An examination of the pharmacodynamics and pharmacokinetics of Levo-alpha-acetylmethadol (LAAM), compared to methadone, in opioid maintenance patients

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Abstract

Methadone is currently the most widely used agent to manage opioid dependence, but clinical experience has highlighted some limitations with its use. In particular, a relatively high proportion of patients complain of breakthrough withdrawal symptoms (non-holding) at apparently adequate methadone doses. Levo-alpha-acetylmethadol (LAAM) is a long acting opioid that is likely to benefit methadone non-holders; however, relatively little is known about its pharmacology at steady state. The primary aim of this thesis was to evaluate LAAM as an alternative maintenance pharmacotherapy to methadone for the treatment of non-holders; subsidiary aims were to elucidate the pharmacodynamics and pharmacokinetics of LAAM and its active metabolites (nor- and dinor-LAAM), and to examine the *in vitro* activity of LAAM, nor- and dinor-LAAM. Sixteen methadone maintenance patients (non-holders=8) were recruited to participate in a randomised, crossover trial of LAAM and methadone. At steady state there were two testing sessions (24 h for methadone and 48 h for LAAM) that featured the concurrent measurement of plasma drug concentrations and both subjective and physiological indices of opioid effect. Cognitive and psychomotor functions were also assessed once during each inter-dosing interval study. Ten age-and gender-matched controls were also tested. The peak magnitude of methadone's and LAAM's effects were similar. Compared to methadone, LAAM was associated with more stable and less severe withdrawal and mood disturbance. The general pattern of symptom complaints and cognitive function was similar for both drugs. Severity of mood disturbance and withdrawal was similar in holders on methadone and LAAM, but was greater in non-holders when they were taking methadone than LAAM. In comparison to plasma (R)-(-) methadone, plasma nor- and dinor-LAAM concentrations fluctuated little over the dosing interval. Furthermore, nor- and dinor-LAAM were both more potent in the guinea-pig ileum bioassay, and had greater affinity for mu opioid receptors in receptor binding studies, than LAAM. In conclusion, LAAM converted methadone non-holders into LAAM holders. It is proposed that it is the relatively flat plasma concentration-time profile for nor- and dinor-LAAM that confer stability of opioid effect, minimising withdrawal. Therefore, LAAM may have a role in selected patients, whose response to methadone is suboptimal.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

David A.L.Newcombe, 31 July, 2006

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Abbreviations, prefixes and symbols

AAG	α_1 acid glycoprotein
ANOVA	Analysis of variance
AUC	Area under the plasma concentration-time curve (AUC) / area under the effect versus time curve during the dosing interval
ARCI	Addiction Research Centre Inventory
B.D	Administered twice daily
BDI	Beck Depression Inventory
C_{max}	Maximum plasma concentration
$C_{min\ (first)}$	Minimum plasma concentration pre-dose
$C_{min\ (last)}$	Minimum plasma concentration post dose
CL	Total systemic plasma clearance
CL/F	Apparent plasma clearance at steady state
CL_R	Renal clearance
CI	Confidence interval(s)
C_{ss}	Steady state plasma concentration
CV	Coefficient of variation (%)
dinor-LAAM	dinoracetylmethadol
EC_{50}	Concentration eliciting 50% of effect
FDA	Food and Drug Administration
HPLC	High performance liquid chromatography
IC_{50}	Concentration inhibiting 50% of ligand binding
i.m	Intramuscular administration
i.v	Intravenous administration
Ki	Inhibition constant
LAAM	Levo-alpha-acetylmethadol
LMP	LAAM maintenance patients
MBG	Morphine Benzedrine Group scale of the ARCI
MG	Morphine Group scale of the ARCI
MMP	Methadone maintenance patients
MMT	Methadone maintenance treatment
MSC	Methadone symptom checklist
NMDA	N-methyl-D-aspartate
nor-LAAM	noracetylmethadol
P	Statistical significance
p*Ka*	Ionisation constant
p.o	Oral administration

POMS	Profile of Mood States
P/T	Peak to trough plasma concentration ratio
r	Correlation coefficient
r^2	Ceofficient of determination
s.c	Subcutaneous administration
sd	Standard deviation
SEM	Standard error of the mean
SOWS	Short Opiate Withdrawal Scale
STAI	State Trait Anxiety Inventory
t½	Half life (distribution phase=t½α: terminal elimination phase=t½β)
t_{max}	Time to reack maximum plasma concentration
Vd	Volume of distribution
q.i.d	Administered four times a day

1. Introduction and Literature Review

Since its introduction in 1965 methadone has been the primary pharmacological intervention for opioid dependence worldwide and at the commencement of the candidature for this thesis was the only opioid drug registered for this purpose in Australia (Joseph, *et al.*, 2000). Methadone maintenance treatment (MMT) has demonstrated efficacy in improving the physical and psychological health of those receiving treatment for opioid dependence (Bell, *et al*., 1997: Gronbladh, *et al.*, 1990; Kreek, 2000; Metzger, *et al*, 1993). Methadone has a number of advantages over other opioids for this purpose because of its high oral bioavailability and relatively long terminal half-life of 24 to 48 hours (Foster, *et al*., 2004; Garrido and Troconiz, 1999). This attribute forms the basis for the orthodox once daily MMT dosage regimen.

To be effective MMT programs should retain patients in treatment, sometimes indefinitely and achieve minimal levels of illicit drug use among those retained (Ball & Ross, 1991; Ward, *et al*., 1999). However, clinical experience with MMT has revealed a number of limitations that threaten the participation of patients in treatment and also in attracting new patients to treatment (Mattick, *et al*., 1998). These limitations revolved around the need for daily clinic attendance, which is disruptive; and individual variations in the pharmacokinetics and response to methadone, such that some patients regularly complain of breakthrough withdrawal (non-holding) at apparent adequate methadone doses, while still others complain of uncomfortable adverse effects directly attributable to methadone (Dyer, *et al*., 1999; Dyer and White, 1997; Goldstein and Judson, 1973; Judson and Goldstein, 1982; Kreek, 1973). These patients may be more likely to dropout out of methadone maintenance treatment. Thus, a number of alternative opioid pharmacotherapies have been evaluated with the aim of helping to attract and retain opioid dependent individuals in treatment.

As many of the limitations of MMT revolve around the necessity for daily dosing and the substantial inter-individual variations in pharmacokinetics, then opioids with a longer duration of action are likely to be useful. The long acting methadone analogue, levo-alpha-acetylmethadol (LAAM), and the partial agonist buprenorphine, have been evaluated and shown to produce equivalent outcomes to methadone (e.g., Johnson, *et al.*, 2000; Ling, *et al*., 1976; 1978). Thus, patients who experience withdrawal on apparently adequate methadone doses, or unacceptable adverse side effects, may benefit from transfer to one of

these medications. Transfer to buprenorphine, however, may precipitate withdrawal in patients who are maintained on methadone doses greater than 30 mg/day (Breen, *et al*., 2003; Bouchez, *et al*., 1998). In contrast, transfer to LAAM is relatively easily achieved (Fudala, *et al*., 1997; Ling, *et al*., 1978; Tennant, *et al*., 1986).

LAAM was approved for use as a treatment for opioid dependence in 1993 (Food and Drug Administration, 1993). Despite this, in comparison to methadone relatively little is known about LAAM's pharmacology, particularly with respect to its pharmacokinetics at steady state. Furthermore, the efficacy of using LAAM as an alternative treatment agent to methadone for those who report the failure of their methadone dose to 'hold' has not been demonstrated (Dyer, *et al*., 1999; Ling, *et al*., 1994). Thus, this thesis describes a comparison of methadone and LAAM in individuals who, during methadone maintenance did (non-holders) and did not (holders) report the failure of their methadone dose to adequately suppress withdrawal across the dosing interval. The study used a randomized crossover design, where patients were maintained on methadone or LAAM for three months and then were crossed over to the alternative drug for three months. This permitted the comparison of steady state pharmacokinetics and pharmacodynamics of each drug across their respective inter-dosing intervals. In addition, *in vitro* assays were undertaken to provide contemporary data to further characterize the relative potency of LAAM and its active metabolites.

This chapter commences with a general overview of opioid dependence and its consequences, followed by an overview of the general pharmacology of methadone, and a discussion of the limitations of MMT. The literature on the development of LAAM as a maintenance pharmacotherapy will then be reviewed followed by reviews on the general pharmacology, pharmacokinetics and pharmacodynamics of LAAM, and finally the experimental rationale and aims of this thesis will be presented.

1.1. Overview of opioid dependence and treatment

Opioid abuse is considered a serious global problem. The United Nations has estimated that during 2000-2001 almost 15 million people worldwide, or 0.4% of the population, aged 15 and above were abusing opioids; this included about 10 million people who used heroin (UNODC, 2003). Hall and colleagues estimated that during 1997 there were 74,000 heroin users in Australia, and that this number has doubled since 1984-87 (Hall, *et al*.,

2000). More recently the 2001 National Drug Strategy Household Survey undertaken in Australia estimated that 2.3 % of the population over the age of 14 years had used opioids in their lifetime, and that 0.5 % of the sample reported using heroin within the last 12 months (AIHW, 2002).

Opioids act to produce a variety of pharmacological effects, the most important of which include alterations in mood (such as euphoria), analgesia, respiratory depression, miosis (pin point pupils), and decreased gastrointestinal motility leading to constipation (Reisine and Pasternak, 1995). The consequence of the repeated administration of opioids is the development of tolerance and physical dependence, which are the result of the adaptation of underlying neural systems to the continual presence of the drug in the body. This is also termed neuroadaptation (Edwards, *et al*., 1981; Trujil and Akil, 1991a). Tolerance refers to the need for increasing doses in order for the individual to experience the desired opioid effect. Physical dependence is characterized by the appearance of unpleasant withdrawal symptoms on cessation of drug use (Jaffe, 1990). Opioid dependent individuals show a pattern of compulsive drug use that is aimed at either maintaining the desired drug effect, and/or to prevent withdrawal symptoms, which often occurs with the awareness of the harmful consequences of such drug use.

1.1.1. Consequences of illicit opioid use

The initial reason for using heroin, or other morphine like opioids, might be to experience the euphoria or the classic 'high' that is experienced soon after intravenous administration or inhalation of the drug. However, tolerance to the effect of short acting opioids usually develops quickly and so individuals may need to use greater amounts of the drug more frequently to achieve the desired effect, or even just to ward off withdrawal symptoms (O'Brien, 1996). As heroin's active metabolite, morphine, has a very short plasma half life (1.5 hrs) and a correspondingly short duration of effect (3 to 5 hrs) a typical heroin user might inject heroin three or four times a day to adequately suppress withdrawal symptoms (Jaffe, 1990). Therefore, users often spend much of their time obtaining and using drugs, often to the neglect of their health; and may also engage in criminal behaviour to finance or acquire their drug supply. Furthermore, opioid dependent users are also more likely to suffer accidents and injuries and be engaged in maladaptive behaviours that result in family dysfunction and domestic violence, and be unemployed. Thus, the repeated use of

opioids results in significant mortality and morbidity amongst users and significant costs to the community (O'Connor and Fiellin, 2000).

Opioid dependent individuals who remain untreated are more likely to die earlier than those in treatment and the general population. The mortality rate for heroin users is between 6 and 20 times that expected for those in the general population of the same age and gender (Darke, *et al.,* 1996; Goldstein and Herrera, 1995; Hser, *et al.*, 2001). Opioid dependent individuals, if they inject, frequently experience overdose with a high risk of death (Darke, *et al.*, 1996). Longitudinal studies have shown that approximately 2 to 3 % of heroin users die each year. Furthermore, over 20 to 30 years more than one-third of untreated heroin dependent individuals are likely to die, predominantly as a result of overdose (Goldstein and Herrera, 1995; Hser, *et al*, 2001). Overdose is commonly the result of opioid-induced respiratory depression that progresses to hypoxia and death. Furthermore, other central nervous system depressants, such as alcohol and benzodiazepines, taken concomitantly with opioids, will act to augment the respiratory depressant effects of the opioid (White and Irvine, 1999). Other causes of premature death may be related to drug-related accidents, suicide, violence, AIDS and both drug-related and other illnesses (Rivara, *et al.*, 1997).

Although opioid users represent only a small proportion of the population, because opioids are predominantly used intravenously, the contribution to the transmission of blood borne viruses, in particular the Human Immunodeficiency Virus (HIV), is significant. The sharing or use of contaminated syringes and needles is a very efficient method of spreading HIV, consequently HIV can spread very rapidly amongst *i.v* drug users (Des Jarlais, 1992; Stimson, 1995). Furthermore, injecting drug users infected with such viruses can be a mode of transmission into the general community via their sexual activity. The United Nations estimates that globally, between 5 and 10% of HIV infections result from injecting drug use (UNDCP, 2002). In the United States 22 % of cumulative cases of AIDS can be directly attributed to injecting drug use (CDCP, 2001). In Australia approximately 8% of HIV diagnoses are in intravenous drug users (NCHECR, 2002). The cost to the community of HIV lies in the high cost of treatment with high rates of premature death.

Injecting drug use is also the major mode of transmission for both hepatitis B and C (Brown and Crofts, 1998; Levine, *et al.*, 1994). Greater than 50 % of those who become

infected with the hepatitis C virus become chronic carriers (Brown and Crofts, 1998), and up to 20 % will develop cirrhosis of the liver over a period of approximately 20 years from time of exposure. Furthermore, hepatocellular carcinoma will develop in one to five percent of those who become chronic carriers (Tong, *et al*., 1995). Although hepatitis C is associated with a lower risk of mortality than infection with HIV, morbidity is significant, and so is the cost to the community (Brown and Crofts, 1998; Sullivan and Fiellin, 2004). Injecting drug use is also associated with other infections, such as skin abscesses, pulmonary infections and endocarditis because of the use of dirty needles (Sullivan and Fiellin, 2004)

Moreover, illicit opioid use is linked inextricably with criminal behaviour in a number of ways. Users commit crime to obtain the necessary funds to purchase drugs, and individuals under the influence of drugs commit crime. It is clear that heroin use results in a significant increase in the frequency of offending. For example, the link between heroin use, property crime and/or robbery in Australia has recently been demonstrated. Fifty five percent of intravenous drug users surveyed in a recent Illicit Drug Reporting Survey self-reported involvement in some form of criminal activity in the month prior to their interview. Property crime, drug dealing, or frauds were respectively the most commonly reported crime (AIHW, 2002). One estimate has put the total cost of heroin related crime in Australia as between AUD $535 million to $1.6 billion per annum (Hall, *et al*., 2000).

1.1.2. General principles of treatment for opioid dependence.

Opioid dependent individuals constitute a heterogeneous group. They have varying social-economic, educational and employment backgrounds and exhibit a diverse pattern of drug use. The combination of physical, psychological and social dimensions makes opioid dependence a particularly complex condition to treat, and all three dimensions need to be addressed to ensure successful rehabilitation.

A common view until relatively recently was of drug dependency as an acute medical condition most appropriately treated by detoxification to return the individual to a drug free state (Ling *et al*., 1997; O'Brien and McClellan, 1996). However, even if the drug dependent individual successfully detoxifies from the drug in question, detoxification does not address the underlying disorder (O'Brien and McLellan, 1996). As will be discussed in some detail in *section 1.2.3.3*, long lasting neural changes take place as a result of the

repeated administration of opioid drugs. These protracted brain changes, and psychological and social difficulties that remain, even after detoxification has finished, put the individual at a great risk of relapse (O'Brien and McLellan, 1996). Hence, more recently the predominant view of opioid dependence is as a chronic relapsing medical condition that requires long-term treatment (Gossop and Marsden, 1996; Ling, *et al.,* 1994; McLellan, *et al*., 2000; O'Brien and McLellan, 1996).

Although abstinence may be the ultimate long-term goal of treatment for opioid dependence, this may not be realistic in the short term and so the aims of treatment need to be matched to the individual's needs and circumstances at the time the individual presents for treatment, but also to be flexible enough to match the changing needs of patients throughout their treatment. Broadly speaking treatment programs have the following goals: (1) to reduce illicit drug use; (2) to reduce the risk of infectious diseases; (3) to improve physical and psychological health; (4) to reduce criminal behaviour; and (5) to improve social functioning, without necessarily stopping drug use all together (Gossop and Marsden, 1996; Hall, *et al*., 1998).

In order to achieve these treatment goals, and because the opioid dependent patient group is so heterogeneous, there is a need for a variety of treatment modalities. Indeed the importance of providing choice of treatment of opioid dependence, and in giving patients greater control over their treatment, has been recognized as pivotal in attracting patients into treatment, and maximizing compliance, treatment retention and effectiveness of treatments for opioid dependence (Gossop and Marsden, 1996; Hartnoll, *et al*., 1980; Mattick, *et al*., 1998; White, *et al*., 1996). There are three main treatment modalities; 1) detoxification, which aims to achieve a drug-free state within a relative short period of time, in order to return the drug user to physiologically normal levels of functioning and from which individuals may progress to more long-term residential forms of treatment; 2) residential rehabilitation (such as therapeutic communities), which provides highly structured programs of counseling and support services in order to facilitate major changes in an individuals life style. These programmes require individuals to reside long term; and 3) maintenance or substitution pharmacotherapies, which entail the prescription of a substance with similar actions as the drug of dependence, but with a lower degree of risk, and which aim to stabilize the physiological and psychological state of the opioid

dependent individual. Full opioid agonists such as methadone and LAAM, and the partial agonist, buprenorphine, are used for this purpose.

Moreover, remaining in treatment for an adequate period of time has been recognized as important for treatment effectiveness (Ball & Ross, 1991; Hubbard, *et al.*, 1989; Marsch, 1998; Simpson, 1979, 1981; Simpson and Sells, 1982, 1990). Research indicates that for most patients the threshold of significant improvement is reached at about three months into treatment (Simpson and Sells, 1982), and indeed more pronounced changes, particularly with regards to reductions in heroin use, is evident in individuals who remain in treatment for greater than one year (Cushman, 1981; Hubbard, *et al.*, 1989). The latter relationship has been found for patients participating in methadone maintenance treatment, therapeutic communities, and outpatient drug-free treatment (Simpson and Sells, 1982). Generally treatment has been found to lead to reductions in illicit drug use, lower rates of criminal activity and improved social outcomes amongst individuals in treatment (Ball & Ross, 1991; Bell, *et al*., 1992; Gottheil, *et al*., 1993; Hubbard, *et al*, 1989; Simpson, 1979, 1981; Simpson and Sells, 1990). Unfortunately, a large proportion of opioid dependent individuals do not seek treatment (Hser, *et al*., 2001), and those that leave treatment prematurely are more likely to relapse to drug use (Des Jarlais, *et al.*, 1981; Dole and Joseph, 1978). Thus, treatment programmes need to adopt strategies to engage and keep patients in treatment. Matching patients to the appropriate treatment has been recognized as an important goal for treatment providers (Gossop, 1987; Gossop and Marsden, 1996; McClellan and Alterman, 1991; Mattick, *et al*, 1998).

1.1.3. Summary

The repeated use of opioids results in significant mortality and morbidity amongst opioid users and significant costs to the community (O'Connor and Fiellin, 2000). The cumulative cost to the community in terms of the costs of medical treatment for untreated opioid users, economic costs due to welfare support, and the costs of crimes committed by these individuals is significant. Engaging opioid dependent individuals in treatment provides an opportunity to address these maladaptive behaviors that result in much of this harm. Treatment aims to reduce the individuals craving for opioids, whether by withdrawing patients from the short acting opioid in question or substituting it for a longer acting oral opioid, such as methadone, which reduces the frequency of injecting drug use, and thus the need to engage in activities to acquire the drug. The provision of treatment

provides the patient with regular contact with treatment agencies, increasing the opportunity for medical and psychosocial intervention. As this thesis is concerned with exploring the utility of using the long acting opioid LAAM as an alternative pharmacotherapy for patients who do not do well on methadone maintenance, the following section will provide a general overview of opioid pharmacology, including opioid receptor pharmacology, the general effects of opioid drugs and will explore in some detail the core features of opioid dependence, i.e., tolerance and dependence.

1.2. Overview of opioid pharmacology

Opium is prepared by drying and powdering the milky juice from the unripe seed capsules of the opium poppy, *papaver somniferum,* and contains more than 20 alkaloids, including morphine and codeine, both of which are active, and thebaine, from which buprenorphine and naloxone are synthesised (Reisine and Pasternak, 1995). The term opiate refers to all the naturally occurring and semisynthetic compounds that are derived from opium. On the other hand the term 'opioid' refers to a more general class of drugs that include all the natural and synthetic compounds, such as pethidine, methadone, and LAAM, as well as endogenous peptides.

Opioid drugs, and endogenous opioid peptides, produce their effects by interacting with protein receptors on cell membranes found throughout the central and peripheral nervous systems. Although opioids as a group are derived from various sources and have different chemical structures, their effects are mediated through their interaction (binding) with three main opioid receptor subtypes – mu (μ), delta (δ) and Kappa (*K*) opioid receptors.

1.2.1. Opioid receptor pharmacology

Studies showing the binding of radioligands in mammalian brain provided the first direct evidence of the existence of stereospecific opioid binding sites (Pert and Snyder, 1973a; Simon, *et al*, 1973; Terenius, 1973). Martin and coworkers (1976) demonstrated the existence of three types of opioid receptor using opioid agonists and antagonists in chronic spinal dogs. They named each receptor according to the drug used to characterise it; μ for morphine, κ for the morphine analog ketocyclazocine and sigma (σ), for which the experimental compound SKF-10, 047 has high affinity (Martin, *et al.,* 1976). These drugs exhibited different pharmacological profiles and could not be replaced by each other in the

suppression of withdrawal in dogs made dependent to one of them, and so the existence of separate receptors was the most plausible explanation. The existence of another receptor type, the delta, δ , (for deferens), was postulated by Lord and colleagues (1977) following their work investigating the activity of opioids in peripheral tissues and was found to have high affinity for enkephalins and endorphins. The delta receptor was soon found to be present in mammalian brain (Gillan and Kosterlitz, 1982). Subsequently the σ receptor has been found to be non-opioid in nature as it preferentially binds phencyclidine and antipsychotic drugs, such as haloperidol, and actions mediated by this receptor are not reversed by the opioid antagonist naloxone (Zukin and Zukin, 1981). To date there is substantial evidence for the existence of the mu, kappa, and delta opioid receptor types (see Brownstein, 1993; Dhawan, *et al.*, 1996; Reissine and Pasternak, 1995; for reviews). Importantly, in the context of opioid dependence, it is the μ opioid receptor that is considered the most important as opioid compounds that have clinical utility as opioid substitution drugs are relatively selective for this receptor subtype.

The existence of other putative opioid receptor types, ie., ε (epsilon), ζ (zeta) and λ (lambda) remains controversial (Connor & Christie, 1999; Dhawan, *et al.*, 1996). Furthermore, some researchers postulate the existence of a number of subtypes of the δ, κ, and μ opioid receptor subtypes; for example, Pasternak and Wood (1986), postulate the existence of μ_1 and μ_2 isoforms of the μ opioid receptor. However, presently the consensus is that there is insufficient evidence to support the existence of such isoforms (Akil, *et al.*, 1998).

Cloning has established that δ, κ, and μ receptors belong to the superfamily of seven–transmembrane spanning G-protein coupled receptors, that act preferentially on pertussis toxin sensitive Gi/Go G-proteins (Connor and Christie, 1999; Dhawan, *et al.*, 1996). There also appears to be some evidence that opioids may exert their effects independently of G-proteins (Dhawan, *et al.*, 1996). A detailed description of the sequence of events that occur as a result of an agonist binding with an opioid receptor are complex and beyond the scope of this review (see Childers, 1991; Connor and Christie, 1999; Di Chiarra and North, 1992; Dhawan, *et al.*, 1996; Koob and Nestler, 1997; Williams, *et al.*, 2001*).* However, in brief, the G protein consists of three subunits, α, β and γ. Activation of the receptor causes the α subunit to uncouple, which then interacts with a number of different intracellular transduction mechanisms to produce changes in neuronal function. Opioid receptor

activation has been shown to produce inhibition of adenylyl cyclase and cAMP formation, which in turn affects phosphorylation and the function of a number of intracellular proteins. Furthermore, neuronal function may also be affected via direct coupling of G proteins to ion channels leading to activation of inward rectifying K^+ channels to reduce Ca^{2+} conductance, and direct inhibition of voltage-gated Ca^{2+} channels. In all cases pre-synaptic reduction in calcium conductance leads to a reduction in neurotransmitter release. Moreover, recently there are reports that opioids also influence the function of cells via activation of protein kinase C, the release of calcium from extracelluar stores, activation of the mitogen-activated protein kinase (MAPK) cascade, and receptor trafficking (e.g., internalization of the receptor) (see review by Williams, *et al.*, 2001). The primary outcome of the binding of an opioid agonist at an opioid receptor is inhibition of neuronal excitability and reduced neurotransmitter release from these neurons. It should be noted, however, that although the primary effect of opioids is inhibitory they may also act indirectly to exert excitatory effects by inhibiting the release of inhibitory neurotransmitters (Williams, *et al.*, 2001). The different patterns of physiological and psychological effects associated with each receptor subtype are due in part to the distribution of opioid receptors in the central nervous system and to different types of interactions with intracellular transduction mechanisms. The reader is directed to a number of reviews that describe the detailed distribution of each opioid receptor type (e.g., Dhawan, *et al*., 1996; Mansour, *et al*., 1986).

In 1996 the International Union of Pharmacologists (IUPHAR) nomenclature committee recommended that the names of the opioid receptors be rationalised to be consistent with the IUPHAR Nomenclature Committee's recommendation for mammalian receptors (Dhawan, *et al.*, 1996). The new nomenclature would use the label OP followed by a subscript number denoting the chronological order it was cloned and sequenced, i.e., the δ receptor (the first cloned) would be OP1, *K* would be OP2, and μ OP3 (Dhawan, *et al.*, 1996). However, as this system has not been widely adopted, and to minimise confusion, this thesis will continue to use the established nomenclature when discussing opioid receptor types.

Moreover, on the basis of their interaction with the opioid receptors described above, opioid drugs can be classified as agonists, partial agonists or antagonists. An agonist is a compound that binds with a receptor and as a consequence maximally stimulates a cascade

of physiological activity. Morphine is considered the prototypical agonist (Emmerson, *et al.*, 1994). A partial agonist, such as buprenorphine, binds to a receptor, but produces submaximal response. On the other hand, an antagonist, such as naloxone, has no intrinsic activity, in that it does not activate intracellular messenger systems, but can block the action of an agonist.

1.2.2. General pharmacodynamic effects of opioid drugs

Opioids exert their most profound effects on the nervous and gastrointestinal systems. These effects are primarily mediated through activation of μ opioid receptors (Reisine and Pasternak, 1995), but may also be mediated through interaction with other opioid sub-receptor types, and indeed other classes of receptors. For example, as well as affinity for μ opioid receptors, morphine has some affinity for κ receptors (Kristensen, *et al.*, 1995; Mignat, *et al.*, 1995); and methadone is not only an agonist at the μ opioid receptor, but also acts as an NMDA receptor antagonist (Ebert, *et al.*, 1995; Gorman, *et al.*, 1997), and inhibitor of noradrenaline and serotonin re-uptake (Codd, *et al.*, 1995). Furthermore, differences in affinity for opioid receptors and pharmacokinetics (e.g., bioavailability, elimination half life, and extent of protein binding) of the drugs concerned will mean that the pharmacodynamic profile, i.e. onset and duration of effects of each drug is likely to differ. With this in mind the pharmacokinetics of methadone and LAAM, the two opioid drugs relevant in the context of this thesis, will be discussed later in this review. Furthermore, chronic administration of opioids will lead to the development of tolerance, which will therefore modify the pharmacodynamic profile compared to that experienced following a single dose of the drug. As such, individual differences in the development of tolerance will also impact on this experience. Generally though, opioids drugs act to produce the following effects.

1.2.2.1. Analgesia

Opioids are thought to modulate the perception of pain by interacting with opioid receptors at both spinal and supraspinal levels and all three opioid receptor types are thought to be involved in analgesia. Mu opioid receptors are involved in supraspinal and spinal mechanisms, and δ and *k* opioid receptors play a role in spinal cord mechanisms of analgesia (Fields, 1993; Yaksh, 1993). Opioids inhibit the transmission of nociceptive information via ascending pathways to limbic areas and higher sensory areas. This is

mediated via μ receptors in the dorsal horn of the spinal cord (Codd, *et al.*, 1995; Fields, 1993). Furthermore, opioids may act directly on ascending pathways at supraspinal sites, for example, at the level of the thalamus and cortex (Fields, 1993; Melzack, 1993). Opioids also increase the activity of descending pathways, originating from brain stem nuclei (such as the midbrain periaqueductal gray and the rostroventral region of the medulla) that result in modulation of the processing of nociceptive information in the dorsal horn of the spinal cord (Fields, 1993; Liebmann, *et al.*, 1994).

1.2.2.2. Alterations in mood

Opioid drugs produce both positive and negative changes in mood states; these include feelings of euphoria and even dysphoria, and mental clouding, which may result in drowsiness and difficulty concentrating. It is the euphoria that may result from the acute administration of many opioids that reinforces repeated self-administration of these drugs and the maintenance of drug seeking behaviour. The neurobiological basis for this phenomenon is not clear. However, there is evidence that activation of the mesolimbic dopamine system may mediate the reinforcing effects of opioids (see reviews by Di Chiara and North, 1992; Koob, 2000; Koob and Bloom, 1988; Koob and Nestler, 1997). The mesolimbic system consists of dopaminergic neurons in the ventral tegmental area (VTA) in the midbrain that project to regions in the forebrain, in particular the nucleus accumbens. The development of euphoria is thought to be due to the increase in activity in dopaminergic neurons in the VTA (Foley, 1993). However, there are recent views that suggest euphoria may not be dopamine mediated, but opioid-mediated instead (see reviews by Levine and Billington, 2004, and, Robinson and Berridge, 2000).

1.2.2.3. Respiratory depression

Respiratory depression is one of the more undesirable side effects of the administration of μ opioid agonists, and apnoea is frequently the major cause of death in opioid overdose (Reisine and Pasternak, 1995). The main centres controlling respiration are located in the brain stem, and include nuclei in the medulla, which include the dorsal respiratory group (DRG) and the ventral respiratory group (VRG); and in the pons. It is not yet clear what the exact function of each of these structures is; the DRG is thought to be responsible for determining respiratory rhythm, while the VRG is thought to be responsible for modifying motor output to the musculature that controls respiration. In addition, the pons is thought

to influence the timing of respiration (Bianchi, *et al.*, 1995; White and Irvine, 1999). Furthermore, peripheral chemoreceptors, located in the carotid and aortic bodies, relay information to the DRG about changes in blood gases. These cells are sensitive to decreases in the partial pressure of oxygen, pO_2, and to a lesser extent to an increase in the partial pressure of carbon dioxide, pCO_2, or decrease in pH (Bianchi, *et al.*, 1995; White and Irvine, 1999). For further details on the physiology of breathing the reader is directed to a review by Bianchi and colleagues (1995).

Opioids act to produce inhibition at all sites that are involved in the control of respiration (White and Irvine, 1999; Yeadon and Kitchen, 1989). Opioids act on peripheral chemoreceptors, via μ opioid receptors, to reduce the sensitivity to changes in blood gases and therefore to decrease nervous output to brain stem respiratory centres. This reduces the capacity of the medullary respiratory centres to alter respiratory parameters (respiratory rate and rhythm) in the presence of abnormal blood gases in order to achieve homeostasis (White & Irvine, 1999). Opioids also directly depress activity of the medullary and pontine respiratory centres, via both μ and δ opioid receptors, which results in direct modulation of respiratory function, in the form of changes in respiratory rate, rhythm and pattern (Martin, 1984; Ressine and Pasternak, 1995).

Moreover, Hurlé and colleagues (1983) reported that exogenously administered opioids differentially affect medullary and pontine respiratory structures responsible for modulating respiration. Pontine structures are more sensitive to opioid induced depression and account for alterations in respiratory rate, whereas depression of medullary structures results in the reduction of tidal volume and carbon dioxide sensitivity. Not withstanding this, there is also evidence that there is a dose-dependent effect of opioids on respiratory function: low concentrations cause decreased tidal volume, whilst higher concentrations cause decreases in both tidal volume and respiratory rate (Morin-Suran, *et al.*, 1984; Santiago and Edelman, 1985; Yeadon & Kitchen, 1989).

In contrast to other opioid effects, such as euphoria, tolerance to the respiratory depressant effects of methadone appears to develop slowly and indeed may not develop completely despite long-term opioid maintenance treatment. Marks and Goldring (1973) and Santiago and colleagues (1977) have shown that tolerance to the respiratory depressant effects of methadone is incomplete; tolerance was complete to the carbon dioxide sensitive

chemoreceptor mechanism, but incomplete to the hypoxia-sensitive chemoreceptor mechanism. Some degree of respiratory depression has been demonstrated in patients who have been maintained on methadone for several months (Dyer, *et al.*, 1999; Gritz, *et al.*, 1975; Martin, *et al.*, 1973; McCaul, *et al.*, 1982), and other studies have shown fluctuation in respiratory rate across methadone's dosing interval with patients exhibiting significant respiratory depression following the administration of their daily methadone dose (Dyer *et al*, 1999; McCaul, *et al.*, 1982).

With regard to the latter point there are a number of important pharmacodynamic interactions that can influence the degree of respiratory depression experienced by patients on opioid maintenance treatment. The co-administration of other μ opioid agonists may result in increased respiratory depression due to increased activity at μ opioid receptors. Furthermore, the co-administration of alcohol (Levine, e*t al.*, 1995) and benzodiazepines (McCormick, *et al.*, 1984) may also synergistically add to the respiratory depressant effects of opioid agonists. The latter drugs mediate their effects via the inhibitory $GABA_A$ receptor complex, which is found in relatively high concentrations in the DRG and VRG, and which is important in the control of respiration (White and Irvine, 1999).

Hence, in summary, patients who have been maintained on long-term opioid agonist treatment may still be at risk of significant respiratory depression following their daily methadone dose. The risk of clinically significant respiratory depression rises dramatically if patients consume opioids in addition to other central nervous depressants, such as alcohol and benzodiazepines, in sufficient quantities.

1.2.2.4. Constriction of pupil (miosis)

One of the most obvious signs of using mu and kappa agonists is pupil constriction (miosis). Humans are very sensitive to the miotic effects of μ opioid receptor agonists (Martin, 1984) and thus miosis is often used as a reliable and objective indicator of opioid effect amongst patients administered methadone and other opioids (Dyer, *et al.*, 1999; Inturrisi and Verebely, 1972; McCaul, *et al.*, 1982). The mechanism of opioid induced miosis is not clear, but most probably involves alteration in autonomic input to the muscles that control pupillary diameter. The dilator muscle of the pupil is innervated by postganglionic sympathetic fibres that use noradrenaline as a transmitter, whereas the constrictor muscle is innervated by parasympathetic fibres that use acetylcholine as a

transmitter (Grunberger, *et al.*, 1990; Kandel, *et al.*, 1991). One proposed mechanism for opioid induced miosis is stimulation of the nucleus of the third cranial nerve, and subsequent increase in cholinergic activity (Jaffe and Martin, 1980). Consistent with this proposal is that miosis can be inhibited by atropine and other muscarinic blocking agents (Jaffe and Martin, 1980). Furthermore, it has also been suggested that opioids might induce miosis by inhibiting central noradrenergic activity (Grunberger, *et al.*, 1990).

There is a strong correlation between the ability of μ opioid agonists to produce analgesia and miosis in humans (Fraser, *et al.*, 1954: Jasinski, 1977). With repeated use partial tolerance to the miotic effects of opioids may occur, however, maintenance patients may still present with constricted pupils (Dyer, *et al*, 1999; Martin, *et al.*, 1973; Martin, 1984, McCaul, *et al.*, 1982; Reisine and Pasternak, 1995; Verebely, *et al.*, 1975), particularly at the time of putative peak plasma methadone concentrations (Dyer, *et al.*, 1999; McCaul, *et al.*, 1982; Ressine and Pasternak, 1995).

1.2.2.5. Cardiovascular effects

Endogenous opioid peptides and receptors have been identified in many central nervous regions associated with central cardiovascular regulation, including the hypothalamus, nucleus tractus solitarius and the intermediolateral nucleus (Fadden, 1993). The cardiovascular effects of opioids are thought to be the net result of opposing increases and decreases in sympathetic activity, mediated by the effect of opioids on different vasomotor centres within the brain. Although, some opioids (e.g., morphine) can be stimulatory by activating a chemoreceptor in the subfornical area, which increases sympathetic tone, opioids are generally depressant by producing an overall depression of neurons in the brain stem, decreasing sympathetic tone and increasing vagal tone. Thus, the overall effect of administering an opioid drug is to produce a modest decrease in peripheral resistance, systolic blood pressure and heart rate (Martin, 1984). Hypotension may occur if an individual changes from a supine to an erect position, possibly due to a modest decrease in peripheral vascular resistance (arteriolar and venous), which produces peripheral vasodilatation (Martin, 1984; Ressine and Pasternak, 1995).

Despite the above, there are equivocal reports in the literature with regard to the cardiovascular effects of opioids, in particular on blood pressure. For example, therapeutic doses of opioids are reported to have little measurable effect on blood pressure, heart rate

and rhythm, particularly if the patient is supine (Jaffe and Martin, 1991). However, others report that opioids (e.g., morphine) may produce a modest decrease in blood pressure and heart rate (Martin, 1984; Reisine and Pasternak, 1986). Interestingly, there are also reports that the administration of opioids, in particular morphine, may be associated with increases in blood pressure (Fadden, 1993; Jasinski, 1981), which is in keeping with morphine's capacity to stimulate sympathetic and arousal mechanisms.

Tolerance to the cardiovascular effects of opioids may develop slowly, but a number of studies have shown patients in maintenance treatment may still exhibit some alteration in cardiovascular function (Gritz, *et al*., 1975; Martin, 1984; McCaul, *et al*., 1982). For example, Gritz and colleagues (1975) showed that the heart rate of patients maintained on methadone (median dose 65 mg: heart rate (HR) = 66 beats/min) was significantly ($p<0.05$) depressed in comparison to abstinent (previously dependent) subjects (HR = 77 beats/min), but was not different to nondrug using subjects (HR = 66 beats/min). There were no significant differences between groups for either systolic or diastolic blood pressure.

1.2.2.6. Constipation

The ability of opioids to produce constipation is an important clinical action, in the context of treating diarrhoea, but is a troublesome side effect following prolonged use, as in the treatment of chronic pain or in opioid substitution therapy. Details of the effects of opioids on the gastrointestinal tract are provided in a review by Kromer (1993). In brief, opioids act directly on the myenteric nervous system, and also on supraspinal receptors, to produce a general decrease in secretions (including gastric, biliary, and pancreatic) and gastrointestinal motility, and a delay in gastric emptying (Kromer, 1993; Reisine and Pasternak, 1995). Opioids have a dual excitatory and inhibitory effect in the small intestine, inhibiting propulsive contractions, while stimulating segmental contractions of smooth muscle (Kromer, 1983). This general decrease in motility causes a delay in the passage of food through the intestines and as a result water is more completely absorbed from bowel contents causing constipation (Ressine and Pasternak, 1995).

Tolerance to the constipating effects of opioids on the gastrointestinal system develops slowly (Kromer, 1993), and may be incomplete so that even after prolonged use constipation may be a troublesome side effect. Thus constipation is one of the more

common and persistent symptom complaints amongst patients who have received long term methadone treatment. Kreek (1973) followed up 214 patients on high dose methadone (80 – 120 mg daily) that were amongst the first cohort to be inducted on to MMT in 1965, and interviewed 92 patients who remained in MMT three years later. Those interviewed reported daily laxative use for an average of eight months during the first year of methadone treatment, and 17 % still reported that constipation was a problem after three or more years of methadone treatment. More recently, Dyer and White (1997) found that 68% of a cohort of 114 patients, whose mean daily methadone dose was 60 mg (sd=28.5 mg), and who had been maintained on methadone for a mean of 326 days (sd=250), reported that constipation was the most frequent symptom complaint.

1.2.2.7. Other effects

Opioids produce a number of other noteworthy effects in humans. These include, the suppression of the cough reflex, nausea and vomiting (Flórez & Hurlé, 1993), and perturbation in neuroendocrine function (Cella, *et al*., 1993). However, these will not be described in any detail in this review.

1.2.3. The core features of opioid dependence - tolerance and dependence

Repeated use of opioid drugs results in the adaptation of the cells and neural systems that are involved in opioid action and which is manifest as the core features of opioid dependence – namely tolerance, physical dependence (withdrawal), and psychological dependence. Tolerance refers to the need for an increase in dose after several administrations in order for the individual to experience the same magnitude of effect (Collett, 1998). Physical dependence, which is not usually manifest until cessation of drug use or where there is a significant reduction in dose, is characterized by the appearance of unpleasant withdrawal symptoms and cravings to use again (Jaffe, 1990). Thus, individuals who are physically dependent on opioid drugs must continually administer these drugs to prevent the occurrence of a constellation of withdrawal signs and symptoms. Psychological dependence refers to a pattern of behaviour characterised by continued intense desire or craving for an opioid drug.

1.2.3.1. Tolerance

The extent of tolerance depends on the magnitude and frequency of drug use, but typically after several months a heavy drug user can self-administer a dose a number of times greater than they initially self-administered. As described previously, tolerance to the effects of opioids generally develops quite rapidly, but not at the same rate or extent (Ling, *et al.*, 1989; Taub, 1982). For instance, tolerance reportedly develops rapidly to the nausea and vomiting, euphoric, sedative and cognitive effects of opioids (Collett, 1998). However, although there are reports that tolerance develops rapidly to the respiratory depressant effects of opioids (Collett, 1998), there are also reports that tolerance to this opioid effect, particularly following chronic administration of methadone, develops slowly and indeed seems to be incomplete (Gritz, *et al.*, 1975; Martin, *et al.*, 1973; Santiago, *et al*., 1977). There are also reports of incomplete tolerance development for the depressant effect of opioids on cardiovascular function (heart rate, systolic and diastolic blood pressure) (Martin, *et al.*, 1973). Furthermore, the constipating effects (Goldstein and Judson, 1973: Kreek, 1973; Longwell, *et al.*, 1979) and miotic effects (Dyer, *et al.*, 1999; Martin, *et al*., 1973; Martin, 1984; Resinne and Pasternak, 1995: Verebely, *et al.*, 1975) continue even after prolonged use of opioids.

Various types of tolerance have been described (see O'Brien, 1996, & Collett, 1998 for a more detailed review). Innate tolerance refers to a reduced sensitivity to the pharmacological effects of a drug that is genetically pre-determined. Pharmacokinetic tolerance occurs where there is an increased capacity of the body to eleminate the drug due to changes in the metabolism (i.e. enhanced ability of hepatic microsomal enzymes to metabolise drugs). In this case changes in the magnitude of drug effect are due to a decrease in the plasma concentration of the drug at the site of action. Learned tolerance occurs as a result of the development of a number of compensatory behavioural skills that act to diminish the magnitude of a drugs effect. Related to this is the finding that the environment in which a person administers the drug can influence the magnitude of tolerance. If the drug is administered in the presence of cues previously associated with drug use then tolerance will be maximal, compared to tolerance experienced in a novel environment, which will be less pronounced (Collett, 1998; Trujillo and Akil, 1991a). Although tolerance is in part due to an increased rate of metabolism, and/or to learning processes, most tolerance is likely due to adaptative changes within the systems affected by the opioid drug in question (i.e., pharmacodynamic tolerance), and thus the proposed

cellular mechanisms of opioid tolerance and dependence will be discussed in more detail later in this section.

Tolerance is an important phenomenon in the clinical context. Inducting a patient onto an opioid substitution agent requires a period of stabilisation whereby the patients receives increasing doses, individualised, until ideally there are no complaints of withdrawal and the patient develops tolerance to the potentially harmful respiratory depressant effects of the opioid drug. Furthermore, with the development of tolerance to the effects of a particular opioid drug the co-administration of pharmacologically similar drugs will result in reduced effectiveness, which is termed *cross-tolerance*.

1.2.3.2. Physical dependence

Physical dependence typically accompanies tolerance, but is usually not manifest until the drug is removed from opioid receptors, either by the user ceasing drug administration or being administered an opioid receptor antagonist, such as naloxone. If the user is physically dependent then ceasing opioid administration will result in a constellation of symptoms that are usually opposite in nature to the acute effects of the drug (O'Brien, 1993; Trujillo and Akil, 1991a), and which are thought to be the result of a lag in re-adaptation of the organ systems affected by opioids (Johnson and Fleming, 1989). As the actions of opioids are generally depressant then withdrawal from opioids results in a general hyperexcitable state (Trujilo and Akil, 1991a), characterized by restlessness, irritability, diarrhoea, piloerection or 'gooseflesh' and feelings of chillness, muscle cramps, increased blood pressure, pupillary dilatation, increase lacrimation and runny nose. Subsequently administering an opioid drug will suppress any signs and symptoms of withdrawal in a dose dependent manner, thus negatively reinforcing the drug user's dependence on the drug (Koob and Nestler, 1997; Martin, 1984).

The nature of the withdrawal syndrome, that is, the onset, severity and duration of symptoms is influenced by a number of factors, including the pharmacokinetics of the drug used (i.e., in particular the elimination half life), the amount of drug used, the duration of drug use, the dose of recently administered opioid, and the general health and personality of the individual user (Farrel, 1994; Jaffe and Martin, 1991). In general, drugs that are more rapidly eliminated, such as heroin, tend to produce withdrawal syndromes that are more intense and shorter in duration than drugs such as methadone, which are more slowly

eliminated from the body (Busto & Sellers, 1986; Farré and Cami, 1991; Jaffe and Martin, 1991). This is because the relatively rapid elimination seen with shorter acting drugs, such as heroin, does not provide sufficient time for reversal of the neuroadaptation that occurs with chronic opioid administration (Busto & Sellers, 1986; Farré and Cami, 1991). Furthermore, if an individual self-administers large doses of a drug for prolonged periods withdrawal tends to be more severe than after shorter periods.

Typically for the heroin user withdrawal signs and symptoms will emerge 4 to 5 hours after the last dose. The early symptoms are subjective in nature, and include anxiety, irritability and craving for the drug. If the individual is unable to procure the drug then physical symptoms, such as yawning, sweating, rhinorrhea, and lacrimation will increase in severity from about 8 to 12 hours (Farrel, 1994; Jaffe, 1990). This is followed by the emergence of other symptoms, such as dilated pupils, muscle and bone aches, hot and cold flushes and tremor, 'gooseflesh', vomiting and increased bowel motility. Increases in blood pressure, pulse, temperature and respiratory rate may also occur (Jaffe, 1990). These symptoms will usually peak in intensity at about 48 – 72 hours following the last dose, and usually disappear by 7-10 days (Jaffe, 1990). However, the absence of the above signs and symptoms of opioid withdrawal does not suggest that physiological equilibrium has taken place, as complete dissipation of symptoms may take months to occur (Jaffe, 1990). For example, there are reports of ex-drug users experiencing a prolonged or 'protracted' withdrawal syndrome, which may last for a number of months or even years (Jaffe, 1990; Kreek, 1992; Martin and Jasinski, 1969). This protracted abstinence syndrome may comprise of a number of behavioural abnormalities, such as restlessness, depression, fatigue, weakness, irritability, craving for opioids, lack of motivation, poor concentration, and poor sleep (Jaffe, 1990; Kreek, 1992), which predisposes the individual to relapse back to drug use (Jaffe, 1990; Kreek, 1992). Furthermore, individuals may experience withdrawal when they return to environments in which they used drugs, as conditioned stimuli elicit certain withdrawal symptoms (O'Brien, *et al.*, 1977; Whitehead, 1972; Wikler, 1968).

Withdrawal from long acting opioids, such as methadone is characterised by a qualitatively similar withdrawal syndrome to that seen with short acting agonists, but which develops more slowly, and is usually less intense (Jaffe, 1990, Martin, *et al.*, 1973). Symptoms of methadone withdrawal generally appear between 24 to 48 hours after the last dose, peak on

the third day and may persist at this level for about three weeks. The severity of symptoms then declines after about six to seven weeks to the intensity of that reported pre-withdrawal (Jaffe, 1990; Martin, *et al*., 1973). As with withdrawal from heroin, a protracted abstinence syndrome may follow (Martin, *et al.*, 1973; Martin & Jasinski, 1969).

Although withdrawal from methadone is less intense than that experienced following withdrawal from shorter acting opioids, its protracted nature is a major disincentive for some patients to enter maintenance treatment (Bell, *et al*., 1995; Eklund, *et al*, 1997; Rosenblum, *et al*., 1991). Fear of detoxification may also impact negatively on some patients experience with methadone withdrawal, although there appears to be a marked discrepancy between their anticipatory fears and that actually experienced by the patient during methadone withdrawal (Eklund, *et al*., 1977). For example, there have been reports of exaggerated anxiety during withdrawal, which is reportedly related to past traumatic detoxification attempts (Hall, 1979). Moreover, studies have shown that certain psychological factors, such as personality (e.g., neuroticism) and mental states (e.g., negative mood states) (Kleber, 1981; Phillips, *et al.*, 1986), and expectations of the degree of distress (Phillips, *et al.*, 1986), may influence the perceived severity of withdrawal and induce cravings for additional opioids (Childress, *et al*., 1994; Kleber, 1981; Phillips, *et al.*, 1986).

The physical manifestations of withdrawal are thought to be mediated, at least in part, by the interaction of the opioid system and other neurotransmitter systems, in particular the noradrenergic system (Gold, 1993). Many of the acute signs and symptoms of opioid withdrawal represent hyperactivity of the noradrenergic system. This is thought to be mediated by the loss of opiate feedback inhibition to the locus coeruleus, a noradrenergic nucleus located at the floor of the fourth ventricle in the anterior pons (Gold, 1993, Koob, 1992; Nestler, 1992). The locus coeruleus contains the major noradrenergic nucleus in the brain, which has wide spread projection to many areas, such as the limbic areas (i.e., hippocampus and hypothalamus) thalamus, and the neocortex, and is responsible for maintaining the organism's state of arousal, attention and autonomic tone (Nestler, 1997). Indirect evidence for the involvement of the noradrenergic system is provided by findings that the alpha-2-noradrenergic agonists, clonidine, and lofexidine, relieve symptoms of acute opiate withdrawal. They are thought to reduce noradrenergic activity by autoreceptor activation and by doing so relieve many of the centrally mediated symptoms

of opiate withdrawal, such as restlessness, cramps etc, but are of limited use in the treatment of more persistent symptoms, such as opiate craving, the inability to concentrate and sleep disturbances (Kleber, *et al.*, 1985; Kreek, 1992).

1.2.3.3. Biological mechanisms underlying tolerance and dependence

Repeated exposure to opioids results in a number of adaptations to receptor mechanisms, intracellular messenger systems, and neuronal circuitry, responsible for mediating opioid effects (Trujillo and Akil., 1991a; Williams, *et al.*, 2001). The mechanisms responsible for such changes may occur concurrently to produce the complex behaviour attributed to opioid dependence and tolerance (Taylor and Fleming, 2001; Williams *et al.*, 2001). The reader is directed to a number of reviews in the area (Johnson and Fleming, 1989; Taylor & Fleming, 2001; Trujilo & Akil., 1991a; Williams, *et al.*, 2001).

Williams and colleagues (2001) identify a number of mechanisms that may account for the changes experienced by the individual during the repeated use of opioids and subsequent cessation of use. These mechanisms include; (1) acute desensitization of opioid receptor to effector coupling and receptor internalisation, that are responsible for short term changes; (2) long-term desensitization of the opioid receptor to effector coupling, and receptor down regulation, that slowly develop and may persist for days even after removal of the opioid agonist; (3) counteradaptations of intracellular messenger systems; and (4) counteradaptations in neuronal circuitry that may account for very long term features of the withdrawal syndrome, such as craving and relapse to drug use. The following discussion will briefly describe each in turn.

Receptor desensitisation and internalisation are thought to contribute to the development of short-term adaptations (e.g., acute tolerance) to opioids, which develop during and abate soon after exposure to opioid agonists (Williams, *et al.*, 2001). Desensitisation involves the functional uncoupling of the opioid receptor from G-protein regulated mechanisms (Trujilo and Akil, 1991a; Williams, *et al.*, 2001). The binding of an agonist to the receptor causes activation of G protein activated protein kinases that phosphorylate the COOH-terminal region of the receptor, which in turn binds with high affinity with the cytoplasmic protein arrestin. Arrestin prevents the re-association of inactive G-proteins with the receptor. Following desensitization receptors may be reactivated, or internalized via endocytosis (Brunemann and Hosey, 1999; Law and Loh, 1999). Internalisation results in

the loss of receptors from the cell surface, a reduction in binding sites, and hence reduced capacity of the agonist to produce a response (Pak, *et al*., 1996; Rogers and El-Fakahany, 1986). There are also reports of phosphorylation-independent mechanisms that may also contribute to μ receptor desensitisation (see Williams *et al*., 2001).

Although receptor desensitisation and internalisation mediate short-term adaptations to opioids, it is not clear if they also contribute to the longer term adaptations that are required for the organism to cope with chronic and increasing use of opioids. Such long term adaptations (hours to days) are purported to include chronic uncoupling of μ receptors from signaling pathways, and down regulation of opioid receptors (Williams, *et al.*, 2001). Early studies found no evidence of a decrease in the number of μ-receptor binding sites following chronic morphine treatment (Werling, *et al*., 1989). However, with improvements in methods that allow more direct assessment of receptor densities (i.e., use of highly selective antibodies), more recent studies have shown receptor down regulation in particular brain regions (Bernstein and Welch, 1998; Law and Loh, 1999; Tao, *et al*., 1998). In addition, there is evidence of the uncoupling of μ-receptors from a number of intracellular pathways, including inwardly rectifying potassium channel currents (Christie, *et al.*, 1987) and calcium channel currents in locus coeruleus neurons (Connor and Christie, *et al*., 1999). However, it is not clear if the latter findings were due to the functional uncoupling of receptors from G proteins or down regulation of surface receptors and whether these mechanisms are clinically relevant (Williams, *et al.*, 2001).

Subsequent to receptor activation a series of counteradaptations come into play to restore normal physiological functioning in the presence of the drug, which can be considered a form of tolerance (Williams, *et al.*, 2001). For example, studies have shown that acutely, morphine acting on δ-receptors produces an inhibition of adenylyl cyclase activity, but with prolonged exposure produces an upregulation of adenylyl cyclase. The increase in adenylyl cyclase activity remained even after the removal of morphine (Brandt, *et al*., 1976; Sharma, *et al.*, 1975). However, more recent studies that examined the effects of chronic morphine on adenylyl cyclase activity in brain and peripheral tissue have produced mixed results (Chakrabarti, *et al*., 1998; Duman, *et al*., 1988; Terwilliger, *et al*., 1991). The use of different cell types, multiple receptors and varying adenylyl cyclase isoforms in such studies makes interpretation of results from these studies difficult (Williams, *et al*., 2001). The contribution of compensatory changes involving other mechanisms, such as

potassium and calcium channels and the neurotransmitter adenosine, to the development of tolerance and dependence, have been considered by other authors, but are beyond the scope of this review (see Williams, *et al*., 2001).

Upon reduction or cessation of opioid use there is a period of time where over-compensation can produce a constellation of signs and symptoms, often the opposite to the acute effects of opioids, which are characteristic of the withdrawal syndrome. As previously mentioned, it is thought that many of the acute signs and symptoms of opioid withdrawal syndrome represent hyperactivity of the noradrenergic system. In part these symptoms are thought to be caused by the rebound increase in adenylyl cyclase in certain noradrenergic rich areas, such as the locus coeruleus (Narita, *et al*., 2001; Nestler, 1992).

Opioid induced changes in the mesolimbic dopaminergic system may also contribute to aspects of the opioid withdrawal syndrome encountered on cessation of opioid use. Acute opioid administration increases cell firing in the VTA and stimulates dopamine release in the nucleus accumbens. Following chronic drug treatment, withdrawal of the opioid produces a significant decrease in release of dopamine in the nucleus accumbens (Koob, *et al*., 1989; Rossetti, *et al*., 1992), which would be consistent with the dysphoria often associated with opioid withdrawal. There are also associated changes in second messenger mechanisms, including a reduction in inhibitory G-protein and an increase in adenylyl cyclase in the nucleus accumbens (Williams, *et al*., 2001). It is recognized that the high rate of relapse to drug use is in part due to the dysphoria associated with withdrawal, and associated negative drive to reinstate drug use (Williams, *et al*, 2001). Long term adaptations in underlying neural systems, responsible for such aspects of opioid dependence, as craving, are now thought to be a major contributor to the chronic relapsing nature of the disorder (Koob and Bloom, 1988; Williams, *et al*., 2001)

Modulation of synaptic activity in those neuronal regions responsible for the core features of opioid dependence is thought to play a role in the development of long lasting brain changes that predispose chronic opioid users to craving and relapse. Such adaptations can be due to either the direct action of opioid agonists on the excitability of cells or on transmitter release, or through opioidergic neurons affecting neuronal activity indirectly, for example by disinhibition (Williams, *et al*., 2001). These adaptations to synaptic and neuronal function are thought to be very similar to those mechanisms considered integral

to memory function, such as long-term potentiation and long-term depression (Williams, *et al*., 2001). Indeed, it has been suggested that modulation of synaptic function in the mesolimbic dopamine system, in particular, by chronic opioid use, might account for compulsive drug seeking behaviour (Williams, *et al*., 2001). Certainly, it is possible that modulation of synaptic activity in other brain areas would account for other features of opioid dependence, but to date this has not been fully explored.

There is also evidence that the development of tolerance may involve nonopioid transmitter systems. The N-methyl-D aspartate (NMDA) receptor has been implicated in the development of acute tolerance (Elliot, *et al.*, 1994; Trujillo and Akil, 1991b). Studies have shown that NMDA receptor antagonists, such as MK-801 (dizocilpine), LY274614, ketamine and dextromethorphan, can inhibit the development of tolerance to the analgesic effects of morphine in rats and also interfere with the development of physical dependence on morphine (Elliot, *et al*., 1994; Mao, *et al*., 1996; Marek, *et al*., 1991; Tiseo and Inturrisi., 1993; Trujilo and Akil., 1991b). Furthermore, it has been shown that tolerance to the effects of morphine can be blocked by inhibition of nitric oxide synthase (Elliot, *et al.*, 1994; Kolesnikov, *et al*., 1993).

1.2.3.4. Summary

In summary, opioids exert their most profound effects on the central nervous and gastrointestinal systems. These effects are predominantly mediated through the μ opioid receptor, and include analgesia, miosis, depressed respiration, changes in mood, and constipation. Repeated exposure to opioid drugs results in a number of adaptations to receptor mechanisms, intracellular messenger systems, and neuronal circuitry responsible for mediating the core features of opioid dependence, i.e., tolerance, dependence, and continuing compulsive drug use. A major goal of the maintenance treatment is to stabilize the physical and psychological state of opioid dependent patients, which is disturbed by the cycles of opioid use and abstinence typically encountered by the individual dependent on short acting opioids.

1.3. Maintenance treatment of opioid dependence

The maintenance treatment of opioid dependence involves the administration of a drug that has similar pharmacological actions to the drug of dependence. Ideally the substituted

drug is effective when administered orally, thereby eliminating the risks associated with intravenous drug use. Furthermore, it is desirable that the drug has a longer duration of action, and flatter plasma concentration-time profile than the original drug of dependence, which permits less frequent administration than typically experienced with short acting opioids, and thus more effective suppression of withdrawal across the inter-dosing interval (Kreek, 1992). This reduced frequency of administration results in less disruption of the normal activities of daily living. Therefore, the primary aims of maintenance treatment are: 1) to suppress the signs of symptoms of opioid withdrawal, and alleviate craving, across the inter-dosing interval of the substituted drug, without causing unacceptable adverse side effects, and 2) at sufficient dosages to confer cross tolerance to the effects of subsequently administered intravenous doses of heroin, or other short acting opioids (Dole and Nyswander, 1966; Levine, *et al.*, 1973; Kreek, 1992; Zaks, *et al*., 1972). The value of maintenance treatment is that it provides the opportunity for the drug dependent individual to engage in treatment, in order to receive both medical and psychosocial intervention, and to reduce their exposure to risk behaviours associated with the intravenous administration of illicit drugs such as heroin. Suitable agents for maintenance treatment are the long acting full opioid receptor agonists, methadone and LAAM, and the partial opioid agonist, buprenorphine.

Globally, methadone is the principal pharmacotherapy utilized in the treatment of opioid dependence, and thus, the following section presents a general review of the pharmacology of methadone and evidence for the effectiveness of methadone maintenance treatment.

1.4. Methadone maintenance treatment

At the start of the candidature for this thesis methadone was the only drug available in Australia for the medical maintenance of opioid dependent individuals. Dole and Nyswander first used methadone as an opioid substitution drug for opioid dependence in 1964 (Dole and Nyswander, 1965). They proposed that individuals dependent on opioid drugs suffered a metabolic deficiency, and thus required a sufficient dose of an opioid drug to stabilize their physiological state (Dole and Nyswander, 1966; 1967). They claimed that sufficiently high doses of methadone ('blockade dose' of 80 to 120 mg/day) would suppress withdrawal and prevent craving across a 24 to 36 hour period, and would provide cross tolerance to the effects of illicitly administered heroin, thus allowing individuals to improve their social functioning (Dole and Nyswander, 1967). To date MMT is one of the

most researched treatment modalities for opioid dependence (For a detailed review of the evidence for the effectiveness of MMT the reader is directed to Ward, *et al*., 1998b). It is because of this that MMT is considered the gold standard against which existing and proposed substitution pharmacotherapies are compared (Ward, *et al.*, 1998b; 1999).

1.4.1. Pharmacology of methadone

Methadone [±6-dimethylamino-4, 4-diphenyl-3-heptanone]; see figure 1.1 for its structure, is a chiral compound, and is administered as a racemate of two stereoisomers, (R)-(-) and (S)-(+) methadone. Methadone is weak base with a pKa-value of 9.2 and is highly lipid soluble (Log P 2.06) (Eap, 2002).

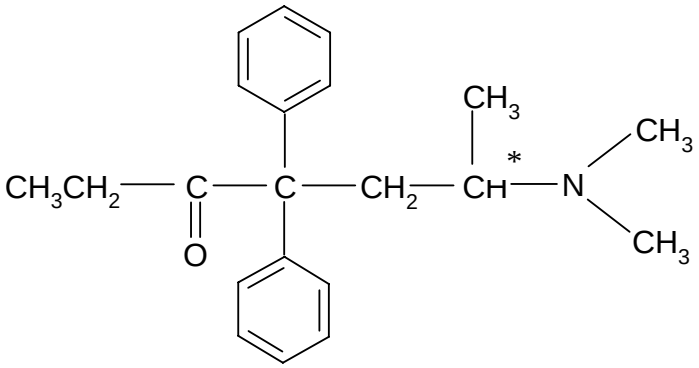

Figure 1-1: Chemical structure of methadone. Asterisk denotes chiral carbon.

1.4.1.1. Receptor target

Early studies examining the affinity of methadone for opioid receptors used the displacement of the relatively non-selective μ opioid receptor antagonist naloxone by (R)-(-) and (S)-(+) methadone, in gross rat brain homogenate. The reported ranges of concentration inhibiting 50 % of [H^3] naloxone binding (IC_{50}) were 4.5 to 30nM (Horng, *et al.*, 1976; Pert & Snyder, 1973a; Pert & Snyder, 1973b), and 250 to 300 nM (Horng, *et al*, 1976; Pert & Snyder, 1973a; Pert & Snyder, 1973b) for (R)-(-) and (S)-(+) methadone, respectively. Horng and colleagues (Horng, *et al.*, 1976; Wong and Horng, 1973) also used [H^3] dihydromorphine to examine the binding affinities of each isomer, and reported very similar IC_{50} concentrations [4.2nM and 200 nM (Wong & Horng, 1976), and 5.0 nM and 130 nM (Horng, *et al*, 1976), for (R)-(-) and (S)-(+) respectively].

More recent studies have examined the binding of racemic- , (R)-(-) and (S)-(+) methadone to opioid receptor subtypes in homogenates prepared from rat, guinea pig, and bovine brain, using ligands that are specific for particular opioid receptor subtypes: that is [H^3]DAMGO for μ, [H^3]DPDPE for δ, and [H^3]U-69,593 for κ receptors (Chen, *et al*., 1991; Codd, *et al*., 1995; Kristensen, *et al*., 1995; Magnan, *et al*., 1982). The reported inhibition constants (K_i) for racemic-methadone were in the range 1.7 - 29 nM (Chen, *et al*., 1991; Codd, *et al*., 1995; Magnan, *et al*., 1982); 15 – 370 nM (Codd, *et al*., 1995; Magnan, *et al*., 1984), and 405 to 1600 nM (Codd, *et al*., 1995; Magnan, *et al*., 1984), respectively for μ, δ and κ opioid receptor subtypes.

Furthermore, the binding affinities of racemic, (R)-(-) and (S)-(+) methadone at μ_1, μ_2, δ, and κ receptors in homogenate prepared from bovine caudate were examined by Kristensen and colleagues (1995). The affinity (IC_{50}) of racemic methadone at μ_1 receptors was twice that for μ_2 receptors (5.7 nM vs 10 nM); while the affinities of (R)-(-) methadone were approximately ten times higher than of (S)-(+) methadone at μ_1 receptors (3 nM vs 26nM) and μ_2 receptors (7 nM vs 88 nM). At the δ receptor (R)-(-) methadone had approximately 20 times higher affinity than (S)-(+) methadone (371 nM vs 9532 nM), while both enantiomers had similar affinities for κ receptors (1332 nM vs 2137 nM). Moreover, Codd and colleagues (1995) reported similar stereoselectivity of methadone enantiomers for opioid receptor subtypes in rat forebrain homogenate. They reported 20 times higher affinity (K_i) for (R)-(-) than (S)-(+) methadone at μ receptors (0.95 nM vs 19.7 nM), similar affinities for either enantiomer at δ receptors (371 nM vs 960 nM respectively) and κ receptors (1 860 vs 1370 nM).

Methadone has also been shown to have non-opioid actions. Codd and colleagues (1995) reported that (R)-(-) methadone was more potent than (S)-(+) methadone in inhibiting the re-uptake of noradrenaline and serotonin in rat brain synaptosmes. Serotonin pathways have been implicated in pain regulation and therefore the these authors suggested that in compounds that possess opioid and monoamine activity, the monoamine activity might act to modulate opioid induced antinociception. There is also evidence that methadone modulates the action of excitatory amino acids, such as glutamate by acting as a non-competitive antagonist at NMDA receptors. Racemic methadone has similar affinity as dextromethorphan, an NMDA antagonist, for the NMDA receptor (Ebert, *et al*., 1995), and the individual enantiomers bind with similar affinity as the racemate to the NMDA

receptor in rat brain preparations (Gorman, *et al*., 1997). As glutamate plays a role in respiratory control, its action may be important in mediating methadone's respiratory effects (White and Irvine, 1999). Furthermore, NMDA antagonists have been associated with impairments in learning and cognitive functioning (Rammasayer, 2001).

The stereoselectivity observed in the binding of (R)-(-) and (S)-(+) methadone at μ receptors is reflected in the effects they produce *in vivo*. In humans (R)-(-) methadone has been shown to be up to 50 times more potent than (S)-(+) methadone (Olsen, *et al*., 1977; Scott, *et al*., 1948). Scott and colleagues showed that in non-drug users the analgesic action of 160 mg of (S)-(+) methadone administered *i.v* was similar to that produced by 3 mg of (R)-(-) methadone. Olsen and colleagues went on to show that 50 and 100 mg oral doses of (S)-(+) methadone produced only slight respiratory depression, whereas 7.5 mg of (R)-(-) methadone produced sustained respiratory depression and miosis. Furthermore, in mice and rats, (R)-(-) methadone has been shown to be between 3 – 30 times more potent in its analgesic action than (S)-(+) methadone following subcutaneous administration (Codd, *et al.*, 1995; Eddy, *et al*, 1952; Smits and Myers, 1974), and between 2.5 – 10 times more potent following oral administration (Eddy, *et al*, 1952; Smits and Myers, 1974). Moreover, although the administration of (S)-(+) methadone (dosage 650 to 1000 mg) alone to humans has been associated with some opioid effect (analgesia, euphoria, slight respiratory depression) (Scott, *et al.*, 1948; Olsen, *et al.*, 1977), it is also associated with adverse side effects, such as nervousness, confusion, and hallucinations (Fraser, *et al*., 1962). Thus, in view of the potency ratio seen in receptor binding studies, and data from *in vivo* studies, it is clear that it is predominantly the action of the (R)-(-) enantiomer at the μ opioid receptor that is responsible for the typical profile of opioid effects experienced by individuals taking methadone.

1.4.1.2. Pharmacokinetics

1.4.1.2.1. Absorption and distribution

Following oral administration the absorption of methadone is rapid, with detectable concentrations in plasma within 15 to 45 minutes (Inturrisi & Verebely, 1972; Kristensen, *et al*., 1996). The time to peak plasma methadone concentrations (T_{max}) varies between individuals, but generally in patients maintained on methadone it occurs at about 2 to 4 hours following the ingestion of the dose (Dyer, *et al.*, 1999; Meresaar, *et al.*, 1981; Mitchell, *et al.*, 2003). The oral bioavailability of methadone is high (estimates up to 90

%; Garrido and Troconiz, 1999), with large inter-individual variability. For example, Meresaar and colleagues (1981) reported a bioavailability value of 79±12 % (mean±sd: range 36 to 119 %) in former opioid addicts administered a single dose of racemic-methadone.

The disposition of methadone is biphasic, with a relatively rapid distribution phase (1-6 hours; Foster, *et al*., 2004; Meresaar, *et al.*, 1981; Wolff, *et al*., 1993; Wolff, *et al*., 1997), and a longer 'terminal' elimination phase. The estimates of methadone's elimination half-life in the literature range from 24 to about 50 hours, with considerable inter-individual differences; for example, chronic dosing studies have reported half-life values of 22±7h (mean±sd: Verebely, *et al.*, 1975) and 52±20h (Angarrd, *et al.*, 1975). Foster and colleagues (2004) recently carried out population modeling of the pharmacokinetics of racemic, (R)-(-) and (S)-(+) methadone in a large number of maintenance patients, and reported a mean (geometric) half-life value for racemic-methadone of 39 hrs (35, 43: 95% CI), and mean half-life values of 51 (45, 57: 95% CI) and 31 (28, 35: 95% CI) for (R)-(-) and (S)-(+) methadone, respectively. Reports of the apparent volume of distribution at steady state (between 2 and 5 L/kg) are greater than physiological volumes, thus indicating the extensive tissue distribution of methadone (Eap, 2002; Garrido & Troconiz, 1999; Somogyi, 2000). Furthermore, chronic dosing studies have reported plasma clearance values ranging from 112±51 ml/min (de Vos, *et al.*, 1995), to 217 ml/min (Wolff, *et al*., 1993). This marked interpatient variability in plasma clearance could result in considerable variation in plasma methadone concentration following administration of the same dose of methadone to different individuals.

The effect of methadone on target tissues is highly dependent on the proportion of unbound methadone in plasma, i.e., the unbound fraction. Methadone binds extensively to plasma proteins, the most important of which is α_1 acid glycoprotein (AAG) (Romach, *et al*., 1981). The extent of plasma binding of methadone has been estimated at about 90% (Abramson, 1982; Romach, *et al.*, 1981). As AAG is an acute phase protein, its concentration in plasma varies according to a number of physiological conditions (Garrido and Troconiz, 1999), which can affect the concentration of unbound drug. For example, during certain stressful conditions there is an increase in AAG concentrations, and this has been shown to the main factor responsible for a lower free fraction of methadone in cancer

patients (Abramson, 1982) and opioid users experiencing withdrawal (Calvo, *et al*., 1996) as compared to healthy volunteers.

1.4.1.2.2. Metabolism and excretion

The primary metabolic pathway of methadone in humans involves *N*-demethylation via cytochrome (CYP) P450 enzymes in the liver and spontaneous cyclization to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). EDDP is the main metabolite of methadone and has no known opioid receptor agonist activity. Although other metabolites, such as α-(-)-normethadone, do possess pharmacological activity, they are only produced in small quantities, and so are unlikely to contribute to methadone's overall opioid activity (Somogyi, 2000). CYP3A4 is the main cytochrome P450 enzyme involved in methadone metabolism to EDDP (Foster, *et al*., 1999; Iribarne, *et al*., 1996; Moody, *et al*., 1997), and its activity is a major determinant of steady state plasma methadone concentrations (Somogyi, 2000). Although other isoforms have been implicated in the biotransformation of methadone [e.g. CYP2D6 (Eap, *et al*., 1997; Yue, *et al*., 1995), CYP1A2 (Yue, *et al*., 1995), CYP2C9 (Foster, *et al*., 1999; Moody, *et al*., 1997), and CYP2C19 (Foster, *et al*., 1999)], their relative importance is unclear at this stage. There is also evidence of substantial inter-individual variability in the activity of these enzymes; for example, there is evidence of up to 30-fold variation between individuals in the expression of hepatic CYP3A4 (Ketter, *et al*., 1995); although *in vivo* inter-individual variability is only about 4- to 6-fold (Wilkinson, 2004). Intestinal CYP3A4 also probably plays a part in the metabolism of methadone, and given that there is up to 11 fold variability in its activity (Ketter, *et al*., 1995), it is possible that variation in intestinal metabolism may affect the bioavailability of methadone (Eap, 2002; Oda and Kharasch, 2001b). Furthermore, auto induction of CYP3A4 activity can occur at the beginning of MMT, resulting in increased plasma clearance, decreased elimination half life, and decreased steady state plasma methadone concentrations particularly during the first month of MMT (Eap, 2002; Rostami-Hodjegan, *et al*., 1999). Thus, some individuals may require alteration of their dosage past induction and stabilization onto methadone maintenance, but this has yet to be confirmed.

The elimination of methadone is primarily by biotransformation in the liver and methadone and its metabolites are mainly excreted in the urine. Urinary excretion of unchanged methadone and EDDP is reported to account for about 40% of the dose ingested, although

considerable inter-patient variability has been reported (Foster, *et al*., 2000; Nilsson, *et al*., 1982a; Verebely, *et al*., 1975). Although renal excretion accounts for a small percentage of total clearance of methadone when urinary pH is neutral, it is increased when urinary pH is below 6 (Nilsson, *et al.*, 1982b). The combined recovery of methadone and EDDP in urine and faeces has been reported to be about 60% of the dose ingested (Nilsson, *et al*., 1982a; Verebely, *et al*., 1975).

1.4.1.2.3. Stereoselectivity in the pharmacokinetics of methadone

To date, only a few studies have examined the pharmacokinetics of the individual enantiomers in maintenance patients at steady state (Foster, *et al*, 2000; Kreek, *et al.*, 1979; Foster, *et al*., 2004). In an early study Kreek and colleagues (1979) administered equal amounts of each isotope labeled methadone enantiomer orally to three methadone maintenance patients. They reported that (R)-(-) methadone had a higher total body clearance, larger volume of distribution, and longer terminal elimination half-life than (S)-(+) methadone (Kreek, *et al.*, 1979). Foster and colleagues (2000), from our research group, examined the disposition of each enantiomer in 18 MMT patients (daily methadone dose ranging from 7.5 to 130 mg) and reported marked stereoselective differences for most pharmacokinetic parameters measured. In comparison to (S)-(+) methadone, (R)-(-) methadone exhibited a significantly greater unbound fraction and total renal clearance, and lower maximum plasma concentration (C_{max}). When protein binding was taken into account (R)-(-) methadone exhibited significantly lower plasma clearance of the unbound fraction, and EDDP formation, and hence greater area under the plasma concentration time curve. There was also greater temporal variation in plasma concentrations across the inter-dosing interval for (S)-(+) than (R)-(-) methadone, as demonstrated in the significantly greater (P<0.0001) ratio of peak to trough plasma concentrations for (S)-(+) (P/T ratio=2.30) than (R)-(-) methadone (P/T ratio=1.81). Importantly there was marked interindividual variation of all pharmacokinetic parameters reported (Coefficient of variation, CV, up to 70%). More recently, Foster and colleagues (2004), in their population modeling of the pharmacokinetics of racemic, (R)-(-) and (S)-(+) methadone, demonstrated that compared to (S)-(+) methadone, (R)-(-) methadone had a greater terminal elimination half-life and volume of distribution, but similar apparent oral clearance. There was considerable variation for all parameters (CV=45%). Such marked stereo selectivity in methadone disposition highlights the need to use stereoselective methadone assays when examining dispositional mechanisms for certain

pharmacodynamic end points as measurement of racemic-methadone may not accurately reflect the plasma concentration of the active (R)-(-) isomer.

1.4.1.2.4. The influence of extrinsic factors on methadone pharmacokinetics

A number of drugs that are commonly co-administered with methadone can alter methadone metabolism and add to inter-individual variability in methadone pharmacokinetics (see Eap, 2002 for a review). Drugs such as rifampicin, phenobarbitol, phenytoin, and carbamazepine, induce CYP3A4 activity, and have been reported to produce a decrease in plasma methadone concentration. This may elicit withdrawal symptoms if plasma methadone concentration falls below a threshold necessary to suppress withdrawal. Conversely, other drugs such as, fluoxetine, flovoxamine, paroxetine, moclobemide, and amitriptyline inhibit one or several CYP isoforms, and have been reported to produce an increase in plasma methadone concentrations, which may, in turn, enhance opioid effects. Moreover, a large proportion of maintenance patients take benzodiazepines, in particular diazepam, concurrently with maintenance pharmacotherapies (Preston, *et al*., 1986). Diazepam, is a CYP3A4 substrate (Ketter, *et al.*, 1995), and has been shown in rat liver homogenates, and human liver microsomes, to inhibit the *N*-demethylation of methadone (Iribarne, *et al*., 1996). *In-vivo* diazepam has been shown to increase the magnitude of some physiological and subjective opioid effects of methadone (Preston, *et al*., 1986). However, the concurrent administration of diazepam has not been shown to alter the plasma concentration-time profile, nor the AUC of methadone (Pond, *et al*., 1982; Preston, *et al*., 1986) and so it is not clear whether a pharmacokinetic mechanism is responsible for these effects.

Pregnancy is also known to alter the metabolism of methadone. Pregnant women exhibit lower plasma methadone concentrations during the final trimester that cannot be attributed to changes in methadone dose and increase in body weight and fluid (Pond, *et al.*, 1985). Commonly pregnant MMT patients require a twice-daily methadone dose. This achieves a more stable plasma concentration-time profile across the inter-dosing interval than that achieved by daily dosing (Ward, *et al*, 1998b), and means that that the plasma methadone concentration is less likely to fall below the threshold concentration required to suppress opioid withdrawal.

Finally, factors such as age, renal and chronic liver disease have been shown to have *no* significant impact on the pharmacokinetics of methadone (see Eap, 2002; Ward, *et al.,* 1998b), and therefore minimal impact on the delivery of MMT.

1.4.1.3. Pharmacodynamic effects during repeated dosing of methadone

The general effects produced by the ingestion of opioid drugs, and the neurobiological basis of such effects, have been previously described in section 1.2.2. The following section will review those studies that have examined the effects of methadone in maintenance patients. It will highlight the parameters that are amenable to measurement, and as such, have been shown to reliably reflect methadone's effects.

In an early study Martin (1984) investigated the physiological effects of methadone administered to six maintenance patients before, during and after treatment with oral methadone. Following 8 weeks of maintenance treatment systolic and diastolic blood pressure, heart rate, pupillary diameter and respiratory rate were all depressed, while body temperature was elevated in comparison to pretreatment. Furthermore, Gritz and colleagues (1975) compared the physiological effects of methadone in ten MMT patients (median dose 65 mg/day; enrolled for a median of 5 months) to ten abstinent patients (abstinent for median of 2 months), and 5 non-drug using controls. Physiological indices were measured in one session and included sublingual temperature, systolic and diastolic blood pressure whilst seated, heart rate and respiratory rate. The mean heart rate of the abstinent group (76.6±9.5: mean±sd) was significantly ($P<0.05$) higher than for both the methadone maintained patients (66±10.9) and control subjects (66.2±6.1), but the methadone and control groups did not differ. Furthermore, the mean respiratory rate for the methadone patients (mean±sd: 13.6±2.8) was significantly ($P<0.01$) lower than the abstinent group (17.4±3.2), but not the controls (16.4±2.3). There were no significant between group differences for blood pressure and temperature.

Several authors have have undertaken repeated measures of methadone's effect in MMT patients across one inter dosing interval. McCaul and colleague (1982) studied the effects of methadone (35 to 80 mg orally), compared to placebo, on seven ambulatory patients for six hours post-dose, and on four sedentary patients (to allow for more sensitive analysis of effects), for four hours post dose. The authors measured pupil diameter, heart rate, blood pressure, temperature, euphoria using the Morphine Benzedrine Group Subscale (MBG) of

the Addiction Research Centre Inventory (ARCI: Haertzen, *et al*., 1963), and mood, using the Profile of Mood States (POMS: McNair, *et al*., 1971). The only objective measure to show a significant change following methadone administration in the ambulatory patients was pupil diameter, which decreased by 0.5 mm in comparison to pre-dose values. MBG scores were generally higher post dose, but this was not a reliable effect for all subjects, and there was no significant effect of methadone administration on any of the POMS sub scales. In comparison, in sedentary subjects methadone administration produced reliable changes for all physiological measures, but not subjective measures. Pupil diameter significantly decreased (1.25 mm) following administration to be at a minimum approximately 90 minutes post dose, and skin temperature increased to a maximum at the same time. With regard to cardiovascular measures, at the end of the 4-hour testing session heart rate was significantly lower than predose (by 6-8 beats/min), and systolic blood pressure had fallen from a mean of 114.6 mmHg predose to be 110.6 mmHg at the end of the session. While baseline respiratory rates were low (12 to 15 breaths/min), respiratory rate decreased significantly (1.2 breaths) to be at a minimum 2 hours post dose.

Dyer and White (1997) investigated the temporal pattern of symptom complaints across a 24-hour inter-dosing interval in 51 MMT patients (mean dose 53.6 mg; range 15-140 mg). They used the methadone symptom checklist (MSC) to record the presence and intensity of symptom complaints; this comprised three groups of 16 items, indicating opioid withdrawal symptoms (e.g., muscle pain, bone and joint pain), direct opioid effects (e.g., constipation, itchy skin, dry mouth) and symptoms that could be characteristic of either withdrawal or direct opioid effect (e.g., excessive sweating, lethargy, sexual problems). Subjects recorded the intensity of symptoms using a 5-point Likert scale; none, mild, moderate, severe and extreme. The MBG was also used to measure positive opioid effect (e.g., euphoria). The instruments were administered immediately prior to the dose, every two hours for 12 hour post dosing, and then once approximately 24 hours after dosing. They found a significant temporal change in withdrawal intensity across the inter-dosing interval. The mean intensity of the 16 withdrawal items (maximum score 64) fell from a high immediately prior to dosing (mean±sd: 11.8±11.8), to a minimum 2 hours post dosing (4.7±7.7), and then increased to peak immediately prior to the next dose (13.6±11.9). Mean MBG scores and some direct effects (itchy skin and itchy nose, pleasant feeling) peaked 2 to 4 hours post dosing, and were minimal at dosing times. Symptoms such as constipation and dry mouth remained stable across the entire dosing interval. They then

used a median split of peak withdrawal intensity to separate subjects into subgroups of holders and non-holders (n=25 in each group). The mean daily methadone dose and methadone dose to weight ratio were higher for non-holders (65.5 mg and 0.88 mg/kg, respectively), than holders (42.2 mg and 0.57 mg/kg, respectively). The mean withdrawal intensity was lower and more stable across the dosing interval for holders, than nonholders. The mean MBG score for nonholders was of the same magnitude as for holders at peak (2 hours post dose), but declined more rapidly than for holders across the dosing interval. This study clearly demonstrated that there is a change in pharmacodynamic response to methadone across the inter-dosing interval, and that this methodology is sensitive enough to be able to identify patients who do not respond well to methadone (non-holders). However, as the authors did not collect plasma it was not possible to determine if differences between holder and nonholders could be attributed to variation in methadone disposition.

In a follow-up study, Dyer and colleagues (1999) carried out a comprehensive examination of the steady state pharmacodynamics and pharmacokinetic of methadone in 18 MMT patients (nine of whom were self reported non-holders) maintained on 7.5 to 130 mg methadone daily. During one 24-hour inter-dosing interval they collected 13 blood samples to measure plasma racemic methadone concentrations, and on 11 of these occasions they measured a number of objective (physiological) and subjective measures of opioid effect. Objective measures included pupil diameter, measured using a standard video camera, and respiratory rate. Subjective measures included: withdrawal severity (using the MSC); euphoria (using the MBG); mood (using the POMS), and pain threshold (a measure of analgesia, operationalised as the threshold at which increasing electrical stimulation of the ear lobe was reported as painful). These measures were also collected from ten non-drug using controls. They reported that temporal changes in plasma racemic-methadone concentrations were inversely related to withdrawal severity, and physiological measures, and directly related to MBG scores and pain threshold values. Specifically, pupil diameter declined from a maximum pre-dose (approximately 5mm) to be at a minimum (3.5-4mm) at 4-5 hours post-dose, and then returned to pre-dose values by 12-hour post dose. Respiratory rate also declined from pre-dose (17 breaths/min) to be at a minimum (13 breaths/minute) 3 hours post dose. Withdrawal severity declined from pre-dose to a minimum at the time of putative peak plasma racemic methadone concentrations (2-3 hours post dose), and then increased again to greater than baseline levels by the end of

the dosing interval. Non-holders exhibited higher withdrawal scores than holders throughout the dosing interval. In contrast to MMT patients control subjects exhibited no such changes in these measures. Mitchell and colleagues (2003), used similar methods as described for the latter study in their comparison of the pharmacodynamics and pharmacokinetics of methadone and slow-release oral morphine, and reported pharmacodynamic data for MMT patients that were consistent with those reported by Dyer and co-workers.

Thus, it is clear from the reviewed studies that MMT patients, even if they are at apparent steady state, may exhibit temporal fluctuation in opioid effect across the 24 hour dosing interval that reflect changes in underlying plasma methadone concentration. Dyer and colleagues (1999) showed that the fluctuation in plasma methadone concentration over the 24-hour dosing interval was significant (mean peak to trough plasma concentration ratio of 1.7), and thus there is significant potential for marked changes in opioid effect and withdrawal across the dosing interval. Hence it is expected that withdrawal severity, pupil diameter, heart rate, blood pressure and respiration will decrease following methadone ingestion. On the other hand, measures of subjective effect, MBG and pain threshold are likely to increase following methadone ingestion.

1.4.2. Effectiveness of methadone maintenance treatment

There is considerable evidence from a number of controlled trials, longitudinal studies and observational studies that methadone substitution treatment is associated with reductions in illicit opioid use, criminal behaviour, deaths due to overdose, and also with reduced risk of spread of HIV/AIDS (Bale, *et al.*, 1980; Ball and Ross, 1991; Dole, *et al*., 1969; Gottheil, *et al*., 1993; Grönbladh, *et al*., 1990; Gunne and Grönbladh, 1981; Newman and Whitehill, 1979; Strain, *et al*., 1993; Vanichseni, *et al*., 1991; Yancovitz, *et al*., 1991). A recent systematic review of all controlled clinical trials demonstrated significant advantages of MMT over non-opioid substitution treatment (such as placebo medication, withdrawal or detoxification, drug free medication, and wait list controls), in terms of retention in treatment, and daily heroin use (Mattick, *et al*., 2002a). The advantage for methadone in reducing criminal activity, and the ability of methadone to prevent deaths was not statistically different from control conditions (Mattick, *et al*, 2002a). However, there are other reports that the death rate is substantially reduced amongst opioid users enrolled in MMT compared to untreated users (Capelhorn, *et al*., 1996; Davoli, *et al*., 1993;

Grönbladh, *et al*., 1990; National Institutes of Health, 1997). For example, Davoli and colleagues (1993) revealed that during the first year after leaving MMT, opioid users were 8 times more likely to die from overdose than if they remained in treatment. From the second to third year out of treatment users were 3 times more likely to overdose than patients who remained in treatment. Furthermore, The National Treatment Outcome Research Study (NTORS), undertaken in the United Kingdom, reported that after one year of treatment (MMT and residential rehabilitation), rates of criminal behaviour by drug users had reduced to approximately half that seen at entry to treatment. These improvements were maintained at two to five year follow-ups, where criminal involvement range from only 20 to 28 % (Gossop, *et al.*, 2000).

Moreover, there is also considerable evidence that methadone maintenance treatment protects opioid dependent patients from HIV (readers are directed to review by Ward, *et al*., 1998a). Such evidence comes from early studies that compared groups in methadone substitution treatment to the general population of untreated drug users (Metzger, *et al*., 1993; Moss, *et al.*, 1994; Serpelloni, *et al*., 1994), plus more recent studies assessing reductions in risk behaviors (e.g., Capelhorn & Ross, 1995). The effectiveness of MMT in this regard is due to a number of factors, not least, its success in retaining patients in treatment, in reducing drug use, and reducing high risk injecting and sexual behaviours. For example, studies have demonstrated reductions in risky behaviors associated with increased spread of blood borne viruses, such as the frequency of injecting (Ball, *et al*., 1988; Ball & Ross, 1991), and sharing injecting equipment (Capelhorn and Ross, 1995; Longshore, *et al.*, 1993). Methadone maintenance programs that advocate the use of methadone dosage greater than 60mg daily and that adopt maintenance rather than abstinence as a goal for treatment are more likely to be effective in reducing the rate of HIV infection amongst injecting opioid users (Ward, *et al*, 1998a). Thus, the protective effect of MMT only holds true for those who remain in MMT and while they are receiving adequate doses of methadone.

There is also good evidence for the cost effectiveness of MMT obtained from studies undertaken in the United Kingdom and USA, which show that the benefits outweigh the cost of providing treatment. For example, a study undertaken in the USA has shown that the six-month costs to society were $21,500 for an untreated drug user, $20,000 for an imprisoned drug user, and $1,750 for a user in outpatient methadone treatment (Yoast, *et*

al., 2001). Furthermore, the NTORS study reports that for every one pound spent on treatment there is a three pound saving in criminal justice processing costs alone (Gossop *et al*., 2000) (For a more detailed review the reader is directed to Ward and Sutton, 1998).

1.4.3. Factors that determine success in MMT

A number of treatment characteristics have been shown to be critical factors in determining successful outcome in MMT. Ball and Ross (1991) showed that the major determinant of successful outcome was the length of time patients remained in treatment; those who dropped out of treatment were more likely to relapse to intravenous opioid use. Some of the program factors that are reported to increase retention in methadone maintenance programs include the adequacy of the methadone dose (Magura, *et al*., 1998; Saxon, *et al*., 1996; Ward, *et al*., 1998b; 1999), degree of staff training, a clinic policy that emphasizes maintenance not abstinence (Bell, *et al*., 1995; Capelhorn *et al*., 1993), and provision of good quality ancillary services, such as counselling and medical services (Ball & Ross, 1991; McLellan, *et al*., 1988). Generally speaking, the model of MMT provided in the randomized controlled trials that have provided evidence for the effectiveness of this treatment have included a range of ancillary services, including counseling, psychosocial services, medical services, and psychiatric care. Thus, the degree that maintenance programs move away from such a model will impact on their effectiveness.

Although many regard MMT as a short-term strategy for the treatment of opioid dependence, the reality is that many patients remain on methadone for many years (Kreek, 1996). The effectiveness of substitution treatment is also evident across a variety of cultural and ethnic groups, and social contexts (Marsch, 1998; Ward, *et al*., 1998b). Hence, it is clear that if patients can be attracted and retained in maintenance treatment, there are significant benefits for the individual concerned and the wider community

1.4.4. Limitations of methadone maintenance treatment

In spite of the well-documented effectiveness of methadone in treating opioid dependence, methadone maintenance is not without limitations (Dyer and White, 1997; Dyer, *et al*., 1999; Ling, *et al*., 1994; Mattick, *et al*., 1998). As methadone is usually administered once daily, patients are required to either attend the clinic on a daily basis to pick up their dose, which can be disrupt an individual's life; or are granted take away privileges, with the resultant risk of diversion (Bell & Zador, 2000). Other limitations relate to the individual

variability in the pharmacokinetics of methadone, and factors related to the variability in response to a given dose (Angaard, *et al*., 1975; Horns, *et al*., 1975) that necessitates an individualised approach to dosing in order to provide a balance between adequate suppression of withdrawal and unacceptable adverse effects. The next section discusses a number of factors that impact on the effective delivery of MMT, including the often unpredictable relationship between methadone dosage, plasma methadone concentration, and therapeutic outcome; the relatively high incidence of persistent side effects amongst MMT patients (Dyer and White., 1997; Judson & Goldstein, 1982; Kreek, 1973; 1978; Longwell, *et al*., 1979; Martin, *et al*., 1973), and complaints of breakthrough withdrawal in patients receiving what appear to be adequate methadone dosages (Dyer and White, 1997; Dyer, *et al*., 1999; Nilsson, *et al*., 1983).

