Doppler ultrasonography and single-fiber laser Doppler flowmetry for measurement of hind limb blood flow in anesthetized horses

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Objective—To use Doppler ultrasonography and single-fiber laser Doppler flowmetry (LDF) to evaluate blood flow in the dependent and nondependent hind limbs of anesthetized horses and to evaluate changes in femoral arterial blood flow and microvascular skeletal muscle perfusion in response to administration of phenylephrine hydrochloride or dobutamine hydrochloride.

Animals—6 healthy adult horses.

Procedure—Horses were anesthetized and positioned in left lateral recumbency. Doppler ultrasonography was used to measure velocity and volumetric flow in the femoral vessels. Single-fiber LDF was used to measure relative microvascular perfusion at a single site in the semimembranosus muscles. Phenylephrine or dobutamine was then administered to decrease or increase femoral arterial blood flow, and changes in blood flow and microvascular perfusion were recorded.

Results—Administration of phenylephrine resulted in significant decreases in femoral arterial and venous blood flows and cardiac output and significant increases in mean aortic blood pressure, systemic vascular resistance, and PCV. Administration of dobutamine resulted in significant increases in femoral arterial blood flow, mean aortic blood pressure, and PCV. Significant changes in microvascular perfusion were not detected.

Conclusion and Clinical Relevance—Results suggest that Doppler ultrasonography and single-fiber LDF can be used to study blood flows in the hind limbs of anesthetized horses. However, further studies are required to determine why changes in femoral arterial blood flows were not associated with changes in microvascular perfusion. (*Am J Vet Res* 2000;61:286–290)

Doppler ultrasonography is a noninvasive method of measuring blood flow in the femoral arteries and veins of anesthetized horses.[1] In addition, analysis of waveforms obtained with Doppler ultrasonography allows calculation of indices that reflect changes in peripheral vascular resistance and other physiologic factors that influence blood flow.[2] One of the limitations of Doppler ultrasonography is that it is restricted to measuring blood flow in larger vessels. Combining Doppler ultrasonography with a technique that measures microvascular perfusion may provide more detailed information on the effects of anesthesia on skeletal muscle blood flow.

Laser Doppler flowmetry (LDF) has been extensively used to measure microvascular perfusion in the skeletal muscles of anesthetized horses.[3-5,a] Laser Doppler flowmetry measures the Doppler shift in frequency of monochromatic laser light scattered by moving RBC within a small (approx 1 mm^3) volume of illuminated tissue. The magnitude and frequency of Doppler-shifted light in the returning LDF signal is proportional to the velocity and number of moving RBC within the illuminated sample volume.[6-8] Because of variations in vascular architecture and optical properties of tissues, calibration of the LDF signal is not possible. Therefore, microvascular perfusion is recorded in arbitrary units.[9,10] Laser Doppler flowmetry, thus, provides a continuous measure of relative perfusion, allowing detection of changes in blood flow over time at a single site.[11]

Initial studies of LDF in anesthetized horses used muscle surface probes to measure relative changes in perfusion within the superficial microvasculature.[4,12,13] However, this method is invasive, because skin incisions are required for insertion of the probe, limiting its use to experimental studies. In more recent studies,[a,b] intramuscular needle probes were used to measure changes in microvascular perfusion of the skeletal muscles of anesthetized ponies. The advantage of intramuscular probes is that they are less invasive and can be used to measure relative changes in perfusion within the muscle itself, where high intramuscular pressures are more likely to cause disruptions in blood flow.

Single-fiber laser Doppler probes, because of their smaller diameter, have the potential to cause less tissue trauma than needle probes, and single-fiber LDF is reported to provide sensitive and repeatable recordings of relative skeletal muscle perfusion in humans and pigs.[14-16] However, to our knowledge, single-fiber Doppler probes have not been used to study skeletal muscle perfusion in horses.

The purposes of the study reported here were to use Doppler ultrasonography and single-fiber LDF to evaluate blood flow in the dependent and nondependent hind limbs of anesthetized horses and to evaluate changes in femoral arterial blood flow and microvascular skeletal muscle perfusion in response to administration of phenylephrine hydrochloride or dobutamine hydrochloride. Doppler ultrasonography was used to measure blood velocity and volumetric flow in the femoral vessels; single-fiber LDF was used to measure microvascular perfusion within the semimembranosus muscles.

