Trendelenburg positioning involves placing a dorsally recumbent body on an incline such that the position of the head is lower than the pelvis; this technique has long been applied to facilitate observation and manipulation of organs in humans during surgery. To the same end, head-down Trendelenburg-like positioning has been applied more recently during surgical procedures in horses, particularly for purposes of laparoscopic examination of the caudal portion of the abdomen. However, in horses, inhaled anesthetic agents such as isoflurane cause intracranial hypertension, which is exacerbated by positioning of the head at a level lower than that of the heart. Moreover, inhaled anesthetic agents cause dose-dependent systemic hypotension and obtund cardiovascular reflexes. Consequently, normal physiologic responses to increased ICP (ie, increases in MAP to maintain CPP) may be compromised. Because inhaled anesthetics also interfere with maintenance of CVR, decreased CPP could decrease CBF and predispose horses to ischemic injury. The purpose of the study reported here was to test the hypothesis that head-down positioning would increase ICP and decrease CPP and that those changes would significantly decrease perfusion to the brain and spinal cord in isoflurane-anesthetized horses.

**Materials and Methods**

**Horses**—Six horses (1 Appaloosa mare, 1 American Paint mare, 3 Thoroughbred mares, and 1 Thoroughbred stallion) were used in this study. Anesthesia was induced with ketamine hydrochloride and xylazine hydrochloride and maintained with 1.57% isoflurane in oxygen. Horses were ventilated to maintain normocapnia. An ICP transducer was placed in the subarachnoid space, and catheters were placed in the left cardiac ventricle and in multiple vessels. Blood flow measurements were made by use of a fluorescent microsphere technique while each horse was in horizontal and head-down positions. Inferential statistical analyses were performed via repeated-measures ANOVA and Dunn-Sidak comparisons.

**Results**—Because 1 horse developed extreme hypotension, data from 5 horses were analyzed. During head-down positioning, mean ± SEM ICP increased to 55 ± 2 mm Hg, compared with 31 ± 2 mm Hg during horizontal positioning; cerebral perfusion pressure was unchanged. Compared with findings during horizontal positioning, blood flow to the cerebrum, cerebellum, and cranial portion of the brainstem decreased significantly by approximately 20% during head-down positioning; blood flows within the pons and medulla were mildly but not significantly decreased. Spinal cord blood flow was low (9 mL/min/100 g of tissue) and unaffected by position.

**Conclusions and Clinical Relevance**—Head-down positioning increased heart-brain hydrostatic gradients in isoflurane-anesthetized horses, thereby decreasing cerebral blood flow and, to a greater extent, increasing ICP. During anesthesia, CNS regions with low blood flows in horses may be predisposed to ischemic injury induced by high ICP. (Am J Vet Res 2008;69:737–743)
bred gelding) were included in the study. Mean ± SEM weight of the horses was 471 ± 22 kg; mean age was 12 ± 3 years. Via physical examination, no cardiorespiratory or neurologic abnormalities were detected in any horse. The University of California, Davis, Animal Use and Care Committee approved the study protocol.

**Anesthesia**—Food was withheld from each horse for 12 hours prior to the experiment, but water was available ad libitum. Following sedation with xylazine hydrochloride (1 mg/kg, IV), anesthesia was induced via administration of ketamine hydrochloride (2 mg/kg, IV). Each horse was intubated with a 26-mm cuffed endotracheal tube. Anesthesia was maintained by use of isoflurane in oxygen delivered via a large-volume anesthesia delivery system; end-tidal isoflurane was maintained at 1.57%, corresponding to 1.2 × MAC for horses. Intermittent positive-pressure ventilation was used to provide a tidal volume of 5 to 6 L (estimated by volume markings on the bellows housing of the large-animal ventilator), which corresponded to a peak inspiratory pressure of 20 to 26 cm H₂O. Respiratory frequency was adjusted to maintain normocapnia. Samples of end-tidal gases were collected by hand in glass syringes, and isoflurane and carbon dioxide concentrations were measured by use of infrared analyzers that were calibrated with multiple certified gas standards. Prior to each microsphere injection, arterial blood samples were collected and respiratory gas partial pressures were measured by use of an automated blood gas analyzer. Lactated Ringer’s solution was administered IV at a rate of 3 mL/kg/h.

