Pharmacokinetics and Pharmacodynamics of Fentanyl in Alpacas after Intravenous and Transdermal Administration

Master’s Thesis

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By

Michael F. Lovasz, DVM

Graduate Program in Comparative and Veterinary Medicine

The Ohio State University

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Thesis Committee:

Richard M. Bednarski, DVM, MS, DACVAA, advisor
Turi K. Aarnes, DVM, MS, DACVAA
Jeffrey Lakritz, DVM, PhD, DACVIM
Phillip Lerche, BVSce, PhD, DACVAA
Abstract

The objective of the study reported here was to determine pharmacokinetics and pharmacodynamics of fentanyl in alpacas after intravenous (IV) and transdermal (TD) administration.

Fentanyl was administered IV (2 µg/kg) or TD (2 µg/kg/hr) in 6 adult alpacas. Samples of venous blood were obtained at predetermined intervals for 24 hours after IV and 96 hours after TD administration to determine plasma concentrations using liquid chromatography-mass spectrometry. Sedation score, HR, RR, and the responses to thermal and mechanical nociception were assessed at each time point.

Maximum plasma concentration (Cmax) of fentanyl was 4.6 ± 1.8 ng/mL after IV administration, clearance was 921 ± 189 mL/kg/hr, volume of distribution was 4.3 ± 1.8 L/kg and elimination half-life was 3.1 hours (range 1.87-7.2 hours). Mild sedation occurred within 5 minutes of IV administration and lasted up to 45 minutes. Apparent excitement occurred in three alpacas following IV fentanyl administration. Limb mechanical threshold and abdominal algometry were significantly increased from baseline at 15 minutes and 45 minutes, respectively. Mean maximum plasma fentanyl concentration was 1.3 ± 0.8 ng/mL, mean residence time was 42 ± 8 hours, and elimination half-life was 16.5 hours (range 10-22 hours) after TD administration. Sedation was mild in three alpacas following TD fentanyl, peaked by 24 hours and lasted up to 60 hours.
Plasma fentanyl concentrations peaked and fell rapidly after IV administration. Uptake of TD fentanyl was absorption dependent, but nearly complete. The behavioral responses to IV fentanyl were inconsistent.
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Vita

1998 ................................................................. AAS Dairy Science, State University of New York Morrisville

2002 ................................................................. AAS Veterinary Technology, LaGuardia Community College

2007 ................................................................. BA Environmental Science, Queens College

2011-2012 ........................................................ Clinical Studies for DVM, Texas A&M University College of Veterinary Medicine

2012 ................................................................. DVM, Ross University School of Veterinary Medicine

2012 – 2013 ........................................................ Small Animal Rotating Internship, The Animal Medical Center

2013 – Present ................................................ Residency, Veterinary Anesthesiology, The Ohio State University

2013 – Present ................................................ Graduate Student, The Ohio State University

Fields of Study

Major Field: Comparative and Veterinary Medicine
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Chapter 1: Review of Literature

1.1 Opioids in Veterinary Medicine

Opioids are used across species for sedation and analgesia. Dose-related side effects are documented and include bradycardia, panting and vomiting in dogs, especially without concurrently administered drugs that can lessen or eliminate these side effects.\(^1\) In addition, opioid administration can lead to respiratory depression.\(^2\) In horses, unwanted dose-related side effects such as ileus and CNS excitation have been observed after opioid administration.\(^3,5\) Despite their negative side effects, the analgesic benefit of appropriate administration of these drugs surpass the possibility of unwanted properties.

Opioids are classified relative to their receptor activity. There are three major opioid receptors throughout the central and peripheral nervous systems, mu, kappa and delta. Opioid binding to these receptors activates membrane associated G proteins. The activation of G\(_{i/o}\) proteins causes hyperpolarization of the cell membrane limiting the cells responsiveness to excitatory signaling.\(^6\) Additionally, opioids contribute to limiting perception of inflammatory pain by blocking the production of prostaglandins.\(^6\) Opioids are administered intravenously, intramuscularly or subcutaneously. Other routes of administration include oral, oral transmucosal, transdermal, epidural, spinal, and topical.\(^1,2,7,8\)
1.1.1 Intravenous Fentanyl in Veterinary Medicine

Fentanyl is a synthetic phenylpiperidine opioid agonist first created by Paul Janssen in 1959. Due to the increased lipophilicity of fentanyl when compared to morphine, fentanyl penetrates the CNS rapidly making the drug 80-100 times more potent.² Despite this sizable difference in potency the absolute receptor affinity for fentanyl is only 1.7 times that of morphine.⁹ Fentanyl citrate: N-phenylethyl-N-[1,(2-phenylethyl) 4 piperidyl] propanamide is the formulation of fentanyl used in veterinary medicine today (Figure 1). Fentanyl compared to morphine in dogs produces less cardiovascular and respiratory depression.¹⁰,¹¹ Unlike morphine fentanyl does not release histamine from mast cells.¹² Fentanyl has been administered intravenously to a variety of species including: adult horses,¹³⁻¹⁵ foals¹⁶, dogs¹⁰,¹¹,¹⁷, cats¹⁸,¹⁹, sheep²⁰, goats²¹,²², swine²³, and alpacas.²⁴,²⁵ Fentanyl has a rapid uptake, distribution, and clearance through tissue in various species. Due to its relatively rapid clearance, fentanyl should be delivered as a constant rate infusion or through multiple intravenous boluses in order to maintain adequate plasma concentrations and sustained therapeutic usefulness.¹²
Dose Related Effects of Fentanyl in Various Species

In mature Thoroughbred and Standardbred horses fentanyl-induced increases in heart rate, respiratory rate, and locomotor activity were observed after 0.01 mg/kg IV.15 Increased locomotor activity was also observed in a later study after 0.02 mg/kg, IV fentanyl.3 After an IV bolus of 8-16 µg/kg of fentanyl, foals (5-13 days old) exhibited ataxia, muscle rigidity, increased locomotor activity, and head pressing. One foal attempted to climb the walls of the stall.16 In a study using adult Thoroughbred horses, a significant increase in heart rate was noted in two horses once plasma fentanyl concentrations reached 7.9 ng/L. Muscle fasciculations in that study were observed in an adult horse with plasma concentrations of 7.8 ng/L. The horse was also observed shifting its weight.29 In unanesthetized spontaneously breathing dogs, plasma concentrations of 5-30 ng/mL were associated with decreases in respiratory rate, heart rate, and cardiac output, increased analgesia was observed with increasing doses and associated plasma concentrations of fentanyl.11 At intravenous fentanyl doses of 2.5 µg/kg and 5 µg/kg, mild restless behavior in the dogs.11 The dogs showed
evidence of sedation with 20 µg/kg dose and finally demonstrated “sleep like behavior” at 40 
µg/kg IV. Goats that received a 2.5 µg/kg IV fentanyl bolus had increased body 
temperatures, vocalization, locomotor activity, and tail twitching. A different study with 
goats using fentanyl delivered as a constant rate infusion for MAC reduction noted post-
operative tail wagging and increased vocalization. The fentanyl rates associated with these 
signs were 15 and 30 µg/kg/hr.

Reports of intravenous administration of fentanyl to alpacas are limited. In a case 
report, a 5-hour-old premature cria with significant respiratory distress was given 5 µg of 
fentanyl intravenously to facilitate the placement of a nasotracheal tube. The tube was 
successfully placed and the cria recovered without complications. A second case report 
described the inclusion of fentanyl along with midazolam and ketamine for balanced 
anesthesia along with isoflurane in a three-year-old alpaca undergoing extensive dental 
surgery. The alpaca received 2 µg/kg of fentanyl in conjunction with 3 mg/kg S-ketamine 
and 0.4 mg/kg midazolam IV. The alpaca was successfully intubated and placed on 
isoflurane. A constant rate infusion (CRI) was started consisting of fentanyl, midazolam, and 
ketamine. The surgical site was blocked locally with lidocaine and bupivacaine. The alpaca 
became bradycardic and hypoxemic. Intermittent positive pressure ventilation was started 
with 5 cm of positive end expiratory pressure and an anticholinergic was administered. The 
hypoxemia and bradycardia resolved and the alpaca recovered uneventfully from anesthesia. 
A 100 µg/hr fentanyl patch was applied and the alpaca received a dose of flunixin meglumine 
(2.2 mg/kg IV).
1.1.1.2 Fentanyl and MAC Reduction

The benefit of combining fentanyl to the anesthetic regimen in various species undergoing general anesthesia has also been evaluated. In horses, a fentanyl plasma concentrations of 13.3 ng/mL reduced MAC of isoflurane by 18%. In another study fentanyl plasma concentrations of 13.9, 20.1, and 24.1 ng/mL failed to change MAC in isoflurane anesthetized horses and were associated with post anesthetic excitement. In swine a fentanyl plasma concentration of 14 ng/mL reduced isoflurane MAC by 25%. Goats receiving a fentanyl CRI of 5, 15, and 30 µg/kg/hr had isoflurane reductions of 28, 41, and 57% respectively.

