Cardiovascular findings in ponies with equine metabolic syndrome

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OBJECTIVE
To determine whether hypertension, high sympathetic tone, resting and exercising arrhythmias, and echocardiographic changes consistent with hypertensive cardiomyopathy were associated with equine metabolic syndrome (EMS) in ponies.

DESIGN
Prospective case-control study.

ANIMALS
19 privately owned ponies with a diagnosis of EMS (history of laminitis, body condition score ≥ 7/9, cresty neck score ≥ 3/5, and abnormal oral sugar test result; cases) and 20 healthy control ponies.

PROCEDURES
Heart rate (HR), noninvasively measured arterial blood pressure (BP), markers of autonomic tone (splenic volume and HR variability), 24-hour and exercising ECGs, and echocardiograms were compared between cases and controls.

RESULTS
Compared with controls, cases had a higher mean ± SD HR (44.5 ± 7.5 beats/min vs 38.6 ± 6.8 beats/min) and median mean left ventricular wall thickness (2.0 cm vs 1.8 cm). No differences were identified between groups in BP, splenic volume, HR variability, and number of premature complexes in ECGs. Mean wall thickness was correlated with BP (r = 0.54), high-frequency power (r = –0.71), and ratio of low- to high-frequency power (r = 0.66). Relative wall thickness was correlated with serum insulin concentration (r = 0.71).

CONCLUSIONS AND CLINICAL RELEVANCE
Ponies with EMS had myocardial hypertrophy that was correlated with insulin response to an oral sugar test, sympathetic and parasympathetic tone, and BP. The heterogeneity and limited sample size of this preliminary study should be considered when drawing conclusions. Cardiovascular changes associated with this syndrome deserve further attention. (J Am Vet Med Assoc 2017;250:1027–1035)

Equine metabolic syndrome is characterized by regionalized or generalized obesity, insulin dysregulation, and an increased risk of laminitis, compared with the risk in unaffected horses.1 Other potential components include changes in the plasma lipid composition,2 alterations in reproductive cycles of mares,3 and hypertension.4 The recent increase in the number of horses in which EMS has been diagnosed may be attributable to changes in their activities and management as well as increasing awareness of owners and veterinarians regarding this condition. This metabolic syndrome in horses is similar in many respects to that in humans but differs in that laminitis is the main component for horses, and a broad spectrum of cardiovascular disease and diabetes mellitus are the main risk factors for humans. In humans, metabolic syndrome is diagnosed on the basis of obesity, dyslipidemia, hypertension, and fasting hyperglycemia5 and is associated with an increase in the risk of cardiovascular abnormalities, including atherosclerosis, endothelial dysfunction, myocardial infarct, stroke, supraventricular and ventricular arrhythmias, cardiac failure, and sudden cardiac death.6

Horses with EMS are suggested to be less prone than humans to development of vascular complications, including atherosclerosis and coronary artery disease, because of differences in lipoprotein metabolism, diet, and lifespan.1,7 The pathophysiologic mechanisms underlying the association of EMS with insulin dysregulation and laminitis in horses are in-

ABBREVIATIONS

ANS  Autonomic nervous system
BP  Arterial blood pressure
EMS  Equine metabolic syndrome
HR  Heart rate
HRV  Heart rate variability
IQR  Interquartile range
LF:HF  Ratio of low-frequency to high-frequency power
LVM  Left ventricular mass
LVWT  Left ventricular wall thickness
MWT  Mean wall thickness
OST  Oral sugar test
RWT  Relative wall thickness
SVPC  Supraventricular premature complex
VPC  Ventricular premature complex
completely understood. Various mechanisms have been investigated, including the effects of low peripheral glucose metabolism on lamellar tissue, accumulation of glycation end products, insulin resistance–associated proinflammatory status, effects mediated by insulin-like growth factor, and vasoactive properties of insulin and subsequent development of hypertension or endothelial damage.\textsuperscript{4,5} Dysfunction of the vascular endothelium is likely to be important in the development of laminitis,\textsuperscript{6} but whether laminitis in the presence of EMS should be interpreted as a cardiovascular, metabolic, or inflammatory condition or a combination of all of these conditions is debatable.

The ANS and cardiovascular system are closely interrelated. Horses intrinsically have high resting parasympathetic tone, and imbalances in autonomic regulation could cause detectable changes. Autonomic imbalances precede insulin resistance in humans with metabolic syndrome,\textsuperscript{9} and overactivation of the sympathetic nervous system has been suggested as one of the possible underlying causes of arrhythmogenesis\textsuperscript{10} and insulin resistance in those affected.\textsuperscript{11,12}

In horses, myocardial hypertrophy and hypertensive heart disease in association with laminitis have been reported,\textsuperscript{13,14} and seasonal increases in blood pressure have been identified in prelaminitic ponies, concurrent with increases in serum insulin and plasma triglycerides concentrations.\textsuperscript{4} However, no studies have been reported that specifically address the cardiovascular components of EMS.

