A neonesthetized horses are notably susceptible to hypotension and reduced cardiac output (CO). Hemodynamic disturbances—in particular, hypotension—in anesthetized horses result in part from the use of inhaled volatile anesthetics that cause negative inotropy and vasodilation.1,2 Hypotension is defined as having a systolic arterial blood pressure (SAP) < 80 mm Hg and mean arterial blood pressure (MAP) < 60 mm Hg.3 Periods of prolonged hypotension have been associated with postanesthetic complications, including myopathy and myelomalacia.4 Several treatment options to mitigate hypotension and hypovolemia are used to restore adequate blood flow to vital organs and to maintain CO, including decreasing inhaled anesthetic delivery, using various strategies to reduce minimum alveolar concentration, administering IV fluids, and providing inotropic or vasopressor medications.1

IV fluid administration allows for maintenance of intravascular volume and improved venous return, and aids in counteracting the hypotension resulting from anesthetic agents. Hypertonic saline (5% to 7.5% sodium chloride [NaCl]) solution (HS) is used to restore CO and arterial blood pressure by increasing plasma volume and stimulating release of arginine vasopressin (AVP).5–8 Administration of HS stimulates AVP release as a result of excitatory inputs from peripheral and central osmoreceptors.6

OBJECTIVE
To compare the effects of 7.2% hypertonic and 0.9% isotonic saline (sodium chloride) solutions on cardiovascular parameters and plasma arginine vasopressin (AVP) concentrations in healthy, isoflurane-anesthetized horses.

ANIMALS
8 healthy horses.

PROCEDURES
In a prospective, randomized, crossover study, horses were anesthetized with isoflurane twice with a 14-day washout period between anesthetic episodes. While anesthetized, horses received a bolus (4 mL/kg) of 7.2% hypertonic saline solution (HS) or 0.9% isotonic saline solution (IS). Heart rate; systolic, mean, and diastolic arterial blood pressures; and central venous and pulmonary artery pressures were measured every 5 minutes; cardiac output was measured by means of thermodilution every 15 minutes. Systemic vascular resistance (SVR) was calculated. Blood samples were collected before and during anesthesia, and plasma AVP concentrations were determined with a validated ELISA. Data were analyzed with repeated-measures ANOVA and Pearson correlations.

RESULTS
HS caused an increase in systolic (P = .003) and mean (P = .023) arterial blood pressures that lasted for 30 minutes. The SVR was increased (P < .001) for 45 minutes with HS compared with the SVR after IS administration. Mean plasma AVP concentration increased (P = .03) 15 minutes after HS administration, with the increase lasting 90 minutes.

CLINICAL RELEVANCE
A bolus of HS resulted in a clinically relevant increase in blood pressure in healthy, isoflurane-anesthetized horses. This effect was attributed to volume recruitment and an increase in SVR. Administration of HS offers an option for improving arterial blood pressure in anesthetized horses.
AVP is an important effector of water balance and secretagogue of the hypothalamic–pituitary–adrenal axis in horses. AVP is synthesized by the magnocellular neurons of the hypothalamus and is stored in secretory vesicles of the neurohypophysis (posterior pituitary gland) until triggered for release. Plasma hypertonicity is a strong stimulator of the hypothalamic osmoreceptors that cause AVP release to reclaim water and maintain extracellular fluid osmolality within homeostatic values. Furthermore, states of hypotension or hypovolemia, in addition to acidosis, pain, hypoxemia, and hypercapnia, result in AVP release. Changes in plasma osmolality (notably sodium shifts) of 1% to 2% have been shown to increase plasma AVP concentrations 2- to 4-fold in rats. In horses, changes in plasma osmolarity and blood volume have been documented to stimulate AVP secretion. Most of these conditions are conditions that anesthetized horses can potentially experience.

