Cardiovascular changes associated with intravenous administration of fumonisin B_1 in horses

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Objective—To determine whether cardiovascular dysfunction is evident in horses with leukoencephalomalacia experimentally induced by administration of fumonisin B_1.

Animals—11 healthy horses of various breeds (body weight, 252 to 367 kg).

Procedure—Horses were randomly assigned to 3 groups and administered fumonisin B_1 daily. Horses received IV injections of 0 (control horses; n = 4), 0.01 (3), or 0.20 mg (4) of fumonisin B_1/kg for 7 to 28 days. Horses were examined daily for evidence of neurologic disease. When neurologic signs consistent with leukoencephalomalacia were evident, horses were anesthetized, and catheters were inserted for evaluation of the cardiovascular system. After recovery from anesthesia, hemodynamic measurements were obtained.

Results—Fumonisin-treated horses with clinical signs of neurologic disease had evidence of cardiovascular dysfunction manifested as decreases in heart rate, cardiac output, right ventricular contractility (assessed by measuring the maximal rate of change of right ventricular pressure), coccygeal artery pulse pressure, and pH and base excess in venous blood as well as increases in systemic vascular resistance, compared with values for control horses. Fumonisin-treated horses with and without clinical signs of neurologic disease also had higher serum and right ventricular sphinganine and sphingosine concentrations than control horses.

Conclusions and Clinical Relevance—An association was detected among fumonisin-induced neurologic disease, increased serum and myocardial sphinganine and sphingosine concentrations, and decreased cardiovascular function in horses. Fumonisin-induced decreases in cardiovascular function may contribute to the pathophysiologic development of leukoencephalomalacia in horses. (*Am J Vet Res* 2002;63:538–545).

Fumonisins are a group of mycotoxins primarily produced by the fungus *Fusarium verticillioides* (formerly *F moniliforme*), 1 of the most prevalent fungi associated with corn intended for consumption by humans and domestic animals.[1] Since their identification in 1988,[2] these toxic fungal metabolites have been implicated in outbreaks of leukoencephalomalacia in horses[3] and pulmonary edema in pigs[4,5] and also have been linked epidemiologically with esophageal cancer in humans.[6] Leukoencephalomalacia has been experimentally reproduced in horses by administering fumonisin B_1 orally[7] and intravenously.[8,9] Horses are considered to be the most susceptible species to fumonisin mycotoxicosis, yet little is known about the mechanism of toxicity in this species.

Fumonisins inhibit sphingosine-*N*-acyltransferase and sphinganine-*N*-acyltransferase,[10] enzymes that are key components in the pathway for de novo sphingolipid biosynthesis. Fumonisin-induced enzyme inhibition results in increased concentrations of free sphinganine and sphingosine in the serum and tissues of horses.[11,12] It is currently believed that altered sphingolipid biosynthesis is responsible for the clinical signs of fumonisin toxicosis. We have reported[13-17] that ingestion of fumonisin-containing culture material or IV administration of fumonisin B_1 causes decreases in heart rate, cardiac output, cardiac contractility, mean arterial pressure, oxygen tension in arterial and mixed-venous blood, and systemic oxygen delivery as well as increases in mean pulmonary artery pressure, pulmonary artery wedge pressure, oxygen consumption, and oxygen extraction ratio in pigs. Our findings indicate that fumonisin-induced pulmonary edema in pigs is attributable to acute left-sided heart failure, which was probably mediated by sphingosine inhibition of the myocardial and L-type calcium channel. Accordingly, we hypothesized that cardiovascular dysfunction would also be evident in horses with leukoencephalomalacia. The objective of the study reported here was to examine the cardiovascular effects of fumonisin B_1 administration in horses to determine whether there was an association among development of neurologic signs of toxicosis, increased serum and myocardial concentrations of sphingosine, and decreased cardiovascular function.

Materials and Methods

Animals—Eleven healthy horses of various breeds that were between 6 and 24 months old and weighed between 252 and 367 kg were obtained from local sources. On arrival at our facility, horses were orally administered a broad-spectrum anthelmintic (ivermectin[a]) and vaccinated IM with tetanus toxoid.[b] Horses were housed separately in stalls

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maintained at an ambient temperature of approximately 21 C. Horses were provided alfalfa several times each day to ensure ad libitum access at all times for the duration of the study. Horses had access to water at all times, but grain and pelleted feed were not fed. Ceftiofur sodium[c] (0.91 mg/kg, IM) was administered daily for the first 3 days after arrival as a prophylactic measure to prevent transportation-related respiratory tract infections. Horses were allowed a minimum of 5 days to acclimate to the stall, diet, and handling procedures before the study was initiated.

The study was approved by our institutional committee on the care and use of laboratory animals. It was part of a larger research project designed to explore the mechanism and dose-response relationship of fumonisin-induced neurologic disease in horses; some results of these experiments have been reported elsewhere.[d,e]

Fumonisin—Fumonisin B_1 was isolated from *F proliferatum* grown on whole corn. Toxin was extracted from the culture medium by use of methanol:water (7:3 [vol:vol]) and purified by use of preparatory liquid column chromatography, using a series of preparative columns alternating between C-18 and cyano phases. Water was removed from the purified fumonisin B_1 by a freeze-drying process. Purity of the final product (as free acid) was determined to be > 95% by analytical liquid chromatography, nuclear magnetic resonance, and mass spectral analysis techniques. Major impurities were determined to be the partial hydrolysis, methyl ester, and amide derivatives of fumonisin B_1. For the study reported here, fumonisin B_1 was dissolved in PBS solution (pH 7.0), and concentration was adjusted to create an administration volume of approximately 10 ml/horse/d.

