EVALUATION OF XENON$^{133}$ WASHOUT FOR MEASUREMENT OF LAMINAR BLOOD FLOW IN THE HORSE

by

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(Under the Direction of John F. Peroni)

## ABSTRACT

Laminitis is a painful, debilitating and life-threatening disease affecting horses worldwide. The disease can arise after local or systemic insults and the pathogenesis is not well understood. Alterations in laminar blood flow have been implicated in the pathogenesis of laminitis; however, controversy exists regarding the nature of these changes. The purposes of this study were to develop and evaluate a new technique for measuring laminar blood flow in horses.

Using a technique to measure cerebral blood flow in humans, xenon$^{133}$ in solution was injected intra-arterially in the metacarpal region of healthy horses and the gamma radiation emitted at the dorsal laminae was recorded.

The results of this study indicate that xenon$^{133}$ solution can be safely injected intra-arterially in healthy horses, and that similar peaks in gamma radiation are recorded at the hoof wall. Consequently, this technique may provide useful information about alterations in laminar blood flow in horses with laminitis.

INDEX WORDS: Xenon$^{133}$, Horse, Laminitis, Laminar blood flow

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# DEDICATION

To my mum, Anita Hatherley, for your unwavering support and encouragement, and for showing me what love, dedication, strength and determination really are.

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# CHAPTER 1

## INTRODUCTION

Laminitis is a condition that can affect horses of all breeds, ages and genders throughout the world. The condition often causes intense pain and lameness that may be sufficiently severe to require humane euthanasia. The pain is attributed to structural changes within the hoof capsule; the tissue that suspends the distal phalanx from the hoof capsule is damaged, allowing the distal phalanx to sink or rotate. This unnatural movement of the distal phalanx disrupts additional tissues within in the hoof capsule including the vasculature and the corium of the sole and coronary band.

Although there are known predispositions to laminitis in horses, the pathogenesis remains controversial and there are several hypotheses proposed for the underlying mechanisms. The disease is most likely multi-factorial, with vascular dysfunction contributing to its pathogenesis, as changes in digital and laminar blood flow have been documented during the prodromal stages of experimentally-induced laminitis. Despite numerous attempts to measure laminar blood flow, contradictory results about the nature of these changes have been reported and a clinically applicable method is lacking.

One of the main limitations in measuring laminar blood flow in the horse is the location of the vessels of interest, as they are protected by the keratinized hoof capsule. The presence of this relatively impenetrable hoof capsule prohibits the use of certain

imaging modalities, such as ultrasonography, that frequently are used to measure peripheral blood flow. In human medicine, measurement of cerebral blood flow similarly is limited by the protection afforded by the relatively impenetrable cranium. This limitation has been overcome by using a xenon$^{133}$ washout technique to measure cerebral blood flow. Therefore, the aims of this study were to develop and evaluate a new method for measuring laminar blood flow in the horse using the xenon$^{133}$ washout technique extrapolated from the human literature.

# CHAPTER 2

# LITERATURE REVIEW

## SECTION 1: OVERVIEW OF LAMINITIS

Laminitis is a disease that affects the tissues that attach the hoof wall to the underlying distal phalanx within the hoof capsule (Herthel and Hood 1999; Sloet Van Oldruitenborgh-Oosterbaan 1999). When these tissues are damaged, they can not counteract the weight of the horse and as a result the distal phalanx displaces distally or rotates within the hoof capsule (Herthel and Hood 1999). This displacement damages the vasculature within the hoof, the corium of the sole and coronary band, and results in lameness. Most commonly, the basement membrane between the dermal and epidermal lamellae is damaged, and histopathologic changes in this area correlate well with the severity of the lameness (Pollitt 1996; Pollitt *et al.* 1998).

Laminitis is a potentially fatal disease due to the persistence and severity of pain, and the structural damage that occurs in affected animals. Horses that survive an acute episode of laminitis frequently require long-term management and are predisposed to recurrent episodes (Hunt *et al.* 1991). In the United States, 13% of horse owners/ operations reported issues with laminitis in the preceding 12 months (USDA 2000) and in

the United Kingdom, a prevalence of 7.1% of 113,000 horses were reported to have suffered laminitis (Hinckley and Henderson 1996).

*Causes of and risk factors for laminitis*

Laminitis can occur secondary to many conditions, including carbohydrate overload; gastro-intestinal disease; severe respiratory disease; hyperlipidaemia; prolonged hyperinsulinaemia; trauma; ingestion of toxins including black walnut wood shavings, avocado leaves or hoary alyssum; metritis and retained placenta (Asplin *et al.* 2010; Hood 1999; Johnson *et al.* 2004; Pollitt and Daradka 1998; Sloet Van Oldruitenborgh-Oosterbaan 1999). Ponies have been reported to be at a significantly greater risk for laminitis than other Equidae (Dorn *et al.* 1975). The reported predisposed age for laminitis varies with one study reporting 4-6 years for ponies and 7-9 years for horses (Dorn *et al.* 1975); however, the results of a prospective multi-center case-controlled study indicated that horses from 5 to 7 and 13 to 31 years of age were at an increased risk for developing acute laminitis (Alford *et al.* 2001). This same study reported that laminitis was more common in mares in comparison to geldings (Alford *et al.* 2001), but other studies have reported no age, breed or sex predispositions to the development of the condition (Polzer and Slater 1997).

*Clinical signs of laminitis*

Both fore feet are usually affected in horses with acute laminitis; however, the feet affected depend on the etiology of the condition and may include the hind feet. Additionally, laminitis may occur in a contralateral limb as a consequence of a severe

non- or minimally-weight bearing lameness (Peloso *et al.* 1996). Clinical signs associated with laminitis include lameness, weight shifting, reluctance to move, increased sensitivity to application of hoof testers and a characteristic stance with the animal's weight preferentially supported over the unaffected limbs, most commonly the hind limbs. Digital pulses palpated at the level of the pastern or fetlock are interpreted as being increased in magnitude, as there is a strong difference between the systolic and diastolic blood pressure in the digital arteries, and the hooves often feel warm in the acute phases of the disease. Other clinical signs may include swelling of the coronary band or a palpable depression at the coronary band possibly in combination with prolapse of the sole at the toe, findings that are suggestive of rotation or sinking of the distal phalanx (Hood 1999).

*Grading of laminitis*

A system for grading the severity of clinical signs of laminitis in horses was established in 1948 (Obel 1948). Grade 1 is characterized by the horse alternately lifting the affected feet; lameness is not evident at a walk, but at a trot a short stilted gait is noted. Grade 2 is characterized by horses that walk with a stilted gait but can still have a foot lifted. Grade 3 is characterized by horses that move very reluctantly and vigorously resist lifting of a foot. Grade 4 is characterized by the horse refusing to move unless forced. These grades are used to monitor progression of clinical disease.

*Experimental induction of laminitis*

Two methods of experimentally inducing laminitis are commonly used to study the

disease. The first method involves administration of black walnut extract (BWE) through a nasogastric tube (Minnick *et al.* 1987) and the second method involves administration of a gruel mix of corn-starch (85%) and wood flour (15%) (Garner *et al.* 1975) or oligofructose (van Eps and Pollitt 2006) through a nasogastric tube effectively causing a 'carbohydrate overload' (CHO) . The main clinical differences between the models are time from administration to clinical signs, the clinical parameters that change, and how closely the model resembles naturally-occurring laminitis conditions. The CHO models can produce profuse watery diarrhea, mild to moderate depression and inappetance, tachycardia and increased rectal temperature and induce Obel grade 3 laminitis by approximately 40 hours (Garner *et al.* 1978; Krueger *et al.* 1986; Moore *et al.* 1979; Sprouse *et al.* 1987) whereas the BWE model does not cause the secondary clinical issues and laminitis is induced more quickly (Adair *et al.* 2000; Eaton *et al.* 1995; Galey *et al.* 1991). Of the two, the CHO models more resemble the clinical condition of laminitis that occurs in horses with grain overload.

*Pathogenesis of laminitis*

The pathogenesis of laminitis is controversial, with several hypotheses being proposed for the underlying mechanisms. These include, the toxic/enzymatic/metabolic theory, the mechanical/ traumatic theory, the inflammatory theory, and the vascular/ hemodynamic theory.

The toxic/enzymatic/metabolic theory is based on the hypothesis that a toxic agent or metabolite alters metabolism within the laminar tissues which affects their structural integrity (Pollitt 1999). The loss of structural integrity is, therefore, a precursor

to vascular and inflammatory alterations that occur during the development of the condition. This theory is supported by increased expression of the genes encoding for disintegrin and metalloproteinase (ADAM) related metalloproteinases (ADAMTS-4 and -5) and increased matrix metalloproteinase (MMP) activity (especially MMP2, MMP9 and MMP 14) in the digit; products of these genes purportedly cause degradation of the laminar tissues (Budak *et al.* 2009; Coyne *et al.* 2009; Johnson *et al.* 1998; Kyaw-Tanner *et al.* 2008; Loftus *et al.* 2007; Loftus *et al.* 2009; Mungall *et al.* 2001; Mungall and Pollitt 1999; Pollitt and Daradka 1998; van Eps and Pollitt 2004). This increase in MMP activity is inducible by exotoxins from *Streptococcus* bacteria in the cecum (Mungall *et al.* 2001). Additionally, a decrease in the expression of the tissue inhibitors of metalloproteinase- 2 (a natural inhibitor of metalloproteinase) has been noted in the laminar tissues of horses with laminitis (Coyne *et al.* 2009).