1.2 Transdermal Fentanyl in Veterinary Medicine

The TD fentanyl patch was created by Janssen Pharmaceutica in 1995 and marketed as Duragesic®. The patch initially contained the drug in a gel matrix, but because of concerns for abuse, the patch was reformulated to contain the drug within its adhesive backing (Figure 2). The patch is sold for human use in strengths designed to deliver 12.5, 25, 50, 75, or 100 µg/hr. Delivery of TD fentanyl into the plasma is via absorption from the TD reservoir in the patch. Through passive diffusion, the drug passes through the stratum corneum, the epidermis, the dermis, and finally into the cutaneous microcirculation and into the blood. Although this route of drug delivery avoids first pass metabolism, a case report in human medicine described a fatal fentanyl overdose in patient following chronic application of multiple patches leading to the severe sickling of red blood cells in the lungs (acute chest syndrome). A major advantage of the transdermal patch is the ability for it to provide a
continuous and sustained plasma concentration and associated analgesia without multiple injections or infusion pumps. Other advantages compared to intermittent bolus administration include the ease of administration and the elimination of drug peaks and sub-therapeutic plasma concentrations.\textsuperscript{1, 2, 12}

The fentanyl patch has been used in various species including horses \textsuperscript{14, 28}, dogs \textsuperscript{30-34}, cats \textsuperscript{36, 39}, llamas \textsuperscript{37}, goats \textsuperscript{21}, pigs \textsuperscript{38}, and sheep.\textsuperscript{20} Maxwell et al. demonstrated in horses weighing 464-585 kg that received 20 mg patches (approximately 0.63 µg/kg/hr) did not increase heart rate, respiratory rate, or locomotor activity.\textsuperscript{14} In a different study, 6 foals weighing 56-74 kg with TD fentanyl patches delivering 100 µg/hr (approximately 1.5 µg/kg/hr) showed no change in heart rate, respiratory rate, temperature, fecal production, urination, or locomotion. The foals tolerated the patch while in place as evident by the patches remaining in place until they were removed by the investigator.\textsuperscript{28}

In a non-surgical study, 100 µg/hr fentanyl patches were applied to eight dogs weighing 29.4 kg (range 26.5-33.6 kg) equal to a dose of approximately 3.3 µg/kg/hr. Significant decreases in heart and respiratory rates were observed following 24 hours of patch application. Seven of the eight dogs showed signs of mild sedation, and decreased activity for up to 72 hours following patch placement. Plasma fentanyl concentrations were 1.01-1.02 ng/mL.\textsuperscript{32}

In a study comparing the analgesic effects of TD to that after epidural morphine, the transdermal group was more vocal and appeared less sedate.\textsuperscript{30} The dogs received a 100 µg/hr fentanyl patch placed (approximately 3.7 µg/kg/hr) 24 hours prior to surgery or received 0.1 mg/kg preservative–free morphine epidurally after the dogs were anesthetized. Pain was assessed prior to surgery, immediately after, and at 6, 18, 30, and 42 hours following surgery. The authors concluded that the post-operative pain was mostly observed within the first
twelve hours following surgery in both groups and that rescue analgesia may be required until adequate fentanyl plasma concentrations are met. Pain scores were consistently decreased in the dogs with fentanyl patches after 12 hours of surgery compared to those in the epidural morphine group. After 24 hours of patch application, the dogs maintained a fentanyl plasma concentration of 0.95 ng/mL. Although the plasma concentration of fentanyl that provides analgesia in dogs has not been determined, the authors speculated that in this study this plasma concentration provided analgesia as assessed by the statistically significant difference in pain scores between dogs in the fentanyl patch group compared to those that received a morphine epidural.

A study in cats investigated the relationship between analgesia and fentanyl plasma concentrations. The cats had a mean body weight of 3 kg and 25 µg/hr patches were applied 24 hours prior to ovariohysterectomy or no surgery. The approximate fentanyl delivery rate was 8.3 µg/kg/hr. The study found that plasma fentanyl was detected following patch application sooner in cats compared to dogs and that peak fentanyl concentrations were reached sooner in cats compared to dogs. Variable plasma concentrations were achieved (approximately 1 ng/mL) between 8 and 12 hours. Although the fentanyl plasma concentration to associated with analgesia in cats has not been determined, the authors were satisfied that 1 ng/mL provided sufficient analgesia determined by a lack of difference between physiologic parameters and behavior scores between the surgical and non-surgical groups.

Transdermal fentanyl patches were applied to sheep at 2.05 µg/kg/hr for 72 hours. Plasma concentrations remained consistent at 0.3 ng/mL following ten hours of application at with no adverse effects or signs of sedation noted. Similar findings were observed in goats.
weighing 40.4 ± 7.5 kg receiving a 50 µg/hr fentanyl patch. The approximate transdermal fentanyl dose was 1.2 µg/kg/hr.21

In eight ten week old pigs weighing 25.2 ± 5.2 kg were subjected to 50µg/hr patches over a three week period of time.38 A comparison of the activity level, weight gain and behavior changes were made to pigs placed under general anesthesia wearing the fentanyl patches compared to a group wearing patches without general anesthesia. After the first anesthesia week, the pigs were given one week wash out, followed by a third week switching groups placed under anesthesia with a fentanyl patches to the group with fentanyl patches only. There were no surgeries performed or noxious stimuli delivered to any of the pigs. The pigs in both groups were monitored via video tape, and their activity level and eating habits were rated and compared. There were no signs of sedation, weight loss or activity changes, however serum concentrations of fentanyl were variable throughout the study. The transdermal fentanyl dose was approximately 2 µg/kg/hr. The authors concluded that the uptake of transdermal fentanyl in growing pigs is unpredictable based on the variation in drug absorption that occurred throughout the study.38 No steady state of fentanyl was attained in either group; Serum concentrations ranged from 0.01-0.99 ng/mL.

Llamas (150 ± 18 kg) were treated with four 75 µg/hr patches (approximately 2 µg/kg/hr). They showed no signs of sedation at any time up to and including the time of placement and after 72 hours. There were no significant changes in heart rate, respiratory rate or body temperature at any time while the patches were in place. Plasma concentrations were sustained at 0.3 ng/mL. A relationship between fentanyl plasma concentrations and analgesia was not yet been determined in the this study.37
1.3 Nociceptive Testing in Veterinary Medicine

Testing for a response to noxious stimuli in animals is challenging. Nociceptive testing is a term used to describe behavioral or physiological responses to a form of adverse stimulation that elicits a reactive response. The test itself should be easily performed, repeatable, and have an identifiably clear end-point. The duration of the stimulation should be limited and should not produce lasting pain, discomfort, or damage to the tested site. Nociceptive threshold testing determines the threshold response to a noxious stimulus. Stimuli can be electrical, mechanical, thermal or chemical. Buccal electrical stimulation, hoof wall electrical stimulation and peripheral nerve stimulation have been used to evaluate nociception in the horse and llama. Tail clamping with a hemostat has been used in the dog and hemostat clamping of the hoof wall has been used in goats, llamas and horses. While nociceptive testing can gauge a response to noxious stimuli, the stimuli provided does not necessarily equate to that associated with surgical pain. This is a limitation when evaluating analgesics for the treatment of surgical pain.
1.3.1 Pressure Algometry

Pressure algometry has been used in various species including the horse, donkey, sheep, dog, cat, human, and llama. Pain pressure threshold is the minimal amount of pressure (force) that produces a response to stimuli that ultimately can induce pain. In one study, Fischer et al. found that 20N (Newtons) is the pressure threshold in men and women. It was noted that in some muscle groups, females had lower threshold responses compared to males.

Pressure algometry devices vary in design. Some use a pressurized cylinder to variably increase the pressure applied to a pin or rod that contacts the skin, and other devices utilize a hydraulically inflatable bladder with blunted pins. Another form of pressure algometry used in a study investigating the efficacy of fentanyl used a mylar balloon for colorectal distension. In one study with llamas, a conventional hoof tester incorporating a force transducer was tested at the withers, mid-neck, and metacarpus. A signal processor was used to increase the force applied.

