The Cardiopulmonary Effects and Pharmacokinetics of Fentanyl in the Dog:
The Influence of Isoflurane Anesthesia
and Sedative Administration during Anesthetic Recovery

by

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ABSTRACT

THE CARDIOPULMONARY EFFECTS AND PHARMACOKINETICS OF FENTANYL IN THE DOG: THE INFLUENCE OF ISOFLURANE ANESTHESIA AND SEDATIVE ADMINISTRATION DURING ANESTHETIC RECOVERY

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University of Guelph, 2013

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The objectives of this study were to determine the cardiopulmonary effects and pharmacokinetics of fentanyl in dogs during isoflurane anesthesia and during anesthetic recovery with or without dexmedetomidine or acepromazine sedation. This was investigated in 7 healthy dogs using a randomized cross over study design. Dogs were given fentanyl as an initial IV loading dose (5 μg/kg) followed by an infusion (5 μg/kg/hr) for 120 minutes during isoflurane anesthesia and for 60 minutes following isoflurane discontinuation. Dogs received IV dexmedetomidine (2.5 μg/kg), acepromazine (0.05 mg/kg) or saline at the time of isoflurane discontinuation. Cardiopulmonary variables were measured and blood samples were obtained at multiple time points during the anesthetic maintenance and recovery phases. Plasma concentrations of fentanyl were measured using HPLC-MS, and subsequent population pharmacokinetic analysis was performed.

During isoflurane anesthesia, fentanyl bolus administration resulted in significant changes in measured cardiopulmonary variables, however, many returned to baseline values during the maintenance of anesthesia. During anesthetic recovery, dexmedetomidine administration resulted in significant increases in PaCO₂, and
decreases in \( \text{PvO}_2 \) and CI. Systemic arterial blood pressures were significantly lower in dogs receiving acepromazine, however CI and \( \text{PvO}_2 \) were significantly higher compared to the other treatments.

Analysis of fentanyl plasma concentrations showed that fentanyl pharmacokinetics best fit a 2-compartmental model, with average concentrations in the treatment groups ranging from 1.6 to 4.5 ng/mL during isoflurane anesthesia, and from 1.6 to 2.0 ng/mL during anesthetic recovery. Plasma concentrations of fentanyl were significantly higher with dexmedetomidine administration compared to the other treatments during the recovery period. Compared to the maintenance phase of anesthesia, anesthetic recovery with dexmedetomidine administration did not significantly change fentanyl pharmacokinetics, while acepromazine administration increased systemic and intercompartmental clearance, and recovery without sedation increased the central volume of distribution and systemic clearance.

In conclusion, recovery from anesthesia with concurrent fentanyl administration, with or without sedation, caused clinically significant alterations in cardiopulmonary function that influenced fentanyl disposition in healthy dogs.
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Finally, I would like to thank my mum, dad and brother for their endless love and inspiration, as well as my dear friends on both sides of the Atlantic.
DECLARATION OF WORK PERFORMED

I declare that with the exception of the items listed below, all the work reported in this thesis was performed by me.

Dr. Carolyn Kerr wrote the initial research proposal and obtained funding for the project. Assistance performing the experiments and collecting data was provided by Dr. Carolyn Kerr, Dr. Wayne McDonell, Nicole Kudo and Mandy Hathway.

Statistical analysis was performed by Gabrielle Monteith.

Dr. Heather Knych performed fentanyl and norfentanyl analysis and contributed to the description of this technique.

Dr. Andrea Edginton performed the pharmacokinetic analysis for the project and contributed to the description of this technique.
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</tr>
<tr>
<td>CL₂</td>
<td>Intercompartmental clearance</td>
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<tr>
<td>CI</td>
<td>Cardiac Index</td>
</tr>
<tr>
<td>CRI</td>
<td>Constant rate infusion</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>CVP</td>
<td>Central venous pressure</td>
</tr>
<tr>
<td>DABP</td>
<td>Diastolic arterial blood pressure</td>
</tr>
<tr>
<td>DO₂</td>
<td>Oxygen delivery</td>
</tr>
<tr>
<td>dP/dt\text{\text{max}}</td>
<td>Maximal rate of pressure change in the left ventricle</td>
</tr>
<tr>
<td>ER</td>
<td>Oxygen extraction ratio</td>
</tr>
<tr>
<td>HPLC-MS</td>
<td>High-performance liquid chromatography-mass spectrometry</td>
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<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>MABP</td>
<td>Mean arterial blood pressure</td>
</tr>
<tr>
<td>MAC</td>
<td>Minimum alveolar concentration</td>
</tr>
<tr>
<td>MPAP</td>
<td>Mean pulmonary artery pressure</td>
</tr>
<tr>
<td>PAOP</td>
<td>Pulmonary artery occlusion pressure</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>Partial pressure of carbon dioxide in arterial blood</td>
</tr>
<tr>
<td>PaO₂</td>
<td>Partial pressure of oxygen in arterial blood</td>
</tr>
<tr>
<td>PvO₂</td>
<td>Partial pressure of oxygen in mixed venous blood</td>
</tr>
<tr>
<td>PVRI</td>
<td>Pulmonary vascular resistance index</td>
</tr>
<tr>
<td>RR</td>
<td>Respiratory rate</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
<td>-----------------------------------------------</td>
</tr>
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<td>SABP</td>
<td>Systolic arterial blood pressure</td>
</tr>
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<td>Stroke index</td>
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<tr>
<td>SVRI</td>
<td>Systemic vascular resistance index</td>
</tr>
<tr>
<td>$t_{1/2\beta}$</td>
<td>Terminal half-life</td>
</tr>
<tr>
<td>$V_1$</td>
<td>Volume of the central compartment</td>
</tr>
<tr>
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CHAPTER 1: Introduction and Objectives

1.1 Introduction

The majority of companion dogs will undergo general anesthesia at least once in their lifetime. In addition to routine procedures and surgery, general anesthesia is increasingly required in canine patients to facilitate advanced diagnostic and therapeutic interventions. Despite the increasing need for general anesthesia, overall canine anesthetic mortality rates have not declined over the last two decades (Clarke & Hall 1990; Dyson et al. 1998, Brodbelt et al. 2008a, Bille et al. 2012). Furthermore, canine mortality rates are over 100 times greater than those in human anesthesia suggesting that there is room for significant improvement (Biboulet et al. 2001, Braz et al. 2006).

A recent study has identified the anesthetic recovery period as the time of greatest anesthetic mortality in dogs (Brodbelt et al. 2008a). This may be related to the anesthetic management practices utilized throughout the anesthetic period; however it may also be a result of management during the recovery period itself. While the specific cause of many of these deaths remains unknown, the majority are cardiorespiratory in origin (Clarke & Hall 1990; Dyson et al. 1998, Brodbelt et al. 2008a). Despite these findings, the physiologic effects of different management practices during the anesthetic recovery period, including drug administration, have received little attention in the literature.

Currently, the inhalant anesthetic isoflurane is one of the agents most commonly used to maintain anesthesia in small animal patients. Since it lacks antinociceptive activity, opioids such as fentanyl, hydromorphone, or morphine are typically administered before and during anesthesia, as well as in the post-operative period, for
pain management. While most opioids produce analgesia, they are also associated with dose-dependent cardiorespiratory depression and may contribute to excitatory behavior during anesthetic recovery. In the latter scenario, administration of sedatives, such as the alpha-2 adrenergic receptor agonist dexmedetomidine or the phenothiazine acepromazine, are recommended to calm the patient (Pascoe 2000).

The physiologic effects of dexmedetomidine, acepromazine, and fentanyl have been studied extensively when used alone; however, there are no studies evaluating their combined effects during recovery from isoflurane anesthesia. The primary goal of the current study was to assess the physiologic effects of sedative administration during recovery from isoflurane anesthesia in dogs under the influence of an opioid analgesic, fentanyl.

*In vitro* investigations have proposed that alpha-2 adrenergic receptor agonists may alter the metabolism of fentanyl. As opioids are known to produce dose-dependent cardiorespiratory depression, a potential etiology leading to anesthetic death, a second goal was to assess the influence of sedative administration on fentanyl disposition during anesthetic recovery.

### 1.2 Objectives

The specific objectives of this research were:

a) To evaluate the cardiopulmonary effects of a fentanyl infusion during isoflurane anesthesia and during recovery from anesthesia, with or without concurrent administration of dexmedetomidine or acepromazine in dogs.
b) To determine plasma fentanyl concentrations resulting from a constant rate infusion of fentanyl during isoflurane anesthesia and during recovery from anesthesia, with or without concurrent administration of dexmedetomidine or acepromazine in dogs.
2.1 Cardiopulmonary effects of anesthetic, analgesic and sedative agents

2.1.a Isoflurane

In general, the inhalant anesthetics currently used in veterinary practice produce dose-dependent cardiopulmonary depression in the dog. The changes in cardiovascular function associated with the different agents are, however, distinct and not uniformly depressant (Steffey & Mama 2007). In a study by Mutoh and colleagues (1997), increasing the inspired concentration of isoflurane in spontaneously breathing dogs resulted in progressive decreases in mean arterial blood pressure (MABP). At 2 times the minimum alveolar concentration (MAC), MABP was 33% lower than conscious baseline values, with corresponding decreases in systemic vascular resistance (Mutoh et al. 1997). Stroke index also decreased by 15% at 2 MAC, however a compensatory increase in heart rate (HR) (31%) also occurred and cardiac index (CI) remained stable. These changes were dose-dependent at 1, 1.5 and 2 MAC. It was speculated that part of the cardiovascular compensatory response demonstrated at 2 MAC may be attributed to the increase in sympathetic tone imparted by increases in the partial pressure of carbon dioxide in arterial blood (PaCO₂), as isoflurane resulted in a dose-dependent respiratory depression (Mutoh et al. 1997). However, the trend of increasing HR and decreasing arterial blood pressure, as well as the values for these variables reported at 2 MAC, are not markedly different in eucapnic mechanically ventilated dogs compared to those breathing spontaneously (Steffey & Howland 1977, Bernard et al. 1990, McMurphy et al. 1996, Johnson et al. 1998, Galloway et al. 2004). This suggests that the stability in
cardiac output observed in healthy dogs with increasing delivered concentrations of isoflurane does not result from elevations in PaCO$_2$ alone.

While the findings of the Mutoh study (1997) are generally consistent with other reports, other researchers have reported decreases in cardiac output and contractility at inspired concentrations of isoflurane in excess of those typically used clinically (Steffey & Howland 1977, Bernard et al. 1990). Further research has established that the mechanism responsible for the direct myocardial depression from isoflurane is, at least in part, from the reduced ability of cardiomyocytes to handle calcium during excitation-contraction coupling (Nakayama et al. 1997).

While the predominant vasodilatory effect of isoflurane has minimal impact on global oxygen delivery (DO$_2$) in healthy dogs at clinically used concentrations, there are few studies evaluating its effects in compromised dogs or those with underlying disease. Experiments evaluating healthy dogs in a state of hypovolemia provide clear evidence that the ability of the cardiovascular system to compensate for isoflurane-induced vasodilation is markedly reduced (Fayyaz et al. 2009). Following withdrawal of 30 mL/kg blood volume, arterial blood pressure, stroke volume and CI dropped precipitously under 1.5% isoflurane anesthesia to values much lower than post-hemorrhage values recorded following anesthetic recovery (Fayyaz et al. 2009). This may be due to a compromised ability to modulate vascular tone, reductions in sympathetic outflow, and a blunted baroreceptor reflex, as HR decreased concurrently with the fall in blood pressure (Seagard et al. 1983, Fayyaz et al. 2009). Although isoflurane does compromise the compensatory physiologic responses to volume loss, it appears to have the least impact when compared to both halothane and sevoflurane in the
dog (Teixeira Neto et al. 2007). It is worth noting that these studies were performed on young, healthy dogs, and the effects of isoflurane in dogs with underlying pathology, while not reported in the literature, are likely to be more profound. The potential for an exaggerated negative cardiovascular response to isoflurane in compromised patients has lead to the promotion of balanced anesthetic techniques, where the addition of other injectable anesthetic drugs may reduce isoflurane requirements and thus the adverse cardiovascular effects.

In addition to cardiovascular effects, isoflurane results in dose-dependent respiratory depression. As with the cardiovascular effects, the magnitude of respiratory change is acceptable in healthy dogs at clinically used concentrations, however depression increases dramatically as concentrations reach and exceed 2 MAC (Mutoh et al. 1997, Galloway et al. 2004). Alterations in ventilation are characterized by decreases in RR, tidal volume and alveolar ventilation, resulting in increases in PaCO$_2$ to 53-66 mmHg at 2 MAC, and an end-tidal CO$_2$ measurement of 69 mmHg at 3 MAC with corresponding decreases in arterial pH (Steffey & Howland 1977, Mutoh et al. 1997, Galloway et al. 2004). Another measure of respiratory depression is the anesthetic index – the concentration of inhalant that produces apnea divided by MAC. Isoflurane is a more potent respiratory depressant than other volatile anesthetics as demonstrated by an anesthetic index of 2.51-2.61 compared to values of 3.45, 3.4 and 2.9 for sevoflurane, methoxyflurane and halothane, respectively (Regan & Eger 1967, Steffey & Howland 1977, Steffey & Howland 1978, Galloway et al. 2004).

The negative effects are often emphasized, however it is worth noting that isoflurane has some beneficial effects on the cardiopulmonary system. Concentrations as
low as 1.0 MAC have been shown to increase the threshold to atrial fibrillation (Freeman et al. 1990). Isoflurane is also known to be an effective means of ischemic preconditioning, reducing reperfusion injury and the degree of myocardial infarction following hypoxic episodes (Raphael et al. 2008). Additionally, isoflurane provides dose-dependent bronchodilation, reducing pulmonary resistance and increasing compliance following bronchoconstrictive challenges (Hirshman et al. 1982, Ishikawa et al. 1998).

2.1.b Fentanyl

Fentanyl citrate is a synthetic pure µ-opioid receptor agonist with excellent analgesic properties. It is routinely used as an intravenous infusion due to its rapid onset and short duration of effect (Ilkiw 1999). As will be discussed, the adverse cardiopulmonary effects of fentanyl when administered alone are minimal at clinical doses in healthy conscious dogs. Because of this, as well as its short-acting and titratable effect, fentanyl is a commonly recommended analgesic for use in both healthy and critical veterinary patients (Anderson & Day 2008).

The most pronounced cardiovascular effect of fentanyl in conscious dogs is a dose-dependent bradycardia, which plateaus at a 50% reduction in HR (Arndt et al. 1984). No changes in HR were noted following a cumulative dose of fentanyl up to 7.5 μg/kg IV over 5 minutes in conscious dogs; however, a 30% decrease was observed 15 minutes after a 15 μg/kg IV bolus (Arndt et al. 1984, Grimm et al. 2005). Heart rate progressively decreases as doses are further increased to the supraclinical range, reaching maximal depression following the cumulative IV administration of 67.5 μg/kg over 15 minutes (Arndt et al. 1984).
The mechanism by which fentanyl exerts this effect has been the focus of many studies and is multifaceted in the dog. There is substantial evidence that modulation of parasympathetic and sympathetic tone, as well as direct cardiac effects, all contribute to the observed decrease in HR. The majority of fentanyl’s negative chronotropic effect is attributable to enhanced parasympathetic outflow. Studies in dogs have consistently demonstrated the attenuation of fentanyl-induced bradycardia following bilateral vagotomy or administration of anticholinergics (Reitan et al. 1978, Flacke et al. 1983). Furthermore, evaluation of neuronal discharge patterns within the cervical vagus identified a selective increase in activity of a subtype of efferent fibers, which were previously identified as cardioinhibitory (Inoue et al. 1980). This has been further corroborated by evaluation of electrical activity within the brain where fentanyl influenced activity in both the nucleus tractus solitarius and nucleus ambiguus indicating an influence on autonomic tone (Laubie & Schmitt 1980). Interruption or antagonism of vagal outflow does not completely eliminate reductions in HR from fentanyl, however, suggesting there may be additional contributing mechanisms (Reitan et al. 1978). Further studies have determined that suppression of sympathetic tone, a direct negative chronotropic effect on the sinoatrial node, and local potentiation of vagal tone also play a small, but notable, role in fentanyl-induced bradycardia in the dog (Laubie & Schmitt 1980, Flacke et al. 1983, Loeb et al. 1984).

The reduction in HR is paralleled by a dose-dependent reduction in cardiac output. Interestingly, cardiac output decreased at lower plasma concentrations than those decreasing HR suggesting changes in contractility, preload or afterload may also occur, however those variables were not measured in the study (Arndt et al. 1984).
Studies evaluating fentanyl in both in vivo and isolated heart models have demonstrated the absence of any effects on contractility. Even following 160 μg/kg IV of fentanyl, a dose greatly exceeding that which would be used clinically, dogs did not demonstrate changes in left ventricular end-diastolic pressure or the maximal rate of pressure change in the left ventricle (dP/dt\text{max}) (Freye 1974). Decreases in the rate and extent of papillary muscle tension development in in vitro preparations occurred at fentanyl concentrations of 10 to 120 μg/mL which would never be achieved clinically even at the highest clinical doses used (Strauer 1972, Motomura et al. 1984). For example, 640 μg/kg of fentanyl resulted in peak plasma concentrations of approximately 375 ng/mL in enflurane anesthetized dogs, which is still 25 times lower than the concentrations required to compromise contractility in the in vitro experiments (Murphy et al. 1983).

A more recent study evaluated the cardiovascular effects of fentanyl following a 15 μg/kg IV bolus in conscious dogs also identified a decrease in CI (Grimm et al. 2005). This decrease was associated with decreases in HR, as well as increased stroke index (SI) and systemic vascular resistance index (SVRI) suggesting that both an increase in afterload and the slower HR may have contributed to the decrease in cardiac output despite increases in preload (Grimm et al. 2005). The collective findings suggest that CI is largely decreased due to a reduction in HR and possibly increases in afterload, despite the preservation of contractility and increases in preload.

Changes in blood pressure following fentanyl administration in conscious dogs have been inconsistent in the literature, with studies demonstrating either progressive increases or decreases with increasing doses. At doses used clinically, MABP did not change significantly, and even decreased slightly, following 5, 10 and 15 μg/kg of
fentanyl IV in conscious dogs (Taneyama et al. 1993, Grimm et al. 2005). In a separate investigation, as doses increased, MABP did not change until a cumulative dose of 27.5 μg/kg had been administered, at which point blood pressure increased by approximately 25% and remained similarly elevated up to the final cumulative dose of 167.5 μg/kg (Arndt et al. 1984). These findings were supported by another study where 20 μg/kg of fentanyl increased MABP by 24% with peak values occurring 10 minutes after administration (Hendrix et al. 1995). The increase in blood pressure in these two studies occurred despite significant bradycardia and apparent sedation suggesting that increases in blood pressure may be due to increases in systemic vascular resistance, but this was not evaluated directly. However, another study reported a transient increase in systemic vascular resistance followed by a more prolonged decrease following fentanyl administration in dogs, which may explain the elevations in blood pressure observed in the other studies (de Castro et al. 1979). A modest increase in systemic vascular resistance was also observed following 15 μg/kg IV along with non-significant mild decreases in MABP and CI; however, the increase in systemic vascular resistance was not statistically significant (Grimm et al. 2005). Overall, at clinically relevant doses, changes in blood pressure following fentanyl administration are minimal in healthy conscious dogs when the drug is administered alone.

Respiratory depression is a significant consequence of fentanyl administration in humans (Bailey et al. 1990). In conscious, healthy dogs, respiratory depression caused by fentanyl has been shown to be dose-dependent, however clinically relevant compromise is not present within the dose range used clinically (2-10 μg/kg) (Lamont & Mathews 2007). Respiratory rate only begins to decline following a cumulative dose of 27.5 μg/kg,
and apnea does not occur even at IV doses as high as 167.5 μg/kg (Arndt et al. 1984). While mild decreases in the partial pressure of oxygen in arterial blood (PaO₂) do occur after 2.5 μg/kg IV, values do not fall below 75 mmHg until a dose of 27.5 μg/kg IV is administered and levels plateau around 50 mmHg in conscious dogs with greater doses (Arndt et al. 1984). The change in oxygenation is accompanied by dose-dependent increases in PaCO₂ that do not exceed 50 mmHg (Arndt et al. 1984). In conscious dogs receiving a single IV bolus (15 μg/kg) or infusion (10 μg/kg/hr) at the highest doses that would be considered for clinical use in conscious dogs, there were no significant changes in RR, PaO₂ or PaCO₂ demonstrating the respiratory safety of this drug in uncompromised conscious dogs (Lemmens et al. 2008; Grimm et al. 2005). As will be discussed, these findings may not apply to patients under inhalant anesthesia, and the respiratory safety of fentanyl depends on the context of its administration.

2.1.c Dexmedetomidine

Dexmedetomidine is the pharmacologically active D-isomer in the racemic mixture, medetomidine. It is a highly selective alpha-2 adrenergic receptor agonist that produces sedation, muscle relaxation and analgesia in conscious dogs (Murrell & Hellebrekers 2005). Accompanying these desired effects are profound cardiovascular changes due to both central and peripheral effects. The most striking cardiovascular effects are increased systemic vascular resistance, bradycardia and decreased cardiac output, which may be profound following intravenous bolus dosing (Pypendop & Verstegen 1998).
Following IV administration, dexmedetomidine activates alpha-2 adrenoreceptors in the systemic and pulmonary vasculature causing calcium influx, vasoconstriction, and an increase in vascular resistance (Xiao & Rand 1989). The period of elevated blood pressure resulting from the peripheral vasoconstrictive effects of the alpha-2 agonists typically lasts 15-45 minutes with clinically recommended sedative doses in the dog (Enouri et al. 2008). A marked reduction in HR is also consistently observed acutely following intravenous alpha-2 adrenergic receptor agonist administration, which is primarily a result of increases in baroreceptor-mediated parasympathetic tone secondary to increases in systemic vascular resistance (Flacke et al. 1990, Pagel et al. 1998). Effects of the alpha-2 agonist on the central nervous system, specifically the locus coeruleus, nucleus of the solitary tract and vagus nucleus, also contribute to the decrease in HR (Bloor et al. 1992, Nicholas et al. 1993). In addition to bradycardia, the shift in autonomic outflow to greater parasympathetic tone likely is responsible for the increase in sinus arrhythmias, first and second degree heart block and the prolonged QT interval that may be observed following medetomidine and dexmedetomidine in dogs (Kuusela et al. 2000, Valtolina et al. 2009).

Cardiac output also drops considerably with dexmedetomidine administration, which can be explained by both decreases in HR, as well as stroke volume. The contribution of HR is supported by the finding that reductions in cardiac output are attenuated by approximately 30% when declines in HR are prevented by the co-administration of an anticholinergic (Bloor et al. 1992). However, autonomically denervated dogs also demonstrated significant reductions in cardiac output with dexmedetomidine administration despite stable HRs, suggesting that additional
mechanisms play a role in reducing cardiac output (Flacke et al. 1990). A direct increase in systemic vascular resistance also seems to contribute as the calcium-channel blocker, nifedipine, as well as peripheral alpha-2 adrenergic receptor antagonists reduce the peripheral vasoconstrictive effect of alpha-2 agonists and significantly improve cardiac output (Bloor et al. 1992, Pagel et al. 1998, Enouri et al. 2008). Studies have consistently demonstrated that dexmedetomidine does not decrease myocardial contractility through direct mechanisms in isolated preparations (Housmans 1990, Flacke et al. 1992). However, centrally mediated reductions in circulating epinephrine and norepinephrine following dexmedetomidine administration can be expected to reduce both positive inotropic and chronotropic effects on the heart (Bloor et al. 1992).

Although cardiac output decreases, there is significant redistribution of blood flow. Dexmedetomidine dosing from 0.1 to 10 μg/kg results in greater preservation of flow to the vital organs (heart and brain) and more significant reductions in flow to the skin, spleen and skeletal muscle; however flow to some vital organs may still decrease (Lawrence et al. 1996, Talke et al. 2000). This decrease in blood flow significantly increases oxygen extraction, however plasma lactate and pH remain within normal limits suggesting that perfusion is adequate to meet global tissue demand (Lawrence et al. 1996, Talke et al. 2000, Ko et al. 2007). Although myocardial perfusion is decreased at low and high IV doses in humans, so is rate-pressure product and myocardial oxygen demand, and ECG analysis does not suggest any evidence of myocardial ischemia (Snapir et al. 2006).

The cardiovascular changes associated with alpha-2 adrenergic receptor agonist administration in dogs are somewhat dose-dependent over the low range of
recommended doses, primarily in relation to duration of effect versus magnitude. Reductions in HR and cardiac output are typically maximal at doses used clinically for sedation, and further increases in dose just prolong the duration of cardiovascular effects (Pypendop & Verstegen 1998). This was demonstrated clearly in a dose-titration study by Pypendop and Verstegen (1998), who demonstrated that in conscious dogs 1 and 2 μg/kg IV of medetomidine reduced HR by approximately 50% and CI by over 60%. The corresponding reductions were only slightly greater and were maximal at 5 μg/kg IV and above. Changes in systemic vascular resistance and blood pressure demonstrate a greater trend toward true dose-dependency with values progressively increasing with higher doses, before gradually decreasing over time (Pypendop & Verstegen 1998). Low-dose constant rate IV infusions of medetomidine at 1, 2 and 3 μg/kg/hr in conscious dogs also demonstrate dose-dependent reductions in HR and CI, and increases in systemic vascular resistance (Carter et al. 2010).

Studies evaluating the respiratory effects of medetomidine and dexmedetomidine in dogs have been consistent in their findings, demonstrating a reduction in RR, stable PaCO₂ and only mild decreases in PaO₂ following typical clinical doses, such as 5 μg/kg dexmedetomidine IV, or medetomidine doses as high as 40 μg/kg IV (Ko et al. 2000, Kuusela et al. 2001, Ko et al. 2007, Alvaides et al. 2008, Enouri et al. 2008, Raekallio et al. 2009). The cyanosis observed in some patients following alpha-2 adrenergic receptor agonist administration is a result of reduced peripheral perfusion and enhanced local oxygen extraction as opposed to impaired arterial oxygenation. This is supported by the observation of a reduction in venous oxygen saturation with no significant change in PaO₂ levels in dogs receiving medetomidine IV (Ko et al. 2007, Enouri et al. 2008).
Although the mean PaO$_2$ does not decrease below 90 mmHg in the majority of experimental studies in dogs, it does decrease slightly from baseline values. It is important to note that the PaO$_2$ of some individuals can drop dramatically, with PaO$_2$ values reported as low as 62 mmHg following 10 μg/kg of medetomidine administered IV, which is not reflected in study averages (Rolfe et al. 2012). The changes in PaO$_2$ in the absence of significant changes in PaCO$_2$ suggest that the changes in blood gases are primarily cardiovascular, as opposed to respiratory, in origin. Specifically, the mild decrease in PaO$_2$ is likely due to an increase in ventilation-perfusion mismatching within the lung.