**Instrumentation**—Each horse was placed in right lateral recumbency on a padded equine hydraulic surgical table for instrumentation and assessment. A 16-gauge, 11.6-cm-long polytetrafluoroethylene catheter was placed percutaneously in the left facial artery, in both the right and left dorsal metatarsal arteries, and in the left ventricle of the heart (accessed via an ultrasound-guided thoracic puncture in the fifth or sixth intercostal spaces). An 18-gauge, 4-cm-long catheter was placed percutaneously in the left facial artery. A 110-cm-long, 6.5-F nylon catheter was placed by use of a modified Seldinger technique through the right jugular vein and advanced into the right atrium, as confirmed by pressure waveform tracings. The catheters in the facial artery and right atrium (central venous) were each connected via high-pressure tubing (filled with heparinized saline [0.9% NaCl] solution) to a strain-gauge transducer; those 2 transducers were calibrated at multiple pressures (spanning the full-scale measurement range) by use of mercury and water manometers, respectively. Heart rate and rhythm were monitored via ECG (base-apex configuration). Physiologic responses were recorded by an electronic data acquisition system. Systolic and diastolic arterial blood pressures represented the 60-second mean of the peaks and nadirs, respectively, of the arterial pressure tracing. Mean arterial blood pressure was the electronic mean of the arterial waveform (ie, MAP) or venous waveform (central venous pressure).

Intracranial pressure was measured by use of a published technique. Briefly, a commercially available catheter-tip transducer was placed in the subarachnoid space through a left parietal craniotomy that was subsequently sealed with bone wax. Transducers were calibrated against a graduated water column to confirm accurate measurements after each experiment. The hydrostatic gradient (mm Hg) at the circle of Willis was calculated as the vertical distance (cm) between the lateral canthus of the eye and the thoracic inlet divided by 1.36. Mean arterial blood pressure at the circle of Willis was calculated as the sum of MAP and the hydrostatic gradient. Cerebral perfusion pressure was calculated as the difference between MAP at the circle of Willis and ICP.

**Physiologic measurements**—For each horse, instrumentation was completed by 1 hour after induction of anesthesia. Right lateral recumbency was maintained, and the horse was either allowed to remain in a horizontal plane or was tipped by use of the hydraulic table into a head-down position such that the lateral canthus of the eye was 30 cm lower than the thoracic inlet. Position order for each experiment was alternated. Horses were allowed 30 minutes for equilibration; therefore, the first physiologic measurements were performed 90 minutes after induction of anesthesia. After pressure measurements were recorded and blood gas samples were collected, glass syringes in separate withdrawal syringe pumps were connected via high-pressure tubing (filled with heparinized saline) to a strain-gauge transducer; arterial blood samples were collected and respiratory gas partial pressures were measured by use of an automated blood gas analyzer. Lactated Ringer’s solution was administered IV at a rate of 3 mL/kg/h.

**Tissue processing and blood flow calculation**—Each horse was euthanized at the end of the study by IV administration of an overdose of pentobarbital; the brain and portions of the thoracolumbar spinal cord were harvested. The cerebrum was digitally separated from the thalamus at the region that grossly corresponded to the internal capsule. The cerebrum was then cut along frontal planes into 10 pieces of equal thickness; each piece was cut into right and left halves for separate analysis. Cerebellar peduncles were cut to separate the cerebellum from the brainstem; the cerebellum was then cut into vermis and right and left hemispheres, each of which was analyzed separately. The thalamus was severed from the midbrain immediately rostral to the cranial colliculus and cut into right and left halves. The colliculi region included transverse midbrain sections that were bounded rostrally by the cranial colliculi and caudally by the cau-
dalar colliculi. The pontine region continued distally to the caudal margin of the cerebellum, and the medulla comprised the remainder of the brainstem to the first cervical segment. All brainstem regions were divided into right and left halves, which were separately analyzed. Tissues of the pituitary gland, thoracic spinal cord, and lumbar spinal cord and a control blood sample (collected prior to any microsphere injections) were also separately analyzed.