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Materials and Methods

Horses—Six adult Thoroughbred and Thoroughbred cross horses (4 castrated males and 2 sexually intact females) weighing between 450 and 650 kg and between 4 and 6 years old were used in the study. The right carotid artery of each horse had been surgically relocated to a subcutaneous position at least 12 weeks before the study. The study protocol was approved by the Ethics Committee of the Animal Health Trust and was performed under a Home Office Project License.

Experimental protocol—Each horse was anesthetized twice, with at least a month between anesthetic episodes. Before each anesthetic episode, food, but not water, was withheld for 12 hours. Horses were sedated with romifidine (0.1 mg/kg, IV), and 5 minutes later, anesthesia was induced with ketamine hydrochloride (2.2 mg/kg, IV). Endotracheal intubation was performed, and horses were hoisted onto a padded operating table and positioned in left lateral recumbency with the upper and lower limbs parallel and the lower limb slightly cranial to the upper limb. Anesthesia was maintained with halothane delivered in oxygen via a large animal anesthetic circuit. End-tidal halothane concentrations were monitored with a calibrated piezoelectric monitor[c] and maintained between 0.9 and 1.0% (1.1–1.2 times minimal alveolar concentration[17]). Intermittent positive-pressure ventilation was performed to maintain $Paco_2$ between 40 and 50 mm Hg.

After horses were anesthetized, a dual-sensor catheter[d] consisting of 2 strain gauge transducers positioned 10 cm apart on an 8F woven polyethylene terephthalate catheter was inserted in the right carotid artery, using the Seldinger technique.[18] The catheter was advanced until pressure waveforms indicated that the distal transducer was positioned in the left ventricle and the proximal transducer was positioned in the aorta. The proximal transducer was used to measure aortic pressure throughout the study. Right atrial pressure was measured by use of a strain gauge transducer[e] mounted on the distal end of a 7F woven polyethylene terephthalate catheter inserted in the right jugular vein, using the Seldinger technique. The catheter was advanced until pressure waveforms indicated the transducer was in the right ventricle. The catheter was then slowly withdrawn until the pressure waveforms indicated that the transducer was in the right atrium. A universal amplifier[f] supplied bridge excitation voltage to each transducer. The analog signal from each transducer was recorded and displayed continuously, using data acquisition software.[g] Transducers were calibrated against a precision mercury manometer at pressures of 0 and 100 mm Hg. Response of the transducers was confirmed to be linear within this measured range.

During the anesthetic period, heart rate was determined from a base-apex electrocardiogram recorded by use of a telemetric system.[h] The voltage output of the electrocardiograph was digitized and recorded by the data acquisition system.

Cardiac output (CO) was calculated from aortic velocity waveforms recorded by use of transesophageal echocardiography.[19] Immediately after horses were anesthetized, a 160-cm 3.75 MHz transesophageal echocardiographic probe connected to an ultrasound system[i] was inserted into the esophagus via the right ventral nasal meatus. The probe was advanced into the esophagus until the long axis of the left ventricular outflow tract and aorta could be imaged. The Doppler sample volume was then placed in the center of the vessel, above the aortic valve. Two-dimensional images of the aorta were obtained, using ultrasound emitted at 3.0 MHz. Images were transferred to acquisition software as a cineloop. Aortic velocity waveforms were recorded, using high pulse repetition frequency mode with ultrasound emitted at 2.5 MHz. Aortic velocity waveforms were digitized and transferred to acquisition software.

Instrumentation was completed within 40 minutes after induction of anesthesia. Baseline data, including PCV, total plasma protein concentration, mean aortic blood pressure, right atrial pressure, and CO were recorded 60 minutes after induction of anesthesia, and Doppler ultrasonography of the left and right femoral vessels and single-fiber LDF at a single site in the left and right semimembranosus muscles were performed.

After baseline measurements were obtained, femoral artery blood flow was decreased by administration of phenylephrine hydrochloride[j] or increased by administration of dobutamine hydrochloride.[k] For each horse, one of these agents was administered during the first anesthetic episode and the other was administered during the second anesthetic episode; order of agent administration was randomized. Agents were administered by use of an automated infusion pump[l] via a cephalic vein catheter.

Phenylephrine was diluted in 5% glucose to a concentration of 20 µg/ml. A loading dose (2 µg/kg of body weight, IV) was administered, followed by IV infusion at a rate of 1 µg/kg/min. Dobutamine was diluted in 5% glucose to a concentration of 0.125 mg/ml and was administered as a constant infusion at a rate of 0.5 µg/kg/min, IV.