1.4.4.1. The relationship between methadone dosage, plasma concentration, and treatment outcome

The importance of adequate dosage in MMT has been highlighted by research that has investigated the relationship between methadone dose and clinical outcomes, such as reduction in illicit drug use, adequate withdrawal suppression, and retention in treatment. With few exceptions evidence from these studies suggests that the effectiveness of methadone is dose related. Methadone doses above a threshold dosage of about 50-60 mg daily were related to better program retention and reductions in illicit drug use when compared to lower dosages (Ball & Ross, 1991; Capelhorn and Bell, 1991; Capelhorn, *et al*., 1993; Del Rio, *et al*., 1997; Fisher & Anglin, 1987; Johnson, *et al.*, 2000; Strain, *et al*., 1993). For example, Ball and Ross found that illicit heroin use declined as a function of increasing methadone dose (10 to 90 mg). Furthermore, Craig (1980) advocated an absolute minimum dosage of 30 mg methadone daily, suggesting that doses lower than this are unsuitable in MMT. All this evidence appears to support the tenets of Dole and Nyswander's (1967) original model of MMT, in which they proposed a dosage protocol of 50-150 mg/daily tailored to individual needs to suppress withdrawal and the effects of subsequently administered intravenous opiates.

Notwithstanding the above evidence, there exists conflicting literature concerning the relationship between dosage and the resultant plasma concentration of methadone. Some authors have shown that there is a linear relationship between dosage and plasma methadone concentration (Bell, *et al*., 1988; Loimer and Schmid, 1992; Wolff, *et al.*,

1991), while others have reported large inter-subject and intra-subject variations in plasma methadone concentrations obtained from patients maintained on the same methadone dose (Angaard, *et al*., 1975; Horns, *et al*., 1975; Kreek, 1973). Even when it is shown that there is a significant relationship between methadone dosage and plasma methadone concentrations, dosage only explains about 50 to 60 % of the variability in concentration of methadone (Eap, *et al*., 2000; Foster, *et al*., 2000). For example, in a study of 18 MMT patients Foster and colleagues (2000) measured plasma (R)-(-) and (S)-(+) methadone steady state concentrations and found a significant correlation between methadone dose and area under the curve (AUC) for (R)-(-) methadone (r^2=0.68, p<0.0001), and (S)-(+) methadone (r^2=0.68, p=0.002). Yet there was still a 4-to 6 fold variation in steady state plasma methadone concentrations when plasma concentration values were normalised to 70 mg racemic methadone. Such variability is likely to reflect inter-individual variability in clearance and CYP3A4 activity.

Nevertheless, many investigators have attempted to identify a threshold plasma methadone concentration necessary for effective maintenance. Values ranging from 50 to 400 ng/ml of racemic methadone has been advocated by investigators (Bell, *et al*., 1988; Dole, 1994; Loimer and Schmid, 1992; Inturrisi and Verebely, 1972; Tennant, *et al.*, 1983; Verebely, *et al*., 1975). Although Dole (1994) advocated a threshold trough plasma methadone concentration of 100 ng/ml, he also suggested that for a methadone dose to effectively block opioid craving a plasma racemic-methadone concentration of above 200 ng/ml should be attained at all times during maintenance treatment. This is consistent with a previous report by Holmstrand and colleagues (1978) that patients on 60 to 85 mg methadone daily who attained plasma steady state racemic-methadone concentrations above 200 ng/ml had more favourable outcome in MMT, as shown by lower illicit drug use and superior psychosocial functioning, than patients with lower plasma racemic-methadone concentrations. Yet in contrast, a number of researchers have reported no clear evidence of a relationship between plasma methadone concentration and clinical outcome on MMT (DeVos, *et al.*, 1996; Horns, *et al.*, 1975; Torrens, *et al*., 1998). Thus, in the aforementioned studies there have been complaints of inadequate withdrawal suppression and adverse opioid effects from MMT patients maintained on a range of methadone doses, and varying plasma racemic methadone concentrations.

The findings outlined in this section have important implications for treatment delivery. That is, despite the evidence to suggest that high dosages are generally related to superior treatment outcome on methadone maintenance (Ball & Ross, 1991; Capelhorn, *et al.*, 1991; Capelhorn, *et al.*, 1993; Del Rio *et al.*, 1997; Fisher & Anglin, 1987; Strain, *et al.*, 1993; Johnson, *et al.*, 2000), variability in methadone pharmacokinetics will mean that some patients maintained on high doses will still experience withdrawal (Dyer, *et al.*, 1999; Tennant, *et al.*, 1983). Therefore, an individual approach to dosing is required to enhance treatment effectiveness and limit adverse effects that might detrimentally affect treatment outcome and retention (Ward *et al.*, 1998b).

1.4.4.2. Symptom complaints during methadone maintenance treatment

As previously mentioned the primary objective of maintenance treatment is to achieve adequate suppression of withdrawal across the inter-dosing interval, without causing unacceptable opioid side effects. There are reports that methadone prescribed in high doses (80-120 mg/daily) for prolonged periods of time (up to 14 years) produces few toxic effects and generally minimal side effects (Kreek, 1973; 1978). However, some MMT patients report a number of symptoms that may be directly attributable to the pharmacological actions of methadone, and/or the interaction of these direct effects and co-existing medical disorders (Crofts, *et al.*, 1993; Ward, *et al.*, 1998d), that may be distressing and which can detrimentally affect treatment outcome.

Generally, many of the common symptom complaints attributable to the direct effects of methadone (e.g., dry mouth, sedation and euphoria) that occur in the initial stabilization phase of treatment will dissipate as tolerance develops and as the patient reaches steady state plasma methadone concentrations. However, some patients may complain of side effects that persist beyond the stabilization phase of MMT (Dyer and White., 1997; Judson & Goldstein, 1982; Kreek, 1973; 1978; Longwell, *et al.*, 1979; Martin, *et al.*, 1973). For example, Kreek (1973) followed up a group of patients maintained on high dose methadone (80 to 120 mg/daily) and reported that even after three years of MMT, 48% of patients still experienced excess sweating, 17% constipation, 16% sleep difficulties, 22% decrease libido and 14% impotence. The most common and persistent side effects reported in the literature include constipation, sweating, painful joints and bones, insomnia, menstrual irregularities and sexual dysfunction (impotence) (Dyer and White, 1997; Judson and Goldstein, 1982; Kreek, 1973; 1996; Longwell, *et al.*, 1979; Schall, *et*

al., 1996). Importantly, some of these symptoms may reflect withdrawal as a consequence of inadequate dosing and/or low plasma methadone concentrations or as a consequence of a rapid decline in plasma methadone concentrations (Dyer, *et al.*, 1999; Schall, *et al.*, 1996) – the latter phenomena are discussed in more detail in the next section. Other reported side effects of methadone ingestion include generalized cognitive impairment (Darke, *et al.*, 2000; Davis, *et al.*, 2002; Mintzer and Stitzer, 2002), and other cognitive changes, such as trouble thinking clearly and confusion (Dyer and White, 1997); weight gain, appetite changes (Dyer and White, 1997) itchy skin (Dyer and White, 1997) and electrocardiogram abnormalities (e.g., QT prolongation (Deamer, *et al.*, 2001; Katchman, *et al.*, 2002). Furthermore, it has been shown that the intensity of some of these direct effects (e.g. itchy skin and nose) varies across the inter-dosing interval, and is most pronounced at the time of putative peak plasma methadone concentration (Dyer and White, 1997).

1.4.4.2.1. The phenomenon of 'not-holding'

A significant proportion of methadone maintenance patients at apparent steady state regularly complain of breakthrough withdrawal during the second half of the inter-dosing interval, that is, they complain that their dose does not adequately 'hold them'. This phenomenon may occur even when an individualised approach to dosing is used and significant patient input about dosing is permitted (Dyer and White, 1997; Dyer, *et al.*, 1999). Although, as previously mentioned, methadone dosages greater than 50 – 60 mg daily (Ball & Ross, 1991; Capelhorn, *et al.*, 1991; Capelhorn, *et al.*, 1993; Del Rio, *et al.*, 1997; Fisher & Anglin, 1987; Johnson, *et al.*, 2000; Strain, *et al.*, 1993), and higher plasma methadone concentrations (Holmstrand, *et al.*, 1978; Dole, 1994), are generally related to superior treatment outcome, there will still be some patients who will complain of withdrawal even when administered apparently adequate dosage (Dyer, *et al.*, 1999; Tennant, 1987). Often such patients have been on maintenance treatment for some months, have requested dose increases because they are experiencing withdrawal, but have decided to reduce their dose because of unacceptable methadone side effects, such as sedation.

A cross sectional survey of 114 participants enrolled in the South Australian Methadone Program showed that up to a third of patients reported the consistent failure of their methadone dose to suppress withdrawal across the 24-hour inter-dosing interval (Dyer and

White, 1997). The group of 'non-holding' patients received a higher mean daily methadone dose (65mg) than those who were reportedly 'holders' (42.5mg). Neither group could be differentiated on gender, age, illicit drug use or time on the methadone program. Furthermore, the non-holders reported a greater severity of opioid withdrawal than the holders (Dyer and White, 1997). Although not representative of patients who participate in the South Australian Methadone Program, this study highlights the possible prevalence of this problem in methadone maintenance programs. It is important, therefore, that this problem is addressed, as persistent complaints of the dose 'not holding' makes it more likely that patients will resort to illicit drug use and dropout out of treatment prematurely (Holmstrand, *et al*., 1978; Schall, *et al*., 1996).

A number of purported mechanisms have been put forward as responsible for the phenomenon of not holding in the context of methadone maintenance. Patients will experience withdrawal at the end of the inter-dosing interval if plasma methadone concentrations fall below a trough or threshold concentration necessary to suppress withdrawal symptoms (Bell, *et al*., 1988; Dole, 1994; Holmstrand, *et al*., 1978). Variability in the activity of CYP3A4, either as a consequence of intrinsic factors (i.e., variability in expression of hepatic CYP3A4; Ketter, *et al*., 1995) or due to induction caused by a number of co-administered drugs (see section 1.4.1.2.4), can increase methadone clearance, thereby shortening the elimination half life of methadone and thus producing lower than expected trough plasma methadone concentrations at the end of the inter-dosing interval. Furthermore, it has been proposed that differences in the degree of withdrawal experienced by nonholders and holders might be due to differences in the rate of decline of plasma methadone concentrations from peak to trough, rather than plasma methadone concentrations falling below some absolute threshold plasma concentration (Dyer, *et al*., 1999; Nilsson, *et al*., 1983; Walton, *et al*., 1978). Two studies have investigated the pharmacokinetic basis of 'non holding' and will be described in detail.

Nilsson and colleagues (1983) examined the disposition of methadone in eight patients designated as therapeutic failures (characterized by complaints of withdrawal, presence of illicit drug in urine, and poor progress with social rehabilitation), who had been enrolled in MMT for between 10 and 31 months, and who were administered a mean methadone dose of 75 mg (range 50–100 mg). A control group of 12 unselected patients (mean dose=45 mg) was also studied. Subjects were admitted to an inpatient facility and on the day of

testing had blood taken 0, 1, 2, 4, 6, 8, 12, 24, and 48 hours after been dosed with methadone. There were no differences between the two groups in steady state plasma methadone concentrations, total clearance of methadone (mean±sd: 104 ± 36 ml/min vs 111 ± 36 ml/min), and renal clearance (mean±sd: 19 ± 10 ml/min vs 13 ± 8 ml/min). However, there were differences in the volume of distribution, with the therapeutic failure group exhibiting a smaller volume of distribution (mean±sd: 2.74 ± 0.96 L/kg) than the control group (mean±sd: 4.2 ± 0.3 L/kg), which resulted in a significant difference in terminal half life values between the groups: therapeutic failures exhibited shorter half lives (mean±sd: 24 ± 2.6 hours) than patients in the control group (mean±sd: 34 ± 7 hours). Hence, therapeutic failures exhibited different plasma methadone concentration-time profiles than patients in the control group, characterised by a higher peak plasma concentration (C_{max}) than control subjects, and more rapid decline in methadone plasma concentrations during the distribution phase. The authors argued that this fluctuation in plasma methadone concentration made it more likely for patients to experience adverse opioid effects (i.e., nodding, respiratory depression) at the time of C_{max} and a prolonged withdrawal period near the end of the inter-dosing interval, when plasma methadone concentrations fell below a threshold concentration. During this time they would be likely to seek illicit opioids to compensate. The treatment strategy they advocated was the use of a shorter dosage interval (i.e., split dosing) or the use of a longer acting opioid drug such as LAAM in order to flatten the plasma methadone concentration-time profile.

Dyer and colleagues (1999), in their investigation of the steady state pharmacodynamics and pharmacokinetic of methadone in MMT patients, also compared the plasma pharmacokinetics of racemic methadone between non-holders and holders. The groups could not be differentiated on any demographic variable, including mean methadone dosage, and peak or trough plasma racemic methadone concentration (249 ng/mL versus 257 ng/mL), or apparent plasma clearance. However, notably the nonholder group exhibited more severe withdrawal and greater temporal variation in withdrawal scores across the dosing interval than holders; withdrawal severity was at its lowest at the time of putative peak plasma methadone concentration for both groups (i.e., 2-3 hours post dose). When the authors excluded data from two patients classified as holders who had tested positive for opioids in the pre-study urine screen, they found a greater maximum rate of decline in plasma racemic methadone concentrations during the period from peak to trough concentrations in non-holders (mean±sd: 74.5±40.4 ng/mL·hr) than holders (mean±sd:

42.1±20.8 ng/mL·hr). They also found a significant correlation between maximum rate of decline in plasma racemic methadone plasma concentration and the mean number of withdrawal symptoms during this period ($r = 0.67$, $P < 0.001$).

Dyer and colleagues went on to demonstrate that the severity of withdrawal (number of symptoms reported) and a number of direct opioid effects, such as, pupil diameter and subjective pain response, were related to plasma methadone concentrations across an inter-dosing interval. They were able to fit the sigmoid Emax model to plasma concentration and effect data and found large slope factors for severity of withdrawal (mean±sd: 5.4±1) and positive opioid defect, MGB (5.1±1), but much smaller slope factors for pain response (2.8±0.7) and pupil diameter (1.2±0.1). The steep plasma concentration-effect relationships for withdrawal and positive opioid effects means that small changes in plasma methadone concentration translate into relatively large changes in response. This would be exaggerated in the non-holders who exhibited greater rate of decline than holders. Despite methadone's long terminal half-life, the fluctuation in the plasma concentration across the 24 h inter-dosing interval is significant, with a mean peak to trough plasma concentration of 1.7 (Dyer, *et al*., 1999), and hence there is potential for marked changes in opioid effect and withdrawal over a dosing interval. These data can be used to provide a pharmacokinetic explanation for the difference found in the severity of withdrawal exhibited by therapeutic failures and control subjects in Nilsson's earlier study (Nilsson, *et al*., 1983). That is, therapeutic failures in their study would be more likely to exhibit withdrawal because of the more rapid rate of decline in plasma methadone concentrations during the period between peak and the 24-hour trough.

The prevalence of non-holding in methadone maintenance is an important clinical issue for, if not addressed, a number of patients are likely to resort to unsanctioned drug use, and/or withdraw from treatment (Holmstrand, *et al*., 1978; Schall, *et al*., 1996). There are several clinical options for these patients. Increasing the methadone dose to achieve higher plasma concentrations may be successful in those patients that have plasma methadone concentrations below the putative threshold necessary to suppress withdrawal. However, for those individuals who are non-holders because of a more rapid rate of decline in plasma methadone concentration, such a strategy would only result in higher peak plasma concentrations and thus the increased likelihood and severity of adverse effects, such as respiratory depression, sedation and constipation, without necessarily addressing the

problem of variability in plasma methadone concentration. It is likely these patients will still experience unacceptable withdrawal at the time of trough methadone concentration (Dyer, *et al.*, 1999; Nilsson, *et al.*, 1983). Another possible option is to administer methadone in divided doses in order to flatten the plasma concentration-time profile across the inter-dosing interval and therefore lessen the rate of decline in plasma concentration of active drug (Dyer, *et al.*, 1999; Walton, *et al.*, 1978). However, this may be impractical where dosing needs to be supervised, which will increase the costs associated with the program; and where take-away privileges are granted, may result in increased diversion of methadone. An alternative to these strategies is to use a maintenance drug with different pharmacokinetics than methadone that lessens the fluctuation in plasma opioid concentrations over the doing interval. The drugs of interest include opioid drugs that have a longer duration of action than methadone, such as the partial agonist buprenorphine, and the full agonist LAAM. These alternatives will be discussed in more detail below.

1.4.4.2.2. Affective states

The incidence of psychiatric disorders, in particular depressive and some anxiety disorders (e.g., generalized anxiety disorder), and mood disorders, is higher among opioid users than their estimated rates in the general population (Ward, *et al.*, 1998c). Although, on the whole, individuals in maintenance treatment exhibit less psychiatric distress than their untreated counterparts (Regier, *et al.*, 1990), there is evidence that depressive disorders and some anxiety disorders are still common (Darke, *et al.*, 1994; Dorus and Senay, 1980; Strain, *et al.*, 1991). Studies that have examined the prevalence rates of depression in opioid dependent patients enrolled in treatment have reported current prevalence rates of between 10 to 20 % (Review by Nunes, *et al.*, 1994). Moreover, Dyer and colleagues (2001) demonstrated that patients in MMT exhibited greater mood disturbance than non-drug using controls. The presence of persistent negative mood states, such as depression, anger and anxiety have been shown to increase the perceived severity of subjective withdrawal symptoms, to induce cravings for opioids (Childress, *et al.*, 1994; Phillips, *et al.*, 1986), and to lead to poor treatment outcome amongst such patients (Kanof, *et al.*, 1993; Unnithan, *et al.*, 1992). Thus, an important goal of maintenance treatment is to normalise mood state across the inter-dosing interval in order to minimise the likelihood of poor treatment outcome.

However, a number of studies have demonstrated fluctuations in mood state among MMT patients following their first dose and at apparent steady state (Dyer, *et al*., 2001; Hiltunen, *et al*., 1999; Price, *et al*., 1975). The administration of methadone to patients exhibiting overt signs of opioid withdrawal at entry to a methadone detoxification program produced rapid and positive changes in mood state, as measured by the POMS (Price, *et al*., 1975). Furthermore, Dyer and colleagues (2001) demonstrated that in MMT patients total mood disturbance fluctuated across the inter-dosing interval in response to plasma methadone concentrations, and was at its lowest at the time of apparent peak plasma methadone concentration (2 to 3 hrs post dosing), but at its greatest at the time of trough plasma methadone concentrations. Furthermore, mood disturbance was particularly pronounced in patients who consistently reported the failure of their methadone dose to hold (Dyer, *et al*., 2001). Thus, those patients who report breakthrough withdrawal during the second half of the inter-dosing interval are likely to experience significant mood disturbance at the time of trough plasma methadone concentrations, and hence are at risk of unsanctioned drug use and poor treatment outcome. The issue of mood disturbance among patients in treatment for opioid dependence is further explored in Chapter Six.

1.4.5. Summary

Methadone was originally selected as a pharmacotherapy for opioid dependence because of its high oral bioavailability and long half-life, which permits once daily oral dosing, with a relatively small fluctuation in peak to trough plasma concentration across the 24 hour dosing interval. There is considerable evidence from a number of sources that MMT confers many benefits to those who remain in treatment. Treatment outcome is generally dosage related, with higher methadone dosages associated with superior treatment outcomes. However, the individual variability in response to a given dose of methadone can pose significant clinical challenges for physicians attempting to find the optimum dose for individual patients. That is, a methadone dose needs to be chosen that is effective in suppressing withdrawal across the inter-dosing interval, without causing unacceptable side effects. Hence an individualised approach to dosing is required to enhance treatment effectiveness and limit adverse effects. However, despite the use of such an approach to dosing, a significant proportion of patients will consistently report breakthrough withdrawal, particularly during the second half of the 24-hour inter-dosing interval. Thus, for individuals who experience such symptoms, either because they exhibit more rapid rate of decline in plasma methadone concentration than expected, and therefore experience

withdrawal symptoms at apparently adequate doses (non-holders), or because they experience adverse effects on doses required to suppress withdrawal, an alternative pharmacotherapy may be required to more effectively manage their dependence.

Moreover, two important methodological issues have emerged from the studies reviewed above (Dyer, *et al*, 1999; Nilsonn, *et al*., 1983). First, methadone is administered as a racemate. As there are marked stereoselective differences in the disposition of each enantiomer (Foster, *et al*, 2000; 2004; Kreek, *et al.,* 1979), the measurement of racemic-methadone may not accurately reflect the plasma concentration of the active (R)-(-) enantiomer. Thus, before one can deduce that there is a pharmacokinetic mechanism responsible for the difference found between nonholders and holders, it will be necessary to repeat the above analyses using the individual enantiomers, in particular the active (R)-(-) enantiomer. Secondly, Dyer and colleagues' (1999) study highlights the importance of collecting both pharmacokinetic and pharmacodynamic data, in particular the measurement of withdrawal severity, across the entire interdosing interval, in order to examine differences between holders and nonholders. Such methodology would be beneficial in examining the efficacy of using alternative pharmacotherapies for treating those patients who find treatment with methadone sub-optimal.

1.4.6. Alternatives to methadone

A number of alternative pharmacotherapies have been investigated that promise to provide increased flexibility to prescribing physicians, and to also improve patient choice, which are likely to help attract patients to maintenance treatment and improve retention (Mattick, *et al.*, 1998). These include heroin (Ghodse, *et al*., 1990; Hartnoll, *et al*., 1980), and slow release oral morphine (Mitchell, *et al*., 2003). However, as many of the limitations of methadone revolve around the necessity to daily dose, and inter-individual variations in pharmacokinetics and response to a given dose, then opioids with a longer duration of action would be desirable alternatives. Such drugs include buprenorphine, a synthetic opioid with mixed agonist and antagonist properties, which can be administered alternate daily to some patients, and the full opioid receptor agonist LAAM, which is the main focus of this thesis. The next section will provide an overview of buprenorphine, highlighting the advantages and disadvantages associated with its use in the treatment of opioid dependence.

1.4.6.1. Buprenorphine

Buprenorphine is a synthetic opioid with both agonist and antagonist properties. It has affinity at all three opioid subtypes, with equal and greater affinity for both μ and κ, than δ opioid receptors (Richards and Sadee, 1985). It is a partial agonist at μ opioid receptors, producing milder, less sedating and less euphoric effects than full opioid agonists (Jasinski, *et al*., 1978; Lewis, 1985), and is also an antagonist at κ opioid receptors.

Buprenorphine is well absorbed when administered via the sublingual route, and has a bioavailability of between 30% to 55% (Bullingham, *et al*, 1982; Kuhlman, *et al.,* 1996); in contrast, when orally administered it has a bioavailability about half that of the sublingual formulation, due to first pass metabolism (Nath, *et al*., 1999). It is extensively metabolized by *N*-dealkylation to nor-buprenorphine, which is an active metabolite (Cone, *et al*., 1984). Following sublingual administration plasma concentrations peak at about 2 to 3 hours post dosing (Jasinski, *et al*., 1978: Lopatko, *et al*, 2003), and it has a variable terminal elimination half-life of approximately 26 hours (9-69 hours) (McAleer, *et al*., 2003). Buprenorphine dissociates very slowly from opioid receptor binding sites, which probably contributes to prolonged duration of action (Mattick, *et al*., 2003; Petry, *et al*., 1999). The latter attribute is also thought to be responsible for the very low level of withdrawal symptoms experienced following abrupt cessation of chronic dosing in comparison to other opioids, such as morphine (Fudala, *et al*., 1990; Lewis, 1985), and the difficulty in antagonizing the effects of buprenorphine once it has bound to opioid receptors (Kreek, 1996; Lehmann, *et al*., 1988).

Buprenorphine's pharmacodynamic profile is complex. Acute administration of buprenorphine produces typical μ opioid receptor effects, including analgesia, euphoria, sedation and miosis (Jasinski, *et al*., 1978; Walsh, *et al.,* 1993). Clinical studies have shown that buprenorphine exhibits ceiling opioid effects and a U shaped dose- response curve for some opioid effects (Jasinski, *et al.,* 1978; Walsh, *et al*., 1994). That is, with increasing doses its opioid effects increase in intensity until a plateau is reached, after which an increase in dose will attenuate the opioid effect. The effects of the doses producing these ceiling effects (8 to 32 mg) were similar to those produced by 60 mg oral methadone, but were more prolonged, lasting up to 48 hours after dosing (Jasinski, *et al.,* 1978). Buprenorphine produces only marginal respiratory depression, and indeed no other serious adverse effects, at doses for which equivalent doses of a full opioid agonist would

be lethal (Walsh, *et al*., 1994). Moreover, a number of studies have demonstrated the feasibility of administering buprenorphine sublingually on an alternate daily dosing schedule (Amass, *et al*., 1994; Fudala, *et al*., 1990; Johnson, *et al.*, 1995). This can be successfully achieved, with some patients, by doubling the normal daily dose on alternate days.

A recent Cochrane review of controlled studies comparing buprenorphine to methadone or placebo, examined the efficacy of using buprenorphine as a maintenance pharmacotherapy (Mattick, *et al*., 2002b). Buprenorphine was superior to placebo in its ability to retain patients in treatment and in suppressing heroin use. Methadone was more likely to retain patients in treatment, especially when considering flexible dosing regimes, which were considered the most similar to actual clinical practice. Methadone was also more effective in suppressing heroin use, especially if high doses (defined as 60-80 mg) were used. However, it was noted that as some patients prefer maintenance at a lower equivalent methadone dose (i.e., at 50 to 60 mg or even lower), such individuals might find buprenorphine treatment suitable. Moreover, the authors further commented that the relative poorer retention rates for buprenorphine, than methadone, may reflect the use of relatively slow induction schedules, and that given the safety profile of buprenorphine, faster induction to higher doses may improve retention.

Thus, buprenorphine possesses many characteristics that make it a suitable alternative to methadone in the maintenance treatment of opioid dependence. It exhibits a superior safety profile than full opioid agonists, reducing the dangers of respiratory depression to individuals in treatment, but also to less tolerant users who may acquire the drug illegally. Its partial agonist properties, and tendency to produce less physical dependence, makes it an ideal initial treatment for individuals who have a relatively mild and short history of dependency on opioids (Ling, *et al*., 1994). Furthermore, as it has a less severe withdrawal syndrome than full opioid agonists, it can be used to aid withdrawal prior to transfer to opioid antagonist treatment (Cheskin, *et al*., 1994). The administration of buprenorphine on an alternate daily dosing schedule to some patients provides a number of advantages over methadone in terms of reducing clinic attendance, reducing the need to provide take-away doses, and reducing the costs of treatment delivery (Ling, *et al*., 1994). Such a dosing regimen would be suitable for stable maintenance patients who are either employed or studying, and who might find daily dosing impractical.

Despite the many benefits outlined above, there are some patients who may not find buprenorphine a suitable alternative. In particular, because of its partial agonists characteristics, patients will not be able to experience the full intensity of opioid effect associated with high dose (> than 60 mg) methadone, and this may be a disincentive for some patients to commence buprenorphine treatment. Furthermore, transfer from methadone to buprenorphine can be problematic for the following reasons. Firstly, as buprenorphine can act as an antagonist in the presence of a full agonist, it is necessary to delay the first dose of buprenorphine until the first signs of withdrawal are experienced to avoid precipitating withdrawal (Bouchez, *et al*., 1998). Secondly, buprenorphine can elicit unacceptable withdrawal in patients taking methadone doses above 30 mg daily (Bouchez, *et al*., 1998; Breen, *et al*., 2003; June, *et al*., 1993; Ling, *et al*., 1994). Therefore, for those patients who consistently report breakthrough withdrawal whilst maintained on high dose methadone, transferring to buprenorphine may not be feasible. In order to achieve the transfer to buprenorphine, non-holders would need to gradually reduce their methadone dose until the target dose is reached (~ 30 mg), during which they are likely to experience an unacceptable increase in withdrawal severity.

Although buprenorphine is a feasible alternative to methadone for many patients, it is clear that there are some individuals who cannot be readily transferred to buprenorphine. For these patients the feasibility of using a long acting full opioid agonist, such as LAAM, that has the potential to provide prolonged suppression of withdrawal, and therefore potential to attract and retain these patients in treatment, should be investigated.

1.5. Levo-Alpha-Acetylmethadol [LAAM]

LAAM was approved by the US Food and Drug Administration in July 1993 for the maintenance treatment of opioid dependence (FDA, 1993). Its approval was hailed as a significant advance in the treatment of opioid dependence as it offered patients and physicians an alternative full opioid agonist treatment to methadone (Ling, *et al*, 1994; Prendergast, *et al*., 1995: Rawson, *et al.*, 1998). The following were put forward in support of its introduction. First, due to its prolonged duration of action, and thus less frequent dosing schedule, there is a reduced need for clinic or pharmacy visits, hence reducing the impact on patients' lives. It was thought LAAM would particularly help those more stable individuals (i.e., employed or students) who would find daily visits

impractical (Rawson and Ling, 1991). Secondly, in the U.S.A federal legislation prohibits take away doses of LAAM, which was considered a distinct advantage in that it would potentially reduce the likelihood of diversion and illicit drug use, thus reducing the incidence of overdose deaths. Thirdly, it was thought the less frequent dosing schedule would translate into cost savings and potentially allow more individuals to participate in maintenance treatment. Finally, it was promoted as potentially more likely to provide stable and effective suppression of withdrawal across the inter–dosing interval, in particular in those individuals who report breakthrough withdrawal before the end of the 24 hour inter-dosing interval for methadone. However, since its approval a number of factors have hindered its uptake into existing opioid treatment services, including regulatory hurdles, and in some U.S clinics, substantial staff resistance to integrate LAAM into existing services (Rawson, *et al*., 1998). More recently, regulatory authorities in both the U.S.A and Europe have recommended either restricted use (Anonymous, FDA, 2001), or switching to alternative treatments (Anonymous, EMEA, 2001), because of concerns raised about clinically significant prolongation of the QTc interval and potential for life threatening cardiac rhythm disorders. The next section reviews studies that evaluated LAAM as a maintenance pharmacotherapy for the treatment of opioid dependence – it will pay particular attention to those studies undertaken prior to the approval of LAAM in 1993. This will be followed by a review of the available data on the clinical pharmacology of LAAM. A summary of the results of the randomised controlled studies and open label studies reviewed is presented in Appendix 1.

1.5.1. Developmental history and key studies in LAAM maintenance treatment

LAAM, a synthetic congener of methadone, was synthesised soon after the Second World War (May and Mosetting, 1948) and was further characterised by Pohland and colleagues in 1949 (Pohland, *et al.*, 1949). Soon after, pre-clinical studies investigated the efficacy of using acetylmethadol and its isomers as substitutes for morphine in the treatment of pain (David and Semler, 1956; Gruber and Baptisti, 1962; Keats and Beecher, 1952). David and colleagues demonstrated that 20-30 mg of racemic alpha-acetylmethadol was effective in relieving chronic pain by both oral and *s.c* routes for up to four to five hours. However, other clinical studies demonstrated the potential for cumulative toxicity (respiratory depression, severe nausea and vomiting, mental confusion, and altered consciousness approaching coma) when the levo-isomer (LAAM) was dosed *s.c* more than once daily (Fraser and Isbell, 1952; Keats and Beecher, 1952). These toxic effects occurred following

administration of doses (20mg/70kg body weight) lower than considered equivalent to 10 mg morphine (Keats and Beecher, 1952). On the basis of this evidence Keats and Beecher concluded that the margin of safety was too narrow for the clinical use of LAAM as an analgesic (Keats and Beecher, 1952). Investigation of LAAM's primary nor-metabolite (nor-LAAM) as an analgesic agent was more promising. It was found to be more potent than morphine and LAAM, and to have fewer side effects than LAAM when taken orally (Gruber and Baptisti, 1962). However, the delayed onset, prolonged duration of action, and potential for development of toxicity, limited the usefulness of these drugs as analgesics. Therefore, further investigation of their usefulness as analgesics was abandoned.

In 1952 Fraser and Isbell (Fraser and Isbell, 1952) documented that up to 60 mg of LAAM, when administered orally, was able to cross substitute for morphine in morphine addicts stabilised on morphine sulphate 60 mg four times daily, and to suppress opioid withdrawal symptoms for more than 72 hours. However, it was not until the late 1960s and early 1970s, with increasing recognition of the limitations of methadone maintenance that interest grew in developing LAAM as an opioid substitution agent. Jaffe and colleagues (Jaffe, *et al*., 1970; 1972) piloted the use of racemic acetylmethadol (administered orally three times a week) in both street heroin addicts and methadone maintained patients. Generally, these trials revealed few differences between racemic-acetylmethadol and methadone on a number of outcome variables; including opioid use, illegal activity, employment rate, and medical safety. Jaffe and colleagues (1970) reported that a number of patients dropped out of their study due to nervousness/anxiety, which they considered psychogenic in origin, but they did not rule out a pharmacological basis, as it was believed that the dextro-isomer in high doses could produce anxiety.

Further controlled trials compared LAAM (dosed three times a week) with daily methadone (Senay, *et al*., 1974; Senay, *et al*, 1977; Zaks, *et al*., 1972), and revealed very similar results to those seen with racemic acetylmethadol. For example, Zaks and colleagues (1972) demonstrated that 80 mg LAAM administered three times per week was equivalent to the daily administration of 100 mg methadone in terms of outcomes on maintenance treatment. Importantly, such studies also confirmed earlier findings that LAAM can be dosed thrice weekly, with adequate suppression of withdrawal across the inter-dosing interval. These initial studies only used small numbers of subjects, but

provided sufficient evidence of the safety and efficacy of LAAM to warrant further large-scale trials.

Unlike methadone, which had been marketed as an analgesic prior to its approval as an opioid substitution agent, LAAM had no approved use, and was not patentable. As a consequence, the further development of LAAM was not taken up by the pharmaceutical industry in the U.S.A. Instead, further development of LAAM was supported by the Special Action Office on Drug Abuse Prevention (SAODAP) and the National Institute on Drug Abuse (NIDA) (Blaine, *et al*., 1981).

Two large multi-centre studies carried out in the mid 1970s were pivotal in providing data regarding the efficacy and safety of LAAM as a maintenance agent in 1100 subjects, of whom 470 received LAAM. The first study, commonly called the VA (Veterans Administration) Cooperative study, was a double-blind, fixed dose study (no dose changes once target dose was reached) in which 430 men were randomised to either LAAM (80 mg) three times a week, with placebo on non-LAAM dosing days, or one of two doses of methadone, either high dose (100mg) or low dose (50 mg) (Ling, *et al*., 1976). The second study conducted by SAODAP, known as the SAODAP Cooperative Study (Ling, *et al*., 1978), primarily tested the feasibility of crossing patients from methadone over to LAAM. This phase III trial was open-label in design in which 636 men stabilised on methadone were crossed over to either the same methadone dose or the LAAM dose equivalent to the methadone dose (using a methadone to LAAM dose conversion ratio of 1:1.1). Subsequent dose adjustments could be made according to individual requests. Both studies were conducted over 40 weeks.

The primary outcome measure for both trials was an index of opiate use obtained from morphine positive urines: an indication of the pharmacological effectiveness of the medication in suppressing drug craving and withdrawal. In both studies LAAM patients performed at least equally as well as those patients on methadone. Indeed, patients in the VA trial had less involvement with illicit opioids (measured by positive urines, staff and patient ratings of drug use), than the high dose methadone group. Furthermore, in both studies there were few differences in a range of other measures of efficacy (retention, staff and patient ratings of psychosocial functioning and staff ratings of patients' treatment acceptance). Interestingly, in the SAODAP trial staff rated LAAM patients superior to

those on methadone on a number of psychosocial dimensions (employment/education, drug abuse, psychiatric problems, and overall adjustment). Patients reported similar side effects on either drug and no serious adverse effects were reported. However, in both trials more patients receiving LAAM (69% for the VA study and 61% for the SAODAP trial) dropped out than patients receiving methadone (48% for high dose methadone group in VA study and 40% for SAODAP study), and in both trials most of these terminations occurred early in treatment. In the VA trial, although there was no trend for patients to drop out earlier from one group than another, those patients receiving LAAM were more likely to drop out due to side effects or reports of 'medication not holding' than any other side effect. In the SAODAP study LAAM patients were significantly more likely to drop out earlier than methadone patients, reporting 'medication not holding' as the primary reason (31 % of cases). In the case of the VA study these early terminations could be attributed to under medication, caused by the slow induction schedule used (Ling, *et al*., 1976), while in the case of the SAODAP trial these early terminations were attributed to the open label nature of the trial and the anxiety over receiving an experimental drug.

A multi-centre phase III trial (Thomas, *et al*., 1979; Whysner and Levine, 1978) was designed to provide LAAM to a large number of patients who were representative of the future intended clinical population. Its focus was on collecting safety data that would be required to provide the FDA with information required to approve a New Drug Application (NDA) (Blaine, *et al*., 1981). It also explored some of the issues raised in the preceding trials, particularly with respect to the dosages of LAAM used in induction and in crossing over from methadone to LAAM. The study incorporated five protocols - the first three were open label and the remaining two double-blinded. LAAM was dosed on Monday, Wednesday, and Friday. The majority of patients (both methadone maintenance and street heroin users) were inducted into an open label trial (Protocol I & II). In protocol I methadone maintenance patients were crossed over to LAAM using a 1.2 to 1.3 ratio of LAAM dose to existing methadone dose, with subsequent increases in LAAM doses as needed. Physicians were encouraged to treat patient complaints of undermedication symptomatically with supplements of methadone if needed during the induction period. In Protocol II MMT patients and street heroin users were randomly allocated to either LAAM or methadone on a 60:40 basis. In protocol III MMT patients were crossed over to LAAM on gradually decreasing methadone dosages and concurrent increases in LAAM dosages. The fourth and fifth protocols utilized different double blind induction schedules of street

heroin users onto LAAM. Findings concerning the safety and efficacy of LAAM were consistent with the findings of preceding multi-site studies, except that fewer patients on LAAM terminated early, probably a reflection of the use of flexible induction protocols and crossover dosages from methadone. Overall 63% of patients receiving LAAM were retained for the whole study period. In all 2.1% of participants were eliminated from the trial because of adverse reactions or side effects, and 0.9% because they reported feeling overmedicated during induction or the switch from methadone. It is important to note that the reporting of this phase III data is limited as it has remained largely unpublished, with the exception of some preliminary data (Marcovici, *et al.*, 1981), and has only been partly reported in research monographs without significant peer review (Blaine, *et al*., 1981; Whysner and Levine, 1978; Thomas, *et al*., 1979).

Further findings from well-controlled and open observational clinical trials conducted in the 1980s consistently supported the previous findings (Freedman & Czertko, 1981; Savage, *et al*., 1976; Tennant, *et al*., 1986). Freedman and Czertko demonstrated the relatively greater efficacy of LAAM over methadone even when administered at low dosages. Forty eight employed heroin users were initially stabilised on methadone for a 16 week control period and then were randomly allocated either to daily methadone (range 9-42 mg) or to LAAM (range 9-20 mg) dosed on Monday, Wednesday, and Friday, with the Friday dose 50% higher than the Monday or Wednesday dose. Patients were crossed over to LAAM using a Methadone to LAAM conversion ratio of 1:1.3. LAAM patients remained in treatment significantly longer than methadone patients (32.4 weeks vs 15.6 weeks, $P<0.02$), and had a significantly lower positive urine rate than methadone patients (28% vs 48%, $P<0.05$). The superior retention rate for LAAM patients was largely attributed to the fact that all patients were employed (compared to the SAODAP study were only 45 % were employed; Ling, *et al*, 1976), and thus, the less frequent clinic visits required by the LAAM dosing regimen would have been particularly advantageous for such patients. LAAM patients also indicated, through their responses to a drug preference questionnaire, that they preferred LAAM on a number of criteria, including 'feeling more normal' and reduced craving for heroin. However, other studies have achieved higher retention rates than those reported for this study; for example the SAODAP study retained 39% of LAAM patients at 40 weeks, in comparison to 25% for this study. Such a discrepancy has been attributed to the use of substantially lower doses (average LAAM

dose = 13.9 mg) than those used in other studies. The authors conclude, however, that LAAM may particularly suit clients who are employed.

Tennant and colleagues (1986) studied 897 patients (male=721; female n=176), either admitted to LAAM maintenance from methadone or propoxyphene maintenance or street heroin use, and presented their clinical experience with these patients for up to 36 months. For the cross over from methadone to LAAM a conversion ratio of 1.1 was used. LAAM (average dose 55 mg; range 20 to 140 mg) was dosed Monday, Wednesday and Friday, with a higher dose (an additional 1-15 mg) on Friday if required. The authors highlighted the need to provide supplemental medication (either methadone or an adrenergic agonist, such as clonidine or guanabenz acetate) during the first three to seven days of induction as the plasma concentrations of active metabolites reached steady state. Thirty nine percent of patients were unsatisfied with LAAM and switched to methadone. Twenty five percent of patients dropped out of treatment within the first thirty days, and only 20 % (n=191) of participants remained in treatment at 12 months, and eight percent at 24 months. At one point 190 participants were surveyed about their drug preference – thirty nine percent stated "that if LAAM was not available, they would attempt drug abstinence or return to heroin before they would enter methadone treatment". The main reasons given for LAAM preference were less clinic attendance (67%, n=128), and that LAAM 'holds better' (43%, n=82). Finally, side effects were not considered severe enough to warrant dropping out of treatment. The authors concluded that LAAM is safe and efficacious for the majority of patients treated.

Few studies have explicitly examined the relationship between LAAM dose and efficacy. Zaks and colleagues (1972), using an open label design, randomly allocated four patients to low dose LAAM (30mg to 40 mg on Monday and Wednesday, and 40 to 50 mg on Friday), six to high dose LAAM (80 mg three times week), and ten to high dose methadone (100mg). At six months eight subjects remained in each group; with equal numbers receiving high dose LAAM (80mg) and low dose LAAM (30-50mg). The percentages of urines positive for opiates were 18.9 % and 2.8% in low and high dose LAAM groups respectively, and 2.0 % from methadone patients. The study found similar clinical outcomes for the high dose LAAM group and methadone group. There were no differences in patient acceptance, withdrawal symptoms, and response to heroin challenges (50 mg). Three of the four patients receiving low dose LAAM complained of withdrawal

at 40 to 48 hours after their last dose. More recently, studies have utilized a randomised double blind design to examine the efficacy of different LAAM dosing regimens (Eissenberg, *et al*., 1997; Oliveto, *et al*., 1998). Eissenberg and colleagues randomly allocated 180 opioid dependent volunteers to one of three dosing regimens for 17 weeks [low dose (n=59): 25 mg, 25 mg, 35 mg/MWF; respectively; medium dose (n=59), 50mg, 50 mg, 70mg/ MWF, respectively; and high dose (n=59), 100mg, 100mg, 140mg/ MWF, respectively]. Although there was no difference in retention across groups, there were significantly more patients achieving at least four weeks of opioid abstinence in the high dose group (n=20, 34 %), than either the medium dose group (n=8, 14 %), or the low dose groups (n=7, 11%). These results are consistent with those found with methadone (Ling, *et al*., 1978; Strain, *et al*., 1993), and indicate that higher LAAM doses are generally required to achieve greater abstinence from illicit opioid use.

In 1984 LAAM received Orphan drug designation in an attempt to attract a sponsor to develop the drug (Finkel, 1984). Renewed interest in LAAM developed in the context of increasing concerns over HIV/AIDS in the U.S; in particular amongst intravenous drug users, and the need to access this population for treatment. In 1991 the extensive existing data concerning the safety and efficacy of LAAM obtained from previous studies were compiled into an IND (Investigational New Drug) submission and submitted to the FDA. In 1993 LAAM became the first new pharmacotherapy to be approved for the treatment of opioid dependence for more than two decades (Food and Drug Administration, 1993). Following this the 'LAAM Labeling Assessment Study' (Fudala, *et al*., 1997) was undertaken to provide post approval data and to test the adequacy of the treatment guidelines, and to refine dosing and induction procedures in a contemporary sample of opioid dependent patients (including HIV positive individuals and females). In the United States maintenance treatment with LAAM usually comprises of three doses per week (Monday, Wednesday and Friday), with some patients requiring an increased dose on Friday to prevent withdrawal over the weekend (Fudala, *et al*., 1997: Johnson, *et al*., 2000). In Australia, LAAM has only been available for research trial purposes, and has been administered on alternate-day (Ritter, *et al*., 2003; White, *et al*., 2002), thrice weekly, a dose every three days and two dose/week dosing regimens (Ritter, *et al*., 2003).

1.5.1.1. Relative treatment effectiveness: LAAM versus Methadone maintenance

Given the variation in methodologies used by the reviewed clinical trials it is difficult to come to a definitive conclusion regarding the relative effectiveness of LAAM versus methadone maintenance. With this in mind two meta-analyses have been recently undertaken to evaluate the efficacy and safety of both pharmacotherapies in the treatment of opioid dependence (Clark, *et al*., 2002; Glanz, *et al*., 1997). Glanz and colleagues evaluated data from 14 randomised controlled trials, one of which utilized racemic-acetylmethadol (Jaffe, *et al.*, 1972), conducted between 1970 and 1982. Clark and colleagues evaluated data from 18 studies (15 randomised controlled studies and 3 controlled prospective studies) that had been conducted between 1974 and 2001. They excluded the initial studies conducted by Jaffe (Jaffe, *et al*., 1970; 1972; Jaffe and Senay, 1971) because they utilized racemic acetylmethadol. With regard to the studies that compared LAAM with different doses of methadone (e.g., Ling, *et al*., 1976), only data associated with the high methadone dose were included. Outcomes were assessed in terms of retention in treatment, illicit drug use, continuous abstinence from opioids, and measures of health/social functioning by both groups.

Both analyses revealed a significant difference in retention between LAAM and methadone that favoured methadone. This difference in retention was more marked in shorter duration studies (3 month vs 6 or 12 month studies; Clark, *et al*., 2002), reflecting the tendency for early termination of patients receiving LAAM. Clark and colleagues reported that there were significantly higher rates of abstinence (defined as at least one 4 week episode of no heroin use; P=0.0003), and significantly greater rates of urine tests negative for opioids (per person per week; P<0.00001) amongst patients receiving LAAM than methadone. In contrast, Glanz and colleagues reported no significant difference in illicit drug use between the two maintenance drugs. They concluded that patients receiving LAAM were more likely to drop out of treatment because of side effects than those receiving methadone (P<0.0001). Clark and colleagues distinguished between those patients who dropped out due to side effects, and dropped out to medication not holding; in both cases significantly more treatment drop outs were seen with patients crossed over to LAAM from methadone than with methadone patients (P=0.001 and P=0.0008, respectively).

The above results would suggest that LAAM and methadone are broadly comparable in the treatment of opioid dependence. The results in regard to superior treatment retention for methadone, and that more patients receiving LAAM dropped out of treatment due to side effects or complaints of medication not holding, should be qualified as they reflect adversely on the efficacy of LAAM. A number of issues related to this discussion were addressed by Clark and Colleagues (2002).

Firstly, in many of the studies reviewed, patients receiving LAAM had the option of dropping out and switching to methadone, while patients receiving methadone, who did not have this option, were more likely to stay in the study (e.g., Ling, *et al*., 1978; Marcovici, *et al*., 1981; Senay, *et al*., 1977). Secondly, it is feasible that blinding was not successful (Clark, *et al*., 2002), and this was discussed by a number of authors (Goldstein and Judson, 1974; Savage, *et al*., 1976). That is, the different pharmacokinetic profile of two medications would have allowed patients to differentiate between the drugs. Methadone produces a 'rush' within 2-3 hours of ingestion due to rapidly rising plasma methadone concentrations, while after LAAM ingestion, patients may not necessarily experience such an intense effect, because of the relatively stable plasma concentration-time profile of LAAM's active demethylated metabolites (see *section 1.5.3.3*: Pharmacokinetics), and if they do, will experience it between 4 to 6 hours after ingestion. Hence, regardless of the blinding, it has been suggested by a number of authors that patients were more likely to drop out when receiving LAAM due to anxiety over receiving an experimental drug (e.g., Ling, *et al*., 1976; Savage, *et al*., 1976).

Thirdly, it is likely that the early termination of patients involvement in the trials, and also the higher overall drop-out rate for LAAM patients noted in the larger studies (VA and SAODAP studies), can be attributed in part to the following factors; the use of slow LAAM induction schedules; the use of low conversion ratios (i.e. 1:1) to calculate the starting dose of LAAM, based on the last methadone dose; and that the Friday dose for many patients was insufficient to prevent withdrawal over the entire weekend (Ling, *et al*, 1976). It is interesting to note that the percentage of early terminators was reduced during the phase III study, in comparison to that seen during the VA and SAODAP studies, possibly due to the use of more flexible induction and crossover schedules. That is, the percentage of early terminators decreased from the VA study (39%) and SAODAP study

(30%) to just 13 % in the Phase III study (8% for street inductees, 15% switched from methadone to LAAM).

Fourthly, another factor concerns the dosing schedules used. A number of flexible dosing studies did not provide dosing details for both methadone and LAAM (e.g., Ling, *et al*, 1978), and so it is difficult to determine dose equivalence between the two drugs, and whether this impacted on retention (Clark, *et al*., 2002). Others reported using LAAM doses that would be considered in the low range (i.e., less than 60 mg thrice weekly) (e.g., Freedman and Czertko, 1981; Jaffe, *et al*, 1970; Senay, *et al*., 1977). Higher methadone doses have been shown to be more effective in the context of maintenance treatment (Strain, *et al*., 1993). Studies have shown the same with LAAM; Zaks and colleagues (1972) reported that high dose LAAM (80mg) was more effective than low dose LAAM, but not 100 mg methadone. Recently it has been shown that higher LAAM doses are more effective than low dose (Eissenberg, *et al*, 1997; Oliveto, *et al*, 1998), in terms of reducing concomitant opioid use.

Finally, in the United States, Federal Law restricts take away LAAM doses, and while this potentially reduces the incidence of diversion and the risks involved with opioid use by naïve users, and in particular children, it has been proposed that the restriction on take away doses may in fact make LAAM less attractive to more stable patients (i.e., those employed or participating in education), who would be eligible to get take away doses on methadone as well (Clark, *et al*, 2002).

Therefore, taking into account the factors discussed above it is clear that LAAM is an effective maintenance treatment for the treatment of opioid dependence. In terms of treatment outcomes, patients on LAAM perform at least equally as well as those on methadone, except that there appears to be a small advantage for LAAM in reducing illicit heroin use. Indeed, recent randomised controlled studies that have compared patient outcomes in LAAM and methadone maintenance (see Appendix 1) have confirmed the latter findings (Johnson, *et al*., 2000; Longshore, *et al*., 2005; White, *et al*., 2002). While it appears in the reviewed studies that patients were more likely to drop out of treatment if they were on LAAM than methadone, the differences in retention rates may not be entirely due to pharmacological factors, but also to staff and patient factors, as well as the novelty of using an experimental drug, such as LAAM.

1.5.2. General pharmacology of the acetylmethadol group of drugs

The acetylmethadols are acetylated synthetic congeners of methadone. Methadone contains a chiral carbon and exists as (R)-(-) and (S)-(+) isomers; therefore, acetylation creates a diastereomer. That is, two pairs of stereoisomers, α and β, are possible. Of these, the α series are the more potent analgesics, and less toxic than the β series (Eddy, *et al*., 1952). LAAM is derived from (S)-(+) methadone, while the dextrorotatory enantiomer (DAAM) is derived from the more active (R)-(-) methadone. Thus, it would be expected that DAAM would be more active than LAAM. Studies investigating the analgesic potency of these compounds in animals have consistently shown this. Chen (1948) demonstrated that DAAM was 5.4 times as active as LAAM when administered *s.c* to rats. Leimbach and Eddy (1954) revealed similar results using the hotplate test in mice; DAAM was about six times as active as LAAM following *s.c* administration (mean ED_{50}: 0.29 and 1.8 mg/kg respectively). Furthermore, LAAM was found to have a delayed onset (23 min vs 9 min) and longer duration of action (190 min vs 127 min) than DAAM. These differences in analgesic potency disappeared following oral administration (Mean ED_{50}: 1.6 and 1.1 mg/kg for DAAM and LAAM, respectively). In the same study LAAM was also found to be less toxic than DAAM in mice, following both *s.c* (mean LD_{50}: 110 vs 72 mg/kg), and oral administration (mean LD_{50}: 173 vs 130 mg/kg). Thus, in early animal studies LAAM was found to be less toxic than DAAM whether given orally or subcutaneously.

Fraser and Isbell (1952) subsequently assessed the pharmacological actions and abuse liability of racemic-acetylmethadol, DAAM and LAAM in humans. They showed that non-tolerant individuals administered a single dose of DAAM (5 to 20 mg *s.c*) reported marked opioid effects (reported as pleasing by the subjects) within 15 minutes of administration, but which were not evident at 24 hours after administration. Subjects reported no evidence of such effects when the same dose of DAAM was administered orally. On the other hand marked and long lasting opioid effects (up to 72 hours after ingestion of the drug), were reported following the oral administration of a single dose of LAAM (30 to 40 mg). Moreover, they also demonstrated that while both DAAM and LAAM could suppress opioid withdrawal symptoms when abruptly substituted for morphine in morphine addicts, the dose of DAAM required for this to occur resulted in toxic side effects, such as marked sedation, dizziness and ataxia (Fraser and Isbell, 1952). Hence, they concluded that DAAM did not have any advantages over methadone, but that

LAAM possibly did, noting its efficacy when administered orally and its prolonged duration of action. On the basis of these early studies LAAM was further investigated as a possible alternative to methadone for the treatment of opioid dependence (eg., Jaffe and Senay, 1971; Senay, *et al*., 1974; 1977). The remaining parts of this section will focus on the pharmacology of LAAM.

1.5.3. Pharmacology of LAAM and its demethylated metabolites

1.5.3.1. Chemistry and structure

Chemically LAAM is levo-alpha-6-dimethylamino-4, 4-diphenyl-3-heptyl acetate (Archer, 1976). The chemical structure of LAAM and its major metabolites can be seen in figure 1.2. LAAM is a weak base with a pKa of 8.98, and is highly lipid soluble with an octanol-to-water partition of 492.6 at pH 7.4 (20^{o}C) (Kaufman, *et al*., 1975). LAAM's prolonged duration of action has been attributed to its slow biotransformation into two active demethylated metabolites, noracetylmethadol (nor-LAAM) and dinoracetylmethadol (dinor-LAAM: Kaiko and Inturrisi, 1975). The octanol-to-water partition of nor-LAAM is 80, and for dinor-LAAM is 42 (Umans and Inturrisi, 1981).

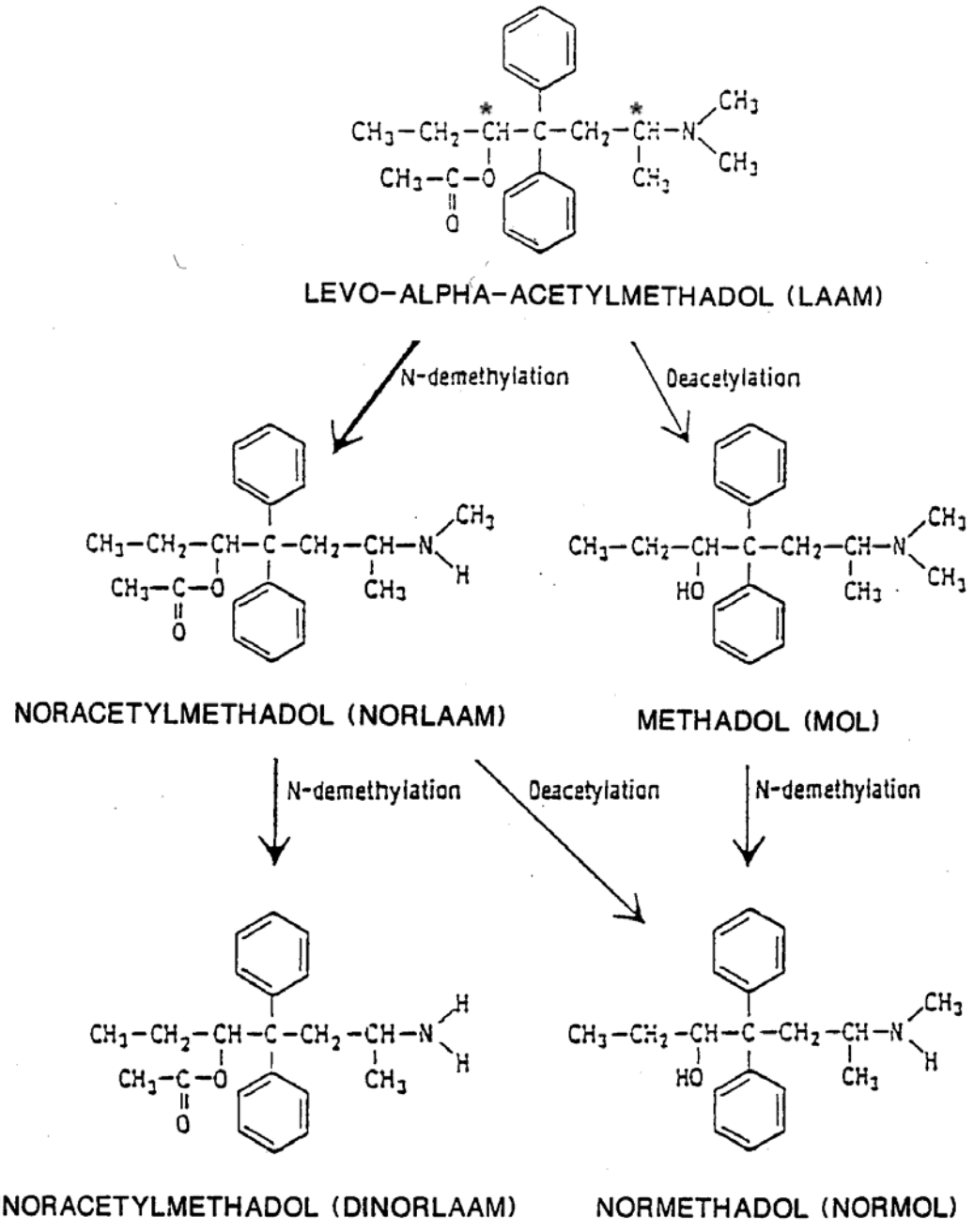

Figure 1-2: Metabolic pathways for LAAM and structural formulas for LAAM, nor-LAAM and nor-LAAM. Note the asterisks on the first structure denote chiral carbons

1.5.3.2. Neuropharmacology.

1.5.3.2.1. Receptor Target.

To date relatively few studies have provided information concerning the relative affinities of LAAM and its metabolites for opioid receptors subtypes. These data are summarised in Table 1.1. Horng and colleagues (1976) used the displacement of the relatively nonselective μ opioid receptor antagonist [^{3}H]naloxone and the μ agonist

[^{3}H]dihydromorphine, by a number of opioids in rat brain homogenate, to demonstrate that both nor- and dinor-LAAM were ten times more potent in displacing [^{3}H] naloxone, and between 20 to 30 times more potent in displacing [^{3}H] dihydromorphine, than LAAM. More recent studies have examined the binding of LAAM, nor- and dinor-LAAM to opioid receptor subtypes in rat and monkey brain homogenates utilizing receptor specific ligands i.e., [^{3}H] DAMGO for μ receptors, [^{3}H] DPDPE for δ receptors, and [^{3}H] U-69, 593 for κ receptors (Bertalmio, *et al*., 1992; Codd, *et al*., 1995; Woods, *et al*., 1991). Codd and colleagues (1995) demonstrated that LAAM was 20 times more selective for μ receptors than δ receptors and 100 more selective for μ than κ receptors in rat brain homogenate. However, they did not examine the relative affinities of nor- and dinor-LAAM for opioid receptors. In addition, they showed that LAAM (K_i=9.86 nM) has similar affinity for the μ opioid receptor as a number of other opioids, including hydrocodone (K_i=11.1 nM) and oxycodone (K_i=8.69 nM), a greater affinity than codeine (K_i=160nM), but less affinity than (-)-morphine (K_i = 1.24) and (R)-(-) methadone (K_i= 0.945 nM). These data suggest that LAAM itself possesses pharmacological activity. Moreover, Bertalmio and colleagues (1992) found nor- and dinor-LAAM were 28 and 14 times more potent than LAAM, respectively, in displacing [^{3}H] DAMGO from μ opioid receptors in monkey cerebral cortex homogenate, but did not investigate the affinity of these ligands for δ and κ opioid receptors. On the other hand, Woods and colleagues, also using monkey brain cortex, investigated the affinity of LAAM for each of the opioid receptor subtypes. They found that LAAM was 70 times more selective for μ than δ receptors and 100 times more selective for μ than κ opioid receptors. Subsequent to the completion of work in this thesis Kharasch and colleagues (2005) showed that nor- and dinor-LAAM were more potent than LAAM in displacing [^{3}H] diprenorphine from Chinese hamster ovary cell membranes expressing human opioid μ receptors.

Table 1-1: Summary of binding affinities of LAAM, nor-LAAM, and dinor-LAAM for opioid receptors.

Ref[1]	Tissue	Ligand	IC_{50} (nM).			
			Opioid receptor [2] Non-selective assay	μ	δ	κ
1	Rat brain	LAAM	33[3] , 35[4]	NR	NR	NR
		NL	2.8 [3], 1.0 [4]	NR	NR	NR
		DNL	3.0 [3],1.6 [4]	NR	NR	NR
2	Monkey cortex	LAAM	NR	34.1[5,]	2259[6]	3564[7]
3	Monkey cortex	LAAM	NR	32, 34[5,9]	NR	NR
		NL	NR	1.1, 1.3[5,9]	NR	NR
		DNL	NR	2.1, 2.5[5,9]	NR	NR
4	Rat brain	LAAM	NR	9.86[5,10]	169 [6,10]	1020 [7,10]
5	Cloned human μ receptors	LAAM	NR	4.72±0.30 [8,10] (mean±sd)	NR	NR
		NL	NR	0.15±0.01 [8,10] (mean±sd)	NR	NR
		DNL	NR	0.37±0.02 [8,10] (mean±sd)	NR	NR

Notes: All data are expressed as mean or replicate values, except for reference number 5. which are mean±sd;. NL=nor-LAAM; DNL= dinor-LAAM; NR= Not reported/ examined Ref = Reference; [1] 1=Horng, et al., 1976; 2=Woods, et al., 1992; 3=Bertalmio, et al., 1992; 4=Codd, et al, 1995; 5=Kharasch, et al., 2005; [2] Non-selective assay; [3] Displacement [^{3}H] naloxone by ligand; [4] Displacement of [^{3}H] dihydromophine by ligand; [5] Displacement of [^{3}H] DAMGO (μ selective) by ligand; [6] [^{3}H] DPDPE (δ selective) by ligand; [7] Displacement of [^{3}H] U69, 593 (κ selective) by ligand; [8] Displacement of [^{3}H] Diprenorphine; [9] Data in the form of replicates; [10] Binding affinity reported in the form of K_I values.

There is a relative lack of data with regard to other receptor targets for LAAM and its demethylated metabolites. Codd and colleagues (1995) demonstrated that LAAM inhibited the reuptake of both noradrenalin (K_i = 67,400 nM) and serotonin (K_i = 1,440 nM) in rat brain synaptosmes, but that this activity was less than that reported for either (R)-(-) or (S)-(+) methadone. Studies investigating the effect of LAAM and its congeners on cardiac function have ascertained that they have low affinity for muscarinic acetylcholine binding sites (Langley, *et al*., 1984). In the latter study the concentration of LAAM and metabolites required to displace 50 % of 125 pM [^{3}H]-quinuclidinyl benziliate (QNB) from rat striatal membranes was 10-20 times higher than for pilocarpine (muscarinic agonist) and 5000 to 8000 times higher than for atropine (muscarinic

antagonist). EC_{50}s were 42, 35, and 24 μM for LAAM, nor-LAAM and dinor-LAAM, respectively. Given that atropine 1μM, but not naloxone, has been shown to antagonize the cardiac depressant effects of LAAM on isolated guinea pig (Stickney, 1977) and rat hearts (Langley, *et al*., 1984) the authors concluded that LAAM might produce some of its cardiac effects through stimulation of muscarinic receptors (Langley, *et al*., 1984). Furthermore, there is recent evidence that LAAM is a potent inhibitor of cloned human cardiac K^+ channel currents and that LAAM is more potent than nor-LAAM in this action (Kang, *et al*., 2003). The latter phenomenon will be discussed in more detail in section 1.5.3.5 (Toxicity).

Moreover, Langley and colleagues (1981) reported that LAAM, like many other opioid agonists (Kuschinsky and Hornykiewicz, 1974) alters dopaminergic activity. They revealed significant increases in [^{3}H]-spiroperidol binding sites in the striata of guinea pigs and mice treated with chronic LAAM (12 mg/kg and 16 mg/kg, for 30 days orally, respectively), as compared to saline treated controls. The LAAM treated animals exhibited an increased responsiveness to apomorphine as evidenced by increase stereotyped behaviour.

1.5.3.2.2. Animal studies *in vitro.*

The activity of LAAM and its nor-metabolites have been investigated in the electrically stimulated guinea pig ileum (GPI) assay (Foldes, *et al*., 1980; Nickander, *et al*., 1974). Such an assay can be used as a convenient model for the study of opioid activity as it makes it possible to look at the relative activity of each compound without contribution of *in vivo* metabolism. Nickander and colleagues (1974) demonstrated that although LAAM was active in this assay, nor-LAAM and dinor-LAAM were 16 and 11 times more active as LAAM, respectively in their ability to depress the electrically induced twitch of a strip of longitudinal muscle stripped from guinea pig ileum. The respective mean inhibitory concentrations (IC_{50}) for LAAM, nor-LAAM and dinor-LAAM were 333 ng/ml (range: 165-671 ng/ml), 20 ng/ml (range: 9-43ng/ml), 28 ng/ml (range: 12-68ng/ml) ng/ml. Foldes and colleagues (1980), in a conference proceeding, reported that nor-LAAM and dinor-LAAM were similarly potent (mean±SEM: 19±6 nM and 15.6±2.5 nM, respectively), but more potent than LAAM (507.5±125nM) in this assay. Hence, these data are consistent with receptor binding data showing the relatively greater potency of nor- and dinor-LAAM than LAAM, but also shows that LAAM has pharmacological activity.

1.5.3.3. Pharmacokinetics of LAAM, nor-LAAM and dinor-LAAM.

Much of the work concerning the pharmacokinetics of LAAM was carried out at the preclinical stage on animals in the 1950s and 1960s (see Archer, 1976 for a review). Since then there have only been six published papers that have comprehensively examined the disposition of LAAM and its demethylated metabolites in humans (Billings, *et al*. 1974; Finkle, *et al*., 1982; Henderson, *et al*., 1977b: Henderson, *et al*., 1977c; Kaiko and Inturrisi, 1975; Walsh, *et al*., 1998). Furthermore, a number of authors have recently investigated drug-drug interactions involving LAAM and the effect they have on the disposition of LAAM and its demethylated metabolites. The latter reports have also presented data that are relevant to this review (Kharasch, *et al*., 2005; McCance-Katz, *et al*., 2006; Moody, *et al*., 2004). Those studies that have examined the disposition of LAAM and its demethylated metabolites in humans are reviewed below.

Using a case study approach Billings and colleagues (1974) examined the disposition of nor-LAAM and dinor-LAAM in the plasma and urine of three male patients who had been maintained on 80 mg methadone daily for 2 to 6 years. Patients received 100 mg of LAAM on a Monday, Wednesday, Friday dosing regimen. Blood was sampled five times over a 48-hour period following the first dose of LAAM and seven times after an arbitrarily selected subsequent dose. Total voided urine was also collected over this time. According to the authors the concentration of LAAM in plasma could not be determined due to methodological limitations. The authors noted the accumulation of both nor- and dinor-LAAM in plasma and urine during chronic dosing, and also noted the relatively constant plasma concentration-time profile for dinor-LAAM following chronic dosing, compared to nor-LAAM, that declined steadily after dosing.

Kaiko and Inturrisi (1975) quantified LAAM and both nor-metabolites in the plasma and urine of eight patients maintained on LAAM 40 – 60 mg three times per week for at least 4 weeks (range 4-25 weeks). The authors collected blood at 4, 8, 24 and 48 hours after the administration of LAAM from these eight patients, and a 48-hour urine sample from an additional 4 patients. The authors also measured pupil diameter at these times and examined the relationship between the plasma-concentration profile of LAAM and its nor-metabolites with the time course of miosis; these results are discussed elsewhere (see Pharmacodynamics, section 1.5.3.4.). The use of so few time points to construct the plasma concentration-time curves leads to a large uncertainty in the interpretation of the

presented results, particularly with respect to estimating the T_{max} of all three compounds. In addition, the authors presented the apparent elimination half-life for LAAM, and the terminal half-life for nor-LAAM, but not the half-life of dinor-LAAM as concentrations did not decline across the sampling period.

Henderson and colleagues (1977b) examined the pharmacokinetics of LAAM in two groups of patients. One group (n=5) had been maintained on 50 mg daily methadone for at least 90 days prior to commencing LAAM and the other (n=5), had a history of heroin addiction, but no prior experience with either methadone or LAAM. LAAM was administered on a Monday, Wednesday, Friday dosing regimen, and for both groups the LAAM dose was increased to a maximum dose of 85 mg (3x week) by the end of the 90-day treatment period. Three patients remained in each group at the end of the 90-day LAAM treatment period time. Blood was sampled over a 48-hour period following the first dose and 72 hours following the 30th dose. A number of pharmacokinetic parameters were reported for LAAM and nor-LAAM, but not dinor-LAAM (see Appendix 2). Many of these parameters were based on data that were below the limit of detection (50ng/ml) and calibration (25ng/ml) of the method used to quantify LAAM and nor-LAAM in plasma.

Finkle and colleagues (1982) examined the disposition of LAAM and metabolites in plasma and urine of 12 male patients who had been maintained on daily methadone (60 – 80 mg) for at least 2 years. Seven patients received 80 mg LAAM irrespective of body weight, and the remaining received doses of 1 mg/kg. Patients received 10 doses of LAAM and had blood sampled over 42 days following the first LAAM dose; including 21 days after the last dose, for a total of 65 samples per patient. A urine sample was collected from each patient every 12 hours throughout the study period. Concentrations of LAAM, nor- and dinor-LAAM in plasma and urine were determined using a gas chromatographic/ chemical ionization-mass spectrometric method, with a lower limit of sensitivity of 5 ng/ml. The authors presented plasma half-life values for LAAM, nor- and dinor-LAAM for each patient for the period following the first dose and last dose. Urinary excretion data for LAAM and deacetylated metabolites was also presented (see Appendix 3).

Walsh and colleagues (1998) carried out a comprehensive examination of the pharmacokinetics and pharmacodynamics of LAAM following a single dose (20 or 40

mg/70 kg body weight) of LAAM administered *i.v*. and orally to nondependent opioid users, using a double blind, double dummy, cross over design. Blood was sampled over a 96-hour period following dosing. Plasma was analyzed for LAAM, nor- and dinor-LAAM using a gas chromatographic/positive ion chemical ionization-mass spectrometric method, which had a lower limit of sensitivity of <5 ng/ml. The authors present estimates of $T_{½}$ for each analyte (LAAM, nor- and dinor-LAAM), apparent total body clearance and bioavailability, which will be discussed later in this review.

Recently, studies have examined the disposition of LAAM and its demethylated metabolites when co-administered with drugs known to either inhibit or induce CYP3A4, the main cytochrome P450 enzyme system thought to be involved in the metabolism of LAAM and nor-LAAM (Kharasch, *et al*., 2005; McCance-Katz, *et al*, 2006; Moody, *et al*, 2004). Moody and colleagues (2004) described the change in plasma concentrations and urinary excretion of LAAM and metabolites following the co-administration of ketoconazole, a potent CYP3A4 inhibitor, and LAAM (single dose of 5 mg/kg) to 13 opioid naïve subjects, compared to placebo. Blood and urine were sampled over 240h and 96h periods, respectively. Kharasch and colleagues (2005), in a three way randomised crossover study where subjects received a single dose of LAAM (0.25 mg/kg *p.o*) and, either rifampicin (to induce CYP3A4 activity), or troleandomycin (to inhibit CYP3A4 activity), or nothing (control condition), examined the disposition of LAAM and its metabolites in blood and urine. Blood and urine were sampled for 96 hours after dosing with LAAM. Furthermore, McCance-Kratz and colleagues (2006) used a within subjects experimental design to determine the effect of delaviradine (DLV; a non-nucleotide reverse transcriptase inhibitor and CYP3A4 inhibitor) on the disposition of LAAM and methadone, in maintenance patients. Blood for determination of plasma LAAM and metabolite concentrations were sampled over a 48-hour inter-dosing interval, once prior to treatment, and again following treatment with DLV.

The next section will discuss the pharmacokinetic parameters reported by the abovementioned authors. For the readers convenience these parameers are summarised in Appendix 2 (General pharmacokinetics) and Appendix 3 (Excretion of LAAM, nor- and dinor-LAAM expressed as per cent of the administered dose).

1.5.3.3.1. Absorption and bioavailability.

Following oral administration LAAM is rapidly absorbed from the gastrointestinal tract, with LAAM and metabolites detectable in plasma within 15 to 30 minutes of ingestion (Finkle, *et al*., 1982; Walsh, *et al*., 1998). Times to reach maximum plasma concentration (T_{max}) for LAAM of approximately 2 hours (Cook, *et al*., 1984), 3.2± 1.1 hours (mean±sd Henderson, *et al*., 1977b), and 4.4 hours (range: 3-6 hours) (Finkle, *et al*., 1982) have been reported in MMT patients administered a single LAAM dose 24 hours after their last methadone dose. In non-dependent heroin users T_{max} values of approximately 2.5 hours (Kharasch, *et al*., 2005; Walsh, *et al*., 1998), and 2.12±0.76 hours (mean±sd; Moody, *et al*., 2004) have been reported after a single LAAM dose. In LAAM maintenance patients T_{max} values of 2.7±1.2 hours (mean±sd; Henderson, *et al*., 1977b) and 1.75 (1.5-2.0) hours (mean, range; McCance-Kratz, *et al*., 2006) have been reported.

With regard to nor-LAAM, T_{max} values of 3.5±1.5 hours (mean±sd, Henderson, *et al*., 1977b), and 5.6 hours (range: 4 to 7 hours; Finkle, *et al*., 1982) have been reported in MMT patients, and approximately 3.5 hours (Walsh, *et al*., 1998), and 3.0±0.8 hours (mean±sd: Kharasch, *et al.*, 2005) in nondependent heroin users after receiving a single LAAM dose. In patients undergoing chronic LAAM treatment T_{max} values for nor-LAAM of 5.3±2.3 hours (mean±sd, Henderson, *et al*., 1977b) and 4 hours (1.5-10) (mean, range; McCance-Katz, *et al*., 2006) have been reported. Precise T_{max} values for dinor-LAAM have been provided by only a few studies. Finkle and colleagues reported a mean T_{max} for dinor-LAAM of 6.6 hours (range 5 to 7h) for eight patients; the remaining four subjects required 24 to 48 eight hours to reach maximum plasma dinor-LAAM concentrations. Furthermore, McCance-Katz and colleagues reported a mean T_{max} of 11 hours (range 1.5 – 36 hours) for dinor-LAAM in LAAM maintenance patients.

Only two studies have examined the oral absorption and bioavailability of LAAM in humans and both have reported relatively moderate bioavailability values for the drug. Cook and colleagues administered simultaneously an oral dose of unlabelled LAAM (15-20 mg) and an equal intravenous dose of ($^{13}C_2$) LAAM to four methadone maintenance patients. From the comparison of the urinary isotope excretion of the administered drugs in these patients the absorption of LAAM was calculated as 48-50%. Comparing the area under the plasma concentration-time curve (AUC) for *i.v*. and orally administered drug

(assuming complete absorption) resulted in an absolute oral bioavailability for LAAM of 45± 6% (mean±SEM).

More recently, Walsh and colleagues (1998) reported mean±sd bioavailability of 48±9% in opioid users after separate administrations of 20 mg oral and *i.v* unlabeled LAAM, and 47±5% after separate administrations of 40 mg oral and *i.v* unlabeled LAAM (Walsh, *et al*., 1998). These authors also reported higher C_{max} and AUC concentrations for nor and dinor-LAAM following oral LAAM administration compared with intravenous administration which is consistent with LAAM being subject to some first pass metabolism. Moreover, these authors tentatively suggest that the kinetics of LAAM itself may be nonlinear, in that the C_{max} for LAAM and AUC did not increase proportionally with increasing dose (20 mg and 40 mg). However, in order to more accurately determine linearity the kinetics of LAAM would need to be examined across a wider dose range.

Hence, there is a time ordered appearance of LAAM, nor-LAAM and dinor-LAAM in plasma that reflects the metabolic pathway of LAAM (see figure 1.2). The appearance of nor-LAAM and dinor-LAAM soon after the peak concentration of LAAM is suggestive of first-pass metabolism soon after LAAM is ingested (Finkle, *et al*., 1982). To date, there are no data on the effect of gastrointestinal pH and motility on the pharmacokinetics of LAAM, in particular absorption.

1.5.3.3.2. Distribution.

The distribution half life ($t_{½\alpha}$) for LAAM has been reported to be 7 hours (Kaiko and Inturrisi, 1975), and 7.1 hours (range: 5.4-8.9 h: Henderson, *et al.*, 1977b) following chronic dosing in patients crossed over from methadone maintenance; and 4.6 hours (3.1-9.9 h: Henderson, *et al*., 1977b) following chronic dosing in patients inducted onto LAAM from street heroin use. Finkle and colleagues utilised a more intensive sampling protocol than the latter studies (i.e., blood sampled over 42 days following the first LAAM dose, for a total of 65 samples per patient) to examine the disposition of LAAM and nor-metabolites in patients previously maintained on methadone. The authors reported that the LAAM concentration-time profile for each individual incorporated an absorption phase, a distribution phase (between 4 and 12 hours), and an elimination phase (between 24 and 72 hours) after the first dose of LAAM. They reported a mean $t_{½\alpha}$ value for LAAM of 2.4 hours (range: 1.1 – 4.5h). Values for the elimination half life ($t_{½\beta}$) for LAAM following

the first and last dose and for nor-LAAM and dinor-LAAM following the last dose were also presented and will be discussed in a later section (see section *1.5.3.3.4:* Half life).

With regard to volume of distribution (Vd), one estimate is available in the literature and is provided in the product information (Roxane Laboratories, 1995). This value (Vd/F ≈20L/kg) was obtained from pharmacokinetic modeling of data obtained from 25 patients maintained on a three times per week (0.94 mg/kg on Monday/Wednesday and 1.125 mg/kg on Friday LAAM *p.o*) dosing regimen over 15 days. A calculated Vd from an *i.v* dose, based on the mean data presented by Walsh and colleagues (1998) is 7.3 L/kg, indicating extensive tissue distribution.

Moreover, there have been reports of the tendency for nor- and dinor-LAAM to accumulate with chronic dosing. Henderson and colleagues (1977b) reported that LAAM did not accumulate with chronic dosing, but that plasma concentration of nor-LAAM and dinor-LAAM increased 5-fold and 13-fold respectively after the thirtieth dose. This would be expected given the relatively short half-life of LAAM ($t_{½\alpha}$ ≈ 4 – 9 h) compared with its demethylated metabolites. In a further study Henderson and colleagues (1977c) described the time course of accumulation of nor-LAAM and dinor-LAAM in 11 male subjects, crossed over from methadone administered daily to LAAM administered alternate daily (0.86 mg/kg LAAM orally). Consistent with their latter study the authors reported that plasma nor-LAAM and dinor-LAAM concentrations increased 3-fold and 4-fold respectively across the sampling period. Plasma concentrations of both nor- and dinor-LAAM appeared to reach steady state at a time between the sixth and ninth dose (between day 11 and 17 days post dosing). The authors concluded that LAAM does not accumulate when administered in doses of 60 mg (approximately 0.86 mg/kg) or less on a 48 hour dosing schedule. The authors did not report any further pharmacokinetic data. Finkle and colleagues (1982) reported that accumulation of nor- and dinor-LAAM occurred in at least half of the subjects participating in their study who were administered 1 mg/kg three times per week, but that this tendency was not predictable. Individual differences in the rate of metabolite accumulation would go some way to explain why some patients find the transfer from methadone less comfortable than others.

Estimates of steady state plasma (C_{ss}) concentrations, and peak to trough plasma concentration ratios for LAAM, nor-LAAM and dinor-LAAM, can be derived from the

data presented by McCance-Katz and colleagues (2006) (see Appendix 2. for C_{min} and C_{max} data). The latter authors presented mean AUC values of 2789 ng·h/ml, 5798 ng·hr/ml, and 4953 ng·hr/ml, for LAAM, nor- and dinor-LAAM, respectively; in maintenance patients administered LAAM on a 48-hour dosing schedule. The estimated C_{ss} values are 58 ng/ml, 121 ng/ml and 103 ng/ml for LAAM, nor-and dinor-LAAM, respectively. Furthermore, the peak-to-trough plasma concentration ratios for LAAM, nor-and dinor LAAM are 5.7, 2.3, and 1.4, respectively. The latter data demonstrate that nor and dinor-LAAM are likely to exhibit more stable plasma-concentration-time profiles than LAAM, in maintenance patients.

1.5.3.3.2.1. Binding in plasma.

There are few available data on the binding of LAAM, nor-LAAM and dinor-LAAM to plasma proteins - being the subject of only two studies in humans (Cook, *et al*., 1984; Toro-Goyco, 1980). The available data indicate that LAAM, nor- and dinor-LAAM bind extensively to plasma proteins (> 90%), predominantly to α_1-acid glycoprotein, but that this binding is readily reversible. Furthermore, nor- and dinor-LAAM compete for the same binding sites (Cook, *et al*., 1984; Toro-Goyco, 1980).

Cook and colleagues (1984) also demonstrated that nor-LAAM had greater affinity for α_1-acid glycoprotein binding sites than did LAAM, and that there were two binding sites, with similar association constants, for dinor-LAAM, in contrast to single binding sites for both LAAM and nor-LAAM. Fluctuations in plasma α_1-acid glycoprotein concentration (as it is an acute phase protein), and individual variation in extent of binding to plasma proteins, would in part, help explain variations in response to LAAM.

1.5.3.3.3. Metabolism and excretion.

LAAM is primarily cleared by hepatic and intestinal metabolic pathways and undergoes first pass metabolism (Oda and Kharasch, 2001b; Walsh, *et al*., 1998). The primary metabolic pathway of LAAM is sequential hepatic *N*-demethylation by cytochrome (CYP) P450 enzymes, first to nor-LAAM and then to dinor-LAAM (Billings, *et al*., 1973; Billings, *et al*., 1974; Finkle, *et al*., 1982; Henderson, *et al*., 1977b) (*See figure 1.2*). Evidence for this is provided by early *in vitro* studies using rat liver homogenates and by *in vivo* studies. McMahon and colleagues (1965) used rat liver homogenates and C^{14} radio-

labeled drug. They revealed that the drug was demethylated by the hepatic microsomal enzyme demethylase and identified both nor- and dinor-LAAM in liver homogenates. Further studies revealed that prior administration of the potent metabolic inhibitor SKF-525A to rodents reduced the analgesic potency of subcutaneously administered LAAM reflecting inhibition of the drugs transformation into active metabolites (Veatch, *et al*., 1964). In contrast, the use of phenobarbitone (a potent inducer of microsomal metabolism) treated rat liver homogenate resulted in increase in microsomal activity and subsequent increase in production of metabolites (McMahon, *et al*., 1965). Moreover, these authors showed that the first demethylation to nor-LAAM occurs at three times the rate of second demethylation to dinor-LAAM. This is would be partly responsible for the prolonged presence of nor-LAAM in plasma and would also provide a pool of nor-LAAM to be biotransformed into dinor-LAAM.

LAAM can also undergo deacetylation to methadol and then subsequent sequential demethylation to normethadol and then to dinor-methadol (Cook, *et al*., 1984; Kaiko and Inturissi, 1975; Kaiko, *et al*., 1975) (see figure 1.2). Methadol and nor-methadol were found in the plasma and urine of rats receiving 5 and 10 mg of oral LAAM (Henderson, *et al*., 1977a), and in the central nervous system and bile of monkeys receiving 2mg/kg of LAAM orally for approximately 30 weeks (Mulé and Misra, 1978). Methadol, nor-methadol and dinor-methadol were found in human urine following 10 doses of LAAM (0.73 - 1.5 mg/kg) (Finkle, *et al*., 1982). Thus, as methadol is analgesically active (Horng, *et al*., 1976), it is possible it would contribute to LAAM's action.

However, the evidence for the presence of these deacetylated metabolites in human plasma is sketchy. There are reports that these compounds do not reach measurable concentrations in human plasma following chronic oral administration of LAAM (Kaiko and Inturrisi, 1975). On the other hand, Cook and colleagues (1984) reported that following a single dose of [^{3}H] LAAM *i.v* to methadone maintenance patients (n=4), that the combined radioactivity from methadol, nor-methadol and dinor-methadol accounted for approximately 10% of total radioactivity present in plasma at a number of different time points (0-48 hrs). Therefore, it is not clear what contribution these metabolites make to the overall pharmacological activity of LAAM following oral dosing in humans.

Studies evaluating the CYP isoforms contributing to the metabolism of LAAM have revealed the primary role of CYP3A4 (Moody, *et al*., 1997; Oda and Kharasch, 2001a), with possible minor contributions from 2C9, 2D6 and 2E1 (Moody, *et al*., 1997), and 2B6, 2C18, 2C19 (Neff and Moody, 2001). In addition, small intestinal CYP3A4 has been shown to *N*-demethylate LAAM and nor-LAAM and therefore may contribute to the pre-systemic clearance of LAAM (Oda and Kharasch, 2001b). The variability in the activity of hepatic and intestinal CYP3A4 (Ketter, *et al*., 1995) may account for variability seen in the pharmacokinetics of and response to LAAM (Oda and Kharasch, 2001a; 2001b).

Moreover, few authors have reported on the excretory profile of LAAM and metabolites in humans (Cook, *et al*., 1984; Finkle, *et al*., 1982; Kaiko, *et al.*, 1975; Kharasch, *et al*., 2005; Moody, *et al.*, 2004). In these studies the mean combined urinary recovery of unchanged LAAM, nor-LAAM and dinor-LAAM has been reported to range from approximately 4 % to 25 % of the administered dose. There are also large inter-individual differences in the proportion excreted (Finkle, *et al*., 1982; Henderson, *et al*, 1977b; Kaiko, *et al*., 1975; Kharasch, *et al*., 2005; see Appendix 3). Kaiko and Inturrisi (1975) demonstrated in LAAM maintenance patients that that the excretion of nor- and dinor-LAAM accounts for greater proportions of the administered dose than unchanged LAAM; with mean recoveries of 8.2 % (range 2.9 to 15 %) for nor-LAAM, and 13.2% (range 6.3 to 18.4%) for dinor-LAAM, compared to 1.8% (range 1.5 to 2.8%) for LAAM. The latter authors also reported that the excretion of methadol accounted for a mean 4.8% (range 2.9 to 6.8%) of the administered dose in the same patients. The pattern of excretion for each patient was similar despite a five-fold difference in administered LAAM dose (20 mg to 100 mg).

In an attempt to determine the mass balance of LAAM, Cook and colleagues (1984) administered a single [^{3}H] radio-labelled dose of LAAM *i.v* to four MMT patients 24 hours after their last methadone dose. The administered LAAM dose was the same as the daily methadone dose usually ingested by patients. Urine and faeces were collected for 168 hours (7 days) following administration of LAAM and the total radioactivity determined by liquid scintillation spectrometry. Recovery of analytes in urine after 7 days amounted to 27±3.4 (mean±sd) % of the total administered radioactivity. Excretion in faeces was even more variable amounting to 17.8±12.0% of the dose in 7 days. The combined excretion of radioactivity in urine and faces ranged from 28.3 to 54 % (mean ±sd: 44.8±14%), and thus mass balance was not achieved. Given the half life values of LAAM

and its *N*-demethylated metabolites (see section *1.5.3.3.4*), a sampling period of up to 28 days (equivalent to approximately four times the mean half life of dinor-LAAM) would be needed to achieve a full recovery of the dose following acute LAAM administration. It is also plausible that the relatively low proportion of the dose recovered in faeces as LAAM and its metabolites may also reflect enterohepatic circulation of these compounds. However, neither biliary excretion nor enterohepatic circulation of LAAM and its metabolites have been verified in humans.

More recently, Moody and colleagues (2004) described the change in excretion of LAAM and metabolites in urine following the co-administration of ketoconazole (to inhibit CYP3A4), and LAAM (single dose of 5 mg/kg) to opioid naïve subjects, compared to placebo. The administration of ketoconazole did not significantly change the volume of urine excreted, but significantly increased the percentage of dose excreted in the form of LAAM, from 0.49 % to 2.74 % (P<0.001), and nor-LAAM, from 2.30 % to 4.97 % (P<0.001), when compared to the placebo session. On the other hand, there was no change in the percentage of dose excreted in the form of dinor-LAAM (6.70% vs 6.16%, respectively). As a result there was an overall significant increase in the percentage of dose excreted from 9.49% following administration of placebo, to 13.9% following the administration of ketoconazole. Moreover, Kharasch and colleagues (2005) demonstrated that the percentage of the total administered oral dose recovered as unchanged LAAM, nor- and dinor-LAAM, was approximately 16% in subjects when they received only LAAM (control condition), but decreased to approximately 2% with CYP3A4 inhibition (pre-treatment with troleandomycin), and increased to approximately 28% with CYP3A4 induction (pre-treatment with rifampicin).

1.5.3.3.3.1. Drug-drug interactions *in vivo.*

Given that there is a large number of compounds that interact with CYP3A4 (Ketter, *et al*., 1995; and see methadone section 1.4.1.2.4), there are potentially many drugs that are likely to alter LAAM pharmacokinetics. Despite this I am aware of only a few published reports of such human drug-drug interactions involving LAAM (Kharasch, *et al*., 2005; McCance-Katz, *et al*., 2006; Moody, *et al*., 2004; a summary of pharmacokinetic data from these reports can be found in Appendix 2.). For example, Moody and colleagues (2004) reported a significant increase in the AUC (mean±sd: 39.6±18 ng·ml/hr vs 219±106 ng·ml/hr: P<0.001) and C_{max} (4.9±1.5 ng/ml vs 16.5±6.7 ng/ml; P<0.001) of LAAM, and a

significant increase in the $AUC_{0\text{-}240}$ (103±42 ng·ml/hr vs 234±100 ng·ml/hr: $P<0.01$) and C_{max} (6.9±1.6 vs 5.3±1.2: $P<0.05$) for nor-LAAM following the concomitant administration of ketoconazole (400mg) and oral LAAM, compared to placebo. There was also a significant increase in the T_{max} for nor-LAAM and dinor-LAAM, and prolongation of the presence of both metabolites in plasma following the administration of ketoconazole, compared to that seen following placebo. The persistence of both nor-metabolites in plasma was associated with a concomitant prolongation of miosis. These data are presented as consistent with the *in vivo* inhibition of the metabolism of LAAM to nor-LAAM and nor-LAAM to dinor-LAAM (Moody, *et al*., 2004), and hence the involvement of CYP3A4 in the metabolism of LAAM. However, as the authors discuss, whether this effect is solely due to the effect of ketoconazole on CYP3A4 is not known at this stage. As discussed above other CYP isoenzymes are involved in the metabolism of LAAM, and ketoconazole, although relatively specific for CYP3A4, at higher concentrations has been shown to inhibit other CYP isoenzymes (Ono, *et al*., 1996; Sai, *et al*., 2000).

A number of reports have been made to the US Food and Drug Administration concerning cardiovascular events associated with the use of LAAM (Deamer, *et al*., 2001; Moody, *et al*, 2004). In some of these cases drugs known to be either inhibitors of CYP3A4 activity (i.e., cimetidine, estrogens, fluoxetine, fluconazole), or known substrates of CYP3A4 (i.e., methadone, buspirone) were also taken. It has been suggested that such drug interactions might have contributed to the prolonged QT intervals exhibited by these patients (Moody, *et al*., 2004; Stimmel, 2001). That is, inhibition of CYP3A4 is likely to result in significant increases in plasma LAAM concentration. As LAAM has been reported to be highly proarryhymic (Kang, *et al*., 2003; Katchman, *et al*., 2002), significant increases in plasma LAAM concentrations could possible cause adverse cardiac effects. The proposed pathophysiology of LAAM induced prolonged QT intervals will be further discussed in section *1.5.3.5*. The prevalence of drug-drug interactions involving LAAM is an important future research concern as the occurrence of such side effects and variability in LAAM disposition will significantly affect the clinical effectiveness of LAAM.