HS causes a rapid increase in plasma osmolality, resulting in fluid shifts from the intracellular space into the extracellular space. HS can thereby expand the blood volume by almost 4 times the infused volume and has the additional benefits of providing mild inotropic, anti-inflammatory, and antiedema effects. However, the inotropic mechanisms of action identified in horses with experimentally induced hemorraghic shock are not fully understood. Pantaleon et al determined that administration of a small volume of hetastarch in combination with HS aided cardiac preload without resulting in overexpansion of the extracellular fluid volume or interstitial edema formation in halothane-anesthetized horses. In halothane-anesthetized dogs, HS administration demonstrated direct myocardial effects resulting from an increase in osmolality, leading to increased contractility, MAP, heart rate, and CO. Further in vitro studies of explanted cardiac tissues from frogs and rabbits have shown that cardiac contractility is directly affected by sodium. Animals with experimentally induced endotoxic shock showed an immediate recovery of blood pressure and increased plasma AVP concentrations after HS administration. In horses with experimentally induced hemorrhagic shock, Schmall et al showed that treatment with HS resulted in a rapid increase in CO, stroke volume, MAP, and pulmonary arterial pressure, with an increase in cardiac contractility for up to 2 hours. Several studies have investigated the effect of hyperosmotic fluids for the treatment of hypovolemia and hypovolemic shock. Moon et al evaluated the effect of a concentrated hypertonic saline–dextran solution on volume expansion in anesthetized euvolemic horses and showed an immediate increase in cardiac index.

It is well documented that HS is an effective treatment for hypotensive states in horses, including shock, septic shock, endotoxemia, and acute hemorrhagic (hypovolemic) shock. A recent study in dehydrated endurance horses demonstrated HS was beneficial, and another study found that HS reduced the short-term risk of anesthesia-related complications. We propose that healthy, euvolemic horses will have rapid cardiovascular and neurohypophyseal responses to HS administration characterized by increases in arterial blood pressure and CO, as well as an increase in systemic AVP concentrations, which have been documented in other species but not confirmed in isoflurane-anesthetized horses. The objective of our study was to compare the effects of 7.2% HS and 0.9% isotonic saline (NaCl) solution (IS) on cardiovascular parameters and AVP concentration in healthy, euvolemic, isoflurane-anesthetized horses. We hypothesized that a 4-mL/kg IV bolus of 7.2% HS would result in a significant increase in arterial blood pressure and CO, with a concomitant increase in plasma AVP concentration, compared with a 4-mL/kg IV bolus of IS.

Materials and Methods

Animals

The study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania (trial registry No. 806775-aaecgb). An a priori power analysis (type I error = 0.05, type II error = 0.2) showed that 8 animals were necessary to detect statistically significant (P < .05) and clinically important (10% increase) changes in MAP and CO with an SD of 15%.

Eight university-owned Thoroughbred horses (5 mares and 3 geldings) were enrolled in the study. All horses were deemed healthy on the basis of results of a preanesthetic physical examination and basic diagnostic testing (packed cell volume, total protein concentration, and L-lactate concentration). Horses were between 8 years and 21 years old (mean ± SD, 14 ± 5.6 years) and weighed between 500 kg and 559 kg (520 ± 20 kg). All horses were of similar phenotype and assigned an American Society of Anesthesiology score of I. Feed and water were withheld for 12 and 4 hours, respectively, prior to each anesthetic event.

Experimental design

This study had a randomized, blinded crossover design. All horses were anesthetized with isoflurane in oxygen twice, with a washout period of 14 days between anesthetic events. For each anesthetic event, horses were assigned randomly to receive a 4-mL/kg IV bolus of 7.2% HS (Nova-Tech Inc) or 0.9% IS (Pfizer). All bolus infusions were administered with a pressurized bag and were completed within 10 minutes. The hospital pharmacist covered up the label on the solution bags with an adhesive bandage so that investigators were blinded to which solution was administered. Each horse had 2 sets of bags, labeled trial 1 and trial 2, representing each of the saline solutions investigated. Each horse received the solutions in a randomized order.