During administration of phenylephrine, a steady state increase in mean aortic blood pressure was achieved within 15 minutes after starting the infusion, and data collection was repeated during this steady state. During administration of dobutamine, a steady state increase in blood pressure was not achieved. Therefore, data collection was repeated 30 minutes after the infusion was started. After the second data collection procedure, catheters were removed, and horses were allowed to recover from anesthesia.

Doppler ultrasonography of femoral vessels—Ultrasonography of femoral vessels was performed using a 7.5 MHz annular phased array transducer connected to an ultrasound system.[i] Femoral vessels were examined by placing the probe over the femoral groove, as proximal as possible in the inguinal region.

Two-dimensional images were obtained by use of ultrasound emitted at a frequency of 5 MHz. Gain and dynamic gray scale were adjusted until vessel walls were clearly delineated from surrounding tissues, and probe position was adjusted until lengths of the femoral artery and vein with distinct parallel vessel walls were imaged within the sector and the angle between the vessel walls and the ultrasound beam was 60 degrees. This angle was used to correct velocity calculations and measured by aligning the angle correction cursor with the vessel axis and measuring the angle between the angle correction cursor and the ultrasound beam. The Doppler sample volume was positioned in the center of the vessel, and size was adjusted to a maximal length of 5 mm. Doppler ultrasonography was then performed in low-pulse repetition frequency mode, using ultrasound emitted at a frequency of 4 MHz. Quality of recorded velocity waveforms was assessed on the basis of clarity of the visual and audible signals. Intermittent positive-pressure ventilation was discontinued while velocity waveforms were recorded to eliminate artifact associated with respiration. Two-dimensional images of the vessels were recorded on videotape. Velocity waveforms were digitized by use of analysis and archiving software[m] and stored on optical disc. An electrocardiogram was recorded simultaneously with images and velocity waveforms.

Single-fiber laser Doppler flowmetry—Laser Doppler flowmetry was performed by use of a dual-channel Oxford array flowmeter.[n] Infrared laser light with a wavelength of 780 nm was generated by 2 stabilized semiconductor laser diodes. Single fiber probes[o] with a diameter of 0.5 mm were used to guide laser light into the semimembranosus muscles of the left and right hind limbs. Probes were calibrated by use of a motility standard consisting of a known concentration of latex spheres undergoing Brownian motion.

Doppler probes were inserted within 5 to 10 minutes

after induction of anesthesia. They were inserted via 20 g polytetrafluoroethylene catheters to a depth of 5 cm and connected via a probe connector to the flowmeter. Probes were secured by taping the coupling bead of the connector to the hub of the catheter. The probe connector was also fixed to an external support to prevent movement. Perfusion, expressed as blood perfusion units, was continuously displayed and recorded by use of data acquisition software.[18] Tissue remittance, a measure of the percentage of the returning LDF signal scattered by RBC, was also recorded.

Data analyses—Vessel diameters were measured from vessel images recorded on videotape, using manual planimetry. Calipers were positioned on the outer edge of the proximal wall and the inner edge of the distal wall, and vessel diameter was calculated as the distance between the calipers. This process was repeated 3 times, and results were averaged to obtain vessel diameter measurements. Vessel diameter was measured at times corresponding to the beginning of the P wave, beginning of the R wave, beginning of the T wave, and midportion of the T-P interval to obtain the average diameter for a single cardiac cycle. This average diameter was subsequently used for estimation of volumetric flow. **Time-averaged mean velocity (TAV)** and volumetric flow were calculated from femoral artery and vein velocity waveforms, using analysis software.[18] Time-averaged mean velocity was calculated by integration of the velocity waveform. Volumetric flow was calculated by multiplying TAV by cross-sectional area, calculated from vessel diameter. Time-averaged mean velocity and volumetric flow were determined for 5 consecutive waveforms, and mean values were calculated.

Microvascular perfusion in the semimembranous muscles was determined by use of analysis software.[18] Tissue remittance was recorded throughout each study to determine whether there were any changes in the percentage backscatter, which would indicate that a probe had moved during the course of a study or that changes in the optical properties of the tissue had occurred because of bleeding or clot formation. If changes in backscatter occurred, data were excluded from the study. Microvascular perfusion was calculated as average blood perfusion units recorded during a 5-minute interval coinciding with Doppler ultrasonography of the femoral vessels.