The purpose of the study reported here was to explore the cardiovascular component of naturally occurring EMS in ponies to determine whether equine clinicians and researchers should consider this aspect of the syndrome. Such information could lead to investigations concerning early recognition, more sensitive diagnostic and monitoring approaches, and more comprehensive therapeutic and preventive strategies. In addition, characterization of cardiovascular abnormalities and ANS dysregulations in affected ponies could be useful from a comparative and translational perspective, providing information regarding whether research findings on exercise, dietary, and pharmacological interventions in affected equids might be applied to affected humans. The hypothesis was that EMS would be associated with hypertension, an increase in sympathetic tone (as evidenced by changes in HRV), a decrease in splenic size, an increase in the frequency of resting and exercise arrhythmias, and echocardiographic changes consistent with hypertensive cardiomyopathy (ie, an increase in MWT, RWT, or LVM).

Materials and Methods

Animals

Ponies with suspected EMS and healthy ponies were identified for inclusion in this case-control study from the hospital records and by collaborating referring veterinarians. Ponies where enrolled between August 2014 and March 2015. Ponies (rather than horses) were included because of the disproportionately larger number of ponies identified during an initial screening of records and communications with collaborating veterinarians. Owners were contacted, informed about the study, and invited to participate. All participating ponies were physically examined, and measurements of plasma insulin concentration (after food withholding for 12 hours) and ACTH concentration as well as an OST\textsuperscript{5} were performed. The study was performed with the approval of the animal care and use committee, and owner consent was obtained.

To be included in the case group, ponies with suspected EMS were required to have had that diagnosis confirmed through a body condition score \(\geq \) 7/9\textsuperscript{16} (ie, fat or extremely fat), a cresty neck score \(\geq 3/5\) (crest of the neck enlarged and thickened to crest large and drooping to 1 side),\textsuperscript{17} resting plasma insulin concentration \(\geq 20 \mu\text{U/mL}\) or serum insulin concentration after OST \(\geq 60 \mu\text{U/mL}\), and a history or clinical evidence of laminitis.\textsuperscript{1,15}

Ponies were selected for the control group to match the case ponies by breed. To be included in this group, ponies were required to have a resting plasma insulin concentration < 20 \(\mu\text{U/mL}\), a plasma insulin concentration after OST < 60 \(\mu\text{U/mL}\), and no history or evidence of laminitis. Whenever possible, control ponies that were housed in the same stables as a respective case pony were selected. Ponies with abnormalities that could be ascribed to a systemic disease other than EMS, ponies that were lame at a walk or had markedly high digital pulses, and ponies with a high basal plasma ACTH concentration (> 10 pmol/L)\textsuperscript{11} were eliminated from both groups.

Measurements

All procedures were performed at the farm where ponies were usually housed. Body weight was estimated by use of a measuring tape, and body condition score\textsuperscript{16} and cresty neck score\textsuperscript{17} were assigned as reported elsewhere. Food was withheld from all included ponies for \(\geq 12\) hours prior to blood sample collection for baseline measurements of serum insulin, blood glucose, and plasma ACTH concentrations. Afterward, an OST was performed by oral administration of corn syrup\textsuperscript{3} (0.15 mL/kg [0.07 mL/lb]) by use of a feeding syringe. A second blood sample was obtained 75 minutes later for measurement of glucose and insulin concentrations. For insulin measurements, blood was collected into serum-separator tubes and for ACTH measurements, blood was collected into tubes containing EDTA. All samples were immediately placed on wet ice until centrifugation at 4°C and 1,400 \(X\) g for 15 minutes. Plasma and serum were separated and stored at \(-20^\circ\text{C}\) until analysis.

Blood glucose concentration was measured immediately on site with a glucometer\textsuperscript{6} in accordance with the manufacturer’s instructions. Serum insulin concentration was measured by means of a solid-phase,
enzyme-labeled chemiluminescent immunometric assay. Plasma ACTH concentration was measured by use of a 2-site immunoradiometric assay previously used in horses and interpreted accordingly.