While changes in ventilation in healthy conscious dogs are minimal, a study by Lerche and Muir (2004) has demonstrated that medetomidine causes centrally mediated respiratory depression. Dose-dependent reductions in RR, tidal volume, minute volume and inspiratory occlusion pressure were demonstrated following medetomidine administration at 5 and 10 μg/kg IV in conscious dogs breathing an increased fraction of carbon dioxide. This respiratory depression in the face of a respiratory stimulus may be explained by the decreased activity of the locus coeruleus, which connects with respiratory centers in the brain (Lerche & Muir 2004). These findings are supported by Nguyen et al. (1992) who evaluated the respiratory effects of 1, 10, 20 and 100 μg/kg of dexmedetomidine IV, and demonstrated a maximal 60% inhibition of the hypercapnic response, which plateaued at 10 μg/kg. However, none of the doses tested impaired the hypoxic ventilatory response (Nguyen et al. 1992). While the central component of respiratory depression from medetomidine alone does not appear to alter respiratory
drive in dogs breathing room air, the concurrent administration of other sedatives or anesthetics may enhance this effect as will be discussed.

**2.1. Acepromazine**

Acepromazine is one of the most widely used sedatives in veterinary medicine, providing sedation and muscle relaxation with only mild cardiopulmonary changes when administered alone to conscious dogs. The most recognized cardiovascular effect is a decrease in arterial blood pressure, attributed to alpha-1 adrenergic receptor antagonism in the vasculature as well as centrally mediated depression of vasopressor reflexes accompanying sedation (Ludders et al. 1983, Farver et al. 1986). The sole administration of acepromazine results in reductions in MABP of 20-25% following doses ranging from 0.05 to 1.1 mg/kg given by either IV or IM routes (Popovic et al. 1972, Coulter et al. 1981, Stepien et al. 1995, Alvaides et al. 2008). While the vasodilatory effects of acepromazine are often discussed, normotensive dogs do not become hypotensive when acepromazine is administered alone at these doses, with lowest recorded mean values for systolic blood pressure (SABP), MABP and diastolic blood pressure (DABP) of 110, 75 and 51 mmHg, respectively (Coulter et al. 1981, Alvaides et al. 2008).

Reports on the effect of acepromazine on HR in the dog have been somewhat variable. The majority of studies have found very mild decreases in HR from the initial point of recording, between 10 to 15 minutes post IV or IM administration, which persists for approximately 2 hours (Popovic et al. 1972, Stepien et al. 1995, Alvaides 2008). One study in dogs reported “tachycardia” 5 minutes following IV administration, however the specific heart rates and changes over time were not reported (Turner et al.
These findings conflict with those from another study where no changes in HR were observed 5 minutes after 0.2 mg/kg acepromazine IV (Farver et al. 1986). The HR response is variable and likely has multiple contributing influences. It is possible that there is an initial reflex increasing HR in response to the decrease in arterial blood pressure, which is then overtaken by a decrease in HR from enhanced parasympathetic outflow or reduced sympathetic tone. This idea is supported by a study conducted by Coulter et al. (1981) which reported a mild increase in HR (from approximately 90 to 110 beats per minute) 3 minutes after IV acepromazine administration, which declined to baseline values by 15 minutes and decreased slightly further for the remainder of the study, with the lowest values still greater than 80 beats per minute.

In addition to HR and blood pressure, cardiac output and stroke volume have also been reported to decrease in conscious dogs given 0.1 and 0.2 mg/kg of acepromazine IV (Farver et al. 1986, Stepien et al., 1995). In a study by Stepien et al. (1995) the decrease in cardiac output was attributed to a 24% reduction in stroke volume, as decreases in HR were not significant. Stroke volume is dependent upon preload, afterload and contractility. In Stepien’s study (1995), indicators of preload such as mean right atrial pressure, left ventricular end-diastolic pressure and pulmonary artery occlusion pressure (PAOP) all tended to decrease, which would support a reduction in preload contributing to the decrease in cardiac output. This occurred together with reductions in dP/dt\text{max}, as well as echocardiographic fractional shortening, aortic acceleration and aortic velocity indicating that reduced contractility also contributed to the observed reductions in stroke volume and cardiac output. The decreases in arterial blood pressure observed in this study suggest the potential of reduced afterload; however, systemic vascular resistance
did not change due to the concurrent decrease in cardiac output. The decrease in cardiac output may be due to direct myocardial depression, a reduction in sympathetic tone from central autonomic effects or simply the sedative effects of acepromazine (Stepien et al. 1995). It is important to mention that while reductions from baseline values did occur, cardiac output was reduced from high values into the normal range for healthy, conscious dogs and did not result in compromised perfusion as the partial pressure of oxygen in mixed venous blood (PvO₂) remained within normal limits (Farver et al. 1986, Stepien et al. 1995, Haskins et al. 2005).

The cardiovascular side effects of acepromazine predominate with less pronounced effects on the respiratory system. When administered to dogs at clinically relevant doses, acepromazine decreases RR, however tidal volume increases and minute volume remains constant (Popovic et al. 1972, Stepien et al. 1995). The literature also consistently reports no effect on the PaCO₂, PaO₂ or arterial pH (Popovic et al. 1972, Farver et al. 1986, Stepien et al. 1995, Alvaides et al. 2008). Perhaps due to the lack of observed respiratory effects, the influence of acepromazine on central respiratory drive in response to hypercapnia and hypoxemia has not been evaluated.

2.2 Cardiopulmonary effects of drug combinations

2.2.a Inhalant anesthetics and opioids

Comparing cardiovascular changes associated with drug administration in conscious versus anesthetized dogs is confounded by variations in the animals’ initial cardiovascular status. It is apparent from the literature that reductions in HR depend largely on pre-existing sympathetic tone, with relatively greater reductions occurring in
those with higher initial sympathetic outflow. For instance, in enflurane/nitrous oxide anesthetized dogs with an average baseline HR of 134 beats per minute, 10 μg/kg of fentanyl IV reduced HR by approximately 60% to an average of 80 beats per minute (Flacke et al. 1983). In resting conscious dogs with baseline HRs of approximately 70 beats per minute, 7.5 μg/kg of fentanyl IV had minimal effect on HR (Arndt et al. 1984). Comparing these findings illustrates that changes are more profound with higher baseline values.

These findings may suggest that there are minimal differences between conscious and anesthetized dogs, however other studies indicate that the cardiovascular depression associated with fentanyl is more profound and longer lasting in dogs under anesthesia with an inhalant agent. Comparing findings by Reitan et al. (1978) with those from Grimm et al. (2005) strongly supports this view as dogs had similar baseline HRs allowing more direct comparisons. Reitan et al. (1978) administered 10 μg/kg of IV fentanyl to halothane anesthetized dogs and reported a decline in HR to 60% of baseline anesthetized values (corresponding to an average of 54 beats per minute) which remained below baseline at the last recorded time point 60 minutes after administration. These changes are greater than those observed by Grimm et al. (2005) where a higher 15 μg/kg IV dose of fentanyl was administered to conscious dogs resulting in a 28% reduction in HR (corresponding to an average of 72 beats per minute) which returned to baseline values within 60 minutes following administration.

In contrast to conscious patients, cardiac contractility is reduced following fentanyl administration in dogs under enflurane anesthesia. This was demonstrated in two separate studies where IV fentanyl doses of 10-50 μg/kg given to dogs under
enflurane anesthesia resulted in a decrease in $\frac{dP}{dt_{\text{max}}}$ of 18-25% compared to baseline anesthetized values (Flacke et al. 1983, Hirsch et al. 1993). Interestingly, one study demonstrated minimal changes in cardiac output despite this reduction (Hirsch et al. 1993), while the other demonstrated a significant decrease of 40% (Flacke et al. 1983). This difference is not readily explained as both studies demonstrated similar reductions in HR and had similar methodology. However, the dogs exhibiting decreases in cardiac output were induced with thiopental an hour prior to data collection, as opposed to inhalant, and were also under concurrent nitrous oxide and enflurane anesthesia, as opposed to enflurane alone. It is possible that the presence of these additional drugs may have resulted in additional cardiovascular depression. The influence of fentanyl on cardiac contractility in dogs has not been evaluated under isoflurane anesthesia.

While the effect of fentanyl on systemic blood pressure appears to be minimal and may actually increase in conscious dogs at moderately high doses, the literature consistently reports a reduction in MABP following fentanyl administration in dogs under halothane, enflurane and isoflurane anesthesia (Reitan et al. 1978, Flacke et al. 1983, Hirsch et al. 1993, Steagall et al. 2006). This is likely due to decreased cardiac output from the reductions in HR and contractility previously mentioned in conjunction with the vasodilatory effects of inhalant anesthetics. In conscious dogs, fentanyl results in a dose-dependent reduction in baroreflex-mediated increases in HR induced by carotid clamping (Freye & Arndt 1979). Thus, when combined with the vasodilation induced by inhalant anesthetics, compensatory increases in HR are impaired and blood pressure drops. However, changes in blood pressure are only partially attributable to reductions in HR, as atropine restored HR in enflurane anesthetized dogs without completely returning
blood pressure to baseline anesthetized values (Hirsch et al. 1993). Another contributing factor may be reductions in sympathetic outflow and vascular tone due to enhanced central depression, as demonstrated by decreased circulating catecholamines following fentanyl administration in enflurane-nitrous oxide anesthetized dogs (Flacke et al. 1983). Ultimately, the combination of inhalant anesthetics and fentanyl appear to result in synergistic reductions in blood pressure in the dog.

In contrast to the minimal respiratory depression caused by fentanyl administration in conscious dogs, those anesthetized with inhalant anesthetics experienced marked respiratory depression, and often apnea following fentanyl administration. This has been demonstrated in both experimental and clinical studies in dogs receiving various inhalant anesthetics. Hug and Murphy (1979) evaluated the ventilatory effects of 10 μg/kg of fentanyl administered IV over 30 seconds to dogs under enflurane anesthesia. Fentanyl resulted in a rapid onset of respiratory depression progressing to apnea in 1.5 minutes. Tidal volume, RR and minute volume then steadily increased toward baseline anesthetized values in 30 to 45 minutes, which paralleled a decline in fentanyl plasma levels. These results are in marked contrast to the effects in conscious dogs reported by Grimm et al. (2005), who demonstrated no change in PaO₂ and PaCO₂ following a 15 μg/kg IV fentanyl bolus.

The respiratory depression caused by concurrent fentanyl and inhalant administration is also present in clinical scenarios and with lower fentanyl doses. After IV premedication with 4 μg/kg of fentanyl and anesthetic induction with propofol, and during maintenance with isoflurane and a fentanyl infusion of 4 μg/kg/hr or greater during orthopedic surgery, dogs experienced periods of apnea ranging from 20 to 90
seconds and end-tidal CO\textsubscript{2} pressures greater than 60 mmHg despite surgical stimulation. Consequently, the majority of dogs were mechanically ventilated (Bufalari et al. 2007). The marked respiratory depression caused by fentanyl under inhalant anesthesia requires vigilant monitoring and the ability to institute positive pressure ventilation if necessary.

While fentanyl can induce significant bradycardia, reductions in blood pressure and hypoventilation, these effects can be readily addressed through anticholinergic administration and mechanical ventilation. Combined with its significant inhalant (MAC) sparing effect, cardiovascular function is further improved and its use as an adjunct in general anesthesia has been strongly advocated (Ilkiw et al. 1993, Steagall et al. 2006, Ueyama et al. 2009). These benefits have secured the use of fentanyl as a staple in modern balanced anesthetic techniques in healthy dogs as well as those with cardiovascular compromise (Williamson et al. 1991, Ilkiw et al. 1993).

2.2.b Inhalant anesthetics and dexmedetomidine

One of the most profound effects of IV dexmedetomidine administration is the significant increase in systemic vascular resistance caused by direct alpha-2 adrenergic receptor activation. The resulting increase in afterload is largely responsible for the initial bradycardia and reductions in cardiac output. Attenuating the initial phase of alpha-2 agonist mediated vasoconstriction with nifedipine, a calcium channel blocker, significantly improves cardiac performance (Bloor et al. 1992). Isoflurane, with vasodilatory properties, also antagonizes the increase in arterial blood pressure induced by alpha-1 and alpha-2 adrenergic receptor activation in dogs (Kenny et al. 1990). This
could, in theory, attenuate the reductions in HR and other adverse cardiovascular effects observed in conscious dogs given dexmedetomidine.

Decreases in the degree of bradycardia can be seen when comparing peak cardiovascular changes following a 1 μg/kg IV bolus of medetomidine in conscious versus isoflurane-anesthetized dogs in separate studies by Pypendop and Verstegen (1998) and Kaartinen et al. (2010), respectively. While the study animals and experimental methodology differ slightly, the expected differences are still illustrated. Specifically, dogs under anesthesia with isoflurane (1.3-1.4% end-tidal concentration) demonstrated lower MABP and higher HRs compared to conscious animals following medetomidine administration (Pypendop & Verstegen 1998, Kaartinen et al. 2010). These findings have been reproduced by studies evaluating the oral, IM and IV use of dexmedetomidine and medetomidine for pre-anesthetic sedation, which demonstrate higher HRs, and lower systemic vascular resistance and arterial blood pressures in dogs once under inhalant anesthesia with isoflurane, desflurane, or halothane/nitrous oxide compared to pre-anesthetic sedated values (Bergström 1988, Kersten et al. 1993, Gómez-Villamandos et al. 2006).

It is tempting to speculate that the reduction in afterload and increase in HR provided by isoflurane would improve cardiac output compared to administration of the alpha-2 agonist alone; however, this is not the case. The dose-dependent cardiovascular alterations caused by isoflurane extend beyond vasodilation and its effect on other parameters with concurrent dexmedetomidine administration, such as CI and contractility, are complex. Despite the discussed differences in HR and blood pressure reported in the Pypendop and Verstegen (1998) and Kaartinen (2010) studies, the
resulting CI was similar. This was also observed in a cross-over study evaluating the
effects of 30 μg/kg dexmedetomidine given to dogs orally 60 minutes before isoflurane
or desflurane anesthesia (Kersten et al. 1993). Delivery of isoflurane at end-tidal
concentrations of 1.29, 1.67 and 2.02% resulted in reductions in MABP and systemic
vascular resistance, as well as increases in HR; however stroke volume and dP/dt max were
significantly lower compared to dexmedetomidine alone resulting in no overall change in
cardiac output at 1.29 and 1.67%. Changes in cardiac output only became significant at
the highest concentration of 2.02% when it declined (Kersten et al. 1993). These effects
appear to be agent specific, as desflurane administration resulted in similar overall
changes, except for significant reductions in cardiac output at all inspired concentrations
compared to sedated values, which were attributable to the greater preservation of
systemic vascular resistance with this inhalant (Kersten et al. 1993).

Interpreting the combined effects of medetomidine or dexmedetomidine and
isoflurane becomes more complex when considering dose and changes over time. While
conscious dogs demonstrate an obvious non-linear dose-effect response reaching
maximal values at lower doses (Pypendop & Verstegen 1998), there appears to be a
greater degree of effect with different doses under isoflurane anesthesia, which may be
due to vasodilation unmasking differential degrees of vasoconstriction (Kuusela et al.
2001, Pascoe et al. 2006, Kaartinen et al. 2010). However, similar to conscious dogs,
higher doses have a more prolonged effect and the timing of isoflurane delivery in
relation to dexmedetomidine administration will ultimately result in variable opposing
Further influencing the cardiovascular effects of alpha-2 agonists under inhalant anesthesia is the ability of the drug to reduce inhalant requirements, and the cardiovascular alterations they produce. In the previously mentioned studies evaluating the combined cardiovascular effects of alpha-2 agonists and inhalant anesthetics, the volatile delivered was held constant at a concentration that what would likely result in a very deep plane of anesthesia (Kersten et al. 1993, Greene et al. 2003, Kaartinen et al. 2010). Despite this, even when reductions in MAC are accounted for, cardiovascular depression is still profound after single high doses are administered, as it is in conscious patients (Bloor et al. 1992). This has lead investigators to explore low dose CRIs in dogs in attempts to minimize cardiovascular changes while maintaining the benefits of MAC reduction, analgesia and the attenuated stress response that it provides when delivered intraoperatively. While hemodynamic stability is improved using this technique compared to large bolus dosing, elevations in blood pressure and systemic vascular resistance with accompanying reductions in HR and cardiac output are still observed in a dose-dependent fashion compared to isoflurane alone, even with surgical stimulation (Pascoe et al. 2006, Lin et al. 2008, Uilenreef et al. 2008). While higher infusion rates impart greater MAC reduction, CI is maintained within normal limits with an IV loading dose of 1 μg/kg followed by a CRI at 1 μg/kg/hr under isoflurane and this infusion rate has been recommended for clinical use in healthy patients (Kuusela et al. 2001, Haskins et al. 2005, Pascoe et al. 2006, Lin et al. 2008, Uilenreef et al. 2008).

The combined effect of alpha-2 adrenergic receptor agonists and isoflurane on respiratory drive has been evaluated directly and the findings are relatively consistent. Nguyen et al. (1992) performed a cross-over study to evaluate the combined respiratory
effects of dexmedetomidine and isoflurane. They observed a significant reduction in the hypercapnic response with 1.5% isoflurane alone, which was significantly enhanced by the addition of 3 μg/kg IV of dexmedetomidine. However, it is important to note that despite this reduction in response to inspired CO₂, PaCO₂ during spontaneous respiration only increased from 36.4 mmHg in isoflurane anesthetized dogs to 41.2 mmHg with the addition of dexmedetomidine. It was also demonstrated that when the MAC-sparing effect of 3 μg/kg dexmedetomidine was accounted for and isoflurane was reduced to 0.37%, the significant inhibition of hypoxic ventilatory drive from isoflurane alone was eliminated completely, presumably because dexmedetomidine does not influence this aspect of central respiratory control (Nguyen et al. 1992). Despite the central impairment of ventilatory responses to CO₂ with the combination of isoflurane and dexmedetomidine, spontaneous respiration with normal inspired gases does not result in clinical hypoventilation. This has also been demonstrated in studies evaluating clinically used medetomidine or dexmedetomidine infusions under isoflurane anesthesia, which demonstrate no significant changes in RR or elevations in PaCO₂ compared to isoflurane alone (Pascoe et al. 2006, Lin et al. 2008).

2.2.c Inhalant anesthetics and acepromazine

Acepromazine is commonly administered for sedation before general anesthesia and upon anesthetic recovery to treat emergence delirium or excitement. The combination of acepromazine administration and isoflurane anesthesia results in concurrent cardiovascular alterations. While healthy dogs are able to maintain MABP above 75 mmHg following the administration of acepromazine or clinical doses of
isoflurane when administered alone (Stepien et al. 1995, Mutoh et al. 1997), the hypotensive effect of these drugs becomes significant when they are administered concurrently (Boström et al. 2002, Boström et al. 2003, Boström et al. 2006). Boström et al. (2002, 2003, 2006) performed a number of experiments in dogs receiving 2% isoflurane following IM premedication with 0.1 mg/kg acepromazine 30 minutes before anesthetic induction with thiopental. This combination resulted in average MABPs of 54-65 mmHg in all studies, compared to an average MABP of 87 mmHg when acepromazine was omitted from the protocol. Blood pressure remained significantly lower in dogs that received acepromazine during the subsequent 120 minutes of anesthesia with isoflurane and increased 30 minutes after anesthetic recovery, but remained lower than those treated with saline (Boström et al. 2003).

Monteiro et al. (2007) also evaluated the cardiovascular effects of acepromazine under isoflurane anesthesia. In this study, dogs were sedated with 0.03 mg/kg acepromazine or given saline IV 15 minutes before anesthetic induction with propofol and subsequent maintenance under 1.8% isoflurane. Consistent with previous findings, acepromazine resulted in lower SABP and MABP compared to saline treated dogs; however, values were higher than those observed in Boström’s studies with average MABPs of 75 and 92 mmHg in acepromazine and saline treated dogs, respectively (Monteiro et al. 2007). The lesser effect observed in this study may be due to lower end-tidal isoflurane concentrations, a difference in induction agents, or the lower dose of acepromazine administered. While acepromazine decreases blood pressure when isoflurane is held constant, this does not reflect its clinical use where the concentration of anesthetic delivered would be reduced due to its MAC reducing effect. When the MAC
reduction provided by acepromazine was accounted for in dogs under halothane anesthesia, blood pressure was higher than with halothane alone (Boyd et al. 1991). It is possible that this is also the case in dogs under isoflurane anesthesia; however, isoflurane and halothane differ in their vascular effects preventing direct extrapolation of these findings (Boyd et al. 1991).

While blood pressure changes due to acepromazine appear to be independent of dose between 0.05 to 1.1 mg/kg in conscious dogs (Popovic et al. 1972, Coulter et al. 1981, Stepien et al. 1995, Alvaides et al. 2008), this may not be the case under inhalant anesthesia. Dogs induced with thiopental and maintained under halothane anesthesia demonstrated dose-dependent reductions in MABP from baseline anesthetized values when acepromazine was administered at 0.05, 0.125 and 0.25 mg/kg IV (Ludders et al. 1983). Similarly, the dose of phenylephrine needed to increase MABP 50% above baseline values increased with increasing doses of acepromazine suggesting dose-dependent alpha adrenergic antagonism (Ludders et al. 1983). While dose effects have not been evaluated with other volatile agents, it is very possible that the same trend is present under isoflurane anesthesia.

The primary concern with hypotension under anesthesia is the presumed reduction in organ perfusion and DO₂ ultimately resulting in organ dysfunction. This concept largely arises from the loss of autoregulation of blood flow to organs, such as the kidney, when MABP drops below 60-70 mmHg in conscious individuals (Kirchheim et al. 1987, Guyton & Hall 2000); however, this threshold has not yet been established under anesthesia. Interestingly, there were no differences in renal blood flow between saline and acepromazine treated dogs under isoflurane anesthesia despite MABPs of 87
and 66 mmHg, respectively (Boström et al. 2003). Furthermore, glomerular filtration rate and relative renal blood flow did not differ among pre-anesthetic measurements, those under acepromazine-isoflurane anesthesia, or 20 hours after recovery from anesthesia, with kidneys receiving 16-17% of cardiac output. Despite these findings, similar elevations in vasopressin, angiotensin II and aldosterone were reported after 120 minutes of anesthesia in both treatment groups indicating reductions in perfusion pressure (Boström et al. 2003). The authors of this study concluded that acepromazine might actually exert a protective effect on renal perfusion despite reductions in blood pressure.

The effect of isoflurane and acepromazine administration on other cardiovascular parameters was also studied by Monteiro et al. (2007). As expected, SABP and MABP were lower in acepromazine compared to saline treated dogs, with no significant differences observed in HR, CI, SI, SVRI or other central pressures measured 90 minutes after anesthetic induction. Despite these findings, $PvO_2$ was lower and oxygen extraction ratio (ER) was higher with acepromazine treatment suggesting that the slightly lower, although not statistically significant, values for CI did result in small reductions in global $DO_2$. Acepromazine administration also significantly impaired the cardiovascular response to dopamine infused IV at rates up to 15 μg/kg/min. Specifically, the dopamine-induced increases in SI, SVRI, CVP as well as SABP, DABP and MABP observed in control dogs were markedly blunted with acepromazine treatment. Despite this, CI was similar between groups due to compensatory increases in HR observed in acepromazine treated dogs suggesting that the baroreflex remains intact (Monteiro et al. 2007). Again, it must be emphasized that all experiments were performed on healthy, euvolemic dogs.
and the ability of compromised animals to compensate under anesthesia is likely further reduced.

While the cardiovascular effects of acepromazine appear to be amplified under isoflurane anesthesia, there is no additional respiratory depression observed with concurrent drug administration. No differences in PaCO$_2$, RR or tidal volume were found in isoflurane-anesthetized dogs treated with acepromazine compared to those undergoing the same anesthetic protocol without its administration (Boström et al. 2003, Monteiro et al. 2007).

2.2.6 Opioids and dexmedetomidine

Studies investigating the combined cardiovascular effects of medetomidine or dexmedetomidine and various opioids have produced variable and conflicting results, which appear to be dose-related. Many studies investigating high doses of medetomidine (between 20 and 60 μg/kg) administered IV or IM demonstrate the predominant cardiovascular effects of the alpha-2 adrenergic receptor agonist, most notably bradycardia, with no further alteration in HR or the majority of other measured cardiovascular variables when an opioid is co-administered. However, other studies report lower systemic blood pressures in dogs given medetomidine/opioid combinations compared to medetomidine alone. Ko et al. (2000) evaluated changes in HR and systemic blood pressure following IM administration of glycopyrrolate (10 μg/kg) and medetomidine (30 μg/kg) with or without 0.2 mg/kg of butorphanol to healthy dogs. While blood pressure remained elevated from baseline in both groups, SABP, DABP and MABP were significantly lower at various time points over 40 minutes in dogs also
receiving butorphanol, without any difference in HR (Ko et al. 2000). The administration of glycopyrrolate reduces opioid-induced decreases in HR and is an obvious confounding factor in this study; however, this trend was also demonstrated by Hellebrekers & Sap (1997). In their study, dogs premedicated with 60 μg/kg of medetomidine demonstrated declines in blood pressure without changes in HR following the IV administration of 2 μg/kg of fentanyl without the use of an anticholinergic (Hellebrekers & Sap 1997). Kuo & Keegan (2004) evaluated these changes more extensively using Swan-Ganz catheters in dogs treated with medetomidine (20 μg/kg), medetomidine (20 μg/kg) and hydromorphone (0.1 mg/kg) or medetomidine (20 μg/kg) and butorphanol (0.2 mg/kg) IV. They confirmed previous findings with lower MABPs in medetomidine/hydromorphone treated dogs compared to those receiving medetomidine alone, and also found no significant differences in HR. Furthermore, they demonstrated reductions in systemic vascular resistance with medetomidine/hydromorphone, with no differences in CI, SI, or central pressures among treatments (Kuo & Keegan 2004).