The mechanical/ traumatic theory of laminitis is supported by the observation that some horses develop laminitis as a result of direct trauma to the hoof rather than as a result of a systemic condition. The most common types of hoof trauma implicated as a cause for laminitis are those for which the horse bears excessive weight on one limb for a prolonged period of time (overload/ support limb laminitis) or those that are linked with excessive concussion such as 'road founder' (Peloso *et al.* 1996). There are several potential mechanisms proposed for traumatic laminitis that include the development of inflammation within the hoof capsule causing vasospasm, which increases capillary hydrostatic pressure and edema formation within the hoof capsule. These local alterations within the digit create a type of compartment syndrome (Peloso *et al.* 1996). Alternatively, the pathologic events may be initiated by physical tearing of the basement

membrane between the dermal and epidermal laminar which initiates an inflammatory response and/or vasospasm that subsequently causes ischemic damage to the laminae (Peloso *et al.* 1996).

The inflammatory theory of laminitis is supported by the presence of leucocyte infiltration and activation (Black *et al.* 2006; Faleiros *et al.* 2011a; Faleiros *et al.* 2009b; Hurley *et al.* 2006; Pollitt 1996; Riggs *et al.* 2007), endothelial activation (Loftus *et al.* 2007), oxidative injury (Yin *et al.* 2009) and increased expression of pro-inflammatory cytokines, mediators and enzymes in the laminar tissue at the onset of laminitis. Although over-expression of similar immunomodulatory agents occurs in both experimental models of laminitis, there are temporal differences in their expression. Many of the inflammatory events appear to peak at or near the onset of lameness in the CHO model, whereas these inflammatory events occur at an earlier time point in the BWE model (Leise *et al.* 2011).

Up-regulation of mRNA for interleukins (IL) (IL-1, IL-6, IL-8, and IL-12), molecule possessing ankyrin-repeats induced by lipopolysaccharide (MAIL), and cyclo-oxygenase-2 has been documented within laminar tissue at the onset of laminitis (Belknap *et al.* 2007; Blikslager *et al.* 2006; Fontaine *et al.* 2001; Waguespack *et al.* 2004a; Waguespack *et al.* 2004b). Additionally, there is increased expression of a potent chemokine (C-X-C motif ligand 1 (CXCL1)) by laminar basal epithelial cells, endothelial cells and interstitial macrophage-like cells that may be important for leukocyte accumulation as early as 1.5 hours after administration of BWE (Faleiros *et al.* 2009a). Horses with CHO-induced laminitis have increases in leukocyte adhesion molecules (E-selectin and intercellular adhesion molecule-1 (ICAM-1)) in their laminae at the onset of

Obel grade 1 lamintis (Leise *et al.* 2011). These increased chemokines and adhesion molecules are consistent with the documented increases in neutrophils, activated macrophages and type 2/"anti-inflammatory" macrophages detected in horses with both BWE and CHO-induced laminitis (Faleiros *et al.* 2011a; Faleiros *et al.* 2009b; Faleiros *et al.* 2011b) in addition to the resident mononuclear inflammatory cells (macrophages and lymphocytes) (Faleiros *et al.* 2011b).

In addition to these changes within the laminar, increased expression of inflammatory cytokine genes occurs concurrently in the skin, lung and liver in the BWE model of laminitis (Stewart *et al.* 2009). Evidence of concurrent inflammatory changes in the laminae and organs distant to the digit suggests that laminitis is a component of a systemic inflammatory response syndrome (SIRS) similar to that documented to occur in septic humans and rodents (Belknap *et al.* 2007; Loftus *et al.* 2007; Stewart *et al.* 2009).

The vascular/hemodynamic theory of acute laminitis is supported by research describing changes in digital and laminar perfusion during the development of the condition. It is proposed that these alterations initiate the local metabolic changes that result in the loss of structural integrity within the digit (Hood 1993). The precise nature of the changes in laminar blood flow remain controversial; vasospasm of the laminar arteries has been proposed (Adair *et al.* 2000; Galey *et al.* 1990; Hood 1993; Hood *et al.* 2001); however, more recently, it has been noted that the laminar veins are predisposed to venoconstriction, which impedes blood outflow from within the hoof capsule (Allen *et al.* 1990; Eaton *et al.* 1995; MacDonald *et al.* 2005; Noschka *et al.* 2009; Noschka *et al.* 2010; Peroni *et al.* 2006). Consequently, there is increased post-capillary resistance and capillary hydrostatic pressure that forces fluid from the capillaries into the laminar

interstitium. When the laminar interstitial pressure exceeds the pressure that is that tolerated by the capillary walls, sufficient external force exists to close the capillaries thereby accentuating the ischemia. The resultant situation is a type of 'compartment syndrome' which has been hypothesized previously (Allen *et al.* 1990).

*Investigations into laminar blood flow in laminitis*

Most likely, the disease is multi-factorial, with vascular dysfunction contributing to its pathogenesis as changes in digital and laminar blood flow have been documented during the prodromal stages of experimentally-induced laminitis (Adair *et al.* 2000; Galey *et al.* 1990; Hood 1993; Hood *et al.* 1990; Robinson *et al.* 1976). However, contradictory results regarding the exact nature of the change in blood flow in laminitis have been reported. These discrepancies may be due to the complexity and unique nature of the vasculature in this area and the difficulties in accurately assessing blood flow beneath the keratinized hoof wall. The laminar vasculature functions at high hydrostatic pressures (Hunt 1991), is maintained under tonic vasoconstriction rather than vasodilation (Bailey and Elliott 1998; Elliott 1997), and laminar capillaries are more permeable to high molecular weight compounds than capillaries elsewhere in the body (Allen *et al.* 1988). Furthermore, information regarding the responses of the large digital vessels cannot be extrapolated to the laminar vasculature as the two vascular beds respond differently to vasoconstrictors *in vitro* (Peroni *et al.* 2006).

Laser doppler flowmetry has been used successfully to measure laminar blood flow in horses (Adair *et al.* 2000). Use of this technique has identified fluctuations in laminar microvascular blood flow during the prodromal stages of BWE-induced laminitis

in horses. During the first two hours after administration of the extract, laminar microvascular blood flow decreases, then increases, followed by a subsequent second decrease that occurs at the onset of clinical signs of laminitis at approximately 8 hours. This technique has limitations, primarily because it requires several 8mm holes to be drilled in the hoof wall to the depth of the junction of the sensitive and insensitive laminar (Adair *et al.* 2000; Adair *et al.* 1994; Adair *et al.* 1997; Castro *et al.* 2010). Additionally, laser doppler flowmetry is sensitive to movement artifacts that can artificially increase recorded values for blood flow. Consequently, its clinical application is limited.

The scintigraphic technique for measuring laminar blood flow involves injection of technetium-$^{99m}$ macroaggregated albumin into the brachiocephalic arterial trunk, intravenous injection of technetium-$^{99m}$ diethylenetriamine pentaacetic acid, and collection of both dynamic radionuclide angiograms and static blood pool images (Trout *et al.* 1990). Results of investigations of laminar blood flow using scintigraphy in horses with experimentally-induced laminitis identified an increase in blood flow at 24 hours (Trout *et al.* 1990). Laminar blood flow was only assessed at 24-hour intervals; the lack of ability to repeatedly measure blood flow at shorter time intervals limits clinical application of this technique. Additionally, the planar images produced using this technique result in poor spatial resolution and limits the ability to differentiate between blood flow in different anatomic locations throughout the foot (Kruger *et al.* 2008).

Arteriography has been used to examine digital blood supply in healthy horses and horses with CHO-induced laminitis. This technique revealed a marked reduction in blood supply and histologic examination of the digits from these horses revealed

microthrombi in the venules of the laminar dermis in 2 of 4 affected horses (Weiss *et al.* 1995). As arteriography was performed in disarticulated feet after CHO-induced laminitis, this is not a clinically applicable method.

Hoof wall surface temperature has been validated and used as an indirect indicator of laminar perfusion in horses with CHO-induced laminitis (Hood *et al.* 2001). The results of these experiments were similar to those of Adair *et al.* (2000) with hoof wall hypothermia being noted during the prodromal stages of laminitis. In contrast, a similar study of CHO-induced laminitis did not document a significant decrease in hoof wall temperature during the prodromal stages of laminitis, but did detect hyperemia at the onset of clinical signs of laminitis (Pollitt and Davies 1998).

More recently, dynamic contrast-enhanced computed tomography has been used to measure laminar blood flow and vascular permeability in anesthetized healthy horses (Kruger *et al.* 2008). Although this technique has produced objective measurements of laminar blood, it has not been used to measure blood flow in horses with laminitis; the necessity for general anesthesia and the consequent small window of time available for data collection limit its usefulness in monitoring alterations in laminar blood flow in horses with either naturally occurring or experimentally-induced laminitis.

In order to define the net vascular changes within the hoof during laminitis and utilize this information to improve management of clinical cases, it is essential to establish a safe, repeatable method for measuring blood flow within the equine digit. Invasive methods of assessing blood flow are not applicable in clinical cases and have the potential to intrinsically alter blood flow. Consequently, they should not be used. In human medicine, cerebral blood flow is measured by a repeatable and safe method of

intra-arterial injection of a radioisotope of xenon. Therefore, investigation into the application of this method for measurement of laminar blood flow in horses is warranted.

