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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/m^2 are excluded.

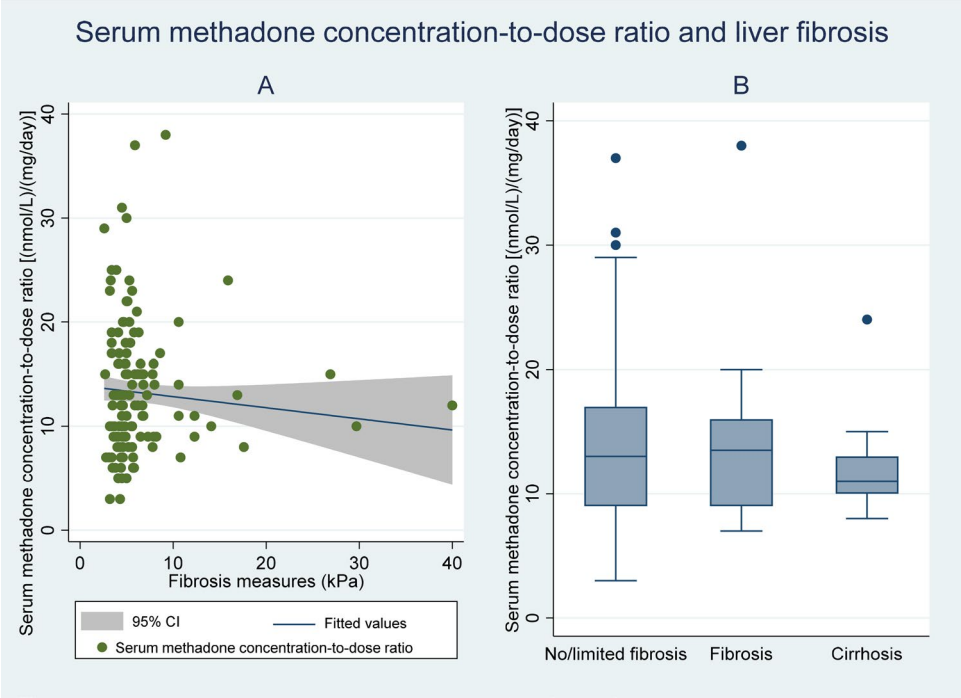

Figure 1. Serum methadone concentration-to-dose ratio [(nmol/L)/(mg/day)], and liver fibrosis measures (kPa) and stages in 155 study participants* on methadone maintenance treatment. Liver stiffness measures: Limited fibrosis: ≤7 kPa; Fibrosis: 7 < kPa < 12; Cirrhosis: ≥12 kPa. *For 192 observations 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/m^2 (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/m^2 showed higher CDR (2.34; 0.22, 4.45; 0.031) compared with those with BMI <25 kg/m^2. 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[1]	
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.
[1]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

No potential conflict of interest was reported by the authors.

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