1.5.3.3.4. Half-life.

The terminal elimination half-life values for LAAM, nor-LAAM and dinor-LAAM in humans have been estimated following repeated administration (Finkle, *et al*., 1982; Kaiko

and Inturrisi, 1975; McCance-Katz, *et al*., 2006) and following a single dose of LAAM (Finkle, *et al*., 1982, Kharasch, *et al*., 2005; Walsh, *et al*., 1998); these half-life values are reported in Appendix 2. With few exceptions (e.g., Finkle, *et al.*, 1982), these values have been estimated from blood sampling protocols that have extended for less than the required 3 to 5 times the calculated half-life. Finkle and colleagues estimated half-life values for all three analytes from a sampling protocol that extended for 21 days after the tenth dose of oral LAAM (subjects either received 80 mg LAAM or 1 mg/kg LAAM). The half-life for LAAM ranged from 14.2 - 104.5 hours (mean = 46.8 hours), for nor-LAAM ranged from 12.9 - 129.6 hours (mean = 62.4 hours) and for dinor-LAAM ranged from 22.5 to 429.9 hours (mean = 174.6 hours). Thus, clearly there will be inter-individual differences for time to steady state concentrations for all analytes, but steady state concentrations of nor and dinor-LAAM should be reached within about one to two weeks, but could take more than one month in some patients.

1.5.3.3.5. Clearance

Walsh and colleagues estimated apparent total body clearance of LAAM following the administration of single doses of LAAM *i.v* and orally. The *i.v* clearance values [mean±sd] were 352±61 ml/hr/kg and 295± 86 ml/hr/kg following 20 and 40 mg LAAM *i.v*, and oral clearance values were 357±61 ml/hr/kg and 296±86 ml/hr/kg following 20 and 40 mg orally, respectively. Furthermore, McCance-Katz and colleagues (2006) estimated oral clearance values prior to and following treatment with the CYP3A4 inhibitor delavirdine (DLV) in LAAM maintenance patients. Prior to treatment with DLV dosing mean clearance values were 26.5 L/h, 12.8 L/h, and 14.9 L/h for LAAM, nor-and dinor-LAAM, respectively. Following treatment with DLV clearance values were 11.4 L/h, 8.12 L/h, and 19.4 L/h for LAAM, nor- and dinor-LAAM, respectively. The significant decrease ($P<0.005$) in the clearance of LAAM and nor-LAAM following DLV treatment is consistent with the role that CYP3A4 plays in the biotransformation of these compounds.

With regard to renal clearance, there is a tendency for clearances of nor-LAAM and dinor-LAAM to be higher than for LAAM, because of their more hydrophilic structures. Kaiko and Inturrisi (1975) determined renal clearance for four maintenance patients who had received a mean LAAM dose of 50 mg (range 30 to 60 mg), three times a week, for at least 4 weeks (range 4 to 25 weeks). The mean±sd renal clearance values for LAAM, nor-LAAM and dinor-LAAM were: 13.8±16 ml/min, 14.03±5.5ml/min and 19.50±19 ml/min

respectively. They reported a pH dependent renal clearance of LAAM and its metabolites, with greater elimination of all these compounds in subjects with acid urine (Kaiko and Inturrisi, 1975). The authors suggest this probably indicates tubular reabsorption of LAAM and its metabolites, which they suggest may in part explain the persistence of nor-LAAM and dinor-LAAM in plasma. Conversely, subjects with alkaline urine tended to excrete less drug via urine and so would be expected to have relatively higher plasma concentrations.

1.5.3.3.6. Summary

Relatively, few studies have examined the pharmacokinetics of LAAM and its demethylated metabolites in humans, particularly at steady state, and thus the disposition of these compounds in humans is not well described. Furthermore, early studies possessed a number of methodological limitations that affect the usefulness of much of the available data; including the use of small subject numbers (Billings, *et al*., 1974; Henderson, *et al*, 1977b), the use of limited blood sampling points across the sampling period, particularly early on in the inter dosing interval, leading to uncertainty in the interpretation of plasma concentration-time profiles for all three compounds (particularly in estimation of T_{max}, C_{max}) (Billings, *et al*., 1974; Henderson, *et al*., 1977b; Kaiko and Inturrisi, 1975); and limitations of the methods used to quantify LAAM, nor- and dinor-LAAM in biological fluids (Billings, *et al*., 1974; Henderson, *et al*., 1977b; Kaiko and Inturrisi, 1975). However, what data are available reveal that there is considerable inter-individual variability in the pharmacokinetics (i.e., C_{max}, $t_{½\beta}$, CL/*F*, and extent of plasma protein binding etc) for LAAM, and its demethylated metabolites. Such individual variability is likely to determine the response to LAAM treatment and hence, as with MMT, an individual approach to LAAM dosing is required. Moreover, the terminal elimination half lives for both metabolites are considerable longer than for LAAM and, thus, at steady state LAAM's demethylated metabolites, in particular dinor-LAAM, exhibit more stable concentration-time profiles than LAAM. Despite the significant variability in the pharmacokinetics of LAAM, nor- and dinor-LAAM, there appears to be a characteristic plasma concentration versus time profile for each analyte at steady state. Plasma concentrations of LAAM increase rapidly following ingestion and peak approximately 2 to 6 hours after dosing and then decline steadily to be at predose concentrations 24 hours after dosing. Nor-LAAM and dinor-LAAM plasma concentrations peak after the parent drug, consistent with the sequential nature of LAAM's metabolic pathway.

1.5.3.4. Pharmacodynamics of LAAM.

The prolonged activity of LAAM following oral administration has been attributed to its *in vivo* conversion to nor- and dinor-LAAM, and the slower clearance of these active metabolites. Early studies reported that LAAM administered parenterally had a more delayed onset of action (4 to 6 hours), than when administered orally (within 90 minutes) (Fraser and Isbell, 1952), which is contrary to the expected rapid response to a parenterally (*s.c* or *i.v*) administered drug (see discussion by Walsh, *et al*., 1998). Therefore, in an attempt to explain this phenomenon many authors have portrayed LAAM as a pro-drug, with little intrinsic activity, instead relying on conversion to active metabolites for its activity (Jaffe, 1992; Jaffe and Martin, 1991). With this in mind this section will briefly review animal data that illustrates the pharmacodynamic profile of LAAM and its active metabolites before describing the typical profile of opioid effects observed following the acute and chronic administration of LAAM in humans.

1.5.3.4.1. Animal studies.

1.5.3.4.1.1. In vivo antinociceptive activity.

The analgesic effects of acetylmethadol LAAM and its metabolites in animals have been investigated by several groups over the years. Much of the published data come from studies of LAAM and its nor-metabolites in rodents which show that LAAM administered parenterally (*s.c* and *i.p*) is between 0.2 and 0.8 times as potent an analgesic as racemic methadone (Codd, *et al*., 1995; Umans and Inturrisi, 1981; Wax, *et al*., 1975). Contrary to the latter data, Leimbach and Eddy (1954) demonstrated that LAAM was approximately equipotent to racemic methadone in the mouse hotplate test following *s.c* administration. The latter authors all demonstrated the greater potency of LAAM than racemic methadone following oral administration.

With regard to the potency of LAAM's demethylated metabolites, nor-LAAM has been found to be 3 – 14 times more potent than LAAM and 2-11 times more potent than dinor-LAAM in a variety of antinociceptive assays (Aceto, *et al*., 1991; Aceto, *et al*., 1992; Smits, 1974; Umans and Inturissi, 1981). Furthermore, dinor-LAAM was found to be approximately equipotent or slightly more potent than LAAM in these studies. The slower onset and longer duration of LAAM than either racemic-methadone or nor- and dinor-

LAAM were noted (Leimbach and Eddy, 1954; Smits, 1974; Wax, *et al*., 1975; Umans and Inturrisi, 1981).

1.5.3.4.1.2. Other pharmacological effects.

A number of investigators have generated data that describe the activity of LAAM, nor-LAAM and dinor-LAAM as μ opioid agonists in animals. The following section only presents a selected review of some of the more salient findings from these studies.

Two early studies conducted by Moreton and colleagues (Moreton, *et al*., 1976; Young, *et al*., 1977) examined the pharmacodynamic profiles (i.e. sleep/wake cycle, patterns of self administration and intensity of withdrawal) of morphine, LAAM and methadone in rats. Both studies used a similar paradigm in which rats, rendered dependent to morphine, were trained to self-administer morphine (10 mg/kg/injection *i.v*) in order to maintain dependence. In the first study (Moreton, *et al*., 1976) morphine was substituted by either methadone (2 mg/kg) or LAAM (1mg/kg). The mean inter-injection interval was greater for LAAM (8.8h±6.8: mean±SEM), than either for methadone (1.4±0.1h), or for morphine (2.5hr±0.1h). The greater mean inter-injection interval for LAAM was presented as indicative of the long duration of action of LAAM in suppressing drug seeking behaviour (i.e., bar pressing). The second study (Young, *et al*., 1977) investigated the behavioural and EEG correlates associated with withdrawal from these drugs. Morphine dependent rats were allocated to either continue self-administering morphine, two other groups either self administered methadone (2 mg/kg per injection), or LAAM (1 mg/kg per injection), and then once stabilised, saline was substituted. The duration of REM sleep time was severely depressed during the first 24-hour period with morphine and methadone, but only moderately suppressed with LAAM. Indices of withdrawal (frequency of lever pressing and head shakes, irritability) were greater for morphine and methadone than for the LAAM group. From these data LAAM had a longer duration of activity than both morphine and methadone, and withdrawal was less severe from LAAM than from both morphine and methadone.

Using a drug self-administration paradigm in morphine dependent rats, Young and colleagues (1979) substituted LAAM, nor-LAAM, or dinor-LAAM (0.5, 1, & 2 mg/kg for each drug) for morphine, to determine their effects on EEG and frequency of drug self-administration. The authors showed that all drugs maintained dependence, producing

dosage related decreases in number of self-administered injections/day. They reported that multiple injections were more frequent following LAAM administration than following nor-LAAM or dinor-LAAM, which they suggest indicated that drug seeking behaviour was reduced following nor-LAAM and dinor-LAAM, but not LAAM administration.

More recently, Vaupel and Jasinksi (1997) described the pharmacodynamic profile of LAAM, nor- and dinor-LAAM in the chronic spinal dog. Singles doses of LAAM (0.25, 1.0, 1.6 mg/kg *i.v*), nor-LAAM (0.05, 0.2, 0.8 mg/kg *i.v*) and dinor-LAAM (0.1, 0.4, 1.6 mg/kg *i.v*) were administered to non-dependent dogs to determine acute agonist effects over the subsequent five hours. All opioids produced dose dependent antinociception (as measured by decreased responsiveness of flexor reflex and skin twitch reflex), and produced uniformly similar morphine-like effects (hypothermia, antinociception and pupil constriction), but differed in potency. The authors concluded, for the latter measures, that nor-LAAM was 6 to 12 times more potent, and dinor-LAAM was 1.5 to 3 times more potent than LAAM. Further studies by these authors examined the ability of these drugs to suppress withdrawal from morphine. Dogs made dependent to a daily dose of morphine (125 mg/day) were administered either morphine (0.5 and 2.0 mg/kg *i.v*), methadone (0.125 and 0.5 mg/kg *i.v*), LAAM (0.75 and 3.0 mg/kg *i.v*), nor-LAAM (0.05 and 0.2 mg/kg *i.v*), or dinor-LAAM (0.1 and 0.4 mg/kg *i.v*) 40 hours after the last dose of morphine. The authors estimated that nor-LAAM was 9 times as potent as LAAM in suppressing withdrawal. Dinor-LAAM was similar in potency as LAAM. It appeared that there was a trend for LAAM to suppress withdrawal more fully than either nor and dinor-LAAM.

The latter results are consistent with other findings from studies that examined the ability of LAAM and its nor-metabolites to suppress opioid withdrawal in rhesus monkeys (Aceto, *et al*., 1992). These authors conducted single dose suppression studies in morphine dependent rhesus monkeys (morphine sulfate, 3 mg/kg *s.c* q.i.d) that were abstinent for 14 to 15 hours and showed definite signs of withdrawal. LAAM, nor-LAAM and dinor-LAAM *s.c* completely suppressed withdrawal. LAAM and dinor-LAAM were assessed as equally potent as morphine, but nor-LAAM was estimated to be six times as potent as morphine or LAAM (Aceto, *et al*., 1991; 1992). The onset of LAAMs action (0.5hr) was somewhat slower, and its duration of action more prolonged (greater than 24 hours) than that of morphine (Aceto, *et al*., 1992). The onset and duration of dinor-

LAAM's action was similar to that of morphine. However, nor-LAAM was estimated to be 6 times as potent as morphine, and its duration of action 2.5 times that of morphine (Aceto, *et al*., 1991).

Moreover, Bertalmio and colleagues (1992) examined the ability of a number of opioid agonists to substitute for the discriminatory and reinforcing stimulus properties of codeine in monkeys. In producing codeine–like effects, methadone was slightly more potent than morphine, while LAAM was less potent. Codeine (1.8 mg/kg), LAAM (3 mg/kg), nor-LAAM (0.1 mg/kg) and dinor-LAAM (0.6 mg/kg) substituted for the codeine cue in this paradigm. The authors also examined the effect of these drugs on self-administration and found that LAAM in doses up to 0.3 mg/kg did not maintain rates of responding substantially higher than those maintained by saline, but that nor-LAAM (0.1 mg/kg per injection *i.v*) maintained rates of responding close to those maintained by codeine (0.3 mg/kg), while dinor-LAAM (0.1 mg/kg per injection *i.v*) maintained responding at rates intermediate between those produced by LAAM and nor-LAAM. These data clearly indicated the relatively greater potency of the nor-metabolites, compared to LAAM.

Data from these latter *in vivo* studies are consistent with the presented *in vitro* data and demonstrate the relatively greater potency of nor- and dinor-LAAM than LAAM, particularly in inducing dependence and suppressing withdrawal in animals. The data also clearly show that LAAM has significant pharmacological activity. The prolonged duration of action of LAAM, compared to other opioids, such as morphine and methadone, was also noted.

1.5.3.4.2. Human studies.

1.5.3.4.2.1. Acute dosing.

Fraser and colleagues carried out the first clinical evaluation of the acetylmethadols, including LAAM, in humans. In their first study (Fraser and Isbell, 1952) the authors demonstrated that following administration of a single oral dose of LAAM (30-40 mg), to former opioid addicts, objective opioid effects (as indicated by miosis, and euphoria) were observed within 90 minutes and peaked by four hours. On the other hand, following the parenteral administration (*i.v* and *s.c*) of LAAM (10-30 mg), detectable drug effects were delayed up to four to six hours, and tended to be less intense than those experienced following oral administration. Reports of euphoria persisted for up to 48 to 72 hours

following all routes of administration. Miosis persisted for up to 48 hours following *i.v* and *s.c* administration, but had almost reached control values by 24 hours following oral administration. A further study by this group (Fraser, *et al*., 1954) produced data that were consistent with these latter findings. They investigated the miotic effects of LAAM, as well as many other analgesics, and showed that LAAM produced miosis more rapidly when given orally, than when administered subcutaneously. The latter evidence is suggestive of the importance of first pass metabolism in mediating the more rapid effects following LAAM ingestion, relative to intravenous administration.

Cook and colleagues, in studies that were designed to investigate the absolute bioavailability of LAAM and nor-LAAM in MMT patients, also measured pupil diameter on eight occasions over the 24 hours following dosing (Cook, *et al*., 1984; Perez–Reyes, 1985). They carried out preliminary studies to determine the effective doses of oral and *i.v* nor-LAAM required to protect patients from opioid withdrawal. Following this four MMT patients (maintained on methadone 30 mg) received simultaneously half of their effective dose of LAAM orally (20 mg) and the other half *i.v* 24 hours after their last methadone dose. For the second study, three MMT patients (35-45mg methadone daily) received simultaneously half of their effective nor-LAAM dose orally (5 mg) and the other half *i.v*. The magnitude of pupil constriction (maximum percent pupillary constriction and $AUC_{0\text{-}24}$ was greater for LAAM (mean±SEM: 39±5%; 1377±156, respectively), than nor-LAAM (28±2%; 863±84, respectively), although these differences were not statistically significant. Furthermore, in these studies the rate of pupil constriction more closely followed the appearance of nor-LAAM (Pearson r=0.993), and to a lesser extent that of dinor-LAAM (r=0.988), than that of LAAM in plasma (r=-0.754). Hence the authors concluded that nor-LAAM was more potent than LAAM and questioned whether LAAM has any *in vivo* pharmacological activity of its own. Notwithstanding the latter result is the finding that the time course of dinor-LAAM was also significantly correlated with the time course of miosis, which suggests that it is the combined activity of both nor-LAAM and dinor-LAAM that is responsible for much of LAAM's overall opioid effect.

More recently, Walsh and colleagues (1998) used a controlled double blind, double dummy crossover design to investigate the effects of single doses of LAAM (20 or 40 mg/70 mg *i.v* or oral) in nondependent opioid users. A number of physiological (heart rate, blood pressure, pupil diameter, respiratory rate and oxygen saturation) and subjective

measures, including visual analog scales (for e.g., "Do you feel any drug effect ?", "Do you like the drug?", "How high are you?"), subscales of the ARCI (including the MBG, PCAG-sedation scale), and adjective rating scales (i.e., withdrawal scale, agonist adjective scale, and Fraser scale, which consists of weighted items indicative of opioid agonist effects) and observer rated measures were taken across a 96 hour sampling period. The magnitudes of response were generally dose related, regardless of route of administration, and the time to reach peak effect for most measures was faster after administration of intravenous than oral LAAM. Miosis and subjective drug effects (e.g., scores on '…any drug effect' and ' … like the drug') were produced within five minutes of the administration of *i.v* LAAM (20 and 40 mg), but peaked much later, within 4 to 5 hours of administration. In contrast, the 20 mg dose of oral LAAM failed to produce any significant change on any subjective measure across the sampling period. The subjective effects of 40 mg of oral LAAM were not apparent for two to three hours following administration and peaked at nine to twelve hours after administration. Following oral LAAM the onset of miosis was delayed in comparison to that seen after *i.v* dosing, occurring within 2.5 h and 1.5 h, for 20 and 40 mg LAAM, respectively, and peaked at 9-12 hours for both doses. The duration of action was dose related. Using pupillary diameter as a representative measure, significant miosis persisted for 36 hours after 20 mg *i.v*, but 72 hour after 40 mg *i.v*. Oral LAAM also produce miosis of long duration, persisting for 48 and 62 hours after 20 and 40 mg, respectively. Except for a significant decrease in heart rate by all dosages (comparing maximum decrease for the session, with placebo), LAAM in the dosages administered produced no other significant physiological effects.

The latter authors concluded, in contrast to earlier reports, that the onset of LAAM is more rapid following oral than parenteral administration (Fraser and Isbell, 1954) and that following intravenous administration of LAAM the onset of opioid effects will be almost immediate. Furthermore, this immediate opioid response is likely to be attributable to the actions of the parent drug, rather than its demethylated metabolites, as these effects occurred when only LAAM was present in plasma, but the metabolites were below the level of detection. These data would suggest that LAAM is not merely an inactive pro-drug. Notwithstanding the latter findings, the authors reported that the magnitude of effect for many measures continued to increase even after plasma LAAM concentrations were

declining, but plasma nor-LAAM and dinor-LAAM concentrations were on the rise, attesting to the potency of these active metabolites.

Moreover, the same group (Eissenberg, *et al*., 1999) recently conducted a formal evaluation of the relative potency of methadone and LAAM in five non-dependent heroin users. They administered single doses of placebo, methadone (15, 30, or 60 mg/70 kg orally), or LAAM (15, 30, or 60 mg/70 kg orally) once weekly. A variety of measures, as described for the previous study undertaken by Walsh and colleagues (1998), were taken over a 12-hour period following administration of the test drug. They also administered naloxone (1.0 mg/kg *i.m*) 24, 72 and 144 h following the administration of the test drug to examine its ability to reverse the effects of the test drugs. Both LAAM and methadone produced dose and time related effects on a number of subjective measures (including 'feeling high', 'any drug effect', good drug effect', and 'like the drug'') and pupillary diameter. Considering two representative measures of opioid agonist effect, i.e., responses on the subject rated measure 'Do you like the drug' and pupil diameter, the average time to peak effect was shorter for methadone (2-4h), than for LAAM (4-8h). Furthermore, LAAM produced a longer duration of subjective effect than methadone, the 30 and 60 mg dose of LAAM producing significant effects that persisted for up to 12 h, compared to 6 to 10 h for the same dose of methadone. The 15 mg dose of both drugs failed to produce any significant change from placebo on subjective measures. Significant miotic effects persisted for up to 12 h following all doses of LAAM, and for the 30 and 60 mg dose of methadone, but only to 9 hour for the 15 mg methadone dose.

The authors went on to examine those measures that met criteria for a valid bioassay (i.e., where dose response functions of LAAM and methadone did not differ with respect to linearity and parallelism, and also were not different from zero) to determine the relative potency of the two drugs on a particular measure. The relative potency estimates were expressed as the dosage of methadone (mg) required to produce the same effect as 1 mg of LAAM. In all cases where the measure met the criteria for a valid bioassay, the relative potency estimate exceeded 1, suggesting that greater than 1 mg methadone was required to produce the same effect as 1 mg of LAAM. For four measures (the subjective scale – 'any drug effect', the agonist adjective scale, the Fraser scale, and pupillary diameter), the relative potency estimate was significantly greater than the 0.8: 1.0 ratio suggested in the LAAM product information (Roxanne Laboratories, 1995). Furthermore, the relative

potency estimate was significantly greater than 1.0 for the Fraser scale, and pupillary diameter, indicating that LAAM was significantly more potent than methadone for these measures. Moreover, the administration of naloxone 24 hours after agonist exposure did not fully reverse the pupillary constriction produced by 60 mg LAAM. Indeed 90 minutes after naloxone administration the significant miosis that was apparent 24 h following all LAAM doses and 60 mg methadone returned and persisted until the end of the session. For 30 and 60 mg LAAM significant pupil constriction persisted until about 72 hours after dosing. The authors concluded that under acute dosing conditions LAAM might be more potent than previously reported in the product information, and in fact, may be more potent than methadone.

1.5.3.4.2.2. Chronic dosing.

Analgesia

As previously mentioned, some of the early clinical studies of the acetylmethadol group of compounds were concerned with their utility as analgesics. Keats and Beecher (1952) evaluated the analgesic potency of LAAM compared to morphine, by administering up to 40 mg/70 kg of body weight in acute postoperative patients. Patients reported modest relief from pain from all LAAM doses, and no delay in analgesic effects. The latter data are not consistent with those previously reported by Fraser and colleagues (1952) of a 4-6 hour delay in euphoria following *s.c* administration of LAAM. The authors concluded that the analgesia resulting from 20 mg LAAM *s.c* was less than that resulting from 10 mg of morphine *s.c*. They also considered LAAM unsuitable as an analgesic because of delayed toxic effects (i.e., coma) that occurred between 12 to 30 h after dosing in four of their patients. However, these patients had received multiple doses of opioid drugs (LAAM plus morphine *s.c*) within about 30 hours. Reportedly one patient had received; '…40 mg LAAM twice in 34h with 10 mg morphine once in this period ' (Keats and Beecher, 1952). Thus, it is clear that the authors had administered opioids to non-tolerant individuals and had not taken into account the relatively long half lives of LAAM's active metabolites, and hence tendency to accumulate when administered more frequently than every 48/72 hours. More recently, Tennant (1983) demonstrated LAAM's effectiveness in treating patients with chronic pain arising from permanent injury or disability. The author presented a series of four case studies where LAAM (60 – 88 mg dosed on M, W, F) had been substituted for short acting opioids (codeine, hydromorphone, and propoxyphene napsylate) that the patients had become dependent upon. In all but one case, patients reported significant reductions in self-reported pain. One patient reported experiencing

insomnia, dysphoria, leg cramps, and nausea, necessitating cessation of LAAM. Therefore, it appears that further evaluation of the LAAM's potential as an analgesic agent is warranted.

Suppression of withdrawal and cross-tolerance

Fraser and Isbell (1952) initially demonstrated LAAM's capacity to cross substitute for morphine in morphine addicts. They abruptly substituted oral LAAM (b.d) for morphine in 10 patients who had been stabilised on 160 to 400 mg daily morphine, and demonstrated that substitution was adequate (i.e., low intensity of opioid withdrawal signs reported), when one mg of LAAM was substituted for every 6 to 8 mg of morphine. They also showed that following the abrupt cessation of LAAM distinct signs of withdrawal did not appear for approximately 48 hours after the last dose, and that patients experienced withdrawal that was similar in course and intensity to that experienced following cessation of methadone. Furthermore, an additional five patients who had previously been stabilised on 60 mg morphine (q.i.d) were placed on 40 mg of LAAM once daily for ten days; they reported no opioid withdrawal symptoms. In these patients the LAAM dose was increased to 60 mg, administered alternate daily for 7 days, then the inter-dosing interval was increased to 72 hours for a further two weeks, and then to 96 hours for a further two weeks. Patients reported mild withdrawal symptoms at 84 hours following dosing, and significant withdrawal symptoms at 96 hours following dosing (note: the authors failed to describe these symptoms).

Moreover, Zaks and colleagues (Zaks, *et al*., 1972) reported that three of four patients who received low dose LAAM (30–40 mg twice weekly, and 50 mg on Friday) exhibited withdrawal symptoms 40 to 48 hours following their Friday dose. All patients (n=5) that received high dose LAAM (80 mg three times a week), and those who received methadone 100 mg daily (n=10) reported no withdrawal symptoms during the inter-dosing interval.

Levine and colleagues (1973) extended these findings by investigating the dose-response relationship for LAAM's ability to suppress withdrawal symptoms, to produce pupil constriction and to provide cross-tolerance to heroin challenges (the latter two points will be discussed later in this section). They inducted seven patients, recently detoxified from methadone, onto LAAM maintenance. Patients were administered LAAM in 10 mg increments on a three day a week dosing regimen, increasing the dose by 10 mg

increments per week until 100 mg was reached. They demonstrated that doses of LAAM above 80 mg three times a week were effective in suppressing abstinence symptoms (i.e., yawning, lacrimation, and a sense of uneasiness) for 72 hours. For doses in the range 50-70 mg abstinence symptoms mild in severity occurred 48-72 hours after the dose was ingested.

Following repeated administration LAAM also exerts its effects by conferring cross-tolerance to the subjective effects (the 'high') of illicitly administered short acting opioids. There have been few well-controlled studies undertaken to evaluate this phenomenon with respect to LAAM. Zaks and colleagues (1972) demonstrated that low dose LAAM (30– 40 mg twice weekly, and 50 mg on Friday) provided cross-tolerance to 25 mg of intravenous heroin administered 24 hours after the LAAM dose, with only mild subjective effects (euphoria) and pupillary constriction reported following 50 mg heroin. High dose LAAM (80 mg three times a week) and methadone (100 mg) conferred complete cross-tolerance to 50 mg intravenous heroin. Moreover, Levine and colleagues (1973) determined the dose response relationship for 'blockade' of the effects of 25 mg *i.v* heroin challenges administered 72 hours after the last dose (Levine, *et al*., 1973). In this study patients who received sequential doses of LAAM from 10 to 100 mg reported no effect of the heroin (i.e., euphoria), but exhibited very slight pupillary constriction whilst maintained on 50 mg LAAM, with complete blockade of heroin-induced opioid effects following 70 mg LAAM.

A more recent, well-controlled study (Houtsmuller, *et al*., 1998) extended these results. Subjects who were randomly allocated to and had been stabilised for 5-7 weeks on either a low dose (25 mg, n=8) or high dose (75 mg, n=8) of LAAM alternate daily, with placebo on intervening days, were administered intramuscular hydromorphone (0, 6 and 12 mg in a double-blind fashion) in ascending dose order, at 24, 48, 72 and 96 hours following ingestion of LAAM. Withdrawal symptoms (self-reported and observer-rated) were measured at 24, 48, 72 and 96 hours following dosing, prior to hydromorphone administration. Hydromorphone produced dose related effects on physiological measures (pupil constriction, and reduction in heart rate, and oxygen saturation) in both groups, but these effects were significantly attenuated in the high dose group. In the low dose group the magnitude of change in pupil diameter following hydromorphone challenges was greater 96 hours than 24 hours following LAAM dosing; there was no time related change in the magnitude of response following hydromorphine challenge in the high dose group.

With regard to subjective measures, there were significant dosed related increases for a number of subject and observer-rated measures (e.g., 'How are you?' and, 'Do you feel any drug effect?') in the low dose, but not the high dose group. Furthermore, at 96 hours following dosing with LAAM the frequency of subject rated withdrawal symptoms (i.e., painful joints, yawning, watery eyes, runny nose, chills/gooseflesh and hot/cold flushes), had increased in both groups in a time related fashion since LAAM dosing (P=0.39). The only significant difference detected between groups was that the low dose group reported more chills than the high dose group. There were no between group differences on any observer rated withdrawal measures (i.e., modified Himmelsbach withdrawal scale). Thus, following 75 mg LAAM withdrawal was considered mild up to 96 hours after dosing. The authors concluded that 75 mg LAAM provides 'opioid blockade and withdrawal suppression for up to 96 hours, whereas 25 mg LAAM is relatively ineffective".

Time course of opioid effects

As miosis is a reliable and objective indicator of opioid effect (Dyer, *et al.*, 1999; Inturrisi and Verebely, 1972; McCaul, *et al.*, 1982), miosis has been utilized by several different investigators to help elucidate the time course of the pharmacological activity produced by LAAM (Kaiko and Inturissi, 1975; Levine, *et al*., 1973). Levine and colleagues (1973) investigated the dose-response relationship for miosis produced by LAAM. They found that although 20 mg of LAAM produced the maximum miotic effect, doses of 80 - 90 mg three times a week was required to produce persistent miosis at 72 hours. This was consistent with their related finding that 80 mg of LAAM successfully suppressed withdrawal symptoms for the 72-hour inter-dosing interval.

Moreover, Kaiko and Inturrisi (1975) investigated the relationship between the time-course of miosis and the underlying plasma time-course of LAAM and its metabolites over a 48-hour period in LAAM maintenance patients (LAAM 40 – 60 mg dosed three times per week). Miosis was at its maximum at 8 hours post dosing with a mean pupillary diameter of 1.8 mm (range 1.1 to 2.7 mm), but at 24 hours had returned to the diameter found at 4 hours post dosing (1.0 mm) post dosing. Even at 48 hours after dosing mean pupil diameter was 0.3 mm (range: -1.7mmm to 1.5 mm). The time-course of miosis was more closely related to the plasma concentration-time course of nor-LAAM ($r = 0.72$, $P < 0.1$) than to LAAM ($r = 0.43$, $P > .1$). The contribution of dinor-LAAM to this opioid effect was not clear due to its relatively constant concentration across the 48-hour period. However,

as mentioned previously, these data are limited as so few time points were used to construct the time course of miosis, which leads to a large uncertainty in the interpretation of the presented results (particularly in determining time of peak effects).

In summary, to date there has been no comprehensive examination of the magnitude and time-course of subjective (level of withdrawal suppression and mood disturbance) and physiological (pupil diameter, blood pressure, heart rate, and respiratory rate) indices of opioid effect across LAAM's inter-dosing interval at steady state. In comparison to the profile of opioid effects expected by an individual following a single dose of LAAM, a number of factors will impact on the response observed during chronic dosing. Firstly, active metabolites of LAAM tend to accumulate with repeated dosing, with the resultant increase in plasma concentration of active drug and the magnitude of response experienced. Secondly, although chronic dosing will result in the development of tolerance to certain opioid effects, there is likely to be individual variation in the degree to which this tolerance will develop, which will, to some extent, influence individual response to LAAM maintenance. Moreover, related to this is the need to determine how therapeutic response (adequate suppression of withdrawal and normalization of mood states) might be affected by individual variability in the pharmacokinetics of LAAM and its metabolites. Hence, there is need to elucidate the steady state pharmacodynamics of LAAM.

1.5.3.4.3. Symptom complaints during LAAM maintenance treatment.

Generally, the side effects reported in the LAAM trials reviewed above were those that are commonly associated with opioid dependence, and included symptoms of under-dosing or withdrawal (e.g., aching bones and joints, yawning, runny nose, watery eyes, muscle cramps, nausea and vomiting, insomnia, diarrhoea, excessive sweating, irritability and anxiety), those of over-medication or intoxication (i.e., feeling high, nodding, dizziness, impotence) (Ling, *et al*., 1976; 1978), and a group of nonspecific symptoms including constipation, excessive sweating, insomnia, impotence, and reduced libido (Fudala, *et al*., 1997; Jaffe, *et al*., 1972; Johnson, *et al*., 2000; Judson and Goldstein, 1982; Judson, *et al*, 1976; Ling, *et al*., 1976; 1978; Savage, *et al*., 1976; Tennant, *et al*., 1986; White, *et al*., 2002). These symptom complaints were similar and were generally no more severe or frequent than those experienced with methadone maintenance (Freedman and Czertko, 1981; Jaffe, *et al*., 1972; Ling, *et al*., 1976; 1978; Tennant, *et al*, 1986). As with

methadone it is anticipated that tolerance will develop to many of the direct effects of LAAM, but that this tolerance will develop at varying rates. Indeed some complaints may persist even after many months of maintenance treatment.

The study that is considered pivotal in demonstrating LAAM's safety was the 'VA' study carried out by Ling and colleagues (1976). In that study there was generally a low frequency of symptom complaints, which were generally mild to moderate in severity. Symptom complaints were more frequent early in the study (associated with induction and stabilization), and decreased in frequency thereafter, consistent with the development of tolerance and steady state conditions (Ling, *et al.*, 1976). The authors reported that generally LAAM patients had the same or fewer severe rankings for side effects than both high dose and low dose methadone, except for excessive sweating, heartburn, numbness of hands and feet, and oedema of extremities (Ling, *et al.*, 1976). In contrast, in the SAODAP study (Ling, *et al*., 1978) LAAM patients were found to experience symptom complaints at a greater frequency than methadone patients, but this was attributed to the fact that patients were not blinded to the treatment they were receiving.

Judson and Goldstein (1982) compared the symptom complaints of patients on stable doses of methadone (mean±SEM 69±5 mg; range: 30 to 100 mg/daily) and LAAM (mean±SEM 63±3mg thrice weekly; range 20 to 84mg) to patients who were no longer participating in maintenance treatment, and to matched non-drug using controls. The authors reported that patients receiving opioid maintenance treatment (LAAM or methadone) generally felt worse in treatment than when the same patients had left treatment and were no longer using opioids. However, when considering the overall pattern and severity of symptom complaints, the methadone patients at steady state reported more severe symptom complaints than patients receiving LAAM. In particular, methadone patients reported more severe rankings for the following complaints, trouble sleeping, feeling sleepy during the day, headache, nausea and vomiting, poor appetite, and painful joints and bones, and constipation.. However, these differences were not tested statistically.

However, investigators have reported a number of negative characteristics of LAAM therapy. A number of investigators reported that some patients taking LAAM experienced increased irritability (Blachly, *et al*., 1972; Ling, *et al*., 1978; Zaks., *et al*., 1972), which led to some patients dropping out of LAAM maintenance treatment (Ling, *et al*., 1978). It

is likely that the latter reaction experienced by patients was due to under-dosing or withdrawal. Moreover, throughout the clinical trials carried out during the 1970s there were severl reports of small numbers of patients complaining of distressing symptoms related to 'stimulation'; i.e., hyperactivity, anxiety and amphetamine-like symptoms (Billings, *et al*, 1974; Jaffe, *et al.,* 1970; Jaffe, *et al*, 1972; Ling *et al*, 1976; Tennant, *et al*., 1986). Since then there have been reports of a bi-phasic phenomenon characterised by complaints of complaints of stimulation on the day the LAAM dose is ingested and relative sedation the day after. Marcovici and colleagues (1981) reported that up to one third of patients administered LAAM resorted to self-administering heroin on the non-dosing day or dropped out of the study because of the distress caused. In two studies, Crowley and colleagues quantified this change in daily activity and mood across the 48-hour period (Crowley, *et al*., 1979; Crowley, *et al*., 1985), and revealed that the activity of patients, as measured by a pedometer (which measured movement), was significantly greater on the day they ingested LAAM than the non-dosing day. In addition patients felt significantly more fatigued on the non-dosing day. More recently, investigators have reported that nervousness, anxiety, and difficulty sleeping were among the most common symptom complaints by patients taking LAAM (Fudala, *et al*., 1997; White, *et al*., 2002).

Nevertheless a number of studies have reported that LAAM is generally well accepted by a significant proportion of patients. Whilst on LAAM some patients reported they felt more 'normal', experienced less 'nodding', felt LAAM provided better heroin blockade and was more effective in reducing craving for heroin than when they were on methadone (Freedman and Czertko, 1981; Savage, *et al*., 1976; Tennant, *et al*., 1986; Trueblood, *et al*., 1978; White, *et al*., 2002). This has been attributed to the relatively consistent and sustained plasma concentration of nor-LAAM and dinor-LAAM across the inter-dosing interval (Blaine, *et al*., 1981).

1.5.3.4.4. Cognitive functioning.

There is a paucity of literature on the neurocognitive effects of LAAM, and to date, there have only been two published reports on the comparative effects of methadone and LAAM on cognitive function in maintenance patients. In an early study, Irwin and colleagues (1976) demonstrated that LAAM maintenance patients (LMP's: mean dose 79.6 mg alternate daily) performed significantly better than a cohort of MMT patients (mean daily dose 93.5 mg) on a battery of tests designed to assess aspects of information processing

and learning (e.g., number learning arithmetic, and number and name checking). In contrast, Grevert and colleagues (1977), found no significant differences in scores for visual memory (memory of location) and verbal material (list of nouns and numbers) between MMT patients (mean daily dosage 52 mg) and LMPs (LAAM dosed 3xweek 50 to 60 mg), who were tested on three occasions, before starting maintenance treatment, one month and three months later. Thus, although there has been significant research on the treatment effectiveness of LAAM as a maintenance pharmacotherapy, there has been little research on the potential cognitive effects of LAAM; indeed the evidence that is available is equivocal. Hence, there is a need to elucidate the comparative effect of methadone and LAAM on cognitive function in maintenance patients – this will be further explored in Chapter 7.

1.5.3.5. Toxicity.

Discussion of the toxicology of LAAM in animals is beyond the scope of this review (see reviews by Borzelleca and colleagues (1994, 1995), and Wolven and Archer (1976)). With regard to humans, as previously discussed, a number of early studies demonstrated the potential for the development of cumulative toxicity when patients received LAAM more frequently than alternate daily (Keats and Beecher, 1952; Fraser and Isbell, 1952). The larger clinical studies (VA, SAODAP, Phase III) that were to follow provided a comprehensive view of the safety of LAAM in maintenance treatment, by assessing liver, kidney functioning, blood chemistry, hematology, and urinalysis, as well as collecting detailed information on the symptom complaints reported by those patients taking LAAM and those taking methadone. The results of the tests performed on those receiving LAAM were similar to those reported for patients on methadone, and showed no systematic or progressive change during any of the studies (Blaine, *et al*., 1981; Ling, *et al*., 1976; 1978; Whysner, *et al*., 1978). Smaller scale studies carried out during the same period presented the same conclusions (Freedman and Czertko, 1981; Karp-Gelernter, *et al*., 1982; Savage, *et al*., 1976; Senay, *et al*, 1974). Furthermore, there were only a few deaths reported in the studies reviewed, and on the whole they were not considered related to LAAM ingestion (Ling, *et al*., 1978; Senay, *et al*., 1974; Tenant, *et al*., 1986).

Tennant and colleagues (1986) failed to identify any long-term toxic effects of LAAM in a subset of patients that had remained in maintenance treatment for up to 36 months. No changes (from baseline) in liver function tests were found in 85 patients at 12 months, nor

20 patients who had been in treatment for longer than two years. Furthermore, of the 41 patients who had been in treatment for 18 to 36 months, radionuclide liver-spleen imaging failed to find evidence of tumor formation, and only eight patients showed evidence of liver disease (enlargement, diffuse disease) commonly seen with early cirrhosis. Moreover, there were few clinically significant findings in those who had ECGs (only one was found to have an abnormality, but had a preexisting valvular disease), EEGs, renal and thyroid function tests.

Following FDA approval of LAAM, Fudala and colleagues (1997) conducted an open-label observational study of LAAM to collect data on safety, and to test treatment guidelines. Six hundred male and female patients were recruited from 26 clinics across the U.S.A. Three deaths were reported, but these could not be attributed to LAAM. Three women became pregnant while taking LAAM; two elected to terminate their pregnancies, while one who received LAAM for one month transferred to methadone and gave birth to premature twins that died soon after birth. The role of LAAM in these deaths could not be determined with any certainty. The authors noted that twenty-four patients were hyperglycemic at some time during the study; twelve of whom had elevated blood sugar or a previous diagnosis of diabetes mellitus at baseline. Blood sugar concentrations returned to normal for six patients during treatment, but remained elevated for the remaining six, and this was considered possibly related to LAAM treatment. Twelve patients also developed histamine or allergic type reaction, which manifested as pruritis plus rash or exacerbation of asthmatic symptoms. Despite these adverse reactions this study concluded that LAAM is safe for use in the maintenance treatment of opioid dependence.

More recently, however, the cardiac effects of LAAM have been raised as a concern. Up to 2001 it was estimated that LAAM had been used on approximately 33,000 patients (Medical and Drug Regulatory Affairs, Roxane Laboratories, 2001). Since 1997 there have been reports of serious cardiac rhythm disorders, including seven known or suspected cases of torsades de pointes, a particular pattern of ventricular fibrillation (Medical and Drug Regulatory Affairs, Roxane Laboratories, 2001). This is a potentially life threatening ventricular cardiac arrhythmia associated with prolonged QT interval (Ben-David and Zipes, 1993). Prior to this it is difficult to be sure of the exact frequency of these events amongst patients that have taken LAAM, as previous events may have gone undetected, and have only recently been reported (Anonymous (FDA), 2001; Deamer, 2001). There

were no reports of cardiac arrhythmia in any of the studies reviewed by Clark and colleagues for their meta-analysis of the efficacy and safety of LAAM; however only two of the reviewed studies measured ECG, and neither specifically measured QT interval (Clark, *et al*., 2002). In 2001, the FDA recommended restricted use of LAAM as a maintenance pharmacotherapy (Anonymous, (FDA) 2001; Schwetz, 2001), and at the same time the European Agency for the Evaluation of Medicinal Products (EMEA) recommended that patients receiving LAAM should be transferred to alternative treatments, and recommended the suspension of the marketing authorisation of LAAM in Europe (Anonymous (EMEA), 2001).

There have been sporadic reports in the literature concerning the tendency of LAAM to be highly proarrhythmic. In early preclinical studies electrocardiogram (ECG) tracings taken from beagle dogs, showed prolonged ST segments immediately following dosing of medium (6.0 mg/kg) and high dose (11.0 mg/kg) LAAM that was associated with bradycardia. Seventy–two hours following dosing the ECG tracing returned to normal (Wolven and Archer, 1976). Towery and Rios (1975) reported that animals administered 4-8 mg/kg of LAAM exhibited ECG changes (ST segment and T waves changes, and alterations in the QRS complex), which reverted to normal on cessation of LAAM. However, these doses were far greater than those recommended in maintenance treatment. Therefore, the latter authors went on to review the ECGs from 430 maintenance patients (with no previous history of cardiovascular disease) who were maintained on either LAAM or methadone for four months. Approximately 15-20 % of patients in each group were also taking tranquilizers and/or antidepressant medications, and baseline ECGs were normal in 84 and 85 % of patients, respectively. Following four months of maintenance treatment a review of ECGs showed no significant changes in any patients. On the other hand clinical studies, where ECGs were repeatedly measured, showed an increase in QTc interval in patients transferred from methadone to LAAM, and this was presented as evidence of the greater proarrhythmic potency of LAAM compared to methadone (Anonymous (EMEA), 2001).

It is proposed that the proarrhythmic action of action of LAAM is mediated through the inhibition of potassium currents in the human heart, a common mechanism by which a number of other proarrhythmic drugs, such as some antiarrhythmics (e.g., quinidine, procainamide), antihistamines (terfenadine), gastrointestinal prokinetics (cisapride), and

antipsychotics (haloperidol, phenothiazines) (DePonti, *et al*., 2001; Faber, *et al*, 1994), mediate prolonged QT interval (Kang, *et al*., 2003). Recent studies, using patch clamp electrophysiological methods have examined the effects of LAAM (Kang, *et al*., 2003; Katchman, *et al*., 2002) and nor-LAAM (Kang, *et al*., 2003) on the human cardiac potassium channel HERG (Human ether-ago-go-related gene) cloned from Chinese hamster ovary cells. These studies have confirmed that LAAM is a potent inhibitor of HERG channel currents (Kang, *et al*, 2003; Katchman, *et al*., 2002). Moreover, Kang and colleagues reported that LAAM (IC_{50} for LAAM block of peak HERG tail currents=3.0 μM: 1.8-5.3μM 95% CI) was more potent in this action than nor-LAAM (estimated IC_{50} 12μM). Methadone was also found to be a potent inhibitor of HERG currents (IC_{50} ~10 μM), but the degree of plasma binding of methadone would reduce the free fraction of methadone and hence its *in vivo* potential to inhibit HERG currents (Katchman, *et al*., 2002).

However, there are reports that very high dose methadone administered to MMT patients (for eg, (daily mean±sd dose 397±283mg, (Krantz, *et al*., 2002); daily mean±sd dose 268±190 mg (Sticherling, *et al*., 2005)) has been associated with prolongation of the QTc interval, and torsades de pointes. The latter authors commented that arrhythmias occurred after co-administration of other agents known to produce prolonged QT intervals, or as a result of hypokalemia, due to excessive alcohol intake (Krantz, *et al*. 2002; Sticherling, *et al*., 2005). Although the stated doses may not be typical of those generally used in the context of maintenance treatment, chronic pain clinics do reportedly use such dosages (Krantz, *et al*., 2002). Furthermore, as in the case of LAAM the level of underreporting is probably quite high.

Although it has been suggested that methadone has the potential to prolong QT interval and thus be proarrhythmic, especially at high doses, it has been suggested that this potential is probably much lower than for LAAM, especially at the doses used for maintenance treatment (Anonymous (EMEA), 2001). Despite this, a recent study has revealed that patients maintained on methadone exhibit demonstrable changes in heart conduction, as measured by QT interval, when compared to treatment entry. Additionally, considerable inter-individual variation in QT intervals was observed (Huber, *et al*., 2001). Moreover, there is a preliminary report that tolerance can develop to this effect of LAAM. In those patients who do develop QT prolongation, the greatest increase in the QTc

interval occurs as the maintenance dose is reached, but thereafter tends to stabilize, even reduce (Ingersoll, *et al*., 2004). Thus, it has been recently suggested that the concerns regarding QT prolongation with LAAM may be exaggerated (Ritter, *et al*., 2003), and therefore it is imperative that further research is conducted to identify the risk factors with regard to QT prolongation occurimng as a result of methadone and LAAM administration.

1.5.3.6. Summary.

In summary, randomised controlled trials have established LAAM's effectiveness as a maintenance agent, particularly with respect to the reduction in illicit heroin use (for e.g. Johnson, *et al*., 2000; Ling, *et al*., 1978; Savage, *et al*., 1976). Like methadone, LAAM is a μ opioid agonist and produces actions that are typical of such drugs, including respiratory depression, euphoria and pupillary constriction (Eissenberg, *et al*., 1999; Fraser and Isbell, 1952; Levine, *et al*., 1973; Walsh, *et al*., 1998). Its main advantage over methadone is its prolonged duration of action, with the suppression of withdrawal and pupil constriction reported to last up to 72 hours following oral administration (Fraser and Isbell, 1952; Houstmuller, *et al*., 1998; Levine, *et al*., 1973). This permits dosing every 48 to 72 hours in the maintenance treatment of opioid dependence. The prolonged activity of LAAM (withdrawal suppression, miosis) has been attributed to its *in vivo* conversion to active metabolites, nor-LAAM and dinor-LAAM (Horng, *et al*., 1976; Nickander, *et al*., 1974; Smits, 1974; Umans and Inturissi, 1981; Vaupel and Jasinski, 1997), which have longer terminal half-lives than LAAM (Finkle, *et al*., 1982; Henderson, *et al*., 1977b). However, the disposition of LAAM in humans is not well described, particularly at steady state. The available data show there is large individual variation in the pharmacokinetics of LAAM. However, it is not clear what effect such individual variations in steady state nor-LAAM and dinor-LAAM plasma concentration-time profiles might have on the therapeutic response to LAAM. Furthermore, to date and to my knowledge, no study has comprehensively examined the time course of opioid effects (i.e., subjective effects such as withdrawal, euphoria and analgesia; and physiological indices, such as miosis, blood pressure) across the 48-hour inter-dosing interval, and also cognitive functioning, under chronic dosing conditions in maintenance patients.

1.6. The present study - experimental rationale and aims.

Since 1965, methadone has become the most widely used agent to manage opioid dependence. MMT programs have demonstrated effectiveness in reducing heroin use and improving the physical and psychological well being of patients engaged in treatment (Bell, *et al*., 1997: Gronbladh, *et al.*, 1990; Kreek, 2000; Metzger, *et al*, 1993). To be effective, MMT should retain patients in treatment, sometimes indefinitely, and achieve minimal levels of illicit drug use amongst those retained (Ward, *et al*., 1999). However, even when doses appear to be adequate, or when doses are reached that induce significant adverse effects, a significant proportion of MMT patients complain of reduced methadone efficacy and withdrawal (non-holding) just prior to their next dose. This non-holding status has been associated with increased illicit drug use and poor psychosocial outcome (Holmstrand, *et al*., 1978), and is likely to contribute to patients withdrawing from treatment.

Dyer and White (1999) demonstrated that 'non-holders' exhibit a greater maximum rate of decline in plasma racemic-methadone concentrations from peak to trough concentrations than holders. They went on to show that in MMT patients, plasma methadone concentration-effect relationships are very steep for both withdrawal and positive opioid effects, such as euphoria (Dyer, *et al*., 1999, 2001). Thus, only small changes in plasma methadone concentration translate into relatively large changes in response, which would be exaggerated in non-holders as they exhibited greater rate of decline in plasma methadone concentrations than holders. Despite methadone's long terminal half life, the fluctuation in the plasma concentration across the 24 h inter-dosing interval is significant, with a mean peak to trough plasma concentration of 1.7 (Dyer, *et al*., 1999), and hence there is potential for marked changes in opioid effect over a dosing interval.

One potential solution to the problem of non-holding at apparently adequate methadone doses is to increase the dose of methadone to achieve higher plasma concentrations; however, this would increase the incidence and severity of adverse effects, such as respiratory depression, sedation and constipation. Another possible option is to administer the methadone in divided doses in order to flatten the plasma concentration-time profile across the inter-dosing interval and therefore lessen the rate of decline in plasma concentration of active drug (Dyer, *et al*., 1999; Walton, *et al*., 1978). However, this may be impractical where dosing needs to be supervised, which will increase the costs

associated with the program; and where take-away privileges are granted, may result in increased diversion of methadone. An alternative to these strategies is to use a maintenance drug with different pharmacokinetics to methadone to lessen the fluctuation in plasma opioid concentrations over the dosing interval. A number of suitable alternatives, such as LAAM, and the partial agonist buprenorphine, have been evaluated and have been shown to produce equivalent outcomes to methadone (for e.g., Johnson, *et al.*, 2000; Ling, *et al*., 1976; 1978). Thus, those patients who experience withdrawal on apparently adequate methadone doses, or unacceptable adverse side effects, may benefit from transfer to one of these medications. Transfer to buprenorphine, however, may precipitate withdrawal in patients who are maintained on methadone doses greater than 30 mg/day (Breen, *et al*., 2003; Bouchez, *et al*., 1998). In contrast, transfer to LAAM is relatively easily achieved (Fudala, *et al*., 1997; Ling, *et al*., 1978; Tennant, *et al*., 1986).

LAAM is converted to two more potent metabolites, nor-LAAM and dinor-LAAM (Horng, *et al*., 1976; Nickander, *et al*., 1974, Smits, 1974; Umans and Inturrisi, 1981); both have longer terminal half-lives than LAAM (Billings, *et al*., 1973, 1974; Henderson, *et al*., 1977b). Therefore, plasma concentrations versus time profiles of the active metabolites are likely to be flatter than can be achieved with methadone, and need to be considered when evaluating the outcomes with LAAM. However, there is a relative paucity of available data on the disposition of LAAM and its effects in maintenance patients. In particular, it is not clear what effect the inter-individual variability of plasma concentration-time profiles might have on therapeutic outcome on maintenance treatment. Moreover, to date, no formal evaluation of the efficacy of using LAAM to treat breakthrough withdrawal (non-holding) amongst MMT patients has been undertaken.

Thus, this thesis describes a comparison of methadone and LAAM in individuals who, during methadone maintenance, did (non-holders) and did not (holders) report the failure of their methadone dose to adequately suppress withdrawal across the dosing interval. The study used a randomized crossover design, where patients were maintained on methadone or LAAM for three months and then were crossed over to the alternative drug for three months. At steady state (unchanged methadone dose for at least 2 weeks, and unchanged LAAM dose for at least 4 weeks), there were two testing sessions (24 hour for methadone and 48 hour for LAAM) that permitted a comparison of the steady-state pharmacodynamics and pharmacokinetics of each drug. During each testing session

concurrent measurements of plasma (R)–(-) methadone concentrations or plasma LAAM, nor-LAAM and dinor-LAAM concentrations and subjective and physiological indices of opioid effect were taken across the inter-dosing interval. In addition, receptor binding studies and a functional assay (depression of the electrically induced twitch of guinea pig ileum) were undertaken to provide contemporary data on the relative potency of LAAM, nor-LAAM and dinor-LAAM.

1.6.1. Aims

The aims of this thesis were:

Aim 1: To further characterise the pharmacokinetic and pharmacodynamic basis of breakthrough withdrawal (non-holding) amongst a cohort of MMT patients.

The first phase of this study was to address a methodological concern raised by Dyer and colleagues (1999). Methadone is usually administered as a racemate, and as there are marked stereoselective differences in the disposition of each enantiomer (Foster, *et al*, 2000; 2004; Kreek, *et al.*, 1979), the measurement of racemic-methadone may not accurately reflect the plasma concentration of the active (R)-(-) enantiomer. Thus, before one can be confident that there is a pharmacokinetic mechanism responsible for the difference found between nonholders and holders on MMT, it is necessary to explore the pharmacodynamic and pharmacokinetic basis for non-holding using the active (R)-(-) enantiomer in any analyses. This aim will be addressed in Chapter 3.

Aim 2: To elucidate the steady state pharmacodynamic and pharmacokinetics of LAAM, nor-LAAM and dinor-LAAM in a cohort of maintenance patients, and, in particular, determine the effect that individual variability in plasma concentration-time profiles of nor-LAAM and dinor-LAAM has on therapeutic outcome (i.e., self reported withdrawal) on maintenance treatment. This aim will be addressed in chapter 4.

Aim 3: To determine whether those who experience reduced therapeutic efficacy (i.e. breakthrough withdrawal and mood disturbance) during the 24 hour methadone inter-dosing interval also experience reduced therapeutic efficacy during the 48 hour LAAM inter-dosing interval, and to characterise the pharmacological basis of any such effect.

This aim will be addressed in chapters 5 through to 7. Chapter 5 will present a comparison of subjective and physiological indices of opioid effect between those who report breakthrough withdrawal (non-holders) on methadone and those who do not (holders) when maintained on methadone and LAAM. Chapter 6 will present a comparison of the temporal change in self reported mood across methadone's and LAAM's inter-dosing interval, and chapter 7 will present a comparison of cognitive and psychomotor function on each drug.

The above-mentioned aims were the primary research aims of the thesis. However, there was a secondary aim, designed to rectify the relative lack of contemporary data characterizing the *in vitro* potency of LAAM, nor-LAAM and dinor LAAM in the literature. Such data would aid in the interpretation of pharmacodynamic data. This will be covered in chapter 8. Thus, the fourth aim was:

Aim 4. To characterise the *in vitro* potency of LAAM, nor-LAAM and dinor-LAAM.

While the bulk of the work reported in this thesis was undertaken prior to the announcement of regulatory changes in response to concerns about LAAM's adverse effects (Anonymous, (FDA), 2001; Anonymous (EMEA), 2001), it is envisaged that the findings reported in this thesis will further inform authorities on the proposed clinical utility of LAAM as an alternative full opioid agonist to methadone. Importantly, the work described herein will explore the utility of using LAAM to treat patients who respond poorly to methadone, and hence explores a potential clinical niche for LAAM.

2. General Methods

2.1. Overview.

The purpose of this chapter is to present an overview of the study methodology and participants that are common to all subsequent chapters concerned with the inter-dosing interval studies. The methods used for the *in-vitro* investigation of the pharmacological activity of LAAM, nor-LAAM, and dinor-LAAM will be presented in chapter 8.

2.2. The South Australian Methadone Program.

In South Australia methadone is prescribed by medical practitioners working either in private practice or through the publicly funded Maintenance Pharmacotherapies Unit (MPU). At the time this study was undertaken the MPU was located at the Warinilla clinic, situated in suburban Adelaide approximately 4 km from the Adelaide central business district. Patients admitted to either the 'private' or 'public' methadone program must meet DSM-IV criteria for opioid dependence (APA, 1994). There are approximately 900 patients on the Public Methadone Program. Although the clinical management of these patients is the responsibility of the Warinilla clinic, in the majority of cases methadone is dispensed through community pharmacies. Patients' take away privileges are determined by staff on the basis of a patient's continual commitment to heroin abstinence (monitored by urinalysis) and evidence of social stability. Normal clinic policy is one of flexible dosing and encourages significant patient input into decisions on dosing (White, *et al*., 1996).

2.3. Study design.

This study was approved by the Royal Adelaide Hospital Research Ethics Committee, Adelaide, South Australia (Protocol No. 980310). It was a randomised, crossover, open comparison of LAAM and methadone. Patients were categorised as holders or non-holders on the basis of their self-report prior to participating in the study. Eligible methadone maintenance patients were randomly allocated to either continue methadone for 3 months or to switch to LAAM for three months, at which time each patient was crossed over to the alternative drug for a further 3 months. This was determined according to a pseudo random schedule so that half of the patients completed methadone first and the other half LAAM first. Patients underwent a testing session in each period (i.e., a 24 hour

methadone inter-dosing study and a 48 hour LAAM inter-dosing study) when they had reached steady state for each drug (unchanged methadone dose for at least 2 weeks and unchanged LAAM dose for at least 4 weeks). Drug-free control subjects were also tested to ensure that any subjective and/or physiological change observed reflected opioid effect. They underwent a 48-hour test session. Only one patient or control subject at a time underwent testing.

2.4. Participants.

2.4.1. Maintenance patients.

Patients were volunteers who were between 18 and 64 years, had been continuously enrolled on the South Australian Methadone Maintenance Program for at least three months, with less than 15 mg dose change during that period, who spoke English as their first language and who could give informed consent. Recruitment was primarily by a brief letter requesting their participation in the study, but also on a few occasions, by me directly approaching prospective patients. Interested patients were asked to attend a preliminary interview with me to discuss the details of the study and to ascertain, using non-directive questioning, the patient's methadone non-holding status. The goal was to have an equal number of patients who were self-reported holders and non-holders on methadone and who had completed both inter-dosing interval studies. Recruitment continued until this was achieved. Patients were given a detailed information sheet to take home with instructions to contact any of the research staff if they had any queries. To ensure that patients met the criteria for admission to the study they received a thorough medical screening, which included blood analysis for liver enzymes and chemistry, assessment of venous access, and an electrocardiograph (ECG). Patients' ECGs were visually checked by a physician (Dr Ian Buttfield at the Warinilla clinic) for the presence of any cardiac arrhythmia, including QTc prolongation.

Patients were excluded from participating in the study if they exhibited any evidence of significant medical ((including positive HIV serology, elevated liver enzymes (i.e., alanine aminotransferase and aspartate aminotransferase > 3 times the upper limit of normal range)) or psychiatric illness, evidence of prolonged QTc interval on ECG or any other cardiac anomaly, i.e., arrhythmia, or clinically significant bradycardia (< 50 beats/minute), were taking any medication that might interfere with methadone and/or LAAM

pharmacokinetics and/or cardiac function, were pregnant or breast feeding or, had any visual or hearing impairment that might affect performance on cognitive tests.

A total of 19 patients (9 males, 10 females) volunteered to participate and met the inclusion criteria for the study. All patients were Caucasian and at the time of the preliminary interview had been participating in methadone maintenance for a median of 46 months (range 3-156 months). The patients were being administered a daily mean (±sd) methadone dose of 73±38 mg (range: 20-150 mg). They reported first using heroin at a mean (±sd) age of 19±3 years. The mean length of consistent heroin use (i.e., no interruptions of greater than one week) prior to commencing the current methadone maintenance program was 3.1±3. (0.17 to 14) years. Table 2.1 presents the demographic details for all patients at the time of the preliminary interview.

Maintenance patients were informed that the information they provided would be treated in confidence, that they were free to withdraw from the study at any time, and that their involvement in the study would not affect their regular treatment.

2.4.1.1. Patient attrition and representativeness of sample.

Two patients (one male and one female) who had been allocated to methadone first, and who had completed the methadone inter-dosing interval study, withdrew from the study soon after commencing LAAM because of adverse effects (i.e., the male patient suffered a skin rash and pruritus, the female patient complained of anxiety). Furthermore, one male subject who been allocated to LAAM first, and who had completed the LAAM inter-dosing interval study, withdrew from the study before crossing over to methadone for non-drug related reasons. Thus, eighteen subjects (8 males, 10 females) completed the methadone inter-dosing interval study and seventeen (8 males, 9 females) completed the LAAM inter-dosing interval study. In total, sixteen subjects (8 holders and 8 non-holders) completed both inter-dosing interval studies. Demographic and clinical details of those patients that completed the methadone inter-dosing interval study will be presented in chapter 3, and those that completed the LAAM inter-dosing interval will be presented in chapter 4.

Table 2-1: Demographic details of methadone maintenance patients (n=19) at time of entry to study.

Age (mean±sd, range)	35±8 (21-48)
Gender (males, %)	9 (47)
Hepatitis C serology (+ve) (n, %)	13 (68)
Highest level education achieved (n, %)	
Year 10 or less	5 (26)
Year 11	6 (32)
Year 12	6 (32)
TAFE/Apprenticeship	2 (11)
University	0
Employment at recruitment (n, %)	
Unemployed/Pension	12 (63)
Unskilled	2 (11)
Skilled/trade	3 (16)
Student	0
Professional	2 (11)
Marital Status at recruitment (n, %)	
Single	9 (47)
Divorced/separated	3 (16)
Has partner (not married)	5 (26)
Currently married	2 (11)

In view of the relatively small sample size utilised in this study it is important to determine the degree it represents the larger population of MMT patients in Australia. The characteristics of the sample used in this study are similar, with the exception of gender ratio, to those recently reported by the National Evaluation of Pharmacotherapies for Opioid Dependence (NEPOD) project undertaken in Australia (Mattick, *et al*., 2001). The NEPOD project pooled data collected in 13 separate clinical trials of pharmacotherapies (buprenorphine, LAAM, naltrexone) for opioid dependence conducted across Australia between 1998 to 2000. This had a combined total of data from 1070 heroin users and 355 methadone patients. The methadone patients in these trials were predominantly male (59%), first used heroin at a mean of 20 (±5) years, had been in MMT for a mean of 53 months, and were unemployed or in receipt of government pension benefits (almost 50%).

The patients' mean methadone dose was 53 mg (±29). Moreover, the characteristics of the sample participating in this study are similar to those reported in the large-scale survey of methadone patients undertaken in South Australia (Dyer and White, 1997).

2.4.2. Drug-free control subjects.

Ten (6 male, 4 female) drug-free control subjects were recruited by word of mouth, and fliers posted around the University of Adelaide campus. Their ages ranged from 21 to 49 years (mean±sd: 35±9.5 years) and their body weights ranged from 60 to 95 kg (mean±sd: 77±12 kg). All were employed on a full time basis or were full time students. They were matched with maintenance patients according to age and gender. One subject reported consuming 20 gms of alcohol daily, while the rest reporting consuming less than 10 gms of alcohol in the four weeks preceding the testing session. Only one subject reported smoking tobacco (on average four cigarettes daily). None reported taking any other psychoactive medication (other than caffeine), or prescribed medication (i.e., benzodiazepines, psychotropic medications) known to affect psychomotor and/or cognitive performance during the month preceding the study. Furthermore, none reported having any visual or hearing impairment that might also affect performance on cognitive tests.

2.5. Drugs and drug administration.

Racemic methadone hydrochloride oral solution (5 mg/ml: Glaxo Wellcome Australia Ltd, Boronia, Victoria, Australia) was administered once daily under supervision. LAAM hydrochloride oral solution (10 mg/ml: gift from Roxane Laboratories, Columbus, OH, U.S.A) was administered every second day (including over the weekend), also under supervision. Doses of each drug were determined on an individual basis as determined by clinic protocol. During the inter-dosing interval studies, opioid agonists were administered, with a glass of water, between 0930 and 1030 am. Patients were not permitted to eat or smoke approximately one hour before or after opioid agonists were administered. All prescribed medications were administered under my direct supervision, and hence complete adherence was assured.

As discussed in chapter one, the use of a number of psychoactive drugs, such as opioids and benzodiazepines, concurrently with maintenance drugs is likely to have significant synergistic effects on pharmacodynamic responses. Therefore, patients were asked to refrain from using any psychoactive drugs (except caffeine or tobacco) during the 24 hours

leading up to each of the inter-dosing interval studies. Predose urine and blood samples were collected to verify the latter. The results of the analysis of these samples, and the implications of these results on study outcomes, are presented in forthcoming chapters. Consumption of tobacco and caffeine is also likely to affect pharmacodynamic responses; however, participants were permitted to use these substances during the inter-dosing interval studies for the following reasons. Firstly, it is likely that abstinence from these substances would manifest in a constellation of withdrawal symptoms that would confound measurement of opioid withdrawal, and, secondly, prohibiting the use of these drugs would have had a negative impact on the ability to recruit for this study, hence affecting the representativeness of the sample.

2.6. Procedures and measures.

2.6.1. Clinical procedures.

2.6.1.1. Transfer between methadone and LAAM.

All stabilisation on LAAM and methadone was carried out at the Warinilla clinic by MPU staff. The starting second daily LAAM dose was 1.1 times the patient's methadone dose, viz, a patient stabilised on 100 mg of methadone daily would be commenced on 110 mg of LAAM every alternate day (Roxane Laboratories, 1995). It is recognized that the product information recommends a conversion factor of 1.2 to 1.3 when transferring patients from methadone to LAAM. However, a conversion factor of 1.1 was used in this study to allow for individual differences in response to the drug and to ensure that the lowest LAAM dose possible was administered**.** The first dose of LAAM was administered 24 hours after the last methadone dose, and patients were required to attend the clinic daily for at least ten days from the commencement of LAAM therapy. On each of these days they were assessed by a counsellor and completed the Short Opiate Withdrawal Scale (SOWS) (Gossop, 1990). If a patient exhibited symptoms of withdrawal or intoxication they were assessed by a medical practitioner, who would alter their dose accordingly. Additional maintenance medication was prescribed according to the following guidelines. If attendance was on a dosing day, the LAAM dose was increased by 5 to 10 mg. However, if attendance was on a non-dosing day, methadone 'top up' doses were administered; these were initially about one third of the patient's pre-LAAM methadone dose, but were gradually decreased across the ten day stabilisation period. Generally, patients did not

require more than four additional methadone 'top up' doses and, indeed, some patients did not require any at all. The final maintenance dose of LAAM was in the range of 40 to 150 mg alternate daily (mean±sd: 80.3 mg (± 43.2 mg)). Following this stabilisation period, patients were expected to return to their community pharmacy to continue collecting their dose. If patients had 'take away' privileges when maintained on methadone they retained these privileges while taking LAAM.

2.6.1.2. Transfer between LAAM and methadone.

Transfer from LAAM back to methadone was generally well tolerated. Initially patients were put back on the same dose of methadone they were on before commencing LAAM. It was administered 48 hour after their last LAAM dose. The clinic protocol for stabilisation on methadone required patients to attend the Warinilla clinic daily for five days from the re-initiation of methadone treatment. Patients filled out the SOWS on each day and could receive a dose change if they reported withdrawal or intoxication.

2.6.2. Inter-dosing interval studies.

The following procedures were used to investigate the pharmacokinetics and temporal change in opioid effects and withdrawal across the inter-dosing intervals for both LAAM and methadone, and were based on the original methods of Dyer and colleagues (Dyer, *et al*., 1999). Patients underwent a testing session in each period (i.e., a 24 hour methadone inter-dosing study and a 48 hour LAAM inter-dosing study) when they had reached steady state for each drug (unchanged methadone dose for at least 2 weeks and unchanged LAAM dose for at least 4 weeks).

All testing sessions were carried out in a quiet air-conditioned (22 ± 2^{0}C), windowless room, under constant illumination (400 lux), at the Royal Adelaide Hospital, Adelaide, South Australia. Lighting conditions were verified on a number of occasions using a digital light meter (Model 610-815, RS Electronics Ltd, United Kingdom). Attempts were made to 'normalise' conditions in this room and so a video tape player and television were available for subjects' use between testing. Participants were permitted to smoke and consume caffeinated beverages. Meals were provided at approximately midday and at 1800 hours. All amenities were easily accessible and I accompanied subjects whenever they left the room.

For both the methadone and LAAM inter-dosing interval studies data collection commenced between 08:30am to 09:30am on the first day of testing. Collection of blood and measurement of pharmacodynamic measures (with the exception of neurocognitive measures) continued until approximately 10:00 am the next morning for those patients participating in the methadone inter-dosing interval study (i.e., 24 h), and the same time on the second day for those patients participating in the LAAM inter-dosing interval study (i.e., 48 h). Patients were transferred to the inpatient residential unit at Warinilla clinic for the intervening nights. This is a locked, secure unit, staffed by trained nursing personnel. This procedure ensured that all patients were treated consistently during the night, and importantly that they did not use illicit psychoactive substances. All transport between the hospital and Warinilla clinic was by taxi and I accompanied the patient at all times.

At the commencement of testing, an intravenous catheter was inserted into a suitable forearm vein. Prior to the administration of the maintenance drug, information concerning demographic factors, past and current drug use (licit and illicit during the preceding month) was collected. At the same time participants also completed the following self-report questionnaires - the Beck Depression Inventory (BDI-II revised: Beck, *et al*., 1996), and the 'trait' anxiety subscale of The State-Trait Anxiety Inventory (Spielberger, *et al*., 1983). The latter measures were completed only once during each inter-dosing interval. Following this, patients were requested to completely empty their bladder; a small sample of this sample was saved for later qualitative analysis for opioids, cannabinoids, sympathomimetic amines and benzodiazepines by the Institute of Medical and Veterinary Science, Adelaide, South Australia. All subsequent urine that was voided during the LAAM inter-dosing interval study was collected (see section 2.6.4.4). A blood sample was also taken for the detection of morphine (a quantitative marker of illicit heroin use) and to screen for a number of benzodiazepines.

Venous blood (7 ml) was taken approximately 0.5 hours before dosing and at the following times after dosing with LAAM: 0.25, 0.50, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24, 36, and 48 hours, and approximately 0.5 hours before dosing and at the following times after dosing with methadone; 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 9, 12, and 24 hours. This resulted in 15 plasma samples for the LAAM inter-dosing interval study and 13 plasma samples for the methadone inter-dosing interval study. I collected all blood samples. The procedures used to treat and test these blood samples will be outlined later in this chapter.

All pharmacodynamic measures were taken approximately 0.5 hours before dosing and at the following times after dosing with LAAM: 1, 2, 3, 4, 6, 9, 12, 24, 36, and 48 hours and approximately 0.5 hours before dosing and at the following times after dosing with methadone: 1, 2, 3, 4, 5, 6, 7, 9, 12, and 24 hours. Control subjects were tested at the following times: baseline and 1, 2, 3, 4, 6, 9, 12, 24, 36, and 48 hours later. The following measures were taken at each of these times. Firstly, participants completed the self-report questionnaires: the Methadone Symptoms Checklist (Dyer and White., 1997), the Morphine Benzedrine Group and Morphine Group sub scales of the Addiction Research Centre Inventory (Haertzen and Hickey, 1987), and the Profile of Mood States (McNair, *et al*., 1971). This was followed by recording of blood pressure, heart rate, and respiratory rate. Third, participants' response to pain induced by electrical stimulation of their ear was measured. Following this, participants were required to complete a tracking task (see section 2.6.3.2.4), and finally had their pupil diameter measured (see section 2.6.3.1.2). All testing was performed with the subject seated in a conformable armchair, except when the subject was required to move to the computer console to carry out the psychomotor task and to a position in the centre of the room for pupillometry. In most cases subjects took no longer than 20 to 30 minutes to complete the battery of tests at each time point.

In addition, participants were administered a battery of neurocognitive tests (see section 2.6.3.2.5) once during each testing session, at the time of putative peak plasma concentrations of opioid drug/s, i.e., at approximately 4 hours after LAAM administration and 2 hours following methadone administration. However, because of time constraints during the first day of the methadone inter-dosing interval study, patients were asked to remain in the hospital testing room for three hours following dosing of methadone on the second day of the methadone inter-dosing interval study to allow for neurocognitive testing. All patients agreed to do this. I administered all self-report questionnaires and neurocognitive tests.

Control subjects participated in a 48-hour testing session. They were tested in the hospital testing room; however, they were not administered opioid agonists, nor did they have blood, or urine collected. They were permitted to go home overnight on days one and two of testing and were asked not to consume recreational drugs, including alcohol, during this time, but were permitted to consume caffeinated beverages and use tobacco.

2.6.3. Measures.

2.6.3.1. Physiological measures.

2.6.3.1.1. Blood pressure, heart rate and respiratory rate.

Blood pressure (mmHg) was measured manually, using a mercury sphygmomanometer. Heart rate was measured at the radial pulse by counting the number of heartbeats in one minute. The subject's same hand was used in both the methadone and LAAM inter-dosing studies. Respiratory rate was the number of breaths taken by subjects in a one-minute period and was measured, without the subject's awareness, by direct observation of the rise and fall of the subject's chest. This was measured approximately one minute after blood pressure. These parameters where measured after subjects had been seated for at least ten minutes.

2.6.3.1.2. Pupil diameter.

Pupillometry was carried out at a premarked area in the centre of the testing room using a standard VHS video camera (Model NV-RX-33, Panasonic, Japan). The lens was positioned approximately 8 cm from the subject's eye. A 20 second recording of the pupil was performed with x 2 magnification. Subjects held a metric ruler immediately below the eye to enable measurement of pupil diameter during television playback. The same eye was used throughout each inter-dosing interval study. Pupil diameter was measured three times at each measurement point during television playback, and the mean pupil diameter recorded. In all cases pupillometry was the last measure to be undertaken.

2.6.3.2. Subjective measures.

2.6.3.2.1. Pain detection and threshold.

Pain was induced by applying an electrical stimulus to one earlobe via an electrode. An electrical stimulator (Grass model S6, Grass Medical Instruments, Mass., U.S.A) supplied this stimulus in the form of square wave pulses of 14 milliseconds duration (frequency 0.7 pulse per second). Electro-conductive gel (Livingstone Conductive Gel, Livingstone International Pty. Ltd., Australia) was used to provide conductance between the ear electrode and the skin. Voltage was increased from zero, in two-volt increments, every 1.4 seconds (range 0 to 100 volts). Subjects where asked to report when they first felt the stimulus (pain detection) and when it became painful (pain threshold). These measures

were recorded in volts. At the commencement of each testing session subjects were familiarised with the procedure. The electrical stimulator controls were hidden from subject view at all time. The same ear was used throughout both inter-dosing studies. Discomfort caused by the electrical stimulation never lasted more than a few minutes after the cessation of testing. The latter methods have previously been used to examine pain responses to methadone across the inter-dosing interval (Doverty, *et al*., 2001; Dyer, *et al*., 1999).

2.6.3.2.2. Measures of subjective positive opioid effect and symptom complaints.

Opioid effect and symptom complaints were assessed using the methadone symptom checklist (a general measure of symptom complaints, including withdrawal) and the MBG and MG subscales of the Addiction Research Centre Inventory, which measure positive opioid effect (euphoria) and specific morphine-like effects, respectively. A description of each questionnaire is given below.

The Methadone Symptoms Checklist (MSC) was developed by Dyer and coworkers (Dyer and White, 1997) and comprises three groups of 16 items, indicating opioid withdrawal symptoms (e.g., headache, runny or stuffy nose, stomach cramps, aches and pains), direct opioid effects (e.g., constipation, dry mouth, pleasant feeling in stomach), and mixed symptoms that could be characteristic of either withdrawal of direct opioid effect (i.e., feelings of weakness, restlessness, vomiting). Subjects recorded the intensity of symptoms using a 5-point likert scale from 'none' to 4 'extreme'. For the purpose of this study the number of withdrawal symptoms reported (range 0–16) was used as a measure of withdrawal severity.

The test-retest reliability of the MSC has been established. Dyer (2000, unpublished PhD thesis) gave the MSC to a group of methadone maintained patients (n =114) to complete. One week later he gave the MSC to a random subsample (n =38) from this group and found that there was significant stability in symptom reporting between the two time points for each symptom complaint (range of correlations: 0.61 – 0.98, $P< 0.001$ in all cases). In addition there was a significant correlation between the total number of symptoms reported at each time point ($r = 0.76$, $P < 0.001$).

The Morphine Benzedrine Group (MBG) subscale and the Morphine Group (MG) subscale of the Addiction Research Centre Inventory. The MBG is an empirically derived measure of euphoric drug effects. It lists sixteen statements of common feelings, such as 'I feel a very pleasant feeling in my stomach', reported to indicate positive opioid effects, i.e., feelings of well-being and euphoria in true (1) - false (0) format, producing a maximum score of 16. The MBG has been used extensively to measure positive opioid effect and has been shown to be reliable and valid (see Haertzen and Hickey, 1987, for a review). In this study, items from the MBG were interspersed with items from the MG to prevent response bias. The MG is a measure of subjective drug effects thought to be specific to morphine. It comprises eight statements in the same format as the MBG, producing a maximum score of eight. There are three statements, such as 'I have been scratching myself', that describe somatic symptoms (i.e., itching) that are specific to morphine like drugs (Haertzen and Hickey, 1987). The remaining statements might describe feelings of intoxication caused by morphine like drugs, such as 'I have been dozing for seconds or minutes'. Although the MG underwent the same validation process as the MBG (see Haertzen and Hickey, 1987), it has not been used as extensively as the latter measure. The MBG was the primary measure of drug-induced euphoria in this study.

2.6.3.2.3. Measures of psychological health status.

General psychological status was assessed using the following standardized questionnaires: POMS (general mood disturbance), BDI-II revised (depression), and STAI (anxiety). Each questionnaire is described in detail in chapter 6.

2.6.3.2.4. Cognitive psychomotor performance.

Cognitive psychomotor performance was assessed using The Occupational Safety Performance Assessment package (OSPAT, Romteck, Perth, WA, Australia). This is a computerised unpredictable tracking task that provides a measure of hand-eye coordination. A more detailed description of this task will be presented in chapter 7.

2.6.3.2.5. Neurocognitive function.

Neurocognitive function was assessed once during each inter-dosing interval study. A number of instruments were chosen to test a range of cognitive functioning, including memory and learning, attention and working memory, information processing and

executive functioning. Drug-free control subjects also underwent neurocognitive assessment. A detailed description of each instrument, and information concerning administration, is presented in chapter 7.

2.6.4. Plasma and urinary drug concentration analysis.

2.6.4.1. Blood collection.

Blood was collected from subjects participating in both the LAAM and methadone inter-dosing interval studies at the times outlined in section 2.6.2. To permit frequent blood collection a 22 gauge, Insyte™, intravenous catheter (Becton Dickinson, Franklin Lakes, N.J., U.S.A), was inserted into a suitable forearm vein by myself or an anaesthetist. A Baxter Interlink® 15.25 cm catheter extension system (Baxter Healthcare Corp, Deerfield, I.L., USA), primed with 0.9 % saline (normal saline), was attached to the intravenous catheter to permit easy collection of blood. The intravenous catheter remained *in situ* for the duration of each testing session (48h for LAAM, 24h for methadone), but in the event that the catheter did not function, blood was collected by venepuncture. The insertion site was frequently observed for signs of thrombophlebitis – no participant suffered any complications arising from the insertion of the intravenous catheter and the collection of blood.

At each blood collection an initial 2 ml of fluid was withdrawn from the intravenous catheter/extension set and discarded to prevent haemodilution by saline remaining in the catheter. A further 7 ml of blood was removed and placed into tubes containing lithium heparin gel (Sarstedt, Ingle Farm, Adelaide, SA, Australia) and centrifuged at 3000 rpm for 20 mins. The plasma (approximately 3 ml) was poured off into separate polypropylene tubes and frozen at –20 ^{0}C for later quantification of drugs. The remaining blood was discarded. The intravenous catheter and extension set were then flushed with 2 ml of normal saline.

2.6.4.2. Plasma LAAM, norLAAM, dinorLAAM and (R)-(-) and (S)-(+) methadone.

LAAM, nor-LAAM and dinor-LAAM concentrations in plasma were quantified using high performance liquid chromatography (HPLC) as described by Menelaou and colleagues (Menelaou, *et al.*, 1999), each with a lower limit of quantification of 10 ng/ml. Inter- and

intra-day variabilities for imprecision and inaccuracy (% CV) were less than 7% in medium and high concentration quality control samples and less than 13 % in low concentration quality control samples. Methadone did not interfere with this method.

(R)-(-) and (S)-(+) methadone were quantified in plasma using HPLC as described by Foster and colleagues (2000). This method had a limit of quantification of 15 ng/ml for each enantiomer and inter- and intra-day variabilities for imprecision and inaccuracy (% CV) of low, medium and high concentration quality control samples were less than 12 %. LAAM and morphine did not interfere with the method. For the purpose of this thesis only (R)-(-) methadone plasma concentrations will be presented.

The above plasma samples were assayed by a number of staff and students in the Discipline of Pharmacology at the University of Adelaide, South Australia. Quantification of LAAM, nor-LAAM and dinor-LAAM in plasma was carried out by Mr Andrew Menelaou (Research Officer) and Marie-Louise Brixen and Bettina Christensen (visiting Master of Sciences (Cand. Pharm) students from the Royal Danish School of Pharmacy). Quantification of (R)-(-) and (S)-(+) methadone in plasma was carried out by Mr Andrew Menelaou.

2.6.4.3. Plasma benzodiazepine and morphine concentrations.

Morphine and a range of benzodiazepines were quantified in the predose plasma sample; if there was insufficient plasma for analysis, the nearest plasma sample to dosing time was used. The presence of morphine in plasma was used as an objective indicator of recent heroin and/or morphine use. Morphine concentration was quantified in plasma using an HPLC-electrochemical detection method that has been previously described (Doverty, *et al*., 2001). The limit of quantification was 0.5 ng/ml. High (20ng/ml) and low (2ng/ml) concentration quality control samples were assayed with each subject's set of plasma samples, and were within 10 and 15% respectively of the nominal concentrations. Methadone and LAAM and metabolites did not interfere with the method. Andrew Menelaou in the Discipline of Pharmacology carried out analysis of the samples. Plasma samples were not analysed for the heroin metabolite 6-mono-acetylmorphine.

In addition, plasma was sent to the Department of Chemical Pathology at the Women's and Children's Hospital, Adelaide, South Australia for determination of the following

benzodiazepines: diazepam, N-desmethyldiazepam, flunitrazepam, oxazepam, temazepam using HPLC. The limit of quantification for diazepam, oxazepam, and temazepam was 28.4, 28.6, 30.0 ng/ml, respectively, and for flunitrazepam was 3.13 μg/ml.

2.6.4.4. Urinary LAAM, nor-LAAM, and dinor-LAAM concentrations

A 48 hour pooled urine sample was collected from 16 patients (8 males, 8 females) who participated in the LAAM inter-dosing interval study. Patients were required to empty their bladder immediately prior to receiving the dose of LAAM. All subsequent urine that was voided was collected and stored in a 5 litre plastic container and kept cool. On completion of testing urine volume and pH was measured, and an aliquot stored at -20^{O}C until analysis. The HPLC method developed to quantify LAAM and metabolites in urine was modified from the method used for plasma analysis (Menelaou, *et al*, 1999). The lower limit of quantification was 10 ng/ml and inter- and intra-day imprecision and inaccuracy (%CV) variabilities were less than 7% in medium and high concentration quality control samples and less than 14% in low concentration quality samples. Mr Andrew Menelaou also carried out this analysis.

2.6.5. Order of testing and duration on maintenance treatment prior to inter-dosing studies.

This study utilized a randomised crossover design where patients' exposure to methadone and LAAM was counterbalanced. That is, half of the sample were randomly allocated to LAAM maintenance first, and the other to methadone maintenance first. While randomization aims to minimise systematic bias there is always the chance that experience with one maintenance drug might affect a patient's response (i.e. due to expectations, patient attitudes, etc) to the other drug. A series of Repeated Measures Analysis of Variance (ANOVA) were undertaken to determine if the order of testing (LAAM: Methadone or Methadone: LAAM) had systematically influenced the pharmacodynamic responses observed during either inter-dosing interval study. These analyses showed no effect of order of testing on any measured response, for either drug (P>0.05; see appendix 4).

Patients were maintained on LAAM for a mean of 53 days (sd=19: range 30 to 86 days) and on methadone for a mean of 46 days (sd=19: range 24 to 90 days; (paired t test: t=0.91,

p=0.38)) prior to participating in the respective testing session. The variation in time taken to participate in the interdosing interval studies reflects individual differences in the time to stabilize on either drug and then to achieve putative steady state (unchanged methadone dose for at least 2 weeks and unchanged LAAM dose for at least 4 weeks). A number of patients required dose increases beyond their initial dose following transfer from methadone to LAAM; these details will be presented in chapter five.

3. Steady State Pharmacodynamics and Pharmacokinetics of (R)–(-) Methadone in Methadone Maintenance Patients

3.1. Introduction.

A number of randomised controlled trials have demonstrated the effectiveness of MMT in improving outcomes for patients and society at large (Gunne and Grönbladh, 1981; Newman and Whitehill, 1979; Strain, *et al*., 1993; Vanichseni, *et al*., 1991; Yancovitz, *et al*., 1991). However, a significant proportion of patients in MMT, despite receiving apparently adequate methadone dosages, or when doses are reached that induce significant adverse effects, complain of reduced methadone efficacy and breakthrough withdrawal (non-holding) just prior to their next dose (Dyer, *et al*., 1999; Dyer and White, 1997). These patients have been designated as therapeutic failures or non-holders (Dyer, *et al*, 1999; Dyer and White, 1997; Nilsson, *et al*., 1983; Schall, *et al*., 1996; Tacke, *et al*., 2001; Tennant, 1987). This non-holding status has been associated with increased illicit drug use and poor psychosocial outcome, and is likely to contribute to patients withdrawing from treatment (Hiltunen, *et al*., 1999; Holmstrand, *et al*., 1978; Schall, *et al*., 1996).

It is not clear what mechanisms are responsible for the phenomenon of non-holding amongst MMT patients. Previously, researchers have postulated that patients complaining of withdrawal during the inter-dosing interval are likely to exhibit more rapid changes in plasma methadone concentrations during the period between peak methadone concentration and trough methadone concentration (i.e., the distribution phase) than those who do not (Dyer, *et al*., 1999; Nilsson, *et al*., 1983). Furthermore, in MMT patients, plasma methadone concentration-effect relationships are very steep for both withdrawal (mean±sd: slope factor=5.4±1) and positive opioid effects, such as euphoria (slope factor=5.1±1) (Dyer, *et al.*, 1999; Dyer, *et al*. 2001). Thus, only small decreases in plasma methadone concentration translate into relatively large changes in response, which would be exaggerated in non-holders as they exhibited greater rate of decline in plasma methadone concentrations than holders. Thus, it is possible that 'non-holding' has a pharmacokinetic basis.

However, confounding the above discussion is that in Australia, as in many other parts of the world, methadone is administered as a racemate that comprises equal proportions of the (R)-(-) and (S)-(+) enantiomers. As there are marked stereoselective differences in the

disposition of each enantiomer (Foster, *et al.*, 2000; 2004; Kreek, *et al.*, 1979), the measurement of racemic-methadone will not accurately reflect the plasma concentration of the active (R)-(-) enantiomer. Thus, before one can be confident that there is a pharmacokinetic mechanism responsible for the difference found between nonholders and holders on MMT, it is necessary to explore the pharmacodynamic and pharmacokinetic basis for non-holding using the active (R)-(-) enantiomer in any analyses.

The purpose of the current study was to examine the steady state pharmacodynamics and pharmacokinetics of (R)–(-) methadone in maintenance patients receiving racemic-methadone. A number of subjective and physiological indices of opioid effect were collected concurrently with frequent blood samples across one 24-hour methadone inter-dosing interval. Patients included those who regularly reported breakthrough withdrawal (non-holders) and those who did not (holders) during the latter half of the 24-hour methadone inter-dosing interval. The specific aims of the work in this chapter were:

- To describe the temporal change in physiological (heart rate, blood pressure, pupil diameter) and subjective (positive opioid effect (MBG), morphine-like effects (MG), and pain detection and threshold, and withdrawal complaints) indices of opioid effect across the 24-hour methadone inter-dosing interval and to compare these responses to that obtained from a group of non-opioid using control subjects.
- To investigate the pharmacokinetic basis of non-holding in MMT patients by comparing the disposition of (R)-(-) methadone between non-holders and holders.

3.2. Hypotheses.

- The temporal profiles of physiological (pupil diameter, heart rate, systolic and diastolic blood pressure, respiratory rate) and subjective (withdrawal, pain threshold, MBG, and MG) indices of opioid effect will be closely related to the underlying plasma (R)-(-) methadone concentration versus time profile.
- At the time of putative peak plasma (R)-(-) methadone concentration the physiological responses of MMT patients will be significantly lower than those obtained from non drug using controls; on the other hand, at the same time, subjective responses (except withdrawal) will be significantly higher than obtained from control subjects.

- Methadone non-holders will exhibit more rapid rate of decline in plasma (R)-(-) methadone concentrations from peak to trough concentrations than holders. Furthermore, in those who experience withdrawal symptoms, the rate of decline of plasma methadone concentrations will determine the severity of these symptoms

3.3. Methods.

The methods and procedures used in the methadone inter-dosing interval study were outlined in detail in chapter two. Briefly, in this study the pharmacodynamics and pharmacokinetics of (R)-(-) methadone were examined in 18 MMT patients, eight of whom were non-holders. Blood was collected pre-dose and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 9, 12, and 24 hours following administration of racemic methadone. Pharmacodynamic responses were recorded at the same time blood was sampled, but no more frequently than hourly. Data were also obtained from ten non-drug using controls and were contrasted with responses from MMT patients. With the exception of data obtained from the cognitive psychomotor task, neurocognitive tests and measures of psychological health status (POMS, BDI-II revised, and STAI), all data collected during the methadone inter-dosing study will be presented in this chapter.

3.3.1. Analyses.

3.3.1.1. Pharmacokinetic analyses.

Pharmacokinetic parameters were derived using noncompartmental methods (Rowland and Tozer, 1995). The area under the (R)-(-) methadone plasma concentration versus time curve under steady state conditions, from 0 to 24 hours (AUC), was calculated using the linear trapezoidal method (Graphpad Prism v3.02 Graphpad Software, CA, USA). Time to reach maximum steady state plasma concentrations (T_{max}), maximum steady state plasma concentrations (C_{max}), minimum steady state plasma concentrations predose ($C_{min(first)}$), and minimum steady state plasma concentrations at the end of the dosing interval ($C_{min(last)}$) were obtained by visual inspection of individual data. Steady state plasma concentrations (C_{ss}) were determined by dividing AUC by the dosing interval (24 hours). AUC, and C_{ss} , C_{max}, $C_{min(first)}$, and $C_{min(last)}$ concentrations were normalised to 70 mg racemic-methadone for each individual. The peak to trough plasma concentration ratio for each individual was calculated by dividing C_{max} by $C_{min(last)}$. Apparent plasma clearance at steady state (CL/F)

was calculated as dose/AUC. As outlined by Dyer and coworkers (1999), the rate of hourly change in plasma (R)-(-) methadone concentration from peak concentration until the trough ($C_{min(last)}$) was calculated for each individual. The relationship between the maximum rate of decline in plasma (R)-(-) methadone concentration and the mean number of self-reported withdrawal symptoms for patients during this period, was determined using a Pearson product-moment correlation coefficient. As an additional index of variability of the plasma (R)-(-) methadone concentration-time profile, the duration (hours) that plasma (R)-(-) methadone concentrations were equal to or above 75% of C_{max} was calculated from visual inspection of individual plasma (R)-(-) methadone concentration-time profiles (Bochner, *et al*., 1999; Gourlay, *et al*., 1997; Leeuwenkamp, *et al*., 1994; Maccarone, *et al*., 1994).

3.3.1.2. Statistical and other analyses.

Demographic data for holders and non-holders (except gender ratio), plasma drug screen results, and pharmacokinetics parameters for holders and non-holders were compared using Student's *t*-test for independent samples. Fisher's exact test was used to compare categorical data. One way repeated measures ANOVAs were used to examine the effect of time since dosing on each pharmacodynamic variable for all patients, and the effect of time during the period baseline to 24 hours, for control subjects. Separate ANOVAs were performed for both the patient and the control group. This was considered appropriate as the protocols used to test methadone patients and control subjects were not equivalent (see section 2.6.2). Within-group comparisons were performed between the pre-dose or baseline measurement and each subsequent measurement point for each group using the post hoc Bonferroni test for selected comparisons (GraphPad Prism v3.02, Graphpad Software, CA, USA).