Experimental protocol—A 16-gauge polyurethane catheter[f] was aseptically inserted into a jugular vein of each horse. This catheter was used to administer treatments and to obtain blood samples for analysis. At least 12 hours after catheter placement, horses were randomly assigned to 1 of 3 groups. Purified fumonisin B_1 was administered IV each morning to horses in 2 groups. Three horses were administered fumonisin B_1 at a dosage of 0.01 mg/kg, and 4 horses were administered fumonisin B_1 at a dosage of 0.20 mg/kg; control horses (n = 4) were administered 10 ml of saline (0.9% NaCl) solution at the same time. Fumonisin dosages were based on results of other studies in which IV administration of 0.125 mg of fumonisin B_1/kg every 24 hours induced leukoencephalomalacia after 9 days in 1 horse[8] and IV administration of 0.1 mg of fumonisin B_1/kg every 24 hours for 16 days followed by IV administration of 0.2 mg/kg every 24 hours for 2 additional days induced leukoencephalomalacia after 18 days in another horse.[9]

Horses were examined daily for neurologic changes by an experienced equine clinician (JHF) who was not aware of treatment assignment for each horse. When abnormal neurologic signs were sufficiently severe to warrant that a horse be euthanatized (day 7 to 9 for horses administered fumonisin at 0.20 mg/kg) or prior to euthanasia of horses at termination of the study (day 28 for horses administered 0.01 mg/kg and days 7 to 9 for control horses), cardiac function and analysis of blood samples were conducted.

Venous blood samples were obtained from a jugular vein and used for blood gas analysis and determination of serum concentrations of sphingolipids and cardiac troponin-I. Each horse was then anesthetized by administration of xylazine hydrochloride[g] (1.0 mg/kg, IV) followed 5 minutes later by administration of ketamine hydrochloride[h] (2.5 mg/kg, IV). After induction, introducer catheters[i] were inserted in the right jugular vein of each horse. Horses were then allowed to recover from anesthesia, and cardiovascular measurements were obtained at least 90 minutes later, by which time hemodynamic values should have returned to preanesthesia values.[18] Blood samples for blood gas analysis were obtained from the pulmonary artery (mixed venous) and facial artery. At the completion of the study, each horse was euthanatized by administration of an overdose of sodium pentobarbital (60 mg/kg, IV).

Cardiovascular measurements—Systolic, diastolic, and mean blood pressures were determined indirectly, by use of an oscillometric technique[j] and a cuff on the coccygeal artery, with the cuff width ranging from 25 to 40% of the tail circumference.[19] The head of each horse was maintained in a position consistent with that of a clinically normal alert horse during blood pressure measurement, and the distance between the scapulohumeral joint and center of the cuff on the coccygeal artery was measured. The actual blood pressure value was calculated as follows:

actual pressure = recorded pressure + (No. of cm between scapulohumeral joint and cuff on coccygeal artery/1.36)

Arterial pulse pressure was defined as arterial systolic pressure minus arterial diastolic pressure. A standard base-apex ECG[k] also was obtained.

Horses were instrumented as described elsewhere[18,20] to enable us to determine **heart rate (HR)**, **cardiac output (CO)**, mean pulmonary artery pressure, and mean right atrial pressure. A 150-cm 7F Swan-Ganz thermodilution catheter[l] was advanced through the introducer catheter in the right jugular vein and successively into the right atrium, right ventricle, and pulmonary artery. Polyethylene tubing was advanced through a second introducer catheter in the right jugular vein into the right atrium. Correct positioning of catheters was determined by evaluating the characteristic pressure waveforms on a strip chart recorder.[m] Pressure measurements were obtained with each horse in a standing position and referenced to the scapulohumeral joint. Cardiac output was measured by the thermodilution technique with the aid of a CO computer.[n] Fifty milliliters of a solution of 5% dextrose (0 C) was injected rapidly through the polyethylene catheter into the right atrium, and the change in temperature of pulmonary artery blood was monitored. Mean value of 5 CO determinations was used as the experimental value for each horse. Following CO determination, the Swan-Ganz catheter and polyethylene tubing were removed from each horse, and a 7F catheter equipped with a tip micromanometer[o] was advanced through the jugular vein and positioned in the right ventricle for recording of right ventricular pressure. The base-apex ECG and right ventricular pressure were digitized at 500 Hz, using a 12-bit microcomputer system, and data were stored on the hard disk of the microcomputer system for subsequent analysis.

Data for right ventricular pressure were analyzed, using a personal computer, commercially available software,[p] and custom-designed software. Right ventricular end-diastolic pressure was defined as the ventricular pressure at the start of the R wave of the QRS complex and represented the mean value for at least 6 consecutive beats. Maximal and minimal rates of change of right ventricular pressure were determined using a 3-point Lagrangian interpolation of digitized pressure data. The maximal rate of change of right ventricular pressure is a reliable variable for determining changes in cardiac contractility in horses.[18,21,22] Rate of right ventricular relaxation was calculated as relaxation half-time.[23] Heart rate was obtained simultaneously with CO determination, and stroke volume was calculated by dividing the CO by HR. Systemic vascular resistance was calculated as follows:

systemic vascular resistance = (mean arterial pressure – central venous pressure)/CO

Blood gas analysis—The pH, P_{O_2}, P_{CO_2}, and hemoglobin concentration were measured,[q] and pH, P_{O_2}, and P_{CO_2} values were corrected for rectal temperature. Plasma bicarbonate concentration, base-excess values, oxygen delivery, oxygen consumption, oxygen extraction ratio, alveolar-arterial oxygen gradient, and physiologic shunt fraction were calculated. Oxygen delivery was calculated as the product of arterial O_2 content and CO and indexed to body weight. Total blood O_2 content was calculated as follows:

(1.39 ml of O_2/g of hemoglobin + dissolved O_2 [equal to 0.3 volume %])/100 mm Hg

Mass-specific oxygen consumption (V_{O_2}) was calculated as the difference between **arterial oxygen content** (Ca_{O_2}) and **mixed-venous oxygen content** ($C\bar{v}_{O_2}$) multiplied by CO and indexed to body weight.[24] Oxygen extraction ratio was calculated as the ratio of the difference in arteriovenous O_2 content in relation to the arterial O_2 content. Room air alveolar-arterial O_2 difference was calculated by use of the alveolar gas equation:

$$P_{AO_2} = P_{IO_2} - (Pa_{CO_2}/R)$$

where P_{AO_2} is the alveolar O_2 tension, P_{IO_2} is the inspired partial pressure of oxygen calculated from the barometric pressure, and R is the respiratory exchange ratio[24]; the value for R was assumed to be 0.8. The physiologic shunt-to-total blood flow ratio was calculated by use of the shunt equation:

$$(Ci_{O_2} - Ca_{O_2})/(Ci_{O_2} - C\bar{v}_{O_2})$$

where Ci_{O_2} is the oxygen content of ideal end-pulmonary capillary blood.[24]

Analysis of sphingolipid and troponin-I concentrations—Serum from jugular venous blood was harvested after centrifugation, and myocardium was collected from the right ventricle of each horse during necropsy. Serum and tissue samples were stored at –20 C and thawed immediately before free sphinganine and sphingosine concentrations were determined as described elsewhere.[15] Minimum detection limits for sphinganine and sphingosine were 3 nM/L for serum and 0.01 nM/g for myocardium. The assay coefficient of variation was 2%.