Sections of CNS tissues were sliced and weighed on a calibrated scale. The sections were placed into a commercially available sample processing unit that contained a filter unit and reservoir from which microspheres were extracted by use of a validated technique. Tissue samples were digested within the filter reservoir with 4M KOH containing 2% Tween 80 and 2 mL of isopropanol alcohol. Tissues were incubated in a 60°C water bath for 48 hours; the filter chambers were then rinsed under suction with a phosphate buffer solution (pH 7.4) and centrifuged dry. Reference blood samples were similarly digested and processed. Sample tubes were then attached to the filter unit, and 2 mL of 2-ethoxyethyl acetate was added; the tubes were capped and centrifuged at 4°C, and the extracted fluorescent dye was collected and sealed in the sample tubes. A control microsphere sample containing approximately 500 microspheres of each color/mL of 2-ethoxyethyl acetate was prepared.

Samples were analyzed by use of a fluorometer and automated data acquisition software. The yellow fluorophore excited at ≈40-nm and emitted at ≈485-nm wavelengths; the persimmon fluorophore excited at ≈540-nm and emitted at 560-nm wavelengths. Samples were diluted to concentrations within a predetermined linear response range for each fluorophore and were analyzed in triplicate by use of matched quartz cuvettes. Blanks containing 2-ethoxyethyl acetate were used to determine zero fluorescence, and the control microsphere sample was used to measure and correct for instrument temporal drift.

Fluorescence signal per milliliter of reference blood flow (ie, $F_{i}$) was calculated via division of the total reference fluorescence signal by the gravimetrically calibrated withdrawal pump rate. Because microspheres only traversed 1 heart valve, it was necessary to verify homogeneous microsphere mixing, which is typically assumed when microspheres traverse 2 heart valves. To achieve verification of homogeneous microsphere mixing, $F_{i}$ values obtained simultaneously from the carotid, facial, and metatarsal arteries were compared; values should be similar when microsphere mixing is sufficient. For all horses included in the study, $F_{i}$ for any arterial sample differed from the mean by ≤10% for both microsphere colors. Blood flow per 100 g of tissue for each section (ie, $Q_{i}$) was calculated by use of a formula as follows:

$$Q_{i} = \sum_{i=1}^{n} \left( \frac{F_{i}}{F_{i} \times \sum_{m=1}^{m} m_{i}} \right) \times 100$$

where $F_{i}$ and $m_{i}$ are the fluorescence signal and mass from the $i$th tissue sample cut into a total of $n$ samples, respectively.

### Data analysis

Paired $t$ tests were used to assess right side-versus-left side differences in regional blood flow. In the absence of significant effects, data for both sides were combined by use of a mass-weighted mean value. Variables were plotted to visually inspect distribution shape; for most physiologic responses, values from 1 horse differed from the mean by more than 2 SD. To maintain assumed normality, statistical analyses were performed on the data from the remaining 5 horses; data for the outlier horse were listed separately.

The effects of body position on regional CBF, ICP, and CPP and on systemic cardiorespiratory responses were analyzed by use of a repeated-measures ANOVA with position order as a covariate. The $df$ for nonspheric data were adjusted by use of the Huynh-Feldt technique, and $P$ values for multiple planned comparisons were adjusted by use of the Dunn-Sidak method. For all analyses, a value of $P < 0.05$ was considered significant.