As the protocols used to test MMT patients and control subjects differed, independent Student *t*-tests were used to determine the significance of differences between these groups for each variable. Between groups comparisons were made at the following measurement points: predose, 1, 2, 3, 4, 6, 9, 12, and 24 hours after dosing for a total of 9 between group comparisons. To reduce the likelihood of type 1 error, Bonferonni adjustment was made to the alpha level (ie. 0.05/ 9 comparisons), so that for these comparisons a probability level of $P<0.0055$ was used to determine significance.

Two way repeated measures ANOVAs were used to examine the effect of time since dosing and methadone holding status on pharmacodynamic responses. Bonferonni post hoc tests were used to determine between group differences when the ANOVA was significant (significance set at $P<0.05$). For repeated measures degrees of freedom were adjusted for violations of the sphericity assumption using Greenhouse Geisser corrections provided by SPSS. To further clarify possible differences between non-holder and holder groups the area under the curve for each pharmacodynamic parameter for each group was calculated using the linear trapezoidal method. The AUCs were compared using independent Student's *t*-test (GraphPad Prism v3.02, Graphpad Software, CA, USA).

Unless otherwise stated, the alpha level for all statistical analyses was set at $P=0.05$. The data were analyzed using SPSS™ for Windows (v.10), unless otherwise stated. SPSS undertakes Levene's test of homogeneity of variance when undertaking t-tests and adjusts the probability accordingly. Coefficients of variation (%CV) were calculated for all pharmacokinetic parameters. All statistical tests were two tailed and data were tabulated as mean ± sd. Where appropriate 95% confidence intervals [CI] were also presented.

3.4. Results.

3.4.1. Participant details.

Eighteen patients participated in the methadone inter-dosing study; table 3.1 presents general demographic details for each individual subject. They were eight males and 10 females who had a mean age of 35.7 years (±7.6 years). They had a mean±sd body weight of 70 kg (± 18 kg) and their mean±sd once-daily methadone dose was 78 mg (±38 mg), which corresponded to a mean methadone dose to body weight ratio of 1.17 mg/kg (±0.65 mg/kg). Thirteen patients smoked tobacco, and six self reported the regular consumption of less than 40 grams of alcohol daily and only one patient reported drinking in excess of 40 grams daily in the month preceding the study. The remaining patients denied drinking alcohol. Seven patients had morphine present in their plasma (see table 3.2 for plasma morphine concentrations), and eight patients had benzodiazepines (diazepam, desmethyldiazepam and oxazepam) present in their plasma at concentrations that were within or greater than the therapeutic range (see notes for table 3.2 for therapeutic ranges). A more detailed list of drug use, other than methadone, for each patient can found in table 3.2. There was general concordance between the presence of benzodiazepines and

morphine in plasma and urine samples. Eight patients identified themselves as holders and another eight as non-holders. Note that patients #17 and #18 (table 3.1) only participated in the methadone interdosing interval study.

Ten drug free volunteers (6 males and 4 females) participated in the control arm of this study – their details were presented in chapter two.

***Table 3-1**: **Demographic details of 18 patients who participated in the 24-hour methadone inter-dosing interval study**.*

Patient No	Gender	Age (years)	Wt (kg)	Dose (mg)	Dose (mg/kg)	MMT (mnth) [1]	Holding Status[2]
1	M	39	96	100	1.04	73	H
2	M	48	70	50	0.71	50	H
3	M	28	85	110	1.29	37	H
4	F	21	47	65	1.38	15	H
5	M	43	79	40	0.51	10	H
6.	M	41	86	80	0.93	12	H
7	F	31	67	120	1.79	74	NH
8	F	25	87	40	0.46	18	NH
9	F	32	44.5	110	2.47	3	NH
10	F	35	51	30	0.59	114	NH
11	F	27	66	150	2.27	36	NH
12	M	43	100	110	1.10	72	H
13	F	41	63	130	2.06	156	NH
14	F	33	63	30	0.48	46	H
15	F	31	58	45	0.78	72	NH
16	M	45	52.5	40	0.78	12	NH
17	M	41	95	60	0.63	120	H
18	F	39	54	100	1.85	96	H
Mean	__	35.7	70	78	1.17	56.4	__
sd	__	7.60	38	38	0.65	44	__

Notes: 1 = Period of maintenance treatment (months) prior to enrolling in study. 2=Holding status - self reported holding status prior to participating in inter-dosing study. H=Holder, NH=Non-Holder. Patient #17 and #18 participated only in the methadone inter-dosing study.

Table 3-2: Drug use for each individual patient that participated in the methadone inter-dosing interval study

Patient No[1]	Prescribed Drugs[2]	Urinalysis Predose[3]	Plasma Morphine[4]	Plasma Benzo[5]	Self Reported drug use[6]
1	Nil	nil	0.0	Nil	Nil
2	1	1	0.0	1	1,2,3
3	Nil	1	0.0	Nil	1,2,3
4	Nil	2	3.4	Nil	1,4
5	Nil	2	54.0	Nil	1,2,4
6	Nil	Nil	0.0	Nil	1
7	1,2	1	0.0	1,2,3	1
8	1	1,2,4	3.7	1	1,2,3,4,6
9	Nil	3,4	0.0	Nil	1,2,5,6
10	Nil	2,4	0.6	Nil	1,4,6
11	3	1,4	0.0	Nil	1,2,6
12	1	1,4	0.0	1,2	1,6
13	1,3	1,2,4	46.6	Nil	1,2,4,6
14	1	1,2,3,4	1.1	1,2,3	1,4,5,6
15	4	4	0.0	Nil	1,2,6
16	5	1,2	15.7	1,2	1,2,3,4
17	1	1,4	0.0	1,2	1,3,4,6
18	1	1	0.0	1	1

Notes: [1]Patients are listed in the same order as in Table 3.1. [2]Prescribed drugs, 1 = diazepam, 2 = oxazepam, 3 = sertraline, 4 = venlafaxine, 5 = alprazolam. [3]Predose urinalysis, 1 = benzodiazepines, 2 = opioids (other than methadone), 3 = sympathomimetic amines, 4 = cannabinoids. [4]Plasma morphine concentration (μg/litre). [5]Presence of the following benzodiazepines in plasma, concentrations within or greater than the quoted therapeutic range (shown in brackets), 1= diazepam (0.7 – 1.8 μmol/L), 2 = desmethyldiazepam (2.0 – 5.0 μmol/L), 3 = oxazepam (1.7 – 7.0 μmol/L), note – no other benzodiazepines were detected. [6]Self-reported drug use in last week, 1 = tobacco, 2 = alcohol, 3 = benzodiazepines (other than prescribed), 4 = opioids (other than methadone), 5 = sympathomimetic amines, 6 = cannabinoids.

The next section will initially present results for all patients, and then will describe results for holder and non-holder comparisons.

3.4.2. Pharmacodynamic responses for all patients.

3.4.2.1. Physiological responses.

Cardiovascular responses (heart rate, systolic and diastolic blood pressure), and pupil diameter and respiratory rate for patients and control subjects are shown in figure 3.1 through to 3.3. Table 3.3 summarises the One Way Repeated Measures ANOVAs used to

determine the significance of change for each physiological variable across the inter-dosing interval. For methadone patients there were significant temporal changes (time since dosing effects) across the 24-hour inter-dosing interval for all physiological parameters depicted ($P<0.005$). In contrast, responses for control subjects, with the exception of heart rate, did not change significantly across the study period. It is noteworthy that post hoc tests revealed no significant within subject differences ($P>0.05$) for heart rate in control subjects. Details of within subject and between group (patients versus control subjects) differences for each physiological parameter will now be considered.

Patients' mean heart rate (figure 3.1) at 6 hours after dosing was significantly (probability level: mean difference; 95 % CI) lower than the heart rate at predose ($P<0.05$; -5.89 beats per minute (bpm); -11.63, -0.15 bpm); in contrast, the heart rate at 24 hours was significantly faster than at predose ($P<0.001$; 9.4; 3.71, 15.18). There were no other within group differences ($P>0.05$) for heart rate. In addition, there were no significant between group differences ($P>0.0055$).

Mean systolic and diastolic blood pressure (figure 3.1) both declined, relative to baseline, following dosing in methadone patients. Mean systolic blood pressure was significantly lower at 3 hours ($P<0.01$; -6.44 mm Hg; -11.88, -1.01mmHg), 5 hours ($P<0.05$; -6.39 mmHg; -11.82, -0.95 mmHg) and 6 hours ($P<0.01$; -7.00 mmHg; -12.44, -1.56 mmHg) after dosing, while diastolic blood pressure measured at 5 hours ($P<0.05$; -7.00 mmHg; -12.98, -1.02 mmHg) and 6 hours ($P<0.05$; -6.17 mmHg; -12.15, -0.18 mmHg) after dosing was significantly lower than the diastolic pressure measured at predose. The mean systolic blood pressure for patients was significantly lower than for controls only at 6 hours after dosing ($P=0.002$; -9.24 mmHg; -14.89, -3.60 mmHg). There were no between group differences for diastolic pressure at any time point ($P>0.0055$).

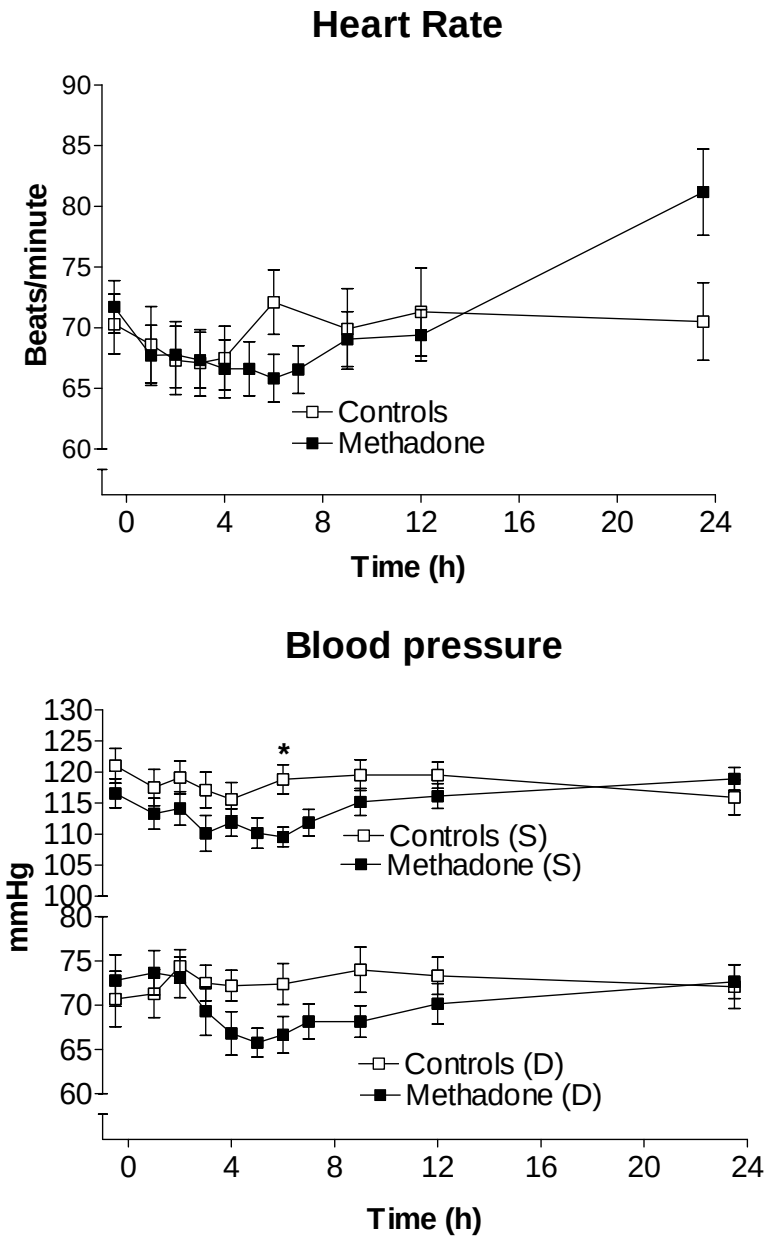

Figure 3-1: Mean (± SEM) heart rate (beats per minute) (upper panel); systolic (S) blood pressure and diastolic (D) blood pressure (lower panel) of methadone patients (closed squares: n=18) who underwent the methadone inter-dosing interval study and for control subjects (open squares: n=10) for the period 0-24 hours. Methadone was administered at zero hours. Asterisks denote results of between group comparisons; * p<0.0055.

The mean respiratory rate of methadone patients (figure 3.2) declined from a maximum at baseline to minimal levels at 3 hours (3.6 breaths/minute below baseline; brpm) after dosing and then slowly returned to baseline rates by the end of the dosing interval. Patients' mean respiratory rate was significantly lower than the predose measurement at 1 hour (P<0.001; -1.94 breaths per minute (brpm); -3.25, -0.64 brpm), 2 hours (P<0.001; -3.33 brpm; -4.63, -2.03 bpm), 3 hours (P<0.001; -3.61; -4.91, -2.31, brpm), 4 hours

(*P*<0.001; -3.44 brpm; -4.75, -2.14, brpm), 5 hours (*P*<0.001; -3.17 brpm; -4.47, -1.87, brpm), 6 hours (*P*<0.001; -2.67 brpm; -3.97, -1.37, brpm), 7 hours (*P*<0.001; -2.50 brpm; -3.80, -1.20, brpm), and at 9 hours (*P*<0.05; -1.50 brpm; -2.8,-0.20, brpm) after dosing.

The mean respiratory rate of methadone patients was significantly lower than that of controls at predose (*P*<0.001, -3.92 brpm; -5.76, -2.09 brpm), and at 1 hour (*P*<0.001; -5.87 brpm; -7.68, -4.06 brpm), 2 hours (*P* <0.001; -7.36 brpm; -8.94, -5.77 brpm), 3 hours (*P*<0.001; -7.83 brpm; -9.49, -6.17 brpm), 4 hours (*P*<0.001; -7.72 brpm; -9.49, -5.95 brpm), 6 hours (*P*<0.001; -6.99 brpm; -8.98, -5.00 brpm), 9 hours (*P*<0.001; -6.52 brpm; -8.71, -4.34 brpm), and 12 hours (*P*<0.001; -6.32 brpm; -8.68, -3.96 brpm), but not at 24 hours (*P*>0.0055) after dosing.

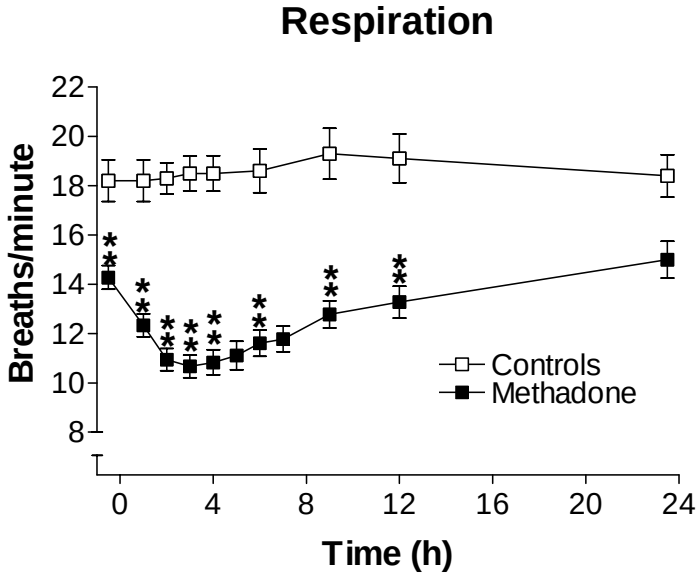

Figure 3-2: Mean (± SEM) respiratory rate (breaths per minute) of methadone patients (closed squares: n=18) who underwent the 24 hour methadone interdosing interval study and of control subjects (open squares: n=10) for the period 0-24 hours. Methadone was administered at zero hours. Asterisks denote significant between group comparisons; ** P<0.001.

Similarly, pupil diameter for methadone patients declined rapidly following dosing to be minimal three hours following dosing (1.4 mm below baseline), and then slowly returned to baseline values by the end of the dosing interval (figure 3.3). Pupil diameter was significantly smaller than the predose pupil diameter at 1 hour (*P*<0.001; -0.64 mm; -1.0, -0.25 mm), 2 hours (*P*<0.001; -1.13 mm; -1.51, -0.74 mm), 3 hours (*P*<0.001; -1.24 mm; -1.62, -0.85 mm), 4 hours (*P*<0.001; -1.21mm; -1.60, -0.82 mm), 5 hours (*P*<0.001; -1.08mm; -1.47, -0.69 mm), 6 hours (*P*<0.001; -0.90mm; -1.29, -0.52 mm), 7 hours

(*P*<0.001; -0.81mm; -1.20, -0.42 mm) and 9 hours (*P*<0.001; -0.57 mm; -0.95, -0.18 mm) after dosing, but not at 12 hours or 24 hours after dosing (p>0.05).

Pupil diameter for methadone patients was significantly smaller than that for control subjects at 2 hours (*P*=0.004; -1.20 mm; -1.96 mm, -0.43 mm), 3 hours (*P*=0.003; - 1.21 mm; -1.98, -0.43 mm), and at 4 hours after dosing (*P*<0.004; -1.33mm; -2.20, -0.46 mm).

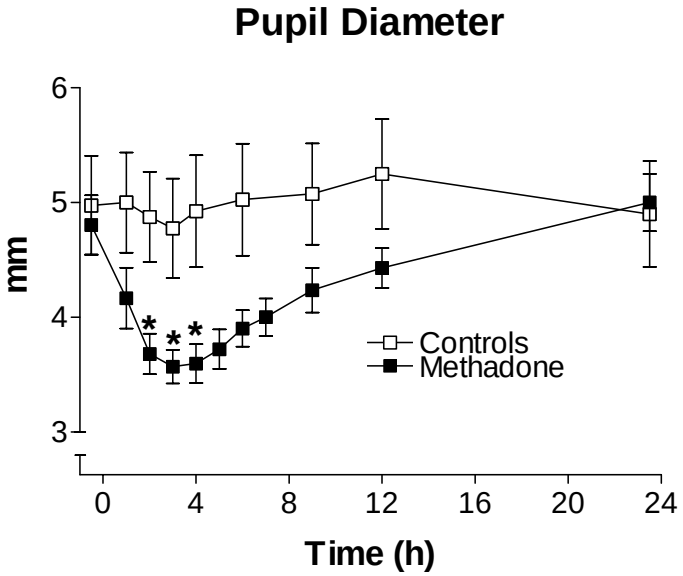

Figure 3-3: Mean (± SEM) pupil diameter (mm) for methadone patients (closed square: n=18) who underwent the 24 hour methadone interdosing interval study and for control subjects (open squares: n=10) for the period 0-24 hours. Methadone was administered at zero hours. Asterisks denote results of between group comparisons; * P<0.0055

Table 3-3: One-Way repeated measures analysis of variance for physiological indices of opioid effect for all methadone patients (n=18) and control subjects (n=10). Values except DF are F ratios

Group	DF	Physiological responses[1]				
		Heart Rate	Systolic BP	Diastolic BP	Pupil	Respiration.
Patient	10, 170	9.23***	4.78***	3.80***	25.67**	18.58**
Controls	8, 72	2.60*	1.90^{NS}	0.63^{NS}	1.63^{NS}	1.84^{NS}

Notes: [1]Heart rate; Systolic BP=Systolic blood pressure; Diastolic BP=Diastolic blood pressure; Pupil= Pupil diameter; Respiration=Respiratory rate. * *P* <0.05, ** *P*<0.01, *** *P* <0.001. NS=Not significant

3.4.2.2. Subjective Responses.

Subjective responses ((Morphine Group (MG) and Morphine Benzedrine Group (MBG) subscales of the Addiction Research Centre Inventory, pain detection and threshold, and number of withdrawal symptoms)), are shown in figures 3.4 through to 3.6. Table 3.4 summarises the One Way Repeated Measures ANOVAs used to determine the significance of change across the inter-dosing interval for each subjective measure. For methadone patients there were significant temporal changes (time since dosing effects) across the 24-hour inter-dosing interval for all subjective measures depicted ($P<0.05$). In contrast, responses for control subjects, with the exception of mean MBG scores, did not change significantly across the study period ($P>0.05$). Details of within subject and between group (patients versus control subjects) differences for each subjective measure will be considered below.

Mean MBG and MG scores for methadone patients and for control subjects during the dosing interval are shown in figure 3.4. Both MBG and MG scores for methadone patients rapidly rose to peak by 2 to 3 hours after dosing and returned to baseline by 12 hours after dosing. For methadone patients mean MBG scores were significantly greater than the mean predose score at 2 hours ($P<0.01$; 2.61; 0.56, 4.67), 3 hours ($P<0.05$; 2.33; 0.28, 4.39) and 4 hours ($P<0.01$; 2.50; 0.45, 4.55) after dosing. Mean MBG scores for control subjects were significantly less than the baseline score at 4 hours ($P<0.05$; -1.8; -3.33, -0.27), 6 hours ($P<0.01$; -2.00; -3.53, -0.47), 9 hours ($P<0.05$; -1.80; -3.33, -0.27). 12 hours ($P<0.001$; -2.60; -4.13, -1.07) and at 24 hours ($P<0.01$; 2.2; 0.67, 3.73).

There were no between group differences for MBG scores ($P>0.0055$). On the other hand, MG scores for methadone patients were significantly greater than those of control subjects at 2 hours ($P=0.001$; 1.69; 0.82, 2.56), 3 hours ($P=0.001$; 1.98; 0.96, 2.96), 4 hours ($P<0.001$, 2.07; 1.07, 3.07), 6 hours ($P<0.001$; 2.11; 1.31, 2.91), 9 hours ($P<0.001$; 1.89; 1.06, 2.72), and at 12 hours ($P=0.001$; 1.62; 0.75, 2.49) after baseline.

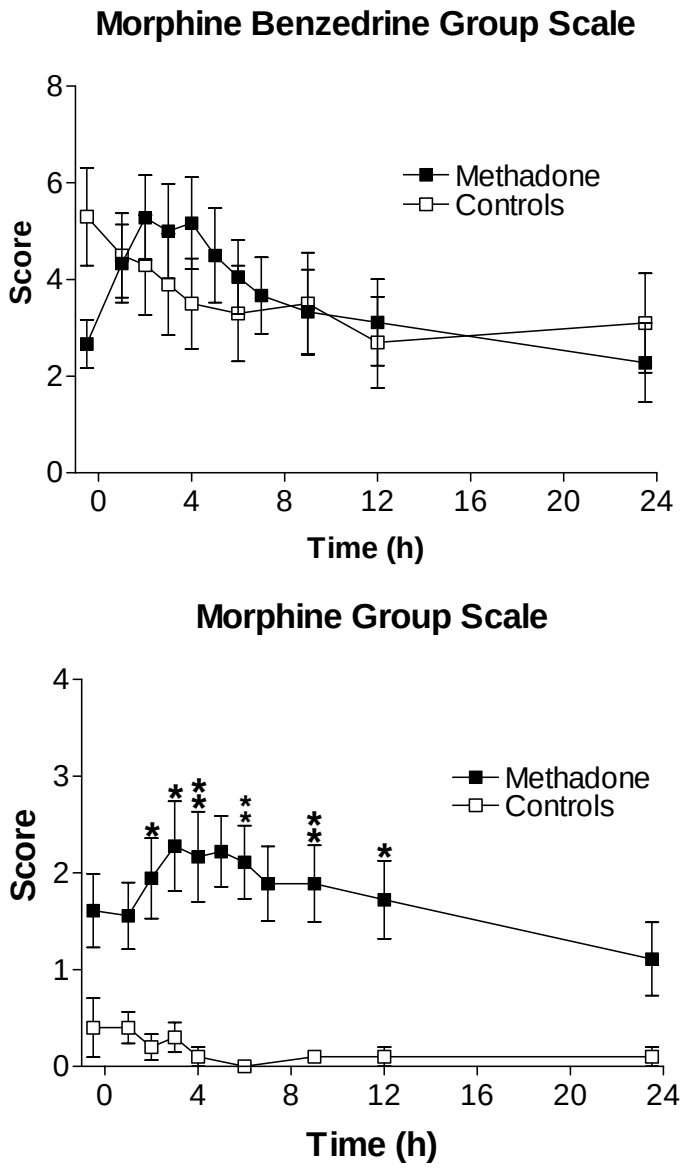

Figure 3-4: Mean (± SEM) Morphine Benzedrine Group Scale scores (upper panel: maximum possible score =16) and Morphine Group scores (lower panel; maximum possible score = 8) for methadone patients (n=18) who underwent the 24-hour methadone interdosing interval study and for control subjects (n=10) for the period 0 to 24 hours. Methadone was administered at zero hours. Asterisks denote results of between group comparisons; * P<0.0055, ** P<0.001.

Mean pain detection and pain threshold scores are shown in figure 3.5. Both mean pain detection and pain threshold were maximal between two to four hours following dosing, returning to baseline or below baseline levels by six hours after dosing. The mean pain detection score at 12 hours post dosing was significantly *lower* than that measured at predose (P<0.01; -4.3 volts; -7.92, -0.75, volts); there were no other within methadone group differences. Pain threshold scores were significantly greater than the predose score

at 2 hours (*P*<0.05; 6.39 volts; 0.94, 11.84, volts), 3 hours (*P*<0.01; 6.57 volts; 1.10, 12.01 volts) and at 4 hours (*P*<0.01; 7.11 volts; 1.7, 12.56 volts) post dosing.

There were no between group differences for either pain detection or pain threshold scores at any time point (*P*>0.0055).

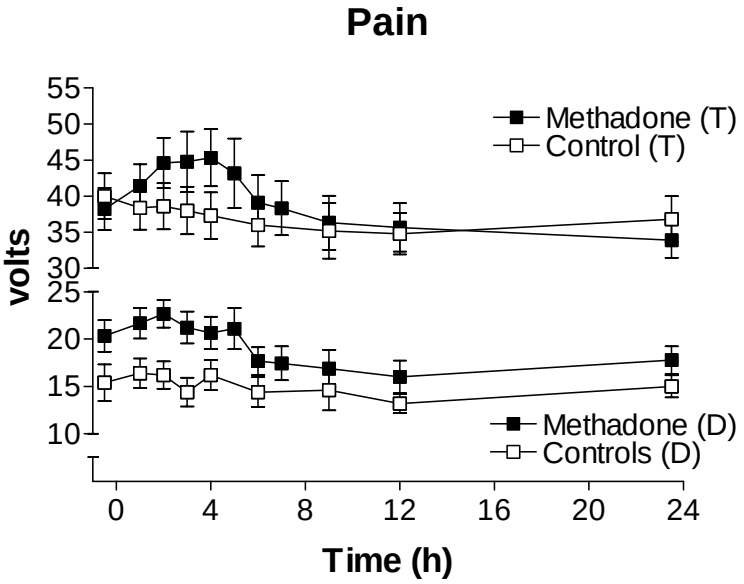

Figure 3-5: Mean (± SEM) pain detection (D) and pain threshold scores (T) (volts) for methadone patients (closed squares: n=18) who participated in the 24 hour methadone inter-dosing interval study and for control subjects (open squares: n=10) for the period 0 to 24 hour. Methadone was administered at zero hours.

Withdrawal scores for methadone patients and control subjects are shown in figure 3.6. Withdrawal scores were at their highest pre-dose, but declined to a minimum between three to five hours after dosing and steadily returned to baseline levels by the end of the dosing interval. Mean withdrawal scores were significantly less than the predose score at 1 hour (*P*<0.001; -3.27; -5.30, -1.25), 2 hours (*P*<0.001; -4.89; -6.91, -2.87), 3 hours (*P*<0.001; -5.44; -7.47, -3.42), 4 hours (*P*<0.001; -5.44; -7.47, -3.42), 5 hours (*P*<0.001; -5.4; -7.47, -3.42), 6 hours (*P*<0.001; -4.61; -6.63, -2.59), 7 hours (*P*<0.001; -3.89; -5.91, -1.87), 9 hours (*P*<0.001; -3.22; -5.25, -1.19), and 12 hours (*P*<0.05; -2.28; -4.30, -0.25) post dosing.

Withdrawal scores for methadone patients were significantly greater than those of control subjects at trough (*P*<0.001; 6.2; 4.1, 8.2), 1 hour (*P*=0.005; 3.2; 1.1, 5.3), 6 hours (*P* =0.004; 2.23; 0.80, 3.70), 9 hours (*P*=0.003, 3.6; 1.4, 5.8), 12 hours (*P*=0.001; 4.7; 2.3,

7.2) and 24 hours (P<0.001, 6.2; 3.6, 8.8) post dosing, but not at any other time point (P>0.0055).

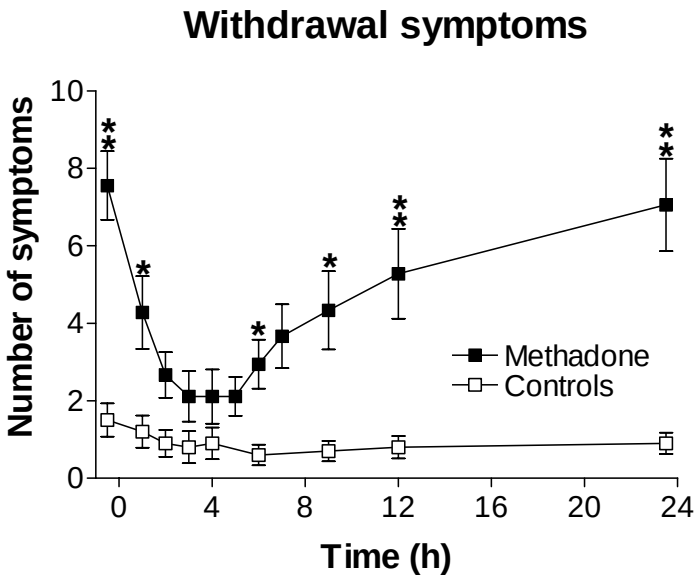

Figure 3-6: Mean (±SEM) withdrawal scores (maximum possible score=16) for methadone patients (closed squares: n=18) who participated in the 24-hour methadone inter-dosing interval study and for control subjects (open squares: n=10) for the period 0 to 24 hours. Methadone was administered at zero hours. Asterisks signify results of between group comparisons; * P<0.0055,** P<0.001.

Table 3-4: One way repeated measures analysis of variance for subjective measures of opioid effect for all methadone patients (n=18) and control subjects (n=10). Values except DF are F ratios.

Group	DF	Subjective measures[1]				
		MG	MBG	Pain Detec.	Pain Thresh.	Withdrawal
Patient	10, 170	2.41*	4.00***	6.37**	8.78**	14.82***
Controls	8, 72	1.32^{NS}	4.24***	0.96^{NS}	1.98^{NS}	1.91^{NS}

Notes: [1]MG=Morphine Group Subscale of ARCI; MBG=Morphine Benzedrine Subscale of ARCI; Pain Detec=Pain Detection; Pain Thresh=Pain Threshold; Withdrawal=Number of Withdrawal Symptoms. * P<0.05, ** P<0.01, *** P <0.001. NS=Not significant (P>0.05).

3.4.3. Steady state pharmacokinetics of (R)-(-) methadone – all patients.

Figure 3.7 shows the mean plasma (R)-(-) methadone concentration-time profile normalised to 70 mg racemic-methadone, for the 18 subjects that participated in the 24-hour methadone inter-dosing study

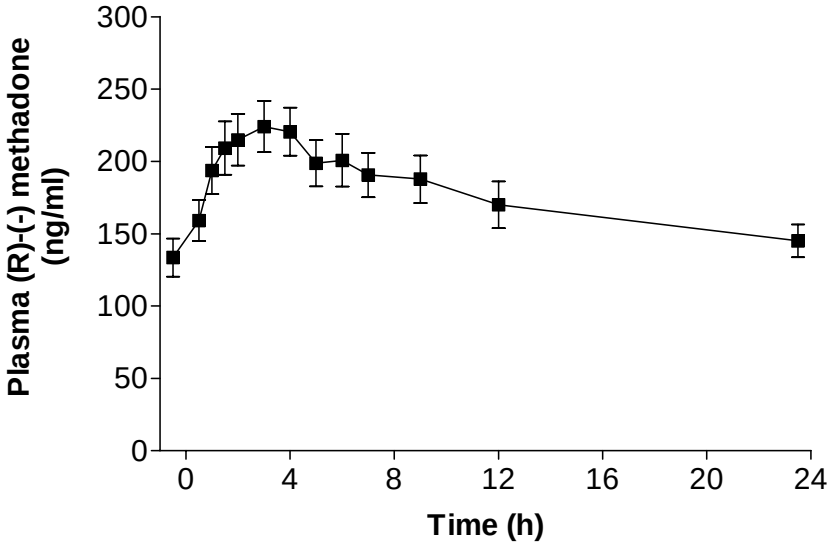

Figure 3-7: Mean (± SEM) plasma (R)-(-) methadone concentration-time profile for 18 maintenance patients during a 24-hour inter-dosing interval. Oral administration of racemic methadone was at zero hours. Data are presented as concentration normalised to a 70 mg racemic methadone dose. 1 ng/ml equal 0.31 nmol/L.

Pharmacokinetic parameters derived from the plasma concentration data are presented in table 3.5. Mean pre-dose ($C_{min(first)}$) and 24 hour post dose ($C_{min(last)}$) plasma (R)-(-) methadone concentrations were not significantly (probability value; mean difference; 95 % CI) different (P>0.05;- 12 ng/ml; -25, 2 ng/ml), indicating that steady state had been achieved. Mean maximum plasma concentration (C_{max}) was achieved 2.7±1.5 hours (mean±sd) after administration of racemic methadone, (R)-(-) methadone plasma concentration then declined steadily across the dosing interval. The ratio of peak to trough ($C_{min(last)}$) plasma concentration was 1.75±0.37 (mean±sd). There was a highly significant positive relationship between plasma (R)-(-) methadone AUC and dose (r^2 = 0.59, P< 0.0002). Furthermore, there was also substantial intersubject variation evident in many of the pharmacokinetic parameters; for example, there was almost four-fold variation in C_{max} , C_{ss}, and plasma AUC across all subjects.

Table 3-5: Pharmacokinetic parameters for (R)-(-) methadone following dosing of between 30 – 150 mg daily racemic methadone in 18 patients who underwent the 24-hour methadone inter-dosing interval study.

Parameter[1]	Mean	Range	%CV[2]
C_{max} (ng/ml)	250	122-450	35
AUC (ng.hr/ml)	4261	1927-8148	35
T_{max} (h)	2.7	1-6	54
$C_{min(first)}$ (ng/ml)	134	43-259	42
$C_{min(last)}$ (ng/ml)	145	64-249	33
C_{ss} (ng/ml)	178	80-340	35
P/T ($C_{max}/C_{min\ (last)}$)	1.75	1.30-2.90	21
CL/ F (ml/min)	154	72-303	38

Notes: [1]C_{max}=maximum plasma concentration, normalised to 70 mg racemic methadone; AUC=area under the plasma concentration-time curve during the 24 hour inter-dosing interval, normalised to 70 mg racemic methadone; T_{max}=time to reach maximum measured steady-state plasma concentration; $C_{min(first)}$ = plasma concentration, normalised to 70 mg racemic methadone, 30 minutes prior to dose; $C_{min(last)}$ = plasma concentration, normalised to 70 mg racemic methadone, 24 hours after dosing; C_{ss} = average steady-state plasma concentration, normalised to 70 mg racemic methadone; P/T=peak to trough plasma concentration ratio (ie, $C_{max}/ C_{min(last)}$); CL/ *F* =apparent plasma clearance at steady-state. [2] Coefficient of variation.

3.4.4. Comparison for holder and non-holder groups.

3.4.4.1. Demographic details.

Methadone patients were categorised as either holder or non-holders on the basis of their self-report prior to participating in this study. Patients (n=16) who completed both the methadone and LAAM interdosing studies are included in the following comparison between holder and non-holder groups, ie. Eight patients were holders and eight non-holders. Table 3.6 presents the demographic details of these groups. Holders were significantly heavier than non-holders (*P*=0.044), but there were no significant differences between holders and non-holders with respect to the daily methadone dose ingested (*P*=0.64), dose per weight ratio (*P*=0.17), age (*P*=0.38), and duration of current enrolment on MMT (*P*=0.33). Fischer's exact test revealed significant differences in the gender ratio between groups ($P<0.05$), but not for the presence of morphine and/or benzodiazepines in plasma tested as part of the drug screen ($P>0.05$).

Table 3-6: Demographic details of methadone patients who completed the methadone interdosing interval study divided into those who reported that they were holders (n=8) or non-holders (n=8).

	Holders	Non Holders	P value[2] (mean difference; 95%CI)
Male/female	6/2	1/7	<0.05
Age (yrs) [range]	37.0±9 [21-48]	33.4±7 [25-45]	0.38 (3.63; -4.91, 12.16)
Dose (mg) [range]	73±32 [30-110]	83±49 [30-150]	0.64 (-10; -54.93, 34.92)
Weight (kg) [range]	78.3±18 [47-100]	61.1±13 [45-87]	0.04 (17.13; 0.49, -33.76)
Dose/weight (mg/kg) [range]	0.9±0.34 [0.48-1.8]	1.4±0.83 [0.48-1.38]	0.17 (-0.47; -1.19. 0.25)
Time on MMT (months)[1]	39±26	61±54	0.33 (-21; -66.51, 24.01)
Positive plasma drug screen (methadone study day) (n)			
Morphine	3	4	>0.05
Benzodiazepines	3	3	>0.05

Notes: Data are mean ± sd unless otherwise indicated. [1] Refers to current enrolment. [2] Comparison between holder and non-holder groups. Fisher's exact test used to compare categorical data. Independent Student's *t*-test used for all other comparisons

3.4.4.2. Pharmacodynamic responses.

Table 3.7 presents the results of the repeated measures analyses of variance comparing pharmacodynamic responses between holders (n=8) and non-holders (n=8). The only significant between group differences found in these analyses was for the number of self reported withdrawal symptoms (P=0.001). This difference is explored later in this section. Consistent with previous analyses that considered all patients, there were significant temporal changes for both holders and non-holders across the 24-hour dosing interval for all of the measured responses ($P \leq 0.041$). Interestingly there was a significant interaction between holding status and time since dosing for heart rate (P=0.037). The heart rate for nonholders (mean±sd: 90±18.43) was faster than for holders (72±4.21) at 24 hours since dosing. However, this difference was not significant (t=2.85, P>0.05).

Table 3-7: Repeated measures analyses of variance for all pharmacodynamic responses, according to holding status: holders (n=8) and non-holders (n=8)

Parameter	Effect[1]	Df [2]	*F* value	*P* Value[3]
Withdrawal	Hold	1,14	17.86	**0.001**
	Time	5,70	21.27	**<0.001**
	Hold x Time	5,70	10.53	**<0.001**
MBG	Hold	1,14	2.18	0.162
	Time	4,50	4.26	**0.006**
	Hold x Time	4,50	1.29	0.287
MG	Hold	1,14	1.85	0.195
	Time	4,60	2.64	**0.041**
	Hold x Time	4,60	1.75	0.150
Pain Detection	Hold	1,14	0.53	0.479
	Time	3,50	4.86	**0.004**
	Hold x Time	3,50	0.66	0.590
Pain Threshold	Hold	1,14	2.51	0.136
	Time	3,40	7.28	**0.001**
	Hold x Time	3,40	0.48	0.683
Diastolic blood pressure	Hold	1,14	0.58	0.456
	Time	5,70	4.23	**0.002**
	Hold x Time	5,70	0.51	0.772
Systolic blood pressure	Hold	1,14	2.66	0.125
	Time	5,70	4.32	**0.002**
	Hold x Time	5,70	0.43	0.816
Heart rate	Hold	1,14	0.62	0.445
	Time	3,40	6.75	**0.002**
	Hold x Time	3,40	3.30	**0.037**
Respiratory rate	Hold	1,14	0.39	0.544
	Time	4,60	20.43	**<0.001**
	Hold x Time	4,60	1.01	0.41
Pupil diameter	Hold	1,14	0.19	0.672
	Time	3,45	15.85	**<0.001**
	Hold x Time	3,45	1.82	0.153

Notes: [1]ANOVA effects=within subjects effect as time since dosing (Time), and between subjects effect as methadone holding status (Hold). [2] Degrees of freedom adjusted using Greenhouse Geisser corrections. Probability value was adjusted accordingly. [3] Significant *P* values in bold text

Figure 3.8 presents the different pattern of withdrawal for non-holders and holders across the 24-hour methadone-interdosing interval. As seen in Table 3.7 there was a significant main effect of group (P=0.001), time (P<0.001), and there was a significant time by group interaction (P<0.001). The number of withdrawal symptoms reported by holders was

relatively consistent across the inter-dosing period. In contrast, there was a greater temporal variation in the number of withdrawal symptoms reported by non-holders. Non-holders reported that withdrawal was at its maximum pre-dose and 24 hours after dosing and was at a minimum between 2 to 5 hours after dosing. The mean number of self-reported withdrawal symptoms was significantly greater for non-holders than holders at predose (P<0.001; -6.25; -10.72, -1.78), one (1) hour (P<0.05; -5.25; -9.72, -0.78), 9 hours (P=0.001; -6.63; -11.09, -2.16), 12 hours (P<0.001; -8.13; -12.59, -3.66) and 24 hours (P<0.001; -9.34; -13.84, -4.91) after dosing.

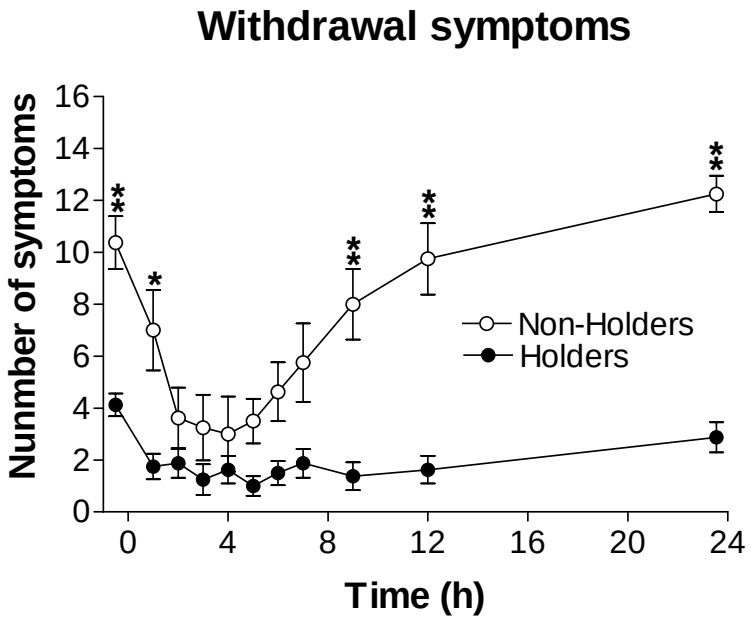

Figure 3-8: Mean (± SEM) withdrawal scores (maximum possible score = 16) for holders (closed circles: n=8) and non-holders (open circles: n=8) who participated in the 24-hour methadone inter-dosing interval study. Methadone was administered at zero hours. Asterisks signify results of between group comparisons; * P<0.05 ** P<0.001.

Table 3.8 presents the area under the curve for each pharmacodynamic parameter for both holder and non-holders groups. The only significant difference found between groups was for the number of self reported withdrawal symptoms. The area under the curve for withdrawal was significantly greater (P<0.001; -160; -219, -101) in non-holders (mean±sd: 210±73 score·h) than holders (50±28 score·h).

Table 3-8: Area under the curve for all pharmacodynamic responses according to holding status: holders (n=8) and non-holders (n=8).

Parameter[1]	Holders	Non-holders	P[2] value, mean difference; 95% CI
Withdrawal (score·h)	50±28	210±73	<0.001; -160; -219, -101
MBG (score·h)	124±95	52±32	0.08; 71.83; -4.05, 147.70
MG (score·h)	59±40	27±26	0.08; 32.38; -3.75, 68.51
Pain Detection (volts·h)	474±235	411±50	0.48; 62.69; -134.78, 260.16
Pain Threshold (volts·h)	1065±428	781±161	0.10; 283.25; -63.71, 630.21
Diastolic blood pressure (mmHg·h)	236±127	228±199	0.93; 7.56; -172.17, 187.28
Systolic blood pressure (mmHg·h)	153±73	145±62	0.81; 8.48; -63.84, 80.79
Heart rate (beats/minute·h)	192±93	182±85	0.83; 9.58; -85.77, 104.92
Respiratory rate (breaths/ minute·h)	44±16	49±21	0.58; -5.31; -25.40, 14.78
Pupil diameter (millimetres·h)	12±6	16±6	0.27; -3.31; -9.48, 2.85

Notes: Data are mean ±sd. [1] The AUC for withdrawal severity, MBG score, and MG score is expressed as score·hours; the AUC for pain detection and pain threshold as volts·hours; the AUC for systolic and diastolic blood pressure as millimeters of mercury·hours; the AUC for heart rate as beats/minute·hours; the AUC for respiratory rate as breaths/minute·hours; and the AUC for pupil diameter as millimeters·hours: [2] Independent Students t-test used to compare holder and non-holder groups.

3.4.4.3. Pharmacokinetic parameters.

The mean plasma (R)-(-) methadone concentration-time profiles normalised to a 70 mg racemic-methadone dose, for the holder and non-holder groups, are shown in figure 3.9.

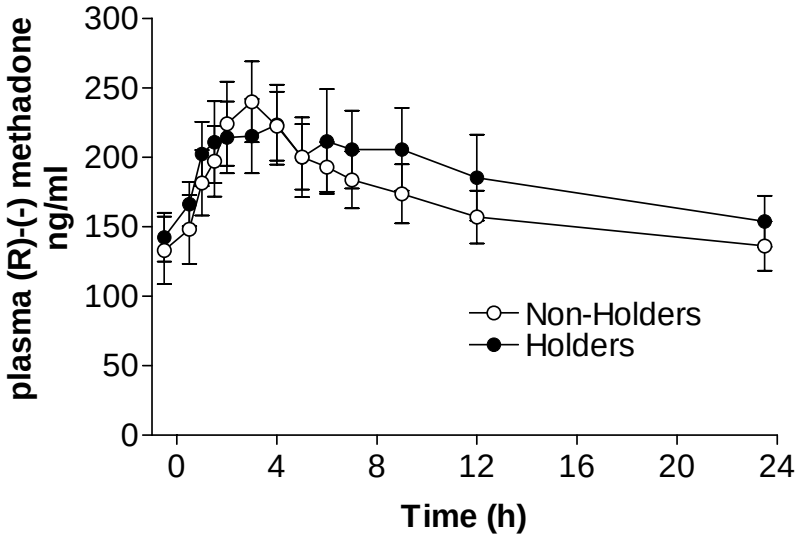

Figure 3-9: Mean (± SEM) plasma (R)-(-) methadone concentration-time profiles for holders (closed circles: n=8) and non-holders (open circles: n=8) during one 24-hour inter-dosing interval. Oral administration of racemic methadone was at zero hours. Data are presented as concentration normalised to a 70 mg racemic methadone dose. 1 ng/ml equals 0.31 nmol/L.

Comparisons between holder and non-holder groups were made on a number of pharmacokinetic parameters derived from the plasma (R)-(-) methadone concentration data, these are presented in table 3.9. No significant differences between holders and non-holders were found with respect to T_{max} (P=0.48), C_{max} (P=0.84), plasma AUC (P=0.56), $C_{min(first)}$ (P=0.75), $C_{min(last)}$ (P=0.50), C_{ss} (P=0.56), and *CL/F* (P=0.62: see table 3.9). Although the mean peak to trough plasma concentration ratio for the holders was 81 % of the mean peak to trough ratio for nonholders, this difference was also not significant (P=0.06, table 3.9). Levene's test for equality of variances showed that non-holders had greater individual variability in P/T ratios than holders (P=0.045). There were no other significant differences, between non-holder and holder groups, in the degree of variability associated with the pharmacokinetic parameters (P>0.05).

The rate of decline in plasma (R)-(-) methadone concentration for each hour from the time of peak concentration to trough was calculated for each individual in each group. The maximum mean rate of decline for non-holders (63.8±66.2 ng.mL/h) was not statistically different than for holders (41.8±50.0 ng.mL/h), (P=0.47). Furthermore, the relationship between the maximum rate of decline in plasma (R)-(-) methadone concentration and the mean number of withdrawal symptoms reported during the period for peak plasma concentration to trough was not significant (r = 0.31, P=0.24). The time, from 0-24 hours, that plasma (R)-(-) methadone concentration was equal to or greater than 75% of C_{max} was estimated from inspection of individual (R)-(-) methadone plasma concentration-time profiles. The time that plasma (R)-(-) methadone plasma concentration was equal to or greater than 75% of C_{max} was significantly greater for holders (14.4 ±6.5hrs: mean ±sd) than for non-holders (6.8±5.5hrs) (P=0.024), thus indicating that non-holders had greater fluctuation in plasma methadone concentrations across the dosing interval than holders.

Table 3-9: Disposition of (R)-(-) methadone for 16 patients following dosing of between 30–150 mg racemic methadone in patients who were studied during the 24-hour methadone inter-dosing interval study. Eight patients were self-reported holders and 8 were self reported non-holders.

Parameter[1]		Mean	Range	%CV [2]	P value[3] (mean difference; 95% CI)
C_{max} (ng/ml)	Holders	246	122-450	41	0.84
	Non-Holders	256	135-359	32	(-9; -108, 90)
AUC (ng.hr/ml)	Holders	4525	2332-8148	39	0.56
	Non-Holders	4060	1927-5684	33	(465; -1222, 2151)
T_{max} (h)	Holders	2.23	1.5-6	62	0.48
	Non-Holders	2.53	1-5	44	(-0.5; -1.58, 0.58)
$C_{min(first)}$ (ng/ml)	Holders	142	63-232	35	0.75
	Non-Holders	133	43-259	52	(10, -55, 74)
$C_{min(last)}$ (ng/ml)	Holders	154	92-249	34	0.50
	Non-Holders	136	63-198	37	(18; -37, 72)
C_{ss} (ng/ml)	Holders	189	97-340	39	0.56
	Non-Holders	169	80-237	33	(19; -51, 90)
P/T($C_{max/Cmin\ (last)}$)	Holders	1.58	1.30-1.80	12	0.063
	Non-Holders	1.95	1.5-2.9	24	(-.38; -.77, 0.02)
CL/F (ml/ min)	Holders	146	72-250	38	0.62
	Non-Holders	162	103-303	42	(-16; -81, 50)
Max decline (ng.mL/h)	Holders	41.81	9.53-161	120	0.47;
	Non-Holders	63.83	9.15-206.5	104	(-22; -85, 41)
T≥75 % C_{max} (h)	Holders	14.38	5.5-24	45	0.024
	Non-Holders	6.82	3-19	80	(7.56; 1.143, 13.98)

Notes: [1]C_{max} = maximum plasma concentration, normalised to an 70 mg racemic methadone dose; AUC = area under the plasma concentration-time curve during the 24 hour inter-dosing interval, normalised to 70 mg racemic methadone; T_{max} = time to reach maximum measured steady-state plasma concentration; $C_{min(first)}$ = plasma concentration, normalised to 70 mg racemic methadone, 30 minutes prior to dose; $C_{min(last)}$ = plasma concentration, normalised to 70 mg racemic methadone, 24 hours after dosing; C_{ss} = average steady-state plasma concentration, normalised to 70 mg racemic methadone; P/T= peak to trough plasma concentration ratio (ie, Cmax/ Cmin(last)); T≥75 % C_{max} = The time plasma (R)-(-) methadone plasma concentration equal to or greater than 75 % $_{Cmax}$; CL/F = apparent plasma clearance at steady-state. [2] Coefficient of variation. [3] Independent t-test used to compare holder and non-holder groups.

3.5. Discussion.

The purpose of this study was to examine the steady state pharmacodynamics and pharmacokinetics of (R)-(-) methadone in MMT patients in order to characterise the pharmacological basis of non-holding. A number of subjective and physiological indices of opioid effect were collected concurrently with frequent blood samples across one 24-hour methadone inter-dosing interval in 18 MMT patients, eight of whom were non-holders. A group of drug- free control subjects was included to ensure that results could be reasonably interpreted as being opioid induced. As hypothesized, the administration of methadone to patients at steady state produced significant temporal changes across the inter-dosing interval for all indices of opioid effect. The time course for self reported withdrawal and physiological indices was the inverse of the plasma (R)-(-) methadone concentration versus time profile: maximal effects and withdrawal suppression occurred at the approximate time of peak plasma (R)-(-) methadone concentration. However, the relationship between the time course of subjective responses and the plasma (R)-(-) methadone concentration versus time profile was less clear. In drug-free subjects, with the exception of MBG scores, there were no significant temporal changes for any parameter. Between group differences (MMT patients vs control subjects) were evident for systolic blood pressure, respiratory rate, pupil diameter, MG scores and withdrawal suppression, and with the exception of withdrawal, were most pronounced at the time of putative peak plasma (R)-(-) methadone concentrations. Between group differences for number of withdrawal symptoms was greatest at times of trough plasma (R)-(-) methadone concentration. Contrary to the finding of Dyer and colleagues (1999) there was no statistical difference between holder and non-holder groups in the rate of decline in plasma (R)-(-) methadone concentration. However, differences were found in the time, from 0-24 hours, that plasma (R)-(-) methadone concentration was equal to or greater that 75% of C_{max} suggesting that nonholders have greater fluctuations in plasma (R)-(-) methadone concentrations than holders.

3.5.1. Pharmacodynamic responses.

Following the administration of methadone there were marked changes in respiratory rate and pupil diameter across the dosing interval that were the inverse of the plasma (R)-(-) methadone concentration versus time profile, with lowest values occurring at the time of putative peak (R)-(-) methadone concentration (2–4 hours after dosing), and highest values

occurring at the time of trough plasma concentrations. With regard to changes in pupil diameter, the pattern and magnitude of results are consistent with those reported following the administration of single doses of oral racemic methadone to drug free subjects (Inturrisi and Verebely, 1972: Olsen, *et al*., 1977), and to MMT patients (Dyer, *et al.*, 1999; Verebely, *et al*., 1975). Thus, the findings of this study are consistent with reports that miosis is a reliable and sensitive marker of opioid effect (Fraser, *et al*., 1954; Olsen, *et al*., 1977; Tress and EL Sobky, 1981).

Furthermore, the temporal change in patients' respiratory rate seen in this study was largely in accord with previous reports (Dyer, *et al.*, 1999; McCaul, *et al.*, 1982). However, the magnitude of maximum respiratory depression in the current study (mean of 3.6 breaths per minute) was relatively greater than demonstrated previously. McCaul and colleagues reported a maximum reduction of 1.5 breaths/minute at two hours after dosing in patients receiving 40 to 80 mg/daily of racemic methadone, while Dyer and colleagues (1999) demonstrated a maximum mean reduction of approximately 2.5 breaths/minute in patients receiving 7.5 to 130mg/daily of racemic methadone. Differences in the methodology used to measure respiratory rate in the current study and that used by McCaul and colleagues make direct comparisons of data difficult. The latter authors measured respiratory rate with a mercury strain gauge stretched across the patients' abdomen, which likely caused discomfort and impeded respiratory effort. In the current study respiratory rate was measured by observing the rise and fall of the patients' chest without their awareness. The methods used in this study were similar to those utilised by Dyer and co-workers (1999) from our research group. In addition, as patients participating in this study, and those of Dyer and colleagues, were on a similar range of methadone doses and had been enrolled in MMT for similar duration, it is likely that observed differences between these studies reflect individual differences in tolerance to the effects of methadone on respiratory function.

Little has been reported in the literature on the effect of orally administered methadone on cardiovascular function in the context of maintenance treatment (Gritz, *et al*, 1975; Martin, *et al*., 1973; McCaul, *et al*., 1982). This study showed that cardiovascular response were less sensitive to changes in plasma (R)-(-) methadone concentration than other physiological indices. Despite this, there were still significant, although relatively small, changes in both heart rate and systolic and diastolic blood pressure for methadone patients

across the 24-hour testing period. The time course of each measure appeared to parallel the plasma (R)-(-) methadone concentration versus time profile, with maximum decreases occurring between 3-6 hours after dosing. The magnitude of change in cardiovascular responses is in keeping with previous available reports (Martin, *et al*., 1973; McCaul, *et al*., 1982). However, except for systolic blood pressure at six hours post dosing, there were no significant differences between MMT patients and control subjects, thus suggesting that these relatively small changes in cardiovascular function are probably of no clinical significance.

The administration of methadone was associated with a decrease in the number of withdrawal symptoms, in comparison to the pre-dose measurement. There was a very strong inverse relationship between the number of self reported withdrawal symptoms and the plasma (R)-(-) methadone concentration-time profile; i.e., withdrawal was at its minimum around the approximate T_{max}, and was at its maximum at trough (predose and 24 hour post dose). This pattern of scores is consistent with that reported by Dyer and colleagues (1999), and attests to the reliability of the finding, that even at steady state and presumably optimum dose, patients can still experience significant temporal fluctuation in severity of withdrawal. If the withdrawal experienced is perceived as sufficiently severe it is conceivable that some patients might use illicit opioids to reduce the severity of the withdrawal.

Subjective reports of positive opioid effect (MBG) and morphine-like effects (MG scores) changed significantly across the inter-dosing interval. Both scores appeared to be directly related to the plasma (R)-(-) methadone concentration-time profile, and peaked at approximate T_{max}. Although the temporal pattern of MBG results is similar to that presented by Dyer and colleagues (1999), in contrast to the latter study, there were no significant differences in MBG scores between patients and control subjects. Furthermore, patients' MBG scores in the current were substantially lower than those reported by these authors. Interestingly, McCaul and colleagues (1982) reported significant inter-individual variation in the magnitude and time course of MBG scores reported by patients maintained on relatively high dose methadone (40-80 mg/daily), with no significant changes in MBG scores for patients maintained on less than 40 mg/daily. In the present study, a few patients reported quite dramatic increases in MBG scores after drug administration. However, the majority reported little change in subjective (euphoria) effect. This might

reflect the development of tolerance to the euphorogenic effects of methadone and/or that the MBG scale did not detect subjective change that might have occurred. That is, the MBG scale, which lists a number of statement of common feelings reported to indicate positive opioid effects, might be subject to different individual interpretations. Indeed, many subjects commented that they felt the phrases were ambiguous and that they had difficulty interpreting their meaning. Given this, it is possible that the MBG scale is not a robust measure of opioid induced euphoria or is culturally inappropriate. With regard to MG scores, in contrast to controls, methadone patients reported significantly more 'morphine like side effects' than control subjects. This result is expected, given that methadone's primary site of action is the μ opioid receptor. As far as I am aware this is the first study of its kind to report the profile of MG scores across the 24-hour methadone inter-dosing interval.

Moreover, there were significant temporal changes in mean pain threshold scores across the inter-dosing interval. Scores for methadone patients peaked between 2 to 4 hours after dosing, were significantly different than the predose measure until 4 hours after dosing, and returned to pre-dose levels by 6 hours. Interestingly, during chronic dosing the analgesic effect of methadone is expected to persist for 6 to 12 hours (Gourlay, *et al*., 1986); my results would support this. The temporal pattern of data is also consistent with that reported by Dyer and colleagues (1999). However, contrary to Dyer and colleagues, the current study failed to find significant differences between methadone patients and control subjects. In the current study control subjects reported substantially greater pain threshold scores (range 35 to 40 volts) than their counterparts in Dyer's study (range 20 to 28 volts). In addition there was greater individual variation in scores for all participants in the current study. Thus no between group differences were evident.

It is noteworthy that morphine (a quantitative marker of recent heroin and/or morphine use) and a range of benzodiazepines were detected in plasma collected from patients participating in this study. The important question is whether the presence of these substances would have influenced the pharmacodynamic parameters measured in this study. All patients with benzodiazepines in their plasma had been prescribed benzodiazepines by their general medical practitioner, and so it is likely that they would be tolerant to the acute effects, such as sedation, of these drugs. Similarly, this would apply to the presence of morphine in plasma. Given that all patients had been enrolled in MMT

for at least 3 months prior to participating in this study, it is likely that they would be cross-tolerant to the effects of additional opioids in plasma. Indeed, Doverty and colleagues (2001) have shown that a small sample of patients on stable doses of methadone (mean±SEM dose 81±25 mg) were cross-tolerant to the antinociceptive effects of additional intravenous morphine [infused to reach two consecutive pseudo steady states (16 and 55 ng/ml)]. In that study, despite increased morphine concentration, patients experienced only small increases in pain threshold (measured by electrical stimulation and cold pressor) when measured at both trough and peak plasma methadone concentrations. The plasma morphine concentrations in the current study (3 to 54 ng/ml) from illicit heroin use are equal to or lower than those achieved by Doverty and coworkers and therefore, I would expect no significant effect of these substances on the measures used in this study.

3.5.2. Steady State Pharmacokinetics of (R)–(-) methadone.

Few studies have described the disposition of (R)-(-) methadone, the important enantiomer with respect to therapeutic outcome, in patients maintained on methadone (Eap, *et al*., 1996, 2000; Foster, *et al.*, 2000; 2004; Hiltunen, *et al.*, 1999; Kreek, *et al*., 1979; Mitchell, *et al*., 2003). Foster and colleagues (2000) have undertaken the most comprehensive investigation to date. They collected blood across one 24-hour inter-dosing interval from methadone maintenance patients at steady state, whose daily dose ranged from 7.5 to 130 mg. The pharmacokinetic parameters (C_{max}, C_{min}, P/T ratio, C_{ss} and CL/*F*) reported in this study for (R)-(-) methadone are largely in keeping with those reported by Foster and co-workers (2000).

Patients were required to be at steady state before participating in this study and so were required to have an unchanged methadone dose for at least two weeks prior to the commencement of testing. Steady state conditions were confirmed by the finding that (R)-(-) methadone concentrations at predose ($C_{min(first)}$) and 24 hour post dose ($C_{min\ (last)}$) were not significantly different. However, despite steady state conditions being verified, and the normalisation of plasma concentration data, there was substantial inter-subject variability of most pharmacokinetic parameters (range of coefficients of variation of 33 to 42%). For example, there was greater than four-fold variation in the C_{max} and C_{ss} and six-fold variation in $C_{min(first)}$ across all individuals participating in this study. This variability is similar to that reported by Foster and colleagues (2000). Moreover, Eap and coworkers (2000) demonstrated a 17-fold difference between the smallest and largest values for (R)-

(-) methadone concentration corrected for weight and dose in patients who did not receive any co-medication, and whose daily methadone dose ranged from 5 to 350 mg/day. The individual variability in methadone disposition means that an individualized approach to dosing is required to achieve a balance between effectively minimizing withdrawal, and minimising adverse side effects for the entire inter-dosing interval.

The degree of inter-individual variability seen in many of the pharmacokinetic parameters would be partly due to inter-subject variability in metabolism (i.e., the activity of cytochrome P450 enzymes). A number of cytochrome P450 isoforms have been shown to metabolise methadone, but CYP3A4 is the predominant P450 isoform involved (Foster, *et al.*, 1999; Iribarne, *et al*., 1996; Moody, *et al*., 1997), and its activity is a major determinant of steady state plasma methadone concentrations (Somogyi, 2000). There is evidence of up to 30-fold variation in the expression of hepatic CYP3A4 (Ketter, *et al*., 1995). Furthermore, *in vivo* it has been shown that there is about 6-fold variability between individuals in the activity of CYP3A4 (Wilkinson, 2004).

It is important to note that patients were excluded from participating in this study if they were known to be taking any drugs, other than tobacco, that are strongly associated with influencing CYP activity. Thirteen patients in the current study reported that they smoked tobacco. Although tobacco smoke has been shown to induce the activity of CYP1A2 (Kalow and Tang, 1991), precluding subjects on the basis of their smoking would have dramatically reduced the number of patients volunteering for this study, and also would have reduced the representativeness of the study sample. Furthermore, it is notable that other medications were taken by study participants, including sertraline (Zoloft®), a selective serotonin reuptake inhibitor, and venlafaxine (Effexor®), a mixed serotonin and noradrenaline reuptake inhibitor, both of which are known to be weak inhibitors of CYP2D6 (Brosen, 1998; Richelson, 1997). Foster and colleagues (1999) demonstrated that CYP2D6 played almost no role in the *N*-demethylation of methadone to EDDP. Based on this evidence it was considered inappropriate to preclude patients on the basis of taking the above drugs.

Moreover, consistent with the findings of Foster and colleagues, there was a significant relationship between plasma AUC and dose for (R)-(-) methadone. That is, despite the extent of interindividual variation seen in metabolic activity, approximately 60% of the

variability in the AUC of (R)-(-) methadone can be explained by variation in dose. It is likely that the remaining variability is due to differences in clearance (Foster, *et al*., 2000).

3.5.3. Holder versus non-holder comparisons.

In this study patients complaining of dose not holding could not be differentiated on any demographic variable recorded (except for the ratio of females to males and body weight), or drug use (plasma morphine and benzodiazepines). There was a significant gender bias in the non-holder group. Since this was an opportunistic study, recruitment continued until there were equal numbers of patients (irrespective of gender) in each group. Therefore, the significance of this gender inequality is not clear. However, it is notable that other studies have not found a gender difference with holders and nonholders (Dyer, and White, 1997: Dyer, *et al*., 1999). With the exception of number of self-reported withdrawal symptoms, there were no differences between non-holder and holder groups on any measured pharmacodynamic response. Non-holders reported significantly more withdrawal symptoms than holders throughout the interdosing interval, except for the period from 2 to 9 hours after dosing, which corresponds to the time of putative peak plasma (R)-(-) methadone concentration. This result was expected and validated the self-reported holding status of patients. Dyer and colleagues (1999) showed a similar relationship between self reported withdrawal score and plasma racemic methadone concentration, but found significant differences between groups throughout the interdosing interval. Furthermore, the mean AUC for withdrawal for non-holders was significantly greater than for holders, attesting to the greater severity of withdrawal experienced by non-holders. It is important to note that despite a similar mean daily methadone dose for the two groups it is unlikely that complaints of greater withdrawal for non-holders are due to inadequate dosing as the South Australian Methadone program uses an individualized approach to dosing and allows considerable patient control over dose.

A major aim of this study was to compare holder and nonholder groups for a number of pharmacokinetic parameters to characterize the pharmacokinetic basis of non-holding. The current study extends that of Dyer and coworkers (1999), who did the same for a cohort of patients whose daily methadone dose ranged from 7.5 to 130 mg, except that they reported results for plasma racemic methadone. In this study plasma AUC and plasma clearance were not significantly different between the holder and non-holder groups, and on the basis of this finding it is likely that bioavailability was not different between the two groups.

The mean C_{max} and T_{max} for (R)-(-) methadone were also similar for both groups. Furthermore, non-holders did not differ from holders on the basis of peak or trough methadone plasma concentration ($C_{min(first)}$ or $C_{min(last)}$) providing greater weight to the argument that there may be limited benefit in using an absolute minimum methadone concentration to judge the adequacy of the methadone dosage regimen (Bell, *et al.*, 1988; Dyer, *et al.*, 1999).

Previous researchers that have examined the pharmacokinetic basis of non-holding have suggested that patients complaining of withdrawal during the inter-dosing interval are likely to exhibit more rapid changes in plasma methadone concentrations than holders (Dyer, *et al*., 1999; Nilsson, *et al*., 1983). Dyer and colleagues demonstrated that the number of withdrawal symptoms reported was significantly correlated with the maximum rate of decline in plasma racemic methadone concentration during the period between peak and trough concentrations. It must be noted Dyer and colleagues only found a significant difference between non-holder and holders, with respect to the rate of decline in plasma racemic methadone concentrations, when they excluded the data from two patients (holders) who had tested positive for opioids in the urine screen. While the current study found that non-holders did exhibit a greater rate of decline in plasma (R)-(-) methadone concentrations than holders, this difference was not significant.

Further analyses were performed to characterise the differences in variability in plasma (R)-(-) methadone concentration-time profiles between the two groups. The percentage of time plasma (R)-(-) methadone concentration was equal to or greater than 75 % of peak plasma concentration (T≥75 % C max) was calculated. This index has previously been used to describe the stability of the plasma time concentration profile produced by sustained release formulations, in comparison to immediate release formulations (Bochner, *et al*., 1999; Leewenkamp, *et al*., 1994; Maccarone, *et al*., 1994). The mean T≥75 %C max was more than double for holders than nonholders, thus indicating that nonholders have greater fluctuation in (R)-(-) methadone plasma concentration across the dosing interval than holders. Clearly more detailed analysis, which is beyond the scope of this study, of the pharmacokinetic differences between holder and nonholders needs to be undertaken to determine the reliability of these findings. Furthermore, there is a need to replicate these findings in a larger sample of MMT patients to determine their reliability. However, it

does appear that nonholding status may have a pharmacokinetic basis, which may be related to distribution, rather than clearance mechanisms.

3.5.4. Conclusions and clinical implications.

A major aim of MMT is to suppress withdrawal across the 24 hour interdosing interval. However, previous studies have shown that despite using an individualized approach to dosing, up to one third of MMT patients report breakthrough withdraw during the dosing interval (Dyer, *et al.*, 1999; Dyer and White, 1997). Consistent with previous studies (Dyer, *et al*., 1999; Nilsson, *et al*., 1983) the present findings suggest that non-holding is associated with greater fluctuation in plasma methadone concentrations across the dosing interval, rather than inadequate daily methadone dosage and plasma methadone concentration. In addition, in MMT patients plasma methadone concentration-effect relationships are very steep for withdrawal (Dyer, *et al*., 1999). Adopting a strategy to reduce the variability in plasma methadone concentration from peak to trough should therefore lessen withdrawal. One such strategy is to split a patient's daily methadone dose (Nilsson, *et al.*, 1983: Walton, *et al*., 1978), and so shorten the dosage interval. However, if patients do not have take way privileges, this may be impractical where dosing needs to be supervised. Another strategy is to use a maintenance drug, such as LAAM, which has different pharmacokinetics to methadone (Ling and Compton, 1997; Nilsson, *et al*., 1983). As the terminal half lives for LAAM and its active metabolites (nor-LAAM and dinor-LAAM) are greater than for methadone, the plasma concentration versus time profiles for these compounds are likely to be flatter. However, as discussed in chapter one, the disposition of LAAM in humans is not well described. Thus, the next chapter will explore in detail the pharmacodynamics and pharmacokinetics of LAAM in a cohort of maintenance patients at steady state.

4. Steady State Pharmacodynamics and Pharmacokinetics of LAAM, Nor-LAAM and Dinor-LAAM in Maintenance Patients

4.1. Introduction.

Randomised controlled trials have established LAAM's effectiveness as a pharmacotherapy in the maintenance treatment of opioid dependence (Freedman and Czertko, 1981; Johnson, *et al*., 2000; Ling, *et al*, 1976;White, *et al*., 2002). Its main advantage over methadone is its prolonged duration of action, with typical opioid effects (miosis and euphoria) and suppression of withdrawal reported to persist for up to 72 hours following a singe dose (Fraser and Isbell, 1952; 1954; Walsh, *et al.*, 1998). This permits dosing every 48 to 72 hours (Fraser and Isbell, 1952) in the maintenance treatment of opioid dependence. The prolonged duration of action of LAAM has been attributed to its *in vivo* biotransformation to active demethylated metabolites, nor-LAAM and dinor-LAAM, which are more potent mu opioid agonists and have longer terminal half-lives longer than the parent drug.

As reviewed in chapter one, there are limited published data on the disposition of LAAM and its demethylated metabolites in humans, particularly at steady state (Billings, *et al*, 1974; Finkle, *et al*., 1982; Henderson, 1977b; Kaiko and Inturrisi, 1975). The available data show that there are large inter-individual variations in the pharmacokinetics of LAAM, nor- and dinor-LAAM. However, it is not clear what effect such individual variations in pharmacokinetics, particularly of nor-LAAM and dinor-LAAM, might have on the therapeutic response to LAAM (i.e., number of withdrawal symptoms). Furthermore, to date, no study has comprehensively examined the time course of opioid effects (i.e., subjective effects such as withdrawal, euphoria and analgesia; and physiological indices, such as miosis, blood pressure, and respiratory rate) across the 48-hour inter-dosing interval, under chronic dosing conditions in maintenance patients. Additionally, before one can formally evaluate the effectiveness of using LAAM to treat MMT patients who report breakthrough withdrawal on methadone, it is first necessary to determine if they can be differentiated from holders with respect to LAAM pharmacokinetics. Such differences would confound any findings regarding the apparent effectiveness of LAAM in treating methadone non-holders.

Therefore, the primary aim of this study was to examine the steady state pharmacodynamics and pharmacokinetics of LAAM, nor- and dinor-LAAM in a cohort of maintenance patients. A number of subjective and physiological indices of opioid effect were collected concurrently with frequent blood samples across one 48-hour inter-dosing interval. The specific aims of the work in this chapter were:

- To describe the temporal change in physiological (heart rate, blood pressure, pupil diameter) and subjective (positive opioid effect (MBG), morphine-like effects (MG), and pain detection and threshold, and withdrawal complaints) indices of opioid effect across the 48 hour LAAM inter-dosing interval and to compare these responses to that obtained from a group of non-opioid using control subjects.
- To determine the steady-state pharmacokinetics (time to maximum plasma concentration, maximum plasma concentration, minimum plasma concentration pre-dose and 48 hours after dosing, steady state plasma concentrations, peak to trough plasma concentration, area under the plasma concentration–time curve, and renal clearance and excretion) of LAAM, nor-LAAM and dinor-LAAM, and the apparent plasma clearance of LAAM, and the degree of inter-individual variability of these pharmacokinetic parameters.

4.2. Hypotheses.

- The temporal profile of physiological (pupil diameter, heart rate, systolic and diastolic blood pressure, respiratory rate) and subjective (withdrawal, pain threshold, MBG, and MG) indices of opioid effect will be closely related to the underlying plasma nor- and dinor-LAAM concentration versus time profiles.
- At the times of approximate putative peak plasma LAAM and nor- and dinor-LAAM concentrations the physiological responses of LAAM patients will be significantly depressed relative to those obtained from non-drug using controls; on the other hand, at the same time, subjective responses will be significantly greater than obtained from control subjects.
- The administration of LAAM will be associated with a significant decrease in the number of self-reported withdrawal symptoms which will remain significantly suppressed across the inter-dosing interval.

- Dinor-LAAM will exhibit a less variable plasma concentration versus time profile, as reflected in a smaller peak to trough plasma concentration (P/T) ratio, than that of nor-LAAM. In turn, both nor-LAAM and dinor-LAAM plasma concentration versus time profiles will be less variable than of LAAM.

4.3. Methods.

The methods and procedures used in the LAAM inter-dosing study were outlined in detail in chapter two. Briefly, in this study the pharmacodynamics and pharmacokinetics of LAAM, nor-LAAM, and dinor-LAAM were examined in 17 patients. Blood was collected pre-dose and 0.25, 0.50, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24, 36, and 48 hours after administration of LAAM. Pharmacodynamic responses were recorded at the same time, but no more frequently than hourly. The data obtained from ten drug free controls were compared to responses obtained from LAAM patients. With the exception of data obtained from the cognitive psychomotor task, neurocognitive tests and measures of psychological health status (POMS, BDI-II revised, and STAI), all data collected during the LAAM inter-dosing study will be presented in this chapter.

4.3.1. Analyses.

4.3.2. Pharmacokinetic analyses.

Pharmacokinetic parameters were derived using noncompartmental methods (Rowland and Tozer, 1995), and were the same as described for (R)–(-) methadone (section 3.3.1.1) except for the following: the areas under the LAAM, nor-LAAM and dinor-LAAM plasma concentration versus time curves, were calculated from 0-48 hours. Steady state plasma concentrations (C_{ss}), for each drug were determined by dividing AUC by the dosing interval (48 hours). AUC, C_{ss}, C_{max}, $C_{min(first)}$, and $C_{min(last)}$, for each drug were normalised to 80 mg LAAM. The peak to trough plasma concentration ratios for LAAM, nor- and dinor-LAAM for each individual were calculated by dividing C_{max} by $C_{min(last)}$. Apparent plasma clearance at steady state (CL/F) for LAAM was calculated as dose/AUC. Renal clearance (CL_R) for LAAM, nor-LAAM and dinor-LAAM was calculated as the amount of each drug recovered in the 0-48 hour urine sample divided by the respective AUC. The percent of LAAM dose excreted in urine was calculated by dividing the number of moles of LAAM, nor-LAAM and dinor-LAAM excreted in urine as the free base by the molar

dose of LAAM. In addition, the duration (hours) that LAAM, nor-LAAM and dinor-LAAM plasma concentrations were equal to or above 75% of the respective C_{max} ($T \geq 75\%$ C_{max}) was calculated from visual inspection of individual plasma concentration-time profiles for methadone holders and non-holders when on LAAM.

4.3.3. Statistical analyses.

Demographic data for methadone holders and non-holders (except gender ratio) when on LAAM, plasma drug screen results, and between group comparison for pharmacokinetic parameters (males and females, and methadone holders and non-holders when on LAAM) were compared using Student's *t*-test for independent samples. Fisher's exact test was used to compare categorical data. Paired *t*-tests were used to compare pharmacokinetic parameters derived from LAAM, nor-and dinor-LAAM concentration data for each individual. Relationships between variables were determined using Pearson's product-moment coefficient.

Repeated measures ANOVA was used to examine the effect of time since dosing and group membership (LAAM patients versus control subjects) on pharmacodynamic responses for all subjects. Analyses exploring within and between group differences used paired and independent *t*-tests, respectively, with Bonferonni adjustment to the alpha level to reduce the likelihood of type I error. Within group comparisons were made between the pre-dose or baseline measurement and each subsequent measurement point for each group, with a probability level of $P<0.005$ used to determine significance (i.e., 0.05/10 comparisons). Between group comparisons were made at each measurement point, with a probability level of $P<0.0045$ used to determine significance (i.e., 0.05/11 comparisons). Further, repeated measures ANOVA were used to examine the effect of time since dosing and methadone self reported holding status on pharmacodynamic responses. For repeated measures degrees of freedom were adjusted for violations of the sphericity assumption using Greenhouse Geisser corrections provided by SPSS.

Unless otherwise stated the alpha level for all statistical analyses was set at $P=0.05$ and all statistical tests were two tailed. The data were analyzed using SPSS™ for Windows (v.10), unless otherwise stated. SPSS undertakes Levene's test of homogeneity of variance when undertaking t-tests and adjusts the probability accordingly. Coefficients of variation (%CV) were calculated for all pharmacokinetic parameters. All statistical tests were two

tailed and data were tabulated as mean ± sd. Where appropriate, 95% confidence intervals [CI] of difference between means were also presented.

4.4. Results.

4.4.1. Patient details.

Seventeen patients participated in the LAAM inter-dosing interval study; table 4.1 presents details of the LAAM dosage, plasma screening results, and self reported drug use for each individual patient at the time of the inter-dosing study. There were eight males and nine females who had a mean age of 34.6 years (±8.0 years) at the time of the study. They had a mean±sd body weight of 71.2 kg (±17.2 kg) and their mean LAAM dose was 80.3 mg (± 43.2 mg), which corresponded to a mean LAAM dose to body weight ratio of 1.17 mg/kg (± 0.75 mg/kg). At the time of this study six (35%) were employed on a full time, part time or casual basis. Fifteen patients self-reported that they smoked tobacco, and six self reported the regular consumption of less than 40 grams of alcohol daily and only two patients reported drinking in excess of 40 grams daily in the preceding week. The remaining patients denied drinking alcohol. Four patients had morphine present in their plasma (see table 4.1 for morphine concentrations), and six patients had benzodiazepines (diazepam, desmethyldiazepam and oxazepam) present in their plasma (see table 4.1). Table 4.1 also presents a detailed list of drug use, other than LAAM, for each patient. With only a few exceptions, there was general concordance between the presence of benzodiazepines and morphine in plasma and urine. Note that patient # 17 (Table 4.1), who was allocated to LAAM first, withdrew from the study soon after completing the LAAM inter-dosing study and did not participate in the methadone inter-dosing study.

Data obtained from the ten drug free volunteers (6 males and 4 females) were compared to data obtained from the maintenance patients.

Table 4-1: Details of LAAM dosage, plasma screening results, and self reported drug use of 17 patients that participated in the LAAM inter-dosing interval study.

Patient No[1]	Dose (mg)	Dose (mg/kg)	Prescribed Drugs[2]	Urinalysis Predose[3]	Plasma Morphine[4]	Plasma Benzo[5]	Self Reported drug use[6]
1	110	1.15	Nil	Nil	0.0	Nil	Nil
2	45	0.66	1,6	1	0.0	1	1,2
3	110	1.16	Nil	1	0.0	Nil	1,2,3
4	65	1.38	Nil	1	0.0	Nil	1
5	40	0.51	Nil	2	65.0	Nil	4
6	75	0.87	Nil	Nil	0.0	Nil	1
7	135	1.80	1,2	1	0.0	1,2,3	1
8	60	0.68	1	1,4	0.0	1	1,2,4,6
9	150	3.23	Nil	1,2,4	0.0	Nil	1,3,4,5,6
10	30	0.59	Nil	4	0.0	Nil	1,6
11	145	2.20	3	1,4	0.0	Nil	1,2,3,6
12	110	1.10	1	1,4	0.0	1,2,3	1,3,6
13	130	2.03	3	4	0.0	Nil	1,2,6
14	30	0.48	1,7	1,2	2.00	1,2	1,2,3,4
15	45	0.74	4	2,4	2.4	Nil	1,2,4,6
16	45	0.83	5	1,2	3.5	1,2	1,2,3,4
17	40	0.57	Nil	4	0.0	Nil	1,6
Mean	80.3	1.17	-	-	-	-	-
sd	43.2	0.75	-	-	-	-	-

Notes: [1]Patients are listed in the same order as in Table 3.1. Patient # 17 (25 year old male) participated only in the LAAM inter-dosing study and withdrew from the study soon after completing the LAAM inter-dosing study. He had been in maintenance treatment for 7 months prior to enrolling in the current study. [2] Prescribed drugs, 1 = diazepam, 2 = oxazepam, 3= sertraline, 4 = venlafaxine, 5 = alprazolam; 6 = sodium valproate; 7 = panadeine forte (each tablet contains 500 mg paracetamol + 30 mg codeine phosphate). [3] Predose urinalysis, 1=benzodiazepines, 2=opioids (other than methadone), 3=sympathomimetic amines, 4=cannabinoids. [4] Plasma morphine concentration (μg/litre). [5]Presence of the following benzodiazepines in plasma, concentrations within or greater than the quoted therapeutic range (shown in brackets), 1= diazepam (0.7 – 1.8 μmol/L), 2 = desmethyldiazepam (2.0 – 5.0 μmol/L), 3 = oxazepam (1.7 – 7.0 μmol/L), note – no other benzodiazepines detected. [6] Self-reported drug use in last week, 1 = tobacco, 2 = alcohol, 3 = benzodiazepines (other than prescribed), 4 = opioids (other than LAAM), 5= ympathomimetic amines, 6 = cannabinoids.

The next section will present results for all patients, prior to describing results for those who described themselves as holders or non-holders when taking methadone.

4.4.2. Pharmacodynamic responses – comparison for all subjects.

4.4.2.1. Physiological responses.

Cardiovascular responses (heart rate, systolic and diastolic blood pressure), and pupil diameter and respiratory rate for LAAM patients and control subjects are shown in figure 4.1 through to 4.3. Table 4.2 summarises repeated measures ANOVA used to examine the effect of time since dosing and group membership (LAAM, controls) on physiological indices of opioid effect. With regard to heart rate (figure 4.1), there was a significant main effect of time since dosing, but no significant effect of group. There was a significant group x time since dosing interaction indicating that changes in heart rate across the inter-dosing interval were significantly different for LAAM than control subjects. Patients' mean heart rate at 1 hour (P=0.003; -4.53 beats per minute (bpm); -7.24, -1.81 bpm), 2 hours (P=0.002; -5.41 bpm; -8.62, -2.21 bpm), 3 hours (P=0.003; -4.24 bpm; -6.85, -1.63 bpm), and 6 hours (P=0.002; -6.47 bpm; -10.11, -2.83) after dosing was significantly lower than the heart rate pre-dose: in contrast the heart rate at 48 hours after dosing was significantly faster than at pre-dose (P=0.001; 9.47 bpm; 4.48, 14.43 bpm). There were no significant within group difference for control subjects (P>0.005).

Furthermore, mean systolic and diastolic blood pressure of LAAM patients and of control subjects are also shown in figure 4.1. For systolic blood pressure there was a significant main effect of time, but not of group. There was no significant interaction between time since dosing and group. Post hoc tests revealed no significant within group differences for the patients (P>0.005), and the control subjects (P>0.005). There were no significant effects of time since dosing or group on diastolic blood pressure.

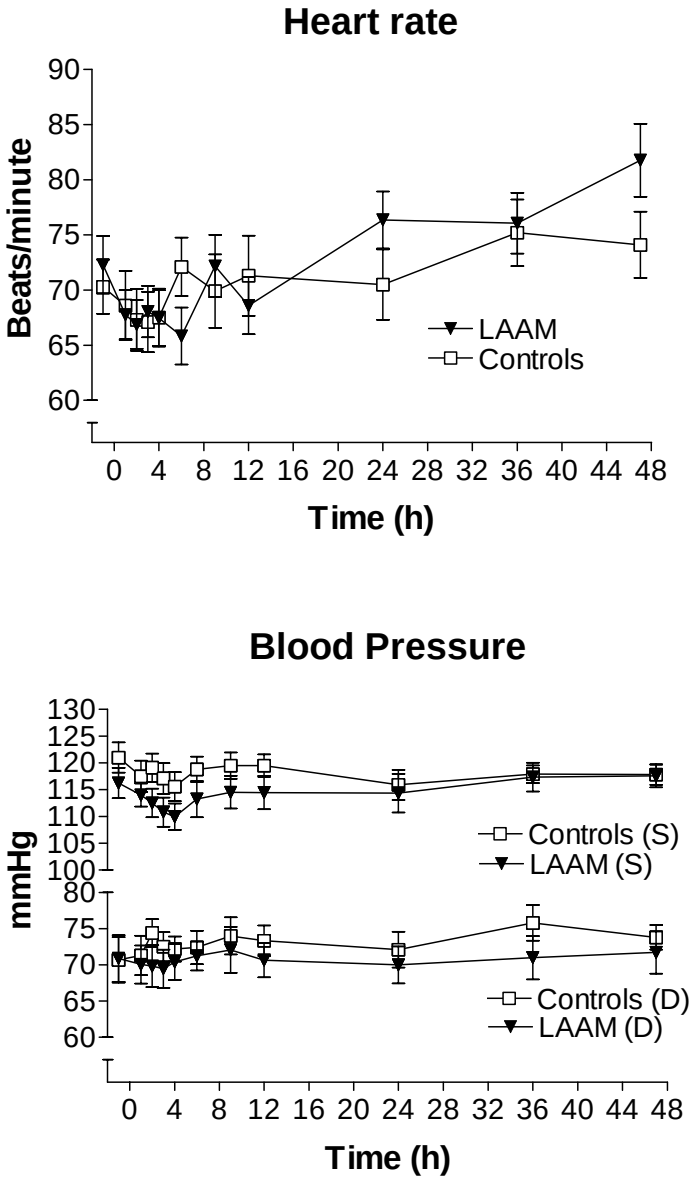

Figure 4-1: Mean (± SEM) heart rate (beats per minute) (upper panel); systolic (S) blood pressure and diastolic (D) blood pressure (lower panel) of LAAM patients (closed triangles: n=17) who underwent the LAAM inter-dosing interval study and for control subjects (open squares: n=10) for the period 0-48 hours. LAAM was administered at zero hours.

Mean respiratory rates for patients and control subjects are shown in figure 4.2. There were significant main effects of group, and of time, and there was also a significant group x time since dosing interaction. The mean respiratory rates of patients declined from a maximum at baseline to minimum levels at 6 hours after dosing (3.82 breaths/minute (brpm) below baseline) and then slowly returned to baseline by the end of the dosing interval. In contrast, respiratory rate for control subjects was relatively constant across the testing interval. Patients' mean respiratory rate was significantly lower than the predose measurement at 1 hour (P=0.001; -1.53 breaths per minute (brpm); -2.01, -1.04 brpm), 2

hours (*P*<0.001; -3.06 brpm; -4.00, -2.12 brpm), 3 hours (*P*<0.001; -3.29 brpm; -4.20, -2.39brpm), 4 hours (*P*<0.001, -3.65 brpm; -4.71, -2.59brpm), 6 hours (*P*<0.001; -3.82 brpm; -4.93, -2.71brpm), 9 hours (*P*<0.001; -3.29 brpm; -4.23, -2.35brpm), 12 hours (*P*<0.001; -2.35 brpm; -3.44, -1.26 brpm), 24 hours (*P*<0.001; -2.76 brpm; -3.78, -1.74. brpm), and at 36 hours (*P* =0.001; 1.71 bpm; 0.80, 2.61 brpm), but not at 48 hours after dosing (*P*>0.005). There were no significant within group differences for control subjects (*P*>0.005).

The respiratory rate of patients was significantly lower than that of control subjects at 1 hour (*P*<0.001; -5.08 brpm; -7.34, -2.82 brpm), 2 hours (*P*<0.001; -6.72 brpm; -8.85, -4.57 brpm), 3 hours (*P*<0.001; -7.15 brpm; -9.15, -5.15 brpm), 4 hours (*P*<0.001; -7.5 brpm; -9.59, -5.51 bpm), 6 hours (*P*<0.001; -7.78 brpm; -10.18, -5.37 brpm), 9 hours (*P*<0.001; -7.95 brpm; -10.25, -5.65 bpm), 12 hours (*P*<0.001; -7.31 brpm; -9.73, -4.90 bpm), 24 hours (*P*<0.001; -6.52 bpm; -8.59, -4.45 brpm), 36 hours (*P*<0.001; -5.96 brpm; -8.31, -3.60 bpm), and 48 hours after dosing (*P*=0.002; -3.92 brpm; -6.25, -1.59 bpm), but not at predose (*P*>0.0045).

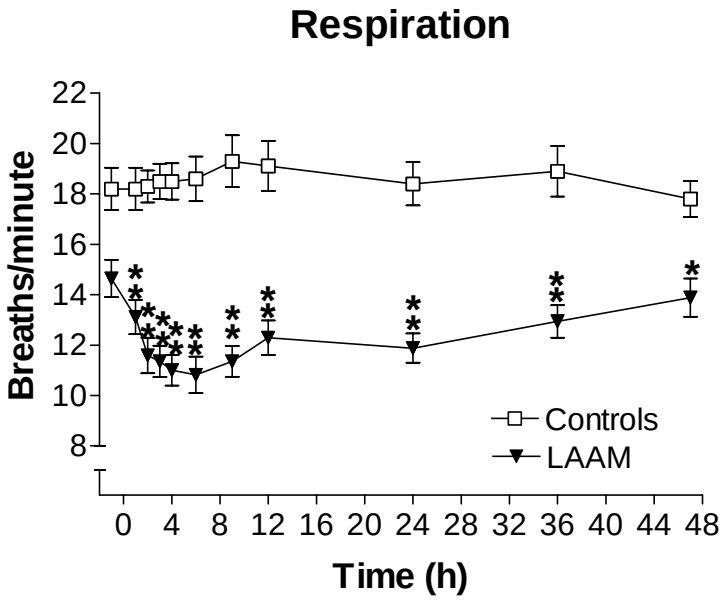

Figure 4-2: Mean (± SEM) respiratory rate (breaths per minute) of LAAM patients (closed triangles: n=17) who underwent the 48-hour LAAM interdosing interval study and of control subjects (open squares: n=10) for the period 0-48 hours. LAAM was administered at zero hours. Asterisks denote significant between group comparisons; * P <0.0045,**P<0.001.

For pupil diameter there were main effects of group and time, and a significant group x time since dosing interaction (figure 4.3). The mean pupil diameter for patients declined from a maximum pre-dose to a minimum between 4 to 6 hours after dosing (mean 0.9 mm

below baseline) and then slowly returned to baseline levels by the end of the dosing interval. In contrast, pupil diameter for control subjects was relatively constant across the inter-dosing interval. Pupil diameter was significantly smaller than the predose pupil diameter at 1 hour (P<0.001; -0.46mm; - 0.61, -0.30 mm), 2 hours (P<0.001; - 0.74 mm; - 0.98, -0.49 mm), 3 hours (P<0.001; -0.85 mm; -1.08, -0.62 mm), 4 hours (P<0.001: - 0.90 mm; - 1.25, - 0.54 mm), 6 hours (P<0.001; - 0.90 mm; - 1.22, - 0.57 mm), 9 hours (P<0.001; -0.81 mm; - 1.16, - 0.46 mm), and 12 hours (P=0.001; - 0.57 mm; - 0.86, - 0.28 mm), but not at 24 hour, 36 hours and 48 hours after dosing (P>0.0045). There were no within group differences for control subjects (P>0.005).