Monofilaments are simple, hand held devices that have been used to test antinociception in both humans and animals. Von Frey filaments were first described in 1922 and continue to be used today. Briefly, the early design consisted of a 50 mm long horse-hair or fiber. A force was applied from the filament to a skin surface at a right angle producing a bend to the fiber to elicit a reaction from the patient. The sensation in humans was described as an itch. Semmes and Weinstein expanded on the monofilament theory by creating monofilaments of varying diameters on different handles. The tip of the fiber changes its contact with the skin depending on how much force is applied as well as the
texture of the surface to which the tip is applied. Modern Von Frey filament sets consist of up to twenty probes capable of producing from 0.008 to 300g of force.

A hand held pressure algometer (Figure 3) eliminates cuffs, multiple pins, gas tanks to build pressure, and computers to program a delivered force. Additionally a hand held algometer can be used to assess nociception at multiple sites. The 1 cm² blunt tip of the hand-held algometer is applied perpendicular to the surface of interest. Pressure as indicated by an incorporated gauge is progressively increased until a response is elicited. Once a response is noted, pressure is discontinued. It has been recommended that at least three measurements per site should be taken, with at least three seconds between elapsed tests. The measurements taken from each anatomic site evaluated are then averaged and a mean and range is calculated. This method has been shown to increase intraexaminer reliability.

Hand held pressure algometry has been successfully used to assess nociception in horses, sheep, cats, and dogs. In a study assessing pressure algometry in Icelandic horses, nine anatomic areas were assessed by two observers. The evaluations were repeated three weeks later, and the results suggested the overall reliability of hand held algometry was moderate to good.

Stubbsjøen et al. used a hand held algometer to assess mechanical thresholds in sheep. Six predetermined anatomic regions were tested. Each region was assessed at five minute intervals for thirty minutes for three consecutive days by two investigators. The investigators found that mechanical thresholds were highly variable with no reproducible results. It was questionable as to whether the sheep were moving their legs as a learned behavior and not in response to noxious stimuli. The researchers felt that using the last two measurements for each region tested yielded the most reliable results. They also recommended an acclimation period of device and subject.
In a study assessing antinociception in cats, a hand held algometer was applied to the surface of either antebrachium. The responses were assessed before and after methadone administration. The study found that peak antinociception occurred prior to when peak plasma methadone concentrations were achieved. This information may be useful in alpacas if they have a similar increase to antinociception and the antinociception were to occur prior to peak fentanyl plasma concentrations.

![Hand-held algometer unit](www.wagnerinstruments.com)

1.4 Thermal Testing

Nociceptive thermal testing has been used in the research and clinical setting. Once a base-line reaction to applied heat is confirmed, a drug related tolerance and elimination of the reaction produced can be studied. The tail flick test is an early form of thermal testing. The tail of a rat is placed in a groove and a focused beam of light directed on the tail. The number of watts and amount of time needed to get a specific response – a tail twitch – is noted. The
rats are or can be treated with analgesics and retested. The Hargreaves test is similar to the tail flick test with the beam of light directed on a rear paw instead of the tail.

Another form of thermal threshold testing is the tail immersion test, where a liquid is either heated to 50-55°C or cooled below 0°C and the tail is placed into the liquid. The response, either immediate withdrawal or not, and the time to which a reaction occurs is documented. The tail immersion test is typically consistent in demonstrating a tail withdrawal response, and a latency from tail withdrawal when rats are treated with drugs that increase nociceptive thresholds. The consistent reactions to variable conditions and treatments makes the tail immersion test favorable with laboratory animal testing.

Hot plate testing uses a porcelain or metal surface onto which a small animal is placed. The surface is heated to a controlled temperature between 50-55°C until an avoidance response occurs. A disadvantage of this test is that some animals show behavioral tolerance that includes a decrease latency and sensitivity both to the heat source and to the antinociceptive agents.

### 1.4.1 Latency to Response to Constant Temperature

A relatively useful form of thermal threshold testing has been described and tested in a variety of species, including horses, dogs, and cats. A light bulb or heat element provides a heat source encased in a metal cylinder that prevents direct contact of the bulb to the skin (Figure 4). A positive response as indicated by any attempt to bite at or look at the region tested, or purposeful movement away from the stimulus is recorded. This is relative to the duration and intensity (watt/second) of heat administration and the resulting skin temperature during heat administration. While an avoidance response to heat is the goal,
damage to the skin must be avoided for the test to be useful and repeatable. Once the control threshold time and temperature is determined the test drug is administered and the test repeated. The latency to response to an intensity that is increased in a linear, ramped manner is again identified. The two times are then compared and an antinociceptive effect is determined.

![Figure 4: Heat Lamp Analgesia Meter](image)

The intensity of heat increases in a linearly fashion (2 watts per second). Heat delivered halogen bulb (5V, 50 W) housed in metal cylinder (3 cm in height x 5.7 cm in diameter).

1.5 Sedation Scoring

Sedation scoring determines whether the effects of a drug are effective in decreasing a patient’s stress, awareness to environmental stimulation, and overall response to nociception. Overly sedate animals may experience unwanted side effects such as respiratory depression and obtundation. Sedation scoring systems have been developed in human medicine and have been modified to fit veterinary medicine behavior models. Commonly used human sedation scales include the Richmond Agitation-Sedation Scale (RASS), the Ramsay Scale, the Minnesota Sedation Assessment Tool (MSAT), and the University of Michigan Sedation Scale (UMSS). The American Society of
Anesthesiologists quantify the quality of sedation in four categories.\(^7\) The first category is “minimal sedation”, in which the patient will respond as expected to verbal stimulation. The second category is “moderate sedation”, where stimulation is responding to either touch or verbal stimulation. With “deep sedation”, the third category, response is limited to painful and repeated stimuli. The fourth category is “general anesthesia”, in which the patient fails to show any arousal despite application of painful stimuli.\(^7\) The simple descriptive scales in veterinary medicine were modeled after those in human medicine. Since animals cannot respond in the same fashion as humans, the sedation levels in animals are determined by evaluating their response to noise, touch, sound, facial expression, overall mentation, ability to stand and or right themselves following drug administration.\(^6,5^\) Sedation scales in veterinary medicine have been used to assess the effects of fentanyl in dogs \(^7\), sheep \(^2\), cats \(^4\), llamas \(^3,4,46\), and alpacas.\(^7\) A sedation scale used in llamas used a scale with levels ranging from 0, 1, 2, 3 and 4.\(^37\) A grade 0 indicates no sign of sedation; grade 1 indicates signs of mild sedation (lowering of the head, protrusion of the lower lip); grade 2 shows signs of sedation with mild ataxia and no recumbency; with grade 3 indicates obvious sedation and recumbency, however the llama can be roused with stimulation; and grade 4 indicates obvious sedation with a failure to respond to stimulation.

1.6 Evaluating Fentanyl in Plasma

Numerous tests are used to detect and quantify fentanyl in the blood and urine in various species. These tests include radioimmunoassay \(^14,18,21,23,30,33,36\), the enzyme-linked immunosorbent assay (ELISA) \(^37\), gas chromatography \(^11,38,74\), and high performance liquid chromatography.\(^16,17,20,26,28,29,35\)
1.6.1 Radioimmunoassay

The radioimmunoassay (RIA) utilizes the specific binding of an antibody to its specific antigen in order to measure either antigen or antibodies.\(^{14}\) For the fentanyl RIA, antibodies coating the RIA collection tubes are specific to fentanyl allowing for plasma detection. It has been demonstrated in horses\(^ {14}\) that the test does not cross react with major metabolites. This is relevant since early immunoassays cross reacted with metabolites in the bovine leading to inaccurate results, so the test should be specific for the parent drug (fentanyl) and not its metabolites. Radioimmunoassay has been used for fentanyl detection in various species including the horse\(^{13, 14}\), goat\(^ {21}\), dog\(^ {30}\) and cat.\(^ {18}\) The limit of quantification (LOQ) is the smallest concentration of a substance that can be accurately detected. The LOQ for fentanyl via RIA was determined to be 0.1 ng/mL in the cat\(^{18}\), dog\(^ {30}\), and goat\(^ {21}\), 0.2 ng/mL in the horse.\(^ {14}\)

1.6.2 Enzyme-Linked Immunosorbent Assay

The ELISA test has been used to determine the concentration of fentanyl present in plasma or serum. This test is based on the competition for limited antibody sites between the drug or its metabolites in the serum sample and the drug-enzyme conjugate. The amount of bound drug is determined by the degree of color change in the substrate. The extent of the color development is inversely proportional to the amount of drug found in the serum. Fentanyl serum detection as low as 0.14 ng/mL using the ELISA has been used successfully demonstrated with llamas.\(^ {37}\)
1.6.3 Gas Chromatography