Studies examining the effects of lower-dose medetomidine or dexmedetomidine combined with fentanyl during inhalant anesthesia report different results. One study evaluated the effects of dexmedetomidine given as 0.1, 0.3, 1.0 or 3.0 μg/kg boluses IV in dogs under enflurane anesthesia while receiving a concurrent fentanyl infusion of 3 or 12 μg/kg/hr or saline as a control (Salmenperä et al. 1994). They found no additional changes in blood pressure, cardiac output or PAOP with dexmedetomidine administration; however, dose-interaction analysis revealed that fentanyl potentiated the bradycardia caused by dexmedetomidine (Salmenperä et al. 1994). There are a number of possible explanations for the discrepancy in the findings reported above. It is possible
that the previously studied high doses of medetomidine created such marked hemodynamic changes that any opioid-induced effects were masked, and the lower doses evaluated in the anesthetized dogs allowed their recognition. It also possible, as previously discussed, that the background of a volatile anesthetic may have augmented changes in HR due to fentanyl resulting in a significant effect.

Low dose medetomidine combined with fentanyl has also been evaluated in conscious dogs. A study by Grimm et al. (2005) comprehensively evaluated the effects of a 15 μg/kg IV fentanyl bolus given to dogs receiving a medetomidine infusion delivered at 1.5 μg/kg/hr. The data indicated a clear trend for further reductions in HR, CI, systemic blood pressure and \( \text{DO}_2 \) following the fentanyl bolus compared to values with the medetomidine infusion alone. While none of these changes reached statistical significance, they may be clinically significant as mean CI values decreased from an already low value of 0.1 L/min/kg with medetomidine to 0.07 L/min/kg following the fentanyl bolus. Additionally, statistically significant increases in pulmonary vascular resistance and mean pulmonary artery pressure (MPAP) were also observed with fentanyl administration (Grimm et al. 2005).

The physiologic mechanisms behind these cardiovascular changes have not been fully explored, but can be speculated. The enhanced of bradycardia seen with concurrent fentanyl and low dose medetomidine or dexmedetomidine administration may be due to greater parasympathetic outflow. It is well documented that the increase in blood pressure caused by alpha-2 adrenergic receptor activation causes bradycardia through the baroreflex and resulting increase in parasympathetic outflow (Sinclair 2003). Opioids also predominantly exert their effects through vagal stimulation and may enhance the
baroreflex response augmenting this bradycardia (Inoue et al. 1980). High doses of medetomidine cause greater increases in systemic vascular resistance and likely parasympathetic drive which may override any additional changes that would be observed from opioid administration, and could explain the difference seen in studies evaluating low versus higher doses of medetomidine and dexmedetomidine (Pypendop & Verstegen 1998).

The findings of decreases in blood pressure and increases in pulmonary vascular resistance are not discussed in the papers that report these findings, but may have an underlying respiratory etiology. As will be discussed below, hypercapnia and reductions in PaO₂ are frequently caused by the concurrent administration of opioids and alpha-2 agonists. While hypercapnia typically causes an increase in blood pressure through enhanced sympathetic outflow, it can have direct vasodilatory effects on various vascular beds, which may predominate in the presence of alpha-2 adrenergic receptor agonist induced sympatholysis ultimately reducing blood pressure (Somers et al. 1989, Nakahata et al. 2003). However, it has also been demonstrated that μ-opioid receptor agonists have direct vasodilatory effects in ex vivo experiments and may contribute to reductions in blood pressure by causing a degree of systemic vasodilation (Kaye et al. 2008). The increases in pulmonary vascular resistance and MPAP with concurrent decreases in left atrial pressure and cardiac output are also not explained but may be due to hypoxic pulmonary vasoconstriction. As will be discussed below, hypoxemia can develop from these drug combinations (Hellebrekers & Sap 1997) and may correspond to areas of hypoxic alveolar gas in dogs breathing room air, which may ultimately cause pulmonary vasoconstriction and increase pulmonary vascular resistance.
In contrast to the variable cardiovascular effects reported in the literature, opioid/alpha-2 agonist combinations reliably increase PaCO$_2$ and decrease PaO$_2$ values beyond that of either drug alone in dogs, with full μ-opioid receptor agonists causing the greatest amount of respiratory depression (England & Clarke 1989, Hellebrekers & Sap 1997, Ko et al. 2000, Kuo & Keegan 2004, Raekallio et al. 2009). These respiratory changes can be marked with PaO$_2$ dropping as low as 55 mmHg in an individual dog and mean CO$_2$ increasing over 53 mmHg (Hellebrekers & Sap 1997, Raekallio et al. 2009). It is important to recall that these respiratory changes are happening in conjunction with profound cardiovascular alterations causing physiologic stress that could contribute to morbidity in some patients.

### 2.2.e Opioids and acepromazine

Interactions between acepromazine and opioid analgesics have not been studied to the same extent as alpha-2 adrenergic receptor agonist combinations, and there are few studies evaluating the combined effects of fentanyl and acepromazine. Combined cardiovascular changes have been evaluated most extensively following the administration of buprenorphine at cumulative doses of 5, 10 and 100 μg/kg IV in dogs sedated with 0.1 mg/kg acepromazine IV (Stepien et al. 1995). This study showed marked stability in cardiovascular function with none of the buprenorphine doses causing further significant changes in cardiac output, stroke volume, MABP, or systemic vascular resistance. There was a trend of dose-dependent reductions in HR following buprenorphine administration, with mean HR declining from 97 beats per minute following acepromazine administration to 71 beats per minute at the highest dose of
buprenorphine, however these changes were not statistically significant (Stepien et al. 1995). Heart rate also declined from a non-sedated average of approximately 114 to 86 beats per minutes following the IM co-administration of 0.03 mg/kg acepromazine and 10 μg/kg buprenorphine in another study; however, statistics were not performed to determine the significance of these findings (Bell et al. 2011). Regardless, it appears that reductions in HR do occur, as would be expected from opioid administration, however the clinical significance of these changes is minimal. Measures of cardiac contractility, such as dP/dt_max, aortic acceleration and left ventricular fractional shortening remained unchanged in acepromazine treated dogs with the addition of 5 and 10 μg/kg of buprenorphine, with only small reductions in dP/dt_max occurring at a cumulative dose of 100 μg/kg (Stepien et al. 1995). This reported stability in cardiovascular function may not be reflective of equivalent doses of fentanyl, as buprenorphine is typically considered a partial μ-opioid agonist with mild cardiovascular effects that plateau, in contrast to fentanyl which is a potent full μ-agonist with demonstrated dose-dependent reductions in cardiovascular function in dogs (Cowan et al. 1977, Arndt et al. 1984). However, predicting cardiovascular outcomes based on receptor activity may be misleading.

Different opioids may interact with a variety of receptors resulting in variable degrees of agonism or antagonism; however, this variability does not necessarily reflect cardiovascular outcomes following concurrent acepromazine and opioid administration. Combining acepromazine (0.05 mg/kg) with either the partial μ agonist, buprenorphine (10 μg/kg), or the full μ agonist, oxymorphone (0.1 mg/kg), IV resulted in very similar decreases in HR and blood pressure over a 90 minute test period despite differences in receptor activity (Jacobson et al. 1994). Similarly, co-administration of morphine (0.5
mg/kg), butorphanol (0.15 mg/kg), or tramadol (2.0 mg/kg) and acepromazine (0.05 mg/kg) IV in dogs resulted in a similar degree of bradycardia, despite marked differences in receptor activities, while methadone (0.5 mg/kg) did not cause any cardiovascular alterations (Monteiro et al. 2009). Furthermore, no significant differences in HR or blood pressure were found with the administration of IV acepromazine (0.22 mg/kg) with either butorphanol (0.22 mg/kg) or oxymorphone (0.22 mg/kg), although average HR tended to be higher in oxymorphine treated dogs (Cornick & Hartsfield 1992).

Studies evaluating the combined effects of opioid agonists with acepromazine administration are limited in the cardiovascular parameters evaluated, often reporting only blood pressure and HR. Despite this, the results seem to suggest mildly synergistic reductions in HR, while reductions in blood pressure are similar to those caused by acepromazine alone (Jacobson et al. 1994, Stepien et al. 1995, Monteiro et al. 2008, Monteiro et al. 2009, Gomes et al. 2011). The trend of lower HRs with an acepromazine/opioid combination compared to either drug alone was demonstrated by Monteiro et al. (2008) who evaluated the cardiovascular effects of methadone (0.5 mg/kg), acepromazine (0.1 mg/kg) or the combination of methadone (0.5 mg/kg) and acepromazine (0.05 mg/kg) IM in dogs. These changes were not statistically significant, but potentially clinically significant trends were present with average HRs of 66 beats per minute with acepromazine and methadone combined, compared to an average of 81 and 89 beats per minute with methadone and acepromazine administered alone, respectively (Monteiro et al. 2008). Most studies evaluating the combination of acepromazine with opioids, such as morphine, butorphanol, tramadol, oxymorphone or buprenorphine, have demonstrated significant reductions in HR beyond that of either acepromazine or the
opioid administered alone (Cornick & Hartsfield 1992, Jacobson et al. 1994, Montiero et al. 2009); however, it has also been demonstrated that reductions in HR following hydromorphone or oxymorphone administration were no different with or without concurrent acepromazine administration (Smith et al. 2001).

Another study evaluated the effects of IV morphine (0.5 mg/kg) or fentanyl (5 μg/kg) following acepromazine (0.05 mg/kg) administration and demonstrated further reductions in HR and SABP compared to acepromazine alone, with fentanyl causing greater changes (Gomes et al. 2011). An interesting finding in this study was the greater decrease in HR, but not blood pressure, demonstrated when combining the same dose of morphine (0.5 mg/kg) with a higher dose of acepromazine (0.1 mg/kg) despite the similar values following lower or higher dose acepromazine alone. This suggests that acepromazine may result in dose-dependent potentiation of the opioid-induced reductions in HR. It is important to note that none of the changes reported in this study were statistically significant, which may reflect an underpowered study, as opposed to a lack of physiologic differences, although observed differences were small (Gomes et al. 2011).

Changes in blood pressure with opioid/acepromazine combinations appear to reflect the effect of acepromazine alone. While there are variable reports regarding blood pressure changes following acepromazine or opioid administration, it depends on the order of drug administration. Studies demonstrate decreases in blood pressure if the opioid was administered first and used for future comparisons, but not if acepromazine was administered first (Jacobson et al. 1994, Stepien et al. 1995, Montiero et al. 2009, Gomes et al. 2011). Furthermore, when the effects of both acepromazine and methadone
were evaluated separately, as well as together, blood pressure was not significantly
different between dogs sedated with acepromazine and those with the
acepromazine/methadone combination (Monteiro et al. 2008). While blood pressure
consistently decreases following the concurrent administration of acepromazine and
opioids, it has been demonstrated that overall blood flow, as measured in the canine
femoral artery, does not change significantly following acepromazine and buprenorphine
co-administration suggesting that perfusion may still be adequate, although this study
does not address regional differences in flow (Nogueira et al. 2012).

The minimal respiratory compromise caused by clinical doses of acepromazine or
opioid analgesics administered separately does not appear to change significantly when
given concurrently. The respiratory effects have largely been reported as changes in RR
with combinations either causing increases or decreases, likely reflecting the prevalence
of opioid-induced panting (Cornick & Hartsfield 1992, Jacobson et al. 1994, Stepien et
al. 1995, Monteiro et al. 2008). When opioid administration does result in an increased
RR, reduced tidal volume and an overall increase in minute volume due to panting,
acepromazine appears to attenuate this effect (Jacobson et al. 1994, Monteiro et al.
2008). The resulting impact on arterial blood gases is generally minimal with mild
elevations in PaCO₂ and reductions in PaO₂ reported. One study reported reductions in
PaO₂ as low as 67 and 69 mmHg following intravenous acepromazine (0.05 mg/kg) with
oxymorphone (0.1 mg/kg) or buprenorphine (10 μg/kg), respectively; however, the
 corresponding baseline values in conscious dogs were 76 and 80 mmHg, which are
already low for the corresponding altitude where the study was completed (Jacobson et
al. 1994). These values are atypical in the literature as other studies report values within
the normal range with changes that do not always achieve statistical significance, and if they do, are not clinically significant (Cornick & Hartsfield 1992, Stepien et al. 1995).

2.3 Fentanyl Pharmacokinetics

2.3.a General Pharmacokinetics

Fentanyl [N-(1-phenethyl-4-piperidyl)propionanilide] is a synthetic opioid whose physiochemical properties give it a unique pharmacokinetic profile among the opioid analgesics (Egan et al. 1993). Fentanyl is one of the most highly lipophilic opioids commonly used in veterinary practice, exceeded only by sufentanil (Björkman et al. 1990, Stoelting & Hillier 2006). The lipophilic nature of fentanyl, molecular size, dissociation constant, and protein binding ultimately result in a rapid onset of effect and a shorter duration of action compared to traditional opiates, favouring its use as an infusion over bolus administration in a clinical setting (Aguado et al. 2011). These properties also affect all aspects of its pharmacokinetics – absorption, distribution, metabolism and elimination.

i. Absorption

The lipophilic nature, small molecular size, and high potency of fentanyl facilitate drug absorption and the attainment of therapeutic plasma concentrations following administration by multiple routes. Many routes of administration have been investigated, however much of the recent focus has been on transdermal and transmucosal delivery. Transdermal absorption from commercially available patches has been investigated widely in multiple species (Kyles et al. 1996, Lee et al. 2000, Maxwell et al. 2003, Ahern
et al. 2010). Slow-release transdermal administration attempts to create more prolonged and stable plasma concentrations, similar to an IV infusion, with the benefit of being non-invasive, convenient and feasible for outpatient analgesia. While these advantages make this method of delivery attractive, the time to attain peak plasma concentrations ($T_{\text{max}}$) is prolonged, the dose is not readily titratable, and plasma concentrations are highly variable and may even be subtherapeutic (Lee et al. 2000).

Transmucosal delivery has been investigated in humans and rabbits demonstrating bioavailability of 52 to 82%, with greater absorption with the free fentanyl base compared to the citrate salt (Streisand et al. 1991, Malkawi et al. 2008). As a result of this favourable absorption, transmucosal fentanyl is used clinically in human patients for analgesia (Farrar et al. 1998); however, because of limitations similar to transdermal administration, as well as the potential for first pass enteric and hepatic metabolism if swallowed, IV infusions still represent the best method of administration during the perioperative period.

**ii. Distribution**

The lipophilicity of fentanyl not only enhances absorption, but also facilitates significant distribution throughout the body despite significant protein binding. Fentanyl has the greatest volume of distribution ($V_d$) when compared to morphine, meperidine, and the fentanyl derivatives (Stoelting & Hillier 2006). The apparent $V_d$ appears to be independent of dose between 6.4 to 640 μg/kg, with reported values between 4.9 and 11.4 L/kg in dogs based on radiolabeled chromatography as well as high-performance liquid chromatography-mass spectrometry (HPLC-MS) (Murphy et al. 1979, Murphy et
al. 1983, Sano et al. 2006). This large $V_d$ is associated with extensive and rapid tissue
distribution with 98% of fentanyl removed from plasma within 5 minutes of
administration (Murphy et al. 1979). This has important implications for distribution to
the primary target site, the central nervous system, and thus the onset of drug effect.

Following an IV fentanyl bolus of 10 μg/kg in dogs, plasma concentrations
dropped rapidly (Hug & Murphy 1979). This corresponded with a rapid increase in
cerebral spinal fluid (CSF) concentrations, which reached near maximal concentrations
in 3 minutes and had equilibrated with plasma concentrations within 20 minutes (Hug &
Murphy 1979). These findings correlate well with peak fentanyl concentrations present in
brain tissue at 10 to 15 minutes following a 12.5 μg/kg IV bolus in normocapeic dogs
(Ainslie et al. 1979). Following the initial distribution phase, the rate of decline in CSF
concentrations paralleled those in plasma; however, CSF concentrations were
approximately half of plasma concentrations likely due to plasma protein binding (Hug
& Murphy 1979). In contrast to these CSF findings, concentrations in brain tissue were
higher than those in serum, which can be explained by the brain/blood partition
coefficient of 4 for fentanyl favouring distribution to lipids in brain tissue as opposed to
CSF (Ainslie et al. 1979, Björkman et al. 1990).

Plasma PaCO$_2$ has been shown to influence the distribution of fentanyl to the
brain, which has particular importance in the face of respiratory depression and
subsequent hypercapnia in anesthetized dogs receiving fentanyl. Ainslie et al. (1979)
reported that fentanyl concentrations in brain tissue peaked earlier, but were lower in
hypercapneic dogs (PaCO$_2$ of 65 mmHg) compared to hypocapneic dogs (PaCO$_2$ of 19
mmHg). Higher PaCO$_2$ likely resulted in enhanced sympathetic tone, increased cardiac
output, cerebral vasodilation and more rapid drug distribution causing the earlier rise in brain concentrations. The lower peak brain concentrations in hypercapneic compared to hypocapneic dogs may be explained by the fairly alkaline pKa value of 8.4 for fentanyl (Stoelting & Hillier 2006), favouring the less permeable ionized form of the drug at a lower plasma pH and decreasing penetration into the brain (Ainslie et al. 1979).

While the rapid distribution to the central nervous system contributes to fentanyl’s rapid onset of effect, rapid redistribution away from target tissues is responsible for its short duration of action (Wegner et al. 2008). This is important to consider when interpreting pharmacokinetics studies, as terminal half-life ($t_{1/2\beta}$) does not reflect therapeutic effect for fentanyl and is variable depending on the duration of the fentanyl infusion as will be discussed.

**iii. Metabolism**

Fentanyl undergoes extensive biotransformation in all species studied, however the metabolic pathways of biotransformation may differ yielding different metabolites (Labroo et al. 1997, Björkman & Redke 2000, Thomasy et al. 2007). In humans, fentanyl predominantly undergoes N-dealkylation of the piperidine moiety producing the metabolite, norfentanyl (Labroo et al. 1997). Norfentanyl appears to have mild agonist activity in *in vitro* assays, but is largely considered an inactive metabolite (Schneider & Brune 1986, Fassoulaki et al. 2010). Other minor pathways in humans include amide hydrolysis and alkyl hydroxylation producing the metabolites despropionylfentanyl and hydroxyfentanyl, respectively (Labroo et al. 1997). While norfentanyl is also extensively produced in the rat, the cytochrome P450 enzymes responsible for its production differ.
Human microsomal assays have identified CYP3A4 as the primary isoform responsible for fentanyl metabolism and norfentanyl production, while the corresponding isoforms in rats sharing significant homology are CYP3A1 and CYP3A2 (Gonzalez et al. 1988, Labroo et al. 1996, Feierman 1996, Feierman & Lasker 1996).

Fentanyl metabolic pathways have not been well explored in the dog. The CYP3A12 enzyme appears to serve the same functional role as the human CYP3A4 isoform in general drug metabolism (Lu et al. 2005), suggesting a potential role for this isoform in canine fentanyl oxidation. However, prior administration of ketoconazole, a CYP3A12 inhibitor, did not influence fentanyl pharmacokinetics in dogs. This may either reflect the robust nature of high extraction ratio drugs, such as fentanyl, or suggest that this isoform does not play a role in canine fentanyl metabolism (KuKanich & Hubin 2010). There are a couple of studies evaluating canine fentanyl metabolites, however they report conflicting results. A single study reported the absence of norfentanyl and despropionylfentanyl in canine plasma by gas chromatography-mass spectrometry following up to 100 μg/kg of fentanyl with sampling times up to 180 minutes (Lin et al. 1981). A later study also used gas chromatography-mass spectrometry to evaluate the urinary excretion of fentanyl metabolites in greyhounds and identified norfentanyl as the primary metabolite, with 4-hydroxyfentanyl and 4-hydroxy-3-methoxyfentanyl as minor metabolites (Russo et al. 2002). These positive findings, together with the known metabolic pathways in other species suggest that norfentanyl is likely the primary metabolite in the dog. This is also supported by indirect evidence. It has been demonstrated that the metabolic pathways for sufentanil biotransformation are very similar among humans, rats and dogs (Lavrijsen et al. 1990). Furthermore, the same
enzyme performs oxidative N-dealkylation in all piperidine opioids, including sufentanil and fentanyl, suggesting that the fentanyl dealkylation and norfentanyl production in rats and humans is also likely present in dogs (Tateishi et al. 1996).

Although the specific metabolic pathways of fentanyl metabolism remain to be completely characterized in the dog, it has been demonstrated that clearance closely matches hepatic blood flow, with an extraction ratio of approximately 1.0, similar to other species (Björkman & Redke 2000). With normal hepatic function, high extraction ratio drugs are more dependent on hepatic blood flow than intrinsic enzyme activity for metabolism and elimination; however, intrinsic enzyme activity becomes more important in pathological conditions such as cirrhosis (Callaghan et al. 1993).

iv. Elimination

The rapid decline in fentanyl plasma concentrations following an IV bolus is largely due to distribution from blood into other tissues. However, it is clear that the significant hepatic metabolism of fentanyl also contributes to these rapid declines as metabolites appear in canine plasma within 2 minutes of administration and peak in 60 minutes (Murphy et al. 1979). The initial steep decline in fentanyl plasma concentrations is followed by a more gradual decrease as there is slow reuptake of drug into the blood from the tissue reservoir with ongoing drug elimination. The lipophilic nature and large $V_d$ of fentanyl result in a large tissue drug reservoir, and a long $t_{1/2\beta}$ relative to other opioids. The reported $t_{1/2\beta}$ of intravenous fentanyl has been reported to be 0.75, 2.7, 3.3 and 6.0 hours in conscious dogs (Kyles et al. 1996, Sano et al. 2006, Little et al. 2008, KuKanich & Hubin 2010). While doses used to determine these values ranged from 10-
50 μg/kg, the variation in $t_{1/2β}$ is more likely due to the use of different dog breeds, detection techniques, and assay detection limits as $t_{1/2β}$ has been shown to be independent of dose from 6.4-640 μg/kg in this species (Murphy et al. 1983). These values are in general agreement with those reported in other species with $t_{1/2β}$ of 1 and 2.2 hours in horses (Maxwell et al. 2003, Thomasy et al. 2007), 3.08 hours in sheep (Ahern et al. 2010), and 4.4 hours in humans (Bentley et al. 1982).

Despite reported values for $t_{1/2β}$ up to 6 hours in dogs, it is important to note that the termination of drug effects occur due to redistribution and duration of analgesia does not correlate with $t_{1/2β}$. For example, morphine has a shorter $t_{1/2β}$ (0.87 hours) than that of fentanyl, but a longer duration of analgesic effect in dogs (Garrett & Jackson 1979, Wegner et al. 2008). The clinical importance of fentanyl’s long $t_{1/2β}$ was demonstrated in a canine study by Hug and Murphy (1979). In their study, three successive 10 μg/kg IV boluses of fentanyl were administered to dogs at 90-minute intervals after the clinical effects of fentanyl had waned, resulting in progressively higher plasma concentrations with each dose. This drug accumulation with repeated dosing should theoretically translate into prolonged context-sensitive half-times when fentanyl is administered as an infusion. This concept is supported by simulated pharmacokinetic modeling based on reported human pharmacokinetic parameters which demonstrated marked increases in context-sensitive half-time with fentanyl infusions exceeding 2 hours (Hughes et al. 1992). This has not been demonstrated in dogs, as there were no statistical differences in $t_{1/2β}$ when dogs received a 10 μg/kg fentanyl bolus followed by an infusion of 10 μg/kg/hr for 1, 3 or 4 hours (Sano et al. 2006). However, total body clearance was lower
with 3 and 4 hour infusions compared to the 1 hour infusion and was suspected to be the result of decreased hepatic blood flow (Sano et al. 2006).

Fentanyl’s high lipid solubility and extensive hepatic metabolism result in very little excretion of the parent molecule making hepatic metabolism the rate limiting step in drug elimination. In dogs, only 4% of the fentanyl is excreted unchanged in the urine within 6 hours of administration independent of dose from 2.5 to 640 μg/kg (Murphy et al. 1983). Human studies report similar results with less than 8% of the administered dose excreted as the parent molecule in the urine and feces over 72 hours (McClain & Hug 1980). The majority of the urinary excretion of both metabolites and unchanged fentanyl occurs between 8 and 24 hours, although radioactivity from fentanyl or its metabolites has been detected in the urine of a human volunteer 6 days following intravenous injection of 500 μg (Hess et al. 1972). These findings highlight the more prolonged elimination period of fentanyl’s metabolites, which are thought to have minimal activity and are of little clinical importance (Fassoulaki et al. 2010).

### 2.3. Alterations in intravenous fentanyl pharmacokinetics by other agents

Drug interactions may be pharmaceutical, pharmacodynamic or pharmacokinetic. Pharmaceutical interactions result from exposure to physically or chemically incompatible agents often before the drugs are administered and may render the drug inactive. Pharmacodynamic interactions occur on a cellular or physiologic level resulting in additive, synergistic or antagonistic adverse or desired clinical effects. This is the principle behind balanced anesthesia, where lower doses of multiple drugs can be used to achieve a desired response through actions on complimentary pathways (Hellyer et al.
Pharmacokinetic interactions can also significantly influence therapeutic effect by influencing the pharmacokinetic profile of a given drug at any stage of absorption, distribution, metabolism or elimination (Beijnen & Schellens 2004). The IV administration of fentanyl eliminates interference with drug absorption. Additionally, because fentanyl metabolites are inactive, elimination has less pharmacologic significance, making distribution and metabolism the most significant areas of pharmacokinetic drug interactions.

**i. Distribution**

Drug distribution depends not only on the physiochemical characteristics of the drug, such as lipid solubility and protein binding, but also physiologic factors, such as body composition, tissue pH, regional blood flow, and cellular mechanisms, such as the P-glycoprotein transporter (Vesell 1974). Thus, the administration of agents that alter plasma protein binding, transport protein function, and distribution of blood flow all have the potential to influence the pharmacokinetics of another concurrently administered drug.