Pupil diameter for patients was significantly smaller than that of control subjects at 2 hours (P=0.002; -1.35 mm; -2.13, -0.56 mm), 3 hours (P=0.002; -1.36; -2.18, -0.55 mm), 4 hours (P=0.001; -1.56 mm; -2.40, -0.71 mm), 6 hours (P<0.001; -1.66 mm; -2.51, -0.80 mm), 9 hours (P<0.001; -1.62 mm; -2.41, -0.83 mm), and 12 hours (P=0.001; -1.41mm; -2.21, -0.61mm), but not at any other time point (P>0.0045).

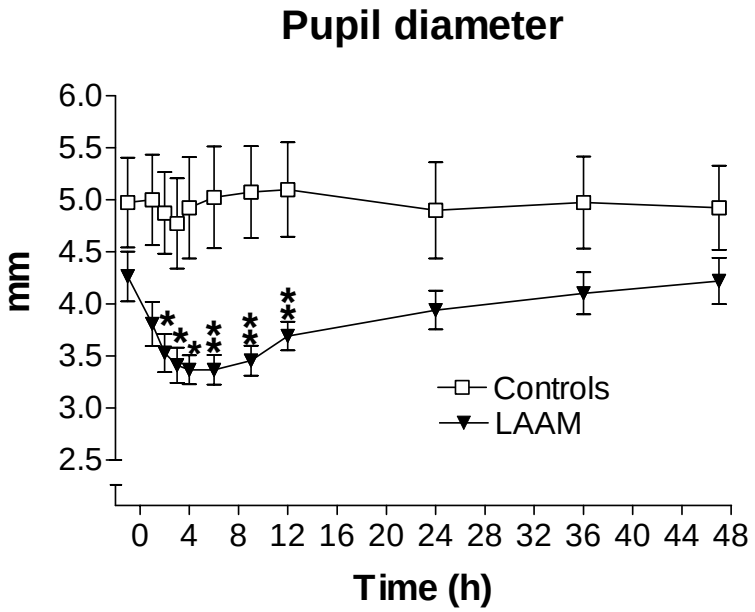

Figure 4-3: Mean (± SEM) pupil diameter (mm) for LAAM patients (closed triangles: n=17) who underwent the 48 hour LAAM interdosing interval study and for control subjects (open squares: n=10) for the period 0-48 hours. LAAM was administered at zero hours. Asterisks denote results of between group comparisons; * P<0.0045,** P<0.001.

4.4.2.2. Subjective responses.

Subjective responses (Morphine Group (MG) and Morphine Benzedrine Group (MBG) subscales of the Addiction Research Centre Inventory, pain detection and threshold, and

number of withdrawal symptoms)) for patients and control subjects are shown in figures 4.4 through to figure 4.6. Table 4.2 summarises the Repeated Measures ANOVAs used to examine within and between group differences for all subjective parameters examined.

Mean MBG scores (figure 4.4) rose to peak by 2 to 3 hours after dosing and declined to baseline scores by 12 hours after dosing. There was a significant main effect of time and group, but the interaction between group and time was not significant. Post hoc tests revealed no significant within group differences for either patients or control subjects (P>0.005), and no between group differences (P>0.0045). With regard to MG scores (figure 4.4) there was a significant effect of group, but not of time. MG scores for LAAM patients were significantly greater than those of control subjects at 2 hours (P=0.002; 1.45; 0.62, 2.23), 4 hours (P<0.001; 1.61; 0.86, 2.35), 6 hours (P=0.001; 1.77; 0.89, 2.65), 9 hours (P<0.001; 1.82; 0.95, 2.70), 12 hours (P=0.001; 1.54; 0.70, 2.40), and at 36 hours (P=0.003; 1.47; 0.56, 2.39), but at no other time point (P>0.0045)

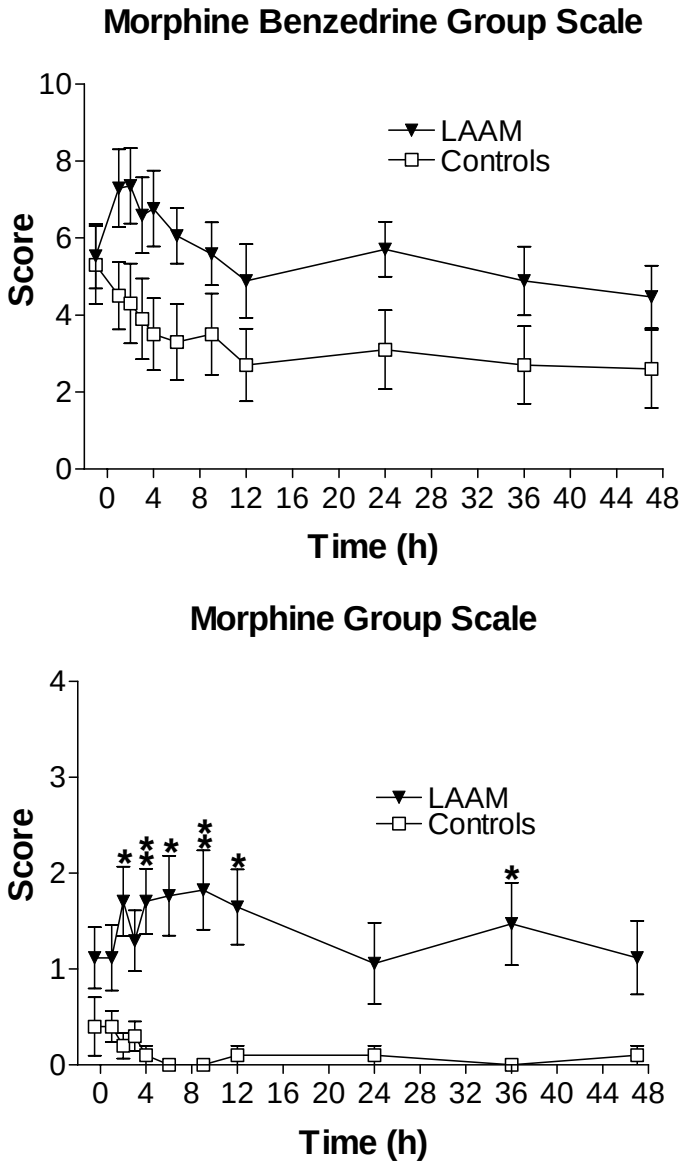

Figure 4-4: Mean (± SEM) Morphine Benzedrine Group Scale scores (upper panel: maximum possible score =16) and Morphine Group Scale scores (lower panel; maximum possible score = 8) for LAAM patients (n=17) who underwent the 48-hour LAAM interdosing interval study and for control subjects (n=10) for the period 0 to 48 hours. LAAM was administered at zero hours. Asterisks denote results of between group comparisons; * P<0.0045, ** P<0.001.

For pain detection (figure 4.5) there were significant main effects of time and of group, but not a significant interaction. There were no significant within group differences for either patients or control subjects (P>0.005), and there were no significant between group differences (P>0.0045). Furthermore, with regard to pain threshold, there were no significant effects of time since dosing or group on pain threshold scores.

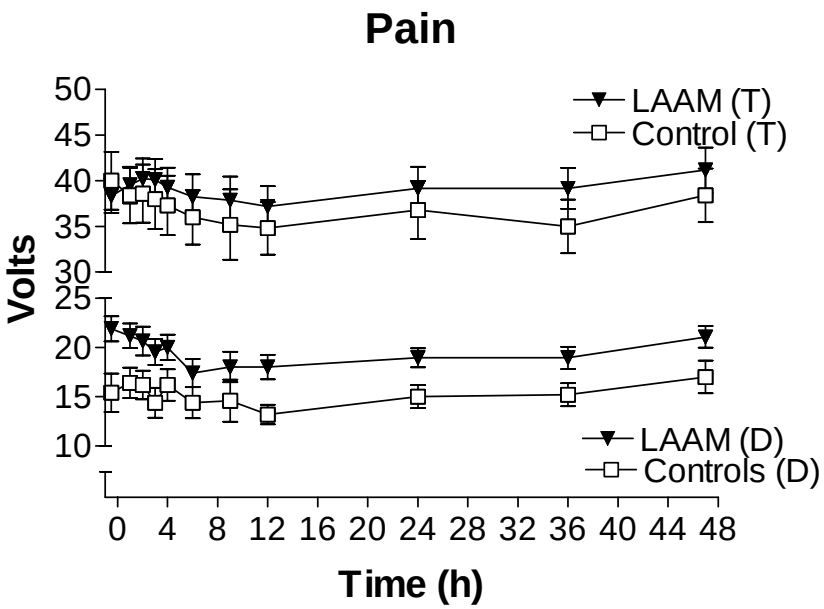

Figure 4-5: Mean (± SEM) pain detection (D) and pain threshold (T) scores (volts) for LAAM patients (closed triangles: n=17) who participated in the 48-hour LAAM inter-dosing interval study and for control subjects (open squares: n = 10) for the period 0 to 48 hour. LAAM was administered at zero hours.

Withdrawal scores for LAAM patients and control subjects are shown in Figure 4.6. There were significant main effects of time since dosing and group on withdrawal scores. There was also a significant interaction between group and time. Mean withdrawal scores for patients were at their highest pre-dose, but rapidly declined to a minimum between 2 to 24 hours after dosing and remained lower than the pre-dose score through to the end of the dosing interval. Mean withdrawal scores were significantly less than the predose score at 1 hour (P=0.001; -2.76; -4.17, -1.36), 2 hour (P<0.001; -3.76; -5.48, -2.05), 3 hours (P<0.001; -4.12; -5.93, -2.30). 4 hours (P<0.001; -3.88; -5.69, -2.07), 6 hours (P<0.001; -3.88; -5.74, -2.02,), 9 hours (P<0.001; -4.06; -5.84, -2.28), 12 hours (P<0.001; -4.3; -6.13, -2.46), 24 hours (P<0.001; -4.18; -5.60, -2.75), 36 hours (P=0.002; -2.52; -3.93, -1.12,), and at 48 hours (P=0.003; -2.59; -4.15, -1.02). In contrast, withdrawal scores for control subjects were stable across the 48 hour testing period (P>0.005).

Withdrawal scores for patients participating in the LAAM interdosing study were significantly greater than those of control subjects at trough (P<0.001; 4.26; 2.14. 6.40), 36 hours (P=0.001; 2.74; 1.21, 4.26), and at 48 hours (P=0.002; 2.58; 1.07, 4.09) post dosing, but at no other time point (P>0.0045).

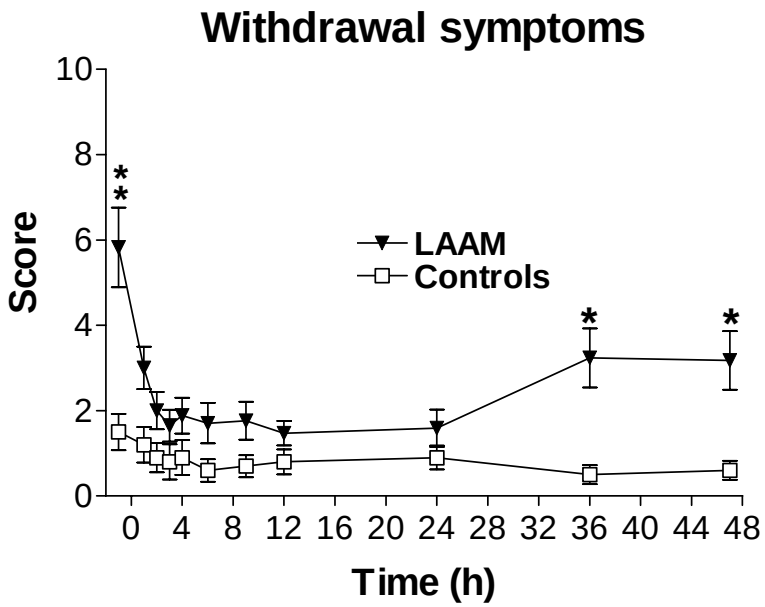

Figure 4-6: Mean (± SEM) withdrawal scores (maximum possible score = 16) for LAAM patients (closed triangles: n=17) who participated in the 48 hour LAAM inter-dosing interval study and for control subjects (open squares: n=10) for the period 0 to 48 hours. LAAM was administered at zero hours. Asterisks signify results of between group comparisons; * P<0.0055, ** P<0.001.

Table 4-2: Repeated measures analyses of variance for physiological and subjective indices of opioid effect for all LAAM patients (n=17) and control subjects (n=10).

Parameter	Effect[1]	Df [2]	*F* value	*P* Value[3]
Heart rate	Group	1,25	0.06	0.82
	Time	6,140	12.5	**<0.001**
	Group x Time	6,140	3.5	**0.004**
Systolic blood pressure	Group	1,25	1.26	0.27
	Time	6,160	2.42	**0.03**
	Group x Time	6,170	1.1	0.39
Diastolic blood pressure	Group	1,25	0.514	0.48
	Time	6,140	0.562	0.75
	Group x Time	6,140	0.296	0.93
Pupil diameter	Group	1,25	9.37	**0.005**
	Time	4,100	9.38	**<0.001**
	Group x Time	4,100	8.95	**<0.001**
Respiratory rate	Group	1,25	42.40	**<0.001**
	Time	6,160	4.74	**<0.001**
	Group x Time	6,160	8.34	**<0.001**
MBG	Group	1,25	4.28	**0.049**
	Time	4,110	4.53	**0.001**
	Group x Time	4,110	1.13	0.35
MG	Group	1,25	10.62	**0.003**
	Time	4,110	0.49	0.75
	Group x Time	4,110	1.43	0.23
Pain detection	Group	1,25	7.6	**0.011**
	Time	6,140	2.8	**0.015**
	Group x Time	6,140	0.63	0.70
Pain threshold	Group	1,25	0.23	0.64
	Time	4,98	1.90	0.12
	Group x Time	4,98	0.45	0.76
Withdrawal	Group	1,25	9.8	**0.004**
	Time	3,75	6.99	**<0.001**
	Group x Time	3,75	3.88	**0.012**

Notes: [1]ANOVA effects= within subjects effect was time since dosing (Time), and between subjects effect was group membership (LAAM patients versus control subjects). [2] Degrees of freedom adjusted using Greenhouse Geisser corrections. Probability value was adjusted accordingly. [3] Significant *P* values in bold text.

4.4.3. Steady state pharmacokinetics of LAAM, nor-LAAM and dinor-LAAM – all patients

Figure 4.7 shows the mean plasma LAAM, nor-LAAM and dinor-LAAM concentration-time profiles normalised to 80 mg LAAM, for the 17 patients that participated in the 48-hour LAAM inter-dosing study.

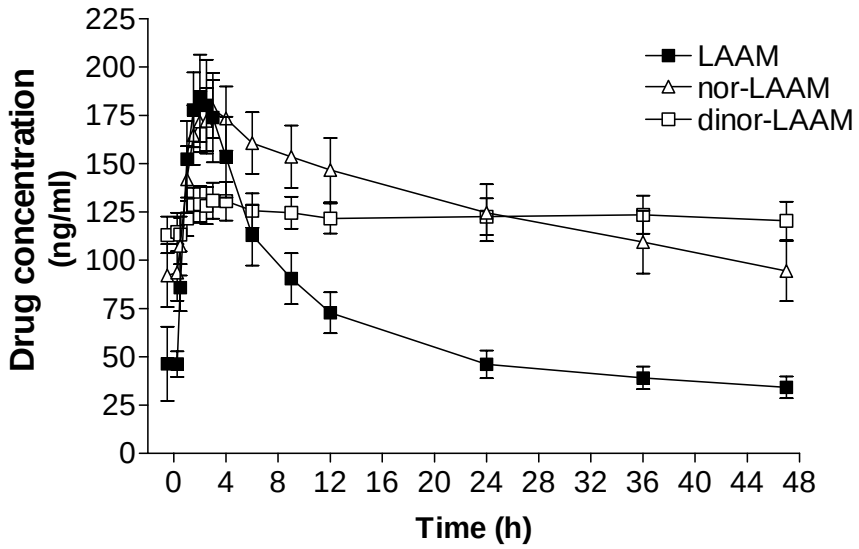

Figure 4-7: Mean (± SEM) plasma LAAM, nor-LAAM and dinor-LAAM concentration-time profiles for 17 LAAM maintenance patients during one 48-h inter-dosing interval at steady state. LAAM was administered at 0 hours. Data are represented as concentration normalised to 80 mg LAAM. 1ng/ml for each analyte equals 0.28, 0.30 and 0.31 nmol/L respectively

Table 4.3 presents mean values for all pharmacokinetic parameters derived from the plasma LAAM, nor-LAAM and dinor-LAAM concentration data. Predose ($C_{min(first)}$) and 48 hours post dose ($C_{min(last)}$) plasma concentrations were not statistically different for LAAM (*P* = 0.44; 10 ng/ml; -17 ng/ml, 38 ng/ml), nor-LAAM (*P* = 0.53;-2ng/ml; -10 ng/ml, 5 ng/ml), and dinor-LAAM (*P*=0.16; -6 ng/ml; -15 ng/ml; 2 ng/ml) indicating that plasma concentrations of all these compounds were at steady state. As evident from figure 4.7 the three analytes displayed markedly different profiles, with dinor-LAAM exhibiting virtually no fluctuation over the dosing interval.

There were significant differences between the mean $C_{min(first)}$ values for nor-LAAM and LAAM (*P* = 0.003; 47 ng/ml; 19 ng/ml, 75 ng/ml), and between the mean $C_{min(first)}$ values for dinor-LAAM and LAAM (*P*<0.001; 68 ng/ml; 43 ng/ml, 93 ng/ml). In contrast, there

was no difference between the mean $C_{min(first)}$ values for dinor-LAAM and nor-LAAM (*P* = 0.108; 21 ng/ml; - 5 ng/ml, 47 ng/ml). Furthermore, there were significant differences between the mean $C_{min(last)}$ values for nor-LAAM and LAAM (*P* < 0.001; 60 ng/ml; 35 ng/ml, 85 ng/ml) and the mean $C_{min(last)}$ values for dinor-LAAM and LAAM (*P* < 0.001, 85 ng/ml; 95 % CI = 69 ng/ml, 100 ng/ ml), but not between the mean $C_{min(last)}$ for dinor-LAAM and nor-LAAM. (*P* = 0.32; 25 ng/ml; 2 ng/ml, 48 ng/ml).

The time taken to reach maximum plasma concentrations (C_{max}) was very similar for LAAM in all 17 patients (mean=2 hours: range 1 to 4 hours: Table 4.3). Nor-LAAM plasma concentration peaked at approximately three hours after dosing. Sixteen subjects achieved maximum nor-LAAM concentrations within 1.5 – 4 hours (mean value = 2.8 hours) while one subject required 6 hours. For dinor-LAAM, the mean time to reach maximum plasma concentration was 15 hours (range 1.5 to 47 hours; Table 4.3). However, eleven subjects did so by nine hours (with a mean value of 3.7 hours), while the remaining six achieved maximum dinor-LAAM plasma concentrations between 24 – 47 hours after dosing. On average LAAM and nor-LAAM attained similar maximum concentrations (*P*=0.63, 8ng/ml; -26ng/ml, 42 ng/ml), while in contrast the mean C_{max} for dinor-LAAM was significantly less than that for LAAM (*P*=0.003;-56ng/ml;-90ng/ml, -23 ng/ml). Furthermore, the mean C_{ss} values for nor-LAAM and dinor-LAAM were a mean of 217% (*P*<0.001) and 222% (*P*<0.001) greater than for LAAM. In contrast, both dinor-LAAM and nor-LAAM attained similar mean steady state plasma concentrations (*P*=0.67) (Table 4.3).

The peak to trough plasma concentration ratio for nor-LAAM (mean±sd: 2.4±1.1) was substantially lower than that for LAAM (6.4±2.5) (*P*<0.001; 3.9; 3.05, 4.79), and the peak to trough plasma concentration ratio for dinor-LAAM (1.2±0.2) was also substantially lower than for LAAM (*P*<0.001; 5.14; 3.91, 6.36). The mean peak to trough plasma concentration ratio for nor-LAAM was also significantly greater than for dinor-LAAM (*P*<0.001; 1.22; 0.71, 1.73). These data indicate a flatter profile concentration versus time profile across the 48-hour inter-dosing interval for both nor-LAAM and dinor-LAAM than LAAM.

The plasma AUC of nor-LAAM was significantly greater than the plasma AUC for LAAM (*P*<0.001; 3063 ng.hr/ml; -4081, -2044 ng.hr/ml), and the plasma AUC for dinor-LAAM

was greater than the AUC for LAAM (P<0.001; -2837; -3385, -2288 ng.hr/ml). In contrast, the plasma AUC values for dinor- and nor-LAAM were not statistically different (P=0.609, 226 ng.hr/ml; -970, 1421 ng.hr/ml). Moreover, there was a significant positive relationship between the weight corrected dose (mg/kg) and plasma AUC of LAAM (r^2 = 0.59, P<0.001), nor-LAAM (r^2 = 0.61, P<0.001), and of dinor-LAAM (r^2 = 0.84, P<0.001).

Inspection of the ranges and coefficients of variation for the pharmacokinetic parameters in Table 4.3 show substantial interindividual variability for LAAM, nor- and dinor-LAAM. This variability was generally greater for LAAM than either nor-metabolite. For example, there was substantial variability in the C_{max} values for LAAM (74 to 484 ng/ ml, %CV = 49: Table 4.4), but less variability in the C_{max} values for nor-LAAM (range: 112 to 351ng/ ml: % CV = 36), and dinor-LAAM (range: 90 to 248 ng/ml: % CV = 27).

There was no significant effect of gender on any pharmacokinetic parameter derived from plasma concentration-time profiles for either LAAM (0.99>P>0.39), nor-LAAM (0.93>P>0.28), or dinor-LAAM (0.71>P>0.20). In addition, Levene's test for equality of variances showed no differences (P>0.05) in variability, between males and females for any pharmacokinetic parameter.

Table 4-3: Pharmacokinetic parameters for LAAM, nor-LAAM and dinor-LAAM following dosing of between 30 – 150 mg LAAM alternate daily in 17 patients who underwent the 48 hour inter-dosing interval study.

Parameter[1]		Mean	Range	%CV [2]
C_{max} (ng/ ml)	LAAM	197	74-485	49
	nor-LAAM	189	112-351	36
	dinor-LAAM	141	90-248	27
AUC (ng.hr/ml)	LAAM	3057	1043-8358	56
	nor-LAAM	6119	3370-13270	50
	dinor-LAAM	5894	3543-11195	30
T_{max}(h)	LAAM	2.00	1.0-4.0	39
	nor-LAAM	3.03	1.30-6.0	35
	dinor-LAAM	15.00	1.50-47.00	112
$C_{min(first)}$ (ng/ml)	LAAM	45	11-313	155
	nor-LAAM	92	26-256	73
	dinor-LAAM	113	48-221	34
$C_{min(last)}$ (ng/ml)	LAAM	35	12-94	62
	nor-LAAM	94	33-254	68
	dinor-LAAM	119	73-244	35
C_{ss} (ng/ ml)	LAAM	65	22-178	56
	nor-LAAM	130	72-282	50
	dinor-LAAM	125	75-238	31
P/T ($C_{max}/C_{min(last)}$	LAAM	6.4	3.6-13.2	39
	nor-LAAM	2.4	1.3-5.9	46
	dinor-LAAM	1.2	1.0-1.7	18
Cl/F (ml/min)	LAAM	557	160-1278	49
$CL/_R$ (ml/min)[3]	LAAM	7.4	2.9-19.0	62
	nor-LAAM	13.4	4.7-27.4	52
	dinor-LAAM	30.1	9.6-53.9	50

Notes: [1]C_{max} = maximum plasma concentration, normalised to 80 mg LAAM; AUC = area under the plasma concentration-time curve, 0-48 hrs, normalised to 80 mg LAAM; T_{max} = Time to reach maximum measured steady-state plasma concentration; $C_{min(first)}$ = plasma concentration 30 minutes, normalised to 80 mg LAAM, prior to dose; $C_{min(last)}$ = plasma concentration, normalised to a 80 mg LAAM, 48 hours after dosing; C_{ss} = Average steady-state plasma concentration, normalised to 80 mg LAAM; P/T= Peak to trough plasma concentration ratio (ie, $C_{max}/ C_{min(last)}$); CL/F =Apparent plasma clearance at steady-state; CL_R = renal clearance. [2] Coefficient of variation. [3] Urine collected from 16 patients-see table 4.4.

The renal clearance (table 4.3) of nor-LAAM was a mean of 197% that of LAAM (P<0.001; -6.00; -8.13, -3.86), and the CL_R of dinor-LAAM was a mean of 442% that of LAAM (P<0.001; -22.71; -29.11, -16.30). Furthermore, the CL_R of dinor-LAAM was a mean of 228 % that of nor-LAAM (P<0.001; -16.71; -21.69, -11.73). Urinary excretion data for LAAM, nor-LAAM and dinor-LAAM are shown in Table 4.4. A 48-hour urine sample was collected for all except one patient. Nor-LAAM and dinor-LAAM consistently accounted for most of the LAAM dose recovered in urine (mean±sd: 7.0 ± 5.8 % and 14.8 ± 6.0 %, respectively). However, the recovery of all three compounds was variable (CV range 41% to 85 %). The total amount of LAAM, nor-LAAM and dinor-LAAM recovered in urine represented (mean±sd) 23.4 ± 11.6 % of the total LAAM dose administered to patients.

Table 4-4: Urinary recovery of LAAM, nor-LAAM and dinor-LAAM following chronic dosing of between 30 to 150 mg LAAM alternate daily in 16 patients enrolled in a maintenance program.

Patient[1]	LAAM Dose (mg/kg)	% Dose recovered			
		LAAM	nor-LAAM	dinor-LAAM	Total Dose
1	1.15	2.3	26.0	25.0	53.3
2	0.66	1.3	5.7	17.0	24.1
3	1.16	4.1	12.6	18.4	35.1
4	1.38	0.4	1.6	6.4	8.5
5	0.51	0.8	2.8	7.2	10.8
6	0.87	1.0	6.2	19.3	26.5
7	1.80	1.3	6.9	20.8	29.0
8	0.68	1.5	5.3	9.3	16.1
9	3.23	2.1	7.9	14.9	25.0
10	0.59	1.9	7.1	20.6	29.6
12	1.10	2.8	8.9	18.5	30.2
13	2.03	0.7	5.5	15.7	21.9
14	0.48	2.5	5.3	16.5	24.3
15	0.74	1.7	3.9	9.1	14.8
16	0.83	0.6	1.6	4.0	6.1
17	0.57	1.1	5.3	13.4	19.8
Mean	1.11	1.6	7.0	14.8	23.4
% *CV*[2]	65	60	82	41	49.1

Notes: [1]Patients are listed in the same order as they are presented in tables 4.1. Patient #11 (see Table 4.1) refused to provide urine. [2] Coefficient of variation.

4.4.4. Pharmacokinetic and pharmacodynamic comparisons for self reported methadone non-holders and holders while on LAAM.

4.4.4.1. Demographic details.

Table 4.5 shows the demographic details of those patients (n=16) that participated in both the methadone and LAAM inter-dosing studies, divided into those who were self reported holders and non-holders (n=8) when taking methadone. The mean age of patients was similar to that reported when they participated in the methadone inter-dosing study (see table 3.6). However, in contrast to that found for the methadone inter-dosing study there was no significant difference in mean body weight between groups. Moreover, there were no significant differences between holders and non-holders with respect to the alternate daily LAAM dose ingested (P=0.39), and dose per body weight (P=0.13). Fischer's exact test revealed no significant differences between groups for the presence of morphine and/or benzodiazepine in plasma (P>0.05)

Table 4-5: Demographic details of patients participating in the LAAM interdosing study divided into those who reported that they were methadone holders (n=8) or methadone non-holders (n=8).

	Holders	Non Holders	*P* value[2] (mean difference; 95% CI)
Dose[1] (mg) [range]	73±34[30-110]	92±52[30-110][	0.39 (0-19; -66.90, 28.2)
Weight (kg) [range]	79.1±18.5 [47-100]	63.3±13.2 [46-88]	0.07 (15.8; -1.62, 33.240
Dose/weight (mg/kg) [range]	0.9±0.34[0.48-1.38]	1.5±0.95[0.59-3.23]	0.13 9-0.60; -1.41, 0.22)
Positive plasma drug screen (LAAM study day) (n)			
Morphine	2	2	>0.05
Benzodiazepines	3	3	>0.05

Notes: Data are mean±sd unless otherwise indicated. [1] refers to alternate daily dosing. [2] Comparison between participants who reported they were holders and non-holders when taking methadone. Fisher exact test used to compare categorical data. Independent Student's *t*-test used for all other comparisons.

4.4.4.2. Pharmacodynamic responses.

Table 4.6 presents the results of the repeated measures ANOVA comparing pharmacodynamic responses between methadone holders and non-holders when on LAAM. There were no between group (holder vs non-holder) differences for any parameter investigated. Consistent with previous analyses that considered the whole group of patients participating in the LAAM inter-dosing study, there were significant temporal changes for holder and non-holders across the 48 hour dosing interval for all responses measured ($P \leq 0.04$), with the exception of MG scores, pain threshold and diastolic blood pressure.

Consistent with the above finding, with respect to self-reported withdrawal it is noteworthy that the $AUC_{0\text{-}48}$ for withdrawal was not significantly different ($P=0.97$; -80.82, 84.06) between holders (mean±sd: 118±67 score.hours) and non-holders (116±85 score.hours).

Table 4-6: LAAM inter-dosing study: Repeated measures analyses of variance for all pharmacodynamic responses, according to holding status [holders (n=8) and non-holders (n=8)].

Parameter	Effect[1]	Df [2]	*F* value	*P* Value[3]
Withdrawal	Hold	1,14	0.18	0.68
	Time	3,39	9.77	**<0.001**
	Hold x Time	3,39	0.48	0.68
MBG	Hold	1,14	0.03	0.87
	Time	4,52	3.41	**0.02**
	Hold x Time	4,52	0.65	0.62
MG	Hold	1,14	1.09	0.32
	Time	4,54	1.21	0.32
	Hold x Time	4,54	0.67	0.66
Pain Detection	Hold	1,14	0.12	0.73
	Time	4,57	3.26	**0.02**
	Hold x Time	4,57	0.50	0.74
Pain Threshold	Hold	1,14	2.98	0.11
	Time	3,49	0.52	0.70
	Hold x Time	3,49	1.76	0.16
Diastolic blood pressure	Hold	1,14	0.40	0.54
	Time	5,74	0.19	0.97
	Hold x Time	5,74	0.55	0.75
Systolic blood pressure	Hold	1,14	1.75	0.21
	Time	6,79	2.44	**0.04**
	Hold x Time	6,79	0.51	0.79
Heart rate	Hold	1,14	0.06	0.82
	Time	5,64	12.5	**<0.001**
	Hold x Time	5,64	0.65	0.65
Respiratory rate	Hold	1,14	1.77	0.21
	Time	5,76	14.21	**<0.001**
	Hold x Time	5,76	1.12	0.36
Pupil diameter	Hold	1,14	0.46	0.83
	Time	3,42	18.36	**<0.001**
	Hold x Time	3,42	0.55	0.66

Notes: [1]ANOVA effects=within subjects effect as time since dosing (Time), and between subjects effect as methadone holding status (Hold). [2] Degrees of freedom adjusted using Greenhouse Geisser corrections. Probability value was adjusted accordingly. [3] Significant *P* values in bold text.

4.4.4.3. Pharmacokinetic parameters.

The mean plasma LAAM, nor-LAAM and dinor-LAAM concentration profiles normalised to 80 mg LAAM, for the holder and nonholder groups, are shown in figures 4.8, 4.9 and 4.10, respectively. Comparison between holder and non-holder groups for each compound were made on a number of pharmacokinetic parameters that were derived from their respective plasma concentration data; these are presented in table 4.7. As seen in figure 4.8 the mean plasma LAAM concentration-time profiles for holders and non-holders were almost identical. There were no significant differences between holders and non-holders for any pharmacokinetic parameter derived from plasma LAAM concentration-time data (Table 4.7). Furthermore, although non-holders exhibited a greater degree of individual variability (coefficient of variation) associated with certain parameters (i.e., C_{max}, AUC, $C_{min(first)}$, $C_{min(last)}$ and C_{ss}), compared to holders, Levene's test for equality of variances showed no significant differences between groups (P>0.05) for any parameter.

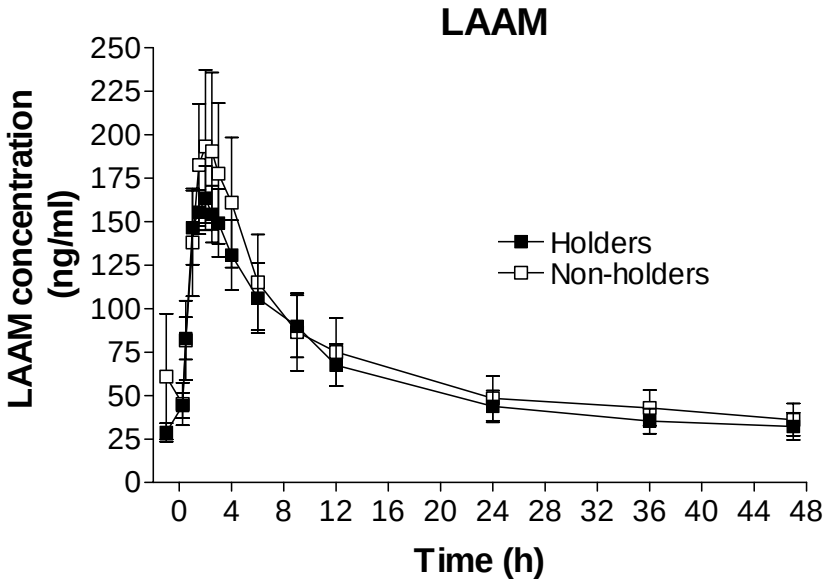

Figure 4-8: Mean (±SEM) plasma LAAM concentrations-time profile for holders (n=8) and non-holders (n=8) during one 48-hour inter-dosing interval. Oral administration of LAAM was a time zero. Data are presented as concentration normalised to 80 mg LAAM.

The mean plasma nor-LAAM concentration-time profiles for holders and non-holders was also very similar (see figure 4.9). There were no significant differences between groups for any pharmacokinetic parameter derived from plasma nor-LAAM concentration-time

data. Furthermore, there were no significant differences between groups in the degree of individual variability present for each pharmacokinetic parameter (Levene's test, $P>0.05$).

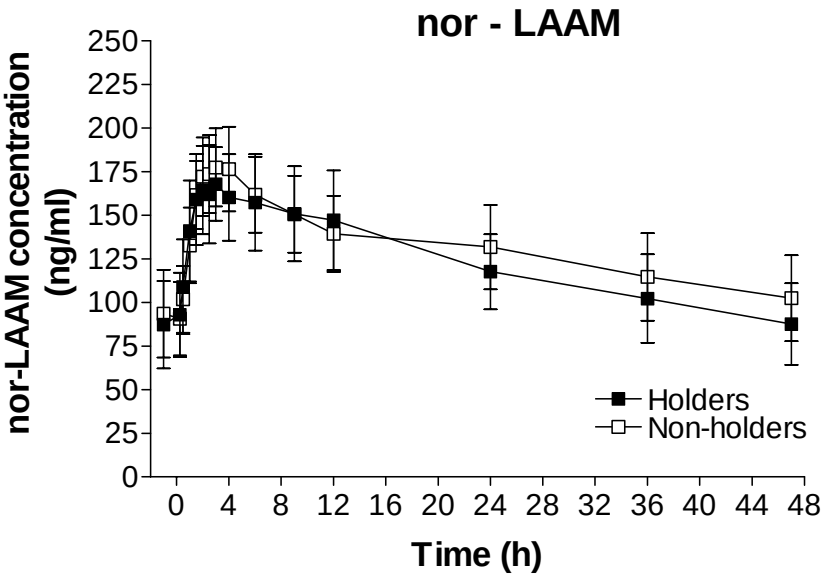

Figure 4-9: Mean (±SEM) plasma nor-LAAM concentrations-time profile for holders (n=8) and non-holders (n=8) during one 48 hour inter-dosing interval. Oral administration of LAAM was a time zero. Data are presented as concentration normalised to 80 mg LAAM dose.

With regard to dinor-LAAM, it appears that non-holders exhibited greater dinor-LAAM concentrations throughout the dosing interval (figure 4.10). However, as shown in Table 4.7 there were no statistically significant differences between groups for any pharmacokinetic parameter derived from plasma dinor-LAAM concentration-time data. Furthermore, with the exception of T≥75% C_{max}, there were no significant between group differences in the degree of individual variability observed for each pharmacokinetic parameter (Levene's test, $P>0.05$). Holders exhibited greater variability than non-holders for T≥75% C_{max} (mean±sd: Holder=36.9±14.1 h vs non-holders 46.6±0.90 h; $P=0.031$). It is also noteworthy that the difference between groups for mean peak to trough ratios for dinor-LAAM neared significance ($P=0.05$) suggesting that non-holders had flatter concentration-time profiles than holders.

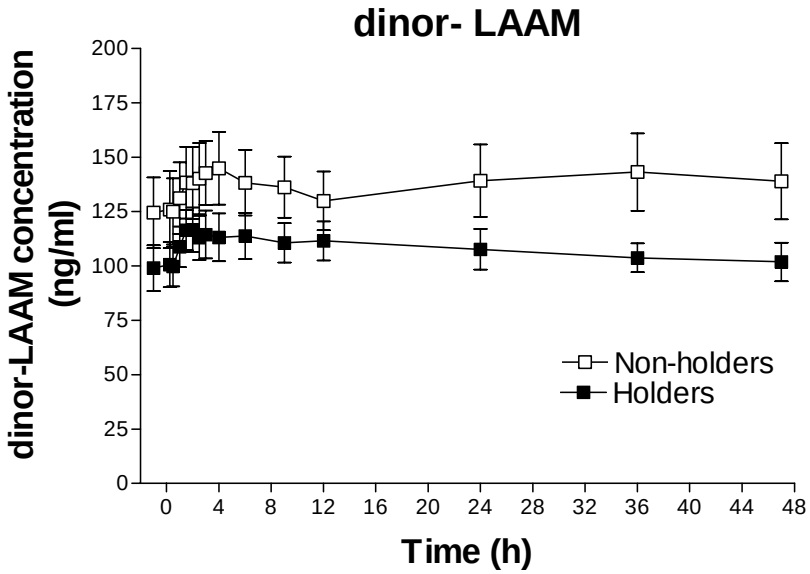

Figure 4-10: Mean (±SEM) plasma dinor-LAAM concentrations-time profile for holders (n=8) and non-holders (n=8) during one 48 hour inter-dosing interval. Oral administration of LAAM was a time zero. Data are presented as concentration normalised to 80 mg LAAM dose.

Table 4-7: Disposition of LAAM, nor- and dinor-LAAM for 16 patients following dosing of between 30 – 150 mg LAAM in patients who were studied during the 48-hour LAAM inter-dosing interval study, according to holding status [Holders (n=8) and non-holders (n=8)].

Parameter[1]		Holder	Non-holder	P value[2] (mean difference; 95% CI)
Cmax (ng/ml)	LAAM	172 (27)	205 (61)	0.50 (-33; -135, 69)
	Nor-LAAM	185 (41)	184 (35)	0.98 (0.5; -75, 76)
	Dinor-LAAM	129 (21)	150 (32)	0.30 (-22; -63, 20)
AUC (ng.hr/ml)	LAAM	2800 (51)	3189 (70)	0.69 (-389; -2409, 1631)
	Nor-LAAM	5864 (56)	6249 (51)	0.82 (-385; -3853, 3084)
	Dinor-LAAM	5156 (21)	6628 (33)	0.11 (-1473; -3329, 384)
Tmax (h)	LAAM	1.6 (32)	2.3 (38)	0.10 (-0.63; -1.38, 0.13)
	Nor-LAAM	3.2 (41)	2.9 (31)	0.58 (0.31; -0.88, 1.51)
	Dinor-LAAM	8.3 (138)	23.2 (83)	0.81 (-14.88; -32, 2.1)
Cmin(first) (ng/ml)	LAAM	30 (38)	61 (168)	0.42 (-31; -108, 48)
	Nor-LAAM	87 (81)	93 (76)	0.87 (-6; -82, 70)
	Dinor-LAAM	99 (30)	124 (37)	0.21 (-25; -67, 16)
Cmin(last) (ng/ml)	LAAM	33 (55)	36 (73)	0.81 (-3; -27, 22)
	Nor-LAAM	88 (75)	102 (68)	0.67 (-15; -88, 58)
	Dinor-LAAM	102 (25)	139 (36)	0.80 (-37; -79, 5)
Css (ng/ml)	LAAM	60 (51)	68 (70)	0.69 (-8; -51, 35)
	Nor-LAAM	125 (56)	133 (51)	0.82 (-8; -82, 66)
	Dinor-LAAM	109 (23)	141 (33)	0.10 (-32; -72, 8)
P/T(Cmax/Cmin (last))	LAAM	6.2 (51)	6.0 (24)	0.92 (0.13; -2.48, 2.74)
	Nor-LAAM	2.7 (55)	2.1 (30)	0.29 (0.62; -0.60, 1.83)
	Dinor-LAAM	1.3 (15)	1.1 (14)	0.05 (0.18; -0.0003, 0.37)
T≥75 % Cmax (h)	LAAM	3.6 (57)	3.4 (33)	0.77 (0.25; -1.55, 2.05)
	Nor-LAAM	15.3 (64)	21.6 (74)	0.34 (-6.31; -20.52, 7.9)
	Dinor-LAAM	36.9 (38)	46.6 (1.9)	0.10 (-9.63; -21.41, 2.157)
CL/F (ml/ min)	LAAM	573 (42)	579 (57)	0.99 (2.6; -305, 310)

Notes: Data expressed as mean (%CV). [1]Cmax = maximum plasma concentration, normalised to 80 mg LAAM; AUC = area under the plasma concentration-time curve 0-48hrs, normalized to 80 mg LAAM; Tmax = time to reach maximum measured steady-state plasma concentration; $C_{min(first)}$ = plasma concentration, normalised to 80 mg LAAM, 30 minutes prior to dose; $C_{min(last)}$ = plasma concentration, normalised to 80 mg LAAM, 48 hours after dosing; C_{ss} = average steady-state plasma concentration, normalised to 80 mg LAAM; P/T= peak to trough plasma concentration ratio (ie, $C_{max}/C_{min(last)}$); T≥75 % C_{max} = The time plasma concentrations were equal to or greater than 75 % C_{max}; CL/ F = apparent plasma clearance at steady-state. [2] Independent t-test used to compare holder and non-holder groups.

4.5. Discussion.

The primary aim of the current study was to describe the steady state pharmacodynamics and pharmacokinetics of LAAM, nor-LAAM and dinor-LAAM in a cohort of maintenance patients. Physiological and subjective indices of opioid effect were collected concurrently with frequent blood samples across one 48-hour inter-dosing interval in 17 maintenance patients. There were significant temporal changes for all physiological parameters (except diastolic pressure), but temporal changes in pupil diameter, and respiratory rate were the most evident. Maximal effects for respiratory rate, miosis, and withdrawal suppression occurred at the approximate time of peak plasma LAAM and nor-LAAM concentrations. Significant effects, relative to baseline, persisted through to 12 hours after dosing for miosis, and through to 48 hours after dosing for respiratory depression and withdrawal suppression. The latter data are indicative of the long duration of action of orally administered LAAM. In contrast, there were no significant changes for any subjective measure across the inter-dosing interval. Between group difference (LAAM patients vs control subjects) were evident for pupil diameter, respiratory rate and MG scores, and were most pronounced at the time of putative peak plasma LAAM and nor-LAAM concentration (2 to 6 hours post dosing). The three analytes displayed different profiles, with dinor-LAAM exhibiting relatively little fluctuation across the inter-dosing interval. There was substantial interindividual variability for all pharmacokinetic variables for the three compounds, which did not appear to adversely affect withdrawal suppression.

4.5.1. Pharmacodynamic responses.

Consistent with LAAM's classification as a μ opioid receptor agonist, the administration of LAAM produced marked temporal changes in respiratory rate and pupil diameter that appeared to be related to the underlying plasma concentrations of LAAM and nor-LAAM. Lowest values for respiratory rate (6 hours post dosing) and pupil diameter (4 to 6 hours post dosing) occurred within the range of reported T_{max} values for LAAM and nor-LAAM (ie., 2 to 6 hours after dosing); values then returned to baseline values by the end of the dosing interval as plasma LAAM and nor-LAAM concentrations were declining. It was difficult to determine the contribution of dinor-LAAM to these effects as it had a flat plasma-concentration–time profile.

Pupil diameter, as previously reported, is one of the more reliable and sensitive markers of opioid effect (Fraser, *et al*., 1954; Tress and El Sobky, 1980), and is therefore a useful indicator of the expected time-course of opioid effects following the administration of LAAM. In the current study pupil diameter decreased rapidly from maximal levels pre-dose to minimal levels four hours after dosing; significant miosis was maintained until 12 hours after dosing. The temporal pattern of results observed in this study is similar to that previously reported following the administration of single doses of oral LAAM to non-drug-using subjects (Fraser and Isbell, 1952), and to nondependent current opioid users (Walsh, *et al*., 1998). In contrast to the current findings, Kaiko and Inturrisi (1975) reported that maximum pupil constriction occurred at eight hours following the administration of oral LAAM in eight maintenance patients at steady state. However, as Kaiko and Inturrisi only examined four time points after dosing (4, 8, 24 and 48 h), they would have been less accurate in their estimation of peak effect than in this study.

Moreover, the magnitude of pupil change observed in this study is somewhat less than that reported by previous authors (Fraser and Isbell, 1952; Kaiko and Inturrisi, 1975). In the present study maximum observed pupil constriction was approximately 1.0 mm. In contrast in the Kaiko and Inturrisi study mean maximum pupil constriction was 1.8 mm. The significance of this difference is not clear, given that so few studies have measured pupil diameter in LAAM maintenance patients at steady state, and that the method of measuring pupil dimeter probably differed. Furthermore, Fraser and Isbell measured pupil diameter in two patients following a single 30 mg dose of LAAM. They reported that maximum pupil constriction occurred at four hours and was 40 % of baseline values (the authors did not report absolute values). The greater magnitude of pupil change seen in the latter study was likely because subjects were not at steady state and tolerance had not developed to the opioid effects of LAAM.

While the respiratory depression following methadone ingestion is well documented (for eg., Dyer, *et al*., 1999; Gritz, *et al*., 1975; Martin, *et al*., 1973), there is a paucity of data concerning the effect of LAAM on respiratory function in humans at steady state, and, in particular, concerning the temporal change in respiratory rate across the interdosing interval. In the current study respiratory depression commenced one hour following dosing, was maximal (reduction of approximately 4 breaths/minute) at 6 hours following

dosing, and persisted through to 36 hours following dosing. Furthermore, patients' respiratory rates were significantly lower than those of control subjects at every time point.

Given that LAAM is a mu opioid receptor agonist, some degree of respiratory depression amongst maintenance patients was expected. However, because of the paucity of data concerning the effect of chronically administered LAAM on respiratory function in humans it is not possible to directly compare these data to those previously collected under similar experimental conditions. Nevertheless, there are data on the effect of single doses of LAAM in opioid naïve and non-dependent opioid users. Walsh and colleagues (1998) reported no significant effect on respiratory rate, or oxygen saturation, of single doses of both 20 and 40 mg LAAM administered intravenously and orally to non-opioid dependent subjects. In contrast, Eissenberg and colleagues (1999) reported that a number of subjects, but not all, who received single oral doses of 60 mg/70 kg LAAM, experienced clinically significant respiratory depression (i.e. <6 breaths per minute and percent blood oxygen saturation <90%), necessitating their termination from the study. Such respiratory depression did not occur after receiving 15 mg/70 kg and 30 mg/70 kg. These data would suggest that in the context of acute dosing there is a dose-dependent effect of LAAM on respiratory function. Needless to say the dramatic perturbations in respiratory function, as seen in the latter mentioned study, would not be expected in the context of maintenance treatment because of the development of at least partial tolerance to these effects of LAAM. However, the findings from the current study suggest that even at steady state, patients receiving a range of LAAM doses (30 – 150 mg alternate daily) are likely to experience some respiratory depression, which may persist across the inter-dosing interval. Whether this respiratory depression is accompanied by associated reductions in oxygen saturation, or other physiological perturbations is not known at this stage. Thus, LAAM maintenance patients need to be made aware of the potential dangers of the concomitant use of other central nervous depressant drugs, such as alcohol and benzodiazepines, which may act synergistically to further depress respiratory function.

With regard to cardiovascular function, as far as I am aware there have been no previous reports on the temporal change in heart rate and blood pressure for LAAM patients at steady state. Previously, Hargreaves and colleagues (1983) measured a range of cardiovascular responses (ECG, blood pressure and heart rate), at week 0, 4 and 20 weeks after the initiation of LAAM maintenance treatment, under a number of different

conditions (supine, 1, 2, 3 minutes following standing, prior to exercise and at the same time points thereafter). They did not report any adverse effects (eg. significant bradycardia or hypotension) of LAAM treatment on cardiac function. In the current study ANOVA showed significant time since dosing effects for both heart rate and systolic pressure, but not diastolic pressure, across the inter-dosing interval. However, post hoc tests revealed that only heart rate, observed early in the interdosing interval, was significantly reduced, in relation to baseline, which corresponded to approximate T_{max} value for LAAM and nor-LAAM (i.e., 2 to 6 hours). However, given the absence of significant differences between patients and control subjects, for any cardiovascular parameter investigated in the current study, it is probable that these changes are of little clinical significance.

As hypothesised, the administration of LAAM was associated with a significant reduction in the number of self-reported withdrawal symptoms relative to baseline. Mean withdrawal scores showed a rapid decline from baseline reaching minimal levels by 2 hours after dosing, and remaining this low (mean scores 1.5 – 2.0) until 24 hours following dosing, only increasing slightly at the end of the dosing period. The mean withdrawal score at predose was relatively higher than that reported at 48 hours post dosing. While it is not evident what caused this discrepancy, it could well reflect variation in the time patients were required to wait for their dose at the beginning of the testing session, such that some patients began to experience an increase in severity of withdrawal; or equally it could reflect variation in anxiety experienced by patients at the start of the testing session. Nevetheless, it is noteworthy that this phenomenon was not evident in those patients participating in the methadone inter-dosing interval study. The prolonged suppression of withdrawal following chronically administered LAAM is consistent with that seen in previous studies that have investigated opioid dependent patients (Fraser and Isbell, 1952; Levine, *et al*., 1973). Moreover, inter-individual variability in withdrawal scores was relatively low, particularly during the period from one to 24 hours post dosing, hence demonstrating effective suppression of withdrawal for most patients.

The profile of mean MBG (euphoria) and MG scores for patients across the inter-dosing interval are indicative of the prolonged and relatively stable profile of effect conferred by LAAM, particularly at steady state. The majority of patients demonstrated remarkably stable individual MBG profiles across the LAAM inter-dosing interval. Furthermore, consistent with LAAM's classification as μ opioid agonist with morphine like actions, MG

scores were significantly greater for patients on LAAM than control subjects from 2 to 12 hours after dosing and then at 36 hours after dosing. The prolonged and relatively stable profiles for both measures are possibly reflective of the stable plasma-concentration profiles of both nor- and dinor-LAAM.

Another possible explanation for the profle of MBG scores seen in this study is that the MBG scale may not be sensitive enough to detect changes in opioid–induced euphoria. Walsh and colleagues (1998) found no significant effect of LAAM on MBG scores in subjects administered single doses of LAAM (20 and 40 mg *i.v* and oral), but found that a number of other subjective ratings of positive opioid effect (eg., if subjects felt any drug effect, 'liking', 'high', and any 'good effects') were significantly elevated following dosing. This might suggest that the latter measures are more sensitive in tapping into such subjective states than the MBG scale. However, this should not discount anecdotal reports from maintenance patients that they feel less of a 'high' on LAAM than on methadone and reports that LAAM has a more consistent effect across the inter-dosing interval (Tennant, *et al*., 1986; Trueblood, *et al*., 1978).

Moreover, there were no significant within or between group differences for pain detection or pain threshold in the current study. These reports are hard to reconcile given the findings of an early study that showed LAAM administered subcutaneously (5 to 20 mg *s.c*) to patients post surgery was modestly effective in relieving pain (Keats and Beecher, 1952). However, unlike patients in the latter study, patients in this study were at steady state, having received LAAM for at least 4 weeks, and would have developed at least partial tolerance to the analgesic effects of LAAM. Furthermore, given the relatively stable pattern of subjective opioid effect reported with LAAM, as reflected in the profiles for MBG and MG scores, patients were less likely to experience the classic opioid high, and to be less sedated than when they were on methadone. Hence, LAAM patients were possibly more alert and consciously 'aware' of somatic symptoms, such as pain, than when they were taking methadone.

Consistent with that reported in the previous chapter, morphine (a quantitative marker of recent heroin/and or morphine use) and a range of benzodiazepines were detected in the plasma of patients participating in the study. I would suggest, as I did in the previous chapter, that the presence of these substances in the plasma of patients participating in this

study would not have a significant effect on the pharmacodynamic measures used in this study.

4.5.2. Steady State Pharmacokinetics of LAAM, nor-LAAM and dinor-LAAM

As far as I am aware this is the first study to comprehensively describe the disposition of LAAM, nor-LAAM, and dinor-LAAM in such a large cohort (n=17) of maintenance patients. Patients in this study were administered a range of LAAM doses (30 to 150 mg alternate daily), which reflects the policy of flexible dosing utilised by the South Australian Maintenance Pharmacotherapy Unit. Plasma concentrations of LAAM, nor- and dinor-LAAM were measured across the 48-hour inter-dosing interval, which allowed for the estimation of a number of important pharmacokinetic parameters for each compound. The maximum and minimum plasma concentrations, the time to maximum plasma concentration, and peak to trough plasma concentration ratios for LAAM, nor- and dinor-LAAM are consistent with data presented by Kaiko and Inturrisi (1975) and McCance-Katz and colleagues (2006).

As with methadone, the degree of inter-individual variability in the disposition of active drug is likely to be an important factor in determining therapeutic response. Therefore, it is noteworthy that despite the verification of steady state conditions and the normalisation of plasma concentrations for each compound, there was considerable inter-individual variability for most pharmacokinetic parameters (range of coefficients of variation 18 to 155 %). For example, there was 3- to 7- fold variation in C_{max} values and 3- to 8- fold variation in AUC values for LAAM, nor- and dinor-LAAM across all participants who participated in this study. This variability is consistent with that reported by previous authors who have examined the pharmacokinetics of chronically administered LAAM (Finkle, *et al.*, 1982; Kaiko and Inturrisi, 1975; McCance-Katz, *et al*., 2006). For example, Finkle and colleagues (1982) reported a greater than nine-fold variation in C_{max} for LAAM (52 to 510 ng/ml), approximately three-fold variation for nor-LAAM (65 to 175 ng/ml) and approximately eight- fold variation for dinor-LAAM (11 to 92 ng/ml). Importantly, the inter-subject variation in plasma concentrations did not adversely affect therapeutic efficacy as all patients reported adequate suppression of withdrawal on the range of LAAM doses administered.

Individual variability in pharmacokinetic parameters, such as CL/F for LAAM and C_{ss} for LAAM, nor-and dinor-LAAM, would be partly due to individual differences in metabolic activity, in particular individual differences in the activity of cytochrome P450 enzymes (Ketter, *et al.*, 1995). Studies have shown the primary role of hepatic and intestinal CYP3A4 in the *N*-demethylation of LAAM and nor-LAAM (Moody, *et al*., 1997; Oda and Kharasch, 2001a, 2001b), which has been shown *in vivo* to have significant inter-individual variability in activity (Wilkinson, 2004). Moody and colleagues (1997; Neff and Moody, 2001) also found preliminary, but inconclusive evidence for the involvement of other P450 enzymes, including CYP2D6 and 2E1, in the metabolism of LAAM. As reviewed in chapter one, a number of therapeutically important drugs have been shown to alter CYP3A4 activity. Although such pharmacokinetic interactions have been described for methadone, the clinical effect of these drugs in patients taking LAAM has not been elucidated. This is an important future research concern, as variability in LAAM disposition will significantly affect the clinical effectiveness of LAAM.

It is important to note, as I did in the previous chapter, that patients participating in this study did not report taking any drug strongly associated with influencing CYP activity. Although one patient was taking sodium valproate (Epilim®), which is a known inhibitor of drug metabolism (Bochner, 2000), there have been reports that valproate has no clinically significant effect on the pharmacokinetics of methadone (Saxon, *et al*., 1989), and so therefore this patient was not precluded from participating in the current study.

The significant positive relationships between weight corrected dose and area under the curve for LAAM, nor-LAAM, and dinor-LAAM suggest that the pharmacokinetics (eg., bioavailability, clearance) of LAAM and its nor-metabolites are linear across the dose range 30 to 150 mg. In contrast, Walsh and colleagues (1998) suggested that the kinetics for LAAM itself maybe non-linear. They found that plasma LAAM AUC increased in a greater than dose proportional fashion following single doses of 20 and 40 mg LAAM (*i.v* and oral), but cautioned that a more formal dose-response evaluation was required to determine the pharmacokinetic profile of LAAM. The data from the current study show that despite the degree of inter-individual variability in metabolic activity, approximately 46%, 62% and 76% of the variability in the AUC of LAAM nor-and dinor-LAAM, respectively, can be explained by variation in LAAM dose. Part of the remaining

variability is likely due to differences in clearance, and in particular, for LAAM and nor-LAAM, clearance to nor-LAAM, and dinor-LAAM, respectively.

Despite the inter-patient variability in plasma concentrations, the plasma-concentration time profiles of LAAM and the two nor-metabolites were reasonably consistent across patients. Plasma concentrations of nor- and dinor-LAAM were significantly greater than plasma concentrations of LAAM prior to dosing ($C_{min(first)}$). On average, LAAM and nor-LAAM attained similar maximum plasma concentrations, which were greater than the C_{max} for dinor-LAAM. In all cases plasma LAAM concentration fluctuated markedly across the dosing interval, rising rapidly after administration and peaking approximately 2 hours later. Plasma LAAM concentrations then declined rapidly, so that by 24 hours they were similar to pre-dose concentrations (below 50 ng/ml) and then remained relatively stable at those concentrations through to the next dose. The degree of fluctuation in plasma LAAM concentrations across the inter-dosing interval was reflected in the relatively large mean peak to trough plasma concentration ratio (mean 6.4: range 3.6 to 13.2). In contrast, both nor- and dinor-LAAM had more stable plasma concentration-time profiles than the parent drug, persisting in plasma across the 48-dosing interval at relatively stable concentrations, as evidenced by their lower peak to trough plasma concentrations. The stable plasma concentration-time profiles for both nor- and dinor-LAAM would contribute significantly to the long duration of action of LAAM.

The sequential nature of the metabolic pathway for LAAM is reflected in the time ordered T_{max} and the predominance of both nor-metabolites, in plasma, over LAAM. Consistent with previous reports, nor-LAAM plasma concentrations peaked approximately one hour after LAAM (Finkle, *et al*., 1982; Henderson, *et al*, 1977b). The mean time to peak concentration for dinor-LAAM was significantly longer than for both LAAM and nor-LAAM; eleven patients achieved maximum dinor-LAAM concentrations by nine hours after dosing (mean value 3.7 hr), while the remaining patients required 24 – 48 hours to reach maximum dinor-LAAM concentration. Moreover, the predominance of both nor-metabolites in plasma over LAAM is reflected in the significantly greater C_{SS} and plasma AUC for both nor- and dinor-LAAM than LAAM.

In the current study the renal clearance (CL_R) of LAAM accounted for approximately 0.5 to 4 % of CL/*F* (apparent plasma clearance) for LAAM, which would be consistent with

LAAM been predominantly cleared by the liver, primarily by conversion to nor-LAAM. The mean CL_R values for nor- and dinor-LAAM obtained in this study are consistent with those reported by Kaiko and Inturrisi, who also reported renal clearances for these compounds (Kaiko and Inturrisi, 1975). However, in contrast, the mean CL_R value reported by the latter study for LAAM (13.8 ml/min) is approximately twice that obtained in the current study (7.41 ml/min). Kaiko and Inturrisi also reported that the renal clearance of LAAM and demethylated metabolites in the four patients they studied was pH dependent, with tubular reabsorption at high urinary pH. One subject in the latter study, whose urinary pH was 5.0, exhibited substantially greater clearance values for all three compounds, but particularly for LAAM, which would explain the disparity between studies. As the range of pHs of urine samples collected in the current study was less (6.45 to 6.76), this relationship could not be examined.

There are relatively few available data on the disposition of LAAM and demethylated metabolites in humans, and in particular in maintenance patients at steady state. In the current study urine was collected from sixteen patients over the entire 48 hour dosing interval. The urinary excretion of LAAM, nor- and dinor-LAAM resulted in a total mean recovery of only 23.4 % of the total LAAM dose administered. Consistent with the extensive biotransformation of LAAM into active metabolites and the predominance of these metabolites over LAAM in plasma, a greater proportion of the original dose was recovered as nor-LAAM (mean 7.0 % of the dose) and dinor-LAAM (mean 14.8 % of the total dose) than LAAM (mean =1.6 % of the dose). These data are in accordance with the few data that are available (Finkle, *et al*., 1982; Henderson, *et al*., 1977b; Kaiko, *et al*., 1975). LAAM can also undergo deacetylation to methadol and then subsequent demethylation to nor- and then to dinor-methadol. However, although these compounds are pharmacologically active (Eddy, *et al*., 1952; Horng, *et al*., 1976; Leimbach and Eddy, 1954), there are equivocal reports regarding whether they reach measurable concentrations in human plasma (Cook, *et al*., 1984; Kaiko, *et al*, 1975) and therefore whether they would contribute to the overall activity of LAAM. Moreover, although the relatively small proportion of the LAAM dose recovered as LAAM, nor- and dinor-LAAM in urine is suggestive of significant non-renal elimination of LAAM, the data on the faecal elimination of LAAM and metabolites are sparse. The only available report is that following *i.v* administration of [^{3}H] LAAM to four MMT patients; only 17.8 % of the total LAAM dose was recovered in faeces over 168 hours (Cook, *et al*., 1984). The combined

excretion of radioactivity in urine and faeces ranged from 28.3 to 54 %. Thus, to date mass balance has not been achieved.

4.5.3. Relationship between plasma concentrations of LAAM and nor-metabolites and physiological indices of opioid effect and withdrawal suppression.

Consistent with previous studies, the time course of LAAM's effect, exemplified by miosis, respiratory depression, and withdrawal suppression, appeared to correspond more closely to the concentration-time profile for nor-LAAM, than for LAAM (Cook, *et al*., 1984; Kaiko and Inturrisi, 1975; Walsh, *et al*., 1998). That is, although peak effects for miosis and respiratory depression occurred within the range of T_{max} values for LAAM and nor-LAAM (i.e., 1 to 6 hours after dosing), values for these physiological parameters remained significantly depressed, relative to baseline, when plasma LAAM concentration had declined to minimum, but plasma nor-LAAM concentrations remained relatively high. Moreover, the number of reported withdrawal symptoms was significantly less than the baseline value through to 48 hours after dosing. At this time plasma LAAM concentration had declined to a minimum, but plasma nor-LAAM concentration was significantly greater than plasma LAAM concentration. It is important to note that it was not possible to determine the apparent contribution of dinor-LAAM to these opioid agonist effects due to its very flat plasma concentration-time profile. However, these data suggest that it is the combined activity of nor-LAAM and dinor-LAAM that is responsible for the long duration of some of pharmacodynamic measures observed in this study.

A number of preclinical and clinical studies have shown that nor-LAAM is more potent than both dinor-LAAM and LAAM (Bertalmio, *et al*., 1992; Nickander, *et al*., 1974; Perez-Reyes, 1985; Smits 1974). However, these assays have also shown dinor-LAAM to be active and to possess greater potency than LAAM (Bertalmio, *et al*, 1992; Horng, *et al*., 1976; Nickander, *et al*., 1974; Perez-Reyes, 1985; Smits, 1974), and thus its contribution to LAAM's overall opioid effect should not be ignored. Furthermore, given the relatively recent findings by Walsh and colleagues (1998) that LAAM possesses significant pharmacological activity when administered *i.v*, it is apparent that all three drugs are likely to contribute to LAAM's opioid effect. It is therefore probable, given the concentration-time profiles for all three drugs, that they all contribute additively to LAAM's peak effect,

but that the long duration of action of LAAM can be attributed to the relatively stable concentration-time profiles of nor- and dinor-LAAM compared to LAAM.

4.5.4. Holder versus non-holder comparisons

This is the first study to directly compare the pharmacokinetics and pharmacodynamics of LAAM in methadone holders and non-holders while they were maintained on LAAM. This was necessary to determine if groups could be differentiated on any pharmacokinetic and/or pharmacodynamic variable that might account for the way in which individuals, in either group, responded to LAAM. Methadone non-holders could not be differentiated on any demographic variable or drug use (presence of morphine or benzodiazepines in plasma), nor on any measure of opioid effect, or self-reported withdrawal, while taking LAAM. The latter result is particularly noteworthy and indicates that for the entire cohort of patients who participated in the current study, LAAM successfully suppressed withdrawal for the duration of the inter-dosing interval. The latter result will be further explored in the next chapter.

With regard to pharmacokinetic parameters, both holder and non-holder groups exhibited very similar mean plasma concentration-time profiles for LAAM, nor- and dinor-LAAM, and did not differ significantly on any pharmacokinetic variable for any drug. Interestingly, it appeared that holders exhibited greater mean peak to trough variation in plasma dinor-LAAM concentrations than non-holders (1.3 vs 1.1). However, this result must be kept in perspective as the peak to trough values were close to unity for both groups.

4.5.5. Conclusions and clinical implications.

In summary, this is the first study to comprehensively describe the steady state pharmacokinetics of LAAM and its two demethylated metabolites and the time-course of some typical opioid effects across the 48-hour dosing interval in such a large cohort of patients. Consistent with other studies (*cf.*, Fraser and Isbell, 1954; Walsh, *et al.*, 1998), the data presented here clearly show that LAAM is long acting and produces measurable opioid effects (i.e., as measured by changes in pupil diameter and respiratory rate) across the 48 hour dosing interval. The pattern of results for the subjective measures, with the exception of self-reported withdrawal, was less clear, with post hoc tests finding no within group differences (relative to baseline) for any response investigated. Importantly, in the

cohort of patients participating in this study, self-reported withdrawal was significantly suppressed for the duration of the 48-hour inter-dosing interval, and was not affected by variability in the measured pharmacokinetic parameters. The pharmacokinetic profile of LAAM, nor-LAAM and dinor-LAAM was complex and was characterised by significantly less fluctuation of plasma nor- and dinor-LAAM concentrations across the inter-dosing interval than plasma LAAM concentrations. The more stable plasma nor- and dinor-LAAM concentration-time profiles are likely to be responsible for the prolonged duration of action, and in particular the prolonged suppression of withdrawal, following the oral administration of LAAM. The previous chapter found that methadone non-holders had more variable plasma (R)-(-) methadone concentration-time profiles than holders. It was hypothesised that lessening the fluctuation in plasma concentration over the dosing interval would help lessen withdrawal in these patients. Hence, transferring methadone non-holders to LAAM may help reduce the severity of withdrawal experienced. The next three chapters will formally evaluate the efficacy of using LAAM to treat self-reported methadone non-holders.

5. Evaluation of LAAM as an Alternative Substitution Treatment for MMT Patients: Part One - Comparative Pharmacodynamics of Methadone and LAAM.

5.1. Introduction.

In chapter three it was established that non-holding on methadone is likely to have a pharmacokinetic basis, characterised by greater fluctuation in plasma (R)-(-) methadone concentration across the interdosing interval for non-holders than for holders. It was therefore hypothesized that lessening the fluctuation in plasma concentration over the dosing interval, by transferring these patients to LAAM, would help lessen withdrawal. An equally important question is whether self reported methadone holders remain holders when transferred to LAAM. Thus, this chapter represents a formal evaluation of LAAM's capacity to adequately suppress withdrawal across the dosing interval for both holders and non-holders.

Furthermore, given that LAAM, and in particular its active demethylated metabolites are potent mu opioid agonists, it is important to determine the magnitude and profile of symptom complaints experienced by LAAM patients, and compare those experienced by the same individuals when maintained on methadone. Generally, the side effects for LAAM reported in the trials reviewed in chapter one were those commonly associated with opioid dependence and withdrawal (for e.g., Fudala, *et al*., 1997; Johnson, *et al*., 2000; Ling, *et al*., 1976; 1978; White, *et al*., 2002). However, there are equivocal reports concerning the severity of specific symptom complaints reported by patients when maintained on methadone and LAAM. Some studies reported that symptom complaints reported by patients maintained on LAAM were generally no more severe than those reported by methadone maintained patients (Freedman and Czertko, 1981; Jaffe, *et al*., 1972; Ling, *et al*., 1976; 1978; Tennant, *et al*., 1986). In contrast, however, Judson and Goldstein (1982) reported more severe rankings for specific symptom complaints by patients maintained on methadone than LAAM. However, they did not test these differences using formal statistical methods.

Given that there is a paucity of data on the comparative effects of methadone and LAAM, the purpose of the present study was to compare the steady state pharmacodynamics of (R)-(-) methadone and LAAM within a cohort of maintenance patients. Patients

participating in this study included those who reported break through withdrawal on methadone (non-holders) and those who did not (holders). As previously described, a number of subjective and physiological indices of opioid effect were collected across the interdosing interval for each drug. The specific aims of the work in this chapter were:

- To determine if self reported non-holders on methadone can become holders when switched to LAAM.
- To determine if self reported methadone holders remain holders when switched to LAAM.
- To compare the temporal change in and magnitude of opioid effect and withdrawal across methadone's and LAAM's inter-dosing interval.
- To determine if the overall prevalence and the severity of specific symptom complaints differs for methadone and LAAM.

5.2. Hypotheses

- Self-reported methadone non-holders will report significantly fewer withdrawal symptoms and specific symptom complaints when maintained on LAAM than when maintained on methadone.
- There will be no significant difference in the number of withdrawal symptoms and specific symptom complaints self reported by methadone holders when maintained on both methadone and LAAM.

5.3. Methods

The methods and procedures used in the methadone and LAAM inter-dosing studies were outlined in detail in chapter two. The pharmacodynamics of LAAM and (R)-(-) methadone were contrasted in the 16 patients (8 self-reported methadone holders and 8 self-reported non-holders) who participated in both the methadone and LAAM inter-dosing studies. Patients participated in the inter-dosing studies when they had reached steady state for each drug (unchanged methadone dose for two weeks and unchanged LAAM dose for at least four weeks). Drug-free control subjects also participated in a 48-hour testing session. Blood was sampled from patients frequently across both the methadone and LAAM inter-dosing intervals, and pharmacokinetic parameters derived from individual plasma concentration-time profiles were reported in previous chapters.

5.3.1. Analyses

The time maintained on each drug prior to participating in the respective inter-dosing study and the number of withdrawal symptoms, for holders and non-holders, when maintained on methadone and LAAM, were compared using Student's t-test for independent samples. Fisher's exact test was used to compare categorical data between holder and non-holder groups, and between patients and control subjects. To compare the overall magnitude of effects of methadone with LAAM on each pharmacodynamic variable, the area under the effect versus time curve over the 24 hour inter-dosing interval for methadone (AUC_{0-24}; to allow 2x AUC_{0-24} to be determined) and the 48 hour inter-dosing interval for LAAM (AUC_{0-48}) were calculated by the trapezoidal rule (GraphPad Prism v3.02, GraphPad Software, CA, USA). The AUC for pupil diameter is expressed as millimetres·hours; for respiratory rate as breaths/minute·hours; for systolic and diastolic blood pressure as millimeters of mercury·hours; for pain detection and threshold as volts·hours; and AUC for withdrawal symptom severity, MBG and MG as score·hours. For each pharmacodynamic measure paired *t*-tests were used to compare the sum of 2x AUC_{0-24} values for methadone with one AUC_{0-48} value for LAAM.

Further analyses were undertaken to determine differences in the peak magnitude of effect between drugs. ANOVA could not be used to directly compare the pharmacodynamic data of patients on methadone with those obtained on LAAM, as testing protocols were not identical. Instead paired *t*-tests were used to directly compare scores from each session at predose, 1 hour, 2, 3, 4, 6, 9, 12, and 24 hours after dosing. Bonferroni adjustment was made to the alpha level for these comparisons (i.e., 0.05/9 comparisons); significance was set at $P<0.0055$.

The frequency with which patients reported specific symptom complaints at least once across the inter-dosing interval for each drug was compared using the McNemar test for related samples. To compare the severity of specific symptom complaints (ie, withdrawal and direct opioid effects) between drugs, the area under the effect versus time curve over the 24-hour inter-dosing interval for methadone and the 48-hour inter-dosing interval for LAAM were calculated by the linear trapezoidal rule (GraphPad Prism v3.02, GraphPad Software, CA, USA). Paired *t*-tests were used to compare the sum of 2x AUC_{0-24} values

for methadone with one AUC_{0-48} value for LAAM. Pearson's product moment coefficients were used to determine relationships between methadone and LAAM dosages and the relevant AUC severity value for each specific symptom complaint. In light of the number of comparisons (n=50) used in the latter correlation analyses the alpha level was set at *P*=0.01.

Unless otherwise stated the alpha level was set at *P*=0.05 and all statistical tests were two tailed and data are tabulated as mean±sd. Data were analysed using SPSS™ for Windows (v.10: SPSS Inc). SPSS undertakes Levene's test of homogeneity of variance when undertaking *t*-tests. And adjust the probability level accordingly. Where appropriate 95% confidence intervals of differences between means were also calculated. Control subject data were not included in analyses, but where possible are included on graphs for comparison purposes.

5.4. Results

5.4.1. General

Table 3.1 and 4.1 present patient demographic details and dosing levels for patients participating in the methadone and LAAM inter-dosing studies, respectively. There were no statistically significant differences between holders and non-holders for any of the demographic attributes, except for gender ratio and the body weight at the time of the methadone interdosing study.

The patients were maintained on LAAM for a mean of 53±19 days (range 30-86 days) and on methadone for a mean of 46±19 (range 24-90 days) (*P*=0.38; 6.69; -9, 22.37) prior to participating in the respective testing session. Furthermore, there was no difference in the time that non-holders and holders were maintained on either LAAM (*P*=0.13; 14.8; -5.11, 34.61), or methadone (*P*=0.31; -10.12; -30.79, 10.55) prior to participating in the respective testing session. Following transfer from methadone to LAAM, and prior to participating in the LAAM inter-dosing study, seven patients (2 holders, 5 non-holders) required dose increases beyond their initial dose, and one patient a decrease in dose. The holders (patients #1 and #6, see table 4.1), who received initial LAAM doses of 110 and 65 mg, respectively, each received a total increase of 10 mg in their LAAM dose. The non-

holders (patients #7, #8, #9, #10, #11 see table 4.1) received initial LAAM doses of 115, 40, 110, 20, and 140, respectively. Their respective LAAM dose increases were 20, 20, 40, 20, and 5 mg (mean increase 19 mg). At the time of the LAAM testing session the mean ratio of the LAAM dose to last methadone dose was 1.05 (range 1.0 to 1.2) for holders, and 1.16 (range 0.97 to 1.5) for non-holders.

5.4.2. Drug use

A summary of the frequency of detection for drugs in urine and plasma for patients participating in both methadone and LAAM studies is shown in Table 5.1. The most commonly detected drugs by urinalysis for both methadone and LAAM were benzodiazepines (56% vs. 62%) and cannabinoids (50% vs. 44%). There was general concordance between methadone and LAAM in the presence of benzodiazepines and cannabinoids in urine. Urinalysis revealed that only two patients had used sympathomimetic amines prior to the methadone-interdosing study, and that none had used these drugs prior to participating in the LAAM study. There were no positive results for cocaine use prior to both studies. Significantly more non-holders than holders had positive results for cannabinoids on both methadone (Fischer's exact test, P=0.04) and LAAM (Fischer's exact test, P=0.04). There were no other differences in the ratio of holders and non-holders showing positive results for any other drug tested by urinalysis during either the methadone or LAAM testing session (P>0.05).

Morphine, a marker of likely heroin use, was detected in plasma of seven patients (four non-holders), at the time of the methadone testing session (18±22 ng/ml), and four patients (two non-holders) at the time of the LAAM testing session (18±31 ng/ml) (see Tables 3.2 and 4.1 for individual plasma morphine concentrations). There was no difference in the ratio of holders and non-holders showing positive results for plasma morphine and benzodiazepines during either the methadone or LAAM testing session (P>0.05).

Table 5-1: Frequency of drug detection in urine and plasma samples taken immediately prior to both the methadone and LAAM inter-dosing studies (n=16), and according to holding status [holders (n=8) and non-holders (n=8)].

	Methadone			LAAM		
	All (n, %)	Holders (n, %)	Nonholders (n, %)	All (n, %)	Holders (n, %)	Nonholders (n, %)
Urine screen						
Methadone	16 (100)	8 (100)	8 (100)	1 (6)	0 (0)	1 (12)
Morphine	7 (44)	3 (38)	4 (50)	5 (31))	3 (38)	2 (25)
Benzodiazepines[1]	9 (56)	4 (50)	5 (63)	10 (62)	5 (63)	5 (63)
Sympathomimetic amines	2 (12)	1 (12)	1 (12)	0 (0)	0 (0)	0 (0)
Cocaine	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cannabinoids	8 (50)	# 2 (25)	# 6 (75)	7 (44)	# 1 (12)	# 6 (75)
Plasma screen						
Morphine	7 (44)	3 (38)	4 (50)	4 (25)	2 (25)	2 (25)
Benzodiazepines	6 (38)	3 (38)	3 (38)	6 (38)	3 (38)	3 (38)

Notes: [1]Diazepam, desmethyldiazepam, oxazepam. # $P<0.05$ Fishers exact test used to compare holder and non-holders.

5.4.3. Pharmacodynamic measures

5.4.3.1. Physiological responses

The effect of LAAM and methadone in patients and non-drug using control subjects is shown for all physiological parameters in figure 5.1 and 5.2. The AUC_{0-24} and AUC_{0-24} x 2 values for methadone and AUC_{0-48} values for LAAM, for each physiological parameter, are presented in table 5.2. The peak magnitude of effect for reduction in heart rate (figure 5.1) and systolic blood pressure (figure 5.1) was similar for both drugs and occurred at 3 to 6 hours after dosing. In contrast, patients' mean diastolic blood pressure was significantly lower when they were maintained on methadone, compared to LAAM, at six hours after dosing (P=0.004; 5.75; 2.15, 9.4). There were no other significant between group differences for diastolic pressure (P>0.0055). The sum of two AUC_{0-24} values for methadone was similar to the AUC_{0-48} value for LAAM for heart rate (P=0.078), and systolic (P=0.86) and diastolic blood pressure (P=0.189: see table 5.2).

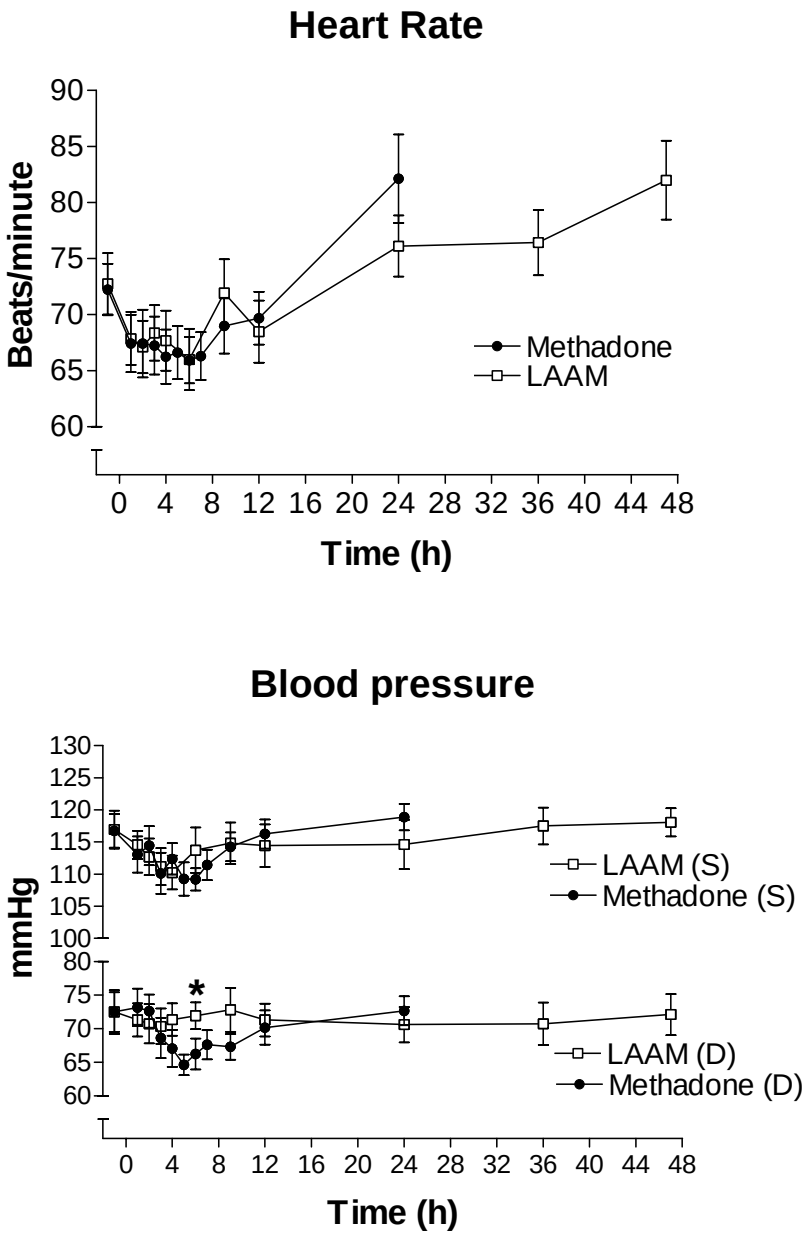

Figure 5-1: Mean (± SEM) heart rate (beats per minute) (upper panel) and systolic (S) blood pressure and diastolic (D) blood pressure (lower panel) in patients (n=16) who underwent one 24-h inter-dosing interval when taking methadone (closed circles) and one 48-h inter-dosing interval when taking LAAM (open squares). Control data not included for the sake of clarity. Asterisk denotes result of between group comparisons: * P<0.0055.

The mean respiratory rate and mean pupil diameter of patients and drug free control subjects are shown in figure 5.2. The peak magnitude of effect was similar for both drugs. Maximal suppression of respiration rate occurred at 3 hours after dosing for methadone, and at 6 hours after dosing for LAAM. For pupil diameter, maximal constriction occurred at 3 hours after dosing for methadone, and 4 to 6 hours for LAAM. There were no

significant between group differences for respiratory rate. However, mean pupil diameter was significantly lower when patients were maintained on LAAM, compared to methadone, at 12 hours (P<0.001; -0.64; -0.88, -0.40), and at 24 hours after dosing (P<0.001; -0.83; -1.10, -0.55). The sum of two AUC $_{0\text{-}24}$ values for methadone was similar to the AUC $_{0\text{-}48}$ value for LAAM for respiratory rate (P=0.61), and pupil diameter (P=0.13; see table 5.2).

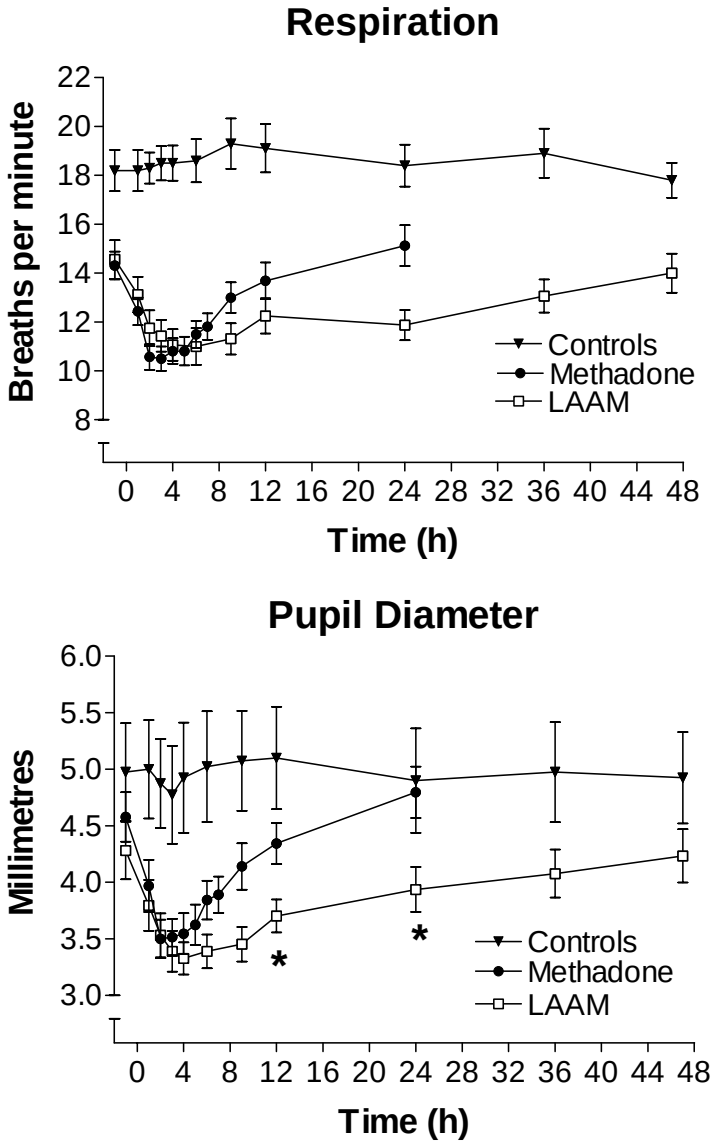

Figure 5-2: Mean (± SEM) respiratory rate (breaths per minute) (upper panel) and pupil diameter (millimeters) (lower panel) in patients (n=16) who underwent one 24-h inter-dosing interval when taking methadone (closed circles) and one 48-h inter-dosing interval when taking LAAM (open squares) and in 10 drug-free control subjects (Closed triangles). * P<0.001 significant differences between patients when maintained on either methadone or LAAM.

5.4.3.2. Subjective responses

Subjective responses ((Morphine Group (MG) and Morphine Benzedrine Group (MBG)) subscales of the Addiction Research Centre Inventory, pain detection and threshold, and number of withdrawal symptoms)), are shown in figures 5.3 through to 5.5. The AUC_{0-24} and AUC_{0-24} x 2 values for methadone and AUC_{0-48} values for LAAM, for each subjective parameter, are presented in table 5.2. The magnitude of increase in MBG scores was greater for LAAM than for methadone. Peak effects occurred 2 to 4 hours after dosing for both drugs (see figure 5.3). In contrast, while MG scores for methadone peaked at 3 hours after dosing, scores for LAAM patients were relatively flat across the dosing interval (see figure 5.3). Despite the latter findings, there were no significant between group differences for either drug across the 0-24 time period ($P<0.0055$). The sum of two AUC_{0-24} values for methadone was similar to the AUC_{0-48} value for LAAM for MBG scores ($P=0.08$), and MG scores ($P=0.28$; see table 5.3).

Mean pain detection and pain threshold scores are shown in figure 5.4. Patients on LAAM exhibited relatively stable mean pain detection and pain threshold scores across the LAAM inter-dosing interval. In contrast, for methadone, both mean pain detection and pain threshold were maximal between two to four hours after dosing. However, there were no significant differences between methadone and LAAM groups across the 0-24 time period ($P<0.0055$). Furthermore, the sum of two AUC_{0-24} values for methadone was similar to the AUC_{0-48} value for LAAM for pain detection ($P=0.63$), and pain threshold ($P=0.77$; see table 5.2).

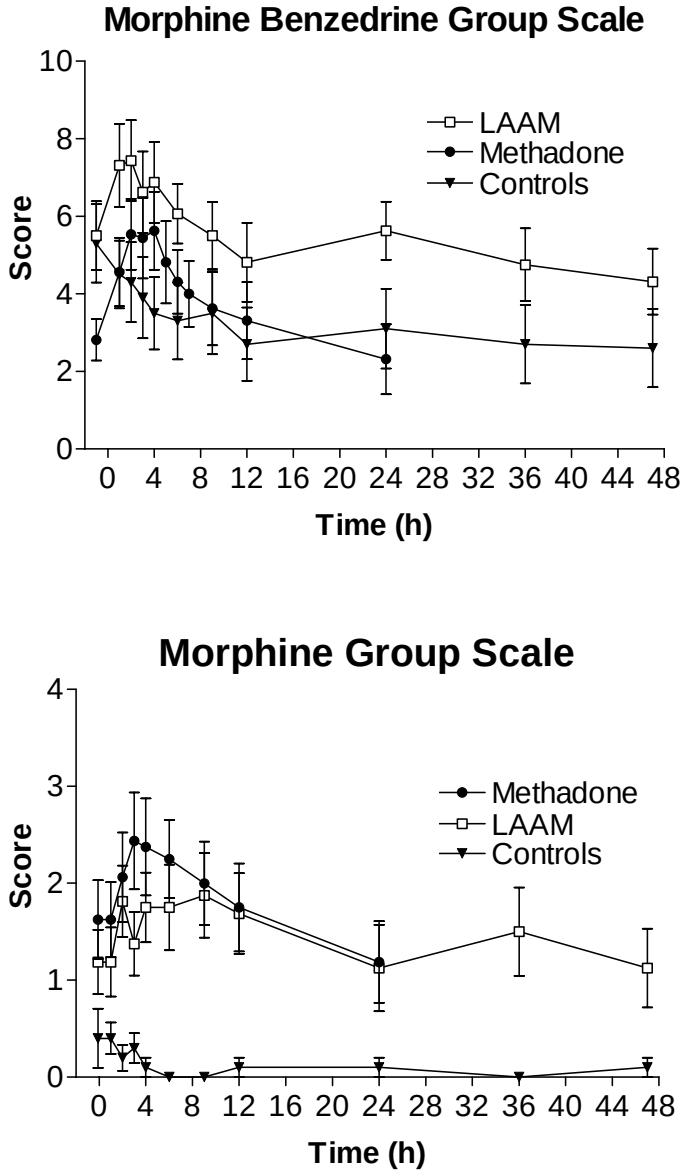

Figure 5-3: Mean (± SEM) Morphine Benzedrine Group Scores (upper panel: maximum possible score =16) and Morphine Group Scale scores (lower panel: maximum possible score =8) in patients (n=16) who underwent one 24-h inter-dosing interval when taking methadone (closed circles) and one 48-h inter-dosing interval when taking LAAM (open squares) and in 10 drug-free control subjects (closed triangles).

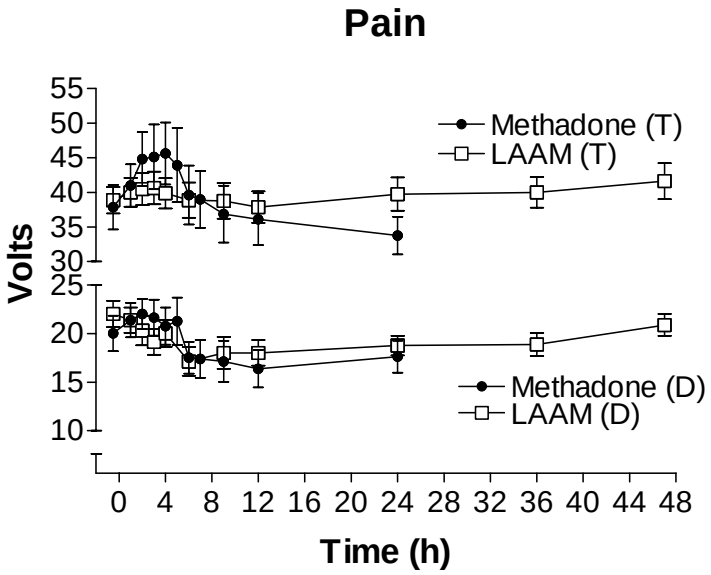

Figure 5-4: Mean (± SEM) pain detection (D) and pain threshold scores (T) (volts) for patients (n=16) who underwent one 24-h inter-dosing interval when taking methadone (closed circles) and one 48-h inter-dosing interval when taking LAAM (open squares). Control data not included for the sake of clarity

Mean withdrawal scores for patients on both LAAM and methadone and for control subjects are shown in figure 5.5. Maximum suppression of withdrawal symptoms was similar for both drugs and occurred at 3 hours after dosing for LAAM, and 4 hours after dosing for methadone. Mean withdrawal scores were significantly greater for patients when taking methadone, compared to LAAM, at 12 hours (P=0.003; -4.1; -6.67, -1.70) and 24 hours (P=0.001; -5.88; -8.89, -2.86) after dosing. Furthermore, the sum of two AUC_{0-24} values in patients when maintained on methadone (259±18 score·hour) was significantly greater than the AUC_{0-48} value for patients when maintained on LAAM (115±73 score·hour, P=0.012: see table 5.2).