Serum troponin concentrations were determined, using a fluorescent immunoassay[r] that used a monoclonal-antibody system that has been validated for horses.[25] Analytical sensitivity of the assay was 0.03 ng/ml.

Statistical analysis—Data were reported as mean ± SD. Data that were not normally distributed were logarithmically transformed or ranked before statistical analysis was performed. A 1-way ANOVA with group as the fixed effect was used for comparison of data. A statistical software package[s] was used for analysis. Values of $P < 0.05$ were considered significant.

Results

Clinical findings—Horses that received fumonisin at a dosage of 0.20 mg/kg were euthanatized between days 7 and 9 after initial administration, because they developed clinical signs of neurologic disease consistent with leukoencephalomalacia. Horses that received fumonisin at a dosage of 0.01 mg/kg did not develop signs of neurologic disease and were euthanatized on day 28. Control horses remained clinically normal until they were euthanatized between days 7 and 9 (paired with the high-dose group). A preliminary report of results of neurologic examinations has been published elsewhere.[a]

Cardiovascular measurements—Fumonisin-treated horses with neurologic signs of toxicosis (0.20 mg/kg, IV) had cardiovascular dysfunction, as indicated by decreases in HR, CO, cardiac contractility (as assessed by maximal rate of change of right ventricular pressure), and coccygeal artery pulse pressure as well as increased systemic vascular resistance (**Table 1**; **Fig 1**). We did not detect changes in mean, systolic, and diastolic pressures in the coccygeal artery, mean pulmonary artery pressure, mean central venous pressure,

Table 1—Mean ± SD cardiovascular values at the termination of the study in control horses administered saline (0.9% NaCl) solution (10 ml, IV, daily for 7 to 9 days), horses administered a low dose of fumonisin B_1 (0.01 mg/kg of body weight, IV, daily for 28 days), or horses administered a high dose of fumonisin B_1 (0.20 mg/kg, IV, daily for 7 to 9 days)

Variable	Control (n = 4)	Fumonisin (0.01 mg/kg; n = 3)	Fumonisin (0.20 mg/kg; n = 4)
Body weight (kg)	308 ± 26	328 ± 59	297 ± 39
Rectal temperature (C)	37.7 ± 1.2	37.7 ± 0.1	37.4 ± 0.6
Respiratory rate (breaths/min)	20 ± 0	25 ± 1	22 ± 4
Heart rate (beats/min)	52 ± 9	47 ± 5	36 ± 6*
Cardiac output (L/ min)	28.0 ± 2.7	29.7 ± 5.3	18.1 ± 3.2*
Cardiac index ([L/min]/kg)	91 ± 8	91 ± 10	63 ± 18*
Stroke volume (ml)	553 ± 90	639 ± 181	507 ± 83
Mean arterial pressure (mm Hg)	94 ± 5	87 ± 15	98 ± 13
Systolic arterial pressure (mm Hg)	124 ± 7	116 ± 7	118 ± 13
Diastolic arterial pressure (mm Hg)	75 ± 3	67 ± 12	89 ± 15
Arterial pulse pressure (mm Hg)	48 ± 5	49 ± 10	29 ± 14*
Systemic vascular resistance ([dynes · s]/cm^5)	266 ± 33	228 ± 10	445 ± 119*
Mean pulmonary artery pressure (mm Hg)	15 ± 4	17 ± 3	16 ± 6
Right ventricular end-diastolic pressure (mm Hg)	12 ± 2	11 ± 6	9 ± 4
Right ventricular dP/dt_{max} (mm Hg/s)	525 ± 113	630 ± 154	356 ± 72*
Right ventricular relaxation rate (ms)	48 ± 5	40 ± 22	44 ± 9
Right ventricular dP/dt_{min} (mm Hg/s)	–392 ± 42	–449 ± 24	–361 ± 88
Mean central venous pressure (mm Hg)	1 ± 3	2 ± 3	1 ± 1

*Value differs significantly ($P < 0.05$) from value for control group.

dP/dt_{max} = Maximal rate of change of right ventricular pressure. dP/dt_{min} = Minimal rate of change of right ventricular pressure.

Signs of neurologic disease were observed only in horses administered the high dose of fumonisin B_1.

right ventricular end-diastolic pressure, and right ventricular relaxation (as assessed by maximal rate of change of right ventricular pressure or rate of right ventricular relaxation). Horses administered fumonisin at a dosage of 0.01 mg/kg did not have evidence of abnormal neurologic signs after 28 days of treatment and had cardiovascular values similar to those of control horses.

The PR interval, QRS duration, and QT interval of the base-apex ECG were similar in all 3 groups (data not shown). Cardiac arrhythmias, other than second-degree atrioventricular block (Mobitz type I), were not observed in treated or control horses.

Blood gas analysis—Jugular venous pH and base excess and mixed venous pH were decreased in horses administered fumonisin at a dosage of 0.20 mg/kg, compared with values for control horses (**Table 2**). Arterial, venous, and mixed-venous oxygen tension; oxygen delivery; oxygen consumption; and oxygen extraction ratios were similar in fumonisin-treated and control horses. Horses administered fumonisin at a dosage of 0.01 mg/kg for 28 days had blood gas values similar to those of control horses.

Analysis of sphingolipid and troponin-I concentrations—Serum concentrations of sphinganine and sphingosine concentrations were increased in fumonisin-treated horses in a dose-dependent manner on the final day of the study (**Table 3**). Sphinganine and sphingosine concentrations also increased in a dose-dependent manner in the myocardium of fumonisin-treated horses.