Displacement from plasma proteins and subsequent increases in circulating free drug could theoretically result in a dramatic increase in drug effect. This concept has been heavily indoctrinated in the study of pharmacology; however, there is little evidence supporting this concept (McElnay & D’Arcy 1983). There may be mild, transient changes in free drug with rapid IV administration of another highly protein bound drug; however, rapid clearance and redistribution will minimize concentrations of free drug and the physiologic impact is ultimately minimal (Rolan 1994). Fentanyl is
approximately 80% protein bound and theoretically susceptible to changes from other highly protein bound drugs such as acepromazine and dexmedetomidine, which are both over 90% bound (Bower 1981, Ballard et al. 1982, Karol & Maze 2000). However in vitro evidence demonstrates that dexmedetomidine does not result in the displacement of a variety of highly protein bound agents from plasma proteins, and that fentanyl protein binding is minimally influenced by many anionic, cationic and uncharged agents (Bower 1981, Karol & Maze 2000). This in vitro data coupled with current opinion, suggests that drug interactions cause minimal displacement from plasma proteins and are clinically insignificant for fentanyl.

P-glycoprotein transport pumps are situated on cell membranes, where they expel their substrates preventing or limiting the movement of compounds to specific sites. They are most notably located on hepatocytes, intestinal epithelium and endothelium of the blood-brain barrier, where they influence the oral bioavailability of drugs as well as their distribution into the central nervous system (Brüggemann et al. 2009). Altered function of these transport pumps from genetic mutations or the presence of inhibitors can prevent the efflux of substrates resulting in dramatic increases in CNS concentrations and central effects that would otherwise be absent (Schinkel et al. 1996, Sadeque et al. 2000). While dexmedetomidine does not appear to interfere with p-glycoprotein activity, phenothiazines have been shown to be effective pump inhibitors (Ramu & Ramu 1992, MacKay et al. 2012). Opioids such as loperamide, methadone, and morphine are substrates for the P-glycoprotein transporter, susceptible to changes in central distribution from modified pump activity; however, fentanyl is not, and instead acts as a moderate P-glycoprotein pump inhibitor itself (Wandel et al. 2002, Armstrong et al.
Thus, drugs causing P-glycoprotein inhibition would not alter the pharmacokinetic profile of fentanyl for this reason alone.

Distribution of blood flow is one of the most significant factors dictating drug distribution within the body. In normal physiologic states, the greatest amount of blood flow is directed to the vessel rich group of tissues including the brain, heart, kidney and splanchnic organs, causing drug concentrations in these tissues to rapidly equilibrate with that of plasma. Drug concentrations in moderately perfused tissues, such as muscle, then increase followed by those in the vessel poor group of tissues, such as fat (Roberts & Freshwater-Turner 2007). Considering this, it is clear how drugs that affect total and regional blood flow will influence the distribution of other drugs.

Centralization of blood flow limits drug exposure to peripheral tissues, which can significantly influence the pharmacokinetic disposition of other concurrently administered drugs. This has been demonstrated in a swine hemorrhagic shock model where the $V_d$ of fentanyl was significantly reduced compared to control subjects resulting in higher plasma concentrations (Egan et al. 1999). As previously outlined, dexmedetomidine has profound cardiovascular effects with blood flow preferentially redistributed to the vessel rich group of tissues (Lawrence et al. 1996, Talke et al. 2000). The administration of clonidine, another alpha-2-adrenergic receptor agonist, resulted in higher alfentanil plasma concentrations than control patients suggesting that $V_d$ was also decreased, although a reduction in clearance cannot be excluded (Segal et al. 1991). A study in healthy human patients provides more direct evidence for alpha-2 adrenergic receptor agonist induced alterations in distribution, as dexmedetomidine significantly reduced the movement of thiopental out of the central compartment. This ultimately
resulted in a 40% lower $V_d$ of thiopental at steady state compared to control patients, and shortened the $t_{1/2\beta}$, as there was no difference in thiopental clearance between groups (Bührer et al. 1994). Since thiopental and fentanyl are both highly lipid soluble, this study is useful for extrapolating changes in fentanyl distribution that may occur with dexmedetomidine administration. However, it does not accurately reflect the influence on drug clearance from alterations in hepatic blood flow as thiopental has a low hepatic extraction ratio and would be less influenced by hepatic perfusion than a high extraction ratio drug, such as fentanyl (Ghonheim & Van Hamme 1978). While this has not been evaluated in the published literature, it has been demonstrated that the administration of atipamezole, and alpha-adrenergic receptor antagonist, increased medetomidine clearance in the dog likely due to enhanced hepatic perfusion (Salonen et al. 1995). This suggests, albeit indirectly, that medetomidine administration reduces hepatic blood flow and may be capable of reducing the clearance of high extraction ratio drugs, such as fentanyl. However, other studies indicate that changes in hepatic blood flow are minimal with alpha-2 agonist administration, potentially due to differences in receptor expression between the systemic and hepatic vasculature (Marteau et al. 1988, Lawrence et al. 1996, Talke et al. 2000).

In contrast to dexmedetomidine, acepromazine and isoflurane result in vasodilation and may be expected to produce opposite pharmacokinetic alterations with greater drug delivery to the periphery; however, this is not supported in the literature. Accompanying the dose-dependent vasodilation and depression in cardiac output induced by isoflurane is a redistribution of blood flow. While the vasodilatory effects do result in an increase in arteriovenous shunting, a greater proportion of cardiac output is directed to
the vessel rich group of tissues; however, due to decreases in cardiac output, total blood flow to various organs is variable and may slightly increase or decrease. This, by necessity, causes a marked decrease in flow to skeletal muscle, skin and fat (Lundeen et al. 1983, Avram et al. 2000).

Although there are no studies evaluating changes in drug disposition due to acepromazine, pharmacokinetic changes resulting from isoflurane administration have been more closely examined. A number of studies have evaluated the pharmacokinetics of fentanyl, alfentanil, lidocaine and thiopental during isoflurane anesthesia in comparison to conscious controls in a number of species (Büch et al. 1991, Thomasy et al. 2005, Feary et al. 2005, Thomasy et al. 2007, Pypendop et al. 2008). These studies consistently demonstrate a reduction in the $V_d$, particularly of the central compartment, as well as decreases in clearance and increases in peak plasma concentrations of the injectable drug during isoflurane anesthesia. The opposing influences of a decreased $V_d$ and clearance have a variable influence on $t_{1/2\beta}$, which is often no different than conscious controls or may even be shorter. These changes were attributed to a decrease in peripheral perfusion and reductions in hepatic perfusion reducing clearance, as both lidocaine and fentanyl have high extraction ratios. Additionally, in anesthetized cats receiving lidocaine, MEGX, one of the metabolites produced through hepatic biotransformation, was lower compared to the conscious state, further supporting the speculation of reduced hepatic blood flow (Thomasy et al. 2005). In contrast, isoflurane anesthetized horses receiving fentanyl showed greater concentrations of PMA, the primary hepatic metabolite of fentanyl in horses, suggesting that any possible changes in
production were masked by the smaller volume of distribution and delayed clearance of this metabolite (Thomasy et al. 2007).

**ii. Metabolism**

Cytochrome P450 enzymes are responsible for the majority of oxidative reactions that occur during drug biotransformation. Although there are over 60 different genes for these enzymes in humans, only 6 isoenzymes are responsible for performing most reactions. Furthermore, 50% of P450 reactions are performed by a single isoenzyme, CYP3A4 (Leucuta & Vlase 2006). The heavy reliance on these pathways results in a high likelihood of metabolic drug interaction if one of the agents causes enzyme induction or inhibition (Leucuta & Vlase 2006). While these interactions can be significant for drugs with low hepatic extraction ratios, the high extraction ratio of fentanyl indicates that it undergoes hepatic flow-dependent clearance, which is traditionally thought to remain robust despite moderate changes in microsomal activity (Callaghan et al. 1993, KuKanich & Hubin 2009). These concepts are generally valid, but not absolute, as pretreatment with troleandomycin, a CYP3A inhibitor, significantly prolonged fentanyl clearance and increased area under the plasma concentration-time curve in a human pharmacokinetic study (Ibrahim et al. 2003). *In vitro* studies have also demonstrated reductions in fentanyl metabolism in the presence of midazolam, ketoconazole, and erythromycin, indicating that multiple agents can interfere with metabolism on a microsomal level (Feierman et al. 1996, Labroo et al. 1997). The metabolic pathways of isoflurane, acepromazine and dexmedetomidine, the potential for enzyme competition, and their induction or inhibition of cytochrome P450 enzymes have
been studied; however, their influence on fentanyl pharmacokinetics in vivo has not been examined as closely in the literature.

Biotransformation of phenothiazine drugs involves a number of oxidative processes performed by cytochrome 450 enzymes, including S-oxidation of the thiazine ring and N-demethylation of a side chain among others (Daniel et al. 2002). The isoforms involved in these pathways are complex, and studies have demonstrated significant variation in the predominant isoforms responsible depending on the specific drug and the species examined (Murray 1992, Daniel et al. 2002, Wójcikowski et al. 2003, Wójcikowski et al. 2010). Although acepromazine metabolism has not been evaluated specifically, the major P450 isoforms responsible for fentanyl metabolism in the rat are not involved in the metabolism of other phenothiazines in this species making competitive inhibition with fentanyl unlikely (Feierman 1996, Daniel et al. 2002). Furthermore, the administration of high dose promazine or chlorpromazine to rats on 3 consecutive days did not influence the function of constitutive P450 enzymes (Murray 1992).

Isoflurane is a stable molecule primarily eliminated through expiration, and undergoes significantly less metabolism than all of the other preceding volatile inhalants (Kharasch et al. 1993). Only 0.2-1.0% of the compound undergoes hepatic biotransformation, specifically oxidation at the alpha carbon site (Davidkova et al. 1988). This oxidation is predominantly the result of cytochrome P450 activity, with the metabolizing isoform consistently demonstrated as CYP2E1 (Kharasch et al. 1993, Kharasch et al. 1999). While the metabolic pathways of isoflurane would not compete with that of fentanyl, its ability to induce or inhibit relevant P450 enzymes is less certain.
In rats, concurrent isoflurane and trifluoroethene (TFE) administration enhances the cytochrome P450 inactivation resulting from TFE administration; however, isoflurane alone does not appear to have a significant effect (Baker et al. 1992). When human and murine hepatic tissue was studied, isoflurane was shown to enhance the metabolism of other halogenated agents by increasing the activity of CYP2B1, CYP2B6 and CYP2C6 (Baker et al. 1995, Ronnenberg et al. 1995). It was further reported that gene expression for a number of drug metabolizing enzymes, including CYP7A1 and CYP2B15, was upregulated following 6 hours of isoflurane anesthesia in rats (Nakazato et al. 2009). Despite these findings, there is no evidence to suggest that isoflurane affects the specific isoform responsible for fentanyl metabolism. Additionally, another study demonstrated no significant difference in CYP2E1 activity in rats exposed to isoflurane compared to those exposed to CO₂ (Plate et al. 2005). Even chronic exposure of mice to 0.5% isoflurane over 9 weeks did not result in any changes in cytochrome b₅ or P450 concentration or rates of metabolism of other halogenated ether volatiles (Rice et al. 1986). Currently there is no conclusive evidence that isoflurane alters fentanyl pharmacokinetics due to microsomal changes, and any changes that do occur may be predominantly from changes in hepatic blood flow and drug distribution.

Medetomidine and its enantiomers not only cause significant changes in blood flow, but they also undergo significant hepatic metabolism increasing the potential for pharmacokinetic interactions. The cytochrome enzymes responsible for medetomidine biotransformation have recently been studied in canine hepatic microsomal preparations, highlighting the primary role of CYP3A enzymes in its metabolism (Duhamel et al. 2010). Furthermore, medetomidine has been reported to have a very high affinity for the
P450 enzymes suggesting the potential to rapidly saturate these pathways (Duhamel et al. 2010). This is of particular significance as CYP3A enzymes are postulated to metabolize fentanyl in the dog, and saturation of this pathway could reduce fentanyl metabolism strictly through competitive binding. However, the specific isoforms (polypeptides) responsible for canine fentanyl metabolism remain uncertain making it difficult to draw conclusions from these findings. Furthermore, fentanyl pharmacokinetics are independent of dose between 6.4 to 640 μg/kg IV suggesting fentanyl metabolic pathways are not readily saturable (Murphy et al. 1983).

In addition to direct competition for metabolic enzymes, dexmedetomidine may result in P450 enzyme inhibition, which could also affect fentanyl metabolism. Dexmedetomidine belongs to a class of drugs called ‘azoles’, some members of which are well-recognized P450 enzyme inhibitors. The ability of antifungal agents in this class to interfere with the metabolism and pharmacokinetics of low-to-medium extraction ratio drugs is well demonstrated in both in vitro and in vivo studies (von Moltke et al. 1996, Hynninen et al. 2009, Tapaninen et al. 2011). Even IV fentanyl has significantly prolonged clearance and increased area under the plasma concentration-time curve following voriconazole and fluconazole pretreatment in humans, despite its high extraction ratio (Saari et al. 2008). This is an important finding as it demonstrates that enzyme inhibition from azoles has the ability to influence fentanyl pharmacokinetics. The alteration is significant, as there are human case reports of enhanced respiratory depression and central opioid effects when patients receiving fentanyl were subsequently administered antimycotic agents, ultimately resulting in morbidity and mortality (Mercadante et al. 2002, Hallberg et al. 2006). While antifungal agents have an
established influence on the metabolism of many coadministered agents, the effect of alpha-2 adrenergic receptor agonists on enzyme inhibition is not as well defined to date.

_In vitro_ studies have demonstrated that the inhibition of P450 enzymes by dexmedetomidine is as potent as many of the antimycotic agents (Kharash et al. 1991). Both dextro and levo isomers of medetomidine inhibit the metabolism of many compounds in rodent and human microsomal preparations, including testosterone, ketamine and alfentanil (Kharash et al. 1991, Pelkonen et al. 1991, Kharash et al. 1992). Furthermore, preincubation does not enhance enzyme inhibition suggesting that maximal inhibition results from the parent molecule and can occur upon initial administration of the drug (Kharash et al. 1992). This competitive inhibition is due to a direct interaction between the dexmedetomidine molecule and the heme atom of the enzyme, suggesting that inhibition is not isozyme-selective (Kharash et al. 1992, Rodrigues & Roberts 1997). However, a number of studies demonstrate variable inhibition of both constitutive and induced P450 isofrom pathways following incubation with dexmedetomidine (Kharash et al. 1992, Pelkonen et al. 1991, Baratta et al. 2010).

While _in vitro_ studies are critical to elucidate molecular pathways, it is undetermined whether all of these findings translate into clinically meaningful pharmacokinetic drug interactions. A study comparing the _in vitro_ inhibition of aminopyrine metabolism produced by dexmedetomidine to _in vivo_ pharmacokinetic changes in rats found a significant discrepancy between assays (Pelkonen et al. 1991). Specifically, changes in aminopyrine clearance were minimal with the administration of high doses of dexmedetomidine despite a very low inhibitory concentration of dexmedetomidine for this compound _in vitro_ (Pelkonen et al. 1991). There is, however, a
mild correlation between dexmedetomidine’s potent inhibition of alfentanil metabolism in human microsomal preparations, and minor elevations in alfentanil plasma concentrations observed with dexmedetomidine administration in human patients (Kharash et al. 1991, Karol & Maze 2000). Studies in dogs are limited, however there is agreement between the findings of in vitro and in vivo studies performed in this species. Cytochrome P450 3A12, the enzyme speculated to be responsible for fentanyl metabolism in dogs, was minimally inhibited by medetomidine in canine microsomal preparations (Baratta et al. 2010). This was corroborated by a study in dogs where pretreatment with ketoconazole, a potent CYP3A12 inhibitor, did not influence fentanyl pharmacokinetics (KuKanich & Hubin 2010). These negative findings suggest that either CYP3A12 does not play a significant role in canine fentanyl metabolism, or that microsomal inhibition is not sufficient to alter metabolism for this high extraction drug.

The lack of understanding of canine fentanyl metabolic pathways, paucity of direct evidence, and tenuous correlation between in vitro and in vivo findings make inferences about the metabolic interactions between dexmedetomidine, acepromazine, and isoflurane with fentanyl difficult at best. While all these drugs, particularly dexmedetomidine, have the ability to alter the pharmacokinetics of fentanyl based on the profound cardiovascular alterations they produce, it remains to be determined as to whether clinical doses of these drugs alter fentanyl pharmacokinetics due to microsomal interactions.
2.4 Anesthetic Recovery Period

2.4.a Definition and characteristics of the recovery period

The recovery period from general anesthesia starts once anesthetic drugs have been discontinued and continues as the patient transitions through progressively lighter planes of anesthesia to the return of consciousness. The popularity of balanced anesthetic techniques and the need for post-operative analgesia often results in the co-existing central and systemic effects of sedatives, analgesic agents and residual inhalant anesthetic while the patient emerges from general anesthesia. As previously discussed, these combinations can result in additive or synergistic cardiopulmonary alterations. Furthermore, these effects are occurring while the patient transitions from 100% inspired oxygen back to room air, and often while equipment used to monitor vital physiologic parameters is removed. When this is considered, it is not surprising that the recovery period is a time of high anesthetic risk (Brodbelt et al. 2008a). Despite this, there are few studies evaluating this phase of anesthesia in dogs.

2.4.b Complications during the recovery period

Complications in the recovery period may involve either vital physiologic systems or central cognitive function. The respiratory system is most commonly involved in post-anesthetic recovery complications, and has been reported to comprise 31% of incidents (Hosgood & Scholl 1998). Reported respiratory complications during recovery in dogs include upper airway obstruction, which is most prevalent in brachycephalic breeds, as well as hypoventilation, hypoxemia, and aspiration of the endotracheal tube or secretions (Clarke & Hall 1990, Dyson et al. 1998, Jackson & Murison 2010). Opioid-
induced respiratory depression is a well-recognized complication in the post-anesthetic period in human patients (Duarte et al. 2009). It is thought to be masked by the stimulation of recovery initially, returning once patients are settled and monitoring is reduced (Hug & Murphy 1979). While opioids alone do not appear to cause significant respiratory compromise in dogs, hypercapnia and hypoxemia have been demonstrated when opioids are combined with other agents as described earlier in this review. Despite this, the significance of this effect on anesthetic recovery has not been reported in dogs.

Primary cardiovascular dysfunction is responsible for 28.6% of post-anesthetic complications, which range from extremes in HR and blood pressure to cardiac arrest (Clarke & Hall 1990, Dyson et al. 1998, Hosgood & Scholl 1998). Changes in HR have been evaluated, with dogs undergoing 3 or more procedures under anesthesia having a greater relative risk of bradycardia and tachycardia than those undergoing 1 or 2 procedures (Hosgood & Scholl 1998). Surveys evaluating complications do not report blood pressure in the immediate anesthetic recovery period, likely due to lack of monitoring, and there is no data reporting the prevalence of hypertension or hypotension at this time. However, hypotension is a commonly reported intra-operative complication and may be responsible for cases of renal failure that develop in the post-operative period (Hosgood & Scholl 1998, Gaynor et al. 1999, Brodbelt et al. 2008a).

Hypothermia, as defined as a body temperature below 37.0 - 37.3°C is a common complication during anesthetic recovery, with 85% of dogs having temperatures below 37.3°C (Dyson et al. 1998, Hosgood & Scholl 1998). Hypothermia is known to cause cardiac dysrhythmias, shivering which increases oxygen demand and may contribute to hypoxemia, increased blood viscosity, and decreased activity of platelets and coagulation.
factors promoting coagulopathy (De Mattia et al. 2012). While it has not been examined in the canine literature, it is possible that these effects contribute to the cardiovascular and respiratory complications described in the previously mentioned studies. In addition, hypothermia results in reduced drug biotransformation and increases the solubility of volatile agents in blood contributing to a prolonged anesthetic recovery, which is another complication described in many canine studies (Lockwood et al. 1997, Dyson et al. 1998, Hosgood & Scholl 1998, De Mattia et al. 2012).

In contrast to prolonged recoveries, dogs may experience excitement when emerging from general anesthesia, requiring restraint or administration of sedative and/or analgesic drugs to adequately control and calm them (Dyson et al. 1998). When analgesia is considered adequate, low doses of either dexmedetomidine or acepromazine have been recommended for sedation on anesthetic recovery to treat this excitement (Lemke 2007). Despite common practice, the administration of sedatives to dogs during this phase of anesthesia has been minimally investigated. Proctor et al. (1991) conducted the only study in this area reporting the cardiopulmonary effects of dexmedetomidine during recovery from enflurane anesthesia in dogs. In this study, dogs received 20 μg/kg of dexmedetomidine orally or placebo an hour before 30 minutes of anesthesia, which was induced and maintained with enflurane. Dogs were then recovered and hemodynamic and respiratory variables were recorded 2 and 7 minutes after extubation. Dexmedetomidine prevented the increases in HR and blood pressure seen on recovery in saline treated dogs. Heart rate, measures of contractility and cardiac output were also significantly lower with dexmedetomidine administration compared to saline controls on anesthetic recovery; however, blood pressure was not statistically different between groups. PaCO₂ was
slightly higher in dexmedetomidine compared to saline treated dogs, but these differences were small and statistically insignificant with values ranging between 32-36 mmHg. Dexmedetomidine treated dogs also demonstrated slightly lower PaO₂ values of 88 and 85 mmHg at 2 and 7 minutes following extubation, however these values were not statistically different from the values of 95 and 89 mmHg seen in saline treated dogs at the same time points. The authors of the study concluded that dexmedetomidine premedication in healthy dogs favourably attenuated the hemodynamic changes seen on anesthetic recovery without causing respiratory depression; however acknowledge that it could cause cardiovascular compromise in patients with limited cardiac reserve or sympathetic tone. While the findings of this study are valuable, the dose, route, and timing of dexmedetomidine administration do not reflect the current practice of administering low-dose intravenous boluses at the time of anesthetic recovery or the effect of concurrent opioid use, and the cardiovascular outcome cannot be extrapolated to these scenarios.

In addition to a range of complications associated with morbidity, the recovery period of anesthesia is also associated with the highest frequency of anesthetic related mortality in dogs (Brodbelt 2008a). A recent prospective multi-center study in the United Kingdom, including both referral and primary care practices, reported that 47% of canine anesthetic-related deaths occurred during the first 48 hours of anesthetic recovery, with 21% in the first 3 hours (Brodbelt 2008a). The most commonly reported causes of anesthetic death in dogs are from primary cardiovascular or respiratory compromise, representing 74% of mortalities, while the specific cause of death is unknown in approximately 20% of cases (Clarke & Hall 1990; Dyson et al. 1998, Brodbelt et al.
Surveys have identified a number of patient factors strongly associated with anesthetic death including increasing ASA physical status classification, old age (based on breed size), body weight under 5 kg, and specific breeds (Hosgood & Scholl 1998, Brodbelt et al. 2008a, Brodbelt et al. 2008b, Bille et al. 2012). Another factor associated with higher risk of death is the use of xylazine, an alpha-2 adrenergic receptor agonist, potentially due to a lack of familiarity with the drug among the veterinarians using it at the time of the study, potential overdoses of other drugs following xylazine administration, and the cardiovascular effects of the drug itself (Clarke & Hall 1990, Dyson et al. 1998). In more recent morbidity and mortality studies, medetomidine was not associated with increased risk in cats or dogs, which may represent a greater understanding of the effects of alpha-2 adrenergic receptor agonists at the time these studies were conducted (Brodbelt et al. 2007, Brodbelt et al. 2008b). The overall risk of anesthetic death in dogs is approximately 0.01-0.1% (Dyson et al. 1998, Gaynor et al. 1999, Brodbelt et al. 2008, Bille et al. 2012), which is over 100 times higher than that observed in modern human anesthesia (Biboulet et al. 2001, Braz et al. 2006). This may be due to less monitoring of physiologic variables, differences in the qualifications of those monitoring anesthesia, or differences in anesthetic management practices, and suggests the need for further research in this area.
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CHAPTER 3: The Cardiopulmonary Effects of a Fentanyl Infusion During Isoflurane Anesthesia and Anesthetic Recovery with Concurrent Acepromazine or Dexmedetomidine Administration in the Dog

3.1 Abstract

Objective: To evaluate the cardiopulmonary effects of fentanyl in dogs under isoflurane anesthesia and recovering from anesthesia without sedation or with dexmedetomidine or acepromazine.

Animals: Seven adult dogs.

Procedures: Dogs received an intravenous loading dose of fentanyl (5 µg/kg) followed by an infusion (5 µg/kg/hr) for 120 min under isoflurane anesthesia and for an additional 60 min after discontinuing isoflurane. Dogs were randomly assigned in a cross over study to receive dexmedetomidine (2.5 µg/kg), acepromazine (0.05 mg/kg) or saline (1 mL) intravenously upon discontinuation of isoflurane. Cardiopulmonary data were obtained during anesthesia and for 90 minutes post treatment during recovery.

Results: Concurrent administration of fentanyl and isoflurane resulted in significant decreases in MABP, HR and CI, and a significant increase in PaCO₂; however all but the latter returned to baseline values prior to discontinuation of isoflurane. During recovery, dexmedetomidine administration resulted in significantly lower HR, CI and PvO₂, and significantly higher arterial blood pressure compared to saline and acepromazine treatments. Acepromazine resulted in significantly lower blood pressure and higher CI and PvO₂ compared to the other treatments. Dexmedetomidine was the only treatment
associated with a decrease in \( \text{PvO}_2 \) and arterial pH, and an increase in \( \text{PaCO}_2 \) relative to baseline values.

Conclusions and Clinical Relevance: Fentanyl resulted in transient pronounced cardiorespiratory effects when administered during isoflurane anesthesia. During recovery from anesthesia while receiving a fentanyl infusion, dexmedetomidine caused significant cardiopulmonary compromise, while acepromazine transiently improved cardiopulmonary performance.