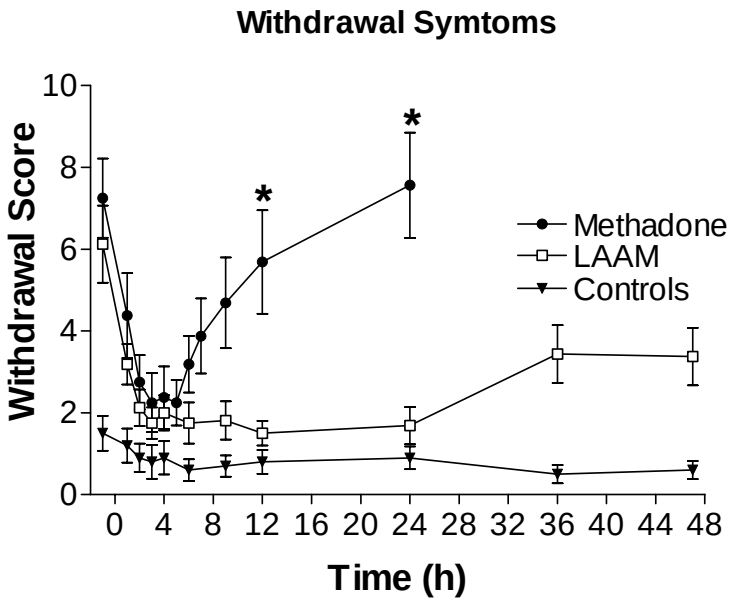

Figure 5-5: Mean (± SEM) withdrawal scores (maximum possible score = 16) in patients (n=16) who underwent one 24-h inter-dosing interval study when taking methadone (closed circles) and one 48-h inter-dosing interval when taking LAAM (open squares) and in 10 drug-free control subjects (closed triangles). * P<0.0055 significant differences between patients when maintained on either methadone or LAAM

In chapter three, when comparing pharmacodynamic parameters between non-holder and holder groups maintained on methadone, it was shown that non-holders could only be differentiated from holders on the basis of the number of self-reported withdrawal symptoms. Furthermore, methadone non-holders when maintained on LAAM could not be differentiated from holders on any pharmacodynamic variable (see chapter 4). Clearly the picture for withdrawal reported by all the patients while maintained on methadone is confounded by self reported methadone holding status. Thus, to test the prediction that methadone non-holders will become a holder when switched to LAAM and that a methadone holder will remain a holder when switched to LAAM, the next section will separately compare the severity of withdrawal exhibited by methadone holders and non-holders when maintained on both drugs.

5.4.3.3. Comparison for holder and non-holder groups - self-reported withdrawal.

Mean withdrawal scores across the inter-dosing interval for methadone holders and non-holders when maintained on LAAM and methadone are shown in Figure 5.6. For methadone holders, withdrawal scores were not significantly different when they were maintained on methadone or LAAM (P>0.005). In contrast, methadone non-holders' scores were significantly greater when they were maintained on methadone compared to LAAM at 9, (P = 0.002; -6.6; -9.9; -3.3), 12 (P = 0.001; -7.9; -11.0, -4.7) and 24 hours (P <0.001; -10.8; -12.7, -8.8) after dosing.

The AUC_{0-24} for withdrawal symptom severity was 210±73 score·hours in non-holders maintained on methadone, and 50±28 score·hours in holders maintained on methadone. The AUC_{0-48} for withdrawal symptom severity was 36±34 score·hours in control subjects, 118±67 score·hours in methadone holders maintained on LAAM and 116±85 score·hours in methadone non-holders maintained on LAAM. The sum of two AUC_{0-24} values in holders maintained on methadone (101±56 score·hours) was similar to the AUC_{0-48} in holders maintained on LAAM (P = 0.62; -18; -98.36, 63.36). In contrast, the AUC_{0-48} in non-holders maintained on LAAM was significantly less than the sum of the two AUC_{0-24} values in nonholders maintained on methadone (420±51 score·hours) (P < 0.001; -330; -419, -187). Thus, LAAM converted methadone non-holders into LAAM holders, but retained the holding status for methadone holders.

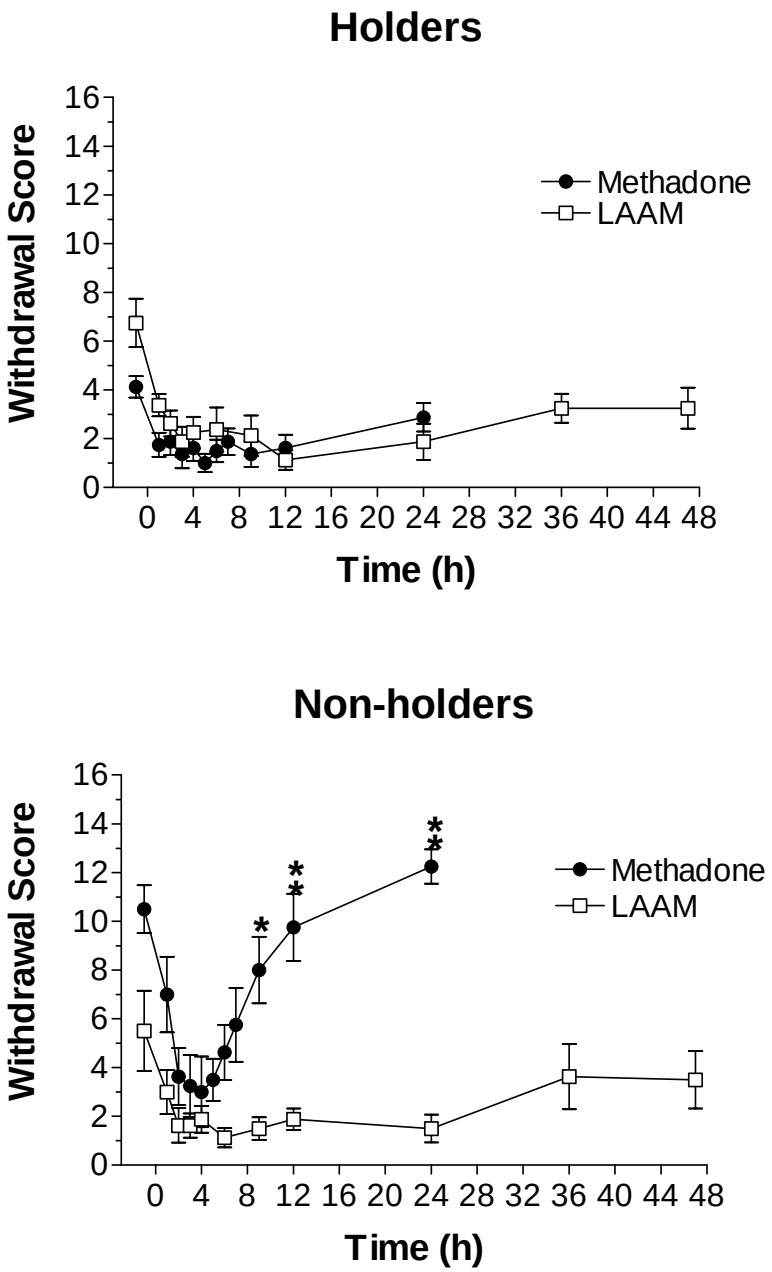

Figure 5-6: Mean withdrawal scores (maximum possible score=16) of eight self reported holders (upper panel) when taking methadone (closed circles) or LAAM (open squares), and 8 self reported non-holders (lower panel) when taking methadone or LAAM. All patients underwent one 24-hour inter-dosing interval study when on methadone and one 48 hour inter-dosing interval study when on LAAM. Values are mean ± SE. * P < 0.0055, ** P < 0.001 significant differences between holder and non-holder groups when maintained on either methadone (upper panel) or LAAM (lower panel).

Table 5-2: Comparison of area under the effect versus time curve for each pharmacodynamic parameter for patients (n=16) who underwent one 24-h inter-dosing interval study when taking methadone and one 48-h inter-dosing interval study when taking LAAM.

Parameter	Methadone		LAAM	P* *(mean diff, 95% CI)*
	AUC_{0-24}	AUC_{0-24}x 2	AUC_{0-48}	
Heart rate (beats/ minute·h)	187±86	374 ±172	284±138	*P*=0.078 (90; -11, 191)
Systolic blood pressure (mmHg·h)	149±65	298±131	288±164	*P*=0.86 (10; -107, 127)
Diastolic blood pressure (mmHg·h)	232±162	464±324	313±230	*P*=0.189 (150; -83, 283)
Pupil diameter (mm·h)	13±5	26±10	20±15	*P*=0.13 (-5.64; -13, 1.82)
Respiratory rate (breaths/min·h)	44±19	88±38	95±48	*P*=0.61 (6.93; -21, 35)
MBG (score min·h)	88±78	175±155	251±137	*P*=0.08 (-76; -160, 9)
MG (score min·h)	43±37	86±73	68±70	*P*=0.28 (18; -12, 48)
Pain detection (volts·h)	442±167	885±335	921±199	*P*=0.63 (-36, -194, 121)
Pain threshold (volts·h)	923±345	1846±690	1884±405	*P*=0.77 (-38; -308, 232)
withdrawal symptoms (score ·h)	129±18	259±197	115±73	***P*=0.012** (144; 36, 252)

Notes: AUC = Area under the effect versus time curve; *P** = Paired *t*-test used to compare AUC_{0-24} x 2 for methadone and AUC_{0-48} for LAAM for each pharmacodynamic measure. Significant *P* values in bold text.

5.4.3.4. Symptom complaints.

The number of patients and control subjects that self reported specific symptom complaints at least once during the inter-dosing studies is shown in table 5.3. During the methadone inter-dosing interval the most commonly reported symptoms were the methadone dose not holding, a number of symptom complaints generally associated with withdrawal (i.e., feeling tired, runny or stuffy nose, yawning, feeling anxious, hot flushes, salivation), a number of direct opioid effects (feeling awake, sweating, nausea), and a number of miscellaneous symptom complaints (decrease appetite, wanting to drink, feelings of weakness, trouble thinking clearly, restlessness), which were reported by greater than 75% of the sample. The symptom complaints reported more frequently by non-holders than holders during the methadone interdosing interval were largely classified as withdrawal

symptoms (e.g., nervousness, headache, feelings of coldness, aches and pains, and tense muscles), but also included two mixed symptom complaints that could be attributable to opioid withdrawal (feeling irritable and dizziness). It is noteworthy that all of the non-holders reported the failure of their methadone dose to hold them on at least one occasion during the interdosing interval. Of the holders, four reported the failure of their methadone dose to hold during the inter-dosing interval. The most commonly reported symptoms for patients when they were maintained on LAAM included feeling tired, hot flushes, feeling awake, dry mouth, sweating, wanting to drink, feelings of weakness, trouble thinking clearly, and restlessness. Non-holders and holders, when maintained on LAAM, could not be differentiated on the basis of the number of symptom complaints.

The mean total number of symptom complaints (total of 52) for patients when they were maintained on methadone (mean±sd: 28.6±8.0) was significantly greater than when they were maintained on LAAM (Paired *t* test: 21.6±8.7; *P*=0.049; 6.4; 0.03, 12.84). The specific symptoms complaints that were reported at significantly higher frequency for methadone than LAAM were the dose not holding, feelings of coldness, and goose pimples, and were most likely to be reported by the methadone non-holders ($P<0.05$). Furthermore, non-holders reported a greater mean number of symptom complaints when taking methadone (34.4 ±4.4) than when taking LAAM (20.37±8.9; *P*=0.007; 14.00; 5.2, 2.8), but holders reported a similar mean number of symptoms on methadone (mean±sd: 21.75±5.3) and LAAM (23±9.0; *P*=0.71; -1.13; -8.04, 5.78). Hence, the reduction in number of symptom complaints seen with patients when taking LAAM is likely due to the reduction of number of symptoms reported by the non-holder group. It is noteworthy that no specific symptom complaint was reported with more frequency by patients on LAAM than on methadone.

Furthermore, the number of control subjects reporting each specific symptom was less than for patients when taking methadone and LAAM. The difference between the methadone and control groups was significant for 24 of these items, and the difference between the LAAM and control groups was significant for 19 items. Some of the more notable symptoms complaints reported with similar frequency (i.e., not statistically different) by the control group and patient groups, included headache, runny or stuffy nose, diarrhoea, feel awake and swelling of feet or ankles.

The comparison of the severity (AUC_{0-24} x 2 for methadone and AUC_{0-48} for LAAM) of those symptom complaints attributable to withdrawal and direct opioid effect, for methadone and LAAM, is shown in Table 5.4. Patients rated 15 symptom complaints (out of the 26 compared between drugs) as similar in severity on both drugs. A few symptoms, attributable to withdrawal, were rated significantly more severe by patients when maintained on methadone than when maintained on LAAM, and included dose not holding, runny or stuffy nose, yawning, feelings of coldness, runny eyes, muscle spasms/ twitching aches and pains, goose pimples, and feeling anxious. Patients also rated the following direct symptoms as more severe on methadone than LAAM (itchy skin and itchy nose). Correlational analyses showed no effect of methadone dosage ($0.96>P>0.037$) or LAAM dose ($0.98>P>0.038$) on any specific symptom complaint. No specific symptom complaint was rated as more severe by patients when maintained on LAAM than methadone

Table 5-3: Number of patients (n=16) and control subjects (n=10) reporting specific symptom complaints at least once during the inter-dosing study period for methadone and LAAM, and during the study period for control subjects; comparisons for all patients (n=16) and methadone holder (n=8) and non-holder(n=8) groups.

	Methadone (*n=16*)			LAAM *(n=16)*			Controls (*n=10*)
	All	Holders	Non holders	All	Holders	Non holders	
Withdrawal							
Dose not-holding	*12	4	8	*4	2	2	Na
Feeling tired	15	7	8	15	8	7	† ^ 5
Nervousness	10	# 2	# 8	7	3	4	† ^ 0
Headache	8	# 1	# 7	8	3	5	2
Runny or stuffy nose	14	7	7	11	6	5	5
Yawning	13	5	8	9	6	3	5
Feelings of coldness	*11	# 3	# 8	*5	2	3	† 2
Stomach cramps	9	4	5	4	2	2	† 0
Runny eyes	9	3	6	7	3	4	† ^0
Muscle spasms/ Twitching	10	# 2	# 8	5	3	2	† 0
Aches and pains	11	# 3	# 8	7	4	3	† 1
Tense muscles	8	# 1	# 7	9	5	4	^1
Goose pimples	*8	2	6	*2	1	1	† 0
Craving	7	2	5	5	2	3	Na
Feeling anxious	12	4	8	10	4	6	†^ 1
Hot flushes	12	5	7	12	6	6	† ^0
Salivation	12	5	7	8	5	3	† ^0
Diarrhoea	1	0	1	0	0	0	0
Direct Opioid Effects							
Constipation	9	4	5	10	5	5	†^ 0
Feel awake	15	7	8	14	6	8	6
Dry mouth	11	6	5	14	7	7	^ 3
Trouble urinating	3	2	1	2	2	0	0
Need to urinate	10	4	6	7	4	3	† 1
Pleasant feeling in stomach	6	3	3	9	5	4	^1
Itchy skin	9	5	4	5	4	1	† 0
Itchy nose	9	6	3	4	3	1	† 0
Swelling of feet or ankles	5	3	2	2	1	1	0
Feeling high	3	1	2	3	3	0	1
Sweating	16	8	8	13	8	5	†^ 0
Nausea	12	4	8	9	4	5	†^ 0

Notes: Values are number of participants reporting specific symptom complaint at least once during the inter-dosing interval for each drug, or 48 hour testing period for control subjects. Na=not applicable; * P <0.05 Methadone vs LAAM (McNemar Test); # P<0.05 holder vs non-holders, (Fischer's exact test); † P<0.05 Methadone vs Control subjects (Fishers exact tests); ^ P <0.05 LAAM vs Control subjects (Fishers exact tests).

Table 5.3: continued.

	Methadone (*n=16*)			LAAM *(n=16)*			Controls (*n=10*)
	All	Holders	Non holders	All	Holders	Non holders	
Mixed/unclear							
Decreased appetite	12	6	6	9	5	4	† ^0
Want to drink (not alcohol)	13	5	8	13	6	7	† ^3
Feelings of weakness	12	4	8	12	5	7	† ^0
Increased appetite	9	4	5	6	3	3	† 0
Trouble thinking clearly	13	5	8	12	6	6	† ^1
Restlessness	12	4	8	13	5	8	†^ 0
Blurred vision	6	3	3	2	2	0	1
Feeling unhappy	10	3	7	9	5	4	† ^1
Feeling irritable	9	# 1	# 8	8	4	4	† ^0
Reduced desire for sex	11	5	6	11	7	4	3
Confusion	10	3	7	6	4	2	† 0
Heart pounding	6	3	3	1	1	0	0
Vomiting	0	0	0	0	0	0	1
Chest pains	2	0	2	2	1	1	0
Bleeding gums	4	3	1	5	3	2	0
Numbness in hands or feet	0	0	0	1	1	0	0
Dizziness	5	# 0	# 5	3	1	2	0
Increased desire for sex	4	1	3	4	2	2	0
Hallucinations	0	0	0	0	0	0	0
Heartburn	6	3	3	1	1	0	0
Want to drink alcohol	2	1	1	3	2	1	1
Pain down left arm	1	1	0	2	1	1	1

Notes: Values are number of participants reporting symptom at least once during the inter-dosing interval for each drug, or 48 hour testing period for control subjects. * P <0.05 Methadone vs LAAM (McNemar Test), # P<0.05 holder vs non-holders (Fischer's exact test), † P<0.05 Methadone vs Control subjects (Fishers exact tests), ^ P <0.05 LAAM vs Control subjects (Fishers exact tests).

Table 5-4: Comparison of area under the effect versus time curve for severity of specific symptom complaints reported by patients (n=16) during the inter-dosing study period for methadone and LAAM.

Symptom Complaint	Methadone		LAAM	*[1]P value (mean difference; 95% CI)[1]*
	AUC_{0-24}	AUC_{0-24}x2	AUC_{0-48}	
Withdrawal				
Dose not-holding	19.5±20.4	39.1±40.9	13.1±34.1	**0.017** (-26.0; -46.7, -5.32)
Feeling tired	27.3±24.0	54.6±47.9	36.0±19.2	0.20 (-18.6; -47.9, 10.8)
Nervousness	9.91±11.9	19.8±23.8	8.58±20.0	0.21 (-11.2; -29.3, 6.82)
Headache	6.84±10.2	13.7±20.4	8.52±14.7	0.30 (-5.17; -15.5, 5.18)
Runny or stuffy nose	20.8±15.7	41.5±31.5	15.7±17.2	**0.009** (-25.8; -44.1, -7.56)
Yawning	16.1±15.9	32.3±31.8	12.3±18.5	**0.045** (-20.0; -39.5, -0.46)
Feelings of coldness	11.0±13.9	21.9±27.9	4.16±7.70	**0.031** (-17.8; -33.7, -1.86)
Stomach cramps	5.69±10.9	11.38±21.7	9.22±15.1	0.73 (-2.16; -15.0, 10.7)
Runny eyes	8.38±11.03	16.8±22.1	3.82±6.34	**0.034** (-12.9; -24.7, -1.13)
Muscle spasms/Twitching	8.69±9.41	17.38±18.8	1.50±3.90	**0.006** (-15.9; -26.4, -5.31)
Aches and pains	16.2±19.9	32.3±39.7	13.9±32.9	**0.004** (-18.4; -30.0, -6.78)
Tense muscles	8.47±13.4	16.9±26.7	4.22±9.28	0.07 (-12.7; -26.8, 1.38)
Goose pimples	8.73±14.3	17.5±28.6	1.69±4.33	0.052 (-15.8;-31.7, 0.12)
Craving	10.0±17.4	20.1±34.9	2.44±5.83	0.054 (-17.6; -35.6, 0.36)
Feeling anxious	16.9±17.8	33.9±35.5	12.0±21.0	**0.014** (-21.9; -38.7, -5.16)
Hot flushes	16.1±14.7	32.2±29.3	22.0±35.0	0.31 (-10.2; -30.9, 10.4)
Salivation	13.9±16.0	27.9±32.1	13.4±20.7	**0.045** (-14.5; -28.7, -0.37)
Direct Opioid Effects				
Constipation	12.1±16.0	24.2±32.1	20.3±25.6	0.67 (-3.9; -23.0, 15.2)
Dry mouth	14.7±21.8	29.4±43.7	26.0±40.3	0.76 (-3.42; -26.9, 20.05)
Need to urinate	5.75±9.12	11.5±18.2	12.6±31.8	0.86 (1.14; -11.9, 14.2)
Pleasant feeling in stomach	4.78±9.95	9.56±19.9	17.2±29.8	0.16 (7.60; -3.38, 18.57)
Itchy skin	4.20±6.56	8.41±13.1	3.14±8.62	**0.028** (-5.27; -9.87, -0.66)
Itchy nose	6.15±8.85	12.3±17.7	3.75±9.51	**0.018** (-8.56; -15.5, -1.65)
Feeling high	3.00±9.85	6.00±19.7	6.34±22.9	0.78 (0.34; -2.21, 2.89)
Sweating	12.8±8.20	25.6±16.4	23.3±18.4	0.62 (-2.30; -11.9, 7.31)
Nausea	8.52±11.24	17.03±22.5	5.77±7.53	0.058 (-11.27; -22.9, 0.41)

Notes: AUC=Area under the effect versus time curve; AUC for each symptom complaint expressed as score•hours. [1] Paired *t*-tests used to compare AUC_{0-24} x 2 values for methadone and AUC_{0-48} values for LAAM. Significant *P* values in bold text. Data are mean±sd.

5.5. Discussion.

This study compared the opioid agonist effects of LAAM and methadone at steady state in a cohort of maintenance patients using a within subjects design. In general, the peak magnitude of methadone and LAAMs effect on physiological and subjective parameters was similar for both drugs. The temporal pattern of self reported withdrawal across the inter-dosing interval differed between drugs; withdrawal increased in severity at the end of the inter-dosing interval for patients when they were on methadone, but remained relatively constant throughout the inter-dosing interval for patients maintained on LAAM. The overall magnitude of effect (AUC_{0-24} X2 for methadone and AUC_{0-48} for LAAM) for all of the pharmacodynamic variables, with the exception of withdrawal, was not statistically different between drugs. Furthermore, patients reported a greater number of symptom complaints when taking methadone than when talking LAAM. The pattern of responses also differed according to self-reported methadone holding status. The holders exhibited adequate withdrawal suppression, and a similar number of symptom complaints, on both methadone and LAAM. In comparison, non-holders exhibited significant reduction in withdrawal at the end of the inter-dosing interval, and more symptom complaints, when taking methadone, than when taking LAAM. Hence, LAAM converted methadone non-holders into LAAM holders. Also important was the finding that methadone holders remained holders when maintained on LAAM.

It was previously hypothesized that, because of methadone's very steep plasma concentration-effect relationship for withdrawal, that flattening the plasma concentration profile over the inter-dosing interval might transform a self-reported methadone non-holder to a holder (Dyer, *et al*., 1999). The results presented here show that this can be achieved by using LAAM on a second daily dosage schedule. The long terminal half lives (Finkle, *et al.,* 1982; Henderson, *et al*., 1977b; Kaiko and Inturissi, 1975) and the relatively flat plasma concentration versus time profile for LAAM's two active metabolites (see section 4.4.3), in particular dinor-LAAM, confer stability of opioid effect and hence minimize withdrawal across the dosing interval. Also important was the finding that holders on methadone remained holders on LAAM, and therefore could benefit from LAAM's less frequent dosing regimen.

When one compares the other pharmacodynamic effects of steady state methadone and LAAM there are few other differences. Mean diastolic blood pressure, at 6 hours after dosing, was significantly lower for patients when taking methadone than when taking LAAM. However, given that this is the only significant difference between drugs for this measure, this finding is probably of little clinical significance. It is noteworthy that the peak magnitude of respiratory depression and pupil constriction, which are frequently used as indicators of opioid effect, were similar for the two drugs at the doses used and the plasma concentrations achieved. The time to peak effect for both respiratory depression and pupil constriction was shorter for methadone (about 2 h), than LAAM (4-6 h), which would correspond with the corresponding range of T_{max} values for methadone and for LAAM and nor-LAAM, respectively. Furthermore, the duration of these opioid effects appeared greater for LAAM than methadone; notably pupil constriction was significantly greater for LAAM than methadone at 12 and 24 hour post dosing. However, when one compares the sum of the two $AUC_{0\text{-}24}$ values for methadone with one $AUC_{0\text{-}48}$ value for LAAM, the overall magnitude of effect was not statistically different for any pharmacodynamic measure, with the exception of self-reported withdrawal.

There is a paucity of available data concerning the comparable pharmacodynamic effects of methadone and LAAM, particularly at steady state. Nevertheless the results reported here are consistent with those presented by Eissenberg and colleagues (1999). They showed that the same dose of oral methadone and LAAM administered under acute dosing and double blind experimental conditions produced at <u>least</u> the same peak magnitude of effect, as assessed by a range of objective and subjective measures. In addition, their results would suggest that patients could expect to experience a similar onset of opioid agonist effects, but delayed peak effects, when maintained on LAAM, than when on methadone.

An additional aim of this study was to explore the prevalence and severity of symptom complaints experienced by patients when taking methadone and LAAM. In keeping with previous reports, the side effect profile for both drugs was similar and generally reflected symptom complaints commonly associated with opioid dependence and/or opioid withdrawal (Freedman and Czertko, 1981; Fudala, *et al*., 1997; Johnson, *et al*., 2000; Ling, *et al.*, 1976; 1978; Tennant, *et al*., 1986). During this study the most common symptoms reported by patients on both drugs were complaints of feeling tired, runny or stuffy nose,

hot flushes, feeling awake, feeling anxious, dry mouth, sweating, trouble thinking clearly, and restlessness, each of which were reported by greater than 70 % of the sample at some stage during the inter-dosing interval for each drug. Despite the similarity in the side effect profile for both drugs, results show that patients experienced significantly more specific symptom complaints when taking methadone (mean±sd: 28.6±8.7) than LAAM (mean 21.6±8.7). The symptoms reported with less frequency by patients when taking LAAM included the dose not holding, feelings of coldness, and goose pimples. The reductions in the total number of symptom complaints for methadone and LAAM were most evident for non-holders than holders. That is, the holders reported a similar number of symptom complaints on both methadone and LAAM, whereas non-holders reported significantly more symptom complaints when taking methadone than when taking LAAM. The symptoms reported more frequently by non-holders, than holders on methadone, included signs of withdrawal, such as, nervousness, headache, runny nose, feelings of coldness, muscles spasms, aches and pains, and tense muscles, and reports of feeling irritable and dizzy.

It is interesting to note that four of the holders, compared to all the non-holders, reported that their methadone dose did not hold at least once during the methadone inter-dosing interval. For each of the holders this complaint occurred only once, just prior to dosing. However, in comparison, all of the non-holders reported the failure of their dose to hold more frequently across the dosing interval. These data would be consistent with the different patterns of withdrawal across the interdosing interval for non-holders compared to holders, when on methadone.

If one looks at the severity of symptom complaints reported by patients, eleven symptom complaints (out of the 26 compared between drugs) were reported as more severe on methadone than LAAM. It is also noteworthy that no specific symptom complaint was reported with more frequency or severity on LAAM than methadone. These findings are consistent with previous reports that symptom complaints of patients maintained on LAAM are generally no more severe than those of patients maintained on methadone (Freedman and Czerko, 1981; Jaffe, *et al*., 1972; Judson and Goldstein, 1982; Ling, *et al*., 1976; 1978; Tenant, *et al*., 1986; White, *et al*., 2002). Moreover, it is noteworthy that nine of the symptoms reported to be more severe on methadone than LAAM reflect withdrawal. Thus, the reduction in the number and severity of symptom complaints reported by patients

when taking LAAM, as compared to methadone, can be largely attributed to the reduction in withdrawal severity experienced by nonholders on LAAM. Hence, both methadone non-holders and holders in this study exhibited adequate withdrawal suppression on LAAM, without a concomitant increase in the severity of other side effects.

Furthermore, previous investigators have reported that a small number of patients maintained on LAAM reporting experiencing distressing symptoms related to 'stimulation', i.e. hyperactivity, anxiety and restlessness (Billings, *et al*., 1974; Jaffe, *et al*., 1970; Ling, *et al*., 1976; Tenant, *et al*., 1986; Zaks, *et al*., 1972) that threatened participation in clinical trials. This study was not designed to directly investigate this phenomenon; nevertheless, the severity of anxiety was not greater for patients when maintained on LAAM compared to methadone. Indeed, patients rated that they felt more anxious on methadone than when on LAAM, but this most probably reflects the greater incidence of complaints of dose not holding during the methadone interdosing interval. However, it is noteworthy that one patient withdrew from the study soon after commencing LAAM, but before participating in the LAAM inter-dosing interval, because of complaints of increased anxiety.

As reviewed in chapter one, the effectiveness of methadone, in the context of maintenance treatment, is generally dose related, with dosages above 50- 60 mg daily related to better program retention and reduction in illicit drug use when compared to lower dosages (e.g., Ball & Ross, 1991; Capelhorn and Bell, 1991; Johnson, *et al*, 2000; Strain, *et al*., 1993). Thus, it is noteworthy that non-holders in this study received a higher than average daily methadone dose (83 mg) than holders (73 mg; see table 3.6). These doses would be in the high dose range described by a number of authors (e.g., Johnson, *et al*., 2000; Ward, *et al*., 1999). Nevertheless there were a few non-holders in this study that received methadone doses as low as 30 mg daily. It is recognized that such low doses might be considered by some clinicians as inadequate to suppress withdrawal. However, these patients had been on MMT for the medium to long term (range 10-114 months: see table 3.1), and had decided to reduce their methadone dose to minimise unacceptable methadone side effects, such as sedation and sweating.

In this study patients were placed on an initial LAAM dose that was 1.1 times their methadone dose. Patients could then request dose increases if needed. It is possible in the

context of a research trial investigating a new treatment that patients might be more likely to request dose increases than during long-term MMT. As a result patients may have received higher equivalent doses of opioid receptor agonist when maintained on LAAM than when on methadone, and this might partly explain the reduction in withdrawal exhibited by non-holders when on LAAM. Although five non-holders received increases in their LAAM dose beyond their starting dose, the final ratio of LAAM to methadone dose for all non-holders, was a mean of 1.16. This ratio of LAAM to methadone is very close to that recommended in the product information to ensure patients are switched from methadone to LAAM on equi-efficacious doses (i.e., 1.2-1.3: Roxanne Laboratories). Hence it is more likely that the significantly better holding seen with non-holders on LAAM is due to the particular pharmacokinetics of the metabolites of LAAM.

Moreover, the frequency of detection for drugs in urine and plasma revealed few significant differences for patients when they participated in either inter-dosing interval study. Although fewer patients tested positive for morphine in their plasma when maintained on LAAM than when on methadone (7 vs 4), this difference was not statistically significant. However, it is conceivable that with a larger sample size that significance may be achieved. A recent meta-analysis of results from previous clinical trials showed that there might be a small advantage for LAAM in reducing illicit heroin use in comparison to methadone in the context of maintenance treatment (Clark, *et al*., 2002). Indeed, reports from previous trials are that patients felt LAAM was more effective than methadone in reducing craving for illicit opioids, which has been attributed to the relatively consistent and sustained plasma concentration of nor-LAAM and dinor-LAAM across the inter-dosing interval (Blaine, *et al*., 1981).

In summary, this is the first study to comprehensively compare the steady state pharmacodynamics of LAAM and methadone within such a large cohort of maintenance patients. When comparing the sum of two $AUC_{0\text{-}24}$ values for methadone to one $AUC_{0\text{-}48}$ value for LAAM for each pharmacodynamic measure, there were no significant differences in the overall magnitude of effect for any measure, with the exception of self reported withdrawal. The general pattern of symptom complaints was similar for patients when maintained on both methadone and LAAM. However, patients reported significantly fewer specific symptom complaints during the LAAM interdosing interval than the methadone inter-dosing interval. As predicted, patients who were self reported non-

holders on methadone exhibited significant reduction in withdrawal and overall number of symptom complaints when maintained on LAAM. Conversely, self reported methadone holders exhibited similar patterns of withdrawal and overall pattern of specific symptom complaints during both the LAAM and methadone inter-dosing interval. These findings show that it is possible to convert a methadone non-holder to a holder with LAAM. It is proposed that it is LAAM's unique pharmacokinetics rather than its intrinsic pharmacodynamics that is instrumental in achieving this outcome. Given that there is link between severity of withdrawal and mood disturbance in methadone patients (Dyer, *et al*., 2001), the next two chapters will determine if treatment with LAAM will also produce improvements in mood and cognitive function.

6. Evaluation of LAAM as an Alternative Substitution Treatment for Methadone Maintenance Patients: Part Two - Comparison of Affective States During the Inter-dosing Interval for Methadone and LAAM.

6.1. Introduction.

The major aim of maintenance treatment for opioid dependence is to stabilize the physiological and psychological state of the opioid dependent patient. The extent to which the latter occurs is largely dependent on the effectiveness of the maintenance drug in suppressing withdrawal across the inter-dosing interval without causing adverse side effects. Opioid withdrawal is associated with negative mood states, such as depression, anger and anxiety (Haertzen and Hooks, 1969; Kanof, *et al*., 1993; Powell, *et al*., 1992; Unnithan, *et al*., 1992). In turn, the presence of such negative mood states increases the perceived severity of subjective withdrawal symptoms, and to also induce cravings for opioids (Brehm and Khantzian, 1997; Childress, *et al*., 1994; Phillips, *et al*., 1986), which motivates continual opioid use and leads to poor treatment outcome (Kanof, *et al*, 1993; Unnithan, *et al*., 1992). Thus, the goal of adequately suppressing withdrawal across the inter-dosing interval is also likely to help normalise mood state.

In the previous chapter it was demonstrated that the magnitude of withdrawal across the inter-dosing interval was significantly less for patients maintained on LAAM than when on methadone. Furthermore, those who complained of break-through withdrawal while on methadone reported significant suppression in withdrawal when transferred to LAAM. The primary aim of this aspect of the study was to examine the patterns of mood states for methadone and LAAM in order to determine if suppression of withdrawal is associated with improvements in affective state. This chapter will begin with a review of the literature regarding the effects of methadone treatment on affective states.

6.1.1. Methadone maintenance treatment and affective states.

Opioid dependent individuals who are not in treatment are more likely to experience significant psychological morbidity (Rounsaville and Kleber, 1985; Ward, *et al*., 1998c). Population surveys in the United States have demonstrated that, in comparison to individuals not diagnosed with an opioid use disorder, opioid dependent individuals were estimated to be five times (odds ratio=5.0) and three times (odds ratio=2.8) more likely to

be diagnosed with an affective disorder and anxiety disorder, respectively (reviewed by Ward, *et al*., 1998c). However, while it is clear that there is a relationship between opioid dependence and affective disorders, the nature of the relationship is not clear. For example, negative mood states (such as depression) may precede drug use (Kanof, *et al*, 1993), but can also occur as a consequence of the stressful lifestyle experienced by many opioid dependent individuals (Ward, *et al*., 1998c).

The presence of psychiatric co-morbidity in opioid dependent individuals on admission to maintenance treatment is associated with poorer psychosocial and medical status that in those presenting without such a diagnosis (Cacciola, *et al*., 2001). In particular, patients with a diagnosis of depression at entry tend to respond more poorly to treatment (Broome, *et al*., 1999; Cacciola, *et al*., 2001; Kosten, *et al*., 1986; Rounsaville, *et al*., 1982, 1986), and are at increased risk of relapse to illicit drug use (Compton, *et al*., 2003; McLellan, *et al*., 1983). In an examination of the relationship between psychiatric co-morbidity and treatment retention amongst patients participating in the Drug Abuse Treatment Outcome Studies (DATOS) carried out in the United States, a diagnosis of depression was associated with early dropout from outpatient MMT (Broome, *et al*., 1999). Thus, the effective treatment of anxiety and depression amongst MMT patients will help to reduce psychological morbidity and the harms associated with continual drug use, as well as improve treatment retention.

Several authors have described a decline in the prevalence and severity of affective states (i.e., in particular depression) amongst MMT patients over time compared to before treatment (Callaly, *et al.*, 2001; Steer and Kotzer, 1980; Strain, *et al*., 1991). Such changes can be attributed to both pharmacological (i.e., suppression of withdrawal) and non-pharmacological (i.e., treatment interventions, such as counseling) aspects of methadone maintenance treatment, in addition to the alleviation of the stress associated with the patients' previous lifestyle (Ward, *et al*., 1998c). Furthermore, methadone has non-opioid mechanisms of action that may have a positive impact on an individual's affective state. That is, methadone is an *in vitro* inhibitor of noradrenaline and serotonin reuptake (Ciofalo, 1974; Codd, *et al*., 1995). Such actions have been associated with antidepressant effects (Ressler and Nemeroff, 2000).

However, there is also evidence that individuals in MMT still exhibit significant psychiatric distress, and in particular that depressive disorders (Darke, *et al*., 1994; Woody, *et al*., 1985; Strain, *et al*., 1991) and anxiety disorders (Darke, *et al*., 1994; Woody, *et al*, 1985) remain common. Furthermore, there is also evidence that certain aspects of methadone's pharmacological profile may have negative affective consequences for some MMT patients. That is, methadone has receptor actions, other than at the μ opioid receptor, which may mediate negative affective consequences (Olsen, *et al*., 1977; Scott, *et al*., 1948). Furthermore, significant fluctuations in mood states occur in response to fluctuations in plasma methadone concentration across the inter-dosing interval (Dyer, *et al*., 2001). These issues will be discussed in more detail in the upcoming sections.

6.1.2. Pharmacodynamic mechanisms – influence on affective states.

As discussed in chapter one, the primary opioid actions (e.g., suppression of withdrawal, euphoria) of methadone are mediated via the μ opioid receptor. (R)-(-) methadone has greater affinity for the μ receptor, relative to (S)-(+) methadone, which is reflected in its greater potency *in vivo* (Olsen, *et al*., 1977; Scott, *et al*., 1948). However, despite lacking potency as an opioid, (S)-(+) methadone may mediate certain adverse effects. The administration of (S)-(+) methadone (dosage 650 to 1000 mg) alone to humans has been associated with some opioid effect (analgesia, euphoria, slight respiratory depression) (Olsen, *et al.*, 1977; Scott, *et al.*, 1948), but it is also associated with adverse side effects, such as nervousness, confusion, and hallucinations that are not necessarily associated with opioid receptor activation, and which increase with chronic dosing (Fraser, *et al*., 1962; Olsen, *et al.*, 1977; Scott, *et al.*, 1948). Hence (S)-(+) methadone may contribute to the adverse effects of methadone maintenance treatment.

Recently, Mitchell and colleagues (2004) measured mood, using the POMS, and withdrawal across the 24 h methadone-interdosing interval in a cohort of 55 MMPs (mean±sd daily dose: 80±48). They used linear regression to determine the degree that variability in pharmacodynamic responses was accounted for by the average plasma concentration (C_{av}) of (S)-(+) methadone, controlling for (R)-(-) methadone C_{av}. (S)-(+) methadone C_{av} accounted for significant additional variance, over that accounted for by (R)-(-) methadone C_{av}, for a number of negative POMS mood subscales (Tension, Fatigue and Confusion). These results suggest that (S)-(+) methadone was likely to be partly responsible for adverse affective effects experienced by these patients.

Moreover, in addition to its primary opioid mediated effects, methadone has non-opioid actions that may impact negatively on mood states. Methadone acts as a noncompetitive antagonist at NMDA receptors (Ebert, *et al*., 1995; Gorman, *et al*., 1997), having similar affinity as dextromethorphan and ketamine for this receptor (Ebert, *et al*., 1995; Gorman, *et al*., 1997), both of which are commonly used NMDA antagonists. Interestingly, the clinical use of such compounds amongst healthy volunteers and patients has been associated with severe mood disturbance (Abi-Saab, *et al*., 1998; Adler, *et al*., 1999; Curran and Monaghan, 2001; Curran and Morgan, 2000). It is conceivable that the NMDA actions of methadone may have negative affective consequences for maintenance patients in the long term, and that this may occur even with adequate withdrawal suppression.

6.1.3. Mood state changes following administration of methadone.

Several studies have demonstrated fluctuations in mood state among MMT patients following a single dose and at presumed steady state (Dyer, *et al*., 2001; Hiltunen, *et al*., 1999; Price, *et al*., 1975). Some studies have shown that the administration of methadone is associated with almost immediate and positive changes in mood (Holloway, 1993; Price, *et al*., 1975). Price and colleagues used the POMS to examine the effect of a single dose of oral methadone (20-40 mg) on mood states in 49 heroin users at entry to a methadone detoxification program. Patients exhibited overt signs of withdrawal, and reported significant mood disturbance at the commencement of the program. Significant improvements in mood states, i.e. reduction in severity of depression and anxiety, and increase in magnitude of vigour, were evident within 45 minutes of methadone ingestion. Holloway (1993) also reported similar dramatic changes in mood in stable MMT patients 90 minutes after the ingestion of their oral methadone dose, compared to that measured 30 minutes prior to dosing. In particular, after dosing, patients reported positive mood (increased vigour, friendliness, and arousal), and a reduction in the intensity of negative mood states, such as anxiety, depression, anger and confusion

6.1.4. Fluctuations in mood states in response to changes in plasma methadone concentration.

More recently, studies have described the changes in mood state across the interdosing interval in MMT patients. Such changes in mood states have been shown to be related to the degree in which methadone was perceived by patients to suppress withdrawal across

the dosing interval. Hiltunen and colleagues (1999) compared objective and subjective signs of well being in two groups of methadone maintained patients who reported either satisfaction (n=25, mean±sd methadone dose=86.2±4.3 mg) or dissatisfaction (n=25; mean±sd dose=69.2±4.0 mg) with their daily methadone dose. The groups could not be differentiated on the basis of trough plasma racemic or (R)–(-) methadone concentrations. Plasma racemic and (R)–(-) methadone concentration–time profiles, for both groups, were also very similar. During the eight hours after dosing the subjective signs of well being (including ratings for anxiety, irritability, and tension) were relatively stable in the satisfied group, but showed significant temporal changes in the dissatisfied group that appeared to be correlated with the plasma methadone time profile.

Moreover, Dyer and colleagues (2001) measured changes in mood in eighteen MMT patients, nine of whom self reported breakthrough withdrawal, across methadone's interdosing interval. The patients filled out the POMS on eleven occasions for 24 hours after dosing. Scores for all the negative mood states measured by the POMS (anger, confusion, depression, fatigue, and tension) were maximal pre-dose, but were minimal at the times corresponding to putative peak plasma methadone concentration. Scores for vigour (positive mood state) were the reverse of that seen for negative mood states, with a trough pre-dose and were maximal at the time of putative peak plasma methadone concentration. Furthermore, the degree of mood change was particularly pronounced in patients who consistently reported breakthrough withdrawal during the inter-dosing interval. Dyer and colleagues also provided a possible pharmacokinetic explanation for this finding. They showed that there was a relatively steep plasma concentration relationship for the Total Mood Disturbance (TMD) score (mean±sd slope factor = 2.2±0.5, n=14), such that a relatively small change in plasma methadone concentration would translate into significant mood change. Hence, as nonholders exhibited greater fluctuation in plasma methadone concentrations across the inter-dosing interval, they were more likely to experience significant mood change than holders. Given that a major objective of methadone maintenance is to stabilize the physiological and psychological state of the opioid dependent patient, the presence of persistent negative mood in MMT patients is particularly problematic

6.1.5. The present study.

The reviewed studies indicate that some MMT patients will experience considerable fluctuations in mood across the inter-dosing interval that appear to be related to the plasma methadone concentration time course. Thus, even with the use of adequate methadone dosages and when MMT patients report that their dose adequately suppresses withdrawal across the inter–dosing interval, patients may still exhibit significant mood disturbance and be predisposed to poor treatment outcomes. The presence of such mood states has been shown to make MMT patients more vulnerable to craving and have been associated with an increased relapse to heroin use (Calsyn, *et al*., 2000; Cummings, *et al*., 1980; Kanof, *et al.,* 1993; Unnithan, *et al*., 1992). As discussed in chapter one, a number of clinical strategies may be utilised to overcome this problem. These include increasing the daily methadone dosage, but this would increase the incidence and severity of adverse opioid effects, such as respiratory depression, and shortening the inter-dosing interval by using split dosing (Nilsson, *et al.*, 1983), which can have practical implications where it is not possible to supervise dosing. As there is a relatively steep plasma concentration-effect relationship for mood disturbance, transferring patients to a long acting opioid agonist, such as LAAM, to lessen the fluctuation in opioid concentrations over the dosing interval, is likely to benefit patients who experience significant mood disturbance that is associated with greater fluctuations in plasma methadone concentration across the inter-dosing interval.

There are other ways in which the pharmacological profile of LAAM differs from methadone and which may have important affective consequences. LAAM has been shown to be significantly less potent than methadone at inhibiting serotonin (mean K_i: 1,440 nM vs 14.1 nM) and noradrenaline uptake (mean K_i; 67, 400 nM vs 702 nM) (Codd, *et al*., 1995). To date no data are available with regard to its activity at the NMDA receptor. Thus, given that LAAM has different pharmacokinetic and pharmacodynamic profiles than methadone, such factors may result in a different pattern of affective responses exhibited by patients when maintained on LAAM than when maintained on methadone.

The purpose of this study was to compare affective states (mood, depression and anxiety) in maintenance patients during the interdosing interval for methadone and LAAM. As previously stated, patients participating in this study included those who reported break-

through withdrawal on methadone (non-holders) and those who did not (holders). The specific aims of the work in this section were:

- To compare depression scores (Beck Depression Inventory-II) and anxiety scores (STAI) for methadone and LAAM, and to determine whether these scores differ for methadone holders and non-holders.
- To compare the temporal change and intensity of mood states across methadone's and LAAM's inter-dosing interval.
- To determine whether the intensity and temporal profile of mood states during the inter-dosing intervals of methadone and LAAM differ for methadone holders and non-holders.

6.1.6. Hypotheses.

- The intensity and temporal fluctuation in the mean Total Mood Disturbance score for methadone and LAAM will be related to the severity and temporal fluctuation in the withdrawal scores for methadone and LAAM, respectively.
- The intensity and temporal fluctuation in mood state scores will be greater for methadone than LAAM. The temporal fluctuation in mood state scores across the methadone inter-dosing interval, and across the LAAM inter-dosing interval, will be closely related to the underlying plasma (R)-(-) methadone, and nor- and dinor-LAAM concentration versus time profiles, respectively.

6.2. Methods.

The subjects and general procedures used in the methadone and LAAM inter-dosing studies were outlined in detail in chapter two. The affective responses for both (R)-(-) methadone and LAAM were contrasted in sixteen maintenance patients (8 self reported methadone nonholders and 8 self reported holders) who participated in both methadone and LAAM interdosing studies. The affective responses in drug-free control subjects were also contrasted to maintenance patients. Maintenance patients filled out the measures of depression (Beck Depression Inventory) and anxiety (State Trait Anxiety Inventory) once only, prior to ingesting their dose, at the commencement of the methadone and LAAM inter-dosing interval studies. Control subjects completed these questionnaires at the start of the testing period. Measures of mood states (POMS) were taken on eleven occasions at the following times; approximately 0.5 hours before dosing and at the following times after

dosing with LAAM: 1, 2, 3, 4, 6, 9, 12, 24, 36, 48 hours and approximately 0.5 hours before dosing and the following times after dosing with methadone: 1, 2, 3, 4, 5, 6, 7, 9, 12, 24. Control subjects filled out the POMS at baseline and at 1, 2, 3, 4, 6, 9, 12, 24, 36, 48 hours later.

6.2.1. Measures.

The Beck Depression Inventory (BDI –II revised) (Beck, et al., 1996) is a self-report measure developed to indicate the presence and severity of depressive symptoms, though it is not designed to diagnose specific depressive disorders. It contains 21 groups of statements, each with 4 statements, that focus on the cognitive distortions that underlie depression (e.g., pessimism, guilt, crying etc.) and a number of performance variables that might be affected (e.g., sleep problems, loss of interest in sex). Respondents selected which statement best reflected the way they had been feeling over the two weeks prior to the testing session, including the day they filled out the questionnaire. The BDI items are rated between 0 and 3, where 0 indicates normal functioning, and 3 indicates severely disrupted functioning. A total depression score is obtained by summing the ratings for each the statement. Possible depression scores range from 0 to 63. As a general guide, a score of between 0-13 is considered asymptomatic; a score of 14 to 19 indicates mild-to-moderate depression; a score of 20 to 28 indicates moderate-to-severe depression; and a score of 29 or more indicates extremely severe depression (Beck, *et al*., 1996).

The State-Trait Anxiety Inventory (STAI: Spielberger, et al., 1983) is a widely used inventory of anxiety. It contains two separate self-report scales for assessing 'state' and 'trait' anxiety; 'state' anxiety refers to transitory feelings of fear or worry that might occur in a given situation, while 'trait anxiety refers to the relatively stable tendency for an individual to respond anxiously to a given situation. In the current study only the 'trait' anxiety was of interest. Individuals who score highly on the T-scale are more likely to respond anxiously to a threatening situation (Spielberger, *et al*., 1983). The 'trait' anxiety scale comprises 20 phrases, such as 'I feel pleasant', and 'I feel like a failure'. Respondents rated their response to each phrase by selecting one of four statements that best described how they generally felt (i.e., 'almost never', 'sometimes, 'often', 'Almost Always'). These are given a weighted score of between 1 and 4. Half of the items are reverse coded and all scores are summed and can range from 20–80, with higher scores indicating higher levels of trait-Anxiety. An asymptomatic score for normal working

adults is considered to be 35 (Spielberger, *et al*., 1983). The STAI is a valid measure in that both scales have been shown to distinguish reliably between psychiatric and nonpsychiatric patients and trait anxiety correlates well with other measures of trait anxiety (Spielberger, *et al*., 1983).

The *Profile of Mood States* (POMS) (McNair, *et al*., 1971) was devised to assess 'transient, fluctuating affective states'. It lists 65 adjectives (e.g., tense, miserable, muddled) or short phrases (e.g., sorry for things done, ready to fight) which are rated by the patient on a five-point scale of 0 (not at all) to 4 (extremely). In this study subjects rated how they felt at the time of filling out the questionnaire (i.e., "Right Now"). The items are scored and grouped into six identifiable subscales that were derived by factor analysis. These include: tension-anxiety (possible score 0-36), depression-dejection (possible score 0-60), anger-hostility (possible score 0-48), fatigue–inertia (possible score 0-28), confusion-bewilderment (possible score 0-28), and vigour-activity (possible score 0-32). Seven POMS items are not included in any of these factor scores. A total Mood Disturbance Score (TMD) can also be obtained by summing the scores across all six subscales (weighting vigour negatively). The TMD represents a "global estimate of affective state" and scores range from -32 to 200, such that high scores indicate more negative mood.

6.2.2. Statistical Analyses.

BDI-II and STAI sores for methadone and LAAM were compared using Student's *t*-tests for paired samples, and these same scores for holder and non-holder groups were compared using Student's *t*-tests for independent samples. The sign test was used to compare the proportion of patients scoring within the asymptomatic, mild-moderate and severe range of scores on the BDI-II scale when maintained on methadone and LAAM.

One way ANOVAs were used to determine the significance of changes across the inter-dosing interval for each mood scale, for patients when they maintained on methadone and LAAM, and for control subjects. For TMD within group comparison were performed between the pre-dose or baseline measurement and each subsequent measurement point for each group using the post hoc Bonferroni tests for selected comparisons (GraphPad Prism v3.02, Graphpad Software, CA, USA). To compare the overall magnitude of effects of methadone with LAAM on each mood scale the area under the effect versus time curve

over the 24 hour inter-dosing interval for methadone (AUC_{0-24}; to allow 2X AUC_{0-24} to be determined) and the 48 hour inter-dosing interval for LAAM (AUC_{0-48}) were calculated by the linear trapezoidal rule (GraphPad Prism v3.02, GraphPad Software, CA, USA). The AUC for TMD and each mood scale is expressed as score·hours. For each mood scale Students *t*-tests for paired samples were used to compare the sum of 2x AUC_{0-24} values for methadone with one AUC_{0-48} value for LAAM, and the AUC values for holder and non-holder groups when maintained on either methadone or LAAM. Students *t*-test for independent samples were used to compare the sum of 2x AUC_{0-24} values for methadone with one AUC_{0-48} value for control subjects and the AUC_{0-48} value for LAAM with the AUC_{0-48} value for control subjects.

Two–way repeated measures ANOVA was used to examine the effect of time since dosing and methadone self reported holding status on each mood scale for patients when they were maintained on methadone and LAAM. For repeated measures, degrees of freedom were adjusted for violations of the sphericity assumption using Greenhouse Geisser corrections provided by SPSS.

Relationships between variables were determined using Pearson's product–moment coefficient. Unless otherwise stated the alpha level for all statistical analyses were set at P=0.05 and all statistical tests were two tailed and data are tabulated as mean±sd. The data were analysed using SPSS™ for Windows (v.10), unless otherwise stated. SPSS undertakes Levene's test of homogeneity of variance when undertaking *t*-tests, and adjusts the probability level accordingly. Where appropriate 95% confidence intervals [CI] of differences between means were also calculated.

6.3. Results.

6.3.1. Depression and Anxiety.

Scores on the Beck Depression Inventory are contrasted for methadone and LAAM in table 6.1. BDI scores were lower for LAAM compared to methadone for the patients as a whole, but this difference was not significant (P=0.115; -5.6; -12.6, 1.15). Mean BDI scores for all patients were within the mild-moderate range for both methadone and LAAM. There were no significant differences between methadone and LAAM in the

proportion of patients scoring within the asymptomatic (6 vs 11), mild to moderate (2 vs 2), or severe (8 vs 3) ranges (sign test *P*=0.51). For non-holders, BDI scores were significantly greater than for holders while on methadone (*P*=0.09; -14.8; -25.2, -4.3), but there was no difference between holders and nonholders on LAAM (*P*=0.88; 0.88; -8.9, 10.7). Furthermore, for holders BDI scores were not significantly different when they were maintained on methadone compared to LAAM (*P*=0.16; 2.3; -1.2, 5.7). In contrast, non-holders' scores were significantly greater when they maintained on methadone compared to LAAM (*P*=0.039; -13.4; -25.8, -09). As expected, BDI scores for patients when on methadone and LAAM were significantly greater than for control subjects (*P*=0.001; 16.7; 8.6, 24.7 and *P*<0.01; 11.1; 6.3, 15.9, respectively).

Table 6-1: Beck Depression Inventory (BDI)-II scores (possible range 0-63) for methadone and LAAM for all patients (n=16) who participated in the methadone and the LAAM interdosing intervals studies ((holders (n=8) and non-holders (n=8)), and for control subjects (n=10).

	All subjects	Holders	Non-holders
Methadone	18.4±12.1	11.0±9.7	25.8±9.8*†
LAAM	12.8±8.8	13.3±10.8	12.4±7.1
Control	1.7±2.1# ^		

Notes: Data are mean±sd. * *P*<0.05 holder vs non-holders (independent *t*-test); † *P*<0.05 methadone vs LAAM (paired *t*-test); # *P* <0.001 methadone vs controls (independent *t*-test); ^ *P* <0.01 LAAM vs controls (independent *t*-test).

STAI Trait anxiety scores are shown for methadone and LAAM in relation to control subjects in table 6.2. For all patients STAI anxiety scores were similar for both methadone and LAAM (*P*=0.912; -0.32; -6.2, 5.5). For non-holders, anxiety scores were significantly greater than for holders when they were maintained on methadone (*P*=0.016; -14.88; -26.6, -3.2), but not when maintained on LAAM (*P*=0.35; -5.5; -17.7, 6.7). Furthermore, for holders anxiety scores were not significantly different when they were maintained on methadone compared to LAAM (*P*=0.17; 4.4; -2.4, 11.2), similarly there was no significant difference in anxiety scores for non-holders when maintained on methadone compared to LAAM (*P*=0.280; -5.00; -15.1, 5.10). STAI anxiety scores for patients when on methadone and LAAM were significantly greater than for control subjects (*P*<0.001; 24.1;16.1, 32.1 and *P*<0.001; 23.8; 15.6, 32.0, respectively).

Table 6-2: STAI trait anxiety scores (possible range 20 – 80) for methadone and LAAM for all patients (n=16) who participated in the methadone and the LAAM interdosing intervals studies ((holders (n=8) and non-holders (n=8)), and for control subjects (n=10).

	All subjects	Holders	Non-holders
Methadone	50.2±13.0	42.8±12.9	57.6±8.34*****
LAAM	49.9±11.3	47.1±9.58	52.6±12.9
Control	26.1±6.62$^{\dagger\,\#}$		

Notes: Data are mean±sd. * $P<0.05$ holder vs non-holders (independent *t*-test); † $P<0.001$ methadone vs controls (independent *t*-test); $^{\#}$ $P<0.001$ LAAM vs controls (independent *t*-test).

6.3.2. Mood states across the inter-dosing interval for all patients.

Mean Total Mood disturbance (TMD) scores for patients on methadone and LAAM across the interdosing interval and for control subjects are shown in figure 6.1. Mean TMD was maximal pre-dosing for both drugs, which decreased to be minimal at three hours after dosing. Thereafter TMD scores increased to baseline values for methadone, but remained relatively stable for LAAM through to the end of the inter-dosing interval.

TMD Scores for both methadone and LAAM showed significant fluctuations over time (($F(10.\ 150)=5.8$, $P<0.0001$), and ($F(10, 150)=2.2$, $P=0.018$), respectively), but remained stable for control subjects ($F(10, 90)=0.6$, $P=0.83$). Mean TMD scores for methadone were significantly lower than the predose score at 1 hour ($P<0.05$; 18.63; -34.7, -2.5), 2 hours ($P<0.001$; 24.13; -40.2, -8.1), 3 hours ($P<0.01$; 22.50; -38.6, -6.4), and 4 hours ($P<0.001$; 23.2; -39.3, -7.1) after dosing, but at no another time point ($P>0.05$). Furthermore, mean TMD scores for LAAM were significantly lower than the predose score at 2 hours ($P<0.01$; 15.31; -28.9, -2.9), 3 hours ($P<0.01$; 15.88; -28.9, -2.9), and 24 hours ($P<0.001$; 18.44; -31.4, -5.5) after dosing, but at no other time point ($P>0.05$). The temporal fluctuations in mean TMD score for both drugs were consistent with changes in plasma concentrations of (R)-(-) methadone (see section 3.4.3) and LAAM's active metabolites (see section 4.4.3), and other pharmacodynamic effects, in particular withdrawal severity, reported in previous chapters.

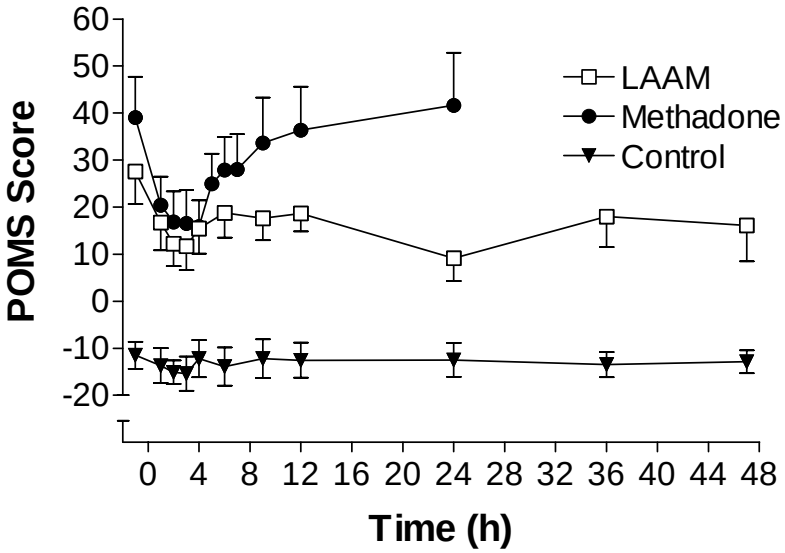

Figure 6-1*: *Mean±SEM Total Mood Disturbance scores for the Profile of Mood States (range of possible scores -32 to 200) in patients (n=16) who underwent one 24-h inter-dosing interval study when taking methadone (closed circles) and one 48-h inter-dosing interval study when taking LAAM (open squares) and in 10 drug free control subjects (closed triangles).

The sum of two AUC $_{0\text{-}24}$ values for TMD in patients when maintained on methadone (mean±sd: 3238±1662 score·hour) was significantly greater than the AUC $_{0\text{-}48}$ value for TMD in patients when maintained on LAAM (2281±933 score·hour, *P*=0.048) (see table 6.4). Hence, the overall magnitude of total mood disturbance was greater in patients when maintained on methadone than when maintained on LAAM. Furthermore, the sum of two AUC $_{0\text{-}24}$ values in patients on methadone and the AUC $_{0\text{-}48}$ value for patients on LAAM, were greater than the $AUC_{0\text{-}48}$ value for control subjects (see table 6.4).

Correlation analysis was used to determine the relationship between the mean AUC for TMD scores and the severity of withdrawal experienced by patients when maintained on either methadone or LAAM. In methadone patients the AUC for TMD was significantly positively correlated with the AUC for withdrawal (*r*=0.68, *P*=0.004). In contrast, in LAAM patients the AUC for TMD was not significantly correlated with the AUC for withdrawal (r=0.49, *P*=0.06).

Table 6.3 summarises the repeated measures ANOVA used to determine the effect of time since dosing on each of the POMS scales. There were significant temporal changes across the inter-dosing interval for each POMS scale, with the exception of confusion, in patients

when maintained on LAAM and methadone. In contrast, scores for each POMS scale in drug free control subjects were stable across time.

Table 6-3: One way repeated measures analysis of variance for each POMS subscale for patients participating in the methadone interdosing study, and the LAAM interdosing study (n=16) and control subjects (n=10). Values except DF are F ratios.

Group	DF	POMS scales[1]					
		Anger	Conf	Depress	Fatigue	Tension.	Vigour
Methadone	10, 150	2.24*	3.06**	2.92**	3.45***	7.83***	4.28***
LAAM	10, 150	1.91*	0.8 NS	4.48***	2.84**	4.29***	2.66**
Control	10, 90	0.88 NS	1.54 NS	1.53 NS	1.53 NS	0.41 NS	1.98*

Notes: [1]Anger; Conf=Confusion; Depress=Depression; Fatigue; Tension; Vigour. * P <0.05, ** P<0.01, *** P <0.001. NS=Not significant

Figure 6.2 presents the temporal pattern for each of the POMS subscales. For the negative mood scales the temporal pattern was similar to that observed for TMD, with mean POMS scores maximal prior to dosing for both drugs, which decreased to be at minimal levels 3 to 4 hours following dosing. Thereafter, scores increased to baseline values for methadone, but remained relatively stable for LAAM through to the end of the dosing interval. As would be expected there was diurnal variation in mean fatigue scores across the inter-dosing interval, with highest scores reported in the evening on each day (12 hours after dosing for methadone and 12 and 24 hours after dosing for LAAM). An inverse pattern was evident for mean vigour scores, with scores peaking at 1 to 2 hours after dosing at higher levels relative to baseline, for both drugs. Mean scores than decreased to below baseline levels by 12 hours post dosing. Thereafter, scores for methadone remained stable across the remainder of the inter–dosing interval. For LAAM there was marked diurnal variation in mean vigour scores that was the inverse of that seen for fatigue, in that lowest scores were evident in the evening.

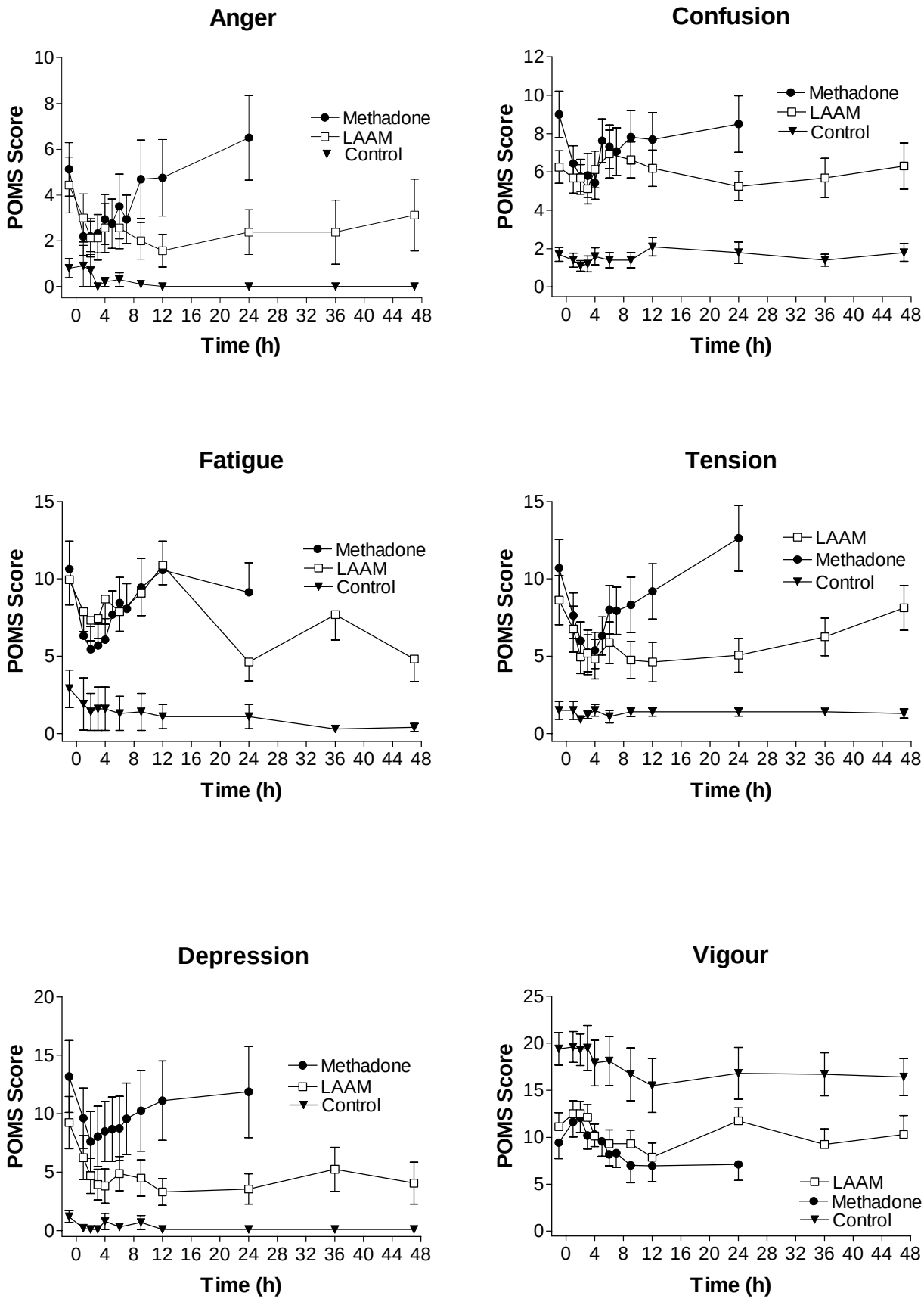

Figure 6-2: Profile of Mood States (POMS) scores during a 24 hour interdosing interval for methadone, and a 48 hour interdosing interval for LAAM (n=16), and for drug-free control subjects (n=10).

The AUC $_{0\text{-}24}$ and AUC $_{0\text{-}24}$ x 2 values in patients maintained on methadone and the $AUC_{0\text{-}48}$ values in patients maintained on LAAM, for TMD and each POMS subscale, are presented in table 6.4. The mean $AUC_{0\text{-}24}$ x 2 value for methadone was significantly greater than the mean $AUC_{0\text{-}48}$ value for LAAM for TMD (P=0.048), and the anger (P=0.047) and tension subscale (P=0.047). There were no other significant between drug differences.

The mean $AUC_{0\text{-}24}$ x2 and $AUC_{0\text{-}48}$ values in methadone and LAAM patients, respectively, for each POMS scale (except for vigour) were significantly greater than the corresponding mean $AUC_{0\text{-}48}$ value in control subjects ($0.001<P\leq 0.025$). The mean $AUC_{0\text{-}48}$ value in control subjects for the vigour subscale was significantly greater than the corresponding score in patients when maintained on methadone and LAAM (P<0.003 and P<0.01, respectively). (See Table 6.4).

Table 6-4: Comparison of area under the effect versus time curve for each POMS scale for patients (n=16) who underwent one 24-h inter-dosing interval study when taking methadone and one 48-h inter-dosing interval study when taking LAAM, and for control subjects (n=10).

POMS Scale[1]	Methadone		LAAM	*Controls*	P[3] *(mean diff, 95% CI)*	P[4] *(mean diff, 95% CI)*	P[5] *(mean diff, 95% CI)*
	AUC_{0-24}	AUC_{0-24}x 2	AUC_{0-48}	AUC_{0-48}	Meth AUC_{0-24}x 2 vs LAAM	LAAM vs Control	Meth AUC_{0-24}x 2 vs Control
TMD[2]	1618±830	3238±1662	2281±933	931±471	**0.048** (-956; -1905, -7.96)	**<0.001** (1350; 691, 2009)	**<0.001** (2306; 1381, 3231)
Anger	113±118	226±236	114±177	4.2±7.11	**0.047** (-112; -222, -1.41)	**0.025** (110; 15.5, 204)	**0.001** (221; 95.7, 347)
Confusion	189±117	379±234	282±160	79.6±58.1	0.16 (-97.3; -236, 41.1)	**0.001** (202; 92.6, 311)	**<0.001** (299; 170, 428)
Depression	264±319	528±638	215±266	9.40±17.3	0.058 (-313; -638, 12.7)	**0.008** (206; 63.6, 348)	**0.005** (518; 178, 858)
Fatigue	223±148	446±295	348±183	45.7±99.3	0.28 (-97.8; -282, 86.4)	**<0.001** (303; 172, 433)	**<0.001** (400; 233, 568)
Tension	230±161	461±322	277±213	65.6±32.6	**0.047** (-184; -364, -3.22)	**<0.001** (211; 96.7, 326)	**<0.001** (395, 223, 568)
Vigour	202±139	404±278	484±246	810±354	0.181 (80.2; -41.6, 202)	**0.01** (-326; -599, -52.9)	**0.003** (-406; -568, -83.7)

Notes: [1] The AUC for each POMS score is expressed as score·hours; [2] TMD=Total Mood Disturbance; [3] Paired *t*-test between the AUC_{0-24}x 2 value for methadone and the AUC_{0-48} value for LAAM; [4] Independent samples *t*-test between the AUC_{0-48} value for LAAM and the AUC_{0-48} value for drug free control subjects; [5] Independent samples *t*-test between the AUC_{0-24}x 2 value for methadone and the AUC_{0-48} value for drug free control subjects; Significant *P* values highlighted in bold. Values are mean±sd

6.3.3. Fluctuations in mood across the inter-dosing interval–Holders vs non-holders.

Table 6.5 and 6.6 presents the results of the repeated measures ANOVA comparing the effect of holding status on each of the POMS scales, for methadone and LAAM, respectively. There were significant between group (holders vs non-holder) differences for TMD (P=0.016), confusion (P=0.036), fatigue (P=0.01) and tension (P=0.009), but no other POMS scale in patients when maintained on methadone. Consistent with previous analyses that considered the whole group of patients, there were significant temporal changes for holders and nonholders across the 24-hour methadone inter-dosing interval for all POMS scales, except anger. Furthermore, as depicted in Figure 6.3 and 6.6 there were significant holding x time since dosing interactions for TMD (P<0.001) and tension (P=0.002), such that the temporal change in POMS scores differed significantly according to holding status. In contrast, there were no significant between group differences, nor significant interactions, evident for any POMS scale in patients when maintained on LAAM (P>0.37). Significant temporal changes across the 48 inter-dosing interval for LAAM were only evident for depression, fatigue, tension and vigour subscales.

Table 6-5: Repeated measures Analysis of variance for all POMS scales, for patients (n=16) participating in the Methadone inter-dosing study according to holding status [holders (n=8) and non-holders (n=8)].

Parameter	Effect[1]	Df [2]	*F* value	*P* Value[3]
Total Mood disturbance	Hold	1, 14	7.49	**0.016**
	Time	4, 50	7.13	**<0.001**
	Hold x Time	4, 50	4.55	**0.005**
Anger	Hold	1, 14	2.01	0.18
	Time	3, 43	2.30	0.09
	Hold x Time	3, 43	1.52	0.22
Confusion	Hold	1, 14	5.38	**0.036**
	Time	5, 64	3.26	**0.013**
	Hold x Time	5, 64	1.97	0.10
Depression	Hold	1, 14	1.53	0.24
	Time	2, 33	3.27	**0.04**
	Hold x Time	2, 33	2.81	0.07
Fatigue	Hold	1,14	8.82	**0.01**
	Time	4, 55	3.73	**0.01**
	Hold x Time	4, 55	2.24	0.08
Tension	Hold	1, 14	9.04	**0.009**
	Time	4, 61	11.13	**<0.001**
	Hold x Time	4, 61	7.31	**<0.001**
Vigour	Hold	1, 14	0.73	0.06
	Time	4, 59	4.56	**0.002**
	Hold x Time	4, 59	2.00	0.104

Notes: [1]ANOVA effects=within subjects effect as time since dosing (Time), and between subjects effect as methadone holding status (Hold). [2] Degrees of freedom adjusted using Greenhouse Geisser corrections. Probability value was adjusted accordingly. [3] Significant *P* values in bold text.

Table 6-6: Repeated measures analysis of variance for all POMS scales, for patients (n=16) participating in the LAAM inter-dosing study according to holding status [holders (n=8) and non-holders (n=8)].

Parameter	Effect[1]	Df [2]	*F* value	*P* Value[3]
Total Mood disturbance	Hold	1, 14	0.015	0.90
	Time	4, 55	2.15	0.09
	Hold x Time	4, 55	0.37	0.82
Anger	Hold	1, 14	0.041	0.84
	Time	3, 48	1.66	0.18
	Hold x Time	3, 48	0.30	0.85
Confusion	Hold	1, 14	0.008	0.93
	Time	3, 49	0.83	0.50
	Hold x Time	3, 49	0.50	0.72
Depression	Hold	1, 14	0.82	0.38
	Time	3, 40	4.33	**0.01**
	Hold x Time	3, 40	0.52	0.66
Fatigue	Hold	1,14	0.022	0.88
	Time	4, 52	2.81	**0.04**
	Hold x Time	4, 52	0.85	0.49
Tension	Hold	1, 14	0.67	0.43
	Time	3, 48	4.21	**0.007**
	Hold x Time	3, 48	0.73	0.56
Vigour	Hold	1, 14	0.73	0.41
	Time	4, 52	2.65	**0.047**
	Hold x Time	4, 52	0.97	0.43

Notes: [1]ANOVA effects=within subjects effect as time since dosing (Time), and between subjects effect as methadone holding status (Hold). [2] Degrees of freedom adjusted using Greenhouse Geisser corrections. Probability value was adjusted accordingly. [3] Significant *P* values in bold text.

The temporal patterns for TMD, confusion, fatigue, and tension across the inter-dosing interval for self-reported methadone holders and non-holders when maintained on LAAM and methadone are shown in Figures 6.3 to 6.6. For each of the POMS scales depicted, the holders showed similar and relatively stable pattern of mood scores across the inter-dosing interval for methadone and LAAM. In contrast, non-holders reported much greater fluctuation in mood scores when maintained on methadone than LAAM. In order to elucidate the differences between holders and non-holders when maintained on methadone and LAAM the AUC_{0-24} x 2 value in holders and non-holders maintained on methadone and the AUC_{0-48} values in holders and non-holders maintained on LAAM were compared (see table 6.7).

The mean TMD scores for holders and non-holders when maintained on methadone and LAAM are shown in figure 6.3. The sum of two AUC_{0-24} values in holders maintained on methadone was similar to the AUC_{0-48} value in holders maintained on LAAM (*P*=0.63; 131; -49, 701). In contrast, the AUC_{0-48} value in non-holders maintained on LAAM was significantly less than the sum of the two AUC_{0-24} values in non-holders maintained on methadone (*P*=0.019; -2044; -3635, -454). Hence non-holders on methadone benefited, in terms of improved overall mood, from transfer to LAAM maintenance.

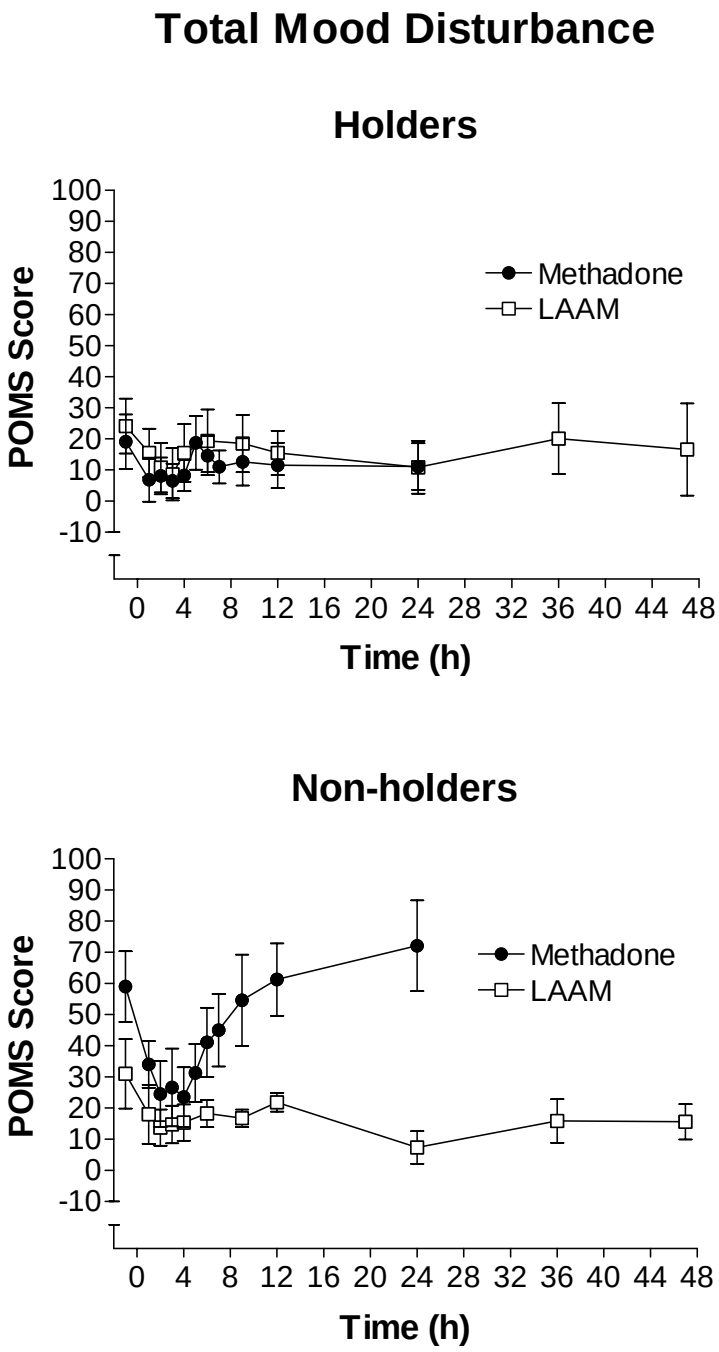

Figure 6-3: Mean TMD scores of eight self reported methadone holders (upper panel) when taking methadone (closed circles) or LAAM (open squares), and 8 self reported methadone non-holders (lower panel) when taking methadone or LAAM. All patients underwent one 24-hour inter-dosing interval study when taking methadone and one 48 hour inter-dosing interval when taking LAAM. Values are mean ± SEM.

The mean confusion scores for holders and non-holders when maintained on methadone and LAAM are shown in figure 6.4. The sum of two AUC_{0-24} values in holders maintained on methadone was similar to the AUC_{0-48} value in holders maintained on LAAM (P=0.37). The AUC_{0-48} value in non-holders maintained on LAAM did not significantly differ from

the sum of the two AUC_{0-24} values in non-holders maintained on methadone. However, the latter difference did approach significance (*P*=0.057). (see table 6.7).

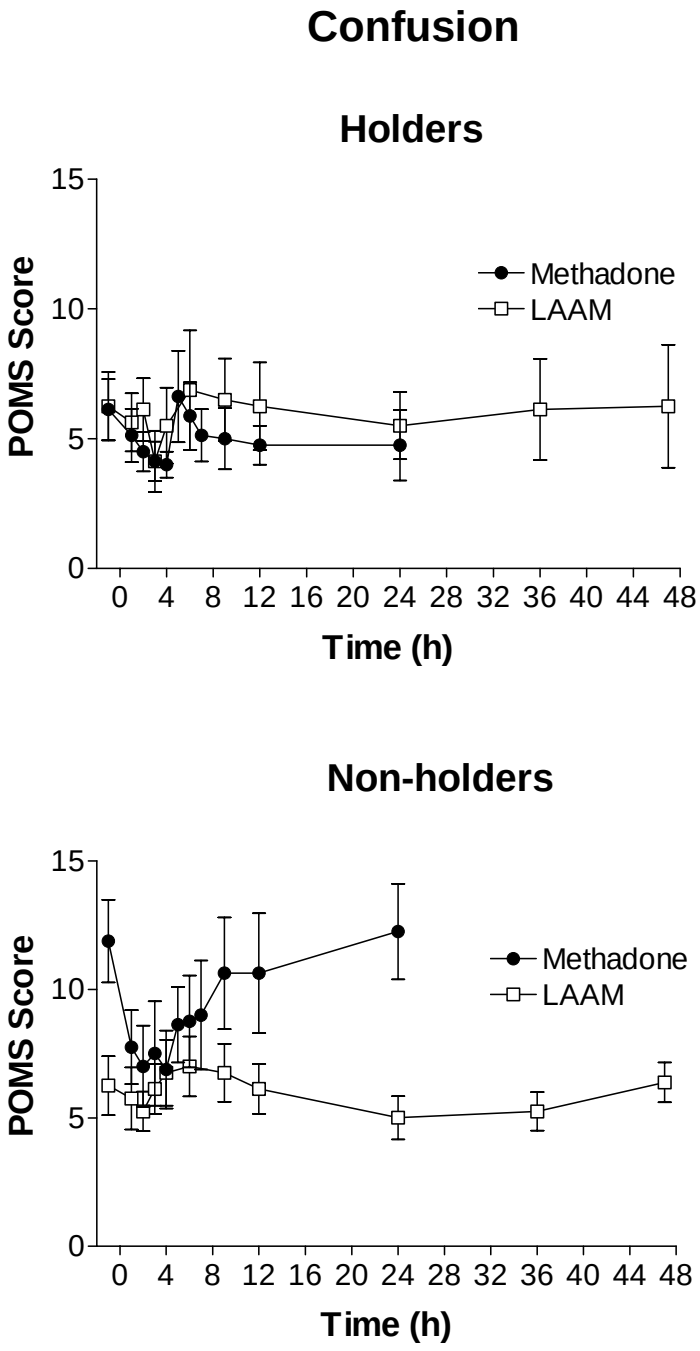

Figure 6-4: Mean Confusion scores of eight self reported methadone holders (upper panel) when taking methadone (closed circles) or LAAM (open squares), and 8 self reported methadone non-holders (lower panel) when taking methadone or LAAM. All patients underwent one 24-hour inter-dosing interval study when taking methadone and one 48-hour inter-dosing interval when taking LAAM. Values are mean ± SE.

The mean fatigue scores for holders and non-holders when maintained on methadone and LAAM are shown in figure 6.5. The sum of two AUC_{0-24} values in holders maintained on methadone was similar to the AUC_{0-48} value in holders maintained on LAAM (*P*=0.14). In

contrast, the AUC_{0-48} value in non-holders maintained on LAAM was significantly less than the sum of the two AUC_{0-24} values in non-holders maintained on methadone (P=0.022). (see table 6.7)

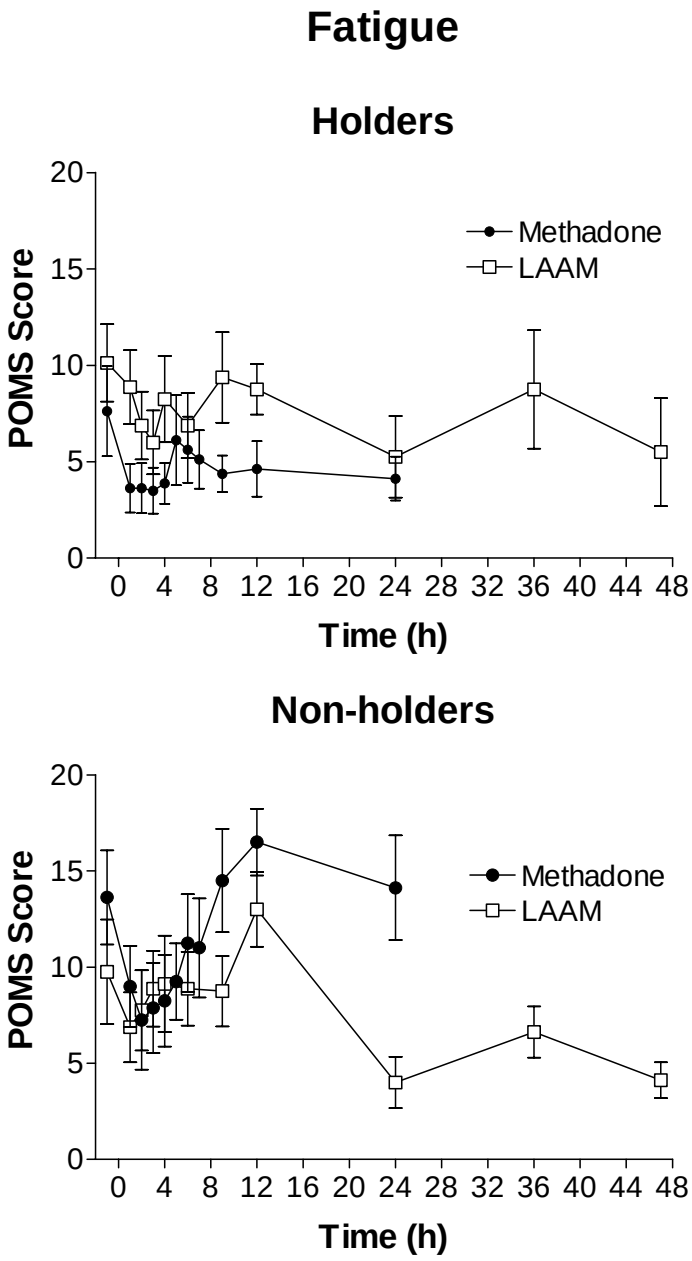

Figure 6-5: Mean Fatigue scores of eight self reported methadone holders (upper panel) when taking methadone (closed circles) or LAAM (open squares), and 8 self reported methadone non-holders (lower panel) when taking methadone or LAAM. All patients underwent one 24-hour inter-dosing interval study when taking methadone and one 48 hour inter-dosing interval when taking LAAM. Values are mean ± SEM.

The mean tension scores for holders and non-holders when maintained on methadone and LAAM are shown in figure 6.6. The sum of two AUC_{0-24} values in holders maintained on methadone was similar to the AUC_{0-48} value in holders maintained on LAAM (*P*=0.56). In contrast, the AUC_{0-48} value in non-holders maintained on LAAM was significantly less than the sum of the two AUC_{0-24} values in non-holders maintained on methadone (*P*=0.026) (see table 6.7) .

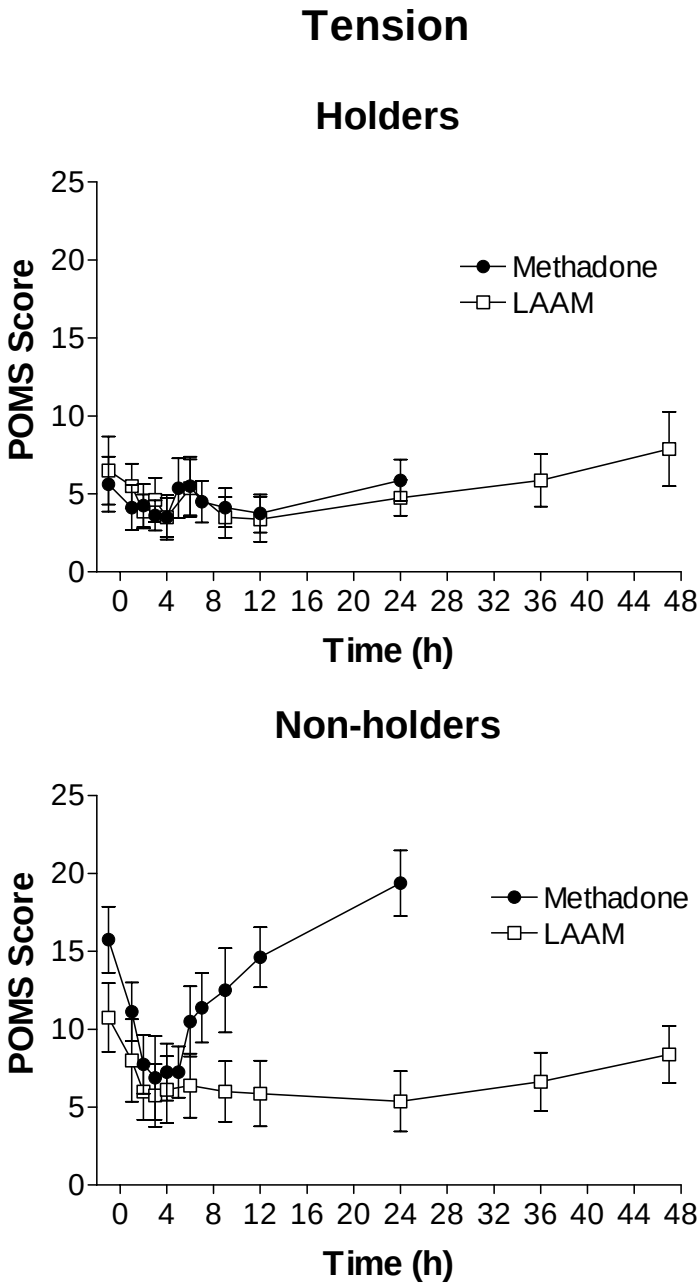

Figure 6-6: Mean Tension scores of eight self reported methadone holders (upper panel) when taking methadone (closed circles) or LAAM (open squares), and 8 self reported methadone non-holders (lower panel) when taking methadone or LAAM. All patients underwent one 24-hour inter-dosing interval study when taking methadone and one 48 hour inter-dosing interval when taking LAAM. Values are mean ± SE

In summary, holders exhibited similar and relatively stable temporal pattern of scores across the inter-dosing interval for both methadone and LAAM, for each POMS scale depicted. In contrast, the non-holders exhibited greater fluctuation of mood when maintained on methadone than LAAM. The results depicted here show that the overall differences seen between methadone and LAAM were likely due to the mood disturbance self reported by the non-holder group.

Table 6-7: Comparison of area under the effect versus time curve for TMD, confusion, fatigue and tension scales for self-reported methadone holders (n=8), and self reported methadone non-holders (n=8) who underwent one 24-h inter-dosing interval study when taking methadone and one 48-h inter-dosing interval study when taking LAAM.

POMS Scale[1]	*Methadone*				*LAAM*		P[3] *(mean diff, 95% CI))*	P[4] *(mean diff, 95% CI)*
	Holders		Non-holders		Holders	*Non-holders*	*Holders*	*Non-holders*
	AUC_{0-24}	AUC_{0-24}x 2	AUC_{0-24}	AUC_{0-24}x 2	AUC_{0-48}	AUC_{0-48}	Meth AUC_{0-24}x 2 vs LAAM AUC_{0-48}	Meth AUC_{0-24}x 2 vs LAAM AUC_{0-48}
TMD[2]	1084±433	2168±865	2154±799	4307±1598	2300±1248	2263±556	0.63 (131; -439, 701)	**0.019** (-2044: -3635, -454)
Confusion	123±60	247±120	256±125	511±250	288±210	276±104	0.37 (41; -60, 142)	0.057 (-236; -481, 9.68)
Fatigue	114±47	228±94	332±132	665±263	354±244	343±111	0.14 (126; -54, 306)	**0.022** (-321; -580, -63)
Tension	115±81	229±161	346±136	693±271	246±161	308±239	0.56 (16, -48, 81)	**0.026** (-384; -706, -62)

Notes: [1] The AUC for each POMS score is expressed as score·hours; [2] TMD=Total Mood Disturbance; [3] Paired *t*-test between the AUC_{0-24}x 2 value in holders when taking methadone and the AUC_{0-48} value in holders when taking LAAM. [4] Paired *t*-test between the AUC_{0-24}x 2 value in non-holders when taking methadone and the AUC_{0-48} value in non-holders when taking LAAM; Significant *P* values highlighted in bold. Values are mean±sd.