Cardiac output was calculated as described.[19] Diameter of the aorta was measured from two-dimensional images of the aorta, using manual planimetry. The **velocity-time integral (VTI)** was calculated as the area under the aortic velocity waveform and was measured by manually tracing the maximal velocity envelope. Five consecutive velocity waveforms were measured, and the average was used for calculation of CO. Cardiac output was calculated as the product of aortic cross-sectional area, VTI, and heart rate. **Mean aortic blood pressure (MABP)** and **right atrial blood pressures (RAP)** recorded simultaneously with aortic velocity waveforms were used to calculate **systemic vascular resistance (SVR)**, using the following equation[20]:

$$SVR = (MABP - RAP)/CO \times 80$$

Because microvascular perfusion recorded by use of LDF is measured in arbitrary units, measurements of blood flow and cardiovascular function during infusion of phenylephrine or dobutamine were expressed as a percentage of the baseline values.

Statistical analyses—Values obtained during infusion of phenylephrine or dobutamine were compared with baseline values by use of Wilcoxon signed rank tests. Values of $P < 0.05$ were considered significant.

Results

Volumetric flows in the femoral arteries and veins of the left (dependent) and right (nondependent) hind limbs were significantly decreased during administration of phenylephrine, compared with baseline values (**Table 1**). Decreases in arterial volumetric flow were accompanied by significant decreases in vessel diameter and TAV, whereas decreases in venous volumetric flow were accompanied by significant increases in vessel diameter and significant decreases in TAV. A significant difference in microvascular perfusion in the right and left hind limbs was not detected. Mean aortic blood pressure, systemic vascular resistance, and PCV were signif-

Table 1—Femoral blood flow and microvascular perfusion of the semimembranosus muscles of 6 anesthetized horses before (baseline) and during infusion of phenylephrine hydrochloride (1 mg/kg/min) or dobutamine hydrochloride (0.5 mg/kg/min)

	Phenylephrine infusion			Dobutamine infusion		
Variable	**Baseline**	**During**	**Change***	**Baseline**	**During**	**Change***
Left hind limb						
Femoral artery						
Diameter (mm)	12.2 (10.8–13.7)	10.7† (9.4–12.2)	87.7 (83.7–90.4)	12.8 (11.6–14.6)	13.0 (11.0–14.2)	102 (94.8–109)
TAV (cm/s)	7.27 (5.96–8.84)	3.95† (2.64–5.47)	55.6 (36.1–91.8)	13.1 (9.91–14.9)	17.0† (13.8–22.1)	131 (98.1–159)
Flow (ml/min)	520 (328–630)	213† (142–309)	42.7 (26.0–69.6)	1,015 (807–1408)	1,364† (964–1989)	136 (92.8–176)
Femoral vein						
Diameter (mm)	16.5 (11.0–22.4)	20.4† (15.6–26.2)	124 (96.0–144.0)	14.2 (9.40–20.3)	14.6 (9.80–20.3)	103 (89.3–123)
TAV (cm/s)	5.20 (2.53–9.49)	1.01† (0.92–1.29)	22.8 (10.1–36.8)	11.2 (5.29–16.8)	14.9 (4.89–43.5)	122 (57.8–259)
Flow (ml/min)	613 (365–1001)	197† (148–297)	36.5 (16.5–52.4)	1,017 (579–2194)	1,164 (363–2389)	121 (62.7–206)
Perfusion (BPU)	4.12 (2.00–7.26)	3.79 (2.13–5.73)	94.7 (79.0–106)	4.31 (2.13–7.74)	3.67 (2.38–5.89)	90.3 (76.1–118)
Right hind limb						
Femoral artery						
Diameter (mm)	13.2 (11.9–14.6)	10.8† (8.40–12.7)	81.8 (70.5–88.9)	13.3 (11.2–15.5)	13.5 (11.7–15.2)	102 (96.8–107)
TAV (cm/s)	6.08 (3.98–11.5)	3.48† (2.81–4.40)	63.2 (38.3–89.8)	8.73 (6.15–11.0)	12.9† (9.82–16.5)	153 (116–229)
Flow (ml/min)	500 (281–900)	190† (126–261)	41.1 (26.8–53.9)	736 (540–1248)	1,140† (732–1593)	1,619 (123–263)
Femoral vein						
Diameter (mm)	18.8 (12.8–24.0)	22.0† (17.5–26.3)	117 (110–137)	16.8 (10.5–19.9)	16.8 (10.5–20.5)	100 (93.9–107)
TAV (cm/s)	8.43 (2.21–14.0)	1.78† (0.75–3.07)	25.8 (9.30–46.1)	13.1 (4.47–22.9)	13.8 (7.29–23.1)	116 (88.2–163)
Flow (ml/min)	1,292 (452–1892)	418† (188–825)	34.7 (12.3–55.4)	1,607 (9826–3059)	1,700 (1073–2380)	118 (77.8–162)
Perfusion (BPU)	7.96 (0.99–17.8)	6.94 (1.15–17.3)	164 (35.9–621)	4.12 (1.42–9.68)	3.76 (1.39–8.60)	98.0 (67.0–162)

Values are given as mean (range). Horses were positioned in left lateral recumbency.