## SECTION 2: XENON$^{133}$

### *Xenon and xenon$^{133}$*

Xenon, a colorless, odorless, inert noble gas, has an atomic number of 54. Xenon$^{133}$ ($Xe^{133}$) is produced during Uranium 235 fission. $Xe^{133}$ is an isotope of xenon (i.e., it contains the same number of protons as xenon (54), but a different number of neutrons (79) than xenon (77)). As a result, $Xe^{133}$ has a different mass number (132.9 atomic mass units) than xenon (131.3 atomic mass units). Furthermore, $Xe^{133}$ is a radioactive isotope (radioisotope) as the atom has an unstable nucleus. Radioisotopes undergo radioactive decay by emitting gamma rays and/or subatomic particles. $Xe^{133}$ has a short half-life of 5.2474 days (Galan 2009) and emits a variety of radiation types (**Table 1**) (Kocher 1981) to form Caesium$^{133}$.

The emission of low-energy gamma radiation is easily detected and can, therefore, be utilized for diagnostic imaging purposes. The specific gamma ray constant for $Xe^{133}$ is 0.51 R/hr-mCi at 1 cm; the use of 0.20 cm of lead will decrease the external radiation exposure by a factor of 1,000.

$Xe^{133}$ is available in gaseous forms in glass vials and the amount is determined by the number of millicuries (mCi), which is a unit of radioactivity. One mCi has $3.7 \times 10^7$ disintegrations per second or $2.22 \times 10^9$ disintegrations per minute. Disintegrations per minute also can be used to measure radioactivity, as they represent the exact number of

atoms of a given radioactive material that decay in one minute. This value is, therefore, calculated using counts per minute from a device with excellent sensitivity and is based on counting efficiency (disintegrations per minute = counts per minute/ efficiency). Counts per minute is another method of measuring radioactivity and is similar to disintegrations per minute, but it reports the number of atoms in a given radioactive material that are detected to have decayed in one minute.

Xenon$^{133}$ diffuses through cell membranes easily, is not metabolised by the body and is eliminated via the lungs. It is chemically and physiologically inert. After an intra-arterial injection into the internal carotid artery in humans, 90% of $Xe^{133}$ is exhaled through the lungs on its first passage. As a result, recirculation of the radionuclide through the cerebral vasculature is minimal (Anderson 1996).

$Xe^{133}$ naturally exists in a gaseous form, but can be mixed with sterile saline to form a solution for injection (Sejrsen 1969). However, gaseous $Xe^{133}$ has poor solubility in aqueous media (0.108ml.ml$^{-1}$ water at a pressure of 100kPa) (Oertel *et al.* 2007). When mixed in solution, it forms a clathrate or lattice in which the water molecules trap the gaseous $Xe^{133}$.

*Making gaseous xenon$^{133}$ into solution*

Injection of $Xe^{133}$ into the cardiovascular system requires it to be mixed in sterile solution rather administered in a gaseous form. Limitations of forming $Xe^{133}$ in solution are its low solubility and its adherence to some types of plastic and rubber. For example, more than 5% of $Xe^{133}$ has been reported to be lost through absorption or diffusion through rubber stoppered glass vials (LeBlanc and Johnson 1971), and more than 80%

has been reported to be lost through the rubber plungers on individual dosing cartridges (Keaney *et al.* 1971). Therefore, it cannot be drawn up into a plastic syringe in advance of use. Xenon$^{133}$ enters saline far more readily at cold temperatures and under increased pressure (DiPiazza and Harbert 1983; Keaney *et al.* 1971; Ladefoged and Anderson 1967) than at room temperature and atmospheric pressure.

There are many proposed methods of forming $Xe^{133}$ in solution (Carroll *et al.* 1973; DiPiazza and Harbert 1983); however, a recently described technique (Oertel *et al.* 2007) involves the use of a sealed, chilled, pressurized, saline-filled chamber within which a glass vial containing $Xe^{133}$ is crushed to release the gas into the solution. This technique has been proven to maximize the concentration of $Xe^{133}$ in solution and minimize $Xe^{133}$ loss (Oertel *et al*, 2007).

*Use of xenon$^{133}$ in humans*

Although $Xe^{133}$ is the most common isotopic gas used in human ventilation scanning (Morris 2005), over the past two decades, numerous studies have been performed in people in which clearance of $Xe^{133}$ has been used to monitor changes in blood flow to the extremities (Bende *et al.* 1983; Sejrsen 1969; Siegel and Wagner 1976; Sorensen *et al.* 1994) and in the cerebrum. Use of this technique for the latter organ has been particularly rewarding, as measurement of blood flow would otherwise be extremely difficult, if not impossible, due to its location within the cranium (Anderson 1996; Fogarty-Mack *et al.* 1996; Joshi *et al.* 2000; Joshi *et al.* 2002; Ko *et al.* 2005b; Young *et al.* 1994; Young *et al.* 1990). The intra-arterial $Xe^{133}$ injection technique used to make

these measurements has proved to be reliable, accurate and safe, and is considered to be the gold standard for assessing changes in cerebral flow in humans (Sousteil *et al.* 2003).

In human medicine, $Xe^{133}$ washout studies are performed by injecting small known amounts of $Xe^{133}$ (1-2mCi in 1ml saline) into a catheter in the internal carotid artery, which is then flushed with a 5-10ml bolus of saline. The washout of $Xe^{133}$ is subsequently recorded under stable conditions for 3 minutes (Ko *et al.* 2005a) using tungsten-collimated cadmium telluride scintillation detectors in a commercial cerebral blood flow collection system (Carolina Medical, King, NC) on the patient's scalp over certain arteries depending on the lesion (Ko *et al.* 2005a). Placement of the detector over the artery is confirmed by fluoroscopy whilst radio-opaque contrast material is injected into the vessel (Ko *et al.* 2005a). The scintillation detector uses a scintillation crystal to detect the photons emitted from the $Xe^{133}$. These photons are then converted into visible light which is subsequently converted into electrical pulses by a photomultiplier tube (Friedman *et al.* 2000). The low-voltage electrical pulses are then modified by a preamplifier and an amplifier to generate pulses that can be detected (Friedman *et al.* 2000). The pulse-height analyzer selects pulses that are at the appropriate energy level for $Xe^{133}$ gamma emission (81keV) to ensure background radiation is not detected (Friedman *et al.* 2000). The count rate meter is then used to quantify the number of counts per unit time, which is subsequently recorded (Friedman *et al.* 2000).

The measurements obtained by the scintillation detectors produce clearance curves that represent the washout of $Xe^{133}$ from the brain. A variety of methods can be used to calculate cerebral blood flow; these include the initial slope index, which utilizes the slope of the first minute of the clearance curve (Olesen *et al.* 1971; Paulson *et al.*

1969; Waltz *et al.* 1972); the stochastic method (Hoedt-Rasmussen *et al.* 1966; Horsch *et al.* 1990; Olesen *et al.* 1971; Waltz *et al.* 1972); and the two-compartment analysis technique, which produces information regarding cerebral blood flow to both the gray and white matter (Hoedt-Rasmussen 1967; Hoedt-Rasmussen *et al.* 1966; Waltz *et al.* 1972). Cerebral blood flow is most commonly calculated using the initial slope method with data collected between 20-80 seconds of tracer washout, giving a value weighted toward gray matter and expressed in millilitres per 100g of brain tissue per minute (Ko *et al.* 2005a). A set of injections is normally performed before and after an event considered likely to alter blood flow, such as completion of an arterial stent (Ko *et al.* 2005a; Ko *et al.* 2005b; Young *et al.* 1990) or pharmacological modification of vascular tone (Fogarty-Mack *et al.* 1996; Joshi *et al.* 2000; Joshi *et al.* 2002). The $Xe^{133}$ washout technique has proved to be reliable, accurate (Sousteil *et al.* 2003) and safe, and is considered the gold standard for assessing changes in cerebral flow after surgical arterial stenting in humans (Ko *et al.* 2005b).

The disadvantages of using the $Xe^{133}$ washout technique include concerns over (i) a 'look-through' phenomenon, which results in overestimation of blood flow, (ii) overestimation of blood flow if measurements are taken in quick succession and (iii) 'Compton scattering'. The 'look-through' phenomenon occurs as the counts per minute recorded represent an area rather than a specific vessel. Thus, a hypoperfused area may be masked because of normal perfusion in over- or under- lying tissues (Donley *et al.* 1975; Hanson *et al.* 1975). Overestimation of cerebral blood flow occurs if measurements are taken before the background levels have completely subsided; in humans, cerebral blood flow measurements taken at 10-minute intervals would be

expected to overestimate blood flow by 5% (Kanno and Uemura 1975). Compton scattering occurs when gamma rays (or x-rays) interact with matter; some of the energy of the ray/ incident particle is transferred to the electron it collides with and changes the wavelength of the ray. Rays of a different wavelength may not be detected by the pulse-height analyzer. This limitation can be overcome by setting the pulse-height analyzer to 75 keV rather than 81 keV (Paulson et al. 1969).

There are many advantages of using $Xe^{133}$ as a tracer to measure blood flow. These include the predictable tissue clearance proportional to blood flow over a variety of flow rates, minimal recirculation due to rapid clearance from the lungs, a short biological half life, low radiation exposure, the need for minimal instrumentation and worldwide availability of $Xe^{133}$ (DiPiazza and Harbert 1983).