Serum concentrations of troponin-I in horses treated with fumonisin B_1 at a dosage of 0.20 mg/kg were similar to those in control horses and horses treated with fumonisin B_1 at a dosage of 0.01 mg/kg (Table 3).

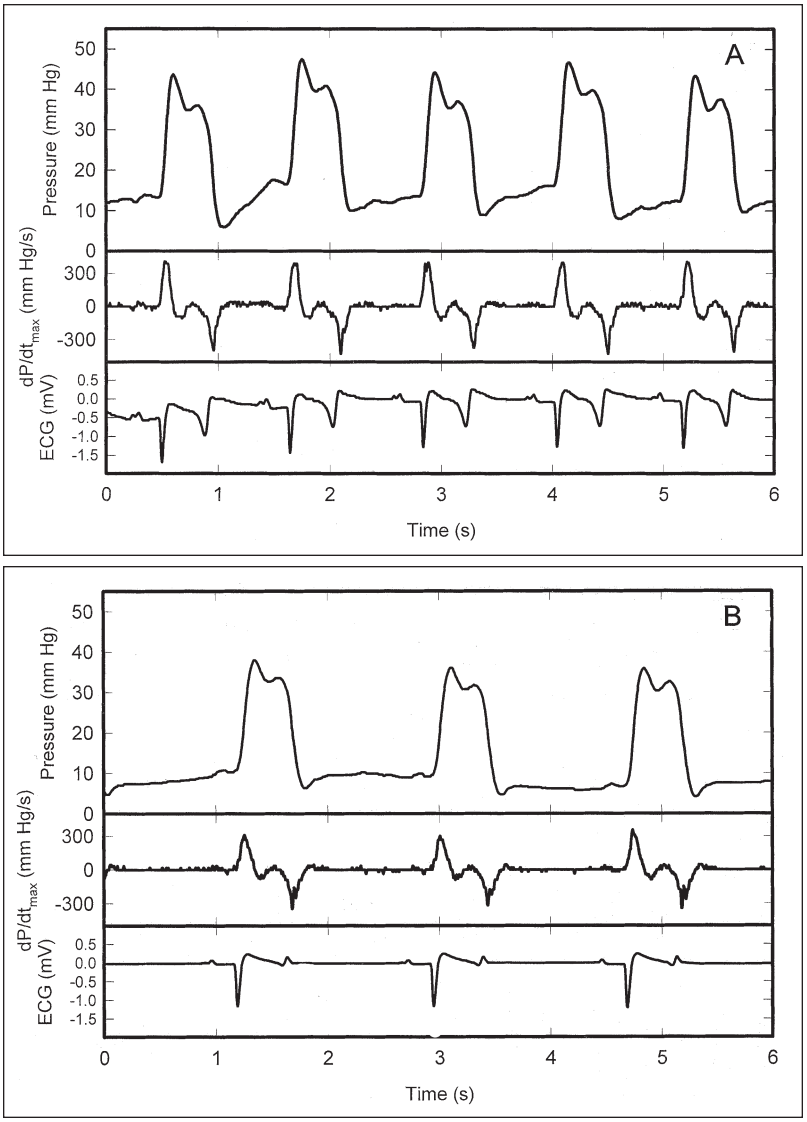

Figure 1—Representative cardiovascular data from a clinically normal control horse (A) and a fumonisin-treated horse (0.20 mg of fumonisin B_1/kg, IV, q 24 h) that had signs of neurologic disease (B). For each horse, right ventricular pressure, maximal rate of change of right ventricular pressure (dP/dt_{max}), and a base-apex ECG are provided.

Table 2—Mean ± SD values for blood pH, gas tension, and oxygen transport at the termination of the study in control horses administered saline solution (10 ml, IV, daily for 7 to 9 days), horses administered a low dose of fumonisin B_1 (0.01 mg/kg, IV, daily for 28 days), or horses administered a high dose of fumonisin B_1 (0.20 mg/kg, IV, daily for 7 to 9 days)

Variable	Control (n = 4)	Fumonisin (0.01 mg/kg;n = 3)	Fumonisin (0.20 mg/kg;n = 4)
Venous pH	7.40 ± 0.01	7.40 ± 0.02	7.36 ± 0.01*
Venous P_{CO_2} (mm Hg)	44 ± 7	50 ± 2	49 ± 2
Venous P_{O_2} (mm Hg)	37 ± 6	31 ± 5	34 ± 5
Venous [HCO_3^-] (m*M*/L)	30.2 ± 1.9	33.2 ± 0.9*	28.7 ± 0.5
Venous base excess (mEq/L)	5.3 ± 1.6	7.5 ± 1.6	2.6 ± 0.8*
Venous [hemoglobin] (g/dl)	10.2 ± 0.9	10.3 ± 0.6	11.2 ± 0.8
Venous hematocrit (%)	28.6 ± 2.4	28.5 ± 1.9	31.8 ± 2.5
Arterial pH	7.43 ± 0.02	7.45 ± 0.04	7.40 ± 0.01
Arterial P_{CO_2} (mm Hg)	38 ± 1	38 ± 6	39 ± 1
Arterial P_{O_2} (mm Hg)	92 ± 12	93 ± 8	88 ± 3
Mixed venous pH	7.37 ± 0.01	7.37 ± 0.01	7.34 ± 0.01*
Mixed venous P_{CO_2} (mm Hg)	49 ± 2	51 ± 3	50 ± 1
Mixed venous P_{O_2} (mm Hg)	29 ± 1	27 ± 2	28 ± 2
Alveolar-arterial O_2 difference (mm Hg)	9 ± 11	7 ± 6	11 ± 3
Oxygen delivery (ml of O_2/min/kg)	13.7 ± 1.7	12.9 ± 1.9	9.6 ± 2.4
Oxygen consumption (ml of O_2/min/kg)	5.9 ± 0.4	6.5 ± 1.2	4.4 ± 1.5
Oxygen extraction ratio	0.43 ± 0.02	0.50 ± 0.04	0.45 ± 0.04
Physiologic shunt-to-total blood flow ratio (%)	2.3 ± 2.8	1.4 ± 1.3	25 ± 0.6

See Table 1 for key.