### Results

In both the horizontal and head-down positions, systemic hemodynamic variables were assessed in all 6 isoflurane-anesthetized horses (Table 1). Although mild decreases in systemic blood pressure were sometimes detected during isoflurane anesthesia, 1 horse developed severe hypotension in the absence of an obvious underlying cause such as systemic illness. Most CBF measurements from this same horse were also outliers from the remaining group mean. Consequently, data for this horse were removed from the main analyses, and descriptive and inferential statistics were calculated by use of data obtained from the remaining 5 horses.

During head-down positioning, the magnitude of the increase in ICP approximated the increase in the brain-heart hydrostatic gradient (22 mm Hg). There was no significant change in MAP, thus, CPP also remained unchanged. Mean heart rate in both positions was 32 beats/min. Mean ± SEM arterial blood gas partial pressures in both positions are compared. During horizontal and head-down positions, PaO$_2$ was 483 ± 32 mm Hg and 472 ± 32 mm Hg, respectively; PaCO$_2$ was 49 ± 2 mm Hg and 51 ± 2 mm Hg, respectively; and arterial blood pH was 7.410 ± 0.027 and 7.409 ± 0.038, respectively. There were no significant differences in either respiratory or end-tidal gas values between body positions.

### Table 1—Mean ± SEM pressure measurements (mm Hg) in 5 isoflurane-anesthetized horses during horizontal and head-down (Trendelenburg-like) positioning. Values for a sixth horse (outlier) are provided in parentheses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Horizontal</th>
<th>Head-down</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP</td>
<td>85 ± 3 (55)</td>
<td>85 ± 3 (79)</td>
</tr>
<tr>
<td>MAP</td>
<td>66 ± 3 (37)</td>
<td>68 ± 2 (60)</td>
</tr>
<tr>
<td>DAP</td>
<td>56 ± 3 (28)</td>
<td>58 ± 3 (51)</td>
</tr>
<tr>
<td>ICP</td>
<td>31 ± 2 (18)</td>
<td>55 ± 2* (49)</td>
</tr>
<tr>
<td>CPP</td>
<td>35 ± 3 (18)</td>
<td>30 ± 3 (53)</td>
</tr>
<tr>
<td>CVP</td>
<td>13 ± 1 (5)</td>
<td>15 ± 2 (5)</td>
</tr>
</tbody>
</table>

*Value is significantly ($P < 0.05$) different from that associated with the horizontal position.

MAP = Mean arterial pressure measured at the heart. SAP = Systolic arterial pressure. DAP = Diastolic arterial pressure. CVP = Central venous pressure.
Although ICP increased approximately 80% during head-down positioning, compared with the value in the horizontal position, tilting only decreased CBF approximately 20% (Figure 1), which must reflect underlying reductions of similar magnitude in CVR because CPP was unchanged by body position in the study reported here. Compared with the findings during horizontal positioning, head-down positioning resulted in significant reductions in CBF in sections of the cerebrum, cerebellum, and cranial portion of the brainstem; however, reductions in CBF in sections of the pons, medulla, or spinal cord were not significant (Table 2). In brain regions with significant changes in blood flow, there was also a significant interaction between body position (horizontal vs head-down) and the order in which these positions were evaluated. Cerebral blood flow was little affected by location within the cerebrum, with the exception that the most rostral sections had higher blood flow than flows in the 2 midcerebral sections. For both body positions, spinal cord blood flow was relatively low, representing only about one fifth of the flow to other CNS tissues.

**Discussion**

In the present study, head-down (Trendelenburg-like) positioning increased ICP and decreased CBF in isoflurane-anesthetized horses, compared with findings during horizontal positioning. However, the magnitude of these changes was not proportional. Because CPP remained relatively unchanged, CBF decreased ≤ 20% in the face of marked intracranial hypertension. This likely...
mirrored a ≤ 20% increase in CVR that, in turn, would have attenuated increases in cerebral blood volume and ICP. Given that cerebral metabolism was unlikely to change with table tilting, this suggests that cerebrovascular autoregulation (the matching of CBF to cerebral metabolic oxygen demand) could be partially preserved in horses anesthetized with isoflurane.