*Use of xenon$^{133}$ in horses*

The $Xe^{133}$ washout technique was used more than 30 years ago in horses to measure blood flow to the extremities during rest and exercise (Stromberg 1972; Strömberg 1973; Stromberg and Norberg 1971; Stromberg 1971). During these investigations, $Xe^{133}$ in solution was injected into the tendons and peritendinous fascia of horses, and its clearance from the tissues was monitored under a variety of conditions (rest, exercise, adrenalin administration, distal limb tourniquet and tendon disease) (Stromberg 1972; Strömberg 1973; Stromberg 1971). Additionally, $Xe^{133}$ was injected 5mm into the coronary corium proximal to the extensor process and its clearance was measured under both resting and exercising conditions (Stromberg and Norberg 1971). Correlations between $Xe^{133}$ washout and thermographic measurement of infra-red

emission were made and good correlation was identified between elevation of temperature and increase in $Xe^{133}$ clearance (Strömberg 1973; Stromberg and Norberg 1971). The disappearance of $Xe^{133}$ suggested an increase in tissue-blood exchange attributed to an increased functioning capillary surface area (Strandell and Shepherd 1967), thus providing a measurement of blood flow (Stromberg and Norberg 1971).

$Xenon^{133}$ clearance has also been used in horses to measure perfusion of skeletal muscles (Weaver *et al.* 1984). $Xenon^{133}$ in solution was injected into the brachiocephalicus, extensor carpi radialis, tongue and semimembranosus muscles in conscious and anesthetized horses, and the clearance of $Xe^{133}$ was monitored. This study documented decreased capillary blood flow in muscles during anesthesia maintained by halothane (Weaver *et al.* 1984).

In light of the excellent safety and reliability of measurement of cerebral blood flow in human medicine using the intra-arterial $Xe^{133}$ technique, and the similarities between measurements of vascular flow beneath the cranium and hoof capsule, this technique may finally provide a safe and repeatable technique for measurement of laminar blood flow in horses.

TABLES

Table 1

Principle Radiation Emission Data from Xenon$^{133}$ (Kocher 1981)

| **Radiation** | **Mean Energy (keV)** | **Mean % per disintegration** |
|---|---|---|
| **Beta-2** | 100.6 | 99.3 |
| **K int. con. Electrons gamma-2** | 45.0 | 53.3 |
| **L int. con. Electrons gamma-2** | 75.3 | 8.1 |
| **M int. con. Electrons gamma-2** | 79.8 | 1.7 |
| **Gamma-2** | 81.0 | 36.5 |
| **KβX-ray** | 35.0 | 9.1 |
| **KαX-ray** | 30.8 | 38.9 |

keV= kilo electron volt

K int. con. Electrons gamma-2= K shell internally converted electrons gamma-2

L int. con. Electrons gamma-2= L shell internally converted electrons gamma-2

M int. con. Electrons gamma-2= M shell internally converted

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# CHAPTER 3

# EVALUATION OF XENON$^{133}$ WASHOUT FOR MEASUREMENT OF LAMINAR BLOOD FLOW IN THE HORSE

## INTRODUCTION

The pathogenesis of laminitis in horses is controversial, with several hypotheses being proposed for the underlying mechanisms. The disease is most likely multi-factorial, with vascular dysfunction contributing to its pathogenesis as changes in digital and laminar blood flow have been documented during the prodromal stages of experimentally-induced laminitis (Adair *et al.* 2000; Hood *et al.* 1990; Robinson *et al.* 1976; Trout *et al.* 1990).

Several methods have been used in attempt to measure laminar blood flow in horses with laminitis, however, contradictory results have been reported (Adair *et al.* 2000; Trout *et al.* 1990). Although blood flow in the more accessible large digital vessels has been investigated, there is evidence that these large conduit vessels respond differently to vasoactive substances than vessels isolated from the laminar tissues (Peroni *et al.* 2006). Therefore, a new approach is needed to accurately assess changes in laminar perfusion during the development of laminitis.

Xenon$^{133}$ ($Xe^{133}$) has been used extensively to measure blood flow in cerebral tissue in humans (Joshi *et al.* 2000; Ko *et al.* 2005a; Ko *et al.* 2005b; Sousteil *et al.* 2003; Young *et al.* 1990). Sets of intra-arterial injections of $Xe^{133}$ are normally performed before and after an intervention expected to alter blood flow (e.g., insertion of an arterial stent or administration of drugs that modify vascular tone). The intra-arterial $Xe^{133}$ injection technique has proved to be reliable, accurate (Sousteil *et al.* 2003) and safe, and is considered to be the "gold standard" for assessing changes in cerebral flow in humans after surgical arterial stenting (Ko *et al.* 2005b).

The hypotheses of this study were: (i) intravenous injection of $Xe^{133}$ is not useful for measuring laminar blood flow in horses, (ii) intra-arterial injection of $Xe^{133}$ in solution can be performed safely in conscious horses, and (iii) intra-arterial injection of $Xe^{133}$ in solution allows repeatable measurement of laminar blood flow in conscious horses.

## MATERIALS AND METHODS

### *Horses selected*

Mature, systemically healthy horses with no history of laminitis or respiratory disease were considered eligible for this study. Horses were included if they did not respond to application of hoof testers, were sound on a lameness evaluation including trotting on hard ground in a straight line, and routine lateromedial and dorsopalmar radiographs (Mark II, Eklin Medical Systems Inc, CA) revealed no evidence of laminitis

(Redden 2003). All experimental protocols were approved by the IACUC at the University of Georgia.

*Horse preparation*

Horses were restrained within stocks and an intravenous over the needle catheter (Milacath, Mila International Inc., KY) was placed sterilely into the left jugular vein; sedation with xylazine hydrochloride (Anased injection, Lloyd laboratories, IA) was used, if necessary.

The proximal medial metacarpal area of a randomly assigned forelimb was clipped and prepped before ultrasonographic imaging of the medial palmar artery at the level of the proximal third of the metacarpus was performed. After prepping the area with chlorhexidine and sterile saline, 1ml of 2% lidocaine (Lidocaine hydrochorlide, Hospira Inc., IL) was injected subcutaneously through a 25G needle. The area was prepped further before a 2" 18G polyurethane over the needle catheter (Surflow IV Catheter, Terumo Medical Corporation, MA) was inserted into the medial palmar artery (Puchalski *et al.* 2006) under aseptic conditions. A previously flushed extension set (20inch Extension set, Medexsupply, NY) with an injection port (Heparin Lock Inj. Site Long, Medical Specialities Distributors Inc., MA) was then attached to the catheter, which was secured with non-circumferential zinc oxide tape and protected with a bandage. Intra-arterial placement was confirmed by measurement of arterial systolic, diastolic and mean and blood pressures (SurgiVet invasive pressure transducer (V6402) and SurgiVet Advisor Vital Signs Monitor, Smiths Medical, OH).

*Xenon$^{133}$ solution preparation*

An instrument, 'the ampoule-crushing device' (Figure 1), specifically designed to crush glass ampoules of $Xe^{133}$ gas and produce $Xe^{133}$ in solution was created from stainless steel based on the description of Oertel *et al.* (2007). Modifications of the originally described device were made to ensure that the device was suitable for the size of $Xe^{133}$ ampoule available and the amount of $Xe^{133}$ necessary for the study. The design allowed 10 mCi and 20 mCi glass ampoules to be broken in sterile saline solution in a gas tight stainless steel cylinder to yield $Xe^{133}$ in solution for intravenous and intra-arterial injection.

A glass ampoule containing gaseous $Xe^{133}$ was obtained from a radiopharmaceutical company (Triad Isotopes Inc., FL) and appropriate measures for ensuring radiation safety were taken. The ampoule-crushing device, the glass ampoule and equipment required for transfer of the ampoule were immersed in 70% ethyl alcohol for five minutes to render them sterile.

The ampoule-crushing device, glass ampoule and equipment for transfer were removed from the ethyl alcohol under aseptic conditions and lavaged with sterile saline. They were then submerged in a sterile saline water bath cooled within an ice bath. Each component of the ampoule-crushing device was systematically agitated, flushed, and wiped with sterile gauze to ensure that all components of the device were devoid of air bubbles. Syringes necessary for drawing up the $Xe^{133}$ in solution were also submerged in the sterile cooled water bath and 1ml sterile saline was aspirated into each syringe while ensuring that no air was present.

The ampoule-crushing device was reassembled whilst submerged in the cooled sterile water bath by inserting the crushing screw into the cylinder until it was firmly in place but not protruding into the main cavity of the device. The ingress and egress ports were closed. The $Xe^{133}$ ampoule was inserted into the cavity of the device to reside where the crushing screw would contact the body of the glass ampoule. Once the $Xe^{133}$ ampoule was appropriately positioned inside the device, the lid of the device was positioned and tightened. The three-way stopcock and a 60mL syringe containing 40mL of saline were attached to the ingress port of the ampoule-crushing device. The device was then removed from the water bath.

The ampoule-crushing device was placed on a counter-top in an area approved for radioactive materials and was inverted so it was positioned on its lid. Steady pressure was applied and maintained on the 60mL syringe attached to the ingress port of the ampoule-crushing device. The ingress port was opened and then the crushing screw was tightened with the system under pressure. The crushing screw was completely closed and when the ampoule was crushed, a reduction in pressure was detected in the syringe attached to the ingress port. Approximately 2 to 4mLs of sterile saline were injected into the device and the ingress port was closed. The device was placed in an ice bath under a laminar flow hood and left undisturbed for a minimum of 4 hours to facilitate formation of $Xe^{133}$ in solution.

