Measurement of equine laminar blood flow and vascular permeability by use of dynamic contrast-enhanced computed tomography

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Objective—To define the reference range for laminar blood flow (BF) and vascular permeability (VPM) in horses without laminitis by use of dynamic contrast-enhanced computed tomography (CT).

Animals—9 adult horses that were not lame and had no abnormalities of the laminae or phalanges detectable via radiographic examination.

Procedures—Each horse was anesthetized by use of a routine protocol. Horses were placed in right or left lateral recumbency with the dependent forelimb in the CT gantry; only 1 limb of each horse was scanned. Serial 10-mm collimated transverse CT images were acquired at the same location every other second for 90 seconds during infusion of ionic, iodinated contrast medium. Custom software was used to estimate BF, VPM, and fractional vascular volume (FVV) in the dorsal, dorsomedial, and dorsolateral laminar regions.

Results—Among the 9 horses’ forelimbs, mean ± SD dorsal laminar BF was 0.43 ± 0.21 mL·min⁻¹·mL⁻¹. Mean dorsomedial and dorsolateral laminar BFVs were 0.26 ± 0.16 mL·min⁻¹·mL⁻¹ and 0.24 ± 0.16 mL·min⁻¹·mL⁻¹, respectively. Mean dorsal laminar VPM was 0.09 ± 0.03 mL·min⁻¹·mL⁻¹. Mean dorsomedial and dorsolateral laminar VPMs were 0.16 ± 0.06 mL·min⁻¹·mL⁻¹ and 0.12 ± 0.06 mL·min⁻¹·mL⁻¹, respectively. Mean dorsal laminar FVV was 0.63 ± 0.20 and dorsomedial and dorsolateral laminar FVV were 0.37 ± 0.14 and 0.34 ± 0.17, respectively.

more quantitative assessment that can be used to evalu-
ate laminar regions. However, both venography and
uclear scintigraphy provide planar images wherein
superimposition can limit the evaluation of anatomic
detail. The measurement of VPM has been less com-
monly performed; we are aware of only 1 study in
which lymphatic flow was used as an indirect measure
of VPM.

Dynamic contrast-enhanced CT is a technique that
capitalizes on the excellent anatomic detail and rapid
acquisition time of CT. Helical CT scanners are able
to generate a series of thin, cross-sectional images in
a single anatomic plane, resulting in a near real-time as-
essment of iodinated contrast agent as it flows into the
region. The iodine concentration in tissue is linearly
related to the recorded change in HUs; therefore, these
data, plotted over time, can be used to estimate regional
blood flow and VPM through mathematical analysis. These DCE-CT measurements have been validated by
use of radioactive microspheres in normal, tumor-infil-
trated, and ischemic brain tissue of rabbits. For the
assessment of peripheral soft tissue tumors in rodents,
DCE-CT estimates of BF and VPM have also compared
favorably with values determined by use of gold stan-
dard techniques.

The purpose of the study of this report was to de-
fine the reference range for laminar BF and VPM in
horses without laminitis by use of an established and
validated DCE-CT technique that had been modified
for application in horses.

Materials and Methods

Horses—Nine university-owned horses were eval-
uated at the University of California, Davis, Veterinary
Medical Teaching Hospital. The horses had no signs
of clinical lameness and no evidence of laminitis was
detected on complete radiographic examination of the
distal portion of the forelimb. The institutional animal
care and use committee approved the experimental pro-
tocols used in the study.

Anesthesia and catheterization—For each horse,
xylazine hydrochloride (0.3 to 0.5 mg/kg, IV) and bu-
torphanol tartrate (0.01 mg/kg, IV) were administered
for sedation and analgesia. Anesthesia was induced with
guaiifenesin (100 mg/kg, IV) and ketamine hydrochlo-
ride (2.2 mg/kg, IV) and maintained with isoflurane
and oxygen. For each horse, ventilation was provided
for the duration of the anesthesia.