Intracranial pressure in healthy, awake horses is minimally influenced by head position. In contrast, 20° Trendelenburg positioning in healthy awake humans increases ICP and decreases CBF. However, in awake human brain tumor patients with higher ICP and lower CBF values of similar magnitude to the ICP and CBF values reported in the present study for isoflurane-anesthetized horizontally positioned horses, 20° Trendelenburg positioning does not change CBF. Presumably, this serves to protect individuals with reduced CBF reserve against further decreases in cerebral perfusion that could ultimately compromise tissue oxygen delivery.

In horses, isoflurane inhalation during horizontal positioning causes dose-dependent decreases in CVR and CPP but no change in ICP when ventilation to maintain normocapnia is provided. In rats, isoflurane impairs normal vascular attempts to maintain constant CBF over a wide range of systemic pressures, particularly at higher anesthetic concentrations and in the subcortical, midbrain, and spinal cord regions. In the present study, we expected that high ICPs associated with head-down positioning in anesthetized horses would decrease CBF within these same regions. However, this was not the case. Instead, decreases in blood flow were detected throughout the cerebrum, cerebellum, and rostral portion of the brainstem, all regions where blood flow was greatest. There was no significant effect of position within the caudal portion of the brainstem and spinal cord where total blood flow was < 50 ml·100 g⁻¹ of tissue·min⁻¹. It is possible that as ICP increases, marginal tissue oxygen delivery to caudal regions of the CNS may account for preferential vasodilation and blood flow preservation within those same areas, analogous to a so-called inverse steal phenomenon.

Brain blood flow measurements for anesthetized horses in lateral recumbency in our study approximated values reported in 1 study of awake ponies but were only nearly 66% to 50% of values determined in other experiments in awake adult ponies and horses, respectively. Moreover, higher CBF values in ponies anesthetized with isoflurane at a concentration similar to that used in the present study have been reported. At least 3 explanations are possible for these differences. First, in the study of CBF in ponies, anesthesia was induced and maintained with isoflurane alone, whereas horses in the present study received ketamine and xylazine for induction of anesthesia followed by isoflurane for maintenance of anesthesia. Ketamine and xylazine have short distribution half-lives that result in clinically short durations of sedative and anesthetic actions, but which belie the existence of much longer terminal elimination half-lives. In fact, xylazine at doses used in the present study decreases MAC and MAP more than 2 hours after administration in horses and could reduce CBF more than during anesthesia with isoflurane alone. The anesthetic dose used in the study reported here was not adjusted for MAC-sparing actions of injectable drugs. In the present study, CBF analyses that included a significant experiment study order covariate (i.e., the head-down position assessed first vs second) support this theory of a temporarily waning drug effect, although a modest temporal effect itself on CPP might have contributed to this result.

A second explanation for differences between CBF findings in ponies and those in horses of the present study is a difference in the method used for CBF measurement. In the study by Manohar et al, a radioactive microsphere technique was used, whereas a fluorescent microsphere technique was used in our study. The same fundamental principles underlie both methods, including an assumption that there is homogeneous microsphere mixing throughout the systemic arterial tree. Because high blood flow in equids makes the retrograde placement of a carotid catheter into the left atrium difficult, left ventricular injection catheters that are accessed either via the carotid artery or percutaneous puncture have been used instead. This means that blood that contains microspheres will only pass through 1 heart valve during injection, thereby creating the potential for microsphere streaming or other inhomogeneity. In the present study, adequate microsphere mixing was verified by comparing multiple reference blood samples collected from arteries in the cranial and caudal portions of each horse's body. Previous studies have instead included comparisons of blood flow in right and left sides of organs to verify mixing. In our experience, this may not be adequate. For example, 2 horses that were evaluated by our group but were not included in the data set for the present study had cerebrum interhemispheric blood flow calculations that differed by ≤ 10%, despite reference blood flow samples from the carotid and femoral arteries that differed by ≥ 50%. Subsequent microsphere mixing in more distal portions of the arterial tree, such as in the circle of Willis, could account for these similar interhemispheric blood flow values in the face of microsphere inhomogeneity at the aortic root.