Table 3—Mean ± SD sphingosine and sphinganine concentrations, sphinganine-to-sphingosine ratio, and troponin-I concentration in serum and cardiac tissues obtained at the termination of the study from control horses administered saline solution (10 ml, IV, daily for 7 to 9 days), horses administered a low dose of fumonisin B_1 (0.01 mg/kg, IV, daily for 28 days), or horses administered a high dose of fumonisin B_1 (0.20 mg/kg, IV, daily for 7 to 9 days)

Variable	Control (n = 4)	Fumonisin (0.01 mg/kg;n = 3)	Fumonisin (0.20 mg/kg;n = 4)
Serum			
Sphinganine (n*M*/L)	3.1 ± 0.5	30.5 ± 14.6*	113.2 ± 29.4*
Sphingosine (n*M*/L)	11.1 ± 1.8	24.2 ± 15.2*	45.0 ± 18.0*
Sphinganine-to-sphingosine ratio	0.27 ± 0.03	1.68 ± 1.12*	2.75 ± 0.85*
Troponin-I (ng/ml)	0.003 ± 0.006	0.003 ± 0.005	0.008 ± 0.005
Cardiac tissues			
Sphinganine (n*M*/g of tissue)†	0.07 ± 0.02	0.49 ± 0.06*	3.11 ± 1.03*
Sphingosine (n*M*/g of tissue)†	0.54 ± 0.08	0.99 ± 0.11*	2.75 ± 1.06*
Sphinganine-to-sphingosine ratio	0.12 ± 0.06	0.50 ± 0.02*	1.31 ± 0.54*

†Wet-weight basis.
See Table 1 for remainder of key.

Discussion

To our knowledge, this is the first study to document that horses with fumonisin B_1-induced neurologic signs have cardiovascular dysfunction characterized by decreases in HR, CO, cardiac contractility, and arterial pulse pressure. Although the pathogenesis of leukoencephalomalacia in horses is unknown, our finding of an association between fumonisin-induced neurologic disease and cardiovascular dysfunction is consistent with suggestions that damage to the cerebral vasculature is involved in the development of leukoencephalomalacia.[26,27] Results of other studies[28,29] support our finding that decreased cardiovascular function plays a role in the development of leukoencephalomalacia in horses, because affected horses can be cyanotic, dyspneic, and edematous,[28] and necropsy of some affected horses has identified pulmonary edema with only minor evidence of hepatotoxicosis.[29]

Horses appear to be more susceptible to cardiotoxic effects of fumonisin, compared to pigs, because cardiovascular depression was induced with lower daily doses of fumonisin B_1 in horses (0.20 mg/kg, IV) than pigs (1.0 mg/kg, IV).[17] Reasons for this increased susceptibility are unknown, but it may be attributable to differences in the pharmacokinetics of fumonisin B_1 or pathophysiologic processes in horses. In pigs, IV administration of purified fumonisin B_1 causes substantial decreases in HR, CO, and cardiac contractility,[17] similar to the results for horses reported here. However, pigs also develop pulmonary hypertension and systemic hypotension,[17] which were not observed in the horses of our study. Although the reason for species differences is not known, it is possible that pulmonary hypertension and systemic hypotension in pigs could be attributable to a direct vascular effect from relatively higher serum concentrations of sphingosine, because in vitro studies involving the use of vascular rings have indicated that serum sphingosine concentrations in pigs that die as a result of fumonisin-induced pulmonary edema are sufficient-

ly high to cause vasoconstriction of the pulmonary artery and vasodilation of the aorta.[t] Pigs treated with fumonisin B_1 (1 mg/kg, IV, for 7 days) had mean ± SD serum sphinganine concentrations of 906 ± 434 n*M*/L and mean sphingosine concentrations of 416 ± 169 n*M*/L,[17] which are approximately 10-fold higher than those detected in the serum of horses from the study reported here. However, milk-fed calves administered purified fumonisin B_1 (1.0 mg/kg, IV, for 7 days) had mean serum sphinganine concentrations of 237 ± 388 n*M*/L and sphingosine concentrations of 44 ± 65 n*M*/L,[30] values that were similar to the concentrations observed in our horses. Cardiovascular dysfunction was not observed in those calves.[30]

Sphingolipid concentrations are difficult to measure in equine serum, because concentrations are low and the efficiency of extraction is poor.[31] Serum sphingolipid assays are further complicated in equine serum, because the concentration of sphinganine and sphingosine can vary by 2- to 3-fold during a period of days for unknown reasons.[11] Nevertheless, the serum sphingolipid concentrations in horses with fumonisin-induced neurologic disease in the study reported here were similar to those reported in horses with leukoencephalomalacia.[11,12] In 1 study,[11] 2 horses were fed a diet containing 44 mg/kg of fumonisin B_1/kg until they died as a result of leukoencephalomalacia. Terminal serum sphinganine concentrations for the 2 horses were 283 and 190 n*M*/L, serum sphingosine concentrations were 226 and 115 n*M*/L, and the sphinganine-to-sphingosine ratio was 1.25 and 1.65, respectively. In a second study,[12] horses were fed fumonisin in the form of culture material prepared from 2 strains of *F verticilliodes*. One group of 2 horses was fed a diet that contained fumonisin (65 to 130 mg/kg; fumonisin-containing culture material produced from *F verticilliodes* isolate AU 2/3). When they died as a result of leukoencephalomalacia, mean serum sphinganine and sphingosine concentrations were 24 and 30 n*M*/L, respectively, and the sphinganine-to-sphingosine ratio was 0.80. A second group of 2 horses was fed a diet containing fumonisin (200 mg of fumonisin/kg; culture material produced from *F verticilliodes* isolate MRC 826). When they died as a result of leukoencephalomalacia, serum sphinganine and sphingosine concentrations were 136 and 71 n*M*/L, respectively, and the sphinganine-to-sphingosine ratio was 1.92.