Gas chromatography is used for separating and analyzing organic compounds in a vaporized phase without decomposition of the analyte. This method is used to analyze volatile substances in the gas phase.\textsuperscript{74} Gas chromatography has been used to demonstrate fentanyl concentrations in dogs.\textsuperscript{11, 74} The LOQ has been reported to be 0.2 ng/mL, compared to liquid chromatography with an LOQ of 0.05 ng/mL.\textsuperscript{16}

1.6.4 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) (Figure 5) is a type of liquid chromatography that is used to separate and quantify compounds that have been dissolved in a solution. High performance liquid chromatography can determine the specific amount of each component in a solution. A solvent travels through a continuously flowing system and is injected with the test analyte. This is known as the mobile phase. The analyte enters a column filled with solid adsorbent material. Upon entering the column at a pressure of 1,000 bar/15 kPSI, the sample enters the stationary phase where analytes are separated by adsorption. The column adsorbent will either be polar or non-polar. A polar hydrophilic analyte will move through a non-polar column quickly. A polar hydrophobic analyte will be adsorbed in the non-polar tube. The more hydrophobic the molecule is, the stronger its bind will be in the stationary phase. Hydrophilic molecules will be eluted first in a hydrophobic stationary stage. Hydrophobic molecules will have increased affinity for the hydrophobic stationary phase.\textsuperscript{78}
Once separated, isocratic or gradient analysis will be performed on the compounds being tested. The analytes will then be exposed to ultraviolet and visible light. Finally a chromatogram will be produced. That shows the absorption peak and retention time. This will help identify the analyte and the chromatogram serves as a quality control for the analysis.\textsuperscript{79}

High performance liquid chromatography was used in this study to analyze the plasma concentrations of fentanyl in alpacas. High performance liquid chromatography has been used in various fentanyl pharmacokinetic studies with various animals, and because the test is able to detect drug at very low concentrations, (0.05 ng/mL), is considered the gold standard.\textsuperscript{16, 17, 26, 80} In a study comparing radioimmunoassay to liquid chromatography mass spectrometry, Thomasy et al. 2008 found that radioimmunoassay overestimated the low fentanyl concentrations and underestimated high fentanyl plasma concentrations in horses.\textsuperscript{80}

Figure 5: High Performance Liquid Chromatography System (Source Image: http://web.nmsu.edu/~kburke/Instrumentation/Waters_HPLCSystem.gif)
Chapter 2: Experimental Study

2.1 Alpacas

This study was conducted on six healthy adult alpacas. There were three intact males and three intact females, with a mean body weight of $60 \pm 21.1$ kg and a mean age of $4.8 \pm 3.2$ years. Alpacas were determined to be healthy based on physical examination, complete blood count, biochemistry profile, and fecal analysis. The alpacas were part of The Ohio State University teaching herd, and were given one week acclimation time when transferred to the experimental stall housing. The Institutional Animal Care and Use Committee of The Ohio State University approved the study (2014A00000132).

2.2 Experimental Design

The study consisted of two trials with a week in between trials. All treatments were administered between 7 and 9 a.m. Baseline parameters were obtained and recorded prior to drug administration and were collected at predetermined intervals throughout the trials. The alpacas were fed hay at normally scheduled times and free choice water was available throughout the study.

Alpacas received 0.6 mg/kg xylazine (Vedco, Inc., St. Joseph, MO, USA) IM for sedation for placement of one TD or two IV jugular catheters at least 12 hours before fentanyl administration. The fiber was removed and the skin was aseptically prepared for
catheter placement. Prior to catheter placement, 1 mL of 2% lidocaine (Vedco Inc., St. Joseph, MO, USA) was injected subcutaneously. A 16 gauge 3.5 inch catheter (Angiocath, Parke, Davis & Company, Sandy, UT, USA) was placed in the left jugular vein for blood collection in both trials. Additionally, an 18 gauge 2.5 inch catheter (Surflo catheter, Terumo Medical Corporation, Elkton, MD, USA) was placed in the right jugular vein in the IV fentanyl citrate (Abbott Laboratories, North Chicago, IL, USA) group.

Fiber along the medial side of the right antebrachium was removed to allow for assessment of mechanical thresholds. Additionally, fiber was removed over both sides of the abdomen for assessment of thermal threshold (left) and mechanical threshold (right). The fiber over the left antebrachium was removed for placement of the transdermal fentanyl patch(es) (Duragesic, Janssen Pharmaceuticals, Inc., Titusville, VJ, USA). An elastic bandage (Elastikon, Johnson and Johnson, New Brunswick, NJ, USA) was placed circumferentially around the limb over the fentanyl patches.

2.3 Monitoring

Heart rate was auscultated with a stethoscope, and visual chest excursions were recorded prior to sample collections. Rectal temperatures were obtained with a digital thermometer prior to collection times in alpacas in the transdermal group.

2.4 Sample Collection

Seven mL of blood was collected from the left jugular catheter prior to (baseline) and at 1, 2, 5, 10, 15, 30, 45, 60, 90 minutes, and 2, 4, 8, 12 and 24 hours after IV fentanyl
administration. Seven mL of blood was collected prior to (baseline) and at 15, 30, and 45 minutes and 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hours after TD fentanyl patch placement. Blood samples were placed in evacuated, heparinized glass tubes, stored on ice, and centrifuged within 1 hour of collection at 4,000 x g for 10 minutes. Plasma was separated and frozen at -80 °C until analysis.

2.4.1 Drug Analysis

Plasma concentrations of fentanyl and norfentanyl were determined using liquid chromatography-triple quadrupole mass spectrometry (Thermo-Dionex "Ultimate" 3000 RSLC system and TSQ Quantum Ultra ESR mass spectrometer, Thermo-Scientific, Waltham, MA, USA). Data acquisition and peak integration were performed (LCQuan version 2.7 (SP1) ThermoFisher Scientific, Waltham, MA, USA). The analyte separation was performed using an Agilent Zorbax C-18 (Agilent Technologies, Santa Clara, CA, USA) extended C-18, 2.1 x 50 mm, 3.5 μM particle size and gradient elution using 0.1% formic acid in water (solvent A) and acetonitrile (Thermo-Scientific, Waltham, MA, USA) with 0.2% formic acid (Sigma Aldrich, St. Louis, MO, USA) (solvent B). Initial conditions were flow rate of 0.35 mL/min with 95% A/5% B, with a linear increase in B to 100% over 3 minutes, held for 1 minute, and returned to 5% B over 0.1 minute with a 1.9 minutes re-equilibration. The column temperature was maintained at ambient temperature (22 ± 2 ºC) and the autosampler maintained sample temperatures at 4°C. Retention times of fentanyl and norfentanyl were 2.56 and 2.16 minutes respectively. Fentanyl (Fentanyl 1.0 mg/mL, Sigma Aldrich (Cerilliant), St. Louis, MO, USA) was monitored in positive electro-spray ionization mode with selective reaction monitoring at m/z 337 → 188. Fentanyl d-5 (Fentanyl d-5 100
µg/mL, Sigma Aldrich (Cerilliant), St. Louis, MO, USA) was used as an internal standard and monitored m/z 341.4 → 188. The primary fentanyl metabolite, norfentanyl (norfentanyl 1.0 mg/mL, Sigma Aldrich (Cerilliant), St. Louis, MO, USA) was monitored at m/z 233.25 → 84.38 and norfentanyl d-5 (norfentanyl d-5 100 µg/mL, Sigma Aldrich (Cerilliant), St. Louis, MO, USA) was used as an internal standard with transition at m/z 238.27 → 84.38. Collision energy for fentanyl was set at 22 (fentanyl) and 23 (fentanyl d-5) and 18 for norfentanyl and norfentanyl d-5. The tube lens was set to 68 V for fentanyl and 80 V for norfentanyl.

Working solutions of the internal standards (fentanyl, fentanyl d-5, norfentanyl, norfentanyl d-5) were prepared by diluting the stock solution with appropriate volumes of methanol (Thermo-Scientific, Waltham, MA, USA). Calibration curves were prepared by serial dilution of stock solutions using methanol to provide concentrations from 0.05 - 100 ng/mL. Quality control (QC) samples (0.5, 1, 5, 50, 100 ng/mL) were prepared in identical fashion. Calibration and quality control samples were prepared in 100 µL of blank alpaca plasma by adding the appropriate amount of each analyte, followed by addition of 10 µL IS solution. Alpaca PK blood samples were handled in identical fashion including 10 µL of IS to each sample. The samples were vortexed for 30 sec and 1 mL of acetonitrile (4 °C) was added to the tube, re-vortexed and centrifuged at 14,000 rpm for 10 minutes at 4 °C. The solvent was removed and dried under nitrogen. After drying, the samples were reconstituted in 100 µL of mobile phase B. Eighty µL of standards, QC samples and unknowns were placed into individual autosampler vials for analysis and 15 µL was injected.