3.2 Introduction

The recovery phase of anesthesia is one of the most challenging anesthetic periods to manage effectively in veterinary patients. This is due to the need to control post-operative pain as well as physiologic and behavioral changes that occur as patients recover from the effects of general anesthetics. It is also a time of high risk; as many as 47% of anesthetic deaths occur within 48 hours of recovery, with 21% of anesthetic related deaths in the first 3 hours (Brodbelt et al. 2008). The most commonly reported causes of death in the post-operative period are respiratory compromise and cardiac arrest, although the inciting cause often remains unknown (Clarke & Hall 1990, Dyson et al. 1998). While the reason for these deaths is not always clear, contributing factors may be the presence of residual or combined drug effects causing cardiopulmonary depression, the transition from high concentrations of inspired oxygen to room air, and a reduction in the monitoring of physiologic variables, all at a time of significant cardiopulmonary change.
Opioids are effective analgesics and their use for pain control in the post-operative period is common practice in veterinary and human medicine (Lipkowski et al. 2004). Fentanyl, a synthetic μ-opioid agonist, is routinely administered as an infusion following an initial bolus because of its pharmacokinetic properties, which include a rapid onset and short duration of action (Ilkiw 1999). Intra-operative use of fentanyl has been advocated for both MAC reduction and analgesia, and infusions are often continued into the post-operative period for continued pain management (Steagall et al. 2006, Aguado et al. 2011). When used alone, fentanyl is associated with a moderate bradycardia that is a result of enhanced parasympathetic tone; however other cardiac and respiratory variables remain within normal ranges (Grimm et al. 2005). It is therefore generally considered safe at clinically recommended doses in the majority of patients.

In addition to opioids, sedatives such as acepromazine or dexmedetomidine are often administered at the time of recovery to control the excitement caused by emergence delirium or dysphoria. While these drugs provide sedation and muscle relaxation, they also produce significant cardiovascular effects. When administered alone, dexmedetomidine causes profound bradycardia, a decrease in cardiac output, first and second degree heart blocks, as well as an increase in systemic vascular resistance: a response typical of other alpha-2 adrenergic receptor agonists at clinically recommended doses in dogs (Kuusela et al. 2000). In contrast, acepromazine at a dose of 0.1 mg/kg IV has been reported to cause a decrease in systemic vascular resistance with subsequent decreases in blood pressure as well as reductions in cardiac output and stroke volume in dogs (Stepien et al. 1995). The combination of these sedatives with opioids further alters cardiopulmonary performance, as their physiologic effects are additive or even
synergistic (Jacobson et al. 1994, Salmenperä et al. 1994, Monteiro et al. 2009). These combined effects, plus any residual effects of volatile anesthetics, may ultimately contribute to morbidity and mortality during recovery from general anesthesia if appropriate patient management and selection is not performed.

Despite the common use of sedatives in combination with opioid analgesics on recovery from anesthesia, there is currently no literature regarding their physiologic effects at clinically relevant doses during this period. The objectives of this study were to evaluate the cardiopulmonary effects of fentanyl, administered as a bolus followed by a CRI during isoflurane anesthesia and with the concurrent administration of acepromazine or dexmedetomidine on recovery from isoflurane anesthesia in dogs. An additional objective was to characterize the cardiopulmonary changes that occur during recovery from anesthesia in the dog receiving only an opioid analgesic. We hypothesized that fentanyl would cause moderate bradycardia, decreases in arterial blood pressure and respiratory depression in dogs under isoflurane anesthesia. We also hypothesized that dexmedetomidine and acepromazine would both result in clinically significant cardiopulmonary alterations on recovery from anesthesia when administered concurrently with fentanyl, with dexmedetomidine having the greatest negative impact.

3.3 Materials and Methods

Animals

Seven intact, male purpose-bred hounds were used. Dogs were 11 to 12 months old with a mean ± SD body weight of 22.3 ± 1.2 kg and were considered healthy on the basis of the medical history, physical examination, complete blood count and serum
biochemistry analysis. Food, but not water, was withheld for 12 hours prior to the experimental period. The study was carried out in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Institutional Animal Care Committee at the University of Guelph.

**Treatment groups**

Dogs were assigned by use of a modified Latin square to receive 1 of 3 treatments in a blinded, randomized cross over design. Treatments were separated by a minimum of 7 days. Treatments included intravenous i) acepromazine\(^a\) (0.05 mg/kg) (Ace), ii) dexmedetomidine\(^b\) (2.5 μg/kg) (Dex), or iii) saline (0.9%, 1 mL). Treatments Ace and Dex were prepared to a final volume of 1 mL using 0.9% saline as the diluent.

**Instrumentation**

For each experiment, a 20-gauge, 4.78-cm catheter\(^c\) was inserted into the cephalic vein. This catheter was used for propofol, fentanyl and fluid administration throughout the study. Anesthesia was induced with propofol\(^d\) and after placement of an appropriately sized endotracheal tube, anesthesia was maintained with isoflurane\(^e\) (1.5-2%) delivered in 100% oxygen via an F-circuit, with the oxygen flow rate set at 60-100 mL/kg/min. An 8.5-F introducer\(^f\) was placed in one of the jugular veins after infiltration of subcutaneous tissues with 0.5 mL of 2% lidocaine hydrochloride\(^g\), and a 7-F thermodilution catheter\(^h\) was advanced through the introducer into the pulmonary artery. Correct catheter placement was verified with fluoroscopy and identification of a pulmonary artery pressure trace. The distal port of the thermodilution catheter was used for mixed-venous blood sampling and measurement of core body temperature, pulmonary artery occlusion pressure, and mean pulmonary artery pressure. The proximal port was used for
measurement of CVP, injection of 5% dextrose solution for measurement of cardiac output, and blood sampling for a separate investigation evaluating the pharmacokinetics of fentanyl. A second 20-gauge, 4.78-cm catheter was placed in the cephalic vein in the opposite limb for subsequent administration of the treatment drug on recovery, and a third 20-gauge, 4.78-cm catheter was inserted in the dorsal pedal artery for direct arterial pressure measurements and collection of arterial blood for gas analysis. All cardiovascular variables were recorded from a multi-parameter monitor\textsuperscript{i}.

\textit{Study Protocol}

Following instrumentation, dogs were positioned in lateral recumbency and isoflurane was adjusted to achieve a stable end-tidal concentration of 1.2\% with dogs breathing spontaneously. End-tidal CO\textsubscript{2} and isoflurane were measured using a side-stream gas analyzer with a sampling rate of 200 mL/min positioned between the endotracheal tube and the anesthetic circuit. Body temperature was maintained between 37\(^\circ\)C and 39\(^\circ\)C using external heat support if needed. Baseline cardiopulmonary variables were then recorded. A 5 \textmu g/kg bolus of fentanyl\textsuperscript{j} was then delivered intravenously over 15 seconds, followed immediately by a CRI at 5 \textmu g/kg/hr. Dogs remained under fentanyl-isoflurane anesthesia for 2 hours, at which point the isoflurane was discontinued (T120). Dogs were then extubated at an end-tidal isoflurane concentration of 0.8\% and administered the assigned treatment at that time (T0\textsubscript{r}). The fentanyl infusion was continued for 60 minutes after treatment administration (T60\textsubscript{r}), and measurements were taken up to 90 minutes after treatment administration (T90\textsubscript{r}). An isotonic balanced solution\textsuperscript{k} was administered to dogs intravenously at 3 ml/kg/hr from T0 to T90\textsubscript{r}. Following the last data collection period, cefazolin\textsuperscript{l} (22 mg/kg) and meloxicam\textsuperscript{m}
(0.1 mg/kg) were administered IV and all catheters were removed. Cardiopulmonary variables were recorded, cardiac output measured and blood collected for gas analysis after instrumentation (baseline-BL), as well as 5, 10, 15, 30, 60, 90, and 120 minutes after fentanyl administration under isoflurane anesthesia, and at 5, 10, 15, 30, 60, 75 and 90 minutes after treatment administration on recovery.

At each data collection point throughout the study, cardiopulmonary variables recorded included CVP, MPAP, PAOP, SABP, DABP and MABP. The zero reference for all pressure measurements was the manubrium. Cardiac output was measured using the thermodilution technique. Briefly, 10 mL of 1-2°C injectate (D5W) was used in each of 3 consecutive measurements. Values within 15% of each other were then averaged to provide the value for that time point. Samples were obtained from the dorsal pedal artery and pulmonary artery, respectively, for blood gas analysis. They were analyzed immediately after collection with an automated blood gas analyzer® with values corrected for body temperature. Heart rate was calculated by counting the arterial pressure trace over a period of 30 seconds and respiration rate was taken from capnograph measurements or counted over 30 seconds after extubation. Cardiac index, SI, SVRI, PVRI, DO₂ and ER were calculated by use of standard equations (Boyd et al. 1991).

Statistics

Statistical analysis was performed using standard statistical software®. Normal distribution of the data were assessed graphically, with residual analysis, and the Shapiro-Wilk test. If necessary, data were log transformed to achieve normal distribution and allow the use of parametric tests. For both maintenance and recovery data, a 3-way ANOVA for repeated measures was applied to determine the interaction of treatment,
time, treatment by time, and carry-over effect from the previous treatment, controlling for the random effects of dog and test period. ANOVA was followed by Dunnett’s or Tukey’s post-hoc analysis (P <0.05).

3.4 Results

Instrumentation and Maintenance Phase

There were no differences among treatment groups and no carry-over effect from previous treatments for propofol dose, the time from anesthetic induction to the collection of baseline data, or any cardiopulmonary variable during the maintenance phase of anesthesia. The means and standard deviations reported for these values were obtained from the average values of the 3 replicate studies for each dog. The mean dose of propofol to induce anesthesia was $5.7 \pm 0.3$ mg/kg (mean ± SD) and the time from anesthetic induction to the collection of BL data was $72 \pm 26$ (mean ± SD) minutes.

All reported cardiopulmonary variables (Tables 3.1 and 3.2) significantly changed following the fentanyl bolus and initiation of the infusion except for SABP, PVRI, and VO$_2$, which were not significantly different from BL values throughout the experimental period. The administration of fentanyl resulted in a significant decrease in HR at 5 min (T5), which gradually returned to values that were not significantly different from BL by T60. There was a corresponding decrease in CI and DO$_2$ at T5 and T10 while SI was significantly higher than BL values from T5-T120. The ER was significantly higher than BL values at T5 only. Systemic vascular resistance index initially increased to values significantly greater than baseline at T5 but was significantly lower than BL values at many time points from T10 to T120. Both DABP and MABP
decreased to values significantly lower than BL at T5 and remained below BL values for the duration of the maintenance phase or until T30, respectively. Central venous pressure, PAOP and MPAP were all significantly higher than BL values between T5-T10 and remained significantly elevated for the duration of the maintenance phase.

Respiratory variables also significantly changed during the maintenance phase of anesthesia following fentanyl administration. The most profound change was the reduction in respiratory rate that occurred immediately after the administration of the fentanyl bolus; however by T15 the average RR was not significantly different than baseline values. Values for PaCO\textsubscript{2} were significantly higher than BL at T5, and remained elevated for the duration of the maintenance phase; however, PaO\textsubscript{2} was only significantly lower than BL values at T10. In contrast, the PvO\textsubscript{2} initially decreased at T5 to values significantly lower than BL before increasing above BL values at multiple time points up to T120.

**Recovery Phase**

Physiologic variables were successfully recorded throughout the recovery period in all dogs with gentle restraint. Some dogs demonstrated signs of excitement including vocalization, paddling and thrashing in the first 10 minutes following treatment administration. These behaviours were present in 3/7 dogs in both the dexmedetomidine and acepromazine treatments, and 5/7 dogs following saline administration.

There were no significant differences among treatments for any of the measured or calculated physiologic variables before treatment administration while dogs were anesthetized (T120). A treatment by time effect was observed for all variables in the recovery period except MPAP, RR and VO\textsubscript{2}.
Throughout the 90 minute post-treatment recovery period, there were significant differences among the treatment groups (Tables 3.3 and 3.4, and Figures 3.1 and 3.2). Heart rate, CI, SI, PVO₂ and DO₂ were significantly lower and ER significantly higher with dexmedetomidine compared to acepromazine and saline treatments throughout most of the post-treatment period. While the dogs receiving acepromazine tended to have higher heart rates than the dogs that received saline, values were not significantly different between these treatment groups at many time points. Cardiac index in the acepromazine treatment was significantly higher compared to the saline treatment at 5 min (T5r), although differences in DO₂ did not achieve statistical significance. The ER was significantly higher in the saline treatment compared to the acepromazine treatment at T5r and T10r.

With respect to systemic arterial pressures, dogs receiving acepromazine had values significantly lower than the dogs receiving dexmedetomidine and saline from T5r to T90r. The dexmedetomidine treatment had higher SABP, DABP and MABP compared to saline administration shortly after administration, however there were no differences between these treatments by T15r.

Central venous pressure and PAOP were significantly higher at T5r in dogs receiving both dexmedetomidine and saline treatment compared to the acepromazine treatment. The differences among groups were present at various time points from T5r to T 60r-90r. While dexmedetomidine tended to have the highest CVP values, they were not significantly different from the saline group except at T30r.

Dexmedetomidine administration was associated with a peak in blood pressures and a concurrent marked elevation in SVRI at T5r. While SVRI steadily declined, values
remained significantly higher than those for both saline and acepromazine treatments from T5r to T75-T90r. In the acepromazine and saline groups, SVRI values did not differ except for higher values in the dogs that received saline at T10r and T15r. Changes in PVRI were not pronounced, however values following dexmedetomidine were higher than acepromazine at T5r, and saline at T10r, up to T60r. Despite this, changes in MPAP were not significant.

Dexmedetomidine caused significant changes in the measured respiratory variables during the recovery phase. Compared to acepromazine and saline treatments, dexmedetomidine values were significantly lower for PaO\textsubscript{2} from T5r – T30r, and significantly higher for PaCO\textsubscript{2} from T5r – T60r.

3.5 Discussion

Fentanyl is commonly administered during inhalant anesthesia to provide analgesia, and reduce inhalant anesthetic requirements (Ilkiw et al. 1993, Steagall et al. 2006). In the current study, a recommended dose of fentanyl for intra-operative and post-operative analgesia administered as a bolus followed by an infusion in dogs concurrently receiving isoflurane resulted in significant decreases in HR, MABP and CI. In a previous report studying awake dogs, despite receiving a three-fold greater intravenous dose of fentanyl administered as a bolus, there were only minor changes in cardiovascular function associated with its administration (Grimm et al. 2005). The difference in findings between these studies is not unexpected, as inhalant anesthetics are well known to result in a magnification of the reduction in HR, blood pressure and cardiac output associated with opioid administration (Kukanich & Papich 2009). The cardiovascular
effects of fentanyl are dose-dependent (Arndt et al. 1984) and it is likely that a lower dose would have resulted in less cardiovascular depression. The bolus and infusion doses of fentanyl selected in this study were based on published plasma concentrations that result in analgesia and clinically significant reductions in inhalant anesthetic requirement (Salmenperä et al. 1994, Robinson et al. 1999). Following initial changes associated with the fentanyl bolus, HR, MABP and CI gradually returned toward baseline values during the CRI of fentanyl in this study. This is likely attributable to a decline in fentanyl plasma levels, but may also have been a result of sympathetic stimulation from the elevation in arterial carbon dioxide levels (Feihl & Perret 1994). Although the cardiovascular effects of fentanyl eventually stabilized, the rapid administration of a fentanyl bolus in anesthetized animals should be done while monitoring HR and arterial blood pressure. In a clinical setting, a slower rate of administration for the initial loading dose or a lower dose could also be considered to reduce the cardiopulmonary impact of fentanyl administration. Administration of atropine can minimize the bradycardia and reduction in cardiac output associated with fentanyl (Ilkiw et al. 1993); however, an anticholinergic was not administered prior to, or concurrently with, fentanyl in the current study to evaluate the effects of the study drugs alone.

As previously mentioned, mu opioid analgesics such as fentanyl are known to reduce inhalant anesthetic requirements (Steagall et al. 2006). To improve cardiopulmonary performance in a patient when an opioid is added to an anesthetic regime, the concentration of inhalant anesthetic delivered could therefore be reduced assuming a constant level of stimulation. In this study, we chose to maintain the dogs at
an end-tidal isoflurane concentration of 1.2% prior to and during the period of fentanyl administration. As in a clinical setting, our goal was to provide an adequate depth of anesthesia while minimizing the cardiopulmonary side effects of the inhalant. Pilot work in healthy dogs found that delivery of 1.2% end-tidal isoflurane was required to permit cardiopulmonary measurements without any dogs responding. In order to minimize potential variability in end-tidal isoflurane concentrations it was elected to maintain the isoflurane at the same level when fentanyl was added to the anesthetic regime. The increase in anesthetic depth due to the fentanyl likely played a role in the observed change in HR, MABP and CI.

While no significant differences in cardiopulmonary variables among groups existed, baseline blood pressure measurements in the dogs in this study were lower than other studies that used mask isoflurane induction prior to instrumentation (Steffey & Howland 1977, Mutoh et al. 1997). Potential contributors to this include the lack of noxious stimulation and differences in study protocol. Specifically, in this study we chose to use propofol for anesthetic induction, which prevented the excitatory phase typically seen with mask inhalant inductions that have commonly been used to evaluate cardiopulmonary effects of inhalants. While it is possible that a low level of hypovolemia may have been present in the dogs in this study we do not believe it contributed to the low blood pressure observed. Instrumentation of the dogs was minimal and hydration status was considered normal prior to each experimental period based on physical examination, physical examination, hematocrit and total protein measurement.

The respiratory effects of fentanyl differ markedly in conscious compared to anesthetized dogs. Following a 15 μg/kg IV bolus of fentanyl, dogs did not demonstrate
significant changes in PaO₂ or PaCO₂ compared to baseline values (Grimm et al. 2005), and doses far exceeding the clinical dose range are required before changes in blood gases occur (Arndt et al. 1984). In contrast, respiratory depression is common following fentanyl administration in anesthetized dogs (Bufalari et al. 2007). Despite anticipating a respiratory depressant effect of fentanyl in dogs receiving isoflurane, we chose not to provide ventilatory support in this study to avoid changes in cardiopulmonary function during the transition from manual to spontaneous ventilation. The period of apnea that occurred produced a maximum average increase in PaCO₂ to 65.5 mmHg, however hypoxemia did not occur since the dogs were inspiring a high oxygen fraction. In a clinical setting, where a patient may have underlying disease or be subjected to positioning that further impairs spontaneous ventilation, one should be prepared to support ventilation if fentanyl is administered as a bolus, reduce the dose, or the rate of administration, particularly if an increase in PaCO₂ is contraindicated.

The anesthetic recovery period is characterized by the emergence through progressively lighter planes of anesthesia to the full return of consciousness. In this study, dogs were extubated at an end-tidal isoflurane concentration of 0.8%. This target was selected over conventional extubation criteria, such as swallowing, to optimize the consistency in the time of sedative administration relative to the effects of residual isoflurane within the patient. In addition, pilot work determined that extubating the dogs at an end-tidal isoflurane concentration of 0.8% was optimal to reduce the excitatory behaviour seen when extubation was delayed until swallowing or signs of resistance to the endotracheal tube were present. The latter occurred at an end-tidal isoflurane concentration of approximately 0.4%. In clinical patients with underlying disease or
surgical trauma, transitioning to room air at an end-tidal isoflurane concentration of 0.8%, or earlier, may have more profound effects on blood oxygenation and supplemental oxygen administration is recommended.

Sedatives are often administered to dogs recovering from general anesthesia to treat excitement caused by dysphoria or emergence delirium (Pascoe 2000). Acepromazine and dexmedetomidine are currently the most commonly used sedatives for this purpose in our veterinary teaching hospital. Due to the different characteristics of sedation produced by these sedatives, determining equivalent sedative doses is difficult. Doses recommended for sedation range from 5 to 50 μg/kg for medetomidine (corresponding to approximately 2.5-25 μg/kg of dexmedetomidine) and 0.025 to 0.2 mg/kg for acepromazine (Bednarski 2007). Controlled studies evaluating doses specifically for post-operative sedation have not yet been performed, however doses generally recommended for this purpose begin at 0.01 mg/kg for acepromazine and range from 2-5 μg/kg for medetomidine (Pascoe 2000). Ultimately, dose selection of these sedative agents for use during anesthetic recovery in this study was based on the high end of clinically used doses that are effective in young, healthy dogs in our clinic setting, as well as from results of preliminary investigations in our laboratory. A comprehensive recovery scoring evaluation was not applied in this study due to the amount of intervention required to ensure collection of cardiopulmonary data. However, both dexmedetomidine and acepromazine administration resulted in a similar frequency of excitatory behaviour, which was less frequent than in dogs that received saline. Further studies evaluating the impact of sedatives on the characteristics of anesthetic recovery in the dog are currently ongoing.
In this study, we showed that at administration of acepromazine and dexmedetomidine at the doses studied, resulted in marked cardiopulmonary changes in dogs receiving fentanyl following isoflurane-based anesthesia. Overall, dexmedetomidine administration was characterized by greater cardiovascular alterations than acepromazine. The cardiovascular effects of dexmedetomidine observed in this study, including bradycardia, decreased cardiac output and increased systemic vascular resistance, are consistent with the effects of dexmedetomidine and other alpha-2 agonists in dogs (Sinclair 2003). The frequently described increase in blood pressure response was also seen as blood pressure initially increased to exceed both other treatments followed by a decline to normal values in 15 to 30 minutes. Despite similar blood pressures among treatments after 15 minutes, other drug effects were still present as HR remained lower and SVRI was significantly higher with dexmedetomidine for at least 75 minutes and likely contributed to the lower SI in this group. Potentially of greatest significance, in combination with the reduction in HR and CI, dogs receiving dexmedetomidine had the lowest global DO\textsubscript{2} and tissue perfusion relative to tissue requirements as indicated by lower PvO\textsubscript{2} and higher ER values.

It is important to recognize that the cardiovascular effects of alpha-2 adrenergic receptor agonists are only somewhat dose-dependent (Pypendop & Verstegen 1998). A dose-titration study in conscious dogs demonstrated that medetomidine administered as low as 1 and 2 μg/kg IV reduced HR by approximately 50% and CI by over 60%. The corresponding reductions were only slightly greater and were maximal at 5 μg/kg and above, with higher doses resulting in a longer duration of effect (Pypendop & Verstegen 1998). Thus, the dexmedetomidine dose of 2.5 μg/kg used in the current study
(approximately equivalent to 5 μg/kg of medetomidine (Kuusela et al. 2001)) may result in maximal cardiovascular depression. Lower doses may provide effective sedation in other patient populations and result in shorter-lived cardiovascular changes, however the magnitude of these effects would be expected to only slightly less or similar to those observed in the current study. The administration of anticholinergics with alpha-2 adrenergic receptor agonists has been explored in dogs and is not commonly recommended due to further reductions in cardiovascular performance, demonstrated by reductions in systolic cardiac function and increased cardiac wall stress (Sinclair et al. 2003). As it is difficult to prevent the cardiovascular changes associated with the alpha-2 adrenergic receptor agonists, the cardiovascular effects of dexmedetomidine should be carefully considered on an individual patient basis and its use avoided during anesthetic recovery in patients where its cardiopulmonary effects would be deleterious.

Interestingly, acepromazine and fentanyl administration transiently enhanced hemodynamic function compared to dogs receiving fentanyl alone during recovery. Stroke index and HR were not statistically different between acepromazine and saline treatments, however there was a trend for HR to be higher with acepromazine resulting in significantly greater CI values 5 minutes after administration. The trend of higher HR in acepromazine treated dogs may be explained by the baroreflex responding to lower MABP caused by peripheral alpha-adrenergic antagonism and enhancing sympathetic outflow (Coulter et al. 1981). Elevated CI coupled with a low SVRI likely resulted in enhanced tissue perfusion, as we observed increases in PvO₂ directly reflecting those of CI indicating less oxygen extraction and more efficient circulation. A previous study by Stepien and coworkers (Stepien et al. 1995) demonstrated a decrease in CI from
conscious baseline values following acepromazine administration at 0.1 mg/kg IV, although values remained within normal physiologic limits (Haskins et al. 2005). This was attributed to a decrease in stroke volume secondary to decreased left ventricular contractility and preload, as HR remained unchanged. These changes were considered to be a result of reduced sympathetic tone and may have been less significant in the current study as a lower dose was used. Despite the larger dose of acepromazine and subsequent buprenorphine administration in the investigation by Stepien and coworkers, systemic arterial blood pressures were still lower in dogs in the current study suggesting additional decreases in blood pressure were due to the use of fentanyl, and potentially the effects of any residual isoflurane in the immediate recovery period.

In addition to causing the greatest ER, dexmedetomidine in combination with fentanyl was the only treatment that resulted in significant ventilatory depression. When administered alone, medetomidine has been shown to decrease RR and inspiratory occlusion pressure, while tidal volume, PaCO2 and PaO2 remain within clinically acceptable limits (Ko et al. 2000, Lerche & Muir 2004). However, opioid-medetomidine combinations have been shown to result in more significant respiratory depression. Values for PaCO2 in the current study were greater than those previously described from opioid-medetomidine combinations (Ko et al. 2000, Grimm et al. 2005, Enouri et al. 2008, Raekallio et al. 2009), which may be attributable to dose or route of administration. Another contributing factor may be additional depression from residual isoflurane, although studies have not shown clinically significant differences in RR or tidal volume in medetomidine compared to medetomidine-isoflurane treated dogs (Lerche & Muir 2004, Lerche & Muir 2006). Although PaCO2 was elevated beyond
normal limits, dogs did not become hypoxemic during the recovery phase. This may be from previous administration of supplemental oxygen, which was continued until the time of extubation. Transitioning patients to room air at earlier time points may have more profound effects on blood oxygenation, and supplemental oxygen administration as well as monitoring with pulse oximetry is recommended during the early recovery period, particularly when dexmedetomidine is administered with an opioid.

Consistent with previous reports, acepromazine in this study had little effect on any of the measured respiratory variables. Previous studies have shown that acepromazine alone causes a decrease in RR but maintenance of minute volume and therefore the effect on PaO₂ and PaCO₂ is minimal (Popovic et al. 1972). When combined with opioids, other studies have demonstrated variable reductions in RR, minute volume, PaO₂, and elevations in PaCO₂ demonstrating a degree of respiratory depression, however this was not present in our study (Cornick & Hartsfield 1992, Jacobson et al. 1994, Stepien et al. 1995). This may be due to lower doses of either acepromazine or opioid used in this study, or the difference in methodology of drug administration. Specifically, other studies administered both the opioid and acepromazine as rapid IV boluses, whereas the fentanyl was delivered as an infusion in the current study and sedatives were administered when fentanyl plasma concentrations had likely declined from initial peak values following the initial bolus.