Echocardiograms were obtained by an experienced operator, who used a portable ultrasonographic device equipped with a 2- to 4-MHz multifrequency sectorial transducer. A single-lead ECG was recorded simultaneously. The mean of 3 nonconsecutive cardiac cycles was obtained to measure all echocardiographic variables. All measurements were performed offline from digitally stored recordings by use of electronic calipers embedded in the ultrasonographic device. The examiner was blinded to pony group when performing measurements. Standard right and left parasternal views were used to obtain 2-D, M-mode, and color Doppler images and assess cardiac echogenicity, size, function, and valvular competence. Two-dimensional variables included pulmonary artery diameter and aortic root diameter measured from right parasternal windows and left atrial size measured from a standard left parasternal window. M-mode measurements, including left ventricular internal diameter during diastole and systole, interventricular septum thickness during diastole and systole, and left ventricular free wall thickness during diastole and systole, were obtained from right parasternal short-axis views of the left ventricle at the level of the chordal attachments. Fractional shortening, MWT, RWT, and LVM were calculated by use of previously reported equations. Values for RWT were compared with reported ranges for horses.

An oscillometric monitor, with the cuff centered over the coccygeal artery around the base of the unclipped tail, was used to noninvasively measure BP. A bladder width-to-tail girth ratio of 0.4 to 0.6 was used in accordance with the instructions of the monitor manufacturer. The BP values were corrected to heart level by multiplying the vertical distance (cm) between the point of the shoulder and the base of the tail by 0.77 mm Hg/cm. The first measurement was discarded, and the mean of 5 consecutive readings was calculated. Heart rate was obtained from the oscillometric device.

Exercising and 24-hour continuous ECGs were recorded by use of a digital telemetry unit with a sampling rate of 500 Hz and self-adhesive electrodes in a modified base-apex configuration. Recordings were digitally stored. A light exercise test was performed, consisting of consecutive 5 minutes of walking, 10 minutes of trotting, 5 minutes of cantering, and 5 minutes of walking on a lunge line, or alternatively, ponies were free lunged or driven at analogous intensities. Exercise tests were modified or not performed if the pony had signs of lameness or had a recent exacerbation of laminitis or when ground conditions were considered unsuitable. During the 24-hour recording, ponies were able to move freely in their stall or paddock and housing and feeding routines were not altered.

Software for ECG analysis was used, and the rhythm was read manually and with the aid of electronic calipers. Arrhythmias were recorded. An SVPC was defined as a complex for which the R-R interval decreased by > 10% (exercise) and > 20% (rest) relative to the previous R-R interval and for which there was no morphological change in the QRS complex. If a complex met this definition but occurred during prominent waxing and waning of the sinus rate, it was classified as sinus arrhythmia. A VPC was defined as a complex for which the R-R interval decreased by > 10% (exercise) and > 20% (rest) relative to the previous R-R interval and for which the QRS complex was obviously abnormal, compared with the previous sinus QRS complex. If a complex had abnormal QRS morphology but did not meet criteria for prematurity, it was classified as idioventricular.

The presence or absence of sinus arrhythmia, defined as continuous and variable decreases and increases of R-R intervals over time, was recorded. Exercise recordings for which < 80% of the rhythm was readable or resting recordings for which < 8 hours were readable were excluded from statistical analysis. The number of abnormal complexes occurring during the 24-hour ECG was reported as the mean number of complexes per hour.

The period between 12:00 am and 4:00 am was used for HRV analysis. Data for ponies during this period that could not be read because of persistent artifact or signal loss were excluded from analysis. The R-R intervals were exported into HRV software. Artifacts, VPCs, SVPCs, second-degree atrioventricular block, and sinus blocks were deleted. Data were included only if ≥ 95% of the original 4-hour segment remained. Time domain variables included SD of RR-intervals and root mean square of successive differences. Frequency domain variables (obtained by means of fast Fourier transformation) included low-frequency power, high-frequency power expressed as normalized units (percentage of the total power), and LF:HF. Frequency for determination of low-frequency power was set at 0.005 to 0.07 Hz, and frequency for determination of high-frequency power was set at 0.07 to 0.6 Hz, as previously described. Nonlinear variables included SDs of the points in the Poincaré plot perpendicular to and along the line of identity. Splenic volume was calculated from transcutaneous measurements obtained with a portable ultrasonographic device equipped with a 3- to 7-MHz large convex array transducer as described elsewhere.

Statistical analysis
Because of the large proportion of Shetland Ponies in the study, 2 sets of statistical analyses were performed: one for all ponies and another for Shetland Ponies alone (because these were considered a more homogeneous group). Statistical software was used for all analyses. The Shapiro-Wilk test was used to determine whether the data were normally distributed. Values of continuous variables were com-
pared between case and control groups by use of the Student t test (for parametric data) or Mann-Whitney U test (for nonparametric data). Binary-coded data were compared between groups by use of the 2-sided Fisher exact test. For cardiovascular variables that differed significantly ($P < 0.05$) between groups, correlations with plausible metabolic mechanism underlying the disease were tested by computation of the Pearson product correlation (r) or Spearman correlation (ρ). Values for MWT were normalized to body weight for this analysis.