To our knowledge, this is the first report of increased sphingosine and sphinganine concentrations in the myocardium of fumonisin-treated horses; however, increases have been reported in pigs with cardiovascular dysfunction.[15,17] Pigs treated with fumonisin B_1 (1 mg/kg, IV, for 7 days) had myocardial sphinganine concentrations of 11 ± 3 n*M*/g of tissue (wet-weight basis) and sphingosine concentrations of 7 ± 1 n*M*/g of tissue.[17] These concentrations represented an 11-fold increase in sphinganine and 7-fold increase in sphingosine concentrations, compared with concentrations for control pigs. However, milk-fed calves administered purified fumonisin B_1 (1 mg/kg, IV, for 7 days) had myocardial sphinganine concentrations of 68 ± 126 n*M*/g and sphingosine concentrations of 19 ± 22 n*M*/g.[30] These concentrations represented a 90-fold increase in sphinganine and 9-fold increase in sphingosine concentrations, compared with concentrations for control calves. The myocardial sphinganine and sphingosine concentrations reported in fumonisin-treated calves are considerably higher than those in pigs or horses with decreased cardiovascular function. Because fumonisin treatment did not produce cardiovascular changes in calves, it can be assumed that calves are more resistant to fumonisin-mediated cardiotoxic effects than horses or pigs. The mechanism responsible for this increased resistance is not known but may be related to differences in sphingolipid metabolism or biochemical alterations among species.

Although the physiologic role of sphinganine is unknown, sphingosine is an important intracellular second messenger.[32] Physiologically relevant concentrations of sphingosine inhibit L-type calcium channels in rabbit myocardial cells, thereby decreasing Ca^{2+}-induced release of calcium from the sarcoplasmic reticulum and cardiac contractility.[33-36] Sphingosine-mediated L-type calcium channel blockade has been observed in ventricular cells of dogs,[34] rats,[35] rabbits,[36] and cats,[37] whereas fumonisin B_1 inhibits calcium channels in atrial tissue of frogs.[38] The mechanism of this sphingosine-mediated L-type calcium channel blockade does not appear to involve effects on bulk surface charge or a voltage-dependent blockade. Instead, sphingosine appears to alter channel gating by increasing the closed time probability of the calcium channel, with the blocking action being related to the polar head groups or the primary amine of sphingosine.[39]

Because of gravitational forces, horses and giraffes are 2 species that are dependent on autoregulation of cerebral blood flow when they lower their head to graze. For instance, pressure in the distal part of the carotid artery increases from 100 to 350 mm Hg when a giraffe lowers its head to drink.[40,41] However this increase in carotid pressure does not create a substantial increase in cerebral blood flow because of the constriction and dilation of cerebral arterioles, which maintain normal cerebral blood pressures.[42,43] It has been documented that L-type calcium channels are the primary regulators of vascular tone in these cerebral arterioles.[44] Because sphingosine is a potent inhibitor of L-type calcium channels,[33-36,39] it is plausible that the cerebral arterioles are preferentially dilated in horses that have consumed fumonisin; thus, a horse is not able to maintain normal cerebral blood pressures when it lowers its head to eat and drink. This would result in vasogenic cerebral edema resulting from the cerebral perfusion pressure increasing to above-normal values.

Horses in the study reported here that had abnormal neurologic signs had increased protein concentrations in the CSF, compared with concentrations in control horses and fumonisin-treated horses that did not have abnormal neurologic signs.[a] This finding is consistent with vasogenic cerebral edema in horses with fumonisin-induced leukoencephalomalacia. Furthermore, magnetic resonance imaging of formalin-fixed brains from horses in this study revealed abnormalities in horses that had abnormal neurologic signs. These changes were characterized by varying degrees of cere-

bral edema, flattening of the cerebral gyri, loss of detail in cerebral sulci and white matter, dilation of the ventricular system, and cerebellar herniation through the foramen magnum.[u] These findings also are consistent with vasogenic cerebral edema in horses with fumonisin-induced leukoencephalomalacia.

If sphingosine is truly the primary mediator of fumonisin-induced cardiovascular dysfunction, it is unclear why calves treated with fumonisin B_1 did not have cardiovascular dysfunction despite having myocardial sphingosine concentrations higher than those in pigs or horses treated with fumonisin. It is possible that there are differences in cellular compartmentalization of sphingosine among species that could explain the differences in susceptibility to fumonisin that have been observed in various species of animals. Another explanation could be that calcium channels from calves are less susceptible to inhibition by sphingosine than calcium channels in horses and pigs. It is also plausible that other sphingolipid metabolites play an important role in fumonisin-induced cardiovascular dysfunction. For example, sphingosine-1-phosphate is an initial product of sphingosine catabolism that has important second-messenger functions.[45] This compound is the primary ligand for the Edg family of receptors including the Edg-1 receptor, which is believed to play an important role in the calcium regulation of cardiac cells.[46] Although our current knowledge is limited, it appears that sphingosine-1-phosphate signaling via the Edg-1 and Edg-3 receptors is also vital for vascular function.[47] Because concentrations of sphingosine-1-phosphate have not been measured in animals administered fumonisin, we can only speculate that it may play a role in fumonisin-induced toxic effects. Therefore, more research is needed to gain a better understanding of the pathophysiologic processes involved in fumonisin-induced cardiovascular dysfunction and fumonisin-induced leukoencephalomalacia.

Troponin-I, -C, and -T form a complex that regulates the calcium-modulated interaction of actin and myosin in striated muscle. Troponin-I from cardiac muscle and troponin-I from skeletal muscle are products of different genes with unique amino acid sequences.[48] Thus, monoclonal antibodies have been developed to cardiac troponin-I that do not cross-react with the skeletal muscle form of troponin-I.[49] In humans, serum concentration of troponin-I is widely accepted as an accurate marker of acute myocardial injury.[48] Although reference ranges for serum troponin-I concentrations have not been established for horses, the fact that similar troponin-I concentrations were detected in fumonisin-treated and control horses in our study suggests that fumonisin-induced cardiovascular dysfunction was not accompanied by myocyte necrosis or injury.

Analysis of results of the study reported here indicates that fumonisin B_1 decreases cardiovascular function in horses. Cardiovascular dysfunction in horses with fumonisin-induced leukoencephalomalacia has many similarities to that observed in pigs with fumonisin-induced pulmonary edema. Therefore, it is possible that the pathophysiologic processes of leukoencephalomalacia in horses and pulmonary edema in pigs involve cardiovascular dysfunction that may be mediated by sphingosine. Additional studies to examine the effects of fumonisin on the systemic and cerebral vasculature of horses appear warranted.