Each horse was positioned in either right or left
lateral recumbency with the dependent forelimb ex-
tended within the gantry of the CT unit. Only 1 limb
of each horse was scanned, and use of the right and
left forelimbs was alternated among the 9 horses. After
positioning, the skin over the median or medial palmar
artery of the dependent limb was clipped and asepti-
cally prepared for catheter insertion by use of a tech-
nique previously described. An 18-gauge, 1.88-inch
(1.8 × 48-mm) catheter was inserted with ultrasound
guidance. The catheter was affixed to the skin with 2-0
polypropylene suture; a 70-cm pressure injector
extension set was attached to connect the catheter to
a continuous infusion pump. Fiducial markers con-
sisting of 3 aluminum bars were affixed to the outer
surfaces of the hoof. The fiducial markers were used
to evaluate and correct for any motion that occurred
during the scan. After acquisition of the described im-
ageing sequences, the catheter was removed from the
artery, the limb was bandaged, and the horse was allowed
to recover unassisted from anesthesia. All horses were
housed in the hospital for 24 hours and monitored for
complications related to the catheterization (heat, signs
of pain, swelling, or changes in digital pulse quality) or
anesthesia (alterations in rectal temperature, pulse, or
respiration).

Data acquisition—A single detector row, helical
CT scanner was used for image acquisition. Latero-
medial and dorsopalmar scout images were acquired
initially. An initial imaging sequence of 5-mm col-
limated contiguous images from the middle of the
first phalanx to the distal extent of the third pha-
lanx was performed. From these scans, a location
was identified that included the deep digital flexor
tendon, collateral ligaments of the distal interpha-
 Langeal joint, and the laminae. Each horse was hy-
perventilated by use of a positive pressure ventilator,
and then the ventilator was turned off for the dura-
tion of the contrast-enhanced scanning procedure,
thereby inducing a single breath-hold for the im-
age acquisition. A 10-mm collimated image was ac-
quired at the chosen location every other second for
90 seconds starting 5 seconds prior to contrast me-
dium injection. Each individual image was acquired
over approximately 0.8 seconds, and 45 images were
obtained. Ionic iodinated contrast medium (400
mg of iodine/mL) diluted 1:1 in physiologic saline
(0.9% NaCl) solution was administered through the
catheter in the medial palmar artery and controlled
with an automated pump at 3 mL/s for 30 seconds
(total volume administered, 90 mL). Computed
tomographic tube output variables were 120 kVp
and 150 mA; the field of view was 17.4 × 17.4 cm,
and the pixel matrix was 512 × 512. A soft tissue re-
construction algorithm was applied.

ROI analysis—From the images obtained, a con-
trast concentration–versus-time curve was generated
from operator-defined ROIs by use of custom soft-
ware in an engineering software program. The ROI
tool was a shapeable ellipse that was drawn on an im-
age within the series that included contrast medium
within the vasculature. The software then applied the
ROI, after correction for any motion identified with
the fiducial markers, to every CT image. To determine
BF to the laminae, separate ROIs were chosen on the
dorsal, dorsomedial, and dorsolateral aspects of the
laminae as well as on an artery in the image; the lat-
ter ROI was used to define the arterial input function
(Figure 1). The arterial input ROI was drawn around
a small artery just medial to the abaxial margin of the
distal sesamoid bone. The laminar ROI was drawn
in the shape of a narrow ellipse with margins that
were closely associated with the areas of laminar BF
whereas the arterial ROI was drawn in the shape of
a small circle to approximate the margins of an ar-
tery within the image. The locations of the ROIs were
consistent and empirically chosen so that the dorsal ROI was located on dorsal midline and the dorsomedial and dorsolateral ROIs approximately 30 degrees medial and lateral from dorsal midline, respectively. The length of each ROI was approximately 1 cm. Blood flow (mL min\(^{-1}\) mL\(^{-1}\)) was calculated by use of measurements obtained from time-versus-contrast enhancement or density curves (Figure 2) for tissue and arterial input ROIs and by use of the Mullani-Gould formula as follows:

\[
BF = \frac{\text{Peak tissue enhancement (HUs)}}{\text{Area under the arterial curve (HUs min)}}
\]