Between 4-24 hours, $Xe^{133}$ in solution was drawn up into syringes from the ampoule-crushing device. The device was removed from the ice bath and a blood filter filled with sterile saline was attached to the egress port. A syringe containing 1ml of cooled sterile saline lacking macroscopic air bubbles was attached to the blood filter on

the egress port of the device. Whilst applying pressure to the 60mL syringe attached to the ingress port, the ingress port and then the egress port were opened. Pressure from the ingress port displaced fluid within the ampoule-crushing device to fill the syringe on the egress port. Whilst maintaining pressure on the ingress syringe, the egress port was closed and then the ingress port was closed. The syringe containing $Xe^{133}$ solution was removed from the ampoule-crushing device and an airtight cap was placed immediately on the syringe. The syringe was then placed within a lead box and the ampoule-crushing device was placed back in the laminar flow hood.

To test the first hypothesis (i), a single 30ml syringe (to obtain sufficient $Xe^{133}$ for approximately a 5mCi injection) was used. For part (ii) of the study, either a single 5ml syringe (to obtain sufficient $Xe^{133}$ for a 1mCi injection) or a 30ml syringe (to obtain sufficient $Xe^{133}$ for a 5mCi injection) was used. For part (iii) of the study, 8-10 3ml syringes (to obtain sufficient $Xe^{133}$ for individual 0.25mCi injections) were used. A 10-minute interval was allowed to elapse between each 3ml syringe aspiration to allow for equilibration of $Xe^{133}$ within the ampoule-crushing device.

*Validation of $Xe^{133}$ in solution*

The syringes containing $Xe^{133}$ solution were individually placed within the dose calibrator (Atomlab Dose Calibrator, Biodex Medical Systems Inc, NY) and the time and mCi $Xe^{133}$ were recorded. The volume required to inject the desired amount of $Xe^{133}$ was calculated, recorded and marked on the syringes containing $Xe^{133}$ solution.

*Baseline recordings*

*Physical examination and laboratory data*

The horses were positioned in stocks in a room approved for the use of gaseous radioactive materials. Baseline parameters were recorded including heart rate, respiratory rate, temperature and systolic, diastolic and mean arterial blood pressures, and blood was taken for packed cell volume, total solids, a complete blood count and serum biochemistry profile.

*Counts per minute at the hoof*

A leaded glass shield was placed between the front limbs and a lead shield was wrapped around the foot to be studied. A 5cm diameter hole that fitted the probe of a portable sodium-iodide Thallium (NaI(Tl)) scintillator (Model 2241-2 Survey Meter, Ludlum Measurements Inc, TX) was positioned midway between the coronary band and the tip of the distal phalanx on the dorsal aspect of the hoof. The scintillator probe was placed adjacent and perpendicular to the dorsal hoof wall through the circular hole in the hoof lead shield and was maintained in this position in a customized stand.

Base line count per minute (c.p.m.) readings were taken for both front feet at 6-second intervals and were recorded directly onto a laptop computer using software purchased from Ludlum Measurements Inc., TX.

*Disintegrations per minute in the systemic circulation*

A 3 syringe technique was used to draw blood from the catheter in the jugular vein to determine baseline values of radiation within the systemic circulation. Two 0.1ml aliquots of blood obtained at the baseline time point were immediately transferred into scintillation cocktail (ScintiSafe Gel, Fischer Sci, TX) and processed using a liquid

scintillator (LS6500 Multi-Purpose Scintillation Counter, Beckman Instruments Inc., CA). The results in disintegrations per minute (d.p.m.) were printed immediately.

*Xenon$^{133}$ Injection*

Immediately after monitoring commenced, the $Xe^{133}$ solution followed by 2.5 mls sterile heparinized saline lacking macroscopic air bubbles were injected into either the intra-venous catheter (Part i) or intra-arterial catheter (Parts ii and iii). The mCi of $Xe^{133}$ and the time of injection were recorded.

Part (i): Single intravenous bolus doses of approximately 5mCi of $Xe^{133}$ were administered (3 injections in 2 horses on 2 separate days).

Part (ii): Single intra-arterial bolus doses of approximately 5mCi of $Xe^{133}$ (6 injections in 2 horses on 6 separate days) or 1mCi (6 injections in 4 horses on 6 separate days) were administered.

Part (iii): Multiple intra-arterial doses of approximately 0.25mCi of $Xe^{133}$ were administered at 5-minute intervals until completion of 5-7 injections (n=7 sets of injections in 4 different horses on 7 separate days).

*Monitoring physical examination and laboratory data*

The following parameters were monitored every 5 minutes during the procedure: rectal temperature, heart rate respiratory rate and systolic, diastolic and mean arterial blood pressures.

*Monitoring counts per minute at the hoof*

Counts per minute at the hoof distal to the intra-arterial injection with $Xe^{133}$ in solution were recorded every 6 seconds for the duration of the procedure. During some

trial experiments, the lead shielding between the front feet and around the foot being studied were removed; this increased the c.p.m. reading at the hoof of interest, supporting the use of the lead shielding.

*Monitoring disintegrations per minute in the systemic circulation*

Radiation in the systemic circulation was measured on serial jugular venous blood samples taken at a maximum of 4-minute intervals. All data were recorded until a minimum of 15 minutes had elapsed after the last intravenous or intra-arterial injection.

*Other monitoring*

Any sedatives administered or time of movement of the horses were recorded.

*After injection of $Xe^{133}$ in solution*

After each experimental procedure, both catheters were removed and discarded following established radiation safety protocols, and the previously catheterized limb was bandaged. Blood was collected from the jugular vein immediately after the experiment and at 24-hour intervals until 72 hours for complete blood counts and serum biochemistry profiles. Horses were monitored closely for signs of swelling at the catheterization site, lameness or colic.

The radioactivity due to the $Xe^{133}$ remaining in the syringes used for injection was measured using the dose calibrator and the amount was recorded. The radiation safety protocol was followed to discard all radioactive waste.

*Statistical analysis*

Means and standard deviations are reported for normally distributed data.

# RESULTS

## *Horses*

Eight horses were used in this study (6 Quarter Horses or Quarter Horse crosses and 2 Warmbloods). Horses weighed between 372 and 600kg and were between 3 and 22 years old.

For part (iii) of the study, four horses (2 Quarter Horses and 2 Warmblood horses) between 4 and 22 years and weighing between 525 and 600 kg were used. Seven data sets from six limbs of these 4 horses were generated. One horse had the left fore limb studied only, 2 horses had both the right and left fore limbs studied, and 1 horse had the left fore limb studied on two separate occasions 40 days apart.

## *Horse preparation*

One horse was sedated with 0.2mg/kg xylazine hydrochloride to facilitate intra-arterial catheterization on one occasion. The sedative was administered more than 2 hours before injection of $Xe^{133}$ in solution. All horses tolerated intravenous and intra-arterial catheterizations well and catheters were easily maintained during the procedure. Trans-cutaneous ultrasonography was used less frequently with increasing experience in intra-arterial catheterization. Preliminary trials confirmed intra-arterial catheters could be maintained for 7 days without complication.

## *Xenon$^{133}$ solution preparation*

The ampoule-crushing device repeatedly allowed formation of $Xe^{133}$ in solution. Additionally, drawing up $Xe^{133}$ in solution was possible using the technique described. In preliminary trials, the importance of eliminating all air from the ampoule-crushing device and equipment used to draw up $Xe^{133}$ in solution (egress port, filter and syringes) was emphasized as in the presence of even small volumes of air, gaseous $Xe^{133}$ did not form $Xe^{133}$ in solution.

*Amount of $Xe^{133}$ in solution*

The amount of $Xe^{133}$ (mCi) aspirated into the syringes was proportional to the volume of solution in the syringes. When less $Xe^{133}$ was required, smaller volumes of solution were aspirated; when low volumes were aspirated from the ampoule-crushing device, the 1st two syringes drawn from the device contained less $Xe^{133}$ than the subsequent syringes. When small amounts of $Xe^{133}$ were needed (e.g., 0.25mCi), the third 3ml syringe contained that amount. Thereafter, the volumes of solution were within 0.5ml of each other when the protocol required 0.25mCi $Xe^{133}$ (part (iii)).

*Baseline recordings*

*Physical examinations and laboratory data*

All horses had normal body temperatures (100.0+/-0.3, Reference range: 98.6-100.9°F), heart rates (33+/-4, Reference range: 28-42 beats per minute), respiration rates (19+/-5, Reference range: 12-24 breaths per minute), packed cell volumes (37.0+/-2.8, Reference range: 25-45%) and total proteins (6.7+/-0.6, Reference range: 5.5-7.5g/dL) at the start of the experiments. All horses had normal systolic blood pressures 124.0+/-23.2,

diastolic blood pressures 99.0+/-8.7, and mean blood pressures 115.0+/-13.4. Complete blood counts and serum biochemistry results were within normal limits.

*Counts per minute at the hoof*

Baseline c.p.m. readings at the hoof (363+/-117) were equivalent for both the study hoof and the contralateral control hoof.

*Disintegrations per minute in the systemic circulation*

Baseline d.p.m. readings in blood from the jugular vein were 97.0+/- 43.0, and were therefore below the threshold for significance (200 d.p.m.).

*Xenon$^{133}$ injections*

All injections were easy to perform through the previously placed catheters. All horses tolerated the injections well and no horses required sedation throughout the experiments.