6.4. Discussion.

The present study compared the presence and severity of depression and anxiety and the intensity and temporal pattern of mood states in patients maintained on methadone and LAAM. Results show that for all patients maintenance on either drug was associated with similar intensity of depression and anxiety. However, while holders exhibited similar levels of depression and anxiety when maintained on either drug, non-holders exhibited greater depression and anxiety than holders when maintained on methadone, but reported significant improvement when switched to LAAM. With regard to mood states, with the exception of fatigue and vigour scores, maintenance on LAAM was associated with greater consistency in mood states than methadone. The overall magnitude of effect (AUC_{0-24} x2 for methadone and AUC_{0-48} for LAAM) for TMD, anger, and tension was significantly greater for methadone than LAAM. Furthermore, the intensity of TMD was positively related to the magnitude of self-reported withdrawal for patients when taking methadone, but not LAAM. The differences in the temporal pattern of TMD, Confusion, Fatigue, and Tension reported by holders and non-holders while maintained on methadone illustrate the importance of withdrawal as a determinant of mood. The self-reported methadone holders, who reported adequate suppression of withdrawal throughout the inter-dosing interval for both methadone and LAAM, also reported a relatively stable profile of mood states when maintained on both drugs. In contrast, the non-holders, who reported adequate withdrawal suppression for LAAM, but not methadone, reported pronounced fluctuations in mood scores while maintained on methadone, but more stable profile of mood when on LAAM.

Despite improvements in psychiatric co-morbidity reported by maintenance patients in past studies (Callaly, *et al*., 2001; Steer and Kotzer, 1980; Strain, *et al*., 1991), the present study demonstrated that maintenance patients are still likely to experience some psychiatric distress. Overall, patients in this study reported statistically similar depression and trait anxiety scores when maintained on both methadone and LAAM, but significantly greater scores than those reported by control subjects. Mean BDI scores for patients on either drug were within the mildly depressed range, which is consistent with previous studies that have utilised heroin dependent individuals seeking MMT (Steer, *et al*., 1980). This is of considerable clinical significance as maintenance patients diagnosed with depression tend to respond more poorly to treatment (Kosten, *et al*., 1986; Rounsaville, *et al*., 1982, 1986).

Interestingly, effective suppression of withdrawal was related to lower depression and anxiety. Holders, who reported similar intensity of withdrawal on both methadone and LAAM, also exhibited similar levels of depression and anxiety when maintained on either drug. On the other hand, non-holders, who reported more severe withdrawal when on methadone than LAAM also exhibited greater depression and anxiety than holders when maintained on methadone, but reported significant improvement when switched to LAAM. To some extent the improvement seen in the BDI-II scores for non-holders when switched to LAAM was likely to reflect the alleviation of physical symptoms that are associated with withdrawal. The BDI-II is heavily loaded with physical items (i.e., agitation, loss of energy, changes in sleep patterns, irritability, concentration difficulty, tiredness or fatigue) and therefore future studies might consider using depression inventories, such as the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983), which was specifically developed for use in patients with somatic co-morbidity. While not denying that physical symptoms are associated with depression, this strategy will permit the differentiation of primary mood disturbance from that associated with changes in plasma methadone concentration that might be evident in non-holders, and the delivery of appropriate treatment strategies.

In accordance with previous research (Dyer, *et al*., 2001; Hiltunen, *et al*., 1999), the current study demonstrated that patients maintained on methadone experienced considerable fluctuation in mood that appeared to be related to the fluctuation in plasma methadone concentrations (see Chapter 3, figure 3.7). In contrast, control subjects exhibited a relatively stable temporal pattern of mood states and the level of mood disturbance was significantly less than that exhibited by maintenance patients. In methadone patients the negative mood states showed an inverse relationship with putative plasma methadone concentrations, reaching a trough at the time of peak plasma methadone concentration and peak at the end of the inter-dosing interval. Vigour scores showed the opposite relationship, peaking at the time of peak plasma (R)-(-) methadone concentration and decreasing to the end of the inter-dosing interval. However, when switched to LAAM, patients showed a more stable pattern of mood states across the inter-dosing interval, with the exception of fatigue and vigour that showed significant diurnal variation. Furthermore, the overall magnitude of effect (AUC_{0-24} x2 for methadone and AUC_{0-48} for LAAM) for TMD, Anger, and Tension was significantly greater for methadone than LAAM.

Furthermore, a major aim of this study was to determine if the intensity and temporal profile of mood states experienced by holders and non-holders differed when they were maintained on methadone compared to LAAM. As reported in chapter three, nonholders could not be differentiated from holders on the basis of any demographic variable (except body weight and gender ratio), methadone dosage, other illicit drug use, nor trough or peak plasma methadone concentrations. However, non-holders exhibited greater fluctuation in plasma methadone concentrations across the inter-dosing interval than holders. Dyer and colleagues (2001) previously demonstrated that there is a relatively steep plasma concentration-effect relationship for TMD in MMT patients. Thus, non-holders are more likely to experience significant changes in mood states across the inter-dosing interval than holders. Therefore it was hypothesised that transferring non-holders to LAAM, to flatten the plasma concentration profile over the inter-dosing interval, should help reduce the severity of mood disturbance experienced by these patients. Results from this study show that non-holders on methadone exhibited greater level of mood disturbance as measured by TMD, Confusion, Fatigue, and Tension, than holders. These differences would be consistent with their greater level of opioid withdrawal previously reported. When methadone nonholders were switched to LAAM they experienced less temporal fluctuation in the intensity of the above-mentioned mood states and significantly less magnitude of effect (AUC_{0-24} x2 for methadone and AUC_{0-48} for LAAM) for TMD, Fatigue and Tension. Also important was the finding that holders experienced similar degree of temporal fluctuation and severity of mood disturbance across the inter-dosing interval when maintained on either drug. These findings are particularly important as the presence of inconsistent and persistent negative mood states has been shown to increase the perceived severity of withdrawal symptoms (Brehm and Khantzian, 1997; Childress, *et al*., 1994; Phillips, *et al*., 1986) and are also associated with relapse to drug use (Kanof, *et al*, 1993; Unnithan, *et al*., 1992). Hence the use of LAAM on a second daily dosage schedule is likely to facilitate stability of mood states in those patients who consistently report breakthrough withdrawal whilst maintained on methadone. The next chapter will examine general cognitive functioning in maintenance patients in order to determine if the transfer from methadone to LAAM can be undertaken without adversely impacting on patients' ability to carry our everyday tasks.

7. Evaluation of LAAM as an Alternative Substitution Treatment for Methadone Maintenance Patients: Part Three - the Comparative Effect of Methadone and LAAM on Psychomotor and Neurocognitive Functioning.

7.1. Introduction.

Although the effectiveness of LAAM as a pharmacotherapy for the treatment of opioid dependence has been well documented (see section 1.5.1), there is a paucity of literature on the neurocognitive effects of LAAM, and particularly on the differential effect of methadone and LAAM on cognitive functioning. Nevertheless, there is anecdotal evidence of differences between the drugs. Previous studies have reported that methadone maintenance patients (MMPs) report a constellation of symptom complaints that might be indicative of the adverse cognitive effects of methadone; such symptom complaints include 'trouble thinking clearly' and 'confusion' (Dyer and White, 1997) and feeling 'forgetful' or 'trouble remembering things' (Grevert, *et al*., 1977; Horns, *et al*., 1975). In contrast, while on LAAM some patients felt less sedated and more 'normal' across the inter-dosing interval than when maintained on methadone (Freedman and Czertko, 1981; Savage, *et al*., 1976; Tennant, *et al*., 1986; Trueblood, *et al*., 1978; White, *et al*, 2002). Thus, when evaluating LAAM as an alternative pharmacotherapy for methadone maintenance patients, particularly those who complain of breakthrough withdrawal during the inter-dosing interval, it is important to also evaluate cognitive function. The design of the current study provided the opportunity to compare cognitive and psychomotor functioning in the same cohort of patients when taking methadone and LAAM. The present chapter presents the results of this comparison, but starts with a review of the available literature.

7.1.1. Cognitive and psychomotor functioning in LAAM and methadone maintained patients.

In the following review the evidence for the effect of LAAM and methadone on cognitive and psychomotor function is grouped according to the broad cognitive domain assessed (i.e., simple motor performance and reaction time, information processing/psychomotor speed, sustained attention/vigilance, immediate memory/attention, learning memory and comprehension). The capacity of maintenance patients to perform complex cognitive and psychomotor tasks is also reviewed. For a wider consideration of the effect of opioids on psychomotor and cognitive functioning in humans, including driving, the reader is directed

to a number of reviews in the area (Chesher, 1989; Lenné, *et al.*, 2000; Zacny, 1995). It should be noted at the outset that there is a paucity of literature, relative to methadone, on the effects of LAAM on cognitive functioning. A summary of the results of the studies reviewed is presented in Appendix 6.

7.1.1.1. Simple motor performance and reaction time.

Reaction time performance incorporates decision time and motor response time, which are both reputedly sensitive to drug effects (Gordon and Appel, 1995). Gordon (1970) compared the performance of 26 MMPs (mean daily dose of 100 mg) to drug free detoxified heroin users and also to opioid free college students (controls) on simple, 2-choice and 4- choice reaction time tasks. All subjects performed equally well or better than control subjects on the simple and 2-choice, but not on the 4-choice task. The author suggested that the difference in reaction times could be ascribed to faster information processing, not motor speed, and attributed between group differences to possible pre-addiction differences between groups, or greater motivation/arousal levels in the MMP group. Furthermore, Kelley and colleagues (1978), using a cross-over experimental design, measured MMPs' performance on a simple and differential reaction time one hour and 24 hours after dosing with methadone (mean 63 mg: range 20-120 mg). They reported that reaction time was relatively stable across time. Curran and colleagues (2001), using a cross-over design, assessed patients' performance on a simple reaction time task pre- and post both at 50% and 100% of their usual stabilisation dose. Times were slightly (but significantly) faster following both doses.

Rothenberg and colleagues (1977) tested MMPs (daily dosage 20 to 70 mg) and drug free control subjects on a simple reaction task on three occasions, each separated by at least one week. Participants were tested before and 2¼ hours after administration of placebo, 5 mg or 10 mg methadone. Furthermore, to address a possible confounder identified by Gordon (1970), that group difference could be due to different levels of motivation, they offered monetary reward to subjects. MMPs were faster than control subjects on pre-testing, and maintained faster reaction times even when monetary reward was offered to control subjects. In contrast, control subjects showed dose dependent slowing of reaction times.

More recently, Specka and colleagues (2000) compared the performance of 54 MMPs (mean daily dosage 93 mg) to a matched control group (matched according to gender, age

and educational level) on a battery of tests designed to assess psychomotor performance and attention that are related to driving aptitude. Subjects were tested approximately one hour after ingesting their methadone dose. On a simple reaction choice task the MMPs tended to react (non significantly) faster, but with more errors than controls. Patients' performance was impaired, relative to controls, on the multiple choice reaction task, particularly on the more difficult trials.

7.1.1.2. Information processing and psychomotor speed.

Psychomotor speed refers to the speed at which mental activities are performed. Impairment clearly shows up in longer performance times on a number of commonly used tests in the absence of a specific motor disability (Lesak, 1995). An early study by Isbell and colleagues (1948) examined the effect of administering increasing doses of methadone (up to 400 mg *s.c*) to formerly dependent opioid users on the speed and accuracy of symbol copying and a number of arithmetic tasks. The rate at which these tasks were performed was not impaired, but the number of errors increased when patients became dependent, relative to the period when subjects were not opioid dependent.

In another early study Irwin and colleagues (1976) compared the cognitive functioning of MMPs (mean daily dose 93.5 mg) who had been maintained on methadone for an average of 18 months, to that of LAAM maintenance patients (LMPs: mean dose 79.6 mg alternate daily) who had been maintained on methadone for an average of 14 months, followed by an average of 8 months on LAAM. The cognitive tasks included number learning, number doubling, an arithmetic test, and the Minnesota Clerical Test that required both number and name checking. MMPs performed significantly worse than LMPs on all cognitive tests administered. The performance of maintenance patients was compared to past control data (no details were provided). The LMP group performed equally as well as a control group made up of drug free college students.

Gritz and colleagues (1975), in the previously described study (section 1.4.1.3), also used a number of tests to examine the effects of methadone on cognitive functioning, relative to the ex opioid users, but not the nondrug using control group. They found no impairment in performance in the Cross-out Test, in which subjects were given 2 minutes to cross out designated numbers on a list, but MMPs were significantly impaired on the 3-minute

Hidden Word Test, in which patients were required to find four-letter words in rows of letters.

A number of studies have used the digit symbol substitution test (DSST) from the Wechsler Adult Intelligence Scale (WAIS: Wechsler, 1955) to examine speed of information processing in MMPs (Appel & Gordon, 1976; Curran, *et al*., 2001; Darke, *et al*., 2000; Gritz, *et al.*, 1975; Mintzer and Stitzer, 2002; Walsh, *et al*., 1994). The DSST is a timed paper and pencil test in which symbols paired with numbers are copied and substituted for the appropriate number (Wechsler, 1955; Wechsler, 1981). Gritz and colleagues, who compared MMPs with ex opioid users, and Appel and Gordon (1976), who compared DSST scores in MMPs (daily dosage 80 – 120 mg) to a group of matched controls, found no between group differences. Interestingly, Walsh and colleagues, who used a wide range of methadone dosages as well as a placebo found no dose related difference in performance. Consistent with the latter findings, more recently Curran and colleagues (2001) reported that performance on the DSST and a Finger Tapping task was unaffected by methadone dose (either 50 or 100 % of usual stabilisation dose).

However, more recently, Mintzer and Stitzer (2002), who examined psychomotor and cognitive functioning in MMPs (mean dosage 67.2 mg) relative to matched non-drug using controls, found MMPs performance on the DSST and also the Trail Making Test (Reitan, 1958) was impaired. The authors recognised that they had not controlled for the time of testing relative to methadone dosing. However, post hoc tests demonstrated no differences in performance on the basis of time of testing relative to dosing (pre- or post). Furthermore, Darke and colleagues (2000) examined cognitive performance in MMPs (mean daily dose 78.6 mg) and a matched sample of non-drug using control subjects using a battery of neuropsychological tests that included the DSST. MMPs were tested immediately prior to their daily dose. Groups did not differ on premorbid intelligence but MMPs' performance on two WAIS subtests – DSST and symbol search – was impaired.

7.1.1.3. Sustained attention (vigilance).

Broadly, attention refers to several different capacities that are related 'aspects of how the organism becomes receptive to stimuli' (Lesak, 1995), and vigilance refers to the ability to maintain concentration over an extended period of time, particularly on a task that is relatively simple or boring. Early studies reported that the repeated dosing of methadone

did not have an adverse effect on sustained attention or vigilance. Kelley and colleagues (1978) demonstrated that performance of MMPs on a 10-minute letter cancellation tests did not differ between one hour and 24 hours after dosing with methadone. Appel (1982) and Rothenberg and colleagues (1977) used different length versions of a similar continuous performance test (CPT) to assess MMPs' performance. The CPT required participants to respond when they detected a target letter (i.e., the letter O) in an ongoing presentation of varying non-target letters (i.e., the letters of the alphabet). Rothenberg and colleagues (1977) also gave drug free control subjects relatively small doses of methadone (5, 10 mg) to determine the acute effects of methadone on performance. Both studies found no impairment in performance between MMPs and control subjects. However, in contrast, Specka and colleagues (2000) demonstrated that the performance of MMPs was impaired, relative to a matched control group, on a 7-minute attention task (under monotonous circumstances), and that MMPs produced more errors than control subjects.

7.1.1.4. Immediate memory.

One of the simplest measures of immediate/working memory is the Digit Span test. This is a subtest of the WAIS (Wechsler, 1955) and requires subjects to reproduce increasing longer strings of digits in the order presented or reversed. Two early studies that used digit span to assess immediate memory found no significant effect of methadone (Gritz, *et al*., 1975; Kelley, *et al*., 1978). However, in contrast, Darke and colleagues (2000) demonstrated that, relative to matched controls, MMPs' performance was significantly impaired on the digit span test.

7.1.1.5. Learning/memory and comprehension, and executive functioning.

There are early accounts of methadone maintenance patients reporting poorer memory (Grevert, *et al*., 1977; Horns, *et al*., 1975). Horns and colleagues estimated the frequency of such complaints in MMPs participating in a 'plasma' level study, and reported that 53 percent of patients, who were asked every week to complete a symptom checklist, checked that they had 'trouble remembering things'. Despite this, relatively few studies have examined the effects of methadone on long-term memory.

Early studies found no effect of long-term methadone on a number of indices of memory function (Gordon, *et al*., 1967; Grevert, *et al*., 1977; Isbell, *et al*., 1948; Lombardo, *et al*., 1976). Lombardo and colleagues (1976) found no difference in WAIS scores between

MMPs on two different methadone dosages (50 vs 80 mg), and no effect of deescalating methadone daily doses (from 80 to 50 mg). However, the absence of a control group makes it difficult to interpret these results. Grevert and colleagues (1977) administered a number of memory tests to assess everyday memory problems ('difficulties remembering where things were', and 'difficulty remembering verbal material') reported by maintenance patients. Patients were 30 MMPs (mean dosage 52 mg; 20-80 mg) and 31 LMPs (LAAM dosed 3xweek 50 to 60 mg), and were tested on three occasions, before starting treatment, one month and three months later. They found no statistically significant between groups difference in scores for visual memory (memory of location) and verbal material (list of nouns and numbers), but noted a trend for both MMPs and LMPs to perform worse on the visual memory task than control subjects at the three month testing session.

The study by Gritz and colleagues (1975) used a broader range of tests to compare the memory and learning capability of MMPs to former heroin dependent control subjects. The authors observed a selective pattern of memory impairment. In tests where no cues were presented (recall of a story, recall of nonsense syllables, and the recall of hard paired associates where words were not related; i.e., *storm* and *nation*), MMPs' performance was impaired, relative to control subjects. In contrast, there was no difference between groups for memory of a story (accessed by recognition), and recognition of a small set of objects amongst a larger set, where cues were present. In addition, there was no difference between groups when recalling easy pairs of words.

More recently, studies have reported equivocal results with regard to the effect methadone has on memory and other higher cognitive functions (Curran, *et al*., 2001: Darke, *et al*., 2000; Davis, *et al*., 2002; Mintzer and Stitzer, 2002; Verdejo, *et al*., 2005). Darke and colleagues (2000) administered a wide range of standardised memory tests to MMPs to assess visual memory (short term and long term), verbal memory (short term and long term), and problem solving, and found that for all domains tested the performance of MMPs was impaired relative to matched controls. However, this study has been criticised on number of accounts (Mintzer and Stitzer, 2002). First, a larger proportion of the patient group reported using benzodiazepines than the control group (40 % vs 10 %, respectively). Benzodiazepines have well documented performance-impairing effects (Curran, *et al*., 1991, 2000). Second, the prevalence of reported head injury in the patient groups was significantly higher than the control group (67% vs 20%, respectively). Both these factors

may have contributed to the poorer performance of MMPs relative to control subjects making it difficult to differentiate the effects of methadone *per se* from the acute effects of other drugs and pre-existing neurological damage. In contrast, Mintzer and Stitzer (2002) reported unimpaired long-term memory, as assessed by the Recognition Memory test and Free Recall Test, amongst MMPs, compared to matched controls. The authors used the above outlined arguments to explain the disparity between their results and those reported by Darke and co-workers.

In an interesting study, Curran and colleagues (2001) used a double-blind design in which the methadone dosage was manipulated across two conditions in twenty MMPs (mean dose 32.65 mg (range: 10-50 mg)). The first group received 50% of their maintenance dose, and the second group received 100% of their daily dose. Memory for prose and psychomotor skills (previously reported in this review) were tested pre- and post-dosing. With regard to memory, the authors reported that while immediate recall of prose was unimpaired after either dose, delayed recall was significantly impaired following 100 % of the dose, but not 50% of the dose. The findings are presented as evidence of the acute effects of methadone on memory.

Two of the more recent studies in the area (Davis, *et al*., 2002; Verdejo, *et al*., 2005) have compared the cognitive performance of MMPs to ex-opioid users. Such a strategy controls for lifestyle and health factors often experienced by opioid users that may either directly or indirectly affect cognitive performance, so that the effect of current methadone administration can be more clearly assessed (Darke, *et al*., 2000; Davis, *et al*., 2002; Verdejo, *et al*., 2005). The study by Davis and colleagues found no differences in the performance of 15 MMPs (dosage not reported), relative to ex-heroin users and healthy controls, on tests designed to assess attention, spatial and verbal learning, full scale IQ and flexibility of thinking. However, they found that MMPs scored significantly less than the ex-heroin users on the Controlled Oral Word Association Test (COWAT), a test of word fluency that is often used to gauge executive dysfunction [1] (Bryan and Luszcz, 2000). They went on to categorise participants as cognitively impaired if their test scores were 2

[1] Executive function refers to superordinate cognitive processes that control and integrate other cognitive activities. They include actions such, planning, using strategies, and monitoring performance. Such function has been linked with the functioning of the frontal lobes (see Bryan and Luszcz, 2000)

or more standard deviations below the mean for published norms and found that the incidence of impaired performance was significantly greater in the methadone group (9/15 cases - 60%) than the control group (1/14 cases – 7%), but not the ex -user group (5/16 cases -31 %). Moreover, Verdejo and co-workers reported impairment of 18 MMPs (mean±sd daily dose 83.82±29.61 mg), relative to ex-heroin users, on tests of processing speed, visuo-spatial attention, working memory, analogical reasoning, and cognitive flexibility (i.e., executive function). They speculated that impairment on tests of working memory and analogical reasoning might reflect the effect of methadone on monaminergic pathways that converge in the frontal lobes, which in turn might influence executive functioning. Although they failed to detect significant between groups differences on all tests of executive functioning (differences were found for the flexibility score for Oral Trails, but not for scores from the Wisconsin Card Sorting Test and the COWAT), participants in both groups (MMPs and ex-opioid users) scored between 0.2 and 2 standard deviations below the mean normative values for these tests. They went on to suggest that this cognitive impairment is likely to influence the daily functioning of MMPs such that they may have, '…difficulty understanding complex instructions, suppressing inappropriate automatic behaviours, dealing flexibly with different sources of information and transferring learned material to real life situations', p. 288.

7.1.1.6. Complex psychomotor/cognitive performance.

A number of comprehensive studies have examined the range of skills thought to be required for driving. Such skills include the division of attention between the task of tracking and a continuous visual search of the environment (Chesher, *et al*., 1989). The reader is directed to a number of detailed reviews of this area (Chesher, 1989; Gordon and Appel, 1995; Lenné, *et al*., 2000).

In a series of eight experiments Robinson and Moskowitz (Moskowitz and Robinson, 1985; Robinson and Moskowitz, 1985) investigated a range of abilities thought to be important for driving. Subjects were 15 MMPs maintained on methadone for at least 6 months (daily dosage range 60 to 110 mg) who were compared to a matched control group of drug free ex-heroin users. Tests were conducted just before and two hours after ingesting their daily methadone dose. The authors reported no effect of methadone on a number of visual tasks, including visual acuity, visual serial search, compensatory tracking, critical tracking (where the task becomes increasing more difficult as

performance improves), and a divided attention task, in which subjects where required to simultaneously perform a visual search and recognition task and a tracking task. In addition, subjects performed a task designed to assess rate of information processing. In this test a visual stimulus (a series of four consonants) presented for a brief interval was followed by a masking stimulus, following which subjects were asked to write down the letters they had seen. If the interval between the target stimulus and mask (the inter-stimulus interval) is brief enough, processing of the stimulus is interrupted. The length of this inter-stimulus interval was therefore taken as a measure of information processing. While methadone did not significantly interfere with this task, MMPs exhibited a slower rate of information processing than the control group. However, the authors generally concluded that MMPs would not be impaired in their ability to perform complex tasks, such as driving.

Chesher and colleagues (1989) used a similar range of tasks to assess the performance of MMPs. Their study utilised four participant groups: a group of stabilised MMPs, methadone 'starters' (patients who had just commenced MMT), methadone 'increasers' (stabilised MMP patients, early in their treatment, who had just received an increase in their dose), and control groups consisting of ex-heroin users, and non-users. The tasks consisted of vigilance, compensatory visual tracking, and divided attention tasks. Following practice sessions, MMPs were tested one hour after they ingested their methadone dose, and controls after an equivalent time. Participants also received challenge doses of alcohol, methadone and diazepam. Overall, there were few effects of methadone on performance, although there was a trend for stabilised MMPs to perform poorly in comparison to control groups. However, the decrement in performance induced by alcohol or diazepam was considerably greater. The authors attributed the differences between methadone and control groups to social and personality characteristics (eg. lower proportion employed) of the patients, rather than to the effects of methadone *per se*.

More recently researchers have demonstrated significant effects of methadone on the performance of certain complex tasks. Berghaus and colleagues (1993) reported that the performance of 13 MMPs (range of doses 17.5 to 60 mg) on attention, comprehension, choice reaction time and tracking tasks (tracking and reaction time) were impaired compared to matched controls. It should be noted that in the latter study there were problems with matching of groups: at baseline testing there were significant differences

between groups on a number of personality dimensions (that are not described) that may have affected performance. Furthermore, in the study by Specka and colleagues (2000), it was demonstrated that MMPs performed more slowly on a tracking task than matched controls, but were more accurate. Moreover, Mintzer and Stitzer (2002) demonstrated that MMPs were impaired on a gambling task (that required decision making) relative to matched controls. Thus, despite earlier evidence to the contrary, recent studies have shown evidence of significant impairment of maintenance patients undertaking more complex cognitive tasks, such as tracking.

7.1.1.7. Summary.

When considered overall, the evidence for the effects of methadone on the cognitive and psychomotor functioning of stabilised methadone patients is inconsistent. The results of most early studies show only minimal impairment of cognitive functioning in MMPs (Appel, 1982; Appel and Gordon, 1976; Gordon, *et al*., 1967; Gordon, 1970; Kelley, *et al*., 1978; Lombardo, *et al*., 1976; Rothenberg, *et al*., 1977). However, these studies suffered from many methodological limitations, including small sample size, lack of appropriate controls, failure to control for concurrent use of other psychoactive drugs, and the use of a limited number of measures (Mintzer and Stitzer, 2002; Verdejo, *et al*., 2005; review by Zacny, 1995). More recently, studies have attempted to address many of the these methodological problems (i.e., Darke, *et al*., 2000; Davis, *et al*., 2002; Mintzer and Stitzer, 2002; Specka, *et al*., 2000; Verdejo, *et al*., 2005) and have clearly demonstrated a wide range of cognitive deficits related to psychomotor speed, attention, memory and executive function that may affect the ability of MMPs to perform daily activities. With regard to LAAM, the evidence is scarce; with only two studies having being identified in the literature and these have provided equivocal data on the effect of LAAM on cognitive functioning.

7.1.1.8. The present study.

As previously discussed, anecdotal reports are suggestive of the differential effect of methadone and LAAM on cognitive functioning (Dyer and White, 1997; Freedman and Czertko, 1981; Savage, *et al*., 1976; Tennant, *et al*., 1986; Trueblood, *et al*., 1978; White, *et al*, 2002), with some patients reporting symptoms (less nodding, feeling more normal) that could be indicative of a clearer mental state when maintained on LAAM. One

possible outcome of the more lucid mental state reported by LAAM patients might be an increased capacity to attend to environmental stimuli. The capacity to effectively attend to stimuli impacts on many other central cognitive tasks, particularly the encoding of information to be recalled, which has an influence on memory (Spreen and Straus, 1991). Thus, it is plausible that maintenance patients when maintained on LAAM will perform better on a range of cognitive tasks than when maintained on methadone. Therefore, the specific aims of the work in this chapter were to compare the effects of methadone and LAAM on psychomotor functioning across the inter-dosing interval for each drug, and also on cognitive functioning, in a cohort of maintenance patients (half who were self-reported methadone non-holders) by administering:

(a). A computerised unpredictable tracking task frequently through the inter-dosing interval for each drug and;

(b). A battery of neuropsychological tests once at the time corresponding to the putative peak plasma concentration of each drug (i.e., 3 to 4 hours post dosing for methadone, and 4 hours post dosing for LAAM)

7.2. Hypotheses.

- Tracking task scores will fluctuate significantly across the methadone and LAAM inter-dosing intervals. The temporal fluctuation in tracking performance across the methadone and LAAM inter-dosing intervals will be closely related to the underlying plasma (R)-(-) methadone, and nor- and dinor-LAAM concentration versus time profiles, respectively.
- Patients when taking LAAM will perform better on cognitive tasks, in particular those assessing attention and memory, than when taking methadone.
- Control subjects will perform better than maintenance patients on cognitive tasks.

7.3. Methods.

The methods and general procedures used in the methadone and LAAM inter-dosing studies were outlined in chapter two. Neurocognitive and psychomotor function were contrasted in 16 patients (8 self-reported methadone holders and 8 self-reported methadone non-holders) who participated in both the methadone and LAAM interdosing studies. Patients participated in the interdosing studies when they had reached steady state for each drug. Ten drug free control subjects also participated in a 48-hour testing session.

7.3.1. Unpredictable tracking.

Assessment of tracking performance was undertaken on eleven occasions for each drug at the following times: approximately 0.5 hours before dosing and at the following times after dosing with LAAM: 1, 2, 3, 4, 6, 9, 12, 24, 36, 48 hours and approximately 0.5 hours before dosing and the following times after dosing with methadone: 1, 2, 3, 4, 5, 6, 7, 9, 12, 24 hours. For control subjects, assessment of psychomotor performance was undertaken at baseline and at 1, 2, 3, 4, 6, 9, 12, 24, 36, 48 hours later.

Cognitive psychomotor performance was assessed using The Occupational Safety Performance Assessment Test (OSPAT, Romteck, Perth, WA, Australia). This is a computerised unpredictable tracking task that participants performed on an IBM computer workstation. The task requires participants to keep a randomly moving cursor in the centre of three concentric circles, using a trackball. Once centred, the cursor moves away to a random position away from the centre and the subject must re-centre the cursor. Participants were seated in front of the computer workstation, and were instructed to use their dominant hand for the test during each inter-dosing interval. A global performance measure for each test is determined by software installed on the computer, and is determined by summing the 'error' distance between the cursor and target and the rate at which the subject adapted to the random changes. This is a measure of eye-hand coordination and incorporates the following cognitive functions: vigilance, information processing, (decision making), and reaction time. It has previously been used to demonstrate performance decrements that occur following fatigue and alcohol consumption (Dawson and Reid, 1997), and following the administration of the pineal hormone melatonin (Rogers, *et al.*, 1998). In the current study scores were expressed as a percentage relative to each individual participants' baseline score. Each test is one minute in duration.

7.3.1.1. OSPAT Pilot Study.

A pilot study was carried out to examine the effect that number of trials, and hence learning, would have on performance on OSPAT. Subjects were a convenience sample of five female and five male graduate students and academic staff recruited from the Department of Clinical and Experimental Pharmacology (currently the Discipline of Pharmacology) at the University of Adelaide (mean age 31.9 years; range 21 to 37 yrs). None reported taking any medication that might have affected psychomotor functioning.

They were seated in front of the computer workstation in a single room with no distractions and were asked to complete 12 successive trials. Testing occurred in the morning in each case. Figure 7.1 shows mean OSPAT performance (%) for 11 trials expressed relative to the first trial.

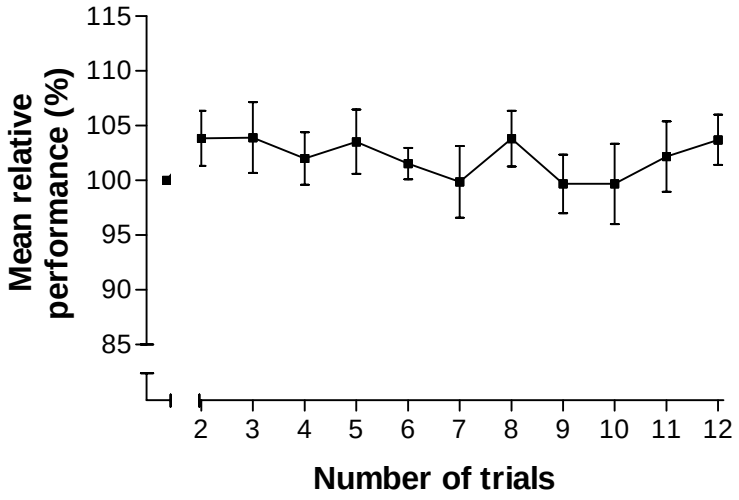

Figure 7-1: Mean OSPAT performance score of ten subjects participating in the pilot study. All subjects performed 12 trials. Performance was expressed as a percentage of the first trial. Values are mean ± SEM.

As can be seen from figure 7.1 relative OSPAT scores increased slightly after the first trial, but thereafter remained relatively stable across the remaining trials. Thus, it appeared that practice made little difference to performance on OSPAT. Based on these data it was decided to instruct participants in the current study to compete five trials at the start of each inter-dosing interval study. The same was asked for control subjects prior to testing. Of the five trials, the first three trials were discounted as practice trials, and the remaining two summed and the mean used as the personal baseline for each individual for that particular inter-dosing interval study. For each subsequent test during the inter-dosing interval study participants performed the OSPAT task twice, with the two scores averaged to provide a mean score

7.3.2. Neurocognitive function

Neurocognitive function was assessed once during each inter dosing interval study, at the time of putative peak plasma concentrations of methadone and LAAM (and metabolites); i.e., at approximately 4 hours after LAAM administration and 2-3 hours following methadone administration. However, due to time constraints during the first day of the

methadone inter-dosing interval study, and also to reduce fatigue, patients remained in the testing room for three hours following dosing of methadone on the second day to allow for neurocognitive testing.

A number of neuropsychological instruments were chosen to examine a range of cognitive functions, including learning and memory (verbal and visual), attention and working memory, information processing and executive functioning. These tests are listed in table 7.1 and described in more detail in the materials section below (section 7.3.2.1). The National Adult Reading Test–Revised (NART-R: Crawford, 1990; 1992), which was used as a measure of pre-morbid functioning, was administered once only to each patient. In most cases the neurocognitive tests that were chosen have parallel forms that permit repeated testing of patients. Where parallel forms were not available the same test was administered to patients during both the LAAM and methadone inter-dosing interval studies. Thus, there were two batches of tests; batch one consisted of the standard version of the tests, and batch two consisted of parallel forms, if available, of the tests contained in batch one. Batch one was administered to patients when they participated in their first interdosing study (LAAM or methadone), and batch two was administered when patients participated in their second inter-dosing study. As patients were randomly allocated to either LAAM or methadone treatment the administration was counterbalanced with respect to drug. Control subjects also underwent neurocognitive assessment. This occurred four hours after the start of the testing: half the control subjects were administered batch one of the neuropsychological tests, and the other half were administered batch two of the neuropsychological tests. A registered clinical psychologist (Dr Patricia Kent, PhD, M Psych) provided advice on the cognitive tests to use, training on the accepted procedures for administration and scoring of all neurocognitive tests. Standardised instructions were provided to each to each participant prior to each test. Neurocognitive testing took approximately 45 minutes to complete.

Table 7-1: List of neuropsychological tests used in neurocognitive assessment of maintenance patients and control subjects

Cognitive Function Assessed	Test	
	Batch 1	Batch 2 (parallel forms)
Premorbid intellectual functioning	NART-R	
Attention and working memory	WMS DS subtest	WAIS-R DS subtest
	TMT A&B	TMT C&D
Verbal memory	RAVLT (Rey)	RAVLT (Crawford)
Visual memory	CFT (Rey)	CFT (Taylor)
Psychomotor/ Processing speed	WAIS-R subtest- DSST	WAIS-R subtest-DSST
Executive function	COWAT	COWAT

Notes: NART-R = National Adult Reading Test-Revised: Note NART-R was administered during the first inter-dosing testing session only; WMS = Wechsler Memory Scale, DS = Digit Span subtest of the WMS; WAIS-R = Wechsler Adult Intelligence Scale-Revised, DS=Digit Span subtest of the WAIS; TMT = Trails Making Test, version A, B, C, D; RAVLT= Rey Auditory Verbal Learning Test, Rey version, Crawford version; CFT= Complex Figure Test, Rey and Taylor versions; COWAT = Controlled Oral Word Association Test

7.3.2.1. Materials.

The following neurocognitive tests were used to assess cognitive and psychomotor functioning in maintenance patients and control subjects.

The National Adult Reading Test (NART) is a widely used measure of pre-morbid intelligence (Crawford, 1992; Frazen, *et al*., 1997; Nelson, 1982). It is based on the common approach of estimating pre-morbid intelligence from an individual's current performance, in this case reading, which is considered relatively resistant to neurological and psychological impairment (Crawford, 1992; Franzen, *et al*., 1997). The test lists 50 single words ordered in increasing difficulty that do not follow rules of normal phonetic pronunciation in English. Subjects are presented with a card with the words printed on either side and are instructed to read each word out loud, taking time to pronounce each word as carefully as possibly, and are scored on the number of words they pronounce correctly. As the majority of the words are short in length and irregular (eg. ache, gauche) subjects cannot use guesswork to pronounce them and therefore must have previous familiarity with the words to score well on the test. Therefore, performance on the test is related to their reading ability (Crawford, 1992). Furthermore, the test demands relatively little attentional resources as it uses simple words and so tends to tap into subjects'

previous familiarity of words, not their current cognitive capacity. Crawford (1990) developed a revised version of the test, the NART-R, by replacing a number of less reliable items with more reliable alternatives – this form was used in the current study. The NART-R has been shown to be a good predictor of WAIS full scale IQ (Crawford, 1992), and so full scale NART-R scores can be converted to WAIS IQ equivalents to provide a more general idea of IQ. The approximate administration time is 10 minutes.

The Digit Symbols Substitution Subtest (DSST) of the Weschler Adult Intelligence Scale-Revised (Wechsler, 1981) is a speeded test that measures individual information processing capacity. Subjects are presented with a key that contains nine numbers, each paired with a symbol, and are required to write the correct symbol under a series of numbers. The number of symbols correctly drawn within a 90 second period determines the score. There is no alternate form of the DSST.

The Digit Span test is a common component of a number of standardised memory batteries (Wechsler, 1955; 1981). It comprises two parts, digits forwards and digits backwards. In digits forwards subjects are verbally presented with a series of numbers over a series of trials. Each trial contains successively more digits (i.e., 3 digits in trial 1 to 8 digits in trial 6) and at the end of each trial subjects are asked to immediately recall the digits presented in that trial. Subjects are given two attempts at each trial level. In digits backward subjects are instructed to reverse the digits presented. The Digit Span test is considered a test of attention and immediate or working memory, as it requires subjects to attend to the information presented, to hold the information for brief time whilst processing it, and then to formulate a response. For the purpose of this study the versions found in the WAIS-R and WMS were used as parallel forms. The only difference between the two tests is that digit span from the WAIS-R contains seven trials and the WMS version 6 trials. Thus for this study only the first 6 trials of the WAIS-R digit span subtest were administered. The measure used in this study was the total Digit Span raw score over the six trials, which has a possible maximum of 24.

The Trailmaking Test (TMT) as devised by Reitan (1958) is an easily administered test of attention, and also complex visual scanning, mental flexibility, and motor function. The test has been found to be highly sensitive to brain damage (Dodril, 1978, O'Donnel, 1983), alcoholism (Grant, *et al.*, 1984; 1987), polysubstance abuse (McCaffrey, *et al.*, 1988), and

prolonged opioid exposure (Mintzer and Stitzer, 2002). The test is administered in two parts – in both cases subjects are asked to complete the test as quickly as possible and are timed. Prior to each test subjects are required to complete a short practice task that is based on the test proper. Part A (Trails A) requires subjects to draw lines to connect consecutively 25 numbered circles that appear randomly on a page. Part B (Trails B) requires subjects to connect 13 circles numbered 1 to 13 and 12 circles lettered A to L in alternating order, i.e., the sequence is 1-A-2-B-3-C etc. The correlation between both parts is reportedly only 0.49, thus suggesting that they measure different functions (Heilbronner, *et al.*, 1991). Trails A is viewed as primarily a visual search task, where the individual must keep track of the numbers while searching for the next circle. Trails B, on the other hand, is more complex where individuals must, in addition to connecting the circles (which is the same motor requirement as Trails A), also alternate between letters and numbers – a form of multiple tracking. It has been said that Trails B is more sensitive to brain damage as it involves more complex information processing (Spreen & Strauss, 1991). A difference score can be calculated by subtracting the time taken to complete Trails B minus the time taken to complete Trails A (b-a) (Lesak, 1995). This removes the speed/motor element from the test evaluation, and can be used as a measure of set shifting /conceptual flexibility (Mintzer and Stitzer, 2002). This score correlates highly with severity of cognitive impairment (Lesak, 1995).

Matarazzo and colleagues (1974), who gave the TMT to young healthy normal males on two occasions 12 weeks apart, reported test retest reliability coefficients of 0.46 and 0.44 for part A and B, respectively. As practice effects may affect subsequent performance on the TMT, desRosiers and Kavanagh (1987) developed Trails C and Trails D as alternate forms of Trails A and Trails B, respectively. These alternate forms can be easily constructed by maintaining the relative position of the circles as in the original form, but by reversing the order of the sequence that must be followed (desRosiers and Kavanagh, 1987). A number of authors have reported adequate alternate form reliabilities (0.66 – 0.95 for TMT Parts A and C, and 0.88 to 0.94 for TMT Parts B and D) using a number of different samples (desRosiers and Kavanagh, 1987; Franzen, *et al.*, 1996). The approximate administration time is 5 – 10 minutes.

The Controlled Oral Word Association Test (COWAT: Benton, 1968; Benton & Hamsher, 1976; Parker and Crawford, 1992; Spreen & Strauss, 1998), otherwise known as the FAS

test is a commonly used test of verbal fluency. Such tests assess the readiness with which individuals can initiate behaviour in response to a novel task and, as such, are used to gauge executive dysfunction (Bryan and Luszcz, 2000; Parker and Crawford, 1992; Lezak, 1995). With the FAS test subjects are asked to produce as many words as they can that begin with the letter *F, A,* and *S* in 60 seconds. The words must have more than three letters and proper nouns, numbers and changing the suffix of the word are not permitted. A warm up trial is provided so that subjects understand the instructions; the letter 'P' is provided, and the trial is stopped as soon as the subject provides three appropriate words. The score is the total number of acceptable words produced in each 60-second trial. The approximate administration time is 5 minutes and no alternate form is available.

The Rey Auditory-verbal learning Test (AVLT; Crawford, *et al*., 1989; Rey, 1964) is a widely used test of verbal memory. It measures immediate memory span, provides a learning curve, measures both short term and long term retention after some intruding activity, susceptibility to interference and recognition memory (Lesak, 1995). The RAVLT is purported to be sensitive to verbal memory deficits in a variety of patient groups (see Lesak, 1995). The test comprises two lists of 15 nouns each (list A and List B). List A is read out loud to the subject for five consecutive trials, each trial is followed by a free recall test. The first free recall test is a measure of immediate word span. After the fifth trial, an interference list (List B) is presented, which is followed by a free recall test. Then an initial delayed recall of list A follows, without the presentation of these words. Following a delay of approximately 20 minutes, during which time other tasks are completed by subjects, another free recall test of List A occurs. Finally, a test of recognition is administered, in which the subject is asked to identify as many words from a list of 50 words that contains all items from List A and List B, as well as words that are semantically similar, or phonemically similar to words in both List A and B. A number of measures can be derived from the RAVLT, including immediate memory span, the total number of words recalled over trials 1-5, short and long term recall, and recognition. According to Lesak (1995), practice effects are prominent, and she recommends not giving the same list twice in succession. Therefore, the current study also used an alternate form developed by Crawford and colleagues (1989). Approximate administration time is normally about 15 minutes.

The Rey-Osterrieth Complex Figure Test (CFT: Osterrieth, 1944; Rey, 1941) permits assessment of visuospatial constructional ability and visual memory (Spreen & Strauss, 1998). The subject is presented with a complex line drawing devised by Rey, or an alternate version such as the Taylor figure (Taylor, 1969; 1979), and is instructed to copy it. The copy trial is said to reflect perceptual, visuospatial, and organizational skills (Spreen & Strauss, 1998). The original and copy are then removed. Following a delay of approximately 20 minutes, during which time other tasks are performed, subjects are asked to draw the figure from memory, thus providing a measure of long-term visual memory. The drawings are coded according to a number of established criteria that assess both quantitative and qualitative aspects of the figure (Spreen & Stauss, 1998). The highest possible score for each figure is 36. With repeated administration of the same figure practice effects have been reported (Spreen and Strauss, 1998), and so the Taylor figure (Taylor, 1969; 1979), an alternate version, was also administered in this study. With regard to the comparability of both the Rey and Taylor figures, reliability coefficients are in the moderate range when the figures have been compared. In healthy normal adults the copy trials for the different forms have been judged as similar in difficulty, but delayed 30 minute recall of the Rey figure is considered harder (by about 5 points) (See Spreen and Strauss, 1998). For this study delayed recall scores for the Taylor figure were adjusted down by 5 points. The CFT is sensitive to the deficits produced by even mild head injury (Leininger, *et al*., 1990).

Figure 7.2 presents the order of administration of the neurocognitive tests used in the current study

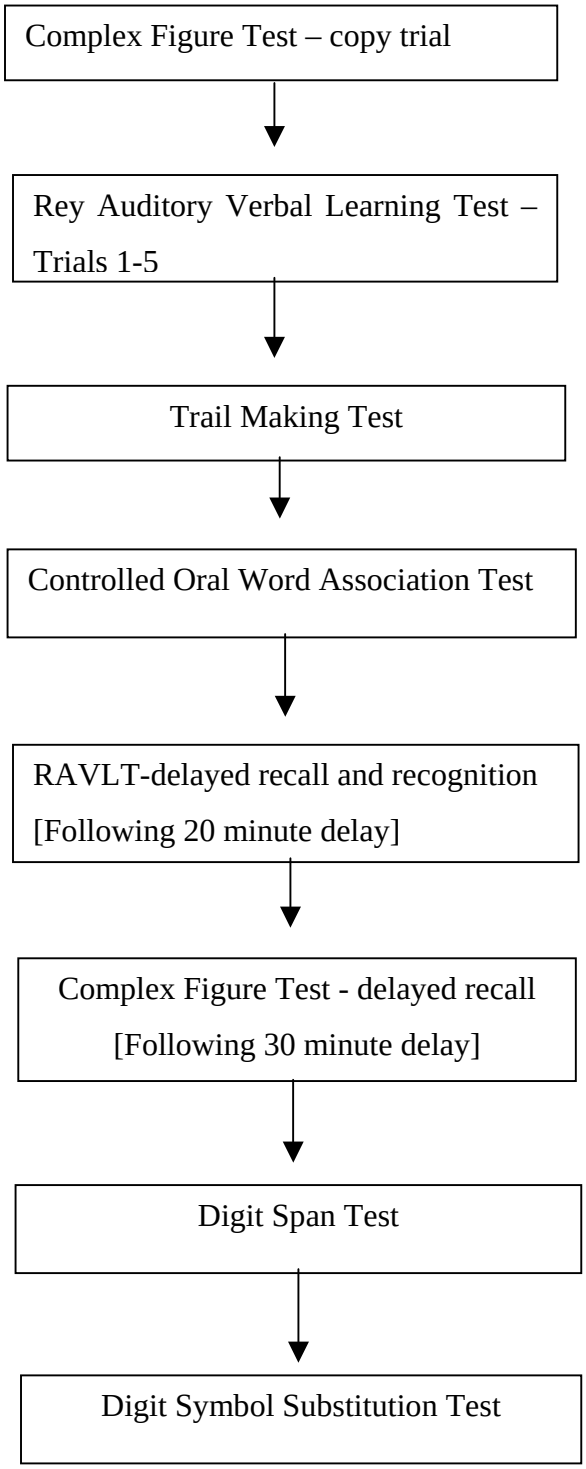

Figure 7-2: Order of presentation of the neurocognitive tests administered to maintenance patients and control subjects

7.3.2.2. Summary of dependent variables derived from neuropsychological tests.

Given the relatively small size utilised in the current study, and the large number of possible dependent variables that can be derived from the administered tests, it was

considered necessary to limit the number of variables examined statistically. They included: premorbid IQ derived from NART-R error score; the copy and delay recall score for the complex figure test; the total number of words recalled over the five trials on the RAVLT, and total number of words recalled in the delayed recall test and recognised in the delayed recognition task on the RAVLT; the time taken to complete parts A and B of the Trail Making Test and the difference in time taken to complete Trails B minus the time taken to complete Trails A (conceptual flexibility measure); the total number of words produced on the COWAT; the total digit span (forwards and backwards); and the number of symbols correctly drawn on the DSST.

7.3.3. Statistical Analyses.

Independent samples *t*-tests were used to contrast demographic data (except categorical variables), neuropsychological variables for maintenance patients and control subjects, and neurocognitive variables according to holding status. Paired *t*-tests were used to contrast neurocognitive variables according to the maintenance drug. Categorical variables were compared between patients and controls using Fischer's exact tests. Pearson correlation coefficients were used to examine linear relationships between paired variables.

Separate one way repeated measures ANOVAs were used to examine the effect of time since dosing on OPSAT performance for patients and control subjects. Following statistically significant ANOVAs, within group comparisons were performed between the pre-dose or baseline measurement and each subsequent measurement point for each group using the post hoc Bonferroni test for selected comparisons (GraphPad Prism v3.02, Graphpad Software, CA, USA). Further analyses were undertaken to determine differences in the peak magnitude of effect for psychomotor performance between drugs. ANOVA could not be used to directly compare data between drugs, as testing protocols were not identical. Instead, paired *t*-tests were used to directly compare scores from each session at predose, 1 hour, 2, 3, 4, 6, 9, 12, and 24 hours after dosing. Bonferroni adjustment was made to the alpha level for these comparisons (i.e., 0.05/9 comparisons); significance was set at $P<0.0055$. OSPAT performance was also compared between maintenance patients and control subjects. Comparisons between the methadone and control sessions were made at the following measurement points: predose, 1, 2, 3, 4, 6, 9, 12, and 24 hours after dosing for a total of 9 between group comparisons; and between the LAAM and control sessions at the following measurement points: predose, 1, 2, 3, 4, 6, 9,

12, 24, 36 and 48 hours after dosing for a total of 11 between group comparisons. The Bonferonni adjusted alpha levels were $P<0.0055$ and $P<0.0045$, respectively.

To compare the overall magnitude of effect of methadone and LAAM on OSPAT performance, the area under the effect versus time curve over the 24 inter-dosing interval for methadone ($AUC_{0-24;}$ to allow 2X AUC_{0-24} to be determined) and the 48 hour inter dosing interval for LAAM (AUC_{0-48}) were calculated by the trapezoidal rule (GraphPad Prism v3.02, GraphPad Software, CA, USA). A paired *t*-test was used to compare the sum of 2X AUC_{0-24} for methadone with one AUC_{0-48} for LAAM. Further, repeated measures ANOVA were used to examine the effect of time since dosing and methadone self reported holding status on psychomotor responses. For repeated measures degrees of freedom were adjusted for violations of the sphericity assumption using Greenhouse Geisser corrections provided by SPSS

Further exploratory correlational analyses were performed to determine the relationship between scores on the STAI (anxiety), and BDI-II (depression), and performance on the neurocognitive tests. Such affective states have been shown to adversely affect performance on tests assessing memory and working memory, in particular the CFT (Meyers and Meyers, 1995; Spreen and Strauss, 1998). The relationship between methadone and LAAM dose, and patient education attainment (years of education completed) and neurocognitive variables was also examined. Given the number of comparisons undertaken it should be recognised that some significant findings, especially if isolated with marginal levels of significance (i.e. $0.01 < P < 0.05$), may represent chance findings.

Unless otherwise stated the alpha level was set at $P=0.05$ and all statistical tests were two tailed and data are tabulated as mean±sd. Data were analysed using SPSS™ for Windows (v.10: SPSS Inc). SPSS undertakes Levene's test of homogeneity of variance when undertaking *t*-tests and adjusts the probability accordingly. Where appropriate, 95% confidence intervals of differences between means were also calculated.

7.4. Results.

7.4.1. General.

Table 7.2 presents a summary of demographics for maintenance patients and control subjects. Patients reported that their mean length of consistent heroin use (i.e., no interruptions of greater than one week) prior to commencing the current methadone maintenance program was 3.1±3 (range: 0.17 to 14) years. Premorbid IQ was estimated from errors on the NART-R. With the exception of years of education, the maintenance patients and control subjects did not significantly differ on any variable.

Table 7-2: Summary of demographic details of maintenance patients (n=16) and drug free control subjects (n=10).

	Maintenance patients (*n=16*)	Control (*n=10*)
Gender (% male, n)	44 % (7)	60 % (6)
Age (mean±sd yrs)	35.2±7.9	34.5±9.6
Education (mean±sd yrs) (range yrs)	11.0±1 (8-14)	14±2 (12-17)*
Employment (% employed, n) [1]	50 (8)	90 (9)
Estimated premorbid IQ (mean±sd) [2]	104±9	110±4

[1] Includes those employed part time and full time students; [2] Premorbid IQ estimated from NART-R error scores; * P <0.001 significant difference between groups; Categorical variables compared between groups using Fischer's exact test, and continuous variables compared using independent groups t-test

Table 7.3 presents a summary of self-reported drug use for maintenance patients and control subjects for the month preceding the inter-dosing testing session. There were no significant differences between the proportion of patients reporting recent use of alcohol or opioids prior to participating in the methadone or LAAM inter-dosing interval study and control subjects. A significantly greater proportion of maintenance patients than control subjects reported recent use of benzodiazepines, cannabis and nicotine prior to undertaking the study (P<0.05). Table 3.2 and Table 4.1 present more comprehensive details of drugs use for patients when undertaking the methadone and LAAM interdosing interval studies, respectively.

Table 7-3: Summary of self reported drug use (% used in last month) in maintenance patients (n=16) for both the methadone and LAAM inter-dosing studies and for drug free control subjects (n=10)

	Maintenance patients		Control Subjects
	Methadone	LAAM	
Opioids (%, n) [1]	44 (7)	31 (5)	0
Benzodiazepines (%, n)	50 (8) [#]	50 (8) [#]	0
Cannabis (%, n)	50 (8) [#]	44 (7) [#]	0
Alcohol (%, n)	56 (9)	50 (8)	90 (9)
Nicotine (%, n)	94 (15) [#]	88 (14) [#]	10 (1)

[1] Opioids- including heroin, morphine and codeine; [#] $P<0.05$ Fischer's exact test used to compare methadone and control groups, and LAAM and control groups.

7.4.2. Psychomotor Performance.

The effect of LAAM and methadone on psychomotor performance in patients and control subjects, as assessed by OSPAT, is shown in figure 7.3. OSPAT data are shown for 15 patients as it was not possible to extract OSPAT data from the computer for patient #4 (see table 3.1 and 4.1). Psychomotor performance appeared to be more depressed following the administration of methadone than LAAM, with maximum suppression occurring at 2 hours after dosing for methadone, and at 6 hours after dosing for LAAM. There were significant temporal changes for methadone ($F(10, 140)=5.15$, $P<0.001$) and LAAM ($F(10, 140)=6.03$, $P<0.001$), but not for control subjects ($P>0.05$). OSPAT scores were significantly greater at 36 hours ($P<0.001$; 1.04; 0.15, 1.9) and 48 hours after dosing ($P<0.001$; 1.2; 0.32, 2.1) than that at baseline for patients when taking LAAM, but there were no significant within group differences for patients when taking methadone.

There were no significant differences in the psychomotor performance of maintenance patients when taking methadone or LAAM at any comparison point ($P>0.0055$). Furthermore, the sum of two AUC_{0-24} values for methadone (mean±sd: 313±181 score·h) was not statistically different to the AUC_{0-48} value for LAAM (244±147 score·h) for psychomotor performance ($P=0.23$; 69; -187, 50). Comparisons were also made between maintenance patients, when undertaking the methadone and LAAM interdosing studies, and control subjects, which revealed no significant between group differences ($P>0.0055$ and $P>0.0045$, respectively).

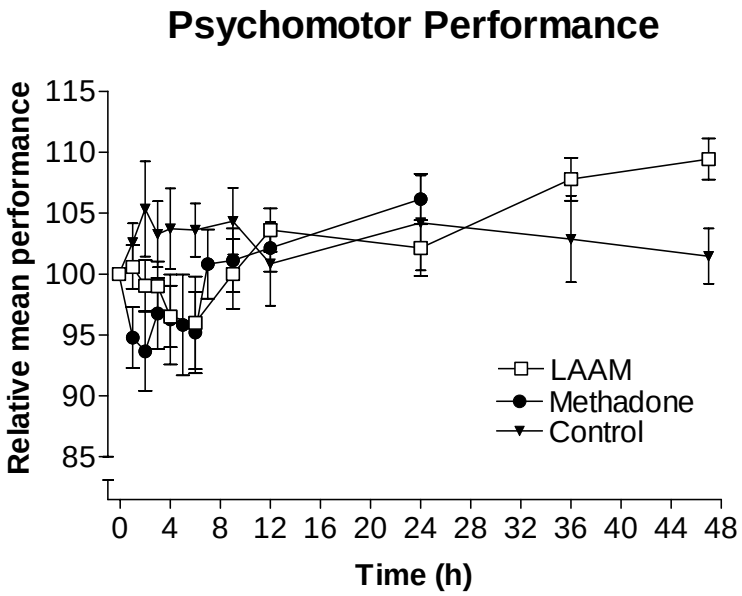

Figure 7-3: Performance (OSPAT) expressed as mean±SEM relative performance to baseline in 15 patients who underwent one 24-h inter-dosing interval study when taking methadone (closed circles) and one 48-h inter-dosing interval study when taking LAAM and in 10 drug-free control subjects (closed triangles).

Two way repeated measures ANOVA, used to compare OSPAT scores between methadone holders and non-holders when on either drug, found no significant between group (holder vs non-holder) differences for either methadone ($F(1,13)=0.29$, $P=0.60$) or LAAM ($F(1,13)= 0.13$, $P=0.69$).

7.4.3. Neurocognitive functioning for all patients.

Table 7.4 provides mean±sd of results from all neuropsychological measures and the results of paired *t*-tests between drug groups (methadone and LAAM), and independent *t*-tests between drug groups and the control group. Patients' performance was significantly worse on the CFT (copy score and delayed recall score) when maintained on methadone than when on LAAM, but was worse on Trails B (and the Trails conceptual flexibility measure (B-A)) when maintained on LAAM than when on methadone. Performance on all other tests did not differ between drugs. With regard to the comparative performance between patients relative to controls: for methadone, performance was impaired relative to controls for all tasks with the exception of the total number of words generated on the COWAT, Digit Span, and Trails B; for LAAM, performance was impaired relative to

controls on all tasks with the exception of the copy trial for the CFT, the total number of words generated on the COWAT, and the time taken to complete the Trails A.

Table 7-4: Mean±sd for neuropsychological measures for maintenance patients (n=16) undergoing the methadone and LAAM interdosing studies, and for control subjects (n=10).

Measures[1]	Methadone	LAAM	Control	*P*[3] (mean diff, 95% CI)	*P*[4] (mean diff, 95% CI)	*P*[5] (mean diff, 95% CI)
CFT [Max score=36]						
Copy	26.44±4.81	29.91±4.11	31.85±3.32	0.002 (-3.47; -5.42, -1.52)	0.005 (-5.41; -9.00, -1.83)	0.22 (-1.94; -5.13, 1.24)
Delayed Recall	13.38±4.71	15.97±5.05	23.30±4.42	0.014 (-2.59; -4.93, -0.26)	<0.001 (-9.93; -13.75, -6.10)	0.001 (-7.33; -11.34, -3.32)
Delayed Recall-adj [2]	10.88±4.03	13.44±5.29	21.30±3.90	0.001 (-2.56; -4.46, -0.67	<0.001 (-10.43; -13.74, -7.12)	<0.001(-7.86; -11.87, -3.86)
RAVLT [# words]						
Trial 1	6.13±1.96	6.19±2.01	8.00±1.83	—	—	—
Trial 2	8.44±2.42	8.06±2.04	10.40±2.37	—	—	—
Trial 3	9.56±2.12	9.44±2.73	11.90±2.13	—	—	—
Trial 4	10.44±2.61	10.19±2.76	12.50±2.07	—	—	—
Trial 5	10.50±2.28	10.88±2.60	13.30±195	—	—	—
Total words trials 1-5	45.19±9.32	44.82±10.76	56.10±8.10	0.89 (0.38; -5.44, 6.19)	0.006 (-10.91; -18.30, -3.52)	0.009 (-11.29; -19.48, -3.10)
Delayed recall	7.69±2.98	7.25±3.26	11.30±3.01	0.53 (0.44; -1.02, 1.89)	0.007 (-3.61; -6.12, -1.11)	0.004 (-4.05; -6.70, -1.40)
Recognition	11.13±3.14	11.44±2.76	14.20±1.23	0.62 (-0.31; -1.61, 0.99)	0.002 (-3.08; -490, -1.25)	0.007 (-2.76; -4.68, -0.85)

Notes: [1] CFT= complex figure test- maximum score of 36 ; RAVLT- Rey Auditory Verbal Learning Test - score is number of words. Crawford version used as alternate form (Crawford et al., 1990); COWAT=Controlled Oral Word Association Test - score is the total number of words produced beginning with the letter *F*, *A* and *S* in sixty seconds; TMT=Trail Making Test - time in seconds to complete task. This study used Trails C and D as alternate forms to Trails A and B, respectively. Digit Span = Total number of digits recalled on Digits Forward and Digit Backwards with a maximum score of 24; DSST=Digit Symbol Substitution Test. Score is number of symbols correctly copied in 90 seconds; [2]Delayed Recall-adj = delayed recall score for Taylor Figure (CFT) adjusted down by 5 points (see section 7.3.2.1). [3] Paired *t*-test between methadone and LAAM; [4] Independent samples *t*-tests between methadone and control groups; [5] Independent samples *t*-tests between LAAM and control groups. Significant *P* values are highlighted in bold

Table 7.4 continued Mean±sd for neuropsychological measures for maintenance patients (n=16) undergoing the methadone and LAAM interdosing studies, and for control subjects (n=10).

Measures[1]	Methadone	LAAM	Control	P[3] (mean diff, 95% CI)	P[4] (mean diff, 95% CI)	P[5] (mean diff, 95% CI)
COWAT [# words]						
FAS total	37.19±9.82	35.25±7.98	45.10±13.80	0.22 (1.94; -1.30, 5.17)	0.10 (-7.92; -17.46, 1.64)	0.06 (-9.85; -20.23, 0.53)
TMT [time in seconds]						
Trails A/C	36.00±11.18	39.63±21.98	27.20±7.61	0.42 (-3.63; -13.00, 5.7)	0.04 (8.80; 0.48, 17.12)	0.051 (12.43; -0.09, 24.94)
Trails B/D	89.88±45.00	118.13±57.08	60.30±22.50	0.02 (-28.25; -50.99, -5.51)	0.07 (29.58; -2.17, 61.32)	0.002 (57.83; 24.68, 91.00)
Trails B-A	52.00±38.53	78.50±58.53	33.10±17.20	0.03 (-26.50; -50.40, -2.59)	0.10 (18.90; -4.03, 41.83)	0.009 (45.40; -12.70, 78.10)
Digit Span [max =24]	15.25±3.13	14.25±2.98	17.30±2.95	0.12 (1.00; -0.28, 2.3)	0.11 (-2.05; -460, 0.50)	0.02 (-3.08; -5.52, -0.58)
DSST [# correct]	51.00±10.92	49.87±10.57	62.00±9.68	0.70 (1.13; -5.09, 7.35)	0.02 (-11.00; -19.72, -2.28)	0.01 (-12.13; -20.77, -3.50)

Notes: [1] CFT= complex figure test- maximum score of 36 , RAVLT- Rey Auditory Verbal Learning Test - score is number of words. Crawford version used as alternate form (Crawford et al., 1990); COWAT=Controlled Oral Word Association Test - score is the total number of words produced beginning with the letter *F*, *A* and *S* in sixty seconds; TMT=Trail Making Test - time in seconds to complete task. This study used Trails C and D as alternate forms to Trails A and B, respectively. Digit Span = Total number of digits recalled on Digits Forward and Digit Backwards with a maximum score of 24; DSST=Digit Symbol Substitution Test. Score is number of symbols correctly copied in 90 seconds; [2]Delayed Recall-adj = CFT delayed recall score for Taylor Figure adjusted down by 5 points (see section 7.2.2.1). [3]Paired *t*-test between methadone and LAAM; [4] Independent samples *t*-tests between methadone and control groups; [5]Independent samples *t*-tests between LAAM and control groups. Significant *P* values are highlighted in bold.

7.4.4. Neurocognitive functioning – Comparisons for holders and non-holder groups.

Table 7.5 and 7.6 show the results of independent *t*-tests comparing performance between self reported holders and non-holders on neurocognitive tests, when taking methadone and when taking LAAM, respectively. The only significant between group differences for patients participating in the methadone inter-dosing interval study (Table 7.5) were for the delayed recall and delayed recognition score for the RAVLT, such that non-holders' performance was superior relative to holders. Interestingly, the between group difference for the total number of words recalled for the RAVLT neared significance (P=0.053), which was also in favour of non-holders. With regard to the relative performance of holders and non-holders when maintained on LAAM (Table 7.6), no significant between group differences were found ($0.83 > P > 0.30$).

Paired *t*-tests were used to separately compare the performance of holders and non-holders between drugs on the delayed recall and delayed recognition score for the RAVLT. For methadone holders and non-holders, the delayed recall score for the RAVLT did not significantly differ when they were maintained on methadone or LAAM (P=0.36; -0.88; -2.99, 1.24; and P=0.08; 1.80; -0.24, 3.74, respectively). In contrast, the delayed recognition score (RAVLT) for holders was significantly greater when taking LAAM (mean±sd: 10.75±2.43) than when taking methadone (8.75±2.43; P=0.005; -2.00; -3.18, -0.82), but for non-holders did not significantly differ between drugs (P=0.10; 1.38; -0.35, 3.10). Thus, on these measures, holders' performance remained stable or improved when they changed to LAAM, but the performance of non-holder's remained stable.

Table 7-5: Mean±sd for all neurocognitive measures, according to holding status (holders =8, non-holders=8), for patients participating in the methadone inter-dosing study.

Measures[1]	Holders	Non-holders	*P** (mean diff, 95% CI)
CFT copy	27.69±4.34	27.00±4.56	0.76 (0.69; -4.09, 5.46)
CFT delayed recall	15.75±3.75	11.56±5.44	0.10 (4.19; -0.82, 9.19)
CFT delayed recall adjusted	12.00±4.07	10.31±4.16	0.43 (1.69; -2.72, 6.1)
RAVLT-Total words	40.75±6.32	49.63±10.06	0.053 (-8.88; -17.88, 0.13)
RAVLT-delayed recall	6.00±2.45	9.38±2.56	**0.017** (-3.38; -6.06, -0.68)
RAVLT-delayed recognition	8.75±2.44	13.50±1.51	<**0.001** (-4.75; -6.92, -2.58)
COWAT	39.25±10.63	35.13±9.17	0.42 (4.13; -6.52, 14.77)
TMT A/C	37.63±15.18	34.38±5.60	0.58 (3..3, -9.02, 15.52)
TMT B/D	94.25±59.09	81.7±31.61	0.61 (12.50; -38.31, 63.31)
Digit Span	15.88±3.52	14.63±2.77	0.44 (1.25; -2.15, 4.65)
DSST	50.38±13.46	51.63±8.58	0.83 (-13.35, 10.85)

Notes: [1]CFT=complex figure test; RAVLT-Rey Auditory Verbal Learning Test; COWAT=Controlled Oral Word Association Test; TMT=Trail Making test. Digit Span = Total number of digits recalled on Digits Forward and Digit Backwards; DSST=Digit Symbol Substitution Test. *P** = Independent *t*-tests used to compare holder and non-holder groups. Significant *P* values in bold text.

Table 7-6: Mean±sd for all neurocognitive measures, according to holding status (holders =8, non-holders=8), for patients participating in the LAAM inter-dosing study

Measures[1]	Holders	Non-holders	*P** (mean diff, 95% CI)
CFT copy	29.25±4.00	30.38±3.89	0.58 (-1.13; -5.34, 3.11)
CFT delayed recall	15.50±5.44	16.13±5.25	0.82 (-0.63; -6.36, 5.11)
CFT delayed recall adjusted	14.25±4.86	12.38±6.01	0.51 (1.88; -3.98, 7.74)
RAVLT-Total words	45.88±11.13	43.75±11.03	0.71 (2.13; -9.78, 14.01)
RAVLT-delayed recall	6.88±2.95	7.63±3.70	0.62 (-0.75, -4.33, 2.84)
RAVLT-delayed recognition	10.75±2.44	12.13±3.04	0.34 (-1.34; -4.34, 2.84)
COWAT	37.38±6.95	33.13±8.84	0.30 (4.25; -4.28, 12.78)
TMT A/C	37.75±17.87	41.50±26.59	0.75 (-3.75; -28.05, 20.55)
TMT B/D	128.38±67.02	107.88±47.44	0.49 (20.50; -41.76, 82.76)
Digit Span	14.63±3.85	13.88±1.96	0.63 (0.75; -2.63, 4.13)
DSST	48.25±11.79	51.63±8.83	0.53 (-3.38; -14.55, 7.80)

Notes: [1]CFT=complex figure test; RAVLT-Rey Auditory Verbal Learning Test; COWAT=Controlled Oral Word Association Test; TMT=Trail Making test. Digit Span = Total number of digits recalled on Digits Forward and Digit Backwards; DSST=Digit Symbol Substitution Test. *P** = Independent *t*-tests used to compare holder and non-holder groups. Significant *P* values in bold text.

7.4.5. Exploratory analyses.

There were few significant relationships between the STAI scores for anxiety, and BDI-II score for depression and the neurocognitive variables for patients when taking methadone. Significant *negative* relationships were found between BDI scores and STAI scores and the delayed recall score (unadjusted) for the CFT (r=-0.65, P=0.006, and r=-0.71, P=0.002, respectively), and Digit Span (r=-0.54, P=0.03, and r=-0.63, P=0.009, respectively). No other significant relationships between BDI scores ($0.18<P<0.67$) and STAI scores ($0.11<P<0.69$) and other neurocognitive variables were found. With regard to patients when taking LAAM, there were no significant correlations between STAI and BDI-II scores, and any of the dependent variables derived from the neurocognitive tests ($0.07<P<0.97$).

Educational level was significantly negatively correlated with the delayed recognition and recall scores on the RAVLT for patients when taking methadone (r=-0.65, P=0.007 and r=-0.68, P=0.003, respectively), but only with the delayed recognition score on the RAVLT when patients were taking LAAM (r=-0.55, P=0.03).

There were no significant relationships between methadone dosage and LAAM dosage and performance on any cognitive test ($0.28 < P < 0.90$, and $0.35<P< 0.97$, respectively).

7.5. Discussion.

The present study compared the effects of methadone and LAAM on neurocognitive and psychomotor functioning in the same cohort of maintenance patients using a within subjects design. A matched group of control subjects also underwent the same testing procedure as patients. Participants completed an unpredictable tracking task eleven times across each inter-dosing interval study. In addition they were administered a battery of cognitive tests once during each inter-dosing study that was designed to assess various cognitive domains. As hypothesised, there were significant temporal changes in tracking performance (OSPAT scores), across both the methadone and LAAM inter-dosing studies. In contrast, tracking performance for control subjects was relatively stable across the study period. Furthermore, tracking performance did not differ for holders and non-holders, when maintained on either drug, and the overall magnitude of effect for tracking performance did not differ between drugs. Maintenance patients' performance on the

neuropsychological tests did not differ when maintained on methadone or LAAM, with the exception of scores on the CFT (performance on LAAM was significantly better than on methadone) and performance on Trails B (completion times were significantly shorter on methadone). Similarly, there were few differences in cognitive performance between holders and non-holders when maintained on either methadone or LAAM. Furthermore, the current study showed that relative to control subjects, patients exhibited a similar pattern of cognitive function (with the exception of performance on the Trail Making Test and Digit Span), when maintained on LAAM and methadone.

7.5.1. Unpredictable tracking performance.

As far as I am aware this is the first study to have examined tracking performance across the inter-dosing interval for both methadone and LAAM. Previous researchers administered these tasks on one or, at the most, on two occasions during the dosing interval (Berghaus, *et al*., 1993; Chesher, *et al*., 1989; Moskowitz and Robinson, 1985; Robinson and Moskowitz, 1985; Specka, *et al*., 2000). The current study design enabled me to examine the impact of the fluctuation in the plasma concentration of opioid on tracking performance. Furthermore, the tracking task used in this study assessed the capacity of maintenance patients to perform a more complex cognitive/psychomotor task than that required when completing each of the individual neuropsychological tests. Such performance may be more informative of the likely effect of these maintenance drugs on patients' ability to engage in more complex everyday activities, such as driving.

Tracking performance across the inter-dosing interval was similarly affected by the ingestion of both drugs. OSPAT scores decreased to be at a minimum at the time of putative peak plasma concentration of LAAM (and metabolites) and (R)-(-) methadone. Thereafter scores increased to be greater than seen at baseline. There were no statistical differences between drugs at any point across the interdosing interval, and the overall magnitude of effect (AUC_{0-24} x2 for methadone and AUC_{0-48} for LAAM) for tracking performance did not differ between drugs. Given the major attentional requirements of the tracking task the decrement in performance seen at the time of putative peak plasma concentrations of both drugs is likely to be, in part, due to sedation caused by the rising plasma concentrations of active opioid drugs, and the consequent 'tuning out' of the patient to external stimuli. In contrast, the improvement in performance at the end of the inter-dosing interval, relative to baseline, for patients when taking both methadone and

LAAM, is likely due to the emergence of withdrawal and heightened arousal. These data would suggest that, despite development of at least partial tolerance to effects of opioids, maintenance patients show significant fluctuation in psychomotor performance across the inter-dosing interval for both LAAM and methadone with greatest impairment evident at the time of putative peak plasma concentration of opioid.

Although this study found no statistically significant differences in the tracking performance of patients when taking either methadone or LAAM, relative to controls, these results need to be considered in light of the conservative alpha level adopted in this study. That is, given an alpha of P=0.05, the tracking performance of patients would have been significantly worse than that of controls at two hours and four hours post dosing for patients when taking LAAM (P=0.04 in each case) and at one hour and two hours post dosing for patients when taking methadone (P=0.04, and P=0.03, respectively); that is at times corresponding to peak plasma concentration of opioid drug. Clearly these results could represent type one error, but could equally represent a real effect. In light of the recent findings by Specka and colleagues (2000) that MMPs performed more slowly on a tracking task than matched controls, and the implications such results might have on determining the capability of maintenance patients to carry out complex tasks, future research should explore this phenomenon further.

7.5.2. Neuropsychological performance.

7.5.2.1. Methadone vs LAAM.

To my knowledge this is the first study to comprehensively examine the comparative effects of both methadone and LAAM on neurocognitive functioning in maintenance patients. A major strength of the current study was the within subject design that held individual differences and pre-existing cognitive deficits constant across drug conditions. In addition, testing was undertaken at the time of putative peak plasma concentration of both drugs, thus controlling for time of dosing.

In one of the few other studies to examine the comparative effects of LAAM and methadone on cognitive functioning, Irwin and colleagues reported that LAAM maintenance patients performed better than MMPs on tasks that assessed the speed and accuracy of information processing and also performance on a learning task (Irwin, *et al.*,

1976). In contrast, the major finding of the current study was the similar performance of patients when taking methadone and LAAM on a wide range of cognitive tasks, including verbal learning and memory (RAVLT), verbal fluency (COWAT), attention and working memory (Trails A, Digit Span), as well as speed of information processing (DSST). However, when taking LAAM, patients demonstrated significantly better performance on the CFT (copy and delayed recall) than when taking methadone; while patients when taking methadone exhibited significantly better performance on both Trails B (completion times shorter) and the related Trail Making measure of conceptual flexibility, than when taking LAAM. The clinical significance of these group differences is unclear given that they are few in number and in the opposite direction, and therefore the following discussion needs to be considered in light of these caveats.

As previously mentioned, the anecdotal reports in the literature of the adverse cognitive effects of methadone, such as feeling 'forgetful' or 'trouble remembering things' (Grevert, *et al*., 1977; Horns, *et al*., 1975), and that patients on LAAM report feeling less sedated and 'clear headed', than when on methadone (Freedman and Czertko, 1981; Savage, *et al*., 1976; Tennant, *et al*., 1986; Trueblood, *et al*., 1978; White, *et al*, 2002), are suggestive of the differential effects of LAAM and methadone on cognition. Given the general finding that patients who report memory problems may have a attention or concentration deficit (Spreen and Straus, 1991), it was hypothesized that while taking LAAM patients would more effectively attend to their environment and that this would translate into improved memory than when taking methadone. The finding in the current study that performance on the Digit Span and Trails A tests, which are considered tests of attention (Van Zomeren and Brouwer, 1992), did not differ between drugs, does not support the latter hypothesis. Nonetheless, it is interesting to note that patients while taking LAAM performed significantly better on the copy trial of the CFT than when taking methadone. The copy trial of the CFT requires that the participant copy the presented figure as accurately as possible; the score obtained represents individual perceptual ability (Spreen and Strauss, 1998). Therefore, as this task involves a significant attentional component it can also be considered to reflect the capacity of the participant to attend, in this case, to visual material.

Only one previous study to date has explored the effect of both drugs on memory function (Grevert, *et al*., 1977). The authors reported no differential effect of methadone or LAAM

on either visual memory (memory of where things are) or verbal memory (memory of lists of nouns and numbers). In contrast, although the current study found that there was no difference between drugs on indices of verbal memory derived from the RAVLT, patients on LAAM reported superior delayed recall of visual material (delayed recall on the CFT) than when on methadone. The latter finding would be consistent with the superior performance on the CFT copy trial for patients on LAAM compared to methadone, particularly if this reflects better attention to visual material for patients when on LAAM. In terms of how this would affect everyday functioning, it is likely that the superior delayed recall of visual material on LAAM would manifest as a better memory of 'where things are' such as everyday objects, e.g., keys and spectacles, than when on methadone.

In the current study patients' performance was significantly worse on Trails B (completion times longer) when taking LAAM than methadone. In this task individuals are required to not only connect circles, as in Trails A, but to also alternate between letters and numbers as they progress from circle to circle, and therefore assesses the capacity of individuals to keep track of more than one stimulus at a time. Furthermore, patients when taking LAAM were impaired on the Trails difference score (i.e., time taken to compete Trails B minus time taken to complete Trails A), suggesting that impairment extended to cognitive flexibility-related executive processes. The design utilised in this study held individual differences constant across testing sessions. In addition, the use of benzodiazepines, which have performance-impairing effects (Curran, *et al*., 1991; Curran, 2000), by patients was also constant across drug testing sessions. Thus the most parsimonious explanation for this finding is that it is a direct effect of LAAM (and/or metabolites) on cognitive function. Given the preliminary nature of this finding further research will need to replicate this finding to ensure its reliability.

7.5.2.2. Maintenance patients vs control subjects.

The current study also separately compared neuropsychological functioning in patients when maintained on either methadone or LAAM, with matched drug free control subjects. The major finding in the current study was the similar pattern of cognitive function (with the exception of performance on the TMT and Digit Span) in patients compared to control subjects when maintained on both drugs. Control subjects were matched to maintenance patients according to premorbid IQ, gender and age, and so between group differences were not attributable to differences in these demographic factors. Furthermore,

neuropsychological performance, when maintained on either drug, was largely unaffected by anxiety (as measured by the STAI), depression, educational attainment, and the dosage of the maintenance drug.

Comparison of patients maintained on methadone vs control subjects

The findings in the current study of slowed information processing speed (DSST), impaired attention and working memory (Trails A), and impaired long term visual (CFT) and verbal memory (RAVLT: recall and recognition) in patients when taking methadone, relative to controls, is consistent with the findings of a number of recent well controlled studies (Mintzer and Stitzer, 2002; Verdejo, *et al*, 2005). Digit Span did not differ for patients when taking methadone, relative to controls, suggesting that their ability to concentrate for brief moments was not significantly impaired. Only a few previous studies have examined the effects of methadone on Digit Span and have reported results that are equivocal with respect to the effect of methadone (Darke, *et al*., 2000; Gritz, *et al*., 1975; Kelley, *et al*., 1978).

As discussed previously, the DSST and Trails A tests incorporate significant motor components (see section *7.3.2.1*: Spreen and Strauss, 1998). Given that an acute effect of methadone is the lengthening of reaction times (see Zacny, 1995, for review), and as testing in the current study was undertaken at the time of putative peak plasma (R)-(-) methadone concentration, it is feasible that the impairment recorded by these tests reflects the acute effect of methadone on processing speed. However, recent evidence would not entirely support this conjecture. Darke and colleagues (2000) demonstrated impaired performance on the DSST in MMPs just before dosing, and more recently Mintzer and Stitzer (2002) showed that performance on DSST in MMPs was not related to the time of methadone dosing. Thus, as discussed by Minzter and Stitzer (2002), in relation to their findings, it may not be possible to differentiate impairment attributable to acute methadone dosing from that attributable to chronic drug use.

Despite impairment on Trails A, patient's performance on Trails B did not differ from that of control subjects. This is despite the fact that Part B is more difficult as it requires a participant to engage in multiple tracking. Furthermore, patients when taking methadone did not exhibit impairment relative to controls on the Trail Making tests' conceptual

flexibility measure (Trails B-A). This measure removes the motor component, as measured by Trails A, from the test evaluation, and suggests that MMPs did not have significant problems shifting their mental set (i.e. letters to numbers) as required by the test. Consistent with the latter result is the finding that performance on the COWAT, a measure of initial word fluency thought to reflect the efficiency of executive processes (Parker & Crawford, 1992), did not differ between methadone patients and control subjects groups. Both lines of evidence suggest that maintenance patients when taking methadone did not exhibit impairment of executive functioning, relative to the control subjects tested.

Comparisons of patients maintained on LAAM vs control subjects

There is a paucity of evidence on the comparative cognitive functioning of maintenance patients on LAAM and drug free control subjects. To our knowledge Irwin and colleagues (1976) reported on the only other study to have compared cognitive functioning in LMPs to controls. The authors reported that LMPs performed equally well on a number of cognitive tests, relative to the past performance of a group of control subjects who had been tested prior to the LMPs. However, the authors did not provide details of this control group, nor the context in which they were tested, and hence there are difficulties in interpreting these results. In contrast, the current study showed that patients when maintained on LAAM exhibited a similar pattern of cognitive function (with the exception of performance on the Trail Making Test and Digit Span), relative to control subjects, as they did when maintained on methadone.

The results from the current study show that there was a trend for the performance of patients on Trails A to be significantly impaired (P=0.051), relative to controls. This coupled with the significant impairment on Digit Span would suggest that attention and working memory were impaired while patients were maintained on LAAM, relative to controls.

Furthermore, patients' performance on Trails B and the Trail Making conceptual flexibility measure were also impaired, relative to controls. These results suggest that patients when maintained on LAAM at the time of testing had problems keeping track with more than one stimulus at the same time. It has been suggested that such a pattern of results may be indicative of impairment of executive functioning (Spreen and Strauss, 1998). This would

manifest in a constellation of behaviours that are likely to influence daily functioning, including difficulty in understanding complex instructions, suppressing inappropriate behaviours, and dealing flexibly with different source of information (Bryan and Luscz, 2000; Verdejo, *et al*., 2005). However, the absence of significant between-group differences on the COWAT, which is widely used to gauge executive dysfunction, would be inconsistent with this hypothesis. Consideration of these results does, nevertheless, raise the following question - given that the same cohort of patients was tested when they were taking each of the drugs, why wasn't a similar pattern of impairment, relative to control subjects, found for maintenance patients when taking methadone? As mentioned earlier in this discussion, one possibility is that such a result might be suggestive of some unique effect of LAAM and/or active metabolites on cognitive function. However, given the preliminary nature of these findings, further research using a larger sample size, and other tests of executive processes (eg. The Wisconsin Card Sorting Test, devised to assess shift of mental set: Lesak, 1995) should be undertaken to elucidate this finding.

The mean CFT copy score for patients when taking LAAM was similar to that achieved by control subjects, thus suggesting no difference between groups in attending to visual material. However, this did not translate into similar performance on delayed recall of visual material. Furthermore, although delayed recall of visual material for patients on LAAM was superior to when they were on methadone, this performance was still significantly worse than that of control subjects. The evidence of significantly impaired long-term visual memory, despite the relatively normal copy score when taking LAAM (and which was evident when patients were taking methadone) is suggestive of some underlying deficit in the process of memory consolidation. There are several factors that could explain the existence of pre-existing impairment in these patients. First, as previously mentioned, a number of maintenance patients participating in the current study were taking benzodiazepines that have acute anterograde amnestic effects (Curran, *et al*., 1991, Curran, 2000). Secondly, although not documented for patients participating in this study, there is evidence of a relatively high prevalence of non-fatal heroin overdoses (Darke, *et al*., 1996; Darke, *et al.*, 2000; McGregor, *et al*., 1998) and head injury in heroin users (Darke, *et al*., 2000) that are likely to result in diffuse brain damage. Such damage would manifest as permanent deficits in a number of cognitive domains, in particular memory, both verbal and visual.

7.5.3. Holder vs non-holders comparisons.

This is the first study to directly compare psychomotor and neurocognitive functioning in methadone holders and non-holders while they were maintained on methadone and LAAM. Psychomotor functioning across the inter-dosing interval, as assessed by OSPAT, did not differ for holders and non-holders when maintained on either drug. Furthermore, cognitive functioning was similar for holders and non-holders when maintained on either drug. The only differences were for delayed verbal recall and recognition indices derived from the RAVLT, such that non-holders' performance was superior to than of holders. When comparing these indices in methadone holders and non-holders when taking methadone and LAAM, it was found that holders' scores remained stable or improved, while for non-holders scores remained stable.

7.5.4. Limitations.

A number of factors may be responsible for the observed differences between the maintenance patients, when maintained on methadone and LAAM, and control subjects. First, maintenance patients were not successfully matched to control subjects according to educational attainment. Education level is an important determining factor in performance on a number of the tests utilised in this study, in particular the RAVLT and TMT (Lesak, 1995; Spreen & Strauss, 1998). Secondly, as patients and controls differed with respect to other drug use, the cognitive deficits seen may be due to differing histories of drug use (Minzter and Stitzer, 2002). Furthermore, a large proportion of patients were taking benzodiazepines at the time of testing, in comparison to none of the control subjects. As benzodiazepines have acute amnestic properties (Curran, 2000), this may also have affected cognitive functioning. Third, as previously discussed, a large proportion of maintenance patients have a history of overdose, and /or head injury, with the increased likelihood of brain damage that may have contribute to the cognitive impairment reported (Darke, *et al*., 2000; Davis, *et al*., 2002). Finally, the limited sample size used in this study needs to be considered when evaluating the findings and care taken not to over generalize the results. Future research should consider and address these limitations.

7.6. Conclusions and clinical implications.

In summary, this is one of the first studies to have comprehensively described the comparative effects of methadone and LAAM on cognitive and psychomotor functioning in maintenance patients. Essentially, patients maintained on either drug exhibited a similar

pattern of cognitive function compared to control subjects. Furthermore, there were few differences in the effects of LAAM and methadone on psychomotor and cognitive functioning in the tested patients. However, results are suggestive of impaired executive functioning in patients transferred from methadone to LAAM that may impact on daily functioning. Further research will need to verify this finding before it can be considered reliable. Importantly cognitive and psychomotor functioning was similar for holders and non-holders when maintained on either drug. Thus, the transfer of methadone non-holders and holders to LAAM is likely to occur without patients experiencing any additional adverse cognitive effects.

8. Characterisation of the Relative *in vitro* Potencies of LAAM, Nor- and Dinor-LAAM

8.1. Introduction

Until quite recently LAAM's opioid action and duration of effect have been attributed to the action of its demethylated metabolites, nor- and dinor-LAAM (Cook, *et al*., 1984; Kaiko and Inturrisi, 1975; Vaupel and Jasinski, 1997). Accordingly, the time course of LAAM's effect, exemplified by miosis, appeared to correspond more closely to the plasma concentration-time profile for nor-LAAM, than for LAAM (Cook, *et al*., 1984; Kaiko and Inturrisi, 1975; Walsh, *et al*., 1998). Hence, reports have attributed little intrinsic activity to LAAM itself (Cook, *et al*., 1984; Jaffe and Martin, 1991; Kaiko and Inturrisi, 1975). However, a relatively recent report suggested that LAAM was active following intravenous administration and therefore may contribute to the early opioid agonist activity of LAAM (Walsh, *et al.*, 1998). As reviewed in chapter 1 (section 1.5.3.2.1.), at the time of carrying out the work for the preceding chapters, few studies had examined the relative *in vitro* potencies of LAAM, nor- and dinor-LAAM with respect to binding and function. Therefore, the work described in this chapter was undertaken to rectify the relative lack of contemporary data characterizing the relative *in vitro* and functional potency of these drugs. It is envisaged that such data might help reconcile the disparate reports regarding the pharmacological activity of LAAM, and its active metabolites, and also may aid in the interpretation of pharmacodynamic data collected as part of this thesis.

In order to address the objectives outlined above, two *in-vitro* models of pharmacological activity, the electrically-stimulated guinea-pig ileum bioassay and receptor binding assay, were used to examine the *in vitro* potency of the drugs of interest. The specific action of opioids on the guinea pig ileum is to produce a dose-dependent inhibition of electrically evoked longitudinal muscle contractions as a result of the depression of acetylcholine release from presynaptic neurons of the myenteric plexus (Cox and Weinstock, 1966; Lord, *et al*., 1977; Paton, 1957). This is usually measured by isometric recording of the longitudinal muscle contraction induced by electrical field stimulation. On the other hand, competition receptor binding studies measure the binding of a single concentration of radiolabeled ligand, in the presence of various concentrations of unlabeled ligand, to determine the affinity of a ligand for a particular receptor, and are usually carried out in tissue homogenate. Bioassays have the advantage over receptor binding assays in allowing

the examination of receptors in their native, functional state. However, unlike receptor binding, the measured response is not a direct measure of the drug-receptor interaction (Smith and Leslie, 1993). Therefore, both *in vitro* assays produce complementary data on the relative potency of drugs.

Thus, the aims of the following set of experiments were:

- To compare the relative potency of LAAM, nor-LAAM, dinor-LAAM in inhibiting the electrically induced twitch of the guinea pig isolated ileum.
- To determine the relative potency of LAAM, nor-LAAM, dinor-LAAM in displacing binding of the μ opioid receptor selective radioligand, [H^3] DAMGO, to opioid receptors derived from rat brain homogenates.

8.2. Materials and Methods

8.2.1. General

All procedures were carried out in accordance with guidelines set down by the National Health and Research Council of Australia for the care and use of laboratory animals and ethics approval was obtained from the institutional animal ethics committee (Adelaide University Ethics No: M6399). Part of the isolated guinea pig ileum work was undertaken by third year Bachelor of Science students (Karen Beard, Emma Clarke, Amanda Jackson, and Tracey Siebert) as the fulfillment of the practical component of a third year pharmacology subject. This work was supervised by myself and Dr Abdallah Salem of the Department of Clinical and Experimental Pharmacology (currently Discipline of Pharmacology).

8.2.2. Animal Housing and maintenance

Male IMVS tri-coloured guinea pigs, from the Institute of Medical and Veterinary Science Animal Resource Centre (Gilles Plains, Adelaide, South Australia), and male Sprague Dawley rats were maintained at the University of Adelaide Medical School Animal House. They were housed in separate rooms and were maintained under conditions of constant temperature (21 ± 2^0C) and 50 % humidity. Room lighting was on a 12-hour light: dark cycle with lights on at 0700 hours and off at 1900 hours. Guinea pigs were supplied with

guinea pig feed and rats with rat chow (New Joint Stockdiet, Ridley Agriproducts, Australia). All animals were offered water *ad libitum*.

8.2.3. Guinea pig ileum assay

8.2.3.1. Drugs and solutions

The physiological fluid used in the organ baths was Krebs solution. This was prepared daily and was of the following composition (in mM): NaCl, 118.4; KCl, 4.7; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; $MgSO_4.7H_20$, 1.2; Glucose, 11.1; $CaCl_2.2H_2O$, 2.5 (All chemicals were obtained from BDH Chemicals, Merck, Pty, Ltd, Sydney Australia). LAAM, nor-LAAM, dinor-LAAM, and racemic-methadone were gifts from the National Institute on Drug Abuse (NIDA, Rockville, MD, USA). Acetylcholine chloride was obtained from Sigma Aldrich (Castle Hill, NSW, Australia). Stock solutions (10^{-2} M) of all test compounds were prepared in filtered de-ionized water (Milli-Q System, Millipore) and kept frozen at – 20^0c until the day required. Drugs were then diluted to the required concentrations with 0.9% NaCl the morning of use and kept on ice until required. The expressed concentrations of all drugs refer to the final molar concentration in the organ bath.

8.2.3.2. Tissue Preparation

Guinea pigs (weighing 350-600 gms) were sacrificed by cervical dislocation and exsanguinated. The ileum was dissected out, the initial and terminal 10 cm discarded, and the remaining tissue gently flushed of gut contents using warm Krebs solution previously bubbled with 95% O_2 and 5% CO_2. Following this, tissue segments were kept in a holding bath that contained Krebs solution, continuously bubbled with 95% O_2 and 5% CO_2 and maintained at room temperature (21 ± 2^0C), until required for experiments.