This method of blood flow estimation has been validated previously against a fluorescent microsphere technique.\(^6\)

Patlak analysis has been previously described\(^8\) to calculate tissue contrast medium clearance VPM (mL min\(^{-1}\) mL\(^{-1}\)) in tissue. Patlak analysis uses a 2-compartment model theory to determine the rate constant of tissue uptake of a tracer between the intravascular and extravascular spaces\(^9\) (Appendix). This technique was originally described for nuclear imaging techniques and has also been applied to DCE-CT.\(^{30-32}\) By mathematical manipulation of the time-density data in relation to an arterial input function, a linear equation is created in which the slope approximates VPM and the y-intercept estimates the fraction of the tissue composed of blood vessels (FVV). For the dorsal, dorsolateral, and dorsomedial laminar, the ROIs were drawn and calculations were performed on 2 occasions by 1 of the authors (EFK). Close inspection of the ROIs and the data produced indicated that ROI drawing was improved in the second set of data. More specifically, better care was taken to exclude avascular cornified tissue and limit ROI boundaries to vascularized laminae in the second set of data. For this reason, statistical analyses were performed on the second measurements only. Blood flow values were adjusted and normalized to the FVV obtained in the Patlak calculations. Fractional vascular volume is reported without units as it represents the proportion of the tissue within the ROI that is vascular.

**Statistical analysis—**All statistical analysis was performed by use of commercially available software.\(^7\) A single-factor ANOVA was used to compare the mean values for BF, VPM, and FVV in the dorsal, dorsomedial, and dorsolateral regions of the laminae. A Student t test was used to identify significant differences among regions when ANOVA results indicated a disparity. Linear regression was performed to determine the relationship between FVV and BF. A value of \(P < 0.05\) was considered significant. Blood flow values were adjusted and normalized by FVV to allow direct comparison of the individual vessel flow in the dorsal laminar region to that of the dorsolateral or dorsomedial laminar region.

**Results**

The technique was completed successfully in all horses. No complication related to catheter placement, contrast agent administration, or anesthesia was detected in any horse.

Values for BF, VPM, FVV, and normalized BF were obtained (Table 1). Blood flows in the dorsal, dorsomedial, and dorsolateral laminar regions were not significantly \((P = 0.06)\) different. The ANOVA identified a significant \((P = 0.03)\) difference in VPM between dorsal and dorsomedial laminar regions. The dorsal laminar VPM was significantly \((P = 0.004)\) less than the dorsomedial laminar VPM. No significant difference in VPM between the dorsal and dorsolateral laminar regions \((P = 0.06)\) or between the dorsomedial and dorsolateral laminar regions \((P = 0.64)\) was detected.

The ANOVA identified a significant \((P = 0.02)\) difference between FVV among the 3 regions. In the dorsal aspect of the lamina, FVV was \(0.67 \pm 0.20\). The dorsal laminar FVV was significantly higher than the dorsomedial laminar FVV \((0.37 \pm 0.14; P = 0.02)\) and dorsolateral laminar FVV \((0.34 \pm 0.17; P = 0.02)\). There was no significant \((P = 0.91)\) difference in FVV between the dorsomedial and dorsolateral laminar regions.

Apparent relationships between FVV and BF in all areas of the hoof were identified (Figure 3). The normalized BF values for the dorsal, dorsomedial, and dorsolateral laminar locations were \(0.67 \pm 0.18\) mL min\(^{-1}\) mL\(^{-1}\), \(0.67 \pm 0.24\) mL min\(^{-1}\) mL\(^{-1}\), \(0.37 \pm 0.13\) mL min\(^{-1}\) mL\(^{-1}\), and \(0.35 \pm 0.12\) mL min\(^{-1}\) mL\(^{-1}\).
Discussion