The onset and duration of cardiovascular effects with dexmedetomidine or acepromazine at the doses administered demonstrated many similarities. The majority of variables reached maximum or minimum values 10 minutes following the administration of either sedative; however the duration of effect differed among variables and between
drugs. The greatest effect on MABP was seen 5 minutes following administration with both sedatives, however the duration of this effect differed between treatments. Following dexmedetomidine administration, values returned within the normal range within 10 minutes and remained stable between 30 to 90 minutes. This is in contrast to acepromazine, where blood pressure remained persistently low for the duration of the experimental period indicating a longer lasting influence on blood pressure. The effect on HR was greatest for both drugs 10 minutes following administration, however the increase seen with acepromazine declined steadily toward normal values over 90 minutes, while the decrease following dexmedetomidine changed very gradually and remained low 90 minutes following administration suggesting that dexmedetomidine may result in longer lasting changes in HR compared to acepromazine. Despite these differences between drugs, their duration seems to be similar with respect to their influence on CI. Cardiac index reached greatest values with acepromazine and lowest values with dexmedetomidine 5 minutes after administration and gradually returned toward the normal range, remaining slightly higher and lower than normal reported values at 90 minutes for acepromazine and dexmedetomidine, respectively. The respiratory depression resulting from concurrent dexmedetomidine and fentanyl administration was slightly slower in onset compared to its cardiovascular effects. Values for PaO₂ were lowest 10 minutes following administration, while elevations in PaCO₂ were maximal between 15 to 30 minutes. Both variables gradually returned to the normal physiologic range at 60 minutes and normalized further following the discontinuation of fentanyl. The discontinuation of fentanyl 60 minutes after sedative administration makes it difficult to discern between cardiovascular changes due to the waning of sedative
effects versus a reduction in drug interactions; however the findings in our study suggest that cardiovascular and respiratory changes will be compromised for at least an hour with dexmedetomidine and fentanyl at the doses administered, and dogs should be monitored more closely during this period. While acepromazine and fentanyl administration on recovery do not result in circulatory or respiratory compromise, it can be expected that blood pressure will be reduced for at least 90 minutes.

A control group, receiving fentanyl alone was included in this study to determine the physiologic changes occurring during recovery from general anesthesia in dogs receiving a clinically relevant dose of fentanyl and to determine the effects of the co-administered sedatives. The cardiopulmonary outcome of anesthetic recovery in the dogs receiving fentanyl alone was a return of respiratory and most cardiovascular variables to referenced normal conscious values. However, CVP, MPAP and PAOP increased and remained higher than reported normal values for most of the recovery period (Haskins et al. 2005). The cardiopulmonary consequences of opioid-induced excitement have not been formally characterized in the dog, but increases in HR, MABP, CI and sympathetic stimulation have been reported accompanying signs of excitement following opioid administration in species more prone to their excitatory effects (Muir et al. 1978, Gaumann et al. 1988, Szöke et al. 1998, Carregaro et al. 2006). This response is not consistent, as a human study demonstrated no change in blood pressure or HR in patients with opioid-induced dysphoria despite elevations in plasma noradrenaline (Rimoy et al. 1994). It is unclear whether the dogs in the present study experienced any cardiovascular stimulation beyond their conscious resting values, as pre-anesthetic baseline values were not obtained. High values for CVP, MPAP and PAOP may be due to increased right and
left ventricular preload from a return of vascular tone following discontinuation of isoflurane and possibly fluid retention. Although a balanced electrolyte solution was delivered at a daily maintenance rate, additional fluids were administered during cardiac output measurements, and both fentanyl and inhalant anesthesia have been shown to decrease urine production (Mackenzie & Donald 1969, Mazze et al. 1974, Anderson & Day 2008) and may have contributed to the elevated pressures observed in the current study by increasing circulating blood volume.

The findings in this study indicate that dexmedetomidine causes significantly greater cardiovascular and respiratory compromise than fentanyl alone or acepromazine with concurrent administration of fentanyl in dogs recovering from isoflurane. In particular, acepromazine increased CI and other measures of perfusion without respiratory compromise providing a hemodynamic benefit on recovery. Fentanyl alone during anesthetic recovery is associated with minimal adverse cardiopulmonary effects. When interpreting these findings, it is important to consider that young, healthy dogs were used in this study. In addition, dogs received oxygen supplementation until the point of extubation (0.8% end-tidal isoflurane) and the effects of either sedative or fentanyl alone may be more extreme in sick or debilitated animals with exhausted catecholamine stores or decreased cardiopulmonary function. Ongoing oxygen supplementation and monitoring of physiologic variables, as well as administration of the lowest effective drug doses are recommended during the anesthetic recovery period to reduce anesthetic risk. Further clinically-based studies will help clarify the cardiopulmonary impact of these sedatives in different patient populations.
3.6 Footnotes

a Atravet; Wyeth Animal Health, ON, Canada
b Dexdomitor; Pfizer Animal Health, QC, Canada
c Insyte-W; Becton Dickinson Infusion Therapy Systems, UT, USA
d Diprivan 1%; AstraZeneca, ON, Canada
e IsoFlo; Abbott Animal Health, IL, USA
f Intro-Flex-Percutaneous sheath introducer kit; Edwards Lifescience LLC, CA, USA
g 2% Lidocaine Hydrochloride Injection; Alveda Pharma, ON, Canada
h Edwards Swan-Ganz; Edwards Lifescience LLC, CA, USA
i S/5 Anesthesia Monitor; Datex-Ohmeda, GE Healthcare, Helsinki, Finland
j Fentanyl citrate; Sandoz Canada Inc, QC, Canada
k Plasma-Lyte A; Baxter, ON, Canada
l Cefazolin; Apotex Inc, ON, Canada
m Metacam; Boehringer Ingelheim, ON, Canada
n Critical Care Xpress; Nova Biomedical, MA, USA
o SAS OnlineDoc 9.2; SAS Institute Inc., NC, USA
### 3.7 Tables

**Table 3.1.** Cardiovascular variables (mean ±SD) in dogs during isoflurane anesthesia (BL) and at various time points (minutes) following a 5 μg/kg IV fentanyl bolus (T0) immediately followed by a CRI (5 μg/kg/hr).

<table>
<thead>
<tr>
<th>Variable</th>
<th>BL</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>93 (11)</td>
<td>56* (14)</td>
<td>63* (15)</td>
<td>74* (20)</td>
<td>85* (21)</td>
<td>86 (19)</td>
<td>88 (23)</td>
<td>88 (22)</td>
</tr>
<tr>
<td>SABP (mmHg)</td>
<td>93 (9)</td>
<td>89 (14)</td>
<td>96 (24)</td>
<td>87 (12)</td>
<td>91 (7)</td>
<td>91 (9)</td>
<td>91 (10)</td>
<td>93 (10)</td>
</tr>
<tr>
<td>DABP (mmHg)</td>
<td>50 (5)</td>
<td>41* (9)</td>
<td>41* (7)</td>
<td>38* (5)</td>
<td>41* (2)</td>
<td>42* (4)</td>
<td>42* (4)</td>
<td>42* (4)</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>60 (5)</td>
<td>52* (11)</td>
<td>53* (8)</td>
<td>51* (6)</td>
<td>54 (3)</td>
<td>54 (5)</td>
<td>55 (5)</td>
<td>55 (5)</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>3 (1)</td>
<td>6* (1)</td>
<td>7* (1)</td>
<td>6* (1)</td>
<td>5* (1)</td>
<td>5* (1)</td>
<td>5* (1)</td>
<td>5* (1)</td>
</tr>
<tr>
<td>MPAP (mmHg)</td>
<td>11 (2)</td>
<td>11 (1)</td>
<td>14* (2)</td>
<td>14* (1)</td>
<td>14* (2)</td>
<td>13* (2)</td>
<td>14* (2)</td>
<td>14* (3)</td>
</tr>
<tr>
<td>PAOP (mmHg)</td>
<td>5 (1)</td>
<td>7* (1)</td>
<td>8* (1)</td>
<td>8* (1)</td>
<td>7* (1)</td>
<td>7* (1)</td>
<td>7* (1)</td>
<td>7* (1)</td>
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<tr>
<td>CI (mL/min/kg)</td>
<td>115 (12)</td>
<td>81* (16)</td>
<td>99* (27)</td>
<td>119 (48)</td>
<td>127 (57)</td>
<td>129 (61)</td>
<td>130 (60)</td>
<td>138 (59)</td>
</tr>
<tr>
<td>SI (mL/beat/kg)</td>
<td>1.24 (0.15)</td>
<td>1.46* (0.21)</td>
<td>1.60* (0.31)</td>
<td>1.60* (0.32)</td>
<td>1.46* (0.31)</td>
<td>1.48* (0.41)</td>
<td>1.46* (0.32)</td>
<td>1.53* (0.35)</td>
</tr>
<tr>
<td>SVRI (dynes.sec/cm²/kg)</td>
<td>80.4 (11.8)</td>
<td>94.0* (26.1)</td>
<td>77.3 (15.1)</td>
<td>65.1* (15.4)</td>
<td>67.3* (17.1)</td>
<td>69.7 (21.4)</td>
<td>71.2 (21.7)</td>
<td>67.4* (24.3)</td>
</tr>
<tr>
<td>PVRI (dynes.sec/cm²/kg)</td>
<td>8.9 (1.82)</td>
<td>7.9 (2.21)</td>
<td>8.4 (1.77)</td>
<td>8.8 (1.56)</td>
<td>8.6 (1.59)</td>
<td>8.5 (1.15)</td>
<td>8.9 (1.22)</td>
<td>8.3 (0.78)</td>
</tr>
</tbody>
</table>

*Statistically different from baseline (BL) (p ≤ 0.05)
Table 3.2. Cardiopulmonary variables (mean ± SD) in dogs during isoflurane anesthesia (BL) and at various time points (minutes) following a 5 μg/kg IV fentanyl bolus (T0) immediately followed by a CRI (5 μg/kg/hr).

<table>
<thead>
<tr>
<th>Variable</th>
<th>BL</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (breaths/min)</td>
<td>16 (7)</td>
<td>2* (3)</td>
<td>7* (5)</td>
<td>9 (2)</td>
<td>16 (6)</td>
<td>17 (8)</td>
<td>18 (9)</td>
<td>23 (19)</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>528.9 (13.9)</td>
<td>513.5 (10.3)</td>
<td>511.7* (10.0)</td>
<td>516.3 (12.4)</td>
<td>522.6 (15.2)</td>
<td>520.9 (8.3)</td>
<td>530.6 (19.5)</td>
<td>531.9 (16.7)</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>45.4 (2.9)</td>
<td>62.4* (4.4)</td>
<td>65.5* (9.7)</td>
<td>59.0* (6.7)</td>
<td>54.8* (4.9)</td>
<td>54.9* (4.9)</td>
<td>55.4* (5.6)</td>
<td>55.9* (5.6)</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.338 (0.016)</td>
<td>7.235*(0.017)</td>
<td>7.218*(0.044)</td>
<td>7.244*(0.036)</td>
<td>7.266*(0.029)</td>
<td>7.282*(0.027)</td>
<td>7.284*(0.026)</td>
<td>7.284*(0.025)</td>
</tr>
<tr>
<td>PvO₂ (mmHg)</td>
<td>72.2 (4.0)</td>
<td>63.2* (4.9)</td>
<td>72.4 (7.4)</td>
<td>79.9 (13.6)</td>
<td>82.4* (21.9)</td>
<td>81.6 (17.1)</td>
<td>86.5* (23.1)</td>
<td>82.9* (16.6)</td>
</tr>
<tr>
<td>ER</td>
<td>0.14 (0.02)</td>
<td>0.22* (0.05)</td>
<td>0.17 (0.06)</td>
<td>0.15 (0.07)</td>
<td>0.15 (0.06)</td>
<td>0.14 (0.05)</td>
<td>0.13 (0.06)</td>
<td>0.14 (0.06)</td>
</tr>
<tr>
<td>DO₂ (mL/kg/min)</td>
<td>22.7 (2.5)</td>
<td>15.6* (2.7)</td>
<td>18.9* (5.8)</td>
<td>22.4 (10.5)</td>
<td>24.3 (13.2)</td>
<td>25.0 (14.2)</td>
<td>26.4 (14.5)</td>
<td>27.8 (14.2)</td>
</tr>
<tr>
<td>VO₂ (mL/kg/min)</td>
<td>3.2 (0.4)</td>
<td>3.2 (0.2)</td>
<td>3.1 (0.6)</td>
<td>2.9 (0.6)</td>
<td>3.0 (0.8)</td>
<td>3.0 (0.3)</td>
<td>2.8 (0.4)</td>
<td>3.2 (0.9)</td>
</tr>
</tbody>
</table>

*Statistically different from baseline (BL) (p ≤ 0.05)
Table 3.3. Cardiovascular variables (mean ± SD) in dogs recovering from isoflurane anesthesia and receiving a fentanyl CRI (5 μg/kg/hr) IV at various time points (minutes) after administration of IV dexmedetomidine (2.5 μg/kg), acepromazine (0.05 mg/kg) or saline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretreatment</th>
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<th>30</th>
<th>60</th>
<th>75</th>
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<tbody>
<tr>
<td>SABP (mmHg)</td>
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<td></td>
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</tr>
<tr>
<td>DEX</td>
<td>100 (15)</td>
<td>165† (13)</td>
<td>151† (13)</td>
<td>138† (12)</td>
<td>130† (12)</td>
<td>127 (15)</td>
<td>134† (15)</td>
<td>139† (18)</td>
</tr>
<tr>
<td>ACE</td>
<td>92 (14)</td>
<td>93‡ (27)</td>
<td>105‡ (25)</td>
<td>106‡ (21)</td>
<td>111‡ (23)</td>
<td>110 (21)</td>
<td>110‡ (19)</td>
<td>116‡ (20)</td>
</tr>
<tr>
<td>SAL</td>
<td>85 (10)</td>
<td>121‡‡ (18)</td>
<td>144† (33)</td>
<td>139† (27)</td>
<td>138† (13)</td>
<td>134† (14)</td>
<td>147‡ (24)</td>
<td>130† (13)</td>
</tr>
<tr>
<td>DABP (mmHg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>43 (4)</td>
<td>97† (10)</td>
<td>89† (13)</td>
<td>80† (10)</td>
<td>70† (12)</td>
<td>65† (12)</td>
<td>67‡ (13)</td>
<td>73† (15)</td>
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<tr>
<td>ACE</td>
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<td>48‡ (13)</td>
<td>54‡ (11)</td>
<td>52‡ (9)</td>
<td>56‡ (10)</td>
<td>51‡ (8)</td>
<td>50‡ (6)</td>
<td>52‡ (7)</td>
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<tr>
<td>SAL</td>
<td>40 (6)</td>
<td>61†‡ (17)</td>
<td>72‡ (21)</td>
<td>72‡ (15)</td>
<td>67† (8)</td>
<td>61† (8)</td>
<td>63† (7)</td>
<td>61 (6)</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
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<td></td>
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</tr>
<tr>
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<td>12† (1)</td>
<td>11† (1)</td>
<td>10† (1)</td>
<td>9† (1)</td>
<td>8† (1)</td>
<td>8‡ (2)</td>
<td>8‡ (2)</td>
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<tr>
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<td>5‡ (3)</td>
<td>4‡ (2)</td>
<td>5‡ (2)</td>
<td>3‡ (1)</td>
<td>3‡ (2)</td>
<td>3‡ (2)</td>
<td>3‡ (2)</td>
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<tr>
<td>SAL</td>
<td>5 (1)</td>
<td>11† (6)</td>
<td>10† (5)</td>
<td>10† (4)</td>
<td>6‡ (3)</td>
<td>6‡ (4)</td>
<td>6 (4)</td>
<td>6 (4)</td>
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<td>MPAP (mmHg)</td>
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<td>20 (3)</td>
<td>20 (2)</td>
<td>19 (2)</td>
<td>17 (3)</td>
<td>18 (2)</td>
<td>17 (2)</td>
<td>17 (3)</td>
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<td>18 (5)</td>
<td>19 (4)</td>
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<td>14 (3)</td>
<td>15 (3)</td>
<td>15 (3)</td>
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<tr>
<td>SAL</td>
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<td>25 (8)</td>
<td>23 (6)</td>
<td>23 (6)</td>
<td>20 (3)</td>
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<td>17 (4)</td>
<td>16 (3)</td>
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<tr>
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<td>8 (1)</td>
<td>17† (2)</td>
<td>15† (1)</td>
<td>14† (2)</td>
<td>12† (2)</td>
<td>10† (2)</td>
<td>10† (2)</td>
<td>10 (3)</td>
</tr>
<tr>
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<td>7 (1)</td>
<td>6‡ (2)</td>
<td>7‡ (2)</td>
<td>9‡ (3)</td>
<td>7‡ (2)</td>
<td>5‡ (3)</td>
<td>6‡ (3)</td>
<td>7 (3)</td>
</tr>
<tr>
<td>SAL</td>
<td>7 (1)</td>
<td>14† (8)</td>
<td>15† (5)</td>
<td>15† (5)</td>
<td>13† (4)</td>
<td>11† (4)</td>
<td>10† (3)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>SI (mL/beat/kg)</td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>DEX</td>
<td>1.64 (0.42)</td>
<td>1.22† (0.35)</td>
<td>1.43† (0.29)</td>
<td>1.34† (0.18)</td>
<td>1.41† (0.23)</td>
<td>1.62† (0.21)</td>
<td>1.72 (0.34)</td>
<td>2.12 (0.32)</td>
</tr>
<tr>
<td>ACE</td>
<td>1.56 (0.46)</td>
<td>2.13‡ (0.40)</td>
<td>1.93‡ (0.25)</td>
<td>2.01‡ (0.35)</td>
<td>2.02‡ (0.34)</td>
<td>1.99‡ (0.26)</td>
<td>2.10 (0.28)</td>
<td>2.04 (0.29)</td>
</tr>
<tr>
<td>SAL</td>
<td>1.35 (0.19)</td>
<td>1.85‡ (0.27)</td>
<td>1.69 (0.37)</td>
<td>1.84‡ (0.47)</td>
<td>2.29‡ (0.37)</td>
<td>1.92‡ (0.44)</td>
<td>1.91 (0.23)</td>
<td>2.06 (0.35)</td>
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<td>PVRI (dynes.sec/cm²/kg)</td>
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<td></td>
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</tr>
<tr>
<td>DEX</td>
<td>7.9 (1.2)</td>
<td>14.1† (8.1)</td>
<td>13.3† (5.6)</td>
<td>13.6† (6.8)</td>
<td>13.1† (4.4)</td>
<td>14.4† (5.1)</td>
<td>10.2 (4.5)</td>
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<tr>
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<td>5.8‡ (1.3)</td>
<td>6.9‡ (2.2)</td>
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<td>6.9 (2.0)</td>
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</tr>
<tr>
<td>SAL</td>
<td>8.8 (0.9)</td>
<td>9.0 (5.0)</td>
<td>7.5‡ (4.1)</td>
<td>6.6‡ (2.2)</td>
<td>6.0‡ (2.8)</td>
<td>7.6‡ (4.3)</td>
<td>7.7 (3.7)</td>
<td>6.6 (2.2)</td>
</tr>
</tbody>
</table>

DEX: Dexmedetomidine; ACE: Acepromazine; SAL: Saline
†Statistically different from acepromazine (p <0.05)
‡Statistically different from dexmedetomidine (p ≤ 0.05)
Table 3.4. Cardiopulmonary variables (mean ± SD) in dogs recovering from isoflurane anesthesia and receiving a fentanyl CRI (5 μg/kg/hr) IV at various time points (minutes) after administration of IV dexmedetomidine (2.5 μg/kg), acepromazine (0.05 mg/kg) or saline.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretreatment</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>75</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>RR (breaths/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>29 (31)</td>
<td>17 (4)</td>
<td>15 (3)</td>
<td>16 (1)</td>
<td>17 (4)</td>
<td>24 (18)</td>
<td>24 (11)</td>
<td>27 (16)</td>
</tr>
<tr>
<td>ACE</td>
<td>21 (18)</td>
<td>21 (7)</td>
<td>30 (14)</td>
<td>24 (9)</td>
<td>30 (6)</td>
<td>52 (37)</td>
<td>51 (27)</td>
<td>50 (36)</td>
</tr>
<tr>
<td>SAL</td>
<td>17 (10)</td>
<td>18 (2)</td>
<td>22 (6)</td>
<td>30 (12)</td>
<td>30 (11)</td>
<td>53 (32)</td>
<td>48 (33)</td>
<td>38 (12)</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>537.5 (20.2)</td>
<td>90.6† (5.6)</td>
<td>86.3† (5.8)</td>
<td>89.4† (6.2)</td>
<td>89.0† (6.9)</td>
<td>98.0 (11.2)</td>
<td>102.8 (6.7)</td>
<td>108.3 (7.4)</td>
</tr>
<tr>
<td>ACE</td>
<td>544.6 (17.6)</td>
<td>120.9‡ (9.4)</td>
<td>117.5‡ (11.0)</td>
<td>121.0‡ (13.6)</td>
<td>111.3‡ (7.2)</td>
<td>103.7 (5.6)</td>
<td>106.5 (8.2)</td>
<td>112.6 (8.4)</td>
</tr>
<tr>
<td>SAL</td>
<td>510.4 (30.5)</td>
<td>114.8‡ (9.5)</td>
<td>110.0‡ (5.5)</td>
<td>108.9‡ (7.2)</td>
<td>101.8‡ (5.8)</td>
<td>102.3 (5.0)</td>
<td>101.5 (7.4)</td>
<td>102.8 (6.8)</td>
</tr>
<tr>
<td>Arterial pH</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEX</td>
<td>7.282 (0.023)</td>
<td>7.299 † (0.029)</td>
<td>7.291 † (0.025)</td>
<td>7.294 † (0.023)</td>
<td>7.305 † (0.038)</td>
<td>7.343 (0.033)</td>
<td>7.370 (0.020)</td>
<td>7.380 (0.012)</td>
</tr>
<tr>
<td>ACE</td>
<td>7.284 (0.034)</td>
<td>7.360 † (0.030)</td>
<td>7.374 † (0.015)</td>
<td>7.378 † (0.014)</td>
<td>7.370 † (0.016)</td>
<td>7.366 (0.017)</td>
<td>7.386 (0.013)</td>
<td>7.405 (0.018)</td>
</tr>
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<td>7.366 † (0.036)</td>
<td>7.354 † (0.028)</td>
<td>7.348 † (0.029)</td>
<td>7.366 (0.024)</td>
<td>7.384 (0.020)</td>
<td>7.389 (0.018)</td>
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<tr>
<td>VO₂ (mL/kg/min)</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>DEX</td>
<td>3.5 (1.1)</td>
<td>4.6 (0.6)</td>
<td>4.8 (0.5)</td>
<td>4.5 (0.5)</td>
<td>4.6 (0.7)</td>
<td>4.4 (0.9)</td>
<td>5.0 (1.0)</td>
<td>5.5 (1.0)</td>
</tr>
<tr>
<td>ACE</td>
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<td>4.8 (1.0)</td>
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<td>5.6 (0.7)</td>
<td>5.4 (0.9)</td>
<td>5.2 (2.5)</td>
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<td>SAL</td>
<td>3.2 (0.4)</td>
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<td>5.7 (1.9)</td>
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</tr>
</tbody>
</table>

DEX: Dexmedetomidine; ACE: Acepromazine; SAL: Saline
†Statistically different from acepromazine (p <0.05)
‡Statistically different from dexmedetomidine (p ≤ 0.05)
3.8 Figures

Figure 3.1. Cardiovascular variables (mean ± SD) HR (A), CI (B), MABP (C) and SVRI (D) in dogs recovering from isoflurane anesthesia and receiving a fentanyl CRI (5 μg/kg/hr) IV at various time points (minutes) after administration of IV dexmedetomidine (2.5 μg/kg) (solid triangle), acepromazine (0.05 mg/kg) (solid square) or saline (open circle).

† Statistically different from acepromazine (p ≤ 0.05)
‡ Statistically different from dexmedetomidine (p ≤ 0.05)
Figure 3.2. Cardiopulmonary variables (mean ± SD) DO₂ (A), PaCO₂ (B), PvO₂ (C) and ER (D) in dogs recovering from isoflurane anesthesia and receiving a fentanyl CRI (5 μg/kg/hr) IV at various time points (minutes) after administration of IV dexmedetomidine (2.5 μg/kg) (solid triangle), acepromazine (0.05 mg/kg) (solid square) or saline (open circle).

† Statistically different from acepromazine (p ≤ 0.05)
‡ Statistically different from dexmedetomidine (p ≤ 0.05)
3.9 References


CHAPTER 4: Pharmacokinetics of a Constant Rate Infusion of Fentanyl in Dogs During Isoflurane Anesthesia and Following Sedative Administration During Anesthetic Recovery

4.1 Abstract

Objectives: To describe fentanyl disposition in dogs receiving a loading dose and constant rate infusion under isoflurane anesthesia, and to evaluate changes in fentanyl and norfentanyl plasma concentrations in dogs recovering from anesthesia receiving an infusion of fentanyl, with or without concurrent acepromazine or dexmedetomidine administration.

Animals: Seven adult dogs

Procedures: All dogs received fentanyl as an IV loading dose (5 μg/kg) followed by a constant rate infusion (5 μg/kg/hr) for 120 minutes during isoflurane anesthesia, and for an additional 60 minutes during the anesthetic recovery period. Dogs were randomized to receive dexmedetomidine (2.5 μg/kg), acepromazine (0.05 mg/kg) or no sedation at the onset of anesthetic recovery in a cross over design. Blood was sampled during the isoflurane maintenance and recovery phases for measurement of fentanyl and norfentanyl plasma concentrations, which were determined using high-performance liquid chromatography-mass spectrometry. Population pharmacokinetic analysis was performed to evaluate the effect of treatment on volume of distribution of the central and peripheral compartments, as well as systemic and intercompartmental clearance.

Results: Average plasma concentrations of fentanyl in the different treatment groups ranged from 1.6 to 4.5 ng/mL during isoflurane anesthesia and from 1.6 to 2.0 ng/mL
during anesthetic recovery. Recovery from anesthesia without sedation increased systemic clearance and the volume of the central compartment for fentanyl causing mild reductions in fentanyl plasma concentrations. Anesthetic recovery with acepromazine administration increased both systemic and intercompartmental clearance and also resulted in mild decreases in fentanyl plasma concentrations. Dexmedetomidine treatment on recovery did not significantly change fentanyl pharmacokinetics or plasma concentrations in relation to those obtained during isoflurane anesthesia and plasma fentanyl concentrations were significantly higher than the other treatment groups at various time points during the recovery period. Norfentanyl concentrations paralleled those of fentanyl for all treatment groups.