*Physical examination and blood work*

The temperature, pulse rate, respiration rate and systolic, diastolic and mean arterial blood pressures and packed cell volumes and total protein concentrations remained within reference ranges during the 40-50 minutes of data collection.

*Counts per minute at the hoof*

Part (i): No changes in c.p.m. were noted at the hoof after intravenous injection of $Xe^{133}$.

Part (ii): A rapid immediate increase and sharp peak followed by a prolonged gradual decrease in c.p.m. at the hoof were noted after each intra-arterial injection of $Xe^{133}$ in solution (Figure 2).

Part (iii) A rapid immediate increase followed by a peak in c.p.m. were observed at the foot 22.8+/- 7.8 seconds after commencing intra-arterial injections. Peak c.p.m. after the 1st injection of 0.25mCi of $Xe^{133}$ was 37190+/- 14322 c.p.m. After this rapid rise and peak in c.p.m., there was a slower decline in values, which diminished further with time and did not return to baseline levels before the subsequent 0.25mCi $Xe^{133}$ injections. With increasing numbers of injections, the peak and trough c.p.m. gradually increased in all horses (Figure 3). In some experiments (n=4), the peak and trough counts began to stabilize after 4-5 injections (Figure 4).

*Disintegrations per minute in the systemic circulation*

Part (i): In one experiment, a bolus intravenous injection of $Xe^{133}$ induced an increase in d.p.m. that was evident within minutes 3-8; the maximum d.p.m. recorded was 251.93.

Part (ii): Radioactivity was detectable in the systemic circulation of all horses after intra-arterial injection of 5mCi, with an average peak in d.p.m. of 688.55+/- 216.22. The d.p.m. remained increased for up to 20 minutes after the injection. Radioactivity was detectable in 2 of the 6 horses after intra-arterial injection of 1mCi of $Xe^{133}$; the average peak d.p.m. was 157.96+/- 93.06.

Part (iii): Disintegrations per minute did not increase from baseline in any horses after repeated injections of 0.25mCi of $Xe^{133}$ at 5-minute intervals (Figure 5).

*Other effects*

In addition to the peaks and troughs in radioactivity at the hoof detected after intra-arterial injections of $Xe^{133}$, there were marked troughs in some recordings that correlated directly with times the horses moved (Figure 2).

*After injection of $Xe^{133}$ in solution*

Physical examination findings were within reference ranges when verified every 6-8 hours for 24-72 hours after injection of $Xe^{133}$ in solution. The results of repeated complete blood count and serum biochemistry assessments after injections were normal in all except one horse, that had 66 band neutrophils/ul and a normal nucleated count of 6600 cells/ul at 24 hours after injection. This abnormality resolved without treatment.

All horses remained sound on the limbs that had been catheterized, although mild swelling on the medial aspect of the metacarpus was present at 24 hours after 15 experiments and moderate swelling was present after 4 experiments. These swellings resolved within 72 hours after application of standing leg wraps.

No radioactivity was noted in the urine or feces at any time point after administration of a single bolus intravenous injection (part i), intra-arterial injection (part ii) or repeated intra-arterial injection of $Xe^{133}$ in solution (part iii).

No radioactivity was noted at the control hooves after the experiment was discontinued.

Upon removal of the intra-arterial catheter, it was noted that radioactivity could still be detected in the catheter; this was presumed to be associated with the high affinity of $Xe^{133}$ for plastics and synthetic materials. In 4 subsequent experiments, radioactivity at the catheter site and hoof was measured 30 minutes after the $Xe^{133}$ bolus had been administered and subsequently after administration of a saline bolus. The radioactivity neither increased at the hoof after administration of the saline bolus nor did it decrease over the catheter.

## DISCUSSION

This manuscript summarizes a technique for the safe formation and administration of intra-arterial $Xe^{133}$ in solution in the distal limb of standing horses. Furthermore, the gamma radiation emitted by $Xe^{133}$ can be detected through the normal hoof wall, thereby providing a potential mechanism for assessing alterations in laminar blood flow in horses with laminitis.

The specially designed ampoule-crushing device successfully eliminated contact between the $Xe^{133}$ and air, thereby allowing $Xe^{133}$ to form a lattice in sterile saline with minimal losses of $Xe^{133}$ (Oertel *et al.* 2007). The importance of eliminating air from the equipment used to form $Xe^{133}$ in solution established in preliminary trials was attributed to the low solubility of $Xe^{133}$ in sterile saline (Oertel *et al.* 2007). To facilitate equilibration of $Xe^{133}$ within the device, a period of at least 4 hours was used between crushing of the $Xe^{133}$ containing ampoule and aspiration of fluid from the ampoule-crushing device (Oertel *et al.* 2007). Equilibration periods in excess of 4 hours have been demonstrated to have no further effect on the concentration of $Xe^{133}$ in solution beyond that measured at 4 hours (Oertel *et al.* 2007).

When large volumes (5-30mls) of fluid were aspirated from the ampoule-crushing device, the expected amount of $Xe^{133}$ was present within the aspirate; however, when small volumes (1ml) were aspirated from the device, the first 2 syringes that were filled in each experiment contained less $Xe^{133}$ than subsequent syringes. This was likely due to the clearance of the saline in the egress port and blood filter before the solution from the chamber was removed. This emphasizes the importance of verifying the amount of $Xe^{133}$

in each aspirate with a dose calibrator to ensure consistency of the amount being injected. Xenon$^{133}$ adheres to some types of plastic and rubber so the time that Xe$^{133}$ is in contact in syringes should be minimized (Keaney *et al.* 1971; LeBlanc and Johnson 1971). The adherence of Xe$^{133}$ to the syringes was inconsequential in this study as repeated measurements of radioactivity remaining in the syringes after injection of Xe$^{133}$ were indicative of accurate delivery of the appropriate amount of Xe$^{133}$ even when low amounts were administered (0.25mCi of Xe$^{133}$). Similarly, while there was evidence of Xe$^{133}$ binding to the intra-arterial catheter, there was no increase in c.p.m. recorded at the hoof after the catheter was flushed with saline. This finding suggests that the Xe$^{133}$ was bound to the catheter but was not released in to the circulation, which may have confounded interpretation of data.

Intravenous and intra-arterial injections of Xe$^{133}$ were tolerated well in all horses, implying an absence of either acute vasculitis or arteritis in response to the injections. Radiation was not detected at the hoof after Xe$^{133}$ in solution was injected into the jugular vein. This was attributed to the rapid clearance of Xe$^{133}$ through the lungs prior to circulation through the body (Anderson 1996). The rapid increase in c.p.m. at the dorsal laminae after intra-arterial injection is associated with the increased delivery of Xe$^{133}$ within the blood to this area. The c.p.m. increased rapidly after intra-arterial injection and then fell slowly. This slow decline could be associated with trapping of dissolved gaseous Xe$^{133}$ within the laminar tissues or could be associated with formation of a steady state flux between the vascular and tissue compartments within the foot as Xe$^{133}$ diffused through cell membranes.

Use of the $Xe^{133}$ washout technique to measure tissue blood flow is reliant on the dose of $Xe^{133}$ used adhering to the first pass principle, wherein less than 5% of the injected radioisotope recirculates after a single pass through the lungs (Anderson 1996). In this study, the larger amounts of $Xe^{133}$ administered (1mCi and 5mCi) caused prolonged increases in the radiation detected at the hoof and detectable radiation in the systemic circulation. The presence of radiation in the systemic circulation after administration of either 1mCi or 5mCi is consistent with recirculation of $Xe^{133}$. The absence of detectable radiation in jugular venous blood after injection of 0.25mCi is consistent with the lack of recirculation of the radioactive tracer after intra-arterial injection of this more appropriate dose.

The qualitative results obtained from the dorsal laminar region of the hoof were repeatable when experiments were performed on the same forelimb, on the contralateral forelimb and in different horses on different days. The results were, however, susceptible to artifacts produced by movement of the horse that resulted in fluctuations in the c.p.m. recorded at the feet. Fortunately, repositioning of the limb to its original orientation allowed resumption of recordings. In humans, the technique is also repeatable and has been used to quantitatively measure cerebral blood flow (Hoedt-Rasmussen *et al.* 1966; Olesen *et al.* 1971; Paulson *et al.* 1969; Potchen *et al.* 1969; Sousteil *et al.* 2003; Waltz *et al.* 1972). This often requires complex calculations and knowledge of exact tissue partitioning coefficients for the tissue of interest (Olesen *et al.* 1971; Paulson *et al.* 1969; Potchen *et al.* 1969). Because the tissue partition coefficient in the equine foot is unknown, quantification of blood flow using this method would be inaccurate. An alternative method that may help to quantify laminar blood flow is based on the ability to

induce a reproducible peak in radiation that exceeds the small amounts in flux between the vascular and tissue compartments. In this system, the steady state rate of flux of $Xe^{133}$ is insignificantly small relative to the dose of $Xe^{133}$ used to generate the peak. As a result, reliable measurements of blood flow using the area under the curve can be made once a consistent time to peak activity, peak height and decay to half-maximal peak height are established with repeated injections of $Xe^{133}$. In the data collected in this study, the steady state flux of $Xe^{133}$ between the vascular and tissue compartments had not been reached as a small (although decreasing) increase in the peak and trough levels persisted between injections. Although it appears likely that large alterations in laminar blood flow could be detected with this technique, it is possible that the technique would be more sensitive in detecting small alterations in laminar flow and that these could be quantified if the baseline peaks and troughs were static. Consideration should, therefore, be given to establishing a method of achieving steady state rate of flux of $Xe^{133}$ within the laminar tissue for quantification of blood flow.