8.2.3.3. Experimental procedure

Strips of ileum (3cm in length) were suspended under 1g tension in organ baths (11-17 ml) filled with Krebs solution at 37^0C which was continuously bubbled with 95% O_2 and 5% Co_2. The ileum preparation was tied via a small loop to a holder while the other end was attached to a transducer. Tissue tension developments were recorded using MacLab computers. The tissue segments were continuously stimulated at 60 to 70 mv, for a duration of 1 msec, once every 10 seconds (0.1 Hz). Tissue viability was assessed by

acetylcholine (10^{-4} M) induced contraction, and non–viable tissue was discarded. For viable tissue segments, the bath was then flushed a number of times until the tissue returned to its normal tone. Electrical stimulation was applied and an equilibration period of 15 minutes was allowed prior to drug addition. One drug concentration was added per tissue segment. To act as positive control and, to relate our data to those previously published, the effect of racemic-methadone was also measured. (R)-(-) and (S)-(+) methadone were not studied because of limited tissue availability. The range of final bath concentrations was 1x 10^{-8} M to 1x10^{-4} M for all drugs tested. The tissue was kept in the bath until maximal inhibition was reached and then discarded. Six tissue segments were used to determine the twitch inhibiting activity at each concentration

8.2.3.4. Data analysis

Data were collected by MacLab (version 3.5, ADInstruments). The twitch inhibiting activity of each drug concentration for each piece of tissue was determined using the following equation:

$$\% \text{ Twitch Inhibition} = \frac{(a - b)}{a} \times 100$$

Where '*a*' is the control twitch height (the mean value of three successive twitches preceding the application of drug), and '*b*' is the minimum twitch height obtained after the application of the drug (mean value of three successive twitches following the application of the drug).

The concentration of each opioid producing a 50% reduction (IC_{50}) in baseline twitch was derived from plots of the percent depression of twitch height (expressed as percentage of baseline) versus concentration data using non-linear regression (Graphpad Prism 3.02, Graph Pad Software, CA, USA). The mean IC_{50} value presented is the mean of six estimated IC_{50} values for each drug. In view of the large variability in IC_{50} values obtained in this study the Kruskal-Wallis nonparametric equivalent to one-way ANOVA was used to determine the significance of differences between mean IC_{50} values for all drugs. *P* values < 0.05 were considered significant

8.2.4. Receptor Binding studies

8.2.4.1. Drugs.

[^{3}H] DAMGO ([^{3}H]-D-Ala^2, MePhe^4, Gly-ol^5 enkephalin; Specific activity=54.5 Ci/mmol) was obtained from NEN Life Science Products (Boston MA, U.S.A) and unlabelled DAMGO from Sigma Aldrich (Castle Hill, NSW, Australia). LAAM, nor-LAAM, dinor-LAAM, racemic-methadone, (R)-(-) methadone and (S)-(+) methadone were gifts from NIDA. Stock solutions (10^{-2} M) of all drugs were prepared in filtered de-ionized water (Milli-Q System, Millipore) and kept frozen at –20^{o}C until required. Fresh dilutions were made each week.

8.2.4.2. Tissue preparation.

The methods used for the preparation of tissue and for receptor binding (see section 8.2.4.3) were based on those described by Kristensen and colleagues (1995). Male Sprague Dawley rats (weighing 300 - 400 g) were stunned by a blow to the head and decapitated. The brain was rapidly dissected and the cerebellum removed prior to homogenization, as this structure is devoid of opioid receptors (Pert and Snyder, 1973a). Each brain was initially placed in 20 volumes of cold 0.32 M sucrose and was gently homegenised using a hand held glass-teflon homogeniser. The homogenate was centrifuged at 4^{o}C for 5 minutes at 2000 x *g*. The supernatant was removed, centrifuged for a further 10 minutes at 30, 000 x g, and the resultant pellet resuspended in 10 volumes of 0.05 M Tris-HCl buffer (pH 7.4 at 4.0 ^{o}C) with several brief pulses from a Jankel and Kunkel Ultra-Turrax homogeniser. During a typical preparation session, four brains were prepared in this manner and then pooled. Aliquots of homogenate sufficient to carry out individual experiments were kept on ice until used or were stored at -80.0 ^{o}C to be used within 24 hours. Protein concentrations were determined using the bicinchoninic assay (Smith, *et al.,* 1985); there was little variation in protein concentration between prepared homogenates (% CV = 6.8%). Prior to use in receptor binding assays the homogenate was quickly thawed, disrupted using the Ultra-Turrax homogeniser, and kept at 4 ^{o}C until used.

8.2.4.3. Receptor Binding experimental procedure

All binding was determined in an incubation mixture containing 500 µl of rat brain homogenate diluted to a final volume of 2 ml with 0.05 M Tris-HCL buffer (pH 7.4), in the

presence of 5 mM $MgSO_4$. Total binding was determined in the presence of 0.7 nM [^{3}H] DAMGO as used by Kristensen and colleagues (1995). Displacement of [^{3}H] DAMGO by each of the test compounds was determined in the presence of six different concentrations (within the range $1x10^{-11}$ to $1x10^{-4}$ M) of each drug. The effects of racemic-methadone, (R)-(-) and (S)-(+) methadone were also measured as controls. Non-specific binding was determined in parallel tubes in the presence of 100 nM unlabelled DAMGO. The assay tubes were incubated for 90 minutes at 25 ^{o} C on a shaking platform. The reaction was terminated by immersing the assay tubes in an ice bath and subsequent vaccum filtration through dry Whatman GF/B filters pre-soaked with 0.5% polyethyleneamine. The filters were then rinsed twice with 8 ml cold tris-HCL buffer and, once dry, transferred to scintillation vials containing 10 ml scintillation fluid (CystoScint, ICN, Australia), left overnight and radioactivity determined by liquid scintillation counting (LS 3801 Beckman Instruments). Experiments were performed in triplicate and 4 to 6 experiments were performed for each competing drug.

8.2.4.4. Data analysis

Specific binding was calculated by subtracting the value for non-specific binding from that for total binding. Displacement curves were drawn for each tested drug. IC_{50} values were derived from each curve, and inhibition constants (K_i) determined by GraphPad Prism (GraphPad Prism v3.02, Graphpad Software, CA, USA) using the Cheng Prusoff equation (Cheng and Prusoff , 1973).

$K_i = IC_{50} / (1+ [\text{radioligand}] / K_d)$.

Where IC_{50} = the concentration of competing ligand that inhibited 50 % of specific binding. The dissociation constant (K_d = 0.28 nM) for DAMGO was obtained from Chen, *et al*., (1991), from our laboratory, who also examined the affinity of a number of opioids to μ opioid receptors using rat brain homogenate. One Way ANOVA with Scheffès post hoc test was used to determine the significance of differences between IC_{50} values and K_i values for LAAM, nor-LAAM, dinor-LAAM and racemic-methadone. P values < 0.05 were considered significant. Results are mean±sd, unless otherwise stated. The data were analysed using SPSS™ for Windows (v.10), unless otherwise stated.

8.3. Results

8.3.1. Guinea pig isolated ileum assay

As can be seen in figure 8.1 all opioid compounds depressed the electrically stimulated twitch of guinea pig ileum in a concentration-dependent manner. Table 8.1. shows the descriptive statistics for the inhibitory effects (IC_{50}) of each compound tested. Kruskal Wallis ANOVA revealed that there were significant differences in mean IC_{50} values for the tested drugs ($\chi(3)^2=11.75$, $P=0.08$). However, in view of the large range of IC_{50} values, in particular for dinor-LAAM, consideration of median values is a more valid indicator of central tendency. With this in mind nor and dinor-LAAM are seen to be equipotent in this preparation, but more potent than LAAM (table 8.1). Furthermore, racemic-methadone was more potent than all other drugs tested.

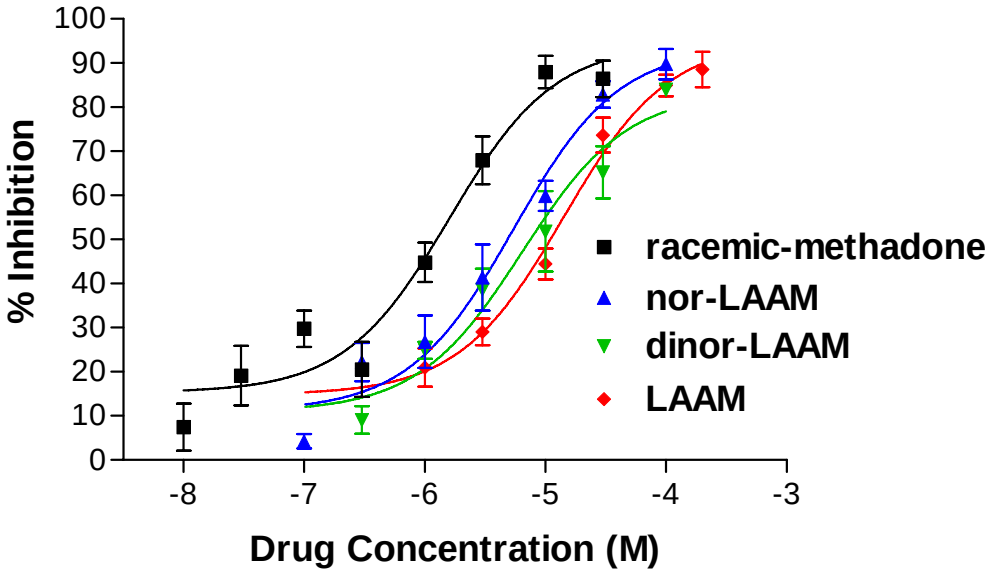

Figure 8-1: Mean percent depression of twitch height as a function of drug concentration for racemic-methadone, LAAM, nor-LAAM and dinor-LAAM. Six tissue segments were used to determine the twitch inhibiting activity at each concentration of each drug tested. Data are mean percent inhibition ±SEM.

Table 8-1: Descriptive statistics for the inhibitory effects (IC_{50}) of racemic–methadone, nor-LAAM, dinor-LAAM, and LAAM on the electrically induced twitch of the isolated guinea pig ileum.

Opioid	[1]IC_{50} (μM)		
	mean	median	sd
racemic-methadone	1.87	1.44	1.43
nor-LAAM	6.95	7.24	4.30
dinor-LAAM	18.34	7.56	24.84
LAAM	15.10	16.12	4.80

Notes: [1]IC_{50} = Concentration of compound producing a 50% inhibition of the electrically induced twitch of the isolated guinea pig ileum. [2] sd=Standard deviation.

8.3.2. Receptor binding

The displacement of [^{3}H] DAMGO by LAAM, nor-LAAM, dinor-LAAM and racemic-methadone is shown in figure 8.2. The displacement curves clearly show that all these drugs inhibited the binding of [^{3}H] DAMGO in a concentration dependent manner. Non-specific binding across all experiments was less than 20% of total binding (mean±sd:19.90±5 %).

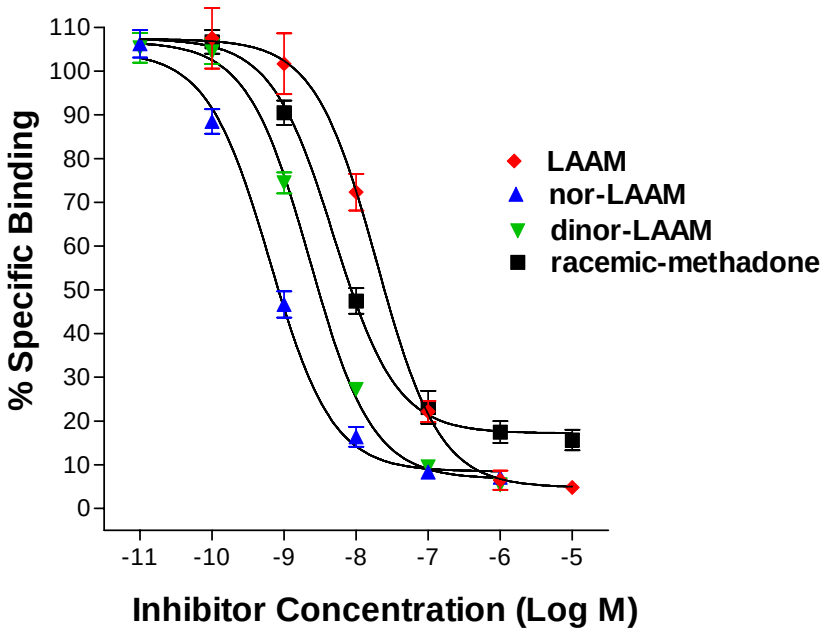

Figure 8-2: Competition displacement curves of [^{3}H]-DAMGO by LAAM, nor-LAAM, dinor-LAAM and racemic-methadone. Data are mean percent inhibition±SEM obtained from 4-6 experiments, each performed in triplicate

The mean IC_{50} and K_I values for all opioids tested are presented in table 8.2. Nor-LAAM and dinor-LAAM were approximately 30 and 10 times more potent than LAAM,

respectively, in displacing [^{3}H]-DAMGO in rat brain homogenates. In turn, nor- and dinor-LAAM were approximately 8 and 3 times more potent than racemic-methadone, respectively. There were significant differences in IC_{50} and K_i values for LAAM, nor-LAAM, dinor-LAAM and racemic-methadone; F (3,14)=160, P<0.001, for both. Post hoc tests revealed that the mean K_i values for nor-LAAM and dinor-LAAM were not significantly different from each other (P=0.45), while the K_i value for nor-LAAM (P=0.004), but not dinor-LAAM (P=0.111) was significantly less than the mean K_i value for racemic methadone. Furthermore, the mean K_i value for LAAM was statistically greater than the K_i value for nor-LAAM, dinor-LAAM and racemic-methadone (see table 8.2. P<0.001). Hence, both nor- and dinor-LAAM had significantly greater affinity for μ opioid receptors than LAAM.

Table 8-2: Binding affinities of LAAM, nor-LAAM, dinor-LAAM, racemic-methadone, (R)-(-) and (S)-(+)-methadone for μ opioid receptors in rat brain homogenates. IC_{50} and Ki values are mean±sd .

Ligand	[1] IC_{50} (nM)	[2] K_i (nM)	[3] R^2	[4] N
LAAM	19.65±2.92	5.61±0.83	0.998±0.000	4
nor-LAAM	0.67±0.13*	0.19±0.04*	0.997±0.002	6
dinor-LAAM	2.29±0.20*	0.64±0.05*	0.998±0.001	4
racemic-methadone	4.94±0.88*	1.41±0.27*	0.998±0.001	4
R-methadone	2.19±0.18	0.63±0.05	0.999±0.000	5
S-methadone	58.04±26.1	16.58±4.72	0.996±0.003	4

Notes:[1] IC_{50} = Concentration of compound required to inhibit 50% of [^{3}H] DAMGO binding; [2] K_i = inhibition constant for displacement of [^{3}H] DAMGO; [3] R^2 Goodness of fit of the non-linear regression line; [4] N= number of experiments, each run in triplicate; *P<0.05, Post hoc Scheffès test versus LAAM (Nb: (R)-(-) and (S)-(+) methadone were not included in comparisons with LAAM).

8.4. Discussion.

These experiments sought to elucidate the relative *in-vitro* potencies of LAAM, nor-LAAM and dinor-LAAM in two different *in-vitro* models of pharmacological activity, i.e., the electrically stimulated guinea-pig isolated ileum and competition receptor binding. Results from both assays show that nor-LAAM and dinor-LAAM were more potent than LAAM. Furthermore, in the guinea pig ileum bioassay racemic-methadone appeared more potent than both metabolites, while in the receptor binding studies nor-LAAM, but not

dinor-LAAM, had significantly greater affinity than racemic methadone. Data from these studies also show that LAAM was pharmacologically active.

To date there has only been one published report on the relative activity of LAAM and its metabolites on the electrically stimulated guinea pig isolated ileum (Nickander, *et al*., 1974). The latter authors demonstrated that nor-LAAM and dinor-LAAM were 16 and 11 times more potent than LAAM. In the current study there was a large range of IC_{50} values for each drug, in particular for dinor-LAAM, which obscured the interpretation of the data. However, the comparison of the median IC_{50} values for these compounds shows that both nor- and dinor-LAAM were more potent than LAAM in this preparation. These results would be in accordance with those presented by Nickander and colleagues. Furthermore, given that the use of the guinea pig ileum assay would have precluded significant biotransformation of LAAM to its active metabolites, it is clear that LAAM is also an active drug, although less potent than its demethylated metabolites. Nevertheless, the mean IC_{50} concentrations of LAAM, nor- and dinor-LAAM in the current study were not of the same order of magnitude as reported by Nickander and colleagues (9.4×10^{-7} M, 5.9×10^{-8} M, 8.6×10^{-8} M, respectively). These differences may be explained by the use of different experimental conditions; in particular the different tissue preparation used in the current study compared to that used by Nickander and colleagues. The latter authors used the isolated myenteric plexus-longitudinal muscle of the guinea pig ileum in their assay, while in the current study used intact strips of guinea pig ileum. While the latter tissue preparation is easier to prepare, it is likely that it would be less sensitive to the effects of opioid agonists as access to receptors would be limited by the intact connective tissue sheath (Smith and Leslie, 1993).

Prior to discussing the results obtained from receptor binding a few methodological comments related to these studies should be made. It should be noted that before using the K_d obtained from Chen and colleagues, I attempted to reconfirm it through saturation binding studies. However, supplies of radioligand ran out before these studies could be completed. Irrespective of this, the rank order of differences in binding affinities (K_i) found in my study is in accordance with past studies (Bertalmio, *et al*, 1992; Horng, *et al*, 1976). Furthermore, this study used the μ-opioid selective radioligand, [^{3}H] DAMGO, to characterise opioid receptors. Codd and colleagues (1995) demonstrated that DAMGO was 230 times more selective for μ receptors (K_i = 0.37 nM) than δ receptors (K_i = 85 nM),

and 570 times more selective for μ receptors than κ receptors (K_i = 210 nM). Thus, unlike studies that utilised radioligands, such as [^{3}H] naloxone, that are relatively nonselective for opioid receptors (Horng, *et al*., 1976), binding affinities obtained in the current studies are more likely to reflect specific μ opioid receptor binding.

In the receptor binding studies nor-LAAM and dinor-LAAM were found to have approximately 30 and 10 times higher affinity, respectively, for μ opioid receptors than LAAM. These results are generally in accord with the results of previous studies that have investigated the relative affinity of these compounds for opioid receptors in rat brain (Horng, *et al*, 1976) and monkey cortex (Bertalmio, *et al*, 1992). Subsequent to the completion of the present receptor binding studies, Kharasch and colleagues (2005) published their findings regarding the relative affinity of LAAM, nor- and dinor-LAAM to Chinese hamster ovary cell membranes expressing recombinant human μ opioid receptors. The mean±sd K_i values for LAAM, nor- and dinor-LAAM for the displacement of [^{3}H] diprenorphine were 4.72±0.30 nM, 0.15±0.01 nM, and 0.37±0.02 nM, respectively, which are similar to, and in the same rank order as, the K_i values found in the current study for the displacement of [^{3}H] DAMGO by each of these compounds.

Racemic-methadone was included as a reference compound in this study as it is considered the gold standard in the substitution treatment of opioid dependence (Ward, *et al*., 1999). The mean K_I concentration of racemic-methadone found in the current binding studies (mean±sd: 1.41±0.27 nM) was in accord with that reported by Codd and colleagues (1995) (mean=1.70 nM). Furthermore, in the current study nor-LAAM was found to have statistically greater affinity (K_I = 0.19 nM), but dinor-LAAM a similar affinity (K_I = 0.64 nM) for μ opioid receptors than racemic-methadone, attesting to their *in vitro* potency. In addition, the mean K_I values for (R)-(-) methadone (mean±sd: 0.63±0.05 nM) and (S)-(+) methadone (mean±sd: 16.58±4.72 nM), reported in the current study, were also in accord with those reported by Codd and colleagues (1995) (0.95 nM and 19.7 nM, respectively).

Notwithstanding the relative *in vitro* potencies of LAAM, nor-LAAM and dinor-LAAM, demonstrated in this chapter, the ultimate contribution of these compounds to the pharmacodynamic effect of LAAM would be dependent on their relative central nervous system concentrations. A combination of passive diffusion and active transport mechanisms are likely to be responsible for determining the resultant brain/blood ratio.

For example, evidence suggests that methadone is a substrate for the efflux transporter p-glycoprotein (P-gp) *in vitro* (Kharasch, *et al*., 2004). As LAAM is structurally similar to methadone, this could affect its brain/blood ratio. However, we do not currently know if LAAM is also a substrate for this transporter. Furthermore, LAAM is more lipophilic (octanol-to-water partition of 492.6: Kaufman, *et al*., 1975), than either nor-LAAM (octanol-to-water partition of 80; Umans and Inturrisi, *et al*., 1981) or dinor-LAAM (octanol-to-water partition of 42; Umans and Inturrisi, 1981), and therefore may more readily enter the brain than its demethylated metabolites.

There are limited animal data to show that LAAM has a high affinity for brain tissue. For example, Mulé and Misra (1978) reported that following the administration of 2 mg/kg (^{3}H) LAAM orally to monkeys, the highest concentration of LAAM was in tissue sampled from the CNS, which was approximately 20 times greater than in CSF. Furthermore, McMahon and colleagues (1965) examined the tissue distribution of radioactivity following the administration of 2.5 mg/kg *i.v* ^{14}C labeled LAAM, or nor-LAAM, to rats. For LAAM, total brain radioactivity was at least two-fold greater than that detected in plasma for up to one hour following administration. Thereafter, radioactivity in brain and plasma appeared to be similar. In contrast, for nor-LAAM, total radioactivity counts in plasma and brain tissue appeared to be similar from 5 minutes to 2 hours following administration. These data, although limited, do show that LAAM has a greater affinity for brain tissue than its demethylated nor- metabolite, and that plasma drug concentrations may not parallel drug concentrations in CNS tissue. Thus, even though nor- and dinor-LAAM have greater *in vitro* potency than LAAM, unchanged LAAM is likely to be distributed to a greater extent to the brain than its active metabolites, and this may counteract any apparent potency differences.

As discussed earlier, the notion that LAAM is merely a prodrug for its active metabolites (Cook, *et al*., 1984; Fraser and Isbell, 1954; Kaiko and Inturrisi, 1975; Vaupel and Jasinski, 1997) has recently been challenged (Walsh, *et al*., 1998). It is now considered probable that the parent drug contributes to the <u>early</u> opioid agonist activity of LAAM (Walsh, *et al*., 1998) and thus, the nature of the LAAM concentration-effect relationships may be more complex than once thought (Kharasch, *et al*., 2005). Researchers utilizing a pharmacodynamic-pharmacokinetic study paradigm have reported that the time course of LAAM's effect, exemplified by miosis, appeared to correspond more closely to the

concentration-time profile for nor-LAAM, than for LAAM (Cook, *et al*., 1984; Kaiko and Inturrisi, 1975; Kharasch, *et al*., 2005). Indeed the results presented in Chapter 4 are consistent with these observations (see section *4.5.3*). These results would be consistent with nor-LAAM's greater *in vitro* potency and relatively flatter plasma-concentration profile, as shown in chapter 4, than LAAM's. Interestingly, there have been equivocal reports concerning dinor-LAAM's possible contribution to LAAM's effect. Although Kaiko and Inturrisi (1975) did not discount the contribution of dinor-LAAM to LAAM's miotic effect, they argued that its relatively stable plasma-concentration profile obscured its contribution. However, Cook and colleagues (1984), while reporting that the rate of pupil constriction closely followed the appearance of nor-LAAM (Pearson r=0.993), reported that it was also highly correlated with the appearance of dinor-LAAM (r=0.988). Therefore, given its *in vitro* potency, and flat plasma concentration-time profile, it is also highly likely that dinor-LAAM would contribute to LAAM's opioid agonist effects, particularly its duration of activity. Moreover, the results of this study demonstrate that LAAM exhibits pharmacological activity, which is suggestive of a role for the parent drug in the opioid effect observed following the administration of LAAM. Given LAAM's plasma concentration-time profile, it is likely, as proposed by Walsh and colleagues (1998), that it would contribute to the early opioid agonist activity of LAAM.

In summary, if one collectively considers the *in vitro* potency data presented in this chapter, with the information discussed above, it can be seen that it is likely that LAAM, nor-LAAM and dinor-LAAM would all contribute to LAAM's opioid effect. I have argued earlier that it is probable, given the plasma concentration-time profile for all three compounds on chronic dosing of LAAM that they would all contribute to LAAM's peak effect, but that the long duration of LAAM would be attributed to the relatively stable concentration-time profiles for nor- and dinor-LAAM, compared to LAAM.

9. General Summary and Discussion.

The principal aim of this thesis was to examine the efficacy of using LAAM to treat MMT patients, some of whom regularly complain of breakthrough withdrawal during the 24-hour methadone inter-dosing interval. Subsidiary aims were to 1) elucidate the pharmacokinetics of LAAM, nor-LAAM and dinor-LAAM at steady state, and to compare the temporal change in opioid effect (eg., miosis, repiratory depression) and withdrawal across methadone's and LAAM's inter-dosing intervals, and 2) to elucidate the *in vitro* potencies of LAAM, nor- and dinor-LAAM with respect to receptor binding studies and function in the electrically stimulated guinea pig ileum assay. The following discussion will commence by reviewing the major findings of the current research.

9.1. Summary of major findings.

Chapter three examined the steady state pharmacodynamics and pharmacokinetics of (R)-(-) methadone in MMT patients in order to characterise the pharmacological basis of break through withdrawal (non-holding). The time course for self-reported withdrawal and physiological indices (pupil diameter and respiratory rate) was the inverse of the plasma (R)-(-) methadone concentration versus time profile (Dyer, *et al*., 1999, 2001; McCaul, *et al*., 1982). In contrast, the relationship between the time course of subjective responses and plasma (R)-(-) methadone concentration versus time profile was less clear.

Methadone non-holders in this study had greater fluctuations in plasma methadone concentration across the dosing interval than holders, as evidenced by the significantly shorter time that plasma (R)-(-) methadone concentrations were above 75% C_{max} (6.82 hours vs 14.28 hours, respectively). Furthermore, the withdrawal profile for self-reported non-holders was the inverse of the plasma methadone concentration versus time profile, with non-holders exhibiting significantly greater withdrawal at the beginning and the end of the methadone inter-dosing interval than the holders. Non-holders in the current study also received a higher average daily methadone dose (83 mg) than holders (73 mg) and did not differ from holders on the basis of peak or trough plasma (R)-(-) methadone concentrations. These findings suggest that non-holding is associated with greater fluctuation in plasma methadone concentration across the dosing interval, rather than inadequate daily methadone dosage and absolute plasma methadone concentration. Future research will need to determine the reliability of these findings in a larger cohort of methadone maintenance patients.

The latter findings have important clinical implications for methadone maintenance patients who consistently report breakthrough withdrawal symptoms during the inter-dosing interval. As previously discussed, one potential solution to the problem of non-holding is to increase the daily dosage of methadone to achieve higher plasma concentrations, and hopefully, reduce the severity of opioid withdrawal symptoms. However, this would increase the incidence and severity of adverse effects, such as respiratory depression, sedation and constipation. In view of methadone's very steep plasma concentration-effect relationship for withdrawal (mean±sd slope factor = 5.4±1; Dyer, *et al*., 1999), and evidence that non-holders exhibit greater fluctuation in plasma (R)-(-) methadone concentration than holders, adopting a strategy to flatten the plasma opioid concentration versus time profile over the inter-dosing interval should help lessen withdrawal in these patients. One strategy is to shorten the inter-dosing interval (by splitting the daily methadone dose), but when this fails, an alternative is to use another maintenance drug with different pharmacokinetics than methadone, such as LAAM, to lessen the fluctuation in plasma opioid concentration over the dosing interval.

The work in chapter four described the pharmacokinetics and pharmacodynamics of LAAM, nor-LAAM and dinor-LAAM in a relatively large cohort (n=17) of maintenance patients. Pronounced temporal changes across the inter-dosing interval were demonstrated for all physiological parameters (excluding diastolic blood pressure). However, temporal changes for pupil diameter and respiratory rates were most evident, with maximal effects occurring at the approximate T_{max} for LAAM and nor-LAAM (i.e., 2 to 6 hours post dosing). Significant effects, relative to baseline, persisted through to 12 hours after dosing for miosis, and through to 48 hours after dosing for respiratory depression. The pattern of results for the subjective measures with the exception of self-reported withdrawal was less clear. As hypothesised, prolonged suppression of withdrawal was evident throughout the 48-hour inter-dosing interval. These data are indicative of the long duration of action of orally administered LAAM.

Furthermore, the steady state pharmacokinetics of LAAM and its demethylated metabolites were examined using non-compartmental methods. The pharmacokinetic profiles of the three analytes were complex and were characterised by significantly less fluctuation of plasma nor- and dinor-LAAM concentrations across the inter-dosing interval than plasma

LAAM concentrations. Indeed, dinor-LAAM exhibited virtually no fluctuation over the 48-hour inter-dosing interval. Despite verification of steady state conditions and dose-normalisation of plasma concentration data, there was substantial inter-individual variability for most pharmacokinetic parameters that was generally greater for LAAM, than nor-LAAM, which in turn was greater than for dinor-LAAM. However, despite this variability, there was a significant relationship between the weight corrected dose and plasma AUC for LAAM, nor- and dinor-LAAM, which suggests that the pharmacokinetics of LAAM and its metabolites are linear across the dose range 30 to 150 mg every second day. Importantly, this inter-individual variability did not appear to adversely impact on therapeutic efficacy as all patients reported adequate withdrawal suppression. Furthermore, urine was also collected from 16 patients over the entire 48 hour LAAM inter-dosing interval. The renal clearance (CL_R) of LAAM accounted for 0.5 to 4 % of CL/F (apparent plasma clearance), which is consistent with LAAM been cleared by the liver, primarily by conversion to nor-LAAM. The urinary excretion of LAAM, nor- and dinor-LAAM only resulted in a mean recovery of 23 % of the total LAAM dose administered, which is also suggestive of significant non-renal elimination of LAAM and metabolites.

Importantly, holders and non-holders could not be differentiated on any pharmacokinetic or pharmacodynamic parameter that might otherwise determine patients' response to LAAM. Verification of the latter was considered important, as any differences detected may have confounded the interpretation of data showing the apparent effectiveness of LAAM in treating methadone non-holders.

Chapters 5 through to Chapter 7 presented an evaluation of LAAM as an alternative substitution treatment to methadone maintenance treatment, in particular for methadone non-holders. It is noteworthy that this is the first study to comprehensively compare the steady state pharmacodynamics of LAAM and methadone. Chapter five compared the magnitude and duration of opioid agonist effects and the prevalence and severity of a number of symptom complaints across the interdosing interval for both (R)-(-) methadone and LAAM. The peak magnitude of methadone and LAAM effects was similar for the two drugs. The time to peak effect for respiratory depression and miosis was shorter for methadone (about 2 hours) than LAAM (4-6 hours), which would be consistent with the range of T_{max} values reported for methadone, and LAAM and nor-LAAM, respectively.

However, when comparing the overall magnitude of effect (AUC_{0-24} x 2 for methadone and AUC_{0-48} for LAAM) for all the pharmacodynamic variables, there were no significant differences between drugs, with the exception of withdrawal. Whilst withdrawal severity for all patients decreased following the administration of both drugs, it increased in severity at the end of the inter-dosing interval for methadone, but remained relatively stable and at low levels during the second half of LAAM's inter-dosing interval. These results suggest that patients are likely to experience a similar onset of opioid agonist effects, but delayed peak effects, when maintained on LAAM, compared to methadone.

Moreover, consistent with past reports, the general pattern of symptoms complaints was similar for patients when maintained on either drug and generally reflected symptom complaints associated with opioid dependence and/or opioid withdrawal (eg., feeling tired, runny or stuffy nose, hot flushes, feeling anxious, dry mouth, sweating) (Freedman and Czertko, 1981; Fudala, *et al*., 1997; Johnson, *et al*., 2000; Tennant, *et al*., 1986). However, patients reported significantly fewer specific symptom complaints during the LAAM inter-dosing interval than the methadone inter-dosing interval. The symptom complaints reported with less frequency by patients taking LAAM included symptom complaints commonly associated with opioid withdrawal, such as the dose not holding, feeling of coldness, and goose pimples.

As previously mentioned, the principal aim of this thesis was to examine the efficacy of using LAAM to treat MMT patients who regularly complain of break through withdrawal symptoms during the 24-hour methadone inter-dosing interval. As predicted, methadone non-holders exhibited significant reductions in the severity of withdrawal and overall number of symptom complaints, while holders exhibited similar patterns of withdrawal and symptom complaints, when switched to LAAM from methadone. The symptom complaints reported significantly more frequently by non-holders, than holders on methadone, were predominantly signs of withdrawal (eg. nervousness, runny nose, feelings of coldness, aches and pains, and muscle spasms). There was no difference in the frequency of symptom complaints reported by holders and nonholders when maintained on LAAM. These results would suggest that it is possible to convert a methadone non-holder to a holder by using second daily LAAM. Also important was the finding that holders on methadone remained holders when transferred to LAAM and so, therefore, would benefit from LAAM's less frequent dosing schedule.

The aim of chapter six was to compare the presence and severity of depression and anxiety in maintenance patients, and also to compare the intensity and temporal pattern of mood states across the inter-dosing interval for methadone and LAAM. For all patients maintenance on methadone or LAAM was associated with a similar intensity of depression and anxiety. Effective suppression of withdrawal was associated with lower depression and anxiety. That is, while holders reported similar intensity of depression and anxiety when maintained on either drug, nonholders exhibited greater depression and anxiety than holders on methadone, but significant improvement when switched to LAAM.

This study demonstrated that mood states in MMPs showed significant temporal fluctuation across the inter-dosing interval. Furthermore, the magnitude of total mood disturbance was significantly correlated with the magnitude of withdrawal reported by patients when on methadone, but not when on LAAM. Notably, patients showed a more stable pattern of mood states across the inter-dosing interval when switched to LAAM. In addition, the overall magnitude of effect (AUC_{0-24} x2 for methadone and AUC_{0-48} for LAAM) for TMD, Anger, and Tension was significantly greater for patients when maintained on methadone than on LAAM. In contrast, drug-free control subjects exhibited a relatively stable temporal pattern of mood states and less severe mood disturbance than maintenance patients. Collectively, these results illustrate the importance of withdrawal as a determinant of mood disturbance.

Given that in MMT patients there is a relatively steep plasma concentration-effect relationship for Total Mood Disturbance (Dyer, *et al*., 2001), it was hypothesized that transferring methadone non-holders from methadone to LAAM, to flatten the plasma concentration profile over the inter-dosing interval, would lessen the severity of mood disturbance experienced by these patients. Consistent with this hypothesis, the intensity of a number of mood states (TMD, Fatigue, and Tension) was significantly reduced in non-holders when transferred to LAAM. As seen with withdrawal severity, holders exhibited similar scores for each mood state when maintained on either drug. Thus, as demonstrated in chapter five for withdrawal severity, it is likely that LAAM's unique pharmacokinetics were instrumental in achieving the observed improvements in mood states.

As reviewed in chapter 7, there is a paucity of available data on the comparative effect of methadone and LAAM on neurocognitive and psychomotor functioning in maintenance patients, In this study there were significant temporal changes in tracking performance across both the methadone and LAAM inter-dosing intervals, but the overall magnitude of effect (AUC_{0-24} for methadone vs AUC_{0-48} for LAAM) for tracking performance did not differ between drugs. Furthermore, tracking performance did not differ for holders and non-holders when maintained on either drug. These data suggest that maintenance patients' ability to perform complex tasks, such as driving, would not be differentially affected by maintenance on either drug or holding status.

With regard to the effects of methadone and LAAM on neurocognitive functioning there were few differences between the effects of the two drugs in the tested patients. Patients when taking LAAM exhibited significantly better performance on the CFT (visual memory) than when taking methadone. On the other hand, results are suggestive of significantly impaired executive functioning (Trails B) in patients transferred from methadone to LAAM that may impact on daily functioning. Further research, using a larger cohort of maintenance patients that permits comparison of cognitive function on each drug, will need to verify this finding before it can be considered reliable. Importantly, cognitive and psychomotor functioning was similar for holders and non-holders when maintained on either drug. Thus, the transfer of self-reported methadone non-holders and holders to LAAM is likely to occur without patients experiencing any additional adverse cognitive effects. Moreover, patients maintained on either drug exhibited a similar pattern of cognitive function compared to control subjects. As this study did not include a comparison group of ex-maintenance patients, it was not possible to determine if the observed impairment in maintenance patients could be ascribed to pre-existing cognitive deficits, as a result of brain injury and/or poly substance use (in particular alcohol), or the maintenance drug *per se*.

Finally, the work described in chapter 8 was carried out to elucidate the *in-v*itro potencies of LAAM, nor-LAAM and dinor-LAAM, with respect to their receptor binding and function. All tested compounds depressed the electrically stimulated twitch of the ileum in a concentration-dependent manner, but nor- and dinor-LAAM were more potent than LAAM in this action. Similarly, in receptor binding studies, nor- and dinor-LAAM showed greater affinity for the μ opioid receptor than LAAM. These data attest to the

pharmacological activity of LAAM, whilst demonstrating that nor- and dinor-LAAM are clearly more potent than the parent drug. These data suggest that it is likely all three compounds contribute to the overall opioid activity experienced following ingestion of LAAM. However, the flatter plasma concentration-time profile for dinor-LAAM (see section 4.4.3) suggests that it likely to be the major contributor to the methadone non-holders becoming holders.

9.2. Limitations

There are several limitations to this work. The current study used an open label crossover design in order to compare the effects of methadone and LAAM in maintenance patients. It is recognised that the use of a double blind methodology would have been preferable, as it would have minimised staff and patient bias and patient expectations. However, while not important for the pharmacokinetic analyses, blinding could potentially have influenced self-reported and observed data. Furhermore, it would have been very difficult to perform such a study because of the problems rendering the two-drug formulations identical in appearance. More importantly, previous researchers, who have conducted studies comparing patients maintained on methadone and LAAM have noted the apparent failure of blinding (Goldstein and Judson, 1974: Savage, *et al*., 1976). These authors suggested that it was likely patients participating in these studies were able to differentiate between the two drugs because of their different pharmacokinetic profiles (i.e., delayed time to peak opioid effect for LAAM, relative to methadone). The use of a crossover design provides another potential source of bias. That is, experience with one opioid maintenance drug may have influenced the pharmacodynamic responses to the other drug. However, as discussed in Chapter 2 (General Methods) post-hoc repeated measures ANOVAs revealed no effect of order of testing on any measured response.

A number of other possible limitations were outlined in previous chapters. In particular, the concomitant use of opioids and benzodiazepines by patients participating in this study had the potential to significantly affect pharmacodynamic measures. However, the frequency of detection for these drugs in the urine and plasma of patients participating in either inter-dosing study was not significantly different. Thus, it is likely that any effects of these additional drugs would be relatively constant across the two inter-dosing studies. Patients were also likely to be tolerant to the acute effects of these drugs. In spite of these identified limitations, in the current study the objective pharmacokinetic and

pharmacodynamic data strongly support the observed subjective (i.e., self-reported withdrawal) responses to the two drugs. In addition, the random allocation to treatment, the cross over design and use of drug-free control subjects removed as much bias in the interpretation of the results as possible.

9.3. Future directions.

The results of the current study clearly demonstrate the benefits of using LAAM in MMT patients who consistently complain of breakthrough withdrawal during the methadone inter-dosing interval, particularly with regard to the significant suppression of withdrawal severity and mood disturbance across the inter-dosing interval when they were switched to LAAM. However, in response to concerns that LAAM can cause clinically significant prolongation of the QTc interval (Deamer, *et al*., 2001), which can be associated with serious arrhythmia, regulatory authorities have recommended either restricted use (Anonymous (FDA), 2001), or switching to alternative treatments (Anonymous (EMEA), 2001). Subsequent to this, Roxanne Laboratories has discontinued the sale and distribution of LAAM (ORLAAM®) in the USA (FDA, 2003) which means that methadone is the only full opioid agonist approved to treat opioid dependence. Hence, the future of LAAM is extremely uncertain.

Nevertheless, since the withdrawal of LAAM from the market a number of authors have expressed their belief that the concerns with QT interval prolongation maybe overstated and, as it is generally equivalent in efficacy to methadone in the treatment of opioid dependence (Jaffe, *et al*., 1970; Jaffe, *et al*., 1972; Johnson, *et al*., 2000; Ling, *et al*., 1994), it should not be withdrawn from the market. Instead they suggest further research should be undertaken to determine the risk factors associated with prolongation of QT intervals occurring as a result of LAAM and methadone administration (Deamer, *et al*., 2001; Longshore, *et al*., 2005; Ritter, *et al*., 2003).

Interestingly, Moody and colleagues (2004) examined information received from the FDA on all cases of adverse cardiac events reported following LAAM ingestion. They received the case histories of 15 patients, all of whom had suffered either prolonged QT interval and/or torsades de pointes. They noted two important trends when examining the case reports that point to possible risk factors that might have contributed to these adverse cardiac events. Firstly, in general, patients received at least one dose per week that was

greater than 100mg; and secondly, a relatively large proportion (6/15) were known to be taking concomitant drugs that are known to be inhibitors (eg., fluoxetine and nevirapine) or substrates (eg., methadone and buspirone) of CYP3A4. As discussed previously, LAAM is a potent inhibitor of cloned human cardiac K^+ channels (Kang, *et al*., 2003). Furthermore, Moody and colleagues (2004) demonstrated that *in vivo* inhibition of CYP3A4 results in increased plasma LAAM concentration. Moody and co-workers speculate that these data are suggestive of the contribution of drug interactions to QT interval prolongation following LAAM ingestion. Furthermore, they proposed that as much of the pharmacokinetic data on LAAM after a single dose is suggestive of a dose-dependent saturation of LAAM metabolism (ie, AUC and C_{max} do not increase proportionally with increasing dose; Finkle, *et al*., 1984; Henderson, *et al*., 1977b; Walsh, *et al*., 1998), the ingestion of larger doses (i.e., 100 mg or over) would result in higher plasma LAAM/nor-LAAM ratios, which in turn would increase the likelihood of adverse cardiac effects. Clearly much of what has been discussed here is speculative that requires verification through systematic evaluation, but nevertheless provides plausible explanations for the adverse cardiac events reported to the FDA.

Thus, in terms of future research directions, the priority, before suggestions for research presented in this thesis can be acted on, is to determine the risk factors (eg. pre-existing cardiac anomalies, drug interactions, and dosage) that are associated with prolongation of QT intervals occurring as a result of LAAM and methadone administration. This will permit informed discussion on the comparative merits and risks of LAAM and methadone maintenance. When such risks have been elucidated, clinical guidelines for the appropriate use of LAAM can be established that would include recommendations from such research. If implemented, these guidelines would substantially reduce the prevalence of the relatively uncommon, but nevertheless life threatening cardiac side effects that have been associated with LAAM use (Longshore, *et al*., 2005; Stimmel, 2001). Hopefully, such research would also encourage renewed interest in using LAAM, which in turn may convince the manufacturers of LAAM to again make it available for the treatment of opioid dependence.

9.4. Conclusions.

In conclusion, the data presented in this thesis clearly show that it is possible to convert a methadone non-holder to a holder with second daily LAAM. Equally important was the finding that methadone holders remained holdes when transferred to LAAM, and so would benefit from LAAM's less frequent dosing schedule. It is proposed that it is the long terminal half lives (Finkle, *et al*., 1982; Henderson, *et al*., 1977b) and the relatively flat plasma concentration time profiles for LAAM's two active metabolites (as shown in chapter 4), in particular dinor-LAAM, that confer stability of opioid effect and hence minimize withdrawal and mood disturbance across the inter-dosing interval. Thus LAAM may have a valuable role in selected patients, whose response to methadone is suboptimal, thereby improving retention in opioid treatment programs. However, the withdrawal of LAAM from the market means that methadone is the only full opioid agonist available to treat opioid dependence and so a substantial proportion of patients may not be receiving the best possible treatment for their opioid dependence. Thus, in light of these findings I would suggest that the use of LAAM be reexamined. Future research studies should be undertaken to elucidate the risk factors associated with prolongation of QT intervals occurring as a result of LAAM and methadone administration. This will permit an informed discussion on the costs and merits of using either drug, which hopefully, may lead to renewed interest in using LAAM as a maintenance pharmacotherapy for the mutual benefit of the opioid dependent individual and society at large.

Appendices

Appendix 1: Randomised control trials and open label trials of LAAM

Randomised control trials and open label studies of LAAM

Study	Design/ duration of study	Intervention/ treatment groups	Participants	Outcomes
Jaffe, et al., 1970	Randomised double blind, parallel group 7 weeks (2 weeks baseline)	[1] Meth (20-55mg) [2] dl-AM (24-66 mg) M/W/F placebo on non-LAAM dosing days . Conversion of meth to LAAM 1:1.2	21 male methadone maintained patients (20 – 90 mg methadone prior to entering study): 9 in group (1), 12 in group (2)	Study retention: 16 patients completed study: four patients in group 2 dropped out on day 1 of study due to complaints of nervousness/anxiety. Other outcomes: There were NS differences between groups in the number of urines positive for morphine; and in the number of patients employed and engaged in illegal activity. dl-AM suppressed withdrawal for up to 72 hours.
Jaffe, et al., 1972	Randomised double blind, parallel group 15 weeks	[1] dl-AM (36-80 mg) M/W/F placebo on non-LAAM day [2] Methadone (30-80 mg) [3] Wait list control group [4] Choice of tx clinic	66 male street heroin users; 19 in group [1]; 15 in group [2]; 16 in each group [3] & [4].	Study retention: 5 (26%) of AM group and 2 (13 %) of methadone group had dropped out at week 15 (NS). Average length of stay for AM dropouts was 5½ weeks, and for methadone dropouts was 10 weeks. 12 (75%) of those referred to other tx clinics dropped out. Other Outcomes: No difference in self reported withdrawal between groups. 12 (80%) of AM patients and 10 (67%) of meth patients were employed at 15 weeks; in comparison to 27% at baseline. 72% of tx weeks for meth and 49% of tx weeks for AM participants were free of positive urine tests (NS between groups). Safety. Generally few side effects reported – the most troublesome was reduced libido. 2 patients reported 'jerking and twitching' of limbs at rest. No toxic effects – liver function/WBC count unchanged across study period.

Notes: Jaffe et al (1970) and Jaffe et al (1972) used racemic-acetylmethadol. AM= acetylmethadol; dl-AM=racemic acetylmethadol; M=Monday; W=Wednesday; F=Friday; tx= treatment; avg=average; bup=buprenorphine; meth = Methadone; neg=negative; SAEs=serious adverse events; sig= Statistically significant; NS=Not statistically significant (P>0.05); T/A =Take-away; WBC=White blood cell.

Randomised control trials and open label studies of LAAM

Study	Design/ duration of study	Intervention/treatment groups	Participants	Outcomes
Zaks, et al., 1972	Randomised, open label, parallel group 26 weeks	[1] meth (100 mg [2] LAAM (80 mg) [3[LAAM (30-50 mg) Inpatients for 1 month until maintenance dose reached. LAAM dosed M, W, F. Meth dosed daily for first month, then 5 take away doses/week on discharge.	20 male patients (with > 2 yr duration of tx) detoxified prior to study; Ten in group [1], 6 in group [2], 4 in group [3].	Study Retention: LAAM: 9/10 completed induction – 1 patient left tx after 3 month, 8/10 completed 6 month tx (4 in group [2] and 4 in group [3]). Meth: 10/10 completed induction – 8/10 completed 6 month tx – one patient left tx at 2 month, the other at 4 months.Other Outcomes: Positive urines were found in 18.9% and 2.8% of urines from low and high dose LAAM, respectively, and 2.0 % of urines from meth patients Safety: All laboratory tests (blood cell count, blood chemistry, fasting blood sugar, urinalysis) were normal before and after study. Liver function (transaminase) abnormal for all subjects, but not considered related to tx. No withdrawal or craving for heroin on high dose LAAM and meth. 3/ 4 patients in Low dose LAAM group complained of withdrawal at 40-48 hour following dosing. 3/9 LAAM patients complained of irritability. 2/9 complained of involuntary jerking of limbs.
Savage, et al., 1976	Randomised, double blind cross-over 6 months (3 months on each drug) 2 year follow-up	[1] Meth-LAAM [2] LAAM-meth Dosages for either study drug not reported. LAAM M, W, F with placebo on non-LAAM day. Conversion meth to LAAM 1:1.3	99 male street heroin users. At start of study: Group 1 n=52; Group 2 n=47 Patients in both groups could earn t/a privileges	Study retention: 60 % (31/47) of meth patients and 34% (16/47) LAAM patients completed first 3 month study – difference not sig diff. Retention rates did not differ for second phase of the study. Side effects were reported as the main reason for terminating treatment (31 % in LAAM group and 22 % in meth group, NS). No specific side effects reported. Other outcomes: Mean % of morphine positive urines for meth and LAAM (5% vs 8%) were not sig diff. Safety: There were no deaths related to drug treatment for up to 2 years following the study in either group. The results of a number of laboratory tests (blood chemistry, hematology etc) and EEG were not different for those who completed either LAAM and meth treatment, and between those who dropped out and completed the study.

Notes: AM= acetylmethadol; dl-AM=racemic acetylmethadol; M=Monday; W=Wednesday; F=Friday; Tx= treatment; avg=average; bup=buprenorphine; meth= Methadone; neg=negative; SAEs=serious adverse events; sig=significant NS=Not significant; T/A =Take-away

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Randomised control trials and open label studies of LAAM

Study	Design and duration of study	Intervention/ treatment groups	Participants	Outcomes
Ling, et al., 1976 'VA' Study	Randomised, double blind parallel group 40 weeks	[1] meth 50 mg [2] meth 100mg [3] LAAM 80 mg M/W/F, placebo on non-LAAM days Dosages fixed for study Each group started on 30 mg of drug, with increments of 10 mg each week until target dose reached.	430 street male heroin users At start of study: 146 in group [1]; 142 in group [2]; 142 in group [3]	Study retention: 42% participants completed study – 69% dropped out of the LAAM group, 58% out of the M-50, and 48 % out of the M-100 group. Most dropped out early in treatment: the time in tx was equivalent across all three groups (~ 81 days). LAAM participants were significantly more likely to drop out due to side effects; and also due to medication not holding. Other outcomes: The M-50 group had a higher positive illicit urine index than either the M-100 and LAAM groups. Safety: No deaths or serious adverse events were recorded. Generally low level of symptoms complaints across all participants. Between group differences in results of blood, and renal and hepatic function tests not considered clinically important.
Senay, et al., 1977	Randomised, open-label, parallel group. 14 weeks	Following one initial dose of methadone patients were randomised to either: [1] meth (mean dose 39.8 mg) [2] LAAM (mean M and W dose = 39.1mg) Methadone pick up 6xdays/week. LAAM dosed M, W, F (10 mg extra). Conversion of meth to LAAM 1:1 Flexible dosing	193 street heroin users with > 2 yrs addiction history. 96 in group [1]; and 95 in group [2] (not including 2 deaths)	Study retention: 62% (n=59) of LAAM and 75% (n=72) of meth patients remained at 14 weeks [NS]. More LAAM patients terminated early from the study – 5 of these patients transferred back to methadone, and thus are not tx dropouts. Other outcomes: The % of urine samples negative for morphine did not differ for either group (LAAM 74% vs meth 72%). There were no sig differences between groups on measures of illegal activity and employment. Safety: There were 2 deaths in the LAAM group – one patient died from a heroin overdose 48hr after receiving their second LAAM dose; the other was murdered. 32% (n=30) of LAAM patients did not report any side effects. The most common side effects reported by other LAAM patients were related to gastro-intestinal complaints (nausea, vomiting, constipation, loss of appetite), nightmares, and decrease libido.

Notes: AM= acetylmethadol; dl-AM=racemic acetylmethadol; M=Monday; W=Wednesday; F=Friday; Tx= treatment; avg=average; bup=buprenorphine; meth= Methadone; neg=negative; SAEs=serious adverse events; sig=significant; NS=Not significant; T/A =Take-away

Randomised control trials and open label studies of LAAM

Study	Design and duration of study	Intervention/ treatment groups	Participants	Outcomes
Freedman & Czertko, 1981	Randomised, blind to dose (not drug), parallel group. Patients maintained on methadone for 16 weeks (control, period) prior to randomization, after which treatment time indeterminate.	[1] meth (avg dose 26.5 mg: 9-42 mg) [2] LAAM (avg dose 13.9mg : 9-20 mg) LAAM dosed M, W, and 50% higher dose on F. Conversion meth to LAAM 1.3 Methadone patients retained take away privileges	48 employed heroin users. 24 in each group.	Retention in study: For the experimental period (post 16 weeks) LAAM patients remained in tx an avg of 32.4 weeks, while meth patients remained in tx on avg 15.6 wks [sig diff p <0.02]. At 52 weeks 5 LAAM patients remained in the study, the last meth patient left at week 48. Other outcomes: LAAM patients had sig less positive urines (all illicit drugs) than methe patients (28% vs 45%). Treatment satisfaction: 20 LAAM Patients completed a drug preference questionnaire on termination – more patients preferred LAAM over meth on 9/15 items, and did not differ on the other items. Reported that taking LAAM interfered less with their daily activities, they felt more normal, healthier, less loaded, and craved and used heroin less. Safety: Results of physical examination, symptom checklist, urinalysis, blood chemistry, hematological investigations were not sig different between groups.
Karp-Gelernter, et al., 1982	Randomised, double-blind, parallel group. 2x2 factorial (Meth vs LAAM, daily vs 3x/week attendance) 40 weeks	[1] meth (3xweek) : [2] meth (daily) :[3] LAAM (3xweek): [4] LAAM (daily) Meth dosed daily – T/A privileges dependent time in tx. LAAM dosed M, W, (5-10 mg higher dose) on F and placebo on non-LAAM dosing days. Conversion to LAAM using 1:1.1 ratio.	95 male stabilised methadone outpatients: 24 in group [1], 22 in group [2], 27 in group [3], 22 in group [4].	Retention in study: 58 % (14/24) of meth group (3x/week) completed study vs 36 % (8/24) in meth group (daily), 30% (8/27) in LAAM group (3 x/week), 27% (6/22) LAAM (daily) [NS]. Other outcomes: Incidence of positive urines (for any illicit drug) did not differ according to drug/ or attendance schedule. Safety: No deaths or adverse events were reported. Results of physical examination, blood chemistry & hematological analysis, and urinalysis revealed no differences between groups. Of patients who dropped out 33 % terminated due to side effects, 29% for other reasons. 33% and 41% of LAAM patients on 3x/week and daily attendance schedules, respectively, dropped out due to side effects [not sig different from meth groups].

Notes: AM= acetylmethadol; dl-AM=racemic acetylmethadol; M=Monday; W=Wednesday; F=Friday; Tx= treatment; avg=average; bup=buprenorphine; meth= Methadone; neg=negative; SAEs=serious adverse events; sig=significant; NS=Not significant; T/A =Take-away.

Randomised control trials and open label studies of LAAM

Study	Design/ duration	Treatment groups	Participants	Outcomes
Fudala, et al., 1997 The LAAM Labeling Assessment Study	Open observational study 12 week induction phase 52 week treatment phase Option of extension until LAAM became commercially available	LAAM only Meth to LAAM conversion ratio of 1:1.2-1.3 LAAM dosed M, W, F (with increased dose if required on F) or alternate daily.	623 males and females; Street heroin users (19%) and methadone maintained.	Study Retention: 71% (440) of patients completed initial 12-week phase - the average weekly dropout was 2.4 % (as high as 4% for first 4 weeks). 35% (216) completed 64 weeks. 31% (191) elected to continue with LAAM into extension phase. Early termination was commonly due to lack of efficacy, n= 87 (i.e. not holding). In phase two administrative discharge commonly reason for termination (n=109). Safety: Three deaths reported, none attributable to LAAM. Three women became pregnant during the study: 2 terminated pregnancies, 1 transferred to methadone after 1 month of LAAM and delivered prematurely – foetus died –LAAM was not ruled out. 41 SAEs were reported by 36 patients – only one related to LAAM (clinic dosing error). 12 SAE s were possibly related to LAAM (e.g., rushing feeling, blurred vision, allergic reaction), 20 not related to LAAM. 6 patients exhibited signs of allergic reaction that were related to LAAM. 6 patients had hyperglycemia that was not evident at baseline, 12 others had raised blood sugar levels at baseline and also during the study. 2 patients exhibited signs of renal impairment (↑ creatinine), and 19 had raised liver enzymes. The most commonly reported side effects included difficulty sleeping, constipation and sweating.
Eissenberg, et al., 1997	Randomised, double blind, parallel group Three different dosing regimens Dose induction phase (1-4 weeks) maintenance phase (5-17 weeks). Dose reduction phase (18-29 weeks)	[1] low dose – 25/25/35 mg [2] medium-dose - 50/50/70 [3] high-dose – 100/100/140 LAAM dosed M, W, F (F dose 1.4 x M & W dose) placebo on non-LAAM dosing day	180 street heroin users (male and female); stratified (race, gender etc) then allocated to groups Group [1] n=62; Group [2] n=59; Group [3] n=59.	Retention in study; Overall 60% (n=108) completed 17 month week induction + maintenance. Trend for slightly lower retention in higher dose group (55%, n=34), than medium dose (66%, n=39), and low dose groups (59%, n=35) [NS]. Other Outcomes: No difference in attendance rates between groups. Percentage of opioid-positive urines less for high dose group (61%), than for both medium dose (68%), and low dose groups (77%). More patients in high dose group (20/59) remained abstinent for 4 consecutive weeks, than in medium dose group (8/59), and low dose groups (7/62) [SIG]. Safety: One death unrelated to medication. At least one side effect reported by 46% (27/59) of patients assigned to high dose groups, 47% (28/59) to medium low dose groups, and 52 % (32/62) to low group. Eight women assigned to high dose LAAM left treatment during induction, six reported symptoms of overmedication (sedation, vomiting). The most common side effects reported were constipation, headache, and irritability.

Notes: AM= acetylmethadol; dl-AM=racemic acetylmethadeol; M=Monday; W=Wednesday; F=Friday; Tx= treatment; avg=average; bup=buprenorphine; meth= Methadone; neg=negative; SAEs=serious adverse events; sig=significant; NS=Not significant; T/A =Take-away.

Randomised control trials and open label studies of LAAM

Study	Design and duration of study	Intervention/ treatment groups	Participants	Outcomes
Johnson, et al., 2000	Randomised, double blind, parallel group. Meth vs LAAM vs buprenorphine (bup) 17 weeks	[1] LAAM (75-115 mg) [2] bup (16-32 mg) [3] meth (high dose) 60-100mg [4] meth (low dose) – fixed at 20 mg LAAM and bup dosed 3x week. M.W, F (with higher doses on F) , placebo on non-dosing day Meth dosed daily.	220 street heroin users; stratified then allocated by random number generation; 55 patients in each group	Retention in study: 53 % of LAAM group, 58 % of Bup group, 73 % of high dose meth group, and 20 % of low dose meth group completed study. Days retained in study (mean ±SE) : LAAM 89±6 days, Bup 96±4 days, high dose meth 105±4 days; low dose meth 70±4 days. Sig difference between LAAM and high dose meth. 8 withdrew from LAAM group due to side effects (effects not reported), and 4 required rescue treatment, i.e. switched to methadone. Other outcomes: Twelve or more consecutive opioid negative urine specimens obtained in 36% of LAAM, 26% of bup, 28% of high dose meth, 8% of low dose meth [sig diff]. No sig diff between groups in self reported opioid use. Safety: No toxic reactions reported. At least one side effect reported by 55% of LAAM patients The most common side effects reported were constipation, nausea, dry mouth.
White, et al., 2002	Randomised, open label, cross-over 6 months (3 months on each drug) then 6 month choice phase	[1] Meth (avg dose end 3 mth 76.6±4.5) [2] LAAM (avg dose at end of 3 mnth 82.1±4.9) LAAM flexible dosing alternate daily, max dose of 140 mg: Meth to LAAM conversion ratio 1: 1.1	62 methadone maintained (avg 42.5 months; avg dose 70.2 mg prior to starting study)	Retention in study: At 6 month 68% (n=42) of patients retained in study. 9 patients dropped out due to LAAM side effects. 11 others withdrew/terminated for non-drug related reasons. Prior to choice phase – 3 patients withdrew from study – 39 chose maintenance drug. 27(69%) chose LAAM, 12 methadone [sig]. Reason given for preferring LAAM, 'held better'(39.3%), fewer side effects 928.5%), less craving (17.9%), fewer pick-up days (14.2%). Other outcomes: LAAM was associated with sig lower rates of self reported heroin use, fewer patients showed positive morphine results from hair analysis. Those who chose LAAM had similar outcomes to those who chose meth on 7/9 of the subscales on the SF-36, sig better outcomes on 'vitality' and 'mental health' subscales. Patients who chose LAAM reported better ratings for LAAM than methadone for - feeling normal, better holding.

Notes: AM= acetylmethadol; dl-AM=racemic acetylmethadeol; M=Monday; W=Wednesday; F=Friday; Tx= treatment; avg=average; bup=buprenorphine; Meth= Methadone; neg=negative; SAEs=serious adverse events; sig=significant; NS=Not significant; T/A =Take-away

Randomised control trials and open label studies of LAAM

Study	Design and duration of study	Intervention/ treatment groups	Participants	Outcomes
Ritter, et al., 2003	Randomised, open label, parallel groups 52 weeks with option of continuing on LAAM thereafter Follow-up assessments at 3, 6, 12 months following admission to trial	[1] Methadone [2] LAAM Flexible LAAM dosing; commence on alternate-daily dosing, thereafter choice of alternate-day/ thrice weekly/ three-day doses/two doses per week.	93 methadone maintained patients (male and female - (avg 26 months maintenance tx). 49 in group [1]; 44 in group [2].	Retention in study: The mean number of days in tx was 337 days for LAAM group and 312 days for meth group [NS]. For LAAM group – at 3 months 95%(n=42) of patients still in tx: 77% (n=34) in LAAM tx, 8 switched to methadone. Two patients dropped out. At 6 months 93% (n=41) still in tx 74%(n=31) in LAAM tx. 2 switched to meth tx, Another patient dropped out. At 12 months 84% remained in tx (n=37). 64% (n=28) in LAAM tx. Meth group - at 12 months 71% (35/49) remained in tx. [NS diff] Other Outcomes: trend for LAAM patients to have lower heroin use than meth patients. At 3 month number days of heroin use in preceding month less for LAAM than meth [sig]. At 6 month more in LAAM group report being abstinent than in meth group [sig]. NS difference at 12 months Safety: There were 7 SAE's in the LAAM group. Three were not related to LAAM (terminated pregnancy, psychotic disorder, appendectomy). The remaining were considered LAAM related – nonfatal overdose, experience of severe withdrawal on reducing LAAM dose; and two dosing errors.
Longshore, et al., 2005	Randomised, open label, parallel groups. Follow-up assessment at 25 weeks.	[1] Methadone [2] LAAM For LAAM group - Induction onto meth initially, than transferred to LAAM on initial dose 1.2 x last meth dose. Flexible dosing	Opioid dependent users randomised to LAAM & Meth on a 2:1 ratio.. [1] = 106 [2] =209	Retention in study: At 26 weeks the mean ±sd number of days in treatment for the LAAM group was 164±37, and for the meth group was 167±36 [NS]. 75% of LAAM patients, and 77% of meth patients remained in treatment, respectively. Drug use: Urine tests – during entire treatment period the positive opioid rate for LAAM group was 46% versus 60% for meth group (P<0.000). At follow-up positive urine rate was significantly lower for LAAM group (39.8%) than meth group (60.2%) (P<0.02). Safety: No adverse events noted

Notes: AM= acetylmethadol; dl-AM=racemic acetylmethadeol; M=Monday; W=Wednesday; F=Friday; Tx= treatment; avg=average; bup=buprenorphine; Meth= Methadone; neg=negative; SAEs=serious adverse events; sig=significant; NS=Not significant; T/A =Take-away

Appendix 2: Summary of previous pharmacokinetic evaluations of LAAM

Summary of previous pharmacokinetic evaluations of LAAM

Reference	Subjects[1]	Dose (mg)	Sampling period (h)	C_{max} (ng/ml)	T_{max} (h)	$t_{½}$ (h)	Other
Billings, et al., 1974	3 MM						
	(a)	100 p.o. 3 x week	0-48h	[2]NL= 50–150 [2]DNL = 110-210	NR	NR	NR
	(ss)	100 p.o. 3 x week	0-48 h	[2]NL=150-400 [2]DNL= 180-340	NR	NR	NR
Henderson, et al., 1977b	Group 1–MM						
	5 (a)	60 p.o.	0-48	L = 127 (±34) NL= 47 (± 6) DNL < LOD	L=3.2 (±1.1) NL=3.5 (±1.9) DNL= NR	L=58[4] NL= 13.3[4] DNL= NR	NR
	3 (ss)	85 p.o. 3 x week	0-72	L = 203 (±39) NL= 236 (± 79) DNL= 275 (est)	L=2.7 (±1.2) NL=NR DNL= NR	L=50[4] NL= 27.7[4] DNL= NR	NR
	Group 2 – ND						
	5 (a)	25 p.o	0-48	[3]NR	[3]NR	[3]NR	NR
	3 (ss)	85 p.o. 3 x week	0-72	L = 229 (±116) NL= 371 (± 82) DNL= 325 (est)	L=3.3 (±1.2) NL=5.3 (±2.3) DNL= NR	L=35[4] NL= 31.5[4] DNL= NR	NR

Notes: Data are expressed as mean±sd or (range); NR=Not reported, L=LAAM, NL=Nor-LAAM, DNL=Dinor-LAAM, LOD = Level of detection, est=estimate, p.o = oral, i.v= intravenous, 3x week = dosed Monday, Wednesday, Friday ;[1] MM= methadone maintained patients, ND= Non opioid dependent, Ss=subjects, (a)= acute single dose, (ss) = steady state dosing, (b) 10th dose; [2] LAAM plasma concentrations not determined due to method limitations. C_{max} estimated from graphs. [3] Plasma concentrations below lower limit of quantification; [4] No index of variability presented or calculable; [5] oral clearance.

Summary of previous pharmacokinetic evaluations of LAAM

Reference	Subjects[1]	Dose (mg)	Sampling period (hr)	C_{max} (ng/ml)	T_{max} (h)	$t_{½}$ (h)	Other
Kaiko & Inturrisi, 1975a.	8 MM (ss)	40 – 60 p.o. 3x week	0-48	L=60 (23-148) NL= 114 (55-193) DNL=57-123	L=4[4] NL=4-8 DNL=NR	L=NR NL=48 (13-78) DNL=NR	NR
Finkle, et al., 1984	12 MM (a)	7 Ss 80 p.o 5 Ss 1mg/kg p.o. 3 x week/ alternate daily	0-72	L= (52-510) NL=(65-175) DNL=(11-92)	L=4.4 (3-6) NL= 5.6 (4-7) for ten Ss; 10 for two Ss. DNL=6.6 h (5-7 h) for 8 Ss; 24-48 h for 4 Ss.	L = 37.5 (21.5-45.8) NL=38.2 (13.5-60.2)	NR
	(b)	7 Ss 80 p.o 5 Ss 1mg/kg p.o. 3 x week/ alternate daily	0-504	NR	NR	L = 46.8 (14.2-104.5) NL=62.4 (12.9-129.6) DNL=174.6 (96.7-429.9)	NR

Notes: Data are expressed as mean±sd or (range); NR=Not reported, L=LAAM, NL=Nor-LAAM, DNL=Dinor-LAAM, LOD = Level of detection, est=estimate, *p.o* = oral, *i.v*- intravenous, 3x week = dosed Monday, Wednesday, Friday ;[1] MM= methadone maintained patients, ND= Non opioid dependent, Ss=subjects, (a)= acute single dose, (ss) = steady state dosing, (b) 10th dose; [2] LAAM plasma concentrations not determined due to method limitations. C_{max} estimated from graphs. [3] Plasma concentrations below lower limit of quantification; [4] No index of variability presented or calculable; [5] oral clearance.

Summary of previous pharmacokinetic evaluations of LAAM

Reference	Subjects[1]	Dose (mg)	Sampling period (hr)	C_{max} (ng/ml)	T_{max} (h)	$t_{½}$ (h)	CL (ml/hr/kg)
Walsh, *et al*., 1998	6 ND males						
	(a)	20 *i.v.*	0-96	L=212 (±78) NL=13 (±2) DNL=9 (±0)	L=0.06(±0.02) NL=4.5 (4.2) DNL= 48 (18.2)	L=14.3 (±4.2) NL=30.3 (±12.7) DNL= 88.9 (±98)	L=352 (±61)
	(a)	40 *i.v.*	0-96	L=756 (±562) NL=26 (±5) DNL=15 (±2)	L=0.06 (±0.02) NL=8.0 (8.3) DNL= 48 (15.2)	L=20.9 (±8.8) NL=37.9 (13.2) DNL= 80.5 (36)	L=295 (±86)
	(a)	20 *p.o.*	0-96	L=39 (±17) NL=26 (±7) DNL=12 (±2)	L=2.5 (±1.0) NL=3.1 (±1.3) DNL= 17.9 (±18)	L=7.9 (±2.9) NL=33.6 (10.3) DNL= 75.6 (38)	L=357 (±61)[5]
	(a)	40 *p.o.*	0-96	L=63 (±20) NL=44 (±10) DNL=19 (±2)	L=2.6 (±0.5) NL=3.9 (±1.8) DNL= 31.0 (±23.5)	L=18.5 (±12.0) NL=23.9 (7.83) DNL= 65.8 (25)	L=296 (±86)[5]
Moody, *et al*., 2004	13 ND						
	(a) + placebo	5mg/70kg *p.o* (54–75 kg)	0-240	L=4.88 (±1.49) NL=6.90 (±1.57 DNL=3.71 (±0.70)	L=2.12 (±0.76) NL=2.38 (±0.75) DNL=3.86 (±1.65)	L=43.1 (±32.6) NL=21.4 (±10.3) DNL=36.9 (±9.7)	NR NR NR
	(a) + Ketoconazole (single 400 mg dose)			L=16.5 (±6.7) NL=5.28 (±1.23) DNL=2.21 (±0.31)	L=2.03 (±0.91) NL=6.48 (±3.48) DNL=48.1 (±19.2)	L=49.6 (±30.0) NL=34.0 (±14.0) DNL=45.7 (±9.2)	NR NR NR

Notes: Data are expressed as mean±sd or (range); NR=Not reported, L=LAAM, NL=Nor-LAAM, DNL=Dinor-LAAM, LOD = Level of detection, est=estimate, *p.o* = oral, *i.v*- intravenous, 3x week = dosed Monday, Wednesday, Friday ;[1] MM= methadone maintained patients, ND= Non opioid dependent, Ss=subjects, (a)= acute single dose, (ss) = steady state dosing, (b) 10th dose; [2] LAAM plasma concentrations not determined due to method limitations. C_{max} estimated from graphs. [3] Plasma concentrations below lower limit of quantification; [4] No index of variability presented or calculable; [5] oral clearance. .

Summary of previous pharmacokinetic evaluations of LAAM

Reference	Subjects[1]	Dose (mg)	Sampling period (hr)	C_{max} (ng/ml)	T_{max} (h)	$t_{½}$ (h)	CL (ml/hr/kg)
Kharasch, *et al*., 2005.	13 ND (a)	0.25 mg/kg *p.o*	0-96	L=14.7(±6.6) NL= 18.7 (±4.8) DNL= 8.36 (±1.42)	L=2.5 (±0.9) NL= 3.0 (±0.8) DNL= 11.8 (±13.4)	L=16.6 (±7.3) NL=18.5 (±4.3) DNL= 45.8 (±13.4)	[8]L= 1410 (±740)
	(a) + rifampicin (600 mg daily x 8 days)	*p.o*		L= 2.0 (±1.1) NL= 3.6 (± 2.7) DNL= 7.10 (±2.02)	L= 1.7 (±(0.9) NL=2.6 (±0.8) DNL= 3.0 (±0.7)	L= 2.4 (±0.7) NL= 3.8 (±0.8) DNL= 9.1 (±3.2)	[8]L= 22320 (±8010)
	(a) + troleandomycin (500 mg BD x 4 days)	*p.o*		L= 39.1 (± 8.6) NL= 14.5 (± 2.5) DNL=5.26 (± 1.14)	L= 2.7 (±0.5) NL= 10.9 (± 14.1) DNL= 74.4 (± 22.6)	L=21.1 (±5.2) NL= 50.6 (±28.0) DNL=114 (±91)	[8]L= 339 (±99)
McCance-Katz, *et al.*, 2006	10 SS Pre-DLV	[6]M/W: [7]79 (9.2) F: [7]104 (11.1) *p.o*	0-48	L=164 (116-249) NL=165 (97-309) DNL=117 (79-186)	L=1.75 (1.5-2.0) NL=4 (1.5-10) DNL=11 (1.5-36)	L= 40 (34-47)	[9]L=26.5 (20.4-36.1) [9] NL=12.8 (9.2-19.9) [9]DNL= 14.9(12.4-18.6)
	Post-DLV	[6]M/W: [7]79 (9.2) F: [7]104 (11.1) *p.o*	0-48	L=272 (190-417) NL=212 (124-393) DNL=92 (66-144)	L=1.75 (0.75-4.0) NL=24 (1.5-48) DNL=14 (0-48)	L= 59 (45-81)	[9]L= 11.4 (9.1-14.8) [9]NL=8.12 (6.2-11.4) [9]DNL=19.4 (15.6-24.9)

Notes: Data are expressed as mean±sd or (range); NR=Not reported, L=LAAM, NL=Nor-LAAM, DNL=Dinor-LAAM, LOD = Level of detection, est=estimate, *p.o* = oral, *i.v*- intravenous, 3x week = dosed Monday, Wednesday, Friday; DLV=delavirdine, Pre= before DLV dose, post = after DLV dose;[1] MM= methadone maintained patients, ND= Non opioid dependent, Ss=subjects, (a)= acute single dose, (ss) = steady state dosing, (b) 10th dose; [2] LAAM plasma concentrations not determined due to method limitations. C_{max} estimated from graphs. [3] Plasma concentrations below lower limit of quantification; [4] No index of variability presented or calculable; [5] oral clearance. [6] M=Monday, W=Wednesday, F=Friday; [7]Mean (standard error); [8] Oral clearance; [9] Oral clearance (L/h).

Appendix 3: Summary of urinary excretion of LAAM and metabolites as a percent of dose administered in the available literature

Summary of urinary excretion of LAAM and metabolites as a percent of dose administered reported in the available literature

Reference	Subjects[1]	Dose (mg)	Sample Collection period (h) [2]	LAAM (% dose)	nor-LAAM (% dose)	dinor-LAAM (% dose)	Other metabolites (% Dose)	Total (% dose)
Billings, et al., 1974	3 MM (ss)	100 p.o.	48	2.1 - 3.4	7.5 – 16.1	14.6-17.3	NA	24.2 – 37.0
Henderson, et al., 1977b	Group 1–MM Males 5 (a)	85 p.o	72	NR	NR	NR	NA	Approx. 6
	3 (ss)	85 p.o	36	NR	NR	NR	NA	Approx. 4
Kaiko, et al., 1975b	4 (ss)	20-100 p.o	48	1.8±0.7	8.2±5.4	13.2±5.2	4.8±5.2[3]	28±10.1
Finkle, et al., 1982								
	2 (a)	80 p.o	72	24.4, 17.3[4]	NR	NR	1.6; 1.7[5]	26.0, 19.0
	8 (b)	0.73-1.5 mg/kg	504	27.9±12.4[6]	NR	NR	2.04±1.2[7]	29.6±13.6

Notes: All data are expressed as mean±sd or range; NR=Not reported; NA-Not anlysed [1]MM= methadone maintained patients, ND= Non opioid dependent, Ss=subjects, (a)= acute single dose, (ss) = steady state dosing, (b)=10th dose; [2] U=urine, F=Faeces; [3] methadol; [4] Data from two patients - Σ LAAM, nor and dinor-LAAM; [5] Data from two patients - Σ Methadol, nor- and dinor-methadol; [6] Σ LAAM, nor and dinor-LAAM; [7] Σ Methadol, nor- and dinor-methadol.

Summary of excretion of LAAM and metabolites as a percent of dose administered reported in the available literature

Reference	Subjects[1]	Dose (mg)	Sample Collection period (h) [2]	LAAM (% dose)	nor-LAAM (% dose)	dinor-LAAM (% dose)	Other metabolites (% dose)	Total (% dose)
Cook, et al., 1984	4 (a)	15 – 40.2 i.v	168	NR	NR	NR	NR	27±3.4
		[^{3}H] Labelled	168 F	NR	NR	NR	NR	17.8±12.0
			168 h U +F	NR	NR	NR	NR	44.8±14
	1 (a)	15 i.v [^{3}H] Labelled	72	2.0	6.0	13.0	2.0	23.0
Moody, et al., 2004	13 (a)	5mg/70kg (54–75 kg)	96					
	placebo			0.49±0.2	2.30±0.8	6.70±1.3	NA	9.49±2.1
	ketoconazole			2.74±1.4	4.97±1.7	6.16±4.1	NA	13.9±4.1
Kharasch, et al., 2005.	13 ND (a)	0.25 mg/kg p.o	96	1.01±0.68	4.82 ± 2.58	10.9 ± 3.3	NR	NR
	+ rifampicin			0.07±0.05	0.28±0.34	2.00 ± 1.03	NR	NR
	+ troleand			4.41± 2.23	11.6±3.7	8.65 ± 1.68	NR	NR

Notes: All data are expressed as mean±sd or range; NR=Not reported; NA-Not anlysed [1]MM= methadone maintained patients, ND= Non opioid dependent, Ss=subjects, (a)= acute single dose, (ss) = steady state dosing, (b)=10th dose; troleand = troleandomycin; [2] U=urine, F=Faeces; [3] methadol; [4] Data from two patients - Σ LAAM, nor and dinor-LAAM; [5] Data from two patients - Σ Methadol, nor- and dinor-methadol; [6] Σ LAAM, nor and dinor-LAAM; [7] Σ Methadol, nor- and dinor-methadol;

Appendix 4: Repeated measures analysis of variance for pharmacodynamics effects to determine if order of testing (LAAM: Methadone, Methadone: LAAM) systematically affected results.

Repeated measures analysis of variance for pharmacodynamic effects to determine if order of testing (LAAM: Methadone, Methadone: LAAM) systematically affected results

Pharmacodynamic response	[1]Effect	[2]DF	[3]P value	[2]Df	[3]P value
		Methadone		LAAM	
Withdrawal (number of symptoms)	Order	1,14	1.1 ^{NS}	1,14	2.50
	Time	3,40	13.9 ***	3,43	10.57* * *
	Order x time	3,40	2.1 ^{NS}	3,43	1.67
MBG	Order	1,14	2.6 ^{NS}	1,14	0.27
	Time	3,47	3.9 ***	4,53	3.54
	Order x time	3,47	0.3 ^{NS}	4,53	1.17
MG	Order	1,14	0.2^{NS}	1,14	0.21
	Time	4,50	2.5^{NS}	4,53	1.25
	Order x time	4,50	1.2 ^{NS}	4,53	1.09
Pain Threshold	Order	1,14	2.1^{NS}	1,14	3.9
	Time	3,50	7.3***	4,60	0.85
	Order x time	3,50	0.5^{NS}	4,60	1.65
Respiratory rate	Order	1,14	0.4 ^{NS}	1,14	1.88
	Time	5,65	22.4***	5,76	14.13* * *
	Order x time	5,65	2.4 ^{NS}	5,76	1.05
Heart Rate	Order	1,14	0.2^{NS}	1,14	0.12
	Time	2,33	5.8**	4,63	12.81* * *
	Order x time	2,33	0.8 ^{NS}	4,63	0.99
Pupil	Order	1,14	0.1^{NS}	1,14	0.05
	Time	3,43	15.4***	3,45	19.10* * *
	Order x time	3,43	1.3^{NS}	3,45	1.13
Systolic BP	Order	1,14	0.08^{NS}	1,14	0.19
	Time	5,64	4.8**	5,77	2.56*
	Order x time	5,64	1.8^{NS}	5,77	1.19
Diastolic BP	Order	1,14	0.6 ^{NS}	1,14	0.34
	Time	10,140	4.4***	5,71	0.19
	Order x time	10,140	0.9^{NS}	5,71	0.38

Notes: [1]ANOVA Effects = Time since dosing (Time) and order of maintenance drug (Order: Methadone, LAAM; LAAM, methadone. [2]Greenhouse-Giesser adjustment to Df for within subject effects. [3]* $p < 0.05$, ** $p < 0.01$, * * * $p < 0.001$, NS= Not significant

Appendix 5: Summary of the studies of the effects of methadone and LAAM on cognitive and psychomotor performance

Studies of the effects of methadone and LAAM on cognitive and psychomotor performance.

Study	Participants	Dose (range)[a]	Tests	Results
Simple motor performance and reaction time				
Gordon, 1970	M/F 27 MMP vs 29 healthy controls (ex-addicts + drug free)	100 mg	a) Visual RT b) Choice visual RT	a) No effect b) No effect
Rothenberg, et al, 1977	M/F 12 MMP vs 12 drug free controls	0, 5, 10 mg to both groups	Visual RT (+/- monetary reward). Tested pre-dose and 2.25 hr after	Healthy volunteers impaired.
Kelley, et al., 1978[b]	M/F 30 MMP	63 mg (20 to 120 mg)	a) Visual RT b) Choice visual RT	a) Nil effect of time b) Nil effect of time
Specka, et al., 2000	M/F MMP 54 vs matched drug free controls	93 mg (10 –240 mg)	a) Simple choice visual RT b) Multiple choice RT	a) Faster, more errors b) Impaired
Curran, et al., 2001[c]	M/F 20 (tested pre and post dose – 50 and 100 % of maintenance dose)	32.65 mg (10-50 mg)	Simple reaction time	Significantly faster following both doses
Information processing/ psychomotor speed				
Isbell, et al., 1948[d]	M only, 15. Formerly opioid dependent, then dependent	0, up to 400 mg/day (SC)	a) Symbol copying; speed, accuracy b) Arithmetic: speed, accuracy	a) Faster speed, impaired accuracy b) Faster speed, impaired accuracy
Irwin, et al., 1976	20 MMP 21 LMP College students (past performance)	Methadone 93.5 (35-190 mg) LAAM 79.6 (12 -140 mg)	a) Number doubling; speed, accuracy b) Minnesota Clerical Test (numbers & name checking); speed, accuracy c). Arithmetic test	MMP significantly poorer performance vs LMP. LMP performance = controls
Gritz, et al., 1975	M only, 10 MMP vs 10 ex-heroin dependent	65 mg	a) DSST b) 3-minute hidden word test c) 2-minute number cancellation	a) No effect b) Impaired c) No effect
Appel & Gordon, 1976	M only, 48 MMP vs 48 controls (ex- and non-users)	80 –120 mg	DSST	No effect

See end of table for notes Table continued

Studies of the effects of methadone and LAAM on cognitive and psychomotor performance.

Study	Participants	Dose (range)[a]	Tests	Results
Information processing/ psychomotor speed continued				
Walsh, et al., 1994	M only, 5 abusers	0, 15-60 mg	DSST	No effect
Darke, et al, 2000	M/F 30 MMP vs 30 matched drug free controls	78.6 mg (15-100)	a) WAIS digit symbol (DSST) b) WAIS symbol search	Impaired
Curran, et al., 2001[c]	M/F 20 (tested pre and post dose – 50 and 100 % of maintenance dose)	32.65 mg (10-50 mg)	a) Finger tapping speed b) DSST	a) No effect b) Improved (practice effects)
Mintzer, et al., 2002	M/F 18 MMP vs 21 matched drug free controls	(Mean±sd) 67.2 mg ±24mg	a) DSST b) Trail making tests; i) time to complete; ii) conceptual flexibility	a) Impaired b) i) impaired; ii) no effect
Verdejo, et al., 2005	M 18 MMP vs 23 matched ex-heroin users	(Mean±sd) 83.82±29.61 mg	Five digit test (processing speed)	Impaired
Sustained attention (vigilance)/ Selective attention				
Rothenberg, et al., 1977.	M/F 12 MMP vs 12 drug free	0, 5, 10 mg to both groups	10 minute choice visual RT (CPT)	No effect
Kelley, et al., 1978[b]	M/F 30 MMP	63 mg (20 to 120 mg)	10 min letter cancellation	No effect
Appel, 1982	M only, 48 MMP vs 48 controls (ex- and non-users)	80 –120 mg	45 min RT (CPT)	No effect
Specka, et al., 2000	M/F MMP 54 vs matched control s	93mg (10 –240 mg)	7 min attention task	Slower, more errors
Curran, et al., 2001	M/F 20 (tested pre and post dose – 50 and 100 % of maintenance dose)	32.65mg (10-50 mg)	Digit cancellation -cross out target number	No effect
Verdejo, et al., 2005	M 18 MMP 23 matched ex-heroin users	83.82±29.61 mg (Mean±sd)	Stroop Colour-word Interference Test - selective attention	No effect

See end of table for notes

Table continued

Studies of the effects of methadone and LAAM on cognitive and psychomotor performance**.**

Study	Participants	Dose (range)[a]	Tests	Results
Immediate memory/attention				
Gritz, et al., 1975	M only, 10 MMP vs 10 ex-heroin dependent	65 mg	a) Digit span b) Picture recognition	a) No effect b) No effect
Kelley, et al., 1978[b]	M/F 30 MMP	63 mg (20 to 120 mg)	Digit span	No effect
Darke, et al, 2000	M/F 30 MMP vs 30 matched drug free controls	78.6 mg (15-100)	Digit span	impaired
Davis, et al., 2002	15 MMP vs 16 ex-opiate users vs 14 pain patients (no opiate use)	Not reported	Test of everyday attention subtests (Map search, Visual elevator)	No effect
Verdejo, et al., 2005	M 18 MMP 23 matched ex-heroin users	83.82±29.61 mg (mean±sd)	a) Letter number sequencing test (WAIS-III) (working memory) b) Oral trails (OT-I) - visuo-spatial attention.	a) No effect b) impaired
Learning, memory and comprehension, executive functioning				
Isbell, et al., 1948[d]	M only, 15. Formerly opioid dependent, then dependent	0, up to 400 mg/day	Otis intelligence	Impaired
Irwin, et al., 1976	20 MMP 21 LMP College students (past performance)	Methadone 93.5 (35-190 mg) LAAM 79.6 (12 -140 mg)	Number learning	MMP significantly poorer performance vs LMP. LMP performance = controls

See end of table for notes Table continued

Studies of the effects of methadone and LAAM on cognitive and psychomotor performance**.**

Study	Participants	Dose (range)[a]	Tests	Results
Learning, memory and comprehension, executive functioning continued				
Grevert, et al., 1977.	M/F 30 MMP, 31 LMP vs 26 matched controls. Tested prior to treatment, 1 and 3 months later	Methadone 52 mg (20-80 mg): LAAM dosed 3 x week [Session 2 = 54 mg (20-75); Session 3=60 mg (15-100 mg)]	a) Matrix memory task: Visual memory-Memory of location b) Recall list of nouns and list of numbers: Verbal memory	a) No difference MMP vs LMP b) No difference MMP vs LMP
Lombardo, et al., 1976[e]	M only, 28 MMP in a) , 25 in b)	a). 50 vs 80 mg b). 80 decreased to 50 mg	a) WAIS b) WAIS	a) No effect b) No effect
Gritz, et al., 1975	M only, 10 MMP vs 10 ex-heroin dependent	65 mg	a) Story recall b) Nonsense syllable learning c) Picture-fact pairing d) Paired associates; easy, hard	a) Impaired recall b) Impaired c) No effect d) Impaired, hard
Gordon, et al., 1967	155 MMP	79 mg (70-100 mg)	WAIS	No effect
Darke, et al, 2000	M/F 30 MMP vs 30 matched drug free controls	78.6 mg (15-100)	a) Visual memory: short term (CFT immediate & VR-I), delayed (VR-II). b) Verbal memory: short term (CVLT & PAL I), long term (PAL II) c) Problem solving, (COWAT, WCST, CFT copy trial)	a) impaired b) impaired c) impaired.
Curran, et al., 2001[c]	M/F 20 (tested pre and post dose – 50 and 100 % of maintenance dose)	32.65 mg (10-50 mg)	Prose recall	i) immediate recall – no effect ii) delayed recall - impaired
Mintzer, et al., 2002	M/F 18 MMP vs 21 matched drug free controls	67.2 mg ±24mg	a) Two back test (working memory) b) Recognition Working memory test c) Free recall test	a) Impaired b) No effect c) No effect

See end of table for notes Table continued

Studies of the effects of methadone and LAAM on cognitive and psychomotor performance**.**

Study	Participants	Dose (range)[a]	Tests	Results
Learning, memory and comprehension, executive functioning continued				
Verdejo, et al., 2005	M 18 MMP 23 matched ex-heroin users	83.82±29.61mg (mean±sd)	a) Oral trails (OT-2) -flexibility b) Similarities subtest (WAIS-III) - Analogical reasoning c) WCST- flexibility d) COWAT (FAS) - flexibility	a) impaired b) impaired c) No effect d) No effect
Complex psychomotor/ cognitive performance				
Mintzer, et al., 2002	M/F 18 MMP vs 21 matched controls	(mean±sd) 67.2 mg ±24mg	Gambling task (decision making)	Impaired
Specka, et al., 2000	M/F MMP 54 vs 54 matched control s	93mg (10–240 mg)	Tracking Task	Slower, more accurate
Bergaus, et al, 1993	13 MMT vs 13 non-user controls	17.5 – 60 mg	Tracking, visual perception, decision making, divided attention (tracking + reaction time)	Impaired on all tests
Robinson & Moskowitz , 1985	M 15 MMP vs 15 ex-heroin users	60-110 mg	Visual serial, masked priming paradigm, divided attention (tracking + visual search)	MMP-slower rate of information processing for priming task – no other effects.
Moskowitz & Robinson, 1985	M 15 MMP vs 15 ex-heroin	60-110 mg	Compensatory tracking, critical tracking	Nil effects
Chesher, et al., 1989	a). 26 MMP b). 10 starter MMP[f] c). 22 increaser MMP[g] d). 19 Control group (ex-users & drugfree)	a) 85 mg (40 – 150) b) 38 mg (15-60 mg) c) 67 mg (40 - 135mg)	Tracking, vigilance and divided attention (tracking and visual search)	No significant differences. Trend towards impaired performance in starter and increaser MMP patients.

See end of table for notes

Table continued

Studies of the effects of methadone and LAAM on cognitive and psychomotor performance**.**

Study	Participants	Dose (range)[a]	Tests	Results
Complex psychomotor/ cognitive performance continued				
Moskowitz & Robinson, 1985	M 15 MMP vs 15 ex-heroin	60-110 mg	Compensatory tracking, critical tracking	Nil effects
Chesher, et al., 1989	a). 26 MMP b). 10 starter MMP[e] c). 22 increaser MMP[f] d). 19 Control group (ex-users & drugfree)	a). 85 mg (40 – 150) b). 38 mg (15-60 mg) c). 67 mg (40 -135 mg)	Tracking, vigilance and divided attention (tracking and visual search)	No significant differences. Trend towards impaired performance in starter and increaser MMP patients.

Notes: Table adapted from Zacny, (1995) pp. 446-7. Unless otherwise indicated maintenance drug was methadone and route of administration for methadone and LAAM was oral; S/C= subcutaneous; M/F = both male and female participated; MMP=Methadone maintenance patients; LMP=LAAM maintenance patients; RT=Reaction time; DSST=Digit Symbol Substitution test; WAIS=Wechsler Adult Intelligence Scale; CPT=Continuous Performance Test; CFT=Complex Figure Test; VR=Visual Reproduction Subtest (Weschler Memory Scale-WMS); CVLT= California Verbal Learning Test; PAL=Paired Associate Learning subtest (WMS); COWAT=Controlled Oral Word Association Test; WCST=Wisconsin Card Sorting Test. **a** Unless otherwise stated dose is expressed as mean (range); **b** performance of MMPs compared at 1 hr versus 25 hr post methadone; **C** Patients randomly allocated two groups to receive either 50% or 100% of stabilisation dose. Assessed pre- and post-dose on 2 separate days; **d** No statistics undertaken, performance during acute challenge and dependent period compared to drug free controls; **e** One group maintained on a daily dose of 50 mg and a second group maintained on a daily dose of 80 mg tested on the WAIS. The group maintained on 80 mg then decreased to a stabilised dose of 50 mg and comparisons made within this group between the two doses; **f** 'starters' , methadone patients who had just commenced MMT; **g** 'increasers', stabilised MMP patients, early in their treatment, who had just received an increase in their dose.

Appendix 6: Publication in support of this thesis

Newcombe, D.A.L, Bochner, F., White, J.M., & Somoggyi, A.A (2004). Evaluation of levo-alpha-acetylmethadol as an alternative treatment for methadone maintenance patients who regularly experience withdrawal: a pharmacokinetic and pharmacodynamic analysis. *Drug and Alcohol Dependence, 76*, 63-72.

Newcombe, D.A.L., Bochner, F. White, J.M. and Somogyi, A.A. (2004) *Evaluation of levo-alpha-acetylmethdol (LAAM) as an alternative treatment for methadone maintenance patients who regularly experience withdrawal: a pharmacokinetic and pharmacodynamic analysis*
Drug and Alcohol Dependence v.76 (1) pp. 63-72 October 2004.

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