**Results**

**Animals**

Nineteen ponies or pony crosses met the criteria for inclusion in the case group containing ponies with EMS; 20 ponies were enrolled in the control group containing healthy ponies. The case group consisted of 13 mares and 6 geldings. Mean ± SD age was 15.1 ± 5.8 years, and median body weight was 225 kg (495 lb; IQR, 197 to 277 kg [435 to 609 lb]). Breeds in the case group included Shetland Pony (n = 13), Welsh Pony (4), fjord-Arabian cross (1), and Connemara Pony (1). The control group consisted of 9 mares, 10 geldings, and 1 stallion. Mean ± SD age was 14.1 ± 5.5 years, and median body weight was 237.5 kg (523 lb; IQR, 126.8 to 287.5 kg [279 to 633 lb]). Breeds in the control group included Shetland Pony (n = 14), Welsh Pony (5), and North American Sport Pony (1). No differences were identified between groups in age, breed, sex, or body weight.

**Measurements**

The case and control groups differed significantly in body condition score, crest neck score, post-OST blood glucose concentration, and pre- and post-OST serum insulin concentration (Table 1). Findings were similar when considering just the Shetland Ponies in both groups.

Echocardiograms were obtained for 38 ponies. Diagnostic images could not be obtained for 1 Shetland Pony in the case group because of large subcutaneous fat deposits. Values for MWT of the left ventricle differed significantly between case and control groups for all ponies ($P = 0.04$) and for Shetland Ponies alone ($P = 0.02$; Table 2). Values for RWT differed significantly ($P = 0.03$) between groups for Shetland Ponies alone. No difference was identified between groups in LVM values. Five case ponies and 2 control ponies had RWT values outside of the reference range. None of the ponies had more than mild valvular regurgitation.

Resting HR was significantly ($P = 0.01$) greater in case ponies than in control ponies (Table 3). Seven of the 19 (37%) case ponies and 3 of the 20 (15%) control ponies in the control group had an HR > 48 beats/min. Systolic ($P = 0.03$) and mean ($P = 0.02$) BP was significantly greater in Shetland Ponies in the case group than in those in the control group. Only 1 pony (in the case group) had BP values that exceeded upper reference limits.19

Exercising (18 case and 20 control ponies) and continuous (19 case and 19 control ponies) ECGs were obtained for 38 ponies. Two ponies (1 case

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**Table 1**—Median (IQR) values of various measurements of privately owned ponies with EMS and healthy control ponies and of Shetland Ponies specifically.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All ponies</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Case</td>
<td>P value</td>
<td>Control</td>
<td>Case</td>
</tr>
<tr>
<td></td>
<td>group (n = 20)</td>
<td>group (n = 19)</td>
<td></td>
<td>group (n = 14)</td>
<td>group (n = 13)</td>
</tr>
<tr>
<td>Body condition score</td>
<td>6 (6–7)</td>
<td>7 (7–7)</td>
<td>&lt; 0.001</td>
<td>6.25 (6–7)</td>
<td>7 (7–7)</td>
</tr>
<tr>
<td>Cresty neck score</td>
<td>2 (2–3)</td>
<td>3 (3–3)</td>
<td>&lt; 0.001</td>
<td>2 (2–3)</td>
<td>3 (3–3)</td>
</tr>
<tr>
<td>Pre-OST glucose (mmol/L)</td>
<td>3.9 (3.6–4.2)</td>
<td>3.8 (3.3–4.4)</td>
<td>0.97</td>
<td>3.8 (3.7–4.1)</td>
<td>3.7 (3.3–4.5)</td>
</tr>
<tr>
<td>Pre-OST insulin (µU/mL)</td>
<td>5.0 (4.6–6.0)</td>
<td>6.1 (5.6–6.8)</td>
<td>0.001</td>
<td>5.0 (4.6–6.1)</td>
<td>6.3 (5.5–7.1)</td>
</tr>
<tr>
<td>Pre-OST insulin (µU/mL)</td>
<td>2.0 (2.0–3.1)</td>
<td>10.5 (3.8–51.7)</td>
<td>&lt; 0.001</td>
<td>2.0 (2.0–2.9)</td>
<td>21.3 (3.3–79.8)</td>
</tr>
<tr>
<td>Post-OST insulin (µU/mL)</td>
<td>8.8 (3.8–25.0)</td>
<td>145.0 (89.9–252.0)</td>
<td>&lt; 0.001</td>
<td>12.2 (4.0–35.1)</td>
<td>201.0 (92.2–259.5)</td>
</tr>
</tbody>
</table>

| Values for glucose represent blood concentrations; values for insulin represent serum concentrations. Values of P < 0.05 were considered significant. Ponies were included in the EMS group if they had a body condition score ≥7/9 and a cresty neck score ≥ 3/5 (crest of the neck enlarged and thickened to crest large and drooping to 1 side).17 |