Analysis of fentanyl and norfentanyl using this method was validated by use of internal standards, calibration standards and defining the linearity of the detector responses over a range of known concentrations. The calibration curves, prepared for each day of analysis
included the range of concentrations in spiked alpaca plasma samples (0, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 100 ng/mL). Quality control samples were 0.05, 0.1, 0.5, 1, 5 and 10 ng/mL. The lower limit of quantitation (LLOQ) for fentanyl was 0.05 ng/mL and norfentanyl 0.1 ng/mL.

The mean ± SD fentanyl concentrations within-run were determined by calculation of the response obtained from the replicates using the corresponding slope and intercept from that run’s standard curve and back-fitting the response recorded for each sample. The method accuracy was determined by defining the relationship between ng of calibrator added to ng of calibrator found from 6 replications of 5 control samples (0.05 - 5 ng/mL) injected in one run on each of 4 days and expressed as a percentage. The method precision is reported as the relative standard deviation (CV%). Calibration curves were linear from 0.05 - 100 ng/mL with correlation coefficients of >0.99. Method accuracy, precision and LLOQ determined for this assay were: fentanyl (96 ± 8.8%, 7 ± 4% and 0.05 ng/mL respectively) and norfentanyl (92 ± 10%, 8 ± 4% and 0.1 ng/mL respectively).

2.5 Assessment of Antinociception

Algometry was assessed using the force one algometer. Thermal threshold was tested using the heat lamp analgesiometer.

2.5.1 Thermal Threshold Testing

A heat lamp analgesiometer (Heat lamp analgesiometer, Columbus International Corporation, Columbus, OH) was used to test superficial analgesia at the region of the right
abdomen in alpacas in both trials. This heat device produces a stimulus delivered via a halogen bulb (5 V, 50 W). The intensity of heat increases in a linearly fashion (2 watts per second). A halogen bulb is encased in a metal cylinder (3 cm x 5.7 cm), which is placed directly on the skin surface. Antinociception was assessed by the recording the time it took from starting the heat source to when the alpaca responded. A reaction was defined as any attempt to bite at the region tested, to look at the tested site, or purposeful movement away from the stimulus. The time that passed until such a described reaction occurred was defined as the thermal threshold. If no reaction occurred after 25 seconds the stimulus was terminated to prevent skin damage. This method has not been validated in alpacas, however it has been used to demonstrate analgesia without causing damage to skin in horses.43

2.5.2 Mechanical Threshold Testing

To evaluate mechanical nociception, a hand held algometer (FDIX25 Algometer, Wagner Instruments, Greenich, CT) was manually applied to the right abdomen and the medial surface of the right antebrachium. The 1 cm² probe tip of the algometer was applied perpendicular to the skin surface. Direct pressure was applied to the surface of the tested region until the alpaca reacted or a maximal force of 5 kg was reached. A positive response was defined as vocalization of the alpaca, turning its head toward the stimulus, moving away from the algometer, or deliberately moving the limb being tested.

2.6 Assessment of Sedation

Sedation scoring was performed before entering the stall just prior to handling. The scoring system utilized has been previously used in llamas 37 and alpacas.73 Briefly, grade 0
indicates no sign of sedation; grade 1 indicates signs of mild sedation (lowering of the head, protrusion of the lower lip); grade 2 shows signs of sedation with mild ataxia and no recumbency; grade 3 indicates obvious sedation and recumbency, however the alpaca can be roused with stimulation; and grade 4 indicates obvious sedation with a failure to respond to stimulation.

2.7 Drug Dosage and Route of Administration

Intravenous fentanyl citrate was administered at a dosage of 2 µg/kg followed immediately by 10 mL 0.9% NaCl to ensure complete intravenous administration. Duragesic® fentanyl patches were applied such that they received a total dose approximating 2µg/kg/hr. If the dosage required based on the animals body weight exceeded that provided by a single patch, addition fentanyl patches were placed to meet the 2 µg/kg/hr target administration dose. After clipping the fiber the skin was wiped with 70% isopropyl alcohol and allowed to dry. The transdermal fentanyl patches were applied to the skin overlying the left antebrachium and left in place for 60 hours. The drug dosage and route of administration were determined based on published studies using similar dosages among various species with favorable results.13, 14, 17- 21, 23, 25, 32, 34, 36, 72, 76

2.8 Study Hypothesis

Our hypotheses were that the pharmacokinetics and pharmacodynamics of fentanyl would be similar to those in other species, and TD and IV administration at these dosages would produce antinociception and sedation in alpacas.
2.9 Statistical Analysis

Plasma concentration versus time (IV and TD data) was subjected to non-compartmental pharmacokinetic analysis. Sedation scores, heart rate (HR), respiratory rate (RR), algometry, and thermal threshold data were examined for normal distribution using the Kolmogorov-Smirnov test. Sedation scores, heart rate, respiratory rate, algometry, and thermal threshold data were not normally distributed and were analyzed using the Kruskal Wallis test. Sedation scores were compared to heart rate, respiratory rate, algometry, and fentanyl plasma concentrations from the same population using the Mann-Whitney U test. \( P \) values \(<0.05\) were considered statistically significant.
Chapter 3: Results

3.1 Pharmacokinetic Results

The plasma drug concentration achieved over time was determined using a non-compartmental model. Maximum plasma fentanyl concentration (Cmax) was $4.6 \pm 1.8$ ng/mL after IV administration (Figure 6). Clearance was $921 \pm 189$ mL/kg/hr, volume of distribution $4.3 \pm 1.8$ L/kg, and mean (range) half-life (terminal phase) of 3.1 (1.87-7.2) hours (Table 1). Plasma norfentanyl was found in a single alpaca at the 90 and 120 minute time points however, both concentrations were below the lower limit of quantification (LLOQ).

Pharmacokinetic parameters comparing male and female alpacas were derived from the statistical moment analysis of plasma concentration versus time data of fentanyl citrate administered intravenously. There was statistically significant differences in average total clearance and average half-life comparing male to female alpacas. Average total clearance in female alpacas reported as $769.4$ mL/kg/hr compared to $1073.4$ mL/kg/hr in males ($P = 0.054$). Average half-life times between male and female alpacas were 1.86 hours and 5.2 hours respectively ($P = 0.027$). Average volume of distribution at terminal phase was $2890$ mL/kg in males, $5731$ mL/kg ($P=0.062$) trending to statistical significance (Table 2).

Actual TD fentanyl dosages ranged from 1.95 to 2.12 μg/kg/hr because multiple fentanyl patches were applied to approximate the targeted 2 μg/kg/hr dose. Following application of TD fentanyl, Cmax of $1.3 \pm 0.8$ ng/mL was detected at $29 \pm 14$ hours (Figure 7). Mean residence time was $42 \pm 8$ hours.
Figure 6: Semilogarithmic plot of plasma concentration versus time after IV administration of fentanyl citrate 2 µg/kg
<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV</th>
<th>TD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; (hr*ng/mL)</td>
<td>2.0 ± 0.4</td>
<td>60 ± 15</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-inf&lt;/sub&gt; (hr*ng/mL)</td>
<td>2.3 ± 0.5</td>
<td>63 ± 14.5</td>
</tr>
<tr>
<td>AUMC&lt;sub&gt;inf_obs&lt;/sub&gt; (hr<em>hr</em>ng/mL)</td>
<td>8.3 ± 6</td>
<td>2550 ± 202</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>4.6 ± 1.8</td>
<td>1.3 ± 0.78</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>—</td>
<td>29 ± 14</td>
</tr>
<tr>
<td>CL (mL/hr/kg)</td>
<td>921 ± 189</td>
<td>—</td>
</tr>
<tr>
<td>V&lt;sub&gt;ss&lt;/sub&gt; (mL/kg)</td>
<td>4310.5 ± 1800</td>
<td>—</td>
</tr>
<tr>
<td>Elimination half-life (hr)</td>
<td>3.1 (1.87-7.2)*</td>
<td>16.5 (10-22)*</td>
</tr>
<tr>
<td>MRT&lt;sub&gt;inf_obs&lt;/sub&gt; (hr)</td>
<td>3.4 ± 1.95</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>MAT (hr)</td>
<td>—</td>
<td>39 ± 8</td>
</tr>
<tr>
<td>F%</td>
<td>—</td>
<td>0.95 ± 0.17</td>
</tr>
</tbody>
</table>

AUC<sub>0-last</sub> = area under the curve from time = 0 to the last quantifiable time point. AUC0-inf = Area under curve extrapolated to infinity; AUMC inf_obs = Area under moment curve, extrapolated to infinity; C<sub>max</sub> = maximum plasma concentration. T<sub>max</sub> = time for maximum plasma concentration. CL = total body clearance. V<sub>ss</sub> = volume of distribution at steady state; MRT = mean residence time (hr); MAT = Mean absorption time (hr); F = bioavailability, determined comparing the AUC<sub>0-∞</sub> after IV and transdermal dosing and normalizing for dose — = Not applicable; * = elimination half-life listed as geometric mean (range).