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[a]Eqvalan paste 1.87%, Merck & Co Inc, Rahway, NJ.
[b]Tetanus toxoid, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
[c]Naxcel, Pharmacia & Upjohn, Kalamazoo, Mich.
[d]Foreman JH, Constable PD, Waggoner AL, et al. Clinical progression of neurological signs and cerebrospinal fluid values in horses with experimentally induced leukoencephalomalacia (abstr). *J Vet Intern Med* 2001;15:286.
[e]Haschek WM, Waggoner AL, Hsiao SH, et al. Morphological alterations in horses induced by intravenous fumonisin B_1 administration (abstr). *Toxicol Sci* 2001;60(1-S):407.
[f]Milacath—extended use, Mila International Inc, Florence, Ky.
[g]Rompun 100 mg/ml injectable, Bayer Corp, Shawnee Mission, Kan.
[h]Ketaset, Fort Dodge Laboratories, Fort Dodge, Iowa.
[i]Desilet Hoffman Introducers, Cook Medical, Bloomington, Ind.
[j]Dinamap model 1255, Critikon Inc, Tampa, Fla.
[k]PageWriter Xli, Hewlett-Packard, Boise, Idaho.
[l]Swan-Ganz thermodilution catheter, Columbus Instruments, Columbus, Ohio.
[m]5/6H Recorder, Gilson Medical Electronics, Middleton, Wis.
[n]Cardiomax-II, Columbus Instruments, Columbus, Ohio.
[o]Product name, Millar Instruments, Houston, Tex.
[p]CONDUCT-PC, Leycom, Maasricht, The Netherlands.
[q]Ciba-Corning 288 blood gas system, Ciba Corning, Medfield, Mass.
[r]Dade Behring Stratus-CS analyzer, Dade Behring, Neward, Del.
[s]SAS release 6.12, SAS Institute Inc, Cary, NC.
[t]Hsiao SH, Constable PD, Smith GW, et al. Effects of sphingosine, sphinganine, and sphingosine-1-phosphate on phenylephrine contracted porcine thoracic aorta and intrapulmonary artery rings (abstr). *Toxicol Sci* 2001;60(1-S):13.
[u]Constable PD, Smith GW, Foreman JH, et al. Equine leukoencephalomalacia: magnetic resonance imaging of affected brains and cardiovascular toxicity following intravenous fumonisin B_1 administration to horses (abstr). *Abstr Fumonisins Risk-Assess Workshop* 2001;13.

References

1. Marasas WFO, Nelson PE. Section Liseola. In: Marasas WFO, Nelson PE, eds. *Toxigenic Fusarium species*. University Park, Pa: Pennsylvania State University Press, 1984;216–252.

2. Gelderblom WCA, Jaskiewicz K, Marasas WFO, et al. Fumonisins—novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl Environ Microbiol* 1988;54:1806–1811.

3. Wilson TM, Nelson PE, Marasas WFO, et al. A mycological evaluation and in vivo toxicity evaluation of feed from 41 farms with equine leukoencephalomalacia. *J Vet Diagn Invest* 1990;2:352–354.

4. Harrison LR, Colvin BM, Greene JT, et al. Pulmonary edema and hydrothorax in swine produced by fumonisin B_1, a toxic metabolite of *Fusarium moniliforme*. *J Vet Diagn Invest* 1990;2:217–221.

5. Osweiler GD, Ross PF, Wilson TM, et al. Characterization of an epizootic of pulmonary edema in swine associated with fumonisin in corn screenings. *J Vet Diagn Invest* 1992;4:53–59.

6. Rheeder JP, Marasas WFO, Thiel PG, et al. *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei. *Phytopathology* 1992;82:353–357.

7. Kellerman TS, Marasas WFO, Thiel PG, et al. Leukoencephalomalacia in two horses induced by oral dosing of fumonisin B_1. *Onderstepoort J Vet Res* 1990;57:269–275.

8. Marasas WFO, Kellerman TS, Gelderblom WCA, et al. Leukoencephalomalacia in a horse induced by fumonisin B_1 isolated from *Fusarium moniliforme*. *Onderstepoort J Vet Res* 1988;55:197–203.

9. Laurent D, Pellegrin F, Kohler F, et al. Fumonisin B_1 in equine leucoencephalomalacia pathogenesis. *Microbiol Aliments Nutr* 1989;7:285–291.

10. Wang E, Norred WP, Bacon CW, et al. Inhibition of sphingosine biosynthesis by fumonisins. Implications for diseases associated with *Fusarium moniliforme*. *J Biol Chem* 1991;266:14486–14490.

11. Wang E, Ross PF, Wilson TM, et al. Increases in serum

sphingosine and sphinganine and decreases in complex sphingolipids in ponies given feed containing fumonisins, mycotoxins produced by *Fusarium moniliforme*. *J Nutr* 1992;122:1706–1716.

12. Goel S, Schumacher J, Lenz SD, et al. Effects of *Fusarium moniliforme* isolates on tissue and serum sphingolipid concentrations in horses. *Vet Human Toxicol* 1996;38:265–270.

13. Smith GW, Constable PD, Bacon CW, et al. Cardiovascular effects of fumonisin in swine. *Fundam Appl Toxicol* 1996;31:169–172.

14. Smith GW, Constable PD, Haschek WM. Cardiovascular responses to short-term fumonisin exposure in swine. *Fundam Appl Toxicol* 1996;33:140–148.

15. Smith GW, Constable PD, Tumbleson ME, et al. Sequence of cardiovascular changes leading to pulmonary edema in swine fed fumonisin-containing culture material. *Am J Vet Res* 1999;60:1292–1300.

16. Constable PD, Smith GW, Rottinghaus GE, et al. Ingestion of fumonisin B_1-containing culture material decreases cardiac contractility and mechanical efficiency in swine. *Toxicol Appl Pharmacol* 2000, 162:151–160.

17. Smith GW, Constable PD, Eppley RM, et al. Purified fumonisin B_1 decreases cardiovascular function but does not alter pulmonary capillary permeability in swine. *Toxicol Sci* 2000;56:240–249.