*Values recorded during infusion of phenylephrine or dobutamine expressed as a percentage of baseline values. †Significantly ($P < 0.05$) different from baseline value.

TAV = Time-averaged mean velocity. BPU = Blood perfusion units.

Table 2—Cardiovascular function before (baseline) and during administration of phenylephrine or dobutamine to 6 anesthetized horses

Variable	Phenylephrine infusion			Dobutamine infusion		
	Baseline	During	Change*	Baseline	During	Change*
Mean aortic blood pressure (mm Hg)	72.0 (56.8–83.2)	101† (79.7–119)	140 (129–153)	80.5 (74.4–93.8)	92.5† (77.8–99.8)	115 (105–130)
Systemic vascular resistance (dyne•s/cm^5)	181 (133–247)	444† (341–557)	256 (149–343)	172 (104–231)	179 (106–259)	102 (84.3–130)
Cardiac output (L/min)	26.9 (14.3–39.2)	13.6† (11.7–15.7)	55.5 (36.0–82.2)	35.0 (20.8–50.5)	37.5 (22.7–60.6)	107 (83.9–120)
PCV (%)	37.5 (35.0–40.0)	53.0† (44.0–61.0)	141 (118–163)	39.2 (38.0–42.0)	45.5† (43.0–48.0)	116 (107–124)
Total plasma protein (g/L)	58.3 (51.0–69.0)	59.0 (52.0–69.0)	101 (96.3–108)	58.8 (53.0–66.0)	58.3 (53.0–67.0)	99.1 (96.5–102)

See Table 1 for key.

icantly increased, and CO was significantly decreased, during administration of phenylephrine (**Table 2**).

Volumetric flow and TAV were significantly decreased, compared with baseline values, in the right and left femoral arteries during administration of dobutamine, but significant changes were not detected in volumetric flow or TAV in the femoral veins (Table 1). A significant difference in microvascular perfusion was not detected. Mean aortic blood pressure and PCV were significantly increased (Table 2). Cardiac output was not significantly changed, although CO did increase in 5 of the 6 horses.

Discussion

In this study, we did not detect any relative change in microvascular perfusion of the semimembranosus muscles, assessed by means of single-fiber LDF, during administration of phenylephrine (1 µg/kg/min) or dobutamine (0.5 µg/kg/min) to anesthetized horses. These findings are consistent with results reported for microvascular perfusion of the triceps muscles during administration of phenylephrine (0.25 to 2 µg/kg/min) or dobutamine (1 µg/kg/min) to anesthetized ponies.[21] However, the lack of change in microvascular perfusion contrasts with the significant changes in femoral blood flow recorded by means of Doppler ultrasonography. Administration of phenylephrine resulted in significant decreases in femoral artery and vein blood flow and CO and significant increases in SVR, MABP, and PCV. Administration of dobutamine resulted in significant increases in femoral artery flow, MABP, and PCV, but did not result in a significant alteration in CO.

Differences between changes in total organ blood flow and microvascular perfusion may arise because of uneven distribution of perfusion within the organ or the effects of hematocrit on microvascular flow.[11] Regional differences in blood flow may arise if specific physiologic and pharmacologic stimuli exert differential effects within the organ.[10] In anesthetized horses, for instance, the physiologic effects of **intracompartmental muscle pressures (ICMP)** may be responsible for regional differences in blood flow in the skeletal muscles. High ICMP in the dependent forelimbs of anesthetized horses have been reported,[22,23] and, although detailed studies of ICMP in the hind limbs of anesthetized horses have not, to our knowledge, been performed, it is presumed that muscles in the dependent hind limb will also have high ICMP. Surprisingly, microvascular perfusion of the semimembranosus muscle was not found to be significantly changed in the dependent or the nondependent limb in response to administration of phenylephrine or dobutamine. This was similar to results of a previous study in which microvascular perfusions in the dependent and nondependent hind limbs were found to be similar.[24] It is possible that ICMP could be high even in the semimembranosus muscle of the nondependent limb because of the weight of the overlying semitendinosus and biceps muscles. Furthermore, elevation of the nondependent limb above the level of the heart would decrease the tolerance of the microcirculation to any increase in tissue pressure by decreasing local arterial blood pressures.[25]