Dynamic contrast-enhanced CT was used in the present study to rapidly and noninvasively evaluate the hemodynamic variables of laminar BF and VPM in horses that did not have signs of laminitis. Results indicated that VPM was significantly lower in the dorsal laminar region, compared with the dorsomedial and dorsolateral laminar regions. However, the consistency of values calculated in our study suggests that interhorse variation of this measure is small. Similarly, the FVV was significantly higher in the dorsal laminar region than the value in either the dorsolateral or dorsomedial laminar region. Conversely, BF did not differ significantly among the 3 regions evaluated, which was likely attributable to the high SD of values. As one might expect, BF is strongly correlated with the vascular density in the ROI. Normalization of blood flow values on the basis of vascular density revealed that the flow in any given vessel was more consistent (evidenced by lower SD values). Furthermore, overall BF differences among the dorsal, dorsolateral, and dorsomedial laminar locations were more related to the number rather than the flow volume of local blood vessels. The implication of these findings in the development and progression of laminitis remains to be elucidated.

Dynamic contrast-enhanced CT has been established as a valid tool to yield quantitative information regarding several BF variables. Modern helical CT scanners can acquire data repetitively from the same physical location by scanning rapidly without table translation. With the administration of commercially available iodinated contrast medium, time-density data are acquired that yield quantitative measures similar to the information previously rendered by radiopharmaceutical tracers used in functional nuclear medicine studies. The advantage of DCE-CT over these scintigraphic methods is that the spatial resolution is far superior, thereby allowing more precise placement of ROIs. In the present study, ROI assignment became more precise with practice and when an effort was made to exclude the cornified, avascular tissue of the hoof wall.

The ability to derive quantitative BF and VPM information via DCE-CT is dependent on several key properties of an intravascular iodinated contrast medium. First, there is a linear relationship between iodine concentration in tissue and the relative density of tissue measured by the CT scanner (in HUs). Further, an iodinated contrast medium mixes rapidly in circulation with the blood. The implications of these findings in the development and progression of laminitis remain to be elucidated.
ing plasma and quickly leaks out of the vascular space into the interstitium, whereas only a small percentage (1% to 2%) enters into intracellular spaces.\textsuperscript{35,36} On the basis of these general properties of iodinated contrast medium, BF can be measured in the early phase of contrast medium ingress into a given region and VPM can be estimated in the later phase.

Blood flow per unit volume of tissue was calculated in the present study by use of the Mullani-Gould relationship, a method of calculation that has been previously validated.\textsuperscript{26} This method makes important assumptions about BF.\textsuperscript{26} It assumes that contrast medium entering the region must pass in a linear fashion through successively smaller vessels and that the maximum contrast medium concentration in tissue precedes the beginning of tissue wash-out or elimination of contrast medium. The ideal arterial contrast medium concentration–versus-time input function has the shape of a delta function (square wave) with instantaneous arrival and departure.\textsuperscript{37} When contrast medium is administered through a peripheral vein, the shape of the arterial contrast concentration–versus-time curve develops a Gaussian (bell) shape as a result of passage through the cardiopulmonary circulation.\textsuperscript{37} In our study, a direct arterial injection was used, which resulted in an arterial time-density curve that more closely resembled a delta function with a very short time to maximum contrast medium concentration.

Vascular permeability is defined as the net flux of fluid from the intravascular space into the interstitial space. The Patlak method of calculation used in the present study is based on a 2-compartment model wherein contrast medium accumulation in the ROI is represented by contrast medium in the intravascular space and the interstitial space.\textsuperscript{27,36} During data collection, a time-density curve is made for the ROI but also for an artery included within the image. The arterial time-density curve provides information regarding the intravascular contrast medium component, which should mirror the plot of vascular contrast medium concentration in the ROI. Vascular permeability is then calculated as the rate constant of contrast medium moving from the intravascular space into the interstitium.

