient, which may influence the total subjective experience and satisfaction with the treatment [10]. The inability to identify some associations in the present study may also be due to the group-level investigation. Following individuals over time with different doses may have revealed some associations. Additionally, we used self-reported responses to a locally developed questionnaire on common subjective adverse effects, not a validated instrument, which could have more accurately captured possible adverse effects.

The participants with higher serum methadone concentrations seemed to use more cannabis. Although the slight association may be due to a type-I error, the finding is of theoretical and clinical interest. A possible mechanism may be a central-acting effect to counterbalance undesirable side effects related to methadone treatment, for instance, an antiemetic effect of cannabis [36]. Some researchers have also suggested an opioid-saving effect of cannabis in patients with opioid dependence [37]. However, use of cannabis was not associated with MMT retention in a review article [38] and could not predict treatment outcomes such as relapse to heroin use or psychosocial functioning. To our knowledge, clinical studies have not yet examined possible associations

between cannabis use and the methadone dose or serum concentration.

Strengths and limitations

Strengths

The prospective nature of our study and the treatment platform allowed us to continuously meet the patients or repeat records if needed. We were thus able to manage the data collection more closely and reduce information bias. This design might be considered as a strength of the study.

Limitations

We were not able to perform all the interviews and blood tests at the same time (delayed follow-up), as some patients did not attend the planned interviews or could not complete the measurements at the same time. As a result, approximately half of those who had completed the primary surveys were eligible to be included in the study. Furthermore, although the study found associations between some subjective symptoms and serum methadone concentrations, the results must be interpreted in light of the relatively small effect sizes. The observed weak to moderate correlations may reflect possible influences of other factors such as concurrent use of illicit drugs or abstinence from these substances, comorbid somatic and psychiatric conditions, or even manipulation of symptoms to receive higher methadone doses. Another limitation may be the small sample size and possibility for some uncovered associations. Further clinical studies are needed to obtain more knowledge in this field.

Conclusions

Subjective opioid withdrawal symptoms – particularly anxiety, bodily pain, restlessness, and shaking (slightly) – and self-reported use of heroin and alcohol were associated with lower serum methadone concentrations. Patients with higher serum methadone concentrations had a tendency to use more cannabis. As the concentration of methadone in serum was related to the patient's self-reported symptoms and use of substances, such symptoms may support the need for dose adjustments. Dividing the dose or converting to other opioids should be considered when dose escalations do not relieve the symptoms. Measurements of serum concentrations can be considered in some aberrant cases as a support to clinical decisions.

Abbreviations

CDR: Concentration-to-dose ratio; CI: Confidence interval; DOT: Direct observed treatment; LMM: Linear mixed model; MMT: Methadone maintenance treatment; OAT: Opioid agonist therapy; SOWS: Subjective opioid withdrawal scale; TDM: Therapeutic drug monitoring

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Authors' contributions

FC, LTF, CO, GH, EML, KAJ and JGB have been involved in design of the study, contributed to implementation and writing protocol. FC, JGB and GH conceived the study and designed it primarily. FC is the principal investigator, led statistical analyses and wrote the first draft for the paper. All authors (FC, LTF, CO, GH, JHV, CFA, EML, KAJ and JGB) participated in interpretation of the data, reviewed the manuscript for intellectual content, and approved the final version of the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript. No additional data are available due to data protection requirements.

Declarations

Ethics approval and consent to participate

The study was approved by the Regional Committee for Medical and Health Research Ethics in Vest Norway (approval No. 2017/297/REK vest). All participants had signed a written informed consent on agreement about using of routine and research data for this purpose and for taking part in the study.

Consent for publication

Participants have consented for publication.

Competing interests

The authors declare that they have no competing interests.

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