No alterations in vital parameters were noted in any horses during the study. The minor changes in the complete blood count of one of the horses was most likely associated with the changes in management and resolved without treatment. The mild or moderate swellings detected around the arterial catheter insertion sites were transient. The potential consequences of intra-arterial catheterization and injections vary from swelling, hemorrhage, arteritis, infection, embolus and thrombus formation (Barr *et al.* 2005) and warrant further investigation using histopathological studies.

The type of scintillation device used in these experiments (NaI (Th)) was different from the cadmium-zinc telluride detectors used in human medicine (Ko *et al.* 2005a; Ko

*et al.* 2005b). The cadmium zinc telluride detectors are the highest resolution room temperature gamma-ray detectors available for isotopic analysis and have demonstrated superior performance than traditional gamma cameras (Dardenne *et al.* 1999; Mueller *et al.* 2003). However, their disadvantages relate to the insufficient coverage of a wide energy range in terms of resolution and efficiency, and their small focus of energy absorption (Feichtinger *et al.* 2004). During human studies in which the detectors can be placed directly over a single vessel of interest, the small area of detection is helpful. In contrast, detection of radiation over a wider area would be important in assessing laminar blood flow in horses. For this reason, consideration should be given to the design of a scintillator probe better suited to detection of gamma rays at 81KeV over the wide curved area of the dorsal hoof wall.

In conclusion, in this preliminary study, 0.25mCi of $Xe^{133}$ solution was safely injected intra-arterially in horses at 5-minute intervals for 5-7 injections. After intra-arterial injection in healthy horses lacking evidence of laminitis, there were similar shaped peaks in c.p.m. recorded at the foot suggestive of similar laminar blood flow. Thus, the $Xe^{133}$ washout technique proved to be a repeatable safe method of assessing laminar blood flow. These findings suggest that this method may be utilized both experimentally and clinically to evaluate laminar blood flow. Future studies are needed to establish the sensitivity of this technique to changes in laminar blood flow, the changes in laminar blood flow that occur during laminitis and to asses the effects of existing and experimental vasoactive pharmaceutical agents used to treat horses with laminitis.

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FIGURES

Figure 1

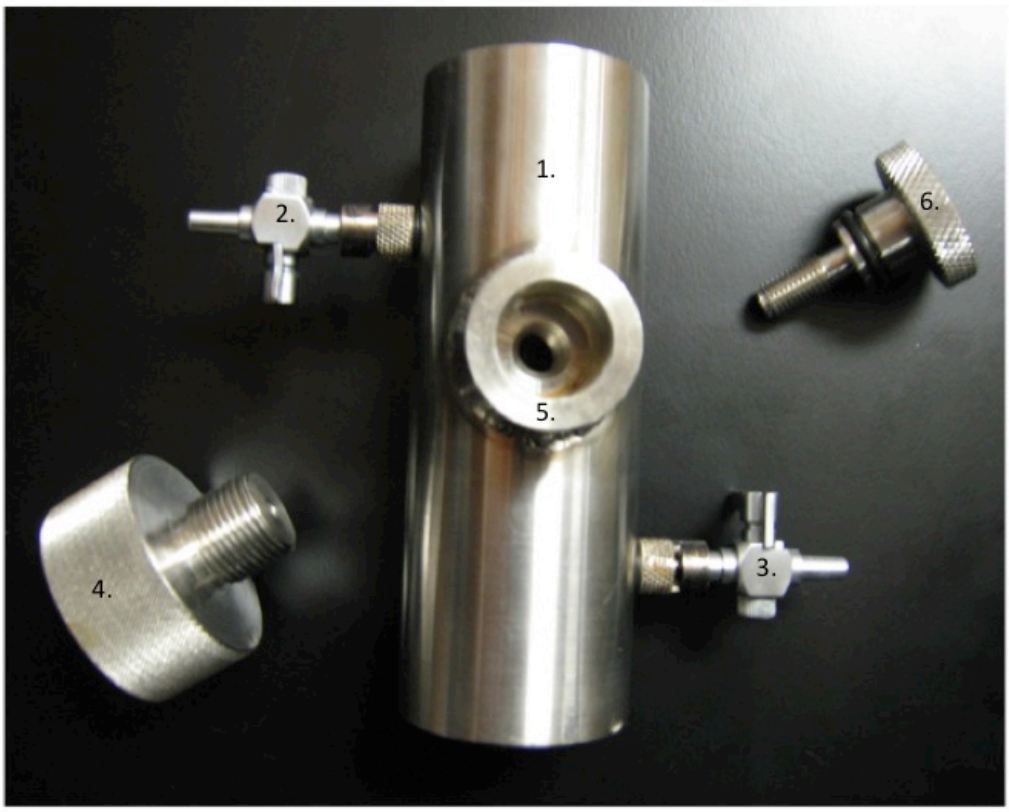


Figure 1

Photograph of the stainless steel ampoule-crushing device used to form Xenon$^{133}$ in solution from gaseous Xenon$^{133}$. 1. Main cylinder of the ampoule-crushing device. 2. Ingress port. 3. Egress port. 4. Lid of ampoule crushing device. 5. Location of position of crushing screw. 6. Crushing screw.

Figure 2

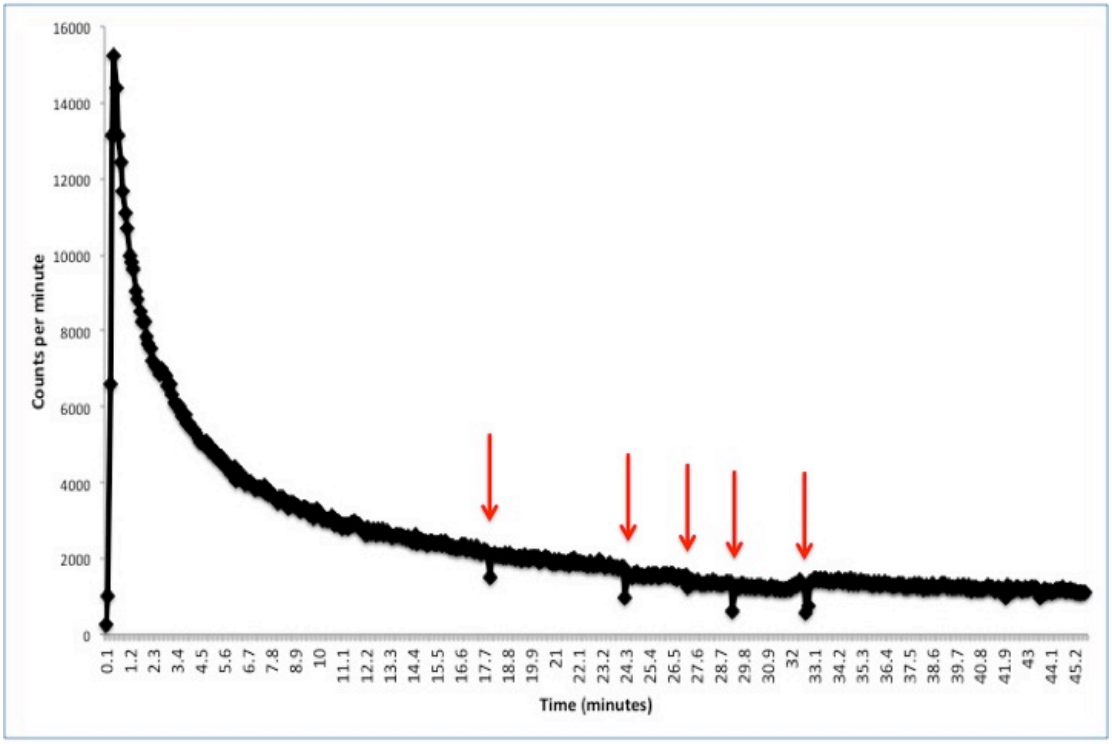


Figure 2

Counts per minute recorded at the hoof after intra-arterial injection of approximately 1mCi of Xenon$^{133}$ in solution (Horse 3). There is a fast rise in counts per minute and a rapid peak. The decline in counts per minute is more gradual and baseline levels are not reached by 45 minutes after intra-arterial injection. The red arrows denote recorded times when the horse moved.