To our knowledge, only 1 attempt at estimation of VPM alterations associated with laminitis in horses has been performed in vivo, and that investigation used lymphatic flow as an indirect measure.\textsuperscript{21} Given that there is histologic evidence to support ischemia-reperfusion as a contributor to laminar damage, it is reasonable to anticipate that both BF and VPM will be altered in this disease.\textsuperscript{9,38} Several reasons exist to predict that VPM in particular will be altered. It is known that small blood vessel pressure increases during the acute phase of laminitis.\textsuperscript{21} Because the vascular supply in the foot operates with high hydrostatic pressures,\textsuperscript{39,40} the capillaries of the foot are relatively permeable to macromolecules,\textsuperscript{41} and the venules of the laminae are apparently more reactive to vasoactive stimulation,\textsuperscript{42} increases in laminar blood vessel pressure would likely force fluid into the interstitium by increasing hydrostatic pressure. Furthermore, arteriovenous anastomoses exist in the dermal lamina and their regulation is not clearly understood; however, if venous hypertension exists, blood is thought to be shunted away from the tissues into venous circulation.\textsuperscript{39} The combination of high hydrostatic pressure with relatively leaky vascular endothelium suggests that the laminae could be prone to abnormalities in VPM. An increased amount of interstitial fluid in the confined physical space of the hoof capsule will potentially further increase interstitial hydrostatic pressure, preferentially affecting the venules, and thereby shunt or increase net fluid flux into the interstitial space. This shunting would be less than the resolving power of CT and could introduce bias. Shunting of contrast medium through these vessels would be interpreted as areas of perfused tissue while actually representing regions with poor cellular perfusion.

Figure 3—Linear regression analysis of dorsal (A), dorsomedial (B), and dorsolateral (C) laminar BF versus FVV derived via DCE-CT from the forelimbs of 9 horses without laminitis (1 limb/horse). For dorsal laminar BF versus FVV, \( y = 0.9178x - 0.1025 \) \( (R^2 = 0.9812) \); for dorsomedial laminar BF versus FVV, \( y = 1.0178x - 1.127 \) \( (R^2 = 0.9829) \); and for dorsolateral laminar BF versus FVV, \( y = 0.9778x - 0.0537 \) \( (R^2 = 0.8614) \).

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In horses, DCE-CT offers a means of BF and VPM evaluation; absolute vascular flow, VPM, and vascular volume values can be assessed, or changes in these variables over time can be monitored. In the present study, this novel, noninvasive technique proved to be repeatable; following normalization for vascular volume, consistent absolute values for BF to laminar regions in the forelimbs of horses were obtained. Vascular permeability values were similarly consistent. We anticipate that this technique will allow for a greater understanding of the pathophysiology of laminitis in horses if applied in research and clinical settings.

References


Following IV administration of contrast medium, the net concentration of contrast medium within an ROI in DCE-CT images at time $t$ ($C(t)$) is made up of the intravascular (IV) and interstitial (IS) components. At early time points, the bulk of transfer of contrast medium is from IV to IS regions. The rate of contrast medium transfer from IV to IS regions can be estimated by an equation as follows:

$$\frac{dQ}{dt} = \alpha - B(t)$$

where $\alpha$ is the blood contrast medium clearance (in HUs) and $B(t)$ is the contribution of blood contrast medium (in HUs) at time $t$. At any given time $t$, the contrast agent transferred into the IS components (in HUs) is estimated by an equation as follows:

$$Q(t) = \alpha \int B(t) dt$$

If the contrast agent transferred into the IS components ($\alpha \int B(t) dt$) is divided by the volume $V$ of the ROI, the contribution of IS contrast medium (in HUs) is determined. The net contribution of contrast agent is determined by adding the IV and IS contrast medium contributions together as follows:

$$C(t) = \frac{\alpha}{V} \int B(t) dt + (FVV)B(t)$$

where FVV is the fractional vascular volume. Division by the contrast medium contribution from blood ($B(t)$) provides an equation as follows:

$$\frac{C(t)}{B(t)} = \frac{\alpha}{V} \int \frac{B(t)}{B(t)} dt + FVV$$

If a plot of $C(t)/B(t)$ versus $\int B(t)/B(t)$ is performed, a straight line with slope $\alpha/V$ (mL•min$^{-1}$•mL$^{-1}$) and $y$-intercept FVV (%) is obtained. Estimated values (in HUs) of $C(t)$ and $B(t)$ can be obtained from the ROIs drawn over the tissue and arterial input in CT images, respectively.