Anesthesia and instrumentation

For each anesthetic event, a 14-gauge, 5.25-inch, polyurethane catheter (MILA Int) was placed aseptically in the left jugular vein. Horses were premedicated with xylazine (0.08 mg/kg, IV), and general anesthesia was induced with ketamine (2.2 mg/kg, IV) and midazolam (0.05 mg/kg, IV). Orotracheal intubation was performed and horses were then
placed in dorsal recumbency on a thick foam pad on a commercial large-animal surgery table and connected to a large-animal rebreathing circuit. Volume-controlled ventilation was initiated (Tafonius large-animal anesthesia workstation; Hallowell EMC). Anesthesia was maintained with isoflurane at an end tidal concentration of 1.3% in oxygen. An 18-gauge catheter was placed aseptically in the facial artery to allow for invasive blood pressure monitoring and arterial blood sampling. Furthermore, two 8F catheter introducers (Arrow International percutaneous sheath introducer kit; Teleflex) were placed separately in the right jugular vein to facilitate placement of balloon-tipped catheters. A standard 7F, 110-cm Swan-Ganz thermodilution pulmonary artery catheter (Criticath; BD Medical) was positioned in the main pulmonary artery, and a second Swan-Ganz catheter was positioned in the right atrium. Correct placement was confirmed by visual inspection of the pressure waveforms. For measurement of CO by thermodilution, ice-chilled 5% dextrose solution (1 mL/15 kg) was injected manually through the catheter into the right atrium at end expiration. The temperature of the injectate was measured with an inline temperature probe, and the temperature change in the pulmonary artery was analyzed with a specialized monitor (Monitor Cardiocap/5, Datex-Ohmeda Inc) to calculate the CO. Five injections were performed over 5 minutes, and the mean of the 3 closest CO values were used. The HR, SAP, MAP, diastolic arterial blood pressure, central venous pressure, and pulmonary artery pressure were measured every 5 minutes. The CO was measured every 15 minutes. Vasoactive medications were specifically not administered to prevent confounding. If MAP was <40 mm Hg, dobutamine (5 μg/kg/min, IV) was to be used as a rescue therapy until blood pressure returned to acceptable limits (MAP > 60 mm Hg).

Data collection
After induction of anesthesia and completion of instrumentation, baseline values were established. The HR, SAP, MAP, diastolic arterial blood pressure, central venous pressure, and pulmonary artery pressure were measured continuously and recorded every 5 minutes for the duration of the experiment. Cardiac output was measured every 15 minutes, and the systemic vascular resistance (SVR) was calculated. Sixty minutes after induction of anesthesia, the experimental treatment solutions were administered. Following each fluid bolus, all cardiovascular parameters were recorded every 5 minutes for 120 minutes. In addition, arterial and venous blood samples were collected for quantification of AVP concentration immediately before induction of anesthesia, during the baseline period, and every 10 minutes for 60 minutes and every 15 minutes from 60 minutes to 120 minutes after bolus administration.

After data collection was completed, the horses were moved into a padded recovery stall. The orotracheal tube was removed and replaced with a nasotracheal tube. Supplemental oxygen was provided with an oxygen demand valve until spontaneous ventilation resumed, and xylazine (0.2 mg/kg, IV) was administered. Horses recovered with the assistance of head and tail ropes. All catheters were removed, and horses were monitored for 24 hours, with a physical examination performed every 6 hours, before they were returned to the teaching and research herd.

Blood sampling and processing
Arterial blood samples (2 mL) were analyzed immediately with a blood gas analyzer (CCA-TS2 blood gas analyzer, Opti Medical Systems) to determine sodium concentration. Venous blood samples were collected into EDTA-containing tubes and plain serum clot tubes. The protease inhibitor aprotinin (Goldbio, Gold Biotechnology) was added to each EDTA blood collection tube individually to allow for AVP sample preservation (500 kU/mL of whole blood) a week before the anesthetic event, and tubes were stored at 4 °C until sample collection. Samples were chilled on ice until all samples for each anesthetic event had been collected; the samples from each anesthetic event were then processed together within 24 hours of collection. The samples were centrifuged at 1,000 g for 15 minutes, and the serum and plasma were aliquoted into 1.5-mL microcentrifuge tubes and frozen at −80 °C until analysis.