Figure 3

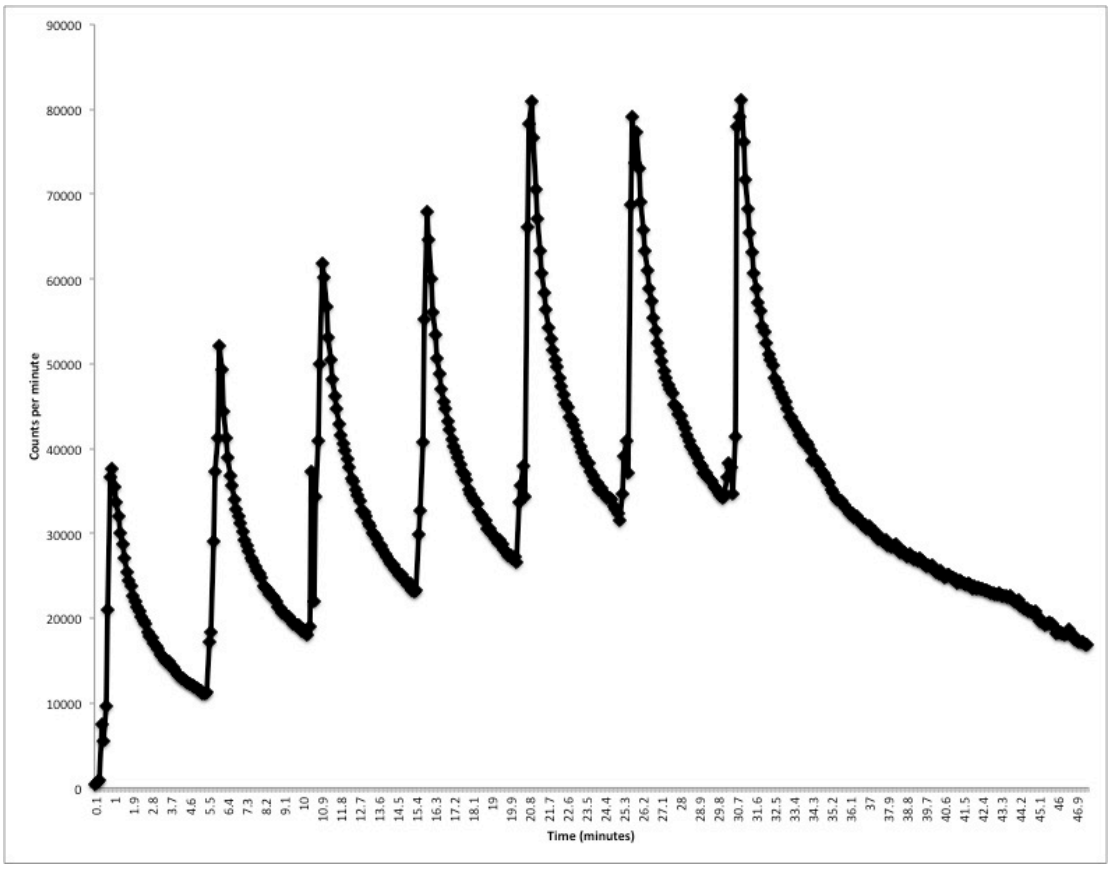


Figure 3

Counts per minute recorded at the dorsal hoof wall after repeated intra-arterial injections of 0.25mCi of Xenon$^{133}$ in solution (Horse 4). Initially, progressive increases in the baseline and peak values were identified. A steady state flux began to occur after 4 injections in this horse.

Figure 4

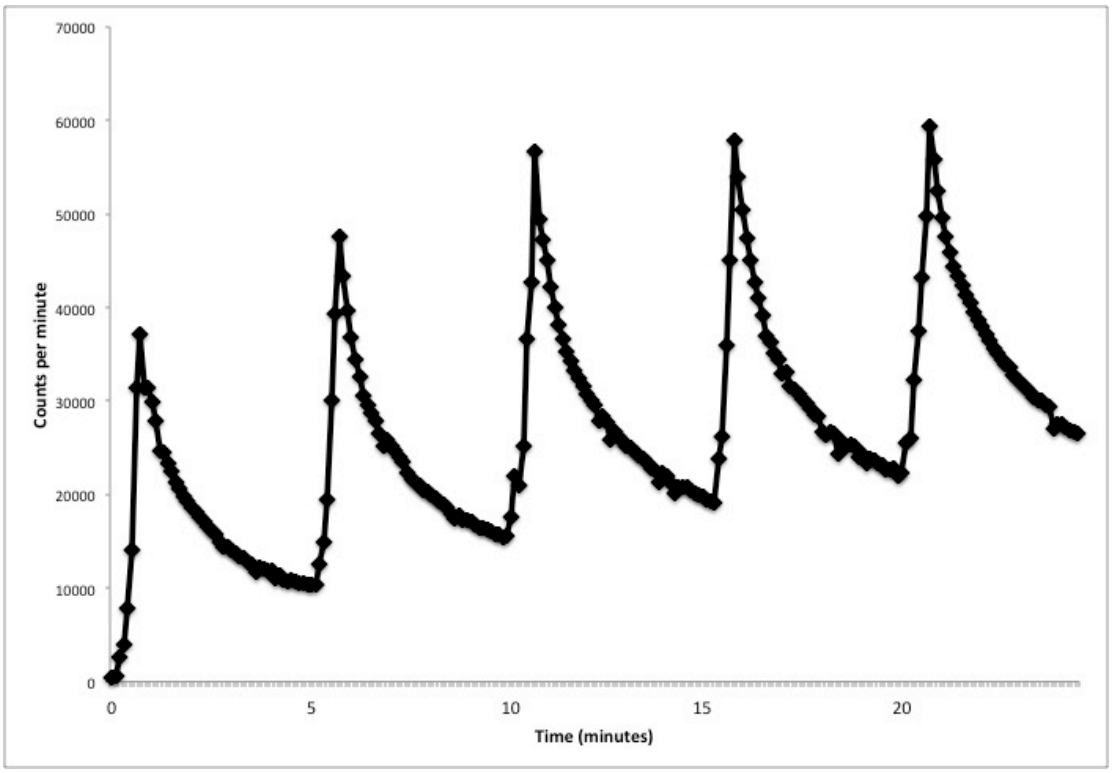


Figure 4

Corrected average counts per minute recorded at the dorsal hoof wall after repeated intra-arterial injections of 0.25mCi of Xenon$^{133}$ in 7 limbs of four horses.

Figure 5

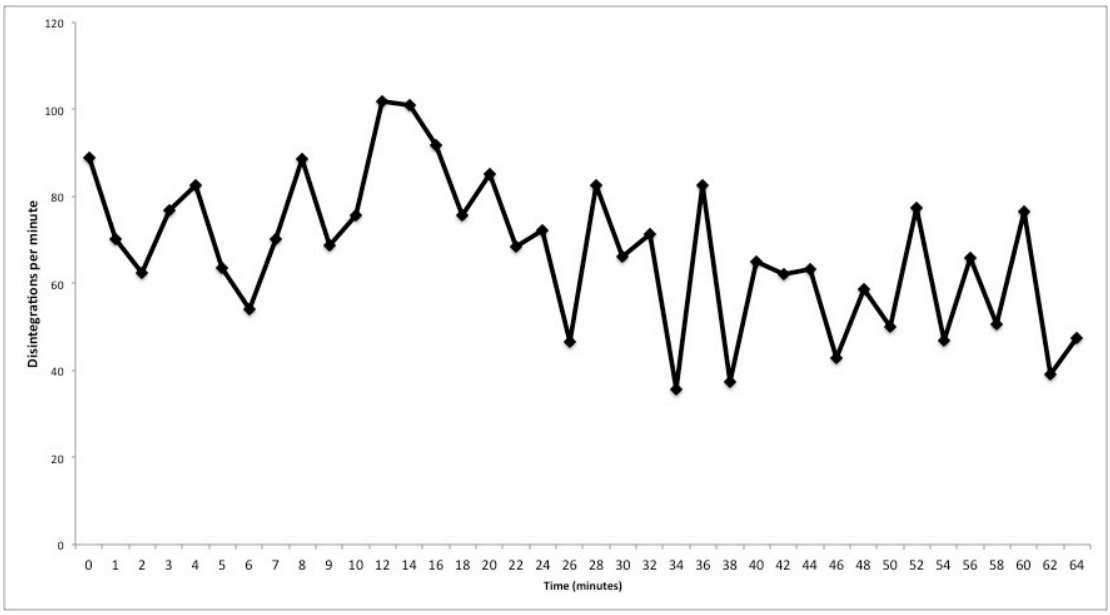


Figure 5

Average disintegrations per minute recorded from the systemic circulation after repeated intra-arterial injections of 0.25mCi of $Xenon^{133}$ in solution (n=7 data sets). All values were below the recognized significant level of 200d.p.m.

# CHAPTER 4

## CONCLUSIONS AND FUTURE DIRECTIONS

The purposes of this study were to develop and evaluate a technique for measuring laminar blood flow in conscious horses. The results obtained during this preliminary study have established that a catheter inserted into the medial palmar artery can be maintained and utilized for the safe intra-arterial administration of $Xe^{133}$ in solution in standing, nonsedated horses. Furthermore, the gamma radiation emitted by $Xe^{133}$ can be detected through the keratinized hoof wall and it creates a consistent profile of a rapid increase and more prolonged decay. This study has also established that a 0.25mCi dose, given singularly or repeated at 5-minute intervals, adheres to the first pass principal with minimal to no recirculation of the tracer. The method described may, therefore, be of value in measuring laminar blood flow in horses in both experimental and clinical scenarios.

Potential improvements to the technique include the use of a multi-crystal array detector, as is used in humans to measure cerebral blood flow, to improve the resolution of the signal from the laminar tissue. Additionally, it may be beneficial to establish quantitative data regarding laminar blood flow, a process that will likely require a steady state rate of flux of $Xe^{133}$ within the laminar tissue. Further investigation into the most

appropriate dose and frequency of doses to achieve steady state flux in the shortest period of time whilst avoiding recirculation is therefore warranted.

The technique described here also requires validation to ensure its sensitivity to changes that may occur in laminar blood flow. This may be achieved by selectively altering laminar blood flow by increasing post-capillary resistance and observing the magnitude of change of c.p.m. at the hoof. This method may then prove helpful to establish the effects of pharmacological agents commonly used to treat laminitis on laminar blood flow, as well as to define the hemodynamic changes at the laminar level in horses with laminitis.