Table 1: Mean ± standard deviation of pharmacokinetic parameters from non-compartmental model after IV and TD administration of fentanyl
Table 2: Individual animal pharmacokinetic parameters from male and female alpacas derived from statistical moment analysis of plasma concentration versus time data of fentanyl citrate administered intravenously.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (mL/hr/kg)</td>
<td>954</td>
<td>1209</td>
<td>1057</td>
<td>926</td>
<td>636</td>
<td>746</td>
<td>0.054</td>
</tr>
<tr>
<td>HL&lt;sub&gt;λz&lt;/sub&gt; (hr)</td>
<td>1.8</td>
<td>1.9</td>
<td>1.9</td>
<td>4.1</td>
<td>4.4</td>
<td>7.2</td>
<td>0.027</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>1.8</td>
<td>1.91</td>
<td>1.95</td>
<td>3.1</td>
<td>4.1</td>
<td>7.3</td>
<td>0.08</td>
</tr>
<tr>
<td>VD&lt;sub&gt;ss&lt;/sub&gt; (mL/kg)</td>
<td>1718</td>
<td>2308</td>
<td>2058</td>
<td>2886</td>
<td>2615</td>
<td>5472</td>
<td>0.153</td>
</tr>
<tr>
<td>V&lt;sub&gt;z&lt;/sub&gt; (mL/kg)</td>
<td>2498</td>
<td>3317</td>
<td>2855</td>
<td>5481</td>
<td>3995</td>
<td>7715</td>
<td>0.062</td>
</tr>
</tbody>
</table>

CL = total body clearance; HL = half-life; VD<sub>ss</sub> = volume of distribution at steady state; MRT = mean residence time (hr); V<sub>z</sub> = volume of distribution during the terminal phase.
Figure 7: Plasma concentration versus time of fentanyl in alpacas after transdermal fentanyl patch administration (2 μg/kg/hr)

3.1 Physiologic Results

There were no significant differences in HR, RR, or sedation score over time after IV or TD fentanyl (Table 3). However, there were observational differences between male and female alpacas achieving levels of either sedation or excitation.
Table 3: Median and range cardiopulmonary variables in 6 healthy alpacas administered fentanyl (2 µg/kg) IV or (2 µg/kg/hr) transdermal patch

<table>
<thead>
<tr>
<th>Time</th>
<th>Heart Rate</th>
<th>Respiratory Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV</td>
<td>TD</td>
</tr>
<tr>
<td>0 min</td>
<td>48 (36-60)</td>
<td>60 (52-80)</td>
</tr>
<tr>
<td>1 min</td>
<td>42 (32-48)</td>
<td>—</td>
</tr>
<tr>
<td>2 min</td>
<td>42 (28-120)</td>
<td>—</td>
</tr>
<tr>
<td>5 min</td>
<td>58 (32-100)</td>
<td>—</td>
</tr>
<tr>
<td>10 min</td>
<td>72 (36-100)</td>
<td>—</td>
</tr>
<tr>
<td>15 min</td>
<td>54 (36-84)</td>
<td>52 (44-80)</td>
</tr>
<tr>
<td>30 min</td>
<td>52 (36-60)</td>
<td>50 (44-80)</td>
</tr>
<tr>
<td>45 min</td>
<td>42 (32-48)</td>
<td>56 (48-80)</td>
</tr>
<tr>
<td>1 hour</td>
<td>48 (36-48)</td>
<td>56 (48-100)</td>
</tr>
<tr>
<td>1.5 hours</td>
<td>46 (36-64)</td>
<td>—</td>
</tr>
<tr>
<td>2 hours</td>
<td>42 (36-60)</td>
<td>54 (44-68)</td>
</tr>
<tr>
<td>4 hours</td>
<td>44 (40-68)</td>
<td>50 (48-60)</td>
</tr>
<tr>
<td>8 hours</td>
<td>48 (40-60)</td>
<td>48 (48-60)</td>
</tr>
<tr>
<td>12 hours</td>
<td>56 (40-68)</td>
<td>60 (48-60)</td>
</tr>
<tr>
<td>24 hours</td>
<td>48 (40-56)</td>
<td>50 (48-64)</td>
</tr>
<tr>
<td>36 hours</td>
<td>—</td>
<td>54 (48-64)</td>
</tr>
<tr>
<td>48 hours</td>
<td>—</td>
<td>60 (48-64)</td>
</tr>
<tr>
<td>60 hours</td>
<td>—</td>
<td>56 (48-60)</td>
</tr>
<tr>
<td>72 hours</td>
<td>—</td>
<td>50 (48-68)</td>
</tr>
<tr>
<td>84 hours</td>
<td>—</td>
<td>54 (48-64)</td>
</tr>
<tr>
<td>96 hours</td>
<td>—</td>
<td>60 (52-60)</td>
</tr>
</tbody>
</table>

— = Not applicable.
3.2 Sedation Results

Sedation after IV fentanyl tended to be minimal with a median sedation score of 1 (range 0 to 1). All female alpacas showed signs of sedation. Sedation, if it occurred, was apparent within 5 minutes of IV fentanyl administration. Sedation scores returned to zero by 45 minutes (range 1 to 45 minutes). Instances of excitation were noted in 3 alpacas, two male and one female after IV fentanyl. Excitation occurred 1 to 3 minutes following the injection and increased the resistance to restraint and handling. Two instances of rearing when the male alpacas were approached were observed. Excitement resolved within 15 minutes. One male alpaca that exhibited excitement was also noted to briefly have a hypermetric gait. Two alpacas that became excited failed to show signs of sedation. One male alpaca that initially showed sedation also showed a brief period of excitation.

Sedation scores of 1 were observed in all 3 males within 24 hours of TD fentanyl application. Duration of sedation was 2 hours in one alpaca and 24 hours in other two. All sedated alpacas returned to a sedation score of 0 by 60 hours. The three female alpacas did not show signs of sedation after TD fentanyl. Excitation was not observed at any time after TD fentanyl patch placement (Table 4).
Sedation data are reported as median (range) values as measured from the time that the fentanyl was administered. Sedation was assessed in real time and scored on a 5-point scale in which 0 = no sedation, 1 = mild sedation, 2 = obvious sedation with mild ataxia and no recumbency, 3 = obvious sedation and recumbency with arousal after stimulation, and 4 = obvious sedation and recumbency.

Table 4: Sedation variables in six healthy alpacas that received fentanyl (2 µg/kg) IV and (2 µg/kg/hr) TD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV (Minutes)</th>
<th>TD (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to onset of sedation</td>
<td>5 (5-30)</td>
<td>24 (24-60)</td>
</tr>
<tr>
<td>Peak sedation score</td>
<td>1 (0-1)</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>Time to peak sedation</td>
<td>5 (1-30)</td>
<td>24 (24-60)</td>
</tr>
<tr>
<td>Time to return to baseline sedation</td>
<td>45 (15-45)</td>
<td>72 (0-72)</td>
</tr>
</tbody>
</table>