Arginine vasopressin ELISA
Plasma AVP concentrations were measured with a multispecies AVP ELISA (Arg-vasopressin ELISA, Arbor Assays). According to the manufacturer’s product information, the assay had a sensitivity of 3.7 pg/mL with a detection range of 4 to 1,000 pg/mL. The assay was highly specific for AVP with no substantial cross-reactivity with oxytocin or AVP analogues (<0.06%). Four-parameter logistic fitting curves (Excel, Microsoft Corp) were used to convert optical densities to concentrations (measured in picograms per milliliter). Standard curves were well-fitted ($R^2 = 0.97$ to 0.99).

Statistical analysis
All analyses were performed with commercial statistical software (Excel, Microsoft Corp; Prism, version 8, GraphPad). Continuous variables were determined to be normally distributed on the basis of visual assessment of Q-Q plots and results of the Shapiro-Wilk test. Hemodynamic data were analyzed with repeated-measures ANOVA followed by the Dunnett multiple comparison test, with a Bonferroni correction applied for all multiple comparisons. A Pearson test was used to test for correlations among sodium concentration, blood pressures, SVR, and AVP concentrations. Plasma AVP concentrations were analyzed with a 4-parameter logistic curve fit. The baseline was set to 1, and each horse was evaluated for percentage change from baseline. For all analyses, values of $P < .05$ were considered significant.

Results
All horses completed both anesthetic events with no adverse events occurring.
Bolus administration of HS resulted in significant increases in mean SAP ($P = .003$) and mean MAP ($P = .023$), compared with baseline values, that lasted for 25 minutes (Figure 1). Mean SVR 15 ($P = .012$), 30 ($P < .001$), and 45 ($P < .001$) minutes after bolus administration were significantly different between the HS and IS treatments. Mean SVR was also significantly ($P < .01$) increased after HS administration, compared with the baseline value, beginning 10 minutes after bolus administration and lasting for 45 minutes. After administration of HS, mean plasma AVP concentrations increased significantly ($P = .03$), compared with values obtained after administration of IS and with baseline values (baseline AVP concentrations, 12.7 ± 6.3 ng/mL [IS treatment]), beginning 10 minutes after bolus administration (Figure 2). Mean AVP concentration peaked at 1,200% of the baseline concentration 75 minutes after HS bolus administration. There was no significant increase in AVP after the IS bolus administration.

For the HS treatment, there were significant ($P < .001$) correlations between arterial plasma sodium concentration and percentage change in plasma AVP concentration ($r^2 = 0.689$), arterial sodium concentration and MAP ($r^2 = 0.633$), MAP and percentage change in plasma AVP concentration ($r^2 = 0.703$), SVR and percentage change in plasma AVP concentration ($r^2 = 0.559$), and SVR and MAP ($r^2 = 0.607$; Figure 3).

Figure 1—Mean ± SD systolic arterial blood pressure (A), mean arterial blood pressure (B), systemic vascular resistance (C), cardiac output (D), diastolic arterial blood pressure (E), and heart rate (F) record in 8 healthy, euvolemic, isoflurane-anesthetized horses for 60 minutes prior to and 120 minutes after administration of a bolus (4 mL/kg) of hypertonic saline (7.2% sodium chloride [NaCl]) solution or isotonic saline (0.9% NaCl) solution. *Values differed significantly ($P < .05$) between treatments.
**Figure 2**—Mean ± SD percentage change in plasma arginine vasopressin (AVP) concentration compared with baseline AVP concentration in 8 healthy, euvoletic, isoflurane-anesthetized horses after administration of a bolus (4 mL/kg) of hypertonic saline (7.2% sodium chloride [NaCl]) solution or isotonic saline (0.9% NaCl) solution (time 0). *Significantly (P < .05) different from baseline concentration and concentration after administration of isotonic saline solution.