Conclusions and Clinical Relevance: Fentanyl administered at the specified doses provided analgesic plasma concentrations of fentanyl for the duration of the infusion. Recovery from isoflurane with acepromazine administration or without sedation altered fentanyl pharmacokinetics in relation to isoflurane anesthesia, while dexmedetomidine did not; however differences in fentanyl concentrations among groups were small and likely not clinically significant. Changes in norfentanyl plasma concentrations are similar to those of fentanyl for all treatment groups suggesting that none of the treatments influenced fentanyl metabolism directly.

4.2 Introduction

Opioid analgesics are routinely administered to dogs during the intra-operative and post-operative period (Steagall et al. 2006, Egger et al. 2007). Short acting opioids are frequently delivered as an infusion following a loading dose to minimize fluctuations
in plasma drug concentrations that would occur following repeated bolus administration, and to provide consistent and titratable analgesia (Sano et al. 2006, Anderson & Day 2008). Fentanyl is a synthetic μ opioid agonist with a rapid onset and short duration of action. It is often used as an intravenous infusion in dogs intra-operatively for both MAC reduction and analgesia, and continued through the anesthetic recovery period for post-operative pain management (Ilkiw et al. 1993, Steagall et al. 2006, Anderson & Day 2008).

In addition to opioid analgesics, dogs may also receive a sedative during recovery from general anesthesia to prevent or treat excitement caused by emergence delirium, opioid dysphoria, or general anxiety (Dyson et al. 1998). Acepromazine and dexmedetomidine have both been recommended for this purpose (Pascoe 2000). While both drugs provide sedation in dogs during anesthetic recovery, their combination with an opioid can result in additive or even synergistic cardiopulmonary depression (Jacobson et al. 1994, Grimm et al. 2005, Monteiro et al. 2008). This is of particular importance during recovery from anesthesia as this has been identified as the time of highest anesthetic mortality in the dog, with the majority of these deaths attributed to primary cardiopulmonary compromise (Clarke & Hall 1990, Dyson et al. 1998, Brodbelt et al. 2008). It is currently unknown whether the physiologic effects resulting from these drug interactions are a result of pharmacodynamic alterations alone, or whether pharmacokinetic interactions also play a role.

The pharmacokinetics of fentanyl following an intravenous loading dose and constant rate infusion (CRI) has been previously evaluated in conscious dogs (Sano et al. 2006). However, pharmacokinetic analysis of a fentanyl CRI has not been performed in
dogs under isoflurane anesthesia despite evidence that inhalant anesthetics significantly alter the pharmacokinetic profile of many drugs (Feary et al. 2005, Thomasy et al. 2005, Thomasy et al. 2007, Pypendop et al. 2008). Dexmedetomidine also causes significant drug interactions, causing potent in vitro inhibition of the CYP450 enzymes involved in the metabolism of a number of anesthetic agents, including alfentanil (Kharash et al. 1991, Kharash et al. 1992). The in vivo implications of these findings have not been thoroughly evaluated; however, dexmedetomidine has been shown to increase alfentanil plasma concentrations in human patients (Karol & Maze 2000). Despite the potential for significant drug interactions, there are currently no published studies evaluating the effects of different sedatives on fentanyl pharmacokinetics. The objectives of the current study are to describe fentanyl plasma concentrations in dogs under isoflurane anesthesia, characterize changes in fentanyl disposition resulting from the elimination of isoflurane and anesthetic recovery, and determine the pharmacokinetic influence of concurrent acepromazine or dexmedetomidine administration at this time. Additionally, concentrations of norfentanyl, the primary metabolite of fentanyl in dogs, were measured to make inferences about the effect of sedatives on drug metabolism. Understanding the influence of other anesthetic agents on fentanyl pharmacokinetics will allow for evidence-based dose selection during times of high anesthetic risk, such as the recovery period of general anesthesia.

4.3 Materials and Methods

Animals

Seven intact male purpose-bred hounds were used in the study. Dogs were 11 to
12 months old with a mean body weight of 22.3 kg (range 20.4 – 24.5 kg). All dogs were considered healthy based on history, physical examination, complete blood count and serum biochemistry analysis. Dogs were housed in individual runs during the experimental period and food, but not water, was withheld for 12 hours prior to induction of anesthesia. The study was carried out in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Institutional Animal Care Committee at the University of Guelph.

**Experimental Design**

A randomized crossover study design was used to evaluate fentanyl pharmacokinetics following three different treatments administered on recovery from anesthesia. Randomization was achieved using a modified Latin square design and a washout period of at least 7 days was instituted between treatments. Each dog underwent the same anesthetic protocol for anesthetic induction and maintenance before treatment administration on recovery.

**Study Protocol**

The study protocol has previously been described in detail (Chapter 3). In brief, for each experiment, a cephalic catheter was placed and anesthesia was induced with propofol. After placement of an endotracheal tube, anesthesia was maintained with isoflurane (1.5-2%) in 100% oxygen to allow instrumentation, including placement of a pulmonary artery catheter, as previously described. The central venous port of the pulmonary artery catheter was used to obtain blood samples for fentanyl and norfentanyl analysis. The cephalic catheter was subsequently used for the administration of fentanyl
and intravenous fluids and a second cephalic catheter was placed for treatment administration on recovery from anesthesia.

Following instrumentation, dogs were positioned in lateral recumbency and connected to a multi-parameter anesthetic monitor to assess cardiovascular status and expired gas composition. Isoflurane was then adjusted to achieve a stable end-tidal concentration of 1.2% with dogs breathing spontaneously throughout the experiment. Dogs were warmed to 37°C before data collection and core temperature was maintained between 37-39°C with active heat support. Once stable end-tidal isoflurane concentrations and normothermia were achieved, a blood sample was obtained for baseline fentanyl and norfentanyl analysis (BL). A 5 μg/kg bolus of fentanyl was then delivered IV over 15 seconds (T0), followed immediately by a continuous rate infusion at 5 μg/kg/hr carried by a balanced electrolyte solution delivered at 3 mL/kg/hr. Dogs remained under fentanyl-isoflurane anesthesia for 120 minutes, at which point the isoflurane was discontinued (T120). Dogs were extubated at an end-tidal isoflurane concentration of 0.8% and administered the assigned treatment at this time (T0r). Treatments were acepromazine (0.05 mg/kg), dexmedetomidine (2.5 ug/kg) or saline control, each prepared to a final volume of 1 mL with physiologic saline and administered IV. The fentanyl infusion continued at the same rate for an additional 60 minutes during the recovery before being discontinued (T60r). Sample collection continued until 30 minutes after fentanyl discontinuation (T90r). Cefazolin (22 mg/kg) and meloxicam (0.1 mg/kg) were administered IV following final sample collection.

At each sampling time, 5 mL of blood was withdrawn prior to collection of 4 mL for fentanyl and norfentanyl analysis. After sample collection, the original 5 mL of
blood was replaced and the catheter was flushed with 8 mL of physiologic saline to partially replace the sampled blood volume. Blood samples were collected during isoflurane anesthesia before fentanyl administration (baseline, BL), at various time points during 120 minutes of general anesthesia (5, 10, 15, 30, 60, 90, 120), and at various time points up to 90 minutes following extubation and anesthetic recovery (5r, 10r, 15r, 30r, 60r, 75r, 90r).

Plasma Fentanyl and Norfentanyl Analysis

Blood samples were transferred to heparinized tubes and centrifuged at 2236 g for 10 minutes. Plasma was collected, transferred into cryogenic storage vials and stored at -80°C until analysis. Fentanyl and norfentanyl were quantified in plasma using liquid chromatography-mass spectrometry after solid phase extraction using standard laboratory protocols.

Analytical reference standards of fentanyl, norfentanyl and fentanyl-d$_5$ were commercially obtained and each prepared in methanol to a concentration of 0.1 mg/ml. Plasma test and calibration samples were processed for analysis by diluting 0.4 mL aliquots with 2 mL 0.1M phosphate buffer, addition of an internal standard (I.S.) solution (5.0 ng/mL fentanyl-d$_5$) and centrifugation (3,000 g x 3 minutes). Solid phase extraction columns (3 mL, 35 mg Cerex Polychrom Clin II) were conditioned sequentially with 2 mL water, 2 mL 1M acetic acid, and 3 mL methanol. The plasma samples were loaded on to the column at a flow rate of 1-2 mL/minute using low-pressure nitrogen gas (N$_2$). The columns were subsequently rinsed with 3 mL of water and 3 mL of methanol. Each column cartridge was dried for 5 minutes using N$_2$ (20 psi). Fentanyl, norfentanyl and I.S., were eluted from the column with 1.7 mL of a 97:3 methanol:ammonium hydroxide
solution. Extracts were dried in an N\textsubscript{2} evaporator, reconstituted in 150 \textmu{}L of the mobile phase and injected (40\textmu{}L) onto the LC-MS/MS system. Linear calibration curves were generated from matrix matched calibrators (ranging from 0.01 – 10 ng/mL) by weighted (1/X) linear regression using the ratio of analyte peak area to internal standard.

Quantitative analyses were performed on a triple quadrupole mass spectrometer\textsuperscript{m} equipped with a liquid chromatography system\textsuperscript{n}. Separation of fentanyl, norfentanyl, and internal standard was performed on a C-18 column\textsuperscript{o} (internal diameter, 10 cm x 2.1 mm; particle size 3 mm) with a linear gradient of acetonitrile (ACN) in water and a constant 0.2\% formic acid at a flow rate of 0.35 ml/minute. The ACN concentration was held at 3\% for 0.4 minutes, ramped from 3-60\% over 8.0 minutes and 60-90\% over 1.0 minute. Detection and quantification employed selective reaction monitoring of LC-MS/MS transitions for the initial product ions for fentanyl, norfentanyl, and fentanyl-d\textsubscript{5}, with mass to charge ratios of 336.8, 233.2 and 342.1, respectively.

Fentanyl and norfentanyl response curves were linear and yielded a correlation coefficient of \geq 0.99. The technique was optimized to provide a minimum limit of quantification (LOQ) of 0.03 ng/mL and a limit of detection (LOD) of 0.01ng/mL for fentanyl and an LOQ of 0.1 ng/mL and an LOD of 0.05 ng/mL for norfentanyl. For fentanyl analysis, the accuracy (percentage of nominal concentration) and precision (percentage relative standard deviation) were 99.5\% and 101\% and 2.5\% and 4\% at 0.04 and 1.5 ng/mL, respectively. For norfentanyl analysis, the accuracy and precision were 88\% and 7.5\% and 92.5\% and 5\% for 0.4 and 1.5 ng/mL, respectively.
Pharmacokinetic Analysis

General Analysis:

Population pharmacokinetic analysis for each treatment was conducted using non-linear mixed effects modeling with the software, Phoenix NLME\(^9\). The population model consists of a structural pharmacokinetic model, a statistical model, and a covariate model that describes the relationship between covariates and model parameters. Systemic clearance (CL\(_1\)), intercompartmental clearance (CL\(_2\)), volume of the central compartment (V\(_1\)), and volume of the peripheral compartment (V\(_2\)) were the parameters selected for estimation. The base structural model was selected by fitting a one, two and three compartment model to observed fentanyl plasma concentrations using the Akaike information criteria value, goodness-of-fit plots and precision of estimates. Estimation used the first order conditional estimation – extended least squares (FOCE – ELS) method with interaction between the between-subject (\(\eta\)) and random (\(\varepsilon\)) effects.

Variability:

The between-subject variability for each structural parameter was modeled using an exponential error model, such that:

\[
P_i = P_{TV} \times \exp(\eta_i)
\]

where \(P_i\) is the parameter value of the \(i\)th subject, \(\eta_i\) is a random deviation describing the difference between \(P_i\) and \(P_{TV}\). \(P_{TV}\) is the typical value of the parameter in the population with no covariates considered. The values of \(\eta_i\) are normally distributed with a mean of 0 and a variance of \(\omega^2\). The residual variability was described using a proportional error model, such that:

\[
C_{ij} = C_{pred,ij} \times (1 + \varepsilon_{pro,ij})
\]
where \( C_{ij} \) is the \( j \)th observed concentration value in the \( i \)th subject, \( C_{\text{pred},ij} \) is the \( j \)th predicted concentration value in the \( i \)th subject and the proportional error value as \( \varepsilon_{\text{pro},ij} \). \( \varepsilon_{\text{pro},ij} \) values are assumed to have a mean of 0 and a variance of \( \sigma^2_{\text{pro}} \).

**Covariate Analysis:**

Covariate analysis was performed to determine the influence of each treatment on fentanyl pharmacokinetic parameters. The treatment covariate (TRT) was defined categorically as either 0 (no treatment effect) or 1 (treatment effect) and influenced the typical value of the fentanyl parameter (\( \theta_{TV} \)) according to the following:

\[
\theta_{TV} = \theta_1 \times \exp(\text{TRT} \times \theta_2)
\]

where \( \theta_1 \) is the value of the parameter with fentanyl alone and \( \theta_2 \) is the fractional change in the value due to the effect of the treatment. TRT was 1 from 120 minutes into the clinical protocol until 210 minutes (protocol completion). Covariate model building used a forward stepwise addition and a reverse deletion approach. The effects of the covariate were assessed based on the change in the objective function value (OFV; \(-2 \log \text{likelihood}\)), visual diagnostics and increased precision of parameter estimates. A covariate effect on the parameter was considered significant (\( p<0.05, \text{df}=1 \)) when the change in the OFV was greater than 3.84 for each added parameter in the forward addition step. A stricter significance level (\( p<0.01 \)) (\( \Delta \text{OFV} > 6.64, \text{df}=1 \)) was used for the backwards deletion step.

**Model evaluation:**

The final model for each treatment was evaluated based on plots of population predicted plasma concentrations vs. conditional weighted residuals and predicted vs. observed individual concentrations. The final models were also subject to non-parametric
bootstrap analysis to assess the robustness of the original parameter estimates. One thousand datasets were generated through random and repeated sampling from the original dataset, with replacement, with each dataset being of equal size to the original dataset. The model was refitted to each new dataset and the mean and 95 percent confidence interval (95%CI) of the parameter estimates were used to assess model robustness.

Statistical Analysis

Differences in fentanyl and norfentanyl plasma concentrations were analysed using standard statistical software. ANOVA for repeated measures was used to evaluate the effects of treatment, time, treatment by time and carry over effect, controlling for the random effects of the individual and treatment period. ANOVA was followed by post-hoc analysis using either Tukey’s or Dunnett’s test. Differences with p≤0.05 were considered statistically significant.

The effect of treatment on parameter estimates was determined with the built-in statistical model in the pharmacokinetic modeling software, which accounts for the random effects of between-subject and within-subject variability. Parameter estimates for the different treatment groups during the maintenance phase were compared using ANOVA to evaluate the effects of treatment and carry over effect, controlling for the random effects of the individual and treatment period. The same ANOVA model was used to compare parameter estimates among treatments in the recovery phase. Differences during the maintenance phase and during anesthetic recovery were considered statistically significant with p≤0.05.
4.4 Results

Fentanyl plasma concentrations collected from baseline (BL) until the end of the experimental period (T90r) were included when formulating the base models for each treatment. A two-compartmental model best fit the fentanyl plasma concentration data and was the base model for all treatments. Each final treatment model was considered robust, as estimates derived from these models were within the 95% confidence interval obtained through bootstrap analysis.

*Fentanyl Disposition During Isoflurane Anesthesia*

Fentanyl and norfentanyl plasma concentrations obtained from initial baseline measurements (BL) until the end of the anesthetic maintenance phase (T120) for each treatment group are displayed in Figures 4.1 and 4.2, respectively. Statistical analysis revealed no significant effect of treatment, treatment by time, or carry over effect from the previous treatment for fentanyl or norfentanyl concentrations during the anesthetic maintenance phase. Fentanyl plasma concentrations increased following the bolus to peak fentanyl concentrations ranging from 5.9 to 2.4 ng/mL at 5 minutes in individual dogs following the bolus administration, with an overall average of 4 ng/mL. Concentrations then steadily declined to an overall average concentration of 1.6 ng/mL in 30 minutes, ranging from 1.1 to 2.4 ng/mL. The CRI maintained these plasma concentrations within a narrow range for the remainder of the maintenance phase under isoflurane anesthesia. Norfentanyl appeared in the plasma of all dogs by 10 minutes after administration and continuously increased throughout the infusion during anesthetic maintenance with isoflurane.
Pharmacokinetic parameters for fentanyl for each treatment group during the maintenance phase are reported in Table 4.1. There were no statistical differences in any parameter among treatment groups during this phase.

Fentanyl Pharmacokinetics During Anesthetic Recovery

Fentanyl and norfentanyl plasma concentrations measured immediately before the discontinuation of isoflurane (T120) until 30 minutes following the discontinuation of fentanyl during anesthetic recovery (T90r) for each treatment group are displayed in Figures 4.3 and 4.4, respectively. Corresponding pharmacokinetic parameters for fentanyl following each treatment are reported in Table 4.2. Basic goodness of fit plots for each treatment model are presented in Figures 4.5, 4.6 and 4.7. Observed plasma concentrations for each dog within a treatment group are plotted with curves of the predicted plasma concentrations based on the final treatment model (Figures 4.5a, 4.6a, 4.7a). These are displayed with corresponding diagnostic plots of population predicted plasma concentrations (Figures 4.5b, 4.6b, 4.7b) and individual predicted plasma concentrations (Figures 4.5c, 4.6c, 4.7c) versus observed plasma concentrations, distributed around the line of identity. Inter-individual and intra-individual (residual) variability for these parameters with each treatment model are reported in Table 4.3.

Recovery from isoflurane with fentanyl without sedative administration (saline control) resulted in a mild decrease in fentanyl plasma concentrations for 30 minutes following the discontinuation of isoflurane before returning to final anesthetized values at T60r. However, concentrations were only significantly lower than final anesthetized concentrations at T15r during the infusion. Fentanyl plasma concentrations steadily declined following the discontinuation of the infusion and were significantly lower than
concentrations observed at T120 at both T75r and T90r. Norfentanyl concentrations decreased slightly during the first 15 minutes of recovery and then remained stable for the duration of infusion and for 30 minutes following the discontinuation of fentanyl.

Pharmacokinetic analysis revealed a 34% increase in CL$_1$ and a 23% increase in V$_1$ of fentanyl in dogs receiving saline during the recovery phase compared to the maintenance phase. The relationship between parameter values during the maintenance phase with those on recovery with saline administration was such that,

\[
\begin{align*}
CL_{1,TV} &= 27.3 \times e^{0.289 \times TRT} \\
V_{1,TV} &= 0.811 \times e^{0.206 \times TRT}
\end{align*}
\]

where TRT equals zero during the maintenance phase (BL until T120), and TRT equals 1 during the recovery phase when no sedative was administered (T120 until T90r). There were no differences in CL$_2$ or V$_2$ on recovery when no sedative was administered compared to values obtained under isoflurane anesthesia. The OFV of the final saline model was 11 points lower than that of the base model indicating that accounting for recovery from anesthesia with saline administration improved the fit of the model. Inter-individual variability was responsible for 25.2-39.4% of the variation in the reported pharmacokinetic parameters in saline treated dogs, while the remaining intra-individual plus residual variability was low at 12.8%. Pharmacokinetic parameters in the recovery phase with no sedative treatment were not significantly different than any parameters with dexmedetomidine treatment; however, V$_1$ was significantly higher and CL$_2$ was significantly lower with no sedative compared to acepromazine treatment.
Acepromazine administration during the anesthetic recovery phase did not result in a significant change in fentanyl plasma concentrations at any time point during the fentanyl infusion compared to concentrations measured under anesthesia at T120. However, there was a trend for a mild decrease in concentrations during the infusion beginning at the onset of recovery, similar to saline. There were no significant differences in fentanyl plasma concentrations between no sedative and acepromazine treatments at any time point. Fentanyl plasma concentrations steadily declined following the discontinuation of the infusion and were significantly lower than concentrations observed at T120 at both T75r and T90r. Norfentanyl concentrations were also lower following acepromazine administration compared to concentrations measured at T120, however decreases were significant at every time point throughout the delivery of the fentanyl infusion and following the discontinuation of fentanyl. Norfentanyl concentrations steadily declined for 15 minutes following the administration of acepromazine and then stabilized, remaining at similar concentrations for the remainder of the infusion and for 30 minutes after it was discontinued. Norfentanyl concentrations in acepromazine treated dogs were not statistically different from those that received no sedative, with the exception of a slightly higher concentration at the final anesthetized time point of T120 before treatment administration.

Pharmacokinetic analysis revealed a 29% increase in CL₁ and a 169% increase in CL₂ of fentanyl in dogs receiving acepromazine during the recovery phase compared to the maintenance phase. The relationship between parameter values during the maintenance phase and those on recovery with acepromazine administration was such that,
where TRT equals zero during the maintenance phase (BL until T120), and TRT equals 1 during the recovery phase with acepromazine administration (T120 until T90r). There were no differences in $V_1$ or $V_2$ on recovery with acepromazine compared to under isoflurane anesthesia. Inclusion of covariates reduced the OFV from the base model by 42 points, indicating a marked improvement from the base model when the effects of acepromazine were accounted for. Inter-individual variability was responsible for 8.6-36.2% of the variation in the reported pharmacokinetic parameters in acepromazine treated dogs, while the remaining intra-individual plus residual variability was low at 6.6%. Volume of the central compartment ($V_1$) was significantly lower with acepromazine treatment compared to saline on recovery; however $CL_2$ was significantly higher with acepromazine compared to both no sedative and dexmedetomidine treatments on recovery.

Dexmedetomidine administration on anesthetic recovery resulted in the highest fentanyl and norfentanyl plasma concentrations of all the treatments. Fentanyl plasma concentrations did not decline following dexmedetomidine, unlike the other treatments, but remained stable with no significant difference compared to concentrations at T120 occurring at any time point during the infusion. Fentanyl concentrations were significantly higher than those following saline administration at T15r and T30r, and significantly higher than those following acepromazine administration at T30r and T60r. As with both other treatments, fentanyl plasma concentrations steadily declined following the discontinuation of the infusion in dexmedetomidine treated dogs and were
significantly lower than concentrations observed at T120 at both T75r and T90r. There were no differences in fentanyl concentrations among treatments following the discontinuation of fentanyl. Norfentanyl concentrations decreased initially following dexmedetomidine administration until T15r; however, concentrations were only significantly lower than those obtained at T120 at T10r. Following this initial decline, norfentanyl plasma concentrations then increased until T90r, with values only significantly higher than pre-recovery values at T75r. Norfentanyl plasma concentrations in dexmedetomidine treated dogs were significantly higher than those treated with acepromazine from T30r onward, and higher than those receiving saline at T75r.

Covariate analysis revealed no difference in fentanyl pharmacokinetics between the anesthetized state and during anesthetic recovery with concurrent dexmedetomidine administration. Inter-individual variability was responsible for 2.1-41.5% of the variation in the reported pharmacokinetic parameters in dexmedetomidine treated dogs, while the remaining intra-individual plus residual variability was low at 6.3%. Pharmacokinetic parameters for fentanyl during recovery did not differ significantly between dexmedetomidine and saline treatments, while \( CL_2 \) was significantly lower with dexmedetomidine than acepromazine during this phase.

4.5 Discussion

This study design was developed to evaluate changes in the concentration of fentanyl and its primary metabolite, norfentanyl, which may occur in a common clinical scenario. Many dogs receive a loading dose and CRI of fentanyl during general anesthesia to provide MAC reduction and analgesia for surgical procedures. In order to
provide ongoing pain management, the infusion is often continued through the anesthetic recovery period when dogs may experience post-anesthetic excitement and also require sedative administration. Determining the effect of sedative administration on fentanyl pharmacokinetics during the anesthetic recovery phase was the primary objective of the study, however blood samples obtained during anesthetic maintenance also allowed the description of fentanyl and norfentanyl disposition in dogs under isoflurane anesthesia.

The administration of fentanyl as a loading dose and infusion in dogs under isoflurane anesthesia resulted in fentanyl plasma concentrations that declined from peak values for approximately 30 minutes. Although plasma concentrations changed little after this time, they did continue to increase slowly. This may be a result of fentanyl reducing hepatic blood flow and systemic clearance, or it may suggest that fentanyl had not yet reached steady state. The latter may be possible as it takes approximately 5 terminal half-lives to achieve steady state, and previously reported values for the terminal half-life of fentanyl in dogs range from 0.75 to 6.0 hours (Murphy et al. 1979, Kyles et al. 1996, Sano et al. 2006). Despite this, plasma concentrations were within the minimum effective analgesic range of 0.95 to 2 ng/mL reported in humans and dogs (Sear 1998, Robinson et al. 1999) suggesting that a 5 μg/kg loading dose followed by an infusion of 5 μg/kg/hr is appropriate for achieving therapeutic fentanyl plasma concentrations. These plasma concentrations would also be expected to provide significant isoflurane sparing effects, as fentanyl plasma concentrations around 1 ng/mL reduce the minimum alveolar concentration (MAC) of enflurane in dogs by 13%. Increasing plasma concentrations further provides a proportionally greater degree of MAC reduction, which plateaus at 65% at concentrations of 30 ng/mL (Murphy & Hug 1982, Salmenperä et al. 1994). The
same trend is seen under isoflurane anesthesia, as a fentanyl loading dose of 5 μg/kg and
infusion at 9 μg/kg/hr resulted in 35% isoflurane MAC reduction in dogs, with higher
doses resulting in further MAC reduction (Hellyer et al. 2001, Steagall et al. 2006,
Ueyama et al. 2009). Fentanyl doses higher than those used in the current study can be
used under anesthesia; however our dosing regime was selected to achieve plasma
concentrations that would be beneficial for reducing isoflurane requirements while
remaining within the range of doses recommended for routine perioperative pain
management in dogs (Pascoe 2000).