3.3 Mechanical threshold results

Scores for abdominal algometry were greater than baseline at 45 minutes and scores for limb algometry were greater than baseline at 15 minutes after IV fentanyl (Table 5) (Figures 8 – 11). There were no differences in abdominal or limb algometry following TD fentanyl compared to baseline values for the same treatment group.
<table>
<thead>
<tr>
<th>Time</th>
<th>Abdominal force (kg)</th>
<th>Limb force (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IV</td>
<td>TD</td>
</tr>
<tr>
<td>0 min</td>
<td>0.6 (0.3-1.0)</td>
<td>1.7 (0.7-3.3)</td>
</tr>
<tr>
<td>1 min</td>
<td>1.0 (0.7-1.3)</td>
<td>—</td>
</tr>
<tr>
<td>2 min</td>
<td>1.3 (0.8-1.7)</td>
<td>—</td>
</tr>
<tr>
<td>5 min</td>
<td>1.4 (0.6-1.6)</td>
<td>—</td>
</tr>
<tr>
<td>10 min</td>
<td>1.3 (0.2-2.1)</td>
<td>—</td>
</tr>
<tr>
<td>15 min</td>
<td>1.4 (1.1-1.5)</td>
<td>1.6 (0.6-2.2)</td>
</tr>
<tr>
<td>30 min</td>
<td>1.3 (0.9-1.8)</td>
<td>1.4 (1.2-2.8)</td>
</tr>
<tr>
<td>45 min</td>
<td>1.1 (0.6-3.9)*</td>
<td>1.7 (1.3-2.3)</td>
</tr>
<tr>
<td>1 hour</td>
<td>1.0 (0.9-1.5)</td>
<td>1.5 (1.2-2.0)</td>
</tr>
<tr>
<td>1.5 hours</td>
<td>1.1 (0.9-1.4)</td>
<td>—</td>
</tr>
<tr>
<td>2 hours</td>
<td>1.1 (0.7-1.5)</td>
<td>1.8 (1.0-3.6)</td>
</tr>
<tr>
<td>4 hours</td>
<td>1.0 (0.8-1.5)</td>
<td>1.6 (1.3-2.0)</td>
</tr>
<tr>
<td>8 hours</td>
<td>0.7 (0.5-0.9)</td>
<td>2.1 (1.1-4.5)</td>
</tr>
<tr>
<td>12 hours</td>
<td>1.1 (0.7-1.6)</td>
<td>1.6 (1.4-2.2)</td>
</tr>
<tr>
<td>24 hours</td>
<td>1.0 (0.6-1.4)</td>
<td>1.9 (0.9-2.3)</td>
</tr>
<tr>
<td>36 hours</td>
<td>—</td>
<td>1.5 (1.3-2.0)</td>
</tr>
<tr>
<td>48 hours</td>
<td>—</td>
<td>1.9 (1.4-2.5)</td>
</tr>
<tr>
<td>60 hours</td>
<td>—</td>
<td>1.4 (1.2-1.9)</td>
</tr>
<tr>
<td>72 hours</td>
<td>—</td>
<td>1.4 (1.2-3.2)</td>
</tr>
<tr>
<td>84 hours</td>
<td>—</td>
<td>1.5 (0.9-2.7)</td>
</tr>
<tr>
<td>96 hours</td>
<td>—</td>
<td>1.4 (0.8-2.1)</td>
</tr>
</tbody>
</table>

— = Not applicable. * = statistically significant from the baseline value for the same treatment group P < 0.05

Table 5: Median and range for abdominal and limb algometry variables in 6 healthy alpacas administered fentanyl (2 µg/kg) IV or (2 µg/kg/hr) transdermal patch
Figure 8: Effects on limb mechanical nociception over time after transdermal fentanyl patch application 2 µg/kg/hr
Figure 9: Effects on abdominal mechanical nociception over time after transdermal fentanyl patch application 2 µg/kg/hr
Figure 10: Effects on abdominal mechanical nociception over time after intravenous fentanyl administration 2 µg/kg. Asterisk indicates a statistical difference from the baseline value for the same treatment group $P < 0.05$. Error Bars: 95% CI.
3.4 Thermal threshold results

There was no significant change in thermal threshold after IV fentanyl. There was a significant increase in thermal threshold over time at 3600 minutes (60 hours) in the transdermal group (p = 0.027). Neither method of testing nociception produced visible tissue damage (Table 6) (Figure 12).
Figure 12: Effects on thermal threshold over time after transdermal fentanyl administration 2 µg/kg/hr. Asterisk indicates a statistical difference from the baseline value for the treatment group P < 0.05
<table>
<thead>
<tr>
<th>Time</th>
<th>IV</th>
<th>TD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>20 (5-25)</td>
<td>18 (1-25)</td>
</tr>
<tr>
<td>1 min</td>
<td>7 (4-25)</td>
<td>—</td>
</tr>
<tr>
<td>2 min</td>
<td>9 (4-25)</td>
<td>—</td>
</tr>
<tr>
<td>5 min</td>
<td>11 (5-25)</td>
<td>—</td>
</tr>
<tr>
<td>10 min</td>
<td>15 (3-25)</td>
<td>—</td>
</tr>
<tr>
<td>15 min</td>
<td>10 (4-25)</td>
<td>5 (2-19)</td>
</tr>
<tr>
<td>30 min</td>
<td>16 (5-25)</td>
<td>15 (9-21)</td>
</tr>
<tr>
<td>45 min</td>
<td>25 (6-25)</td>
<td>4 (3-23)</td>
</tr>
<tr>
<td>1 hour</td>
<td>23 (4-25)</td>
<td>13 (6-25)</td>
</tr>
<tr>
<td>1.5 hours</td>
<td>23 (3-25)</td>
<td>—</td>
</tr>
<tr>
<td>2 hours</td>
<td>10 (1-25)</td>
<td>6 (1-25)</td>
</tr>
<tr>
<td>4 hours</td>
<td>10 (4-21)</td>
<td>12 (2-19)</td>
</tr>
<tr>
<td>8 hours</td>
<td>15 (3-25)</td>
<td>16 (8-25)</td>
</tr>
<tr>
<td>12 hours</td>
<td>12 (7-25)</td>
<td>23 (17-25)</td>
</tr>
<tr>
<td>24 hours</td>
<td>17 (4-25)</td>
<td>14 (3-25)</td>
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<tr>
<td>36 hours</td>
<td>—</td>
<td>23 (14-25)</td>
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<tr>
<td>48 hours</td>
<td>—</td>
<td>7 (6-24)</td>
</tr>
<tr>
<td>60 hours</td>
<td>—</td>
<td>25 (20-25)*</td>
</tr>
<tr>
<td>72 hours</td>
<td>—</td>
<td>12 (4-22)</td>
</tr>
<tr>
<td>84 hours</td>
<td>—</td>
<td>19 (8-22)</td>
</tr>
<tr>
<td>96 hours</td>
<td>—</td>
<td>25 (4-25)</td>
</tr>
</tbody>
</table>

— = Not applicable. * = statistically significant difference

Table 6: Median and range of thermal threshold variables in 6 healthy alpacas administered fentanyl (2 µg/kg) IV or (2 µg/kg/hr) transdermal patch. Asterisk indicates a statistical difference from the baseline value for the same treatment group P < 0.05
Chapter 4: Discussion

Plasma concentrations of fentanyl declined rapidly after IV dosing. Fentanyl was rapidly distributed, and eliminated after IV administration. The plasma clearance was similar to dogs\textsuperscript{17} and goats\textsuperscript{20} the volume of distribution was greater in alpacas compared to that in goats\textsuperscript{20} and horses.\textsuperscript{14} There was a significant difference between male and female alpacas in the elimination half-life of the terminal portion of the concentration versus time curve. While there was no significant difference in clearance between male and female alpacas, there was evidence to support a wider volume of distribution in the females when compared to the males. Since the elimination half-life is related to the clearance and volume of distribution, prolonged half-life in females compared to males suggests the wider volume of distribution prolongs the residence of fentanyl in camelids. Since the drug is rapidly cleared, a difference in VD produces a difference in the elimination rate. These observations are underpowered with only three intact male and female alpacas in each group. A power analysis indicates a sample size of twelve alpacas, six intact males and females would be an adequate number of animals to power these observations.

Plasma fentanyl concentrations after TD patch were first observed 30 minutes after placement of patch in one alpaca, 120 minutes in two alpacas and at 240 minutes in the remaining three alpacas. Mean absorption time for fentanyl was $39 \pm 8$ hours demonstrating that absorption of fentanyl through skin into plasma was rate limiting. The amount of drug that was systemically absorbed in the transdermal group was lower owing to the time required for absorption. The time it took the majority of alpacas to have a plasma
concentration of 1.0 ng/mL was 24 to 48 hours. Maximum plasma fentanyl concentrations peaked at 29 ± 16 hours after TD application with plasma concentrations that remained above the LLOQ (0.05 ng/mL) were detectable for up to 72 hours. Interestingly all three of the female alpacas reached peak fentanyl plasma concentration at 24 hours and two of the males at 48 hours. The final male reached peak fentanyl plasma concentrations at 8 hours. In llamas, Grubb et al. reported peak fentanyl plasma concentrations at 12 hours, with peak plasma concentrations of 0.3 ± 0.08 ng/mL.\textsuperscript{37} Transdermal uptake is variable among species with horses reaching peak plasma concentrations between 8 and 12 hours after patch application \textsuperscript{14} while other species such as dogs and cats reach peak plasma concentrations between 12 and 14 hours.\textsuperscript{30, 18} Plasma fentanyl levels were inconsistent and variable after patch application in our alpacas. Plasma concentrations were as high as 0.5 ng/mL within 1 hour of patch application in one alpaca and undetectable in the others until 120 minutes. Peak plasma concentrations were also highly variable with one alpaca having a peak plasma concentration of 3.0 ng/mL at 8 hours and 5 ranging between 0.89 and 1.2 ng/mL at 24 hours.