**Figure 3**—Scatterplots of plasma sodium concentration versus mean arterial blood pressure (MAP; A), plasma sodium concentration versus percentage change in plasma arginine vasopressin concentration (AVP conc) compared with baseline concentration (B), MAP versus percentage change in plasma arginine vasopressin concentration (C), systemic vascular resistance (SVR) versus percentage change in plasma arginine vasopressin concentration (D), and SVR versus MAP (E) in 8 healthy, euvoletic, isoflurane-anesthetized horses administered a bolus (4 mL/kg) of hypertonic saline (7.2% sodium chloride) solution. In each plot, the solid line represents the best-fit correlation.
No other cardiovascular parameters changed significantly after bolus administration of HS or IS (Figure 3 and Supplementary Table S1).

**Performance of the AVP ELISA**

Intra-assay and interassay coefficients of variation for equine samples were < 12%. Samples were run in duplicate. Equine samples showed linear dilutional parallelism up to a dilution of 1:8 ($R^2 > 0.98$), demonstrating that the assay could detect equine AVP over a wide range of concentrations.

**Discussion**

In our study, administration of 7.2% HS resulted in rapid increases in arterial blood pressures (SAP and MAP) that were associated with increases in SVR, arterial plasma sodium concentration, and plasma AVP concentration. These results indicate that increases in blood pressure in response to HS bolus administration in healthy horses are mediated by rapid intravascular volume expansion combined with AVP-mediated vasocostriction.26,27

Endogenous AVP release is stimulated after administration of HS as a result of hypernatremia and increased plasma osmolarity.28–30 HS has four main effects on the cardiovascular system: rapid increase in circulating volume, initial vasoconstriction stimulated by osmoreceptors mediating a vagal reflex, transient vasodilation, and increased cardiac contractility.31 Increases in MAP after HS administration occur as a result of fluid shifts to the intravascular compartment caused by an increase in plasma osmolarity as well as an increase in vasomotor tone as a direct result of AVP’s effects on V1 receptors and AVP-mediated effects on the sympathetic nervous system.32 In a previous study33 in dogs, infusion of HS over 20 minutes resulted in increased plasma osmolality and sodium concentration, with an associated increase in plasma AVP concentration. Intracarotid infusion of HS triggers AVP secretion secondary to the increase in plasma osmolarity, supporting the neurohypophysial’s response to HS and implying the presence of osmoreceptors in the common carotid arteries.34

It has been previously suggested that supraphysiologic plasma AVP concentrations are required to induce significant changes in MAP in dogs and humans.35 This was also the case in the horses in our study. Administration of IS (154 mEq sodium/L) increased AVP concentration to 200% of the baseline concentration, indicating that even modest increases in plasma sodium concentration can stimulate AVP release, but MAP and SVR did not change in these horses.

A study36 involving euvolemic animals showed an increase in arterial pressure by approximately 12% after administration of 7.2% HS (5 mL/kg). In hypovolemic and hypotensive animals, HS administration resulted in rapid restoration of arterial blood pressure, aortic blood flow, and cardiac contractility.37 In our study, the HS bolus resulted in increased SVR, which has also been shown in normovolemic and hypovolemic conditions.38 This change in SVR was the main reason for the significant increase in blood pressure without affecting other cardiovascular parameters seen in our study.

Vasopressin causes vasoconstriction via various mechanisms, including activation of V1 vascular receptors, activation of ATP-sensitive potassium channels, activation of nitric oxide, and potentiation of other vasoconstrictive agents.39,40 The associated vasocostriction results in increased SVR as well as increased MAP, with significant correlations among AVP, SVR, and MAP. The importance of AVP in hemodynamic regulation in several disease states has been documented. In sepsis, vasodilation and vasoplegia are caused by proinflammatory cytokine–mediated downregulation of V1 receptors, increased inducible nitric oxide synthase activity, and refractoriness of adrenergic receptors to catecholamines.41 The cardiovascular effects of endotoxic shock commonly discussed are hypotension and decreased SVR; however, direct myocardial depression can occur, which results in an increased risk of pulmonary and systemic edema formation following large-volume fluid resuscitation with crystalloid solutions.16,27 HS aids in preservation of preload and limits vascular leakage resulting from leukocyte–endothelial reactions.16