Third, CBF measurements in isoflurane-anesthetized ponies could be higher than CBF values in isoflurane-anesthetized horses of the present study because MAP in the ponies was 15 mm Hg higher than that of the horses. At specific ICP and CVR values, greater MAP will result in increased CPP and thus increased CBF. The converse is also true. In our study, 1 horse became profoundly hypotensive (MAP, 37 mm Hg); accordingly, CPP and CBF were approximately 40% lower in this horse, compared with findings in the remaining normotensive horses, indicating loss of cerebrovascular autoregulation.

Anesthetic agents cause dose-dependent cardiovascular depression. Horses, by virtue of their size, are susceptible to development of postanesthetic myopathy as a consequence of sustained systemic hypotension. Consequently, positive inotropes or vasopressors are routinely administered to horses during anesthesia to treat hypotension. Yet, these same drugs may also have the potential to interfere with normal cerebrovascular autoregulation in anesthetized horses.
For example, in isoflurane-anesthetized dogs, the positive inotropes dopamine and dobutamine both cause a dose-dependent decrease in CBF, possibly as a result of redistribution of cardiac output to the heart, muscles, and splanchic organs. In isoflurane-anesthetized rabbits, the vasopressors norepinephrine and phenylephrine both cause dose-dependent increases in ICP and CBF. Whether adrenergic receptor agents induce similar effects in horses, a species that inherently has high intracranial elastance and ICP, during isoflurane anesthesia, remains unknown. Certainly, differences detected between the normotensive horses and the outlier hypotensive horse in the present study could indicate a large interaction between baseline systemic blood pressure and the hemodynamic effects of body positioning.

However, one important question persists: are the regional CNS blood flow measurements determined in the study reported here cause for concern? If CBF values in horses in the awake and anesthetized states are similar, then concerns might be allayed. Cerebral blood flow values associated with an awake state are known for many other species, including humans (mean CBF 68 ml/100 g·min⁻¹), 2 dogs (49 ml/100 g·min⁻¹), 3 sheep (81 ml/100 g·min⁻¹), 4 pigs (81 ml/100 g·min⁻¹), 5 and cats (50 ml/100 g·min⁻¹). 6 Among horses in the horizontal position in the present study, CBF measurements were within the lower end of this range of reported values. However, mean values for most sections of the cerebrum, thalamus, colliculi, and pontine regions during head-down tilting decreased to lower values than those previously reported for equids and other species. Therefore, even modest decreases in CBF might predispose certain brain regions to ischemia, perhaps causing overt cortical damage or contributing to a less than ideal recovery from inhaled anesthesia.

Similar comparisons provide compelling evidence for spinal hyperperfusion as well. Although we are unaware of any published data regarding equids, mean spinal cord blood flow values in an awake state have been reported for dogs (15 ml/100 g·min⁻¹), 7 sheep (14.5 ml/100 g·min⁻¹), 8 rats (51 ml/100 g·min⁻¹), 9 and pigs (75 ml/100 g·min⁻¹). 10 In the present study, spinal cord blood flow in isoflurane-anesthetized horses was 9 ml/100 g·min⁻¹; this comparatively smaller value could be a consequence of increased ICP. The implication of this finding is that horses have a reduced capacity to withstand further decreases in spinal cord blood flow, such as those resulting from increased sinus pressure and decreased spinal perfusion pressure during dorsal recumbency; thus, some horses could be at risk for postanesthetic hemorrhagic myelopathy or myelomalacia.

Findings of the present study indicated that increasing the heart-brain hydrostatic gradient in horses caused relatively small decreases in CBF; provided that CPP gradients also remained relatively constant. Nevertheless, given the low baseline measurements in isoflurane-anesthetized horses, body positions that cause even small decreases in CNS blood flow might still contribute to ischemic injury, particularly within the spinal cord.

References