The variability in TD fentanyl plasma concentrations was similar to that found in sheep\textsuperscript{20}, goats\textsuperscript{21}, horses\textsuperscript{14}, pigs\textsuperscript{38} and llamas\textsuperscript{37} and is likely multifactorial. Application sites were clipped and the skin could have been damaged prior to patch application although no irritation was visible. Inflammation or micro breaches to the skin could have affected absorption. Application sites were also cleansed with alcohol prior to patch application, potentially altering TD uptake although the alcohol was allowed to dry prior to patch placement. The skin of the alpaca is thick over the back, which could affect absorption, however, patches were placed at an area of thin skin in the alpacas, which should result in improved absorption.\textsuperscript{32, 82, 83} The chosen site of placement (medial antebrachium) may have
been damaged by dermatologic conditions common to camelids (skin mites). These alpacas were not examined for skin mites.

Fentanyl produced minimal sedation in alpacas after 2 μg/kg IV and the degree of sedation was inconsistent. After administration of 2 μg/kg IV fentanyl, the sedative effects were rapid and short lived, offering potentially limited use as a premedication. The transdermal patch resulted in sedation in 3 of 6 alpacas after 24 hours of application.

The excitement observed in three alpacas could potentially be due to the rapid injection of the drug. Opioid dysphoria following intravenous fentanyl administration has been described in horses. In one equine MAC reduction study using fentanyl, target fentanyl plasma concentrations were 16, 24 and 32 ng/mL. Not only did the study fail to find a MAC reduction at these plasma concentrations, MAC increased by 1.2% for plasma fentanyl concentrations of 24.1 ng/mL. An unidentified number of horses from the study showed signs of excitement during the recovery period by attempting to stand prematurely, showing circling behavior followed by numerous falls. Once standing, mean plasma fentanyl concentrations were 5.70 ± 2.25 ng/mL. In standing adult horses, muscle fasciculations and increased locomotor activity was noted at fentanyl plasma concentrations of 7.8 μg/l. A study with escalating fentanyl bolus administration in foals showed similar signs of excited behavior when fentanyl plasma levels were 7.9 μg/l ± 6.6 μg/l. The foals also showed signs of head pressing and one excited foal tried climbing up the walls of its stall two minutes after drug administration. Another phenomenon to consider is opioid induced hyperalgesia, which causes nociceptive sensitization and consequently makes people more sensitive to a painful stimulus while taking opioids.

A dose response or chronic administration study could determine if sedation can be achieved without adverse effects. The sedation achieved was limited to standing quiet
behavior, which allowed for handling and examination with little to no resistance. One alpaca with a plasma concentration of 3.0 ng/mL at 8 hours after TD patch placement showed hyperactive behavior consisting of circling in the stall and hypersalivation. This behavior lasted until 8 hours later when the plasma concentration was determined to be 1.2 ng/mL. Plasma concentrations ranged from 1.5 to 3.9 ng/mL when limb tremors were observed. At fentanyl plasma concentrations between 1.6 and 1.9 ng/mL excitement behavior and increased activity were observed. Episodes of rearing occurred at fentanyl plasma concentrations between 0.4 and 2.2 ng/mL. Excitement was not anticipated prior to starting the study therefore an adjusted sedation scale was not used. This was a limitation of the study. The overall sedation may be over-reported since signs of excitement were reported as a sedation score of 0. A more precise scoring system, incorporating -1, -2, -3, and -4 to detail signs of excitement may have elucidated these differences. A scale in dogs recently prosed in dogs used a scale numbered 1-7. A grade 1 is very excitable, grade 2 moderately excitable, grade 3 slightly excitable, grade 4 normal, grade 5 subtle signs of sedation, grade 6 moderate signs of sedation and grade 7 very sedate.84

Alpacas treated with TD fentanyl had similarly variable pharmacodynamics results. A sedation score of 1 was recorded in 3 of 6 alpacas by 24 hours of patch placement. While signs of sedation were not observed in 3 alpacas, overall handling of, and performing physical examinations on, these animals became notably easier 24 to 48 hours following patch placement.

Minimum plasma fentanyl concentrations associated with analgesia have not been established in the sheep, horse, goat or llama. Analgesia was demonstrated between dogs that received a fentanyl patch compared to those dogs that did not based on the difference of
mean pain scores when fentanyl plasma concentrations were greater than 0.95 ng/mL following orthopedic surgery.\textsuperscript{30}

The use of TD fentanyl in alpacas for analgesia is not supported by the results of the study reported here. Overall, there were no consistent instances of antinociception in either the IV or TD group of alpacas. There were no statistically significant instances of antinociception demonstrated by the TD group using the algometer. There was one statistically significant increase to thermal threshold at 60 hours following TD application. The significant increases in algometry scores at 15 minutes after IV fentanyl administration were associated with peak plasma concentrations and peak sedation scores. The alpacas resisted having their extremities examined at all time points (before and after IV fentanyl administration), therefore this suggests that abdominal algometry more reliably assesses nociception although there was no consistent, statistically significant findings between the two methods. The results of limb algometry were more variable and subjectively seemed to stress the alpacas. The alpacas anticipated algometry and would move their limbs prior to being examined. Abdominal algometry was easier to perform and was not resisted by the alpacas.

There were no increases in algometry thresholds in the TD group of alpacas at plasma concentrations of 0.5 ng/mL and above. Two alpacas did not react to limb algometry at time points when peak plasma concentrations (1.3 ± 0.8 ng/mL) were measured (12 hours after patch application).

Antinociception measured at both antebrachial and abdominal sites yielded inconsistent results. Abdominal algometry appears to have more potential to assess nociception in alpacas based on the resistance to limb handling described herein. None of the alpacas in this study had surgery and therefore surgical pain and the efficacy of fentanyl for
treating such pain was not evaluated. Further study of IV fentanyl in alpacas to determine optimum dosing is warranted as a decrease in dosage may provide sedation without excitation.

There were only two significant time points indicating antinociception in the IV group and none were found in the TD group. These limited findings are not enough to state fentanyl administration in either group reliably demonstrated antinociception based on out testing methods. Additional studies in alpacas in experiencing clinical/surgical pain may provide a better evaluation of efficacy by evaluating fentanyl administration for the treatment of surgical pain and associated inflammation which is unlike the simulation provided in this study.

A limitation was the relatively small number of alpacas used. Some alpacas seemed more affected by IV fentanyl administration, displaying excitement, ataxia and sedation in rapid succession. These observations are underpowered and until additional studies are conducted, it cannot be concluded with certainty that there should be a dose reduction or adjustment in rate of drug administration. By comparison, none of the alpacas that none of the transdermal group showed excitation with a peak plasma concentration of 3.0 ng/mL. These findings should support the decision for future IV studies with a decreased IV dose.

Despite two of the alpacas reacting less to being handled, only half of the alpacas showed any signs of sedation in the transdermal group. The TD patch treated alpacas were not apprehensive to handling, thermal nociception testing, or algometry for up to 72 hours. Once the patches were removed and an additional 12 hours had passed, placing a halter on and examining the alpacas was subjectively more difficult than when the patches were in place.
During the study, the males seemed to demonstrate more instances of excitement and subjectively appeared more sedate than the females. Gender differences in rats, trout, chickens, ferrets, dogs and goats have demonstrated that sex hormones have an effect on drug metabolism. While an unexpected find, these behavior discrepancies are underpowered to be categorized as sexually determined. Interestingly, clinical use of TD fentanyl in alpacas has not been associated with observations of excitement or respiratory depression.

The pharmacokinetics for IV fentanyl administration reported in this study were similar to the horse, cat, dog, sheep, and goat. The pharmacokinetics for TD administration were similar to the sheep, goat and llama. The pharmacokinetics for TD fentanyl administration were variable among alpacas. This variability in TD fentanyl uptake is consistent with findings in the pig, llama, sheep and goat. Following IV and TD fentanyl administration, alpacas failed to demonstrate adequate antinociception. Our hypothesis that the alpaca would have similar pharmacokinetics compared to other species has been proven accurate. However, antinociception was infrequently demonstrated in either group leading us to reject the hypothesis that TD and IV would produce antinociception in alpacas following a 2 µg/kg IV bolus of fentanyl or after placement of a 2 µg/kg/hr TD patch.
References