18. Grubb TL, Benson GJ, Thurmon JC, et al. Effects of xylazine/ketamine on cardiac function and serum ionized calcium in horses. *J Vet Anaesth* 1997;24:6–11.

19. Geddes LA, Chaffee V, Whistler SJ, et al. Indirect mean blood pressure in the anesthetized pony. *Am J Vet Res* 1977;38:2055–2057.

20. Vischer CL, Foreman JL, Constable PD, et al. Hemodynamic effects of thyroidectomy in sedentary horses. *Am J Vet Res* 1999;60:14–21.

21. Nollet H, Van Loon G, Deprez P, et al. Use of right ventricular pressure increase rate to evaluate cardiac contractility in horses. *Am J Vet Res* 1999;60:1508–1512.

22. Grubb TL, Benson GJ, Foreman JH, et al. Hemodynamic effects of ionized calcium in horses anesthetized with halothane or isoflurane. *Am J Vet Res* 1999;60:1430–1435.

23. Constable PD, Muir W, Sisson D. Clinical assessment of left ventricular relaxation. *J Vet Intern Med* 1999;13:5–13.

24. West JB. Gas exchange. In: West JB, ed. *Pulmonary pathophysiology—the essentials*. 3rd ed. Baltimore: Williams & Wilkins Co, 1987;19–41.

25. O'Brien PJ, Landt Y, Ladenson JH. Differential reactivity of cardiac and skeletal muscle from various species in a cardiac troponin I immunoassay. *Clin Chem* 1997;43:2333–2338.

26. Wilson BJ, Maronpot RR, Hildebrandt PK. Equine leukoencephalomalacia. *J Am Vet Med Assoc* 1973;163:1293–1294.

27. Marasas WFO, Kellerman TS, Pienaar JG, et al. Leukoencephalomalacia: a mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. *Onderstepoort J Vet Res* 1976;43:113–122.

28. Kellerman TS, Marasas WFO, Pienaar JG, et al. A mycotoxicosis of equidae caused by *Fusarium moniliforme* Sheldon. A preliminary communication. *Onderstepoort J Vet Res* 1972;39:205–208.

29. Bela F, Endre B. Occurrence of the equine leukoencephalomalacia (ELEM) caused by fumonisin-B_1 mycotoxin in Hungary. *Magyar Allatorvosok Lapja* 1996;8:484–487.

30. Mathur S, Constable PD, Eppley RM, et al. Fumonisin B_1 increases serum sphinganine concentration but does not alter serum sphingosine concentration or induced cardiovascular changes in milk-fed calves. *Toxicol Sci* 2001;60:379–384.

31. Riley RT, Hinton DM, Chamberlain WJ, et al. Dietary fumonisin B_1 induces disruption of sphingolipid metabolism in Sprague-Dawley rats: a new mechanism of nephrotoxicity. *J Nutr* 1994;124:594–603.

32. Jefferson AB, Schulman H. Sphingosine inhibits calmodulin-dependent enzymes. *J Biol Chem* 1988;263:15241–15244.

33. Sabbadini RA, Betto R, Teresi A, et al. The effects of sphingosine on sarcoplasmic reticulum membrane calcium release. *J Biol Chem* 1992;267:15475–15484.

34. Dettbarn CA, Betto R, Salviati G, et al. Modulation of cardiac sarcoplasmic reticulum ryanodine receptor by sphingosine. *J Mol Cell Cardiol* 1994;26:229–242.

35. McDonough PM, Yasui K, Betto R, et al. Control of cardiac Ca^{2+} levels. Inhibitory actions of sphingosine on Ca^{2+} transients and L-type Ca^{2+} channel conductance. *Circ Res* 1994;75:981–989.

36. Webster R, Sabbadini RA, Dettbarn CA, et al. Sphingosine effects on the contractile behavior of skinned cardiac myocytes. *J Mol Cell Cardiol* 1994;26:1273–1290.

37. Oral H, Dorn GW, Mann DL. Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-α in the adult mammalian cardiac myocyte. *J Biol Chem* 1997;272:4836–4842.

38. Sauviat MP, Laurent D, Kohler F, et al. Fumonisin, a toxin from the fungus *Fusarium moniliforme* Sheldon, blocks both the calcium current and the mechanical activity in frog atrial muscle. *Toxicon* 1991;29:1025–1031.

39. Yasui K, Palade P. Sphingolipid actions in sodium and calcium currents of rat ventricular myocytes. *Am J Physiol* 1996;270:C645–C649.

40. Goetz RH, Warren JV, Gauer OH, et al. Circulation of the giraffe. *Circ Res* 1960;8:1049–1058.

41. Van Citters RL, Franklin DL, Vatner SF. Cerebral haemodynamics in the giraffe. *Trans Assoc Am Physicians* 1969;82:293–303.

42. Van Citters RL, Kempler WS, Franklin DL. Blood flow in the giraffe carotid artery. *Comp Biochem Physiol* 1968;24:1035–1042.

43. Faraci FM, Heistad DD. Regulation of large cerebral arteries and cerebral microvascular pressure. *Circ Res* 1990;66:8–17.

44. Michelakis E, Tewari K, Simard JM. Calcium channels in smooth muscle cells from cerebral precapillary arterioles activate at more negative potentials than those from basilar artery. *Pflugers Arch* 1994;426:459–461.

45. Olivera A, Spiegel S. Sphingosine kinase: a mediator of vital cellular functions. *Prostaglandins* 2001;64:123–134.

46. Nakajima N, Cavalli AL, Biral D, et al. Expression and characterization of Edg-1 receptors in rat cardiomyocytes: calcium deregulation in response to sphingosine-1-phosphate. *Eur J Biochem* 2000;267:5679–5686.

47. Hla T. Sphingosine-1-phosphate receptors. *Prostaglandins* 2001;64:135–142.

48. Adams JE III, Bodor GS, Davila-Roman G, et al. Cardiac troponin I: a marker with high specificity for cardiac injury. *Circulation* 1993;88:101–106.

49. Bodor GS, Porter S, Landt Y, et al. The development of monoclonal antibodies and an assay for cardiac troponin-I with preliminary results in suspected myocardial infarction. *Clin Chem* 1992;11:2203–2214.