Perfusion pressure is the difference between MABP and ICMP. Therefore, if ICMP increases or MABP decreases, perfusion pressure will decrease. As perfusion pressure continues to decrease, it will eventually reach the critical closing pressure, resulting in collapse of the capillaries and decreased nutrient blood flow in affected regions.[26,27] Thus, blood flow within the skeletal muscles of the hind limb would preferentially be distributed away from regions of high ICMP to regions with low ICMP. Although the presence of arteriovenous shunts within the skeletal muscles remains controversial, communications between arteries and veins in the connective tissues surrounding the muscle fibers have been observed in some species when capillaries are obstructed by high tissue pressures.[26,28] If horses have similar arteriovenous shunts, they likely would divert blood flow away from areas of high ICMP, resulting in an uneven distribution of blood flow.

Studies in other species have demonstrated that once a capillary has collapsed, the perfusion pressure required to open the capillary is substantially higher than the perfusion pressure observed when capillary flow ceased.[26] Thus, although dobutamine was able to increase femoral artery blood flow in the horses described in the present study, the dosage may have been insufficient to produce detectable changes in microvascular perfusion in regions of high ICMP. If this were the case, any increase in femoral artery blood flow would be distributed to capillaries in regions of low ICMP or to arteriovenous shunts. In the triceps muscles of ponies, a significant increase in microvascular perfusion was not observed until dobutamine was administered at dosages > 2.5 µg/kg/min.[21]

Previous studies of microvascular perfusion of skeletal muscle in anesthetized horses, measured by use of single-fiber LDF, found relative changes in perfusion at single intramuscular sites in the dependent and nondependent limbs.[3-5,a] However, if regional differences in the distribution of blood flow or arteriovenous shunts are present in the hind limbs of anesthetized horses, measurement of microvascular perfu-

sion at multiple intramuscular sites may be necessary to determine the effects of pharmacologic agents.[10,11]

Differences between changes in femoral artery blood flow and microvascular perfusion may also be caused by alterations in PCV. Increases in microcirculatory PCV and associated increases in viscosity are detrimental to microcirculatory perfusion, and it has been suggested that these detrimental effects alone could alter the distribution of perfusion within organs.[29,30] By increasing viscosity, the increased PCV observed during administration of dobutamine to the horses described in the present report could have prevented changes in microvascular perfusion that would normally have accompanied an increase in femoral artery blood flow.

Furthermore, microvascular flux recorded by means of LDF is proportional to RBC velocity and the number of RBC in the sample volume of the Doppler probe.[6-8] As a result, increases in PCV have been observed to produce linear increases in calculated flux when velocity is constant.[30] Thus, it is possible that the expected decreases in microvascular perfusion associated with decreases in TAV observed during phenylephrine administration could have been masked by increases in PCV. As many of the agents used clinically to improve blood pressure in anesthetized horses, such as phenylephrine, dobutamine, and dopexamine, increase PCV,[21] a technique for measuring microvascular perfusion in anesthetized horses that is not affected by changes in PCV may be preferable.

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[c]Lamtec 605, pneuPAC Ltd, Luton, UK.

[d]Gaeltec Ltd, Dunvegan, Isle of Skye, Scotland.

[e]Millar Instruments Inc, Houston, Tex.

[f]Gould Electronics, Gould Inc Recording Systems, Valley View, Ohio.

[g]Pon-e-mah digital acquisition, analysis & archive systems, Linton Instrumentation, Diss, Norfolk, UK.

[h]Nihon Khodon Europe Ltd, Brentword, Middlesex, UK.

[i]Vingmed CFM 800A, GE Ultrasound, Bedford, UK.

[j]Phenylephrine Injection, BP Knoll Ltd, Nottingham, UK.

[k]Dobutrex, Eli Lily Co Ltd, Basingstoke, Hampshire, UK.

[l]Graseby 500, Graseby Medical Ltd, Colonial Way, Watford, UK.

[m]Echopac, Vingmed Sound, Horten, Norway.

[n]Oxford Optronix, Magdalen Cntr, Oxford Science Pk, Oxford, UK.

[o]SF100 fibre optic probes, Oxford, Magdalen Cntr, Oxford Science Pk, Oxford, UK.

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