Hypotensive animals have increased concentrations of AVP with MAPs remaining low for as long as 12 hours during experimentally induced septic shock.42 In septic shock, there is a reduction in the endogenous release of catecholamines, which are important in maintaining a minimally acceptable MAP and adequate organ perfusion.43 Furthermore, a reduction in ATP in vascular smooth muscle cells results in hypotension and vasodilation.11 Profound hypotension is also documented in conscious dogs with experimentally induced hemorrhagic shock after specific blockade of V1 receptors, confirming the suspected role of AVP in hemorrhagic shock.44

Vasopressin analogs have been administered in humans experiencing septic shock to increase blood pressure, decrease catecholamine requirements, and improve renal function.45,46 Increased plasma AVP concentrations have been associated with an increased risk of death in foals, dogs, and people because of an initial strong stress response, transitioning to adrenal exhaustion, resulting from overstimulation in response to severe or prolonged disease.4,44 HS administration, which is well documented in horses with severe gastrointestinal disease and horses with circulatory or distributive shock, would have a different AVP response.15,25,30 Ludders et al47 demonstrated that horses with colic had high plasma AVP concentrations that remained stable over the anesthetic period, in contrast to control horses, which had an initial sharp decrease followed by a more gradual decrease over the anesthetic period. This could be related to the maximal response of the neurohypophysis and subsequent depletion of AVP stores resulting in steady concentrations despite the additional stress of surgery and anesthesia.47 Although we measured comparable...
baseline AVP concentrations when horses were given HS versus IS, plasma AVP concentration increased up to 390 pg/mL in horses that received HS, indicating a rapid and strong response to plasma hyperosmolality during anesthesia.

We speculate that critically ill catecholamine-depleted horses (eg, horses with colic, colitis, or endotoxemia) may require greater doses of HS to stimulate an AVP release that could promote vasconstriction and renal water retention.\textsuperscript{25,42} This may be explained by the biphasic release of AVP, which has been demonstrated in shock conditions. In early shock states, there is a rapid AVP release to maintain organ perfusion, followed by a reduction in AVP concentration secondary to pain, hypoxia, acidosis, hypotension, and neurohypophysial store depletion.\textsuperscript{35} Hypotension and decreases in intravascular volume are potent stimuli to endogenous AVP release.\textsuperscript{43,44} HS administration in animals with experimentally induced hypovolemic and septic shock have demonstrated intravascular volume expansion, improvement of myocardial contractility, enhanced tissue perfusion, and better modulation of cellular pathways.\textsuperscript{45} Additional studies on critically ill equine patients may provide further clarification of HS dosing and AVP response in these cases.

Our study had some limitations with regard to limited hormonal assays and endocrine analysis. Horses were determined to be healthy on the basis of a physical examination and routine bloodwork. None of the horses displayed a phenotype for pituitary pars intermedia dysfunction. No other hormones associated with the regulation of blood pressure and vascular fluid volumes such as atrial natriuretic peptide, plasma renin activity, adrenomedullin, or aldosterone were quantified at single time points or dynamically.\textsuperscript{13,48,50} Impairment of the hypothalamic–pituitary–adrenal axis would have potentially influenced individual responses to HS administration. The impact of other neurohormonal factors on water retention and regulation of blood pressure in the horses of this study remains unclear. Osmolarity was not calculated because urea and glucose concentrations were not measured, and we did not have the ability to perform freezing point depression osmometry. Information on osmolarity would have provided further clarification on the complex relationship between the renal and cardiovascular systems with fluid homeostasis.\textsuperscript{48} In addition, we did not have an isoflurane-anesthetized control group that did not receive either HS or IS to explain individual variations associated with instrumentation and fluid administration.

In conclusion, bolus infusion of 7.2% HS to healthy isoflurane-anesthetized horses caused increases in SAP and MAP. These effects were a result of increased SVR, likely from rapid volume expansion, combined with hypernatremia-induced AVP release resulting in vasoconstriction. Administration of HS offers an additional means to improve blood pressure in isoflurane-anesthetized horses, at least on a short-term basis. Further studies are needed to assess whether similar effects are observed in clinical cases.

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Supplementary Material

Supplementary materials are posted online at the journal website: avmajournals.avma.org