A number of studies have been performed to directly investigate the effect of
inhalant anesthesia on the pharmacokinetics of various agents. Results from these studies
are consistent, demonstrating smaller volumes of distribution and lower clearance rates in
the anesthetized versus the conscious state (Feary et al. 2005, Thomasy et al. 2005,
pharmacokinetics has previously been evaluated in the horse and also demonstrated this
trend, however only reductions in clearance were statistically significant (Thomasy et al.
2007). While the current study was not designed to directly compare fentanyl
pharmacokinetics in conscious dogs to those under anesthesia, the trend of lower
volumes of distribution and systemic clearance rates during anesthesia can be seen by
comparing parameter values from this study with those from a similar study performed in
conscious dogs (Sano et al. 2006). In conscious dogs, the reported volume of the central
compartment and total body clearance rates were 1.5 L/kg, and 46.9 to 77.9 mL/min/kg,
respectively (Sano et al 2006), both larger than the corresponding central compartment
volumes and systemic clearance rates in the current study, which ranged from 0.694 to
0.811 L/kg and 27.3 to 37.7 mL/min/kg, respectively. Inferences about the effect of isoflurane on fentanyl pharmacokinetics can also be made from the current study by comparing parameters determined under isoflurane anesthesia with those following anesthetic recovery in the saline control group. Systemic clearance and volume of the central compartment were both significantly lower during anesthesia compared to anesthetic recovery further supporting other findings; however, it is important to recognize that recovery from general anesthesia represents more than simply the elimination of isoflurane.

The recovery period is a dynamic time when patients undergo many physiologic changes that can influence drug disposition. Clearing the inhalant anesthetic not only changes cardiopulmonary function through removal of its direct physiologic effects, but also allows the return of consciousness with potential disorientation and sympathetic stimulation, which can further influence cardiopulmonary performance. Administering a sedative on recovery may attenuate the cardiovascular changes associated with disorientation; however, they cause marked cardiopulmonary alterations of their own. Fentanyl has a hepatic extraction ratio of approximately 1.0 (Björkman & Redke 2000), with metabolism being more dependent on hepatic blood flow than intrinsic liver enzyme activity. Thus, changes in hepatic perfusion resulting from changes in cardiac output and regional blood flow are expected to significantly alter fentanyl pharmacokinetics. This has indeed been demonstrated in a porcine hemorrhagic shock model where cardiac output was closely correlated with changes in fentanyl plasma concentrations (Egan et al. 1999). In addition to fentanyl and norfentanyl plasma concentrations, cardiopulmonary variables were also recorded in the current study and are reported in the previous chapter.
Although hepatic blood flow was not specifically evaluated, CI was determined and can help explain the observed differences in fentanyl plasma concentrations among treatments.

Recovery from isoflurane without sedation (saline control) resulted in an initial decline in fentanyl plasma concentrations corresponding to increases in both $V_1$ and $CL_1$. Cardiac index (CI), as demonstrated in the previous chapter, significantly increased during recovery in saline treated dogs and likely contributed to these findings. Increases in CI can be attributed to the elimination of isoflurane, which causes dose-dependent cardiopulmonary depression, as well as disorientation, as evidenced by mild vocalization and uncoordinated movements in many of the dogs. The presence and degree of this behaviour was highly variable, resulting in variability in CI index, and ultimately greater variability in fentanyl plasma concentrations. The increase in CI can explain the observed increase in $CL_1$ through increased hepatic perfusion and drug elimination. Similarly, an increase in CI likely contributed to the increase in $V_1$ seen during recovery from increased perfusion of peripheral tissues, such as skeletal muscle (Hartman et al. 1992).

Recovery from anesthesia without sedation not only increased CI, but also resolved the respiratory acidosis present under isoflurane. The change in pH would favour the non-ionized diffusible form of the drug, as fentanyl is a weak base (Stoelting & Hillier 2006), and subsequently facilitate the diffusion of fentanyl into peripheral tissues also increasing $V_1$. This phenomenon has been previously demonstrated in mechanically ventilated dogs under anesthesia, where peak fentanyl concentrations were lower in the brain tissue of hypercapneic compared to hypocapneic dogs (Ainslie et al. 1979).
Acepromazine administration during anesthetic recovery resulted in a fentanyl plasma concentration profile very similar to that observed in dogs receiving no sedative. Average plasma concentrations of fentanyl initially declined and remained low for the remainder of the infusion, but these changes were not significant. However, when variation and individual changes were accounted for with pharmacokinetic analysis, significant increases in both $\text{CL}_1$ and $\text{CL}_2$ compared to values under isoflurane anesthesia were identified and explain this trend. CI index also increased in dogs receiving acepromazine on recovery, and trended higher than both other treatments for at least 30 minutes. It is not surprising that elevations in CI increased both clearance rates, as hepatic perfusion likely increased causing more rapid fentanyl clearance from systemic circulation, and as $\text{CL}_2$ is highly dependent on cardiac output and blood flow (Henthorn et al. 1992). While $\text{CL}_2$ following acepromazine administration was significantly higher than with both other treatments, $\text{CL}_1$ did not achieve statistical significance, and may reflect the need for greater power in the study, as opposed to a lack of true difference between treatment groups. The respiratory acidosis observed under isoflurane anesthesia also resolved with acepromazine treatment, similar to saline, however this did not have a statistically significant effect on either volume of distribution.

Dexmedetomidine administration during anesthetic recovery resulted in higher plasma concentrations than with both other treatments, as fentanyl plasma concentrations remained stable instead of declining. Because fentanyl plasma concentrations during recovery with dexmedetomidine did not differ from those during isoflurane anesthesia, there was no treatment covariate effect for any pharmacokinetic parameter, and thus values for both clearance rates and volumes of distribution were the same during the
maintenance and recovery phases. Following recovery with acepromazine or saline, CI improved from the depressed values caused by isoflurane-fentanyl anesthesia resulting in declines in fentanyl plasma concentrations. In contrast, dexmedetomidine administration caused further decreases in CI compared to anesthetized values. This may be expected to reduce hepatic blood flow and clearance, resulting in elevations in fentanyl plasma concentrations; however, the stability of fentanyl pharmacokinetics suggests that hepatic perfusion was maintained. While dexmedetomidine does cause dramatic reductions in CI, there is also a significant redistribution of blood flow favouring hepatic perfusion. While flow to peripheral tissues, such as skin and skeletal muscle is reduced, liver blood flow is maintained or even increased in dogs under halothane-fentanyl anesthesia and in conscious sheep (Lawrence et al. 1996, Talke et al. 2000). If fentanyl were in steady state equilibrium at the time of dexmedetomidine administration, the reduction in peripheral blood flow with unchanging hepatic blood flow would have little influence on fentanyl plasma concentrations. Although less fentanyl move into the peripheral compartment, less would return into the blood and plasma concentrations would be expected to remain stable, as observed in the current study.

As a flow-limited drug, fentanyl clearance theoretically depends primarily on hepatic blood flow, with little influence from moderate changes in liver enzyme activity. While this is generally valid, administration of drugs that inhibit the enzyme responsible for fentanyl metabolism cause significant elevations in fentanyl plasma concentrations, indicating that fentanyl pharmacokinetics are subject to microsomal changes induced by other agents (Ibrahim et al. 2003, Saari et al. 2008). Evidence suggests that isoflurane and acepromazine do not inhibit the activity of enzymes responsible for fentanyl
metabolism (Rice et al. 1986, Murray 1992); however, there is a body of in vitro research demonstrating that dexmedetomidine is a potent inhibitor of many cytochrome enzymes, including the isoform responsible for fentanyl metabolism in many species, CYP3A4 (Kharash et al. 1991, Kharash et al. 1992, Rodrigues & Roberts 1997). Additionally, mild elevations in alfentanil metabolism have been observed in humans receiving dexmedetomidine and correspond with in vitro inhibition of alfentanil metabolism; however, it is uncertain whether these changes are due to changes in hepatic blood flow or microsomal activity (Kharash et al. 1991, Karol & Maze 2000). The cytochrome isoform speculated to be involved in canine fentanyl metabolism, CYP3A12, is minimally inhibited by dexmedetomidine in vitro, however fentanyl metabolic pathways have not been fully characterized in the dog, and the clinical significance of this finding is unknown (Baratta et al. 2010). To evaluate the contribution of microsomal inhibition to the observed fentanyl plasma concentrations, plasma concentrations of norfentanyl, the primary fentanyl metabolite in the dog (Russo et al. 2002), were also evaluated in the current study. Changes in norfentanyl plasma concentrations paralleled those of fentanyl during the infusion for all treatment groups similarly, suggesting that norfentanyl was being produced at the same rate and that any potential changes in microsomal activity did not influence fentanyl disposition in the current study.

The primary objective of this study was to evaluate changes in fentanyl and norfentanyl concentrations during recovery from general anesthesia with or without concurrent sedative administration. While the study design allowed us to meet this objective, it limited our ability to distinguish between the effects of isoflurane elimination and disorientation on anesthetic recovery. Despite this, plasma
concentrations for fentanyl and norfentanyl and some important pharmacokinetic parameters could still be reported providing clinically meaningful data. Administering the infusion for 60 minutes following recovery from anesthesia was sufficient to demonstrate identifiable trends in fentanyl plasma concentrations following each treatment. Extending the duration of the infusion would allow for further assessment of the duration of pharmacokinetic influence of acepromazine and dexmedetomidine; however, given that the differences in fentanyl plasma concentrations were small at the time when sedatives were exerting maximal cardiovascular and sedative effects, differences in plasma concentrations among groups are not likely to be relevant at further time points.

Despite marked differences in the cardiopulmonary effects of acepromazine and dexmedetomidine, the corresponding changes in fentanyl and norfentanyl plasma concentrations following concurrent fentanyl and sedative administration were small. While it is possible that small changes may have greater than expected physiologic effects due to the synergistic nature of drug interactions, differences of this magnitude within the observed plasma concentrations do not significantly influence cardiopulmonary performance in dogs receiving fentanyl alone (Arndt et al. 1984). Based on these findings, it is likely that the degree of cardiopulmonary change observed when fentanyl is co-administered with sedatives during anesthetic recovery is primarily attributable to pharmacodynamic mechanisms. This suggests that any reduction in the required fentanyl infusion rate following sedative administration is reflective of the additional analgesic effects provided by neuroleptic combinations, as opposed to changes in plasma concentrations and drug pharmacokinetics.
4.6 Footnotes

a. Insyte-W; Becton Dickinson Infusion Therapy Systems, UT, USA
b. Diprivan 1%; AstraZeneca, ON, Canada
c. IsoFlo; Abbott Animal Health, IL, USA
d. S/5 Anesthesia Monitor; Datex-Ohmeda, GE Healthcare, Helsinki, Finland
e. Fentanyl citrate; Sandoz Canada Inc, QC, Canada
f. Plasma-Lyte A; Baxter, ON, Canada
g. Atravet; Wyeth Animal Health, ON, Canada
h. Dexdomitor; Pfizer Animal Health, QC, Canada
i. Cefazolin; Apotex Inc, ON, Canada
j. Metacam; Boehringer Ingelheim, ON, Canada
k. Cerilliant, TX, USA
l. Cerex Polychrom Clin II; Cera, CA, USA
m. TSQ Quantum Ultra; Thermo Scientific, CA, USA
n. Model 1100; Agilent Technologies, CA, USA
o. Mac-Mod Analytical, PA, USA
p. Pharsight; Certara, MO, USA
q. SAS OnlineDoc 9.2; SAS Institute Inc., NC, USA
### 4.7 Tables

**Table 4.1.** Pharmacokinetic parameters for fentanyl during isoflurane anesthesia for each treatment group before treatment administration. Parameter estimates obtained from final treatment models using non-linear mixed effects modeling are displayed beside estimates and the 95% confidence interval (95% CI) obtained through bootstrap analysis. There were no significant differences in parameter values among groups (P<0.05).

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<td>$V_1$ (L/kg)</td>
<td>0.811</td>
<td>0.785</td>
<td>(0.544-0.978)</td>
<td>0.694</td>
<td>0.691</td>
<td>(0.639-0.758)</td>
<td>0.766</td>
<td>0.772</td>
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<td>$V_2$ (L/kg)</td>
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<td>5.42</td>
<td>(3.49-11.3)</td>
<td>3.17</td>
<td>3.11</td>
<td>(2.25-3.60)</td>
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<td>$Cl_1$ (mL/min/kg)</td>
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<td>26.0</td>
<td>(7.1-37.7)</td>
<td>37.7</td>
<td>37.9</td>
<td>(34.4-42.4)</td>
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<td>64.1</td>
<td>(46.8-89.2)</td>
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<td>(51.9-68.2)</td>
<td>70.1</td>
<td>72.9</td>
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Table 4.2. Pharmacokinetic parameters for fentanyl during recovery from isoflurane anesthesia with or without concurrent sedative administration. Parameter estimates obtained from final treatment models using non-linear mixed effects modeling are displayed beside estimates and the 95% confidence interval (95% CI) obtained through bootstrap analysis.

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<td>$V_1$ (L/kg)</td>
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<td>0.987 (0.843-1.141)</td>
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<td>$V_2$ (L/kg)</td>
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<td>5.42 (3.49-11.3)</td>
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<td>$Cl_1$ (L/min/kg)</td>
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<td>39.2 (28.0-80.9)</td>
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<tr>
<td>$Cl_2$ (L/min/kg)</td>
<td>62.7†</td>
<td>64.1 (46.8-89.2)</td>
<td>163.7*‡</td>
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</tbody>
</table>

* Significantly different from the anesthetic maintenance phase (p≤0.05)
† Significantly different from acepromazine (p≤0.05)
‡ Significantly different from dexmedetomidine (p≤0.05)
Table 4.3. Measures of inter-individual and intra-individual variability for each final treatment model

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<td><strong>Inter-individual Variability</strong></td>
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<tr>
<td>$\omega^2_{V1}$ (variability)</td>
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<td>0.00742 (8.6%)</td>
<td>0.0147 (12.1%)</td>
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<td>$\omega^2_{V2}$ (variability)</td>
<td>0.0955 (30.9%)</td>
<td>0.131 (36.2%)</td>
<td>0.173 (41.5%)</td>
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<td>$\omega^2_{CL1}$ (variability)</td>
<td>0.0815 (28.5%)</td>
<td>0.0482 (22.0%)</td>
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<td>$\omega^2_{CL2}$ (variability)</td>
<td>0.155 (39.4%)</td>
<td>0.0782 (28.0%)</td>
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<td><strong>Intra-individual Variability</strong></td>
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<td>$\sigma^2_{pro}$ (residual error)</td>
<td>0.128 (12.8%)</td>
<td>0.0661 (6.6%)</td>
<td>0.0628 (6.3%)</td>
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$\omega^2$ represents the variance for the parameter. Inter-individual variability was calculated as the square root of the variance multiplied by 100%. $\sigma^2_{pro}$ represents standard deviation of the proportional error. Intra-individual error (residual error) was calculated by multiplying the standard deviation by 100%.
4.8 Figures

Figure 4.1. Mean ± SD plasma concentrations of fentanyl for each treatment group during isoflurane anesthesia in dogs before (BL) and for 120 minutes following the administration of fentanyl as a loading dose (5 μg/kg) followed immediately by a constant rate infusion (5 μg/kg/hr).
Figure 4.2. Mean ± SD plasma concentrations of norfentanyl for each treatment group during isoflurane anesthesia in dogs before (BL) and for 120 minutes following the administration of fentanyl as a loading dose (5 μg/kg) followed immediately by a constant rate infusion (5 μg/kg/hr).
Figure 4.3. Mean ± SD plasma concentrations of fentanyl for dogs in each treatment group receiving a constant rate infusion of fentanyl (5 μg/kg/hr) for 60 minutes during the anesthetic recovery period. Concentrations were measured immediately before recovery from 120 minutes of isoflurane anesthesia (T120), and for 90 minutes following treatment administration during recovery. Letters represent significant differences from concentrations taken at T120 for acepromazine (a), dexmedetomidine (b) or saline (c) (p≤0.05).

† Significantly different from acepromazine (p≤0.05)
‡ Significantly different from dexmedetomidine (p≤0.05)
Figure 4.4. Mean ± SD plasma concentrations of norfentanyl for dogs in each treatment group receiving a constant rate infusion of fentanyl (5 μg/kg/hr) for 60 minutes during the anesthetic recovery period. Concentrations were measured immediately before recovery from 120 minutes of isoflurane anesthesia (120), and for 90 minutes following treatment administration during recovery. Letters represent a significant difference from concentrations taken at T120 for acepromazine (a), dexmedetomidine (b) or saline (c) (p≤0.05).

† Significantly different from acepromazine (p≤0.05)
‡ Significantly different from dexmedetomidine (p≤0.05)
Figure 4.5. Diagnostic plots evaluating the final pharmacokinetic treatment model for saline. A) Observed plasma concentrations for each dog plotted with curves of the predicted plasma concentrations based on the final treatment model. B) Population predicted plasma concentrations versus observed plasma concentrations. C) Individual predicted plasma concentrations versus observed plasma concentrations.
Figure 4.6. Diagnostic plots evaluating the final pharmacokinetic treatment model for acepromazine. A) Observed plasma concentrations for each dog are plotted with curves of the predicted plasma concentrations based on the final treatment model. B) Population predicted plasma concentrations versus observed plasma concentrations. C) Individual predicted plasma concentrations versus observed plasma concentrations.
Figure 4.7. Diagnostic plots evaluating the final pharmacokinetic treatment model for dexmedetomidine. A) Observed plasma concentrations for each dog are plotted with curves of the predicted plasma concentrations based on the final treatment model. B) Population predicted plasma concentrations versus observed plasma concentrations. C) Individual predicted plasma concentrations versus observed plasma concentrations.
4.9 References


Murphy MR, Hug CC. The anesthetic potency of fentanyl in terms of its reduction of enflurane MAC. *Anesthesiology* 1982;57:485-488.


CHAPTER 5: General Discussion and Conclusions

Our growing understanding of pre-emptive analgesia, MAC reduction, and drug pharmacology has led to the use of multi-modal anesthetic, sedation and pain management strategies in canine patients. These balanced techniques have been advocated since drug combinations may have additive or synergistic sedative and analgesic effects, allow for lower individual drug doses, and potentially reduce the adverse effects associated with each drug (Ilkiw 1999). However, the enhancement of desired effects is not independent from adverse effects and there is a growing body of evidence to suggest that anesthetic, sedative and analgesic drug combinations increase cardiorespiratory depression (Salmenperä et al. 1994, Monteiro et al. 2008, Raekallio et al. 2009). These effects are particularly relevant during the recovery phase of anesthesia, when the effects of multiple drugs are present and patient monitoring may be reduced. Considering this, it is not surprising that the highest proportion of canine anesthetic complications and fatalities occur during anesthetic recovery (Brodbelt et al. 2008). In spite of this, the cardiopulmonary effects of sedative, analgesic and anesthetic drug combinations have not been studied during this phase of anesthesia.

The overall objective of this research was to characterize the cardiopulmonary effects of different drug combinations used in a common clinical scenario; specifically, anesthetic maintenance with isoflurane and fentanyl, and continued fentanyl administration with concurrent acepromazine or dexmedetomidine administration during anesthetic recovery. Furthermore, fentanyl pharmacokinetic analysis was performed to assess the pharmacodynamic and pharmacokinetic contributions to these effects.
The findings in this study demonstrate that all of the drug combinations evaluated affected cardiovascular performance. The cardiopulmonary effects of fentanyl administered during isoflurane anesthesia were most profound following bolus administration, with many parameters returning towards baseline during the subsequent period of infusion. During anesthetic recovery with a fentanyl infusion, the co-administration of dexmedetomidine caused the greatest cardiopulmonary impairment, while acepromazine caused the greatest increases in measured indices of cardiac performance without respiratory depression. These cardiopulmonary changes were associated with distinct changes in fentanyl disposition; however, because of the small magnitude of change, the clinical significance of these trends is likely minimal.

A factor that likely affected experimental outcome was the dose selected for each drug used in the study. While the study was designed to evaluate a common clinical scenario, the drug doses selected may differ from those commonly used in practice. When drugs are combined for clinical use the doses of each are often reduced, which may mitigate adverse effects; however, the concentration of isoflurane was held constant with the addition of fentanyl, and the fentanyl infusion rate during the recovery period was unchanged with the addition of sedative agents in the current study. Additionally, premedication was not administered to dogs before the induction of anesthesia, as would typically be done clinically, and may have contributed to the high sedative doses used during the recovery period. Nevertheless, the protocol selected allowed us to clearly assess the pharmacokinetics and cardiopulmonary effects of fentanyl, and to isolate the influence of the treatments administered on anesthetic recovery without the confounding influence of an additional drug. The lack of pre-anesthetic sedation may also have
increased the incidence of excitatory behaviour during anesthetic recovery and contributed to the sedative dose requirements in the study. Although these doses are considered high for use during the recovery period (Pascoe 2000), they are within the dose range recommended for general sedation in dogs (Bednarski 2007). Currently, there are no studies evaluating dose requirements for post-anesthetic sedation or characterizing the resulting sedation using comprehensive sedation scoring schemes, and the findings of this research may mark the beginning of investigation in this area.

Statistical analysis of cardiopulmonary data revealed many significant differences among treatments for many variables, as changes were often large. In contrast, statistical analysis of fentanyl plasma concentrations and pharmacokinetic variables during the anesthetic recovery period did not always reveal expected statistical differences despite the clear trends observed. This may reflect insufficient power in the study, as opposed to the absence of a treatment effect. Increasing the number of dogs in the study would address this by increasing the ability to detect statistical significance with small differences; however, the importance of statistical versus clinical significance must be considered. The observed differences in variables describing fentanyl disposition among treatment groups were often small and, as discussed in chapter 4, the clinical significance of these findings is questionable.

A potential limitation of this study is that the findings apply to a homogenous population of young, healthy dogs, and may not be reflective of other patient populations. However, as with any study design, the strengths of controlled experimental research must be weighed against evaluating a more relevant but variable population of clinical cases. In order to comprehensively evaluate cardiopulmonary variables, invasive
monitoring equipment was required and precluded the use of clinical patients undergoing anesthesia. If a similar experiment was performed in clinical patients there would be limitations to the cardiovascular variables recorded; however, valuable pharmacokinetic data could still be obtained using population pharmacokinetics. The benefit of population pharmacokinetic analysis over more basic pharmacokinetic modeling is that the determination of variability allows for more robust analysis of data obtained from a diverse patient population, or when few samples are obtained. Additionally, the influence of different variables, such as drug dosage, age, presence of hepatic disease, ASA status, or gender, could all be evaluated as covariates with population pharmacokinetic modeling.

Despite potential limitations, the current study identified drug combinations that cause significant cardiorespiratory depression and may contribute to overall anesthetic morbidity and mortality in dogs. Additionally, clinical recommendations have been made elsewhere in the thesis, emphasizing the importance of vigilant patient monitoring and ongoing supportive care during the recovery period. It is my hope that this research will increase awareness of current management practices, improve the safety of canine anesthesia, and provide a foundation for further research in this area.

References


**APPENDICES**

Table A.1. Fentanyl plasma concentrations in individual dogs (A) and mean ± SD values (B) under 1.2 % end-tidal isoflurane anesthesia following an intravenous loading dose of 5 μg/kg immediately followed by a constant rate infusion of 5 μg/kg/hr during the maintenance phase for each of 3 treatment trials. Note: Bruno did not have data from the saline treatment.

A.

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B.

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Table A.2. Norfentanyl plasma concentrations in individual dogs (A) and mean ± SD values (B) under 1.2 % end-tidal isoflurane anesthesia following an intravenous loading dose of 5 μg/kg immediately followed by a constant rate infusion of 5 μg/kg/hr during the maintenance phase for each of 3 treatment trials. Note: Bruno did not have data from the saline treatment. Blank cells represent erroneous measurements that were removed.

### A.

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Table A.3. Fentanyl plasma concentrations in individual dogs receiving a constant rate infusion of 5 μg/kg/hr for 60 minutes into the anesthetic recovery period for each of 3 treatment trials. Data were collected for 90 minutes following anesthetic recovery. Pre-recovery (120) samples were taken after 120 minutes of isoflurane/fentanyl anesthesia before anesthetic recovery and treatment administration. Note: Bruno did not have data from the saline treatment. Blank cells represent erroneous measurements that were removed.

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Table A.4. Mean ± SD fentanyl plasma concentrations in dogs receiving a constant rate infusion of 5 μg/kg/hr for 60 minutes into the anesthetic recovery period for each of 3 treatment trials. Data were collected for 90 minutes following anesthetic recovery. Pre-recovery samples (120) were taken after 120 minutes of isoflurane/fentanyl anesthesia before anesthetic recovery and treatment administration.

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<td>1.95† (0.27)</td>
<td>1.99† (0.30)</td>
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<td>1.68‡ (0.12)</td>
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<td>0.95* (0.07)</td>
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* Statistically different than pre-recovery (120) values  
† Statistically different than acepromazine within a time point  
‡ Statistically different than dexmedetomidine within a time point
Table A.5. Norfentanyl plasma concentrations in individual dogs receiving a constant rate infusion of 5 μg/kg/hr for 60 minutes into the anesthetic recovery period for each of 3 treatment trials. Data were collected for 90 minutes following anesthetic recovery. Pre-recovery (120) samples were taken after 120 minutes of isoflurane/fentanyl anesthesia before anesthetic recovery and treatment administration. Note: Bruno did not have data from the saline treatment. Blank cells represent erroneous measurements that were removed.

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Table A.6. Mean ± SD norfentanyl plasma concentrations in dogs receiving a constant rate infusion of 5 μg/kg/hr for 60 minutes into the anesthetic recovery period for each of 3 treatment trials. Data were collected for 90 minutes following anesthetic recovery. Pre-recovery (120) samples were taken after 120 minutes of isoflurane/fentanyl anesthesia before anesthetic recovery and treatment administration.

<table>
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<td>0.23</td>
<td>0.21*</td>
<td>0.23</td>
<td>0.27†</td>
<td>0.27†</td>
<td>0.31*†</td>
<td>0.28†</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.04)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Acepromazine</td>
<td>0.26</td>
<td>0.22*</td>
<td>0.22*</td>
<td>0.20*</td>
<td>0.21*†</td>
<td>0.21*†</td>
<td>0.21*†</td>
<td>0.20*†</td>
</tr>
<tr>
<td></td>
<td>(0.03)</td>
<td>(0.05)</td>
<td>(0.06)</td>
<td>(0.04)</td>
<td>(0.03)</td>
<td>(0.04)</td>
<td>(0.01)</td>
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<tr>
<td>Saline</td>
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<td>0.24</td>
<td>0.24</td>
<td>0.24‡</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>(0.05)</td>
<td>(0.03)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.05)</td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.06)</td>
</tr>
</tbody>
</table>

* Statistically different than pre-recovery (120) values
† Statistically different than acepromazine within a time point
‡ Statistically different than dexmedetomidine within a time point