<table>
<thead>
<tr>
<th>Variable</th>
<th>All ponies</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Case</td>
<td>P value</td>
<td>Control</td>
<td>Case</td>
</tr>
<tr>
<td></td>
<td>group (n = 20)</td>
<td>group (n = 18)</td>
<td></td>
<td>group (n = 14)</td>
<td>group (n = 12)</td>
</tr>
<tr>
<td>MWT (cm)</td>
<td>1.8 (1.5–2.0)</td>
<td>2.0 (1.8–2.2)</td>
<td>0.04</td>
<td>1.5 (1.4–1.9)</td>
<td>1.9 (1.8–2.1)</td>
</tr>
<tr>
<td>RWT (g)</td>
<td>0.48 ± 0.05</td>
<td>0.51 ± 0.06</td>
<td>0.06</td>
<td>0.49 ± 0.05</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>966.6 (582.3–1379.0)</td>
<td>1,083.1 (936.7–1,667.8)</td>
<td>0.24</td>
<td>659.1 (448.0–1,320.9)</td>
<td>1,039 (878.0–1,092.7)</td>
</tr>
</tbody>
</table>

Nonparametric data (MWT and LVM) are reported as median (IQR). Parametric data (RWT) are reported as mean ± SD.

See Table 1 for remainder of key.
and 1 control pony) were driven, and all others were lunged or exercised in hand. The exercise protocol was modified for 20 ponies (12 case and 8 control ponies) because of behavioral issues or surface conditions (n = 12) or lameness (8), and 5 recordings were excluded because of poor quality recording.

No difference was identified in the median number of premature complexes detected during exercise between control ponies (0 [range, 0 to 7]) and case ponies (0 [range, 0 to 105]). Four ponies (3 case ponies and 1 control pony) had arrhythmias during exercise. Arrhythmias consisted of ventricular ectopy in 2 case ponies, with 2 single VPCs in the first pony and complex ventricular ectopy consisting of 65 VPCs (including couplets and triplets) and idioventricular beats (n = 40) in the second pony. Fourteen SVPCs were detected in 1 case pony, and 7 SVPCs were detected in 1 control pony. No significant differences were identified between groups in the number of premature complexes during 24-hour continuous recordings (Table 4). Ten of 31 (32%) ponies (5 case and 5 control ponies) had >1 SVPC or VPC/h during Holter ECG monitoring. Six continuous ECG recordings (representing 5 control ponies and 1 case pony) were excluded because of poor quality.

No significant differences in HRV variables and HR derived from the continuous ECG recording were identified between groups (Table 5). Seventeen recordings (representing 10 control and 7 case ponies)

Table 3—Values for BP and HR at rest for the ponies in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All ponies</th>
<th>Shetland Ponies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n = 20)</td>
<td>Case group (n = 19)</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>119.2 ± 14.4</td>
<td>127.2 ± 16.0</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>69.6 (63.7–81.9)</td>
<td>77.3 (73.7–82.8)</td>
</tr>
<tr>
<td>Mean BP (mm Hg)</td>
<td>88.6 (80.0–103.1)</td>
<td>96.3 (89.7–98.9)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>38.6 ± 6.8</td>
<td>44.5 ± 7.5</td>
</tr>
</tbody>
</table>

Nonparametric data (diastolic BP and mean BP) are reported as median (IQR). Parametric data (systolic BP and HR) are reported as mean ± SD.

Table 4—Median (range) number of various type of arrhythmias per hour derived from 24-hour continuous ECG recordings of the ponies in Table 1.

<table>
<thead>
<tr>
<th>Arrhythmia type</th>
<th>All ponies</th>
<th>Shetland Ponies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n = 14)</td>
<td>Case group (n = 17)</td>
</tr>
<tr>
<td>SVPC</td>
<td>0.46 (0–9.21)</td>
<td>0.66 (0.05–3.96)</td>
</tr>
<tr>
<td>VPC</td>
<td>0 (0–2.27)</td>
<td>0 (0–0.21)</td>
</tr>
<tr>
<td>Second-degree atrioventricular block</td>
<td>0 (0–0.51)</td>
<td>0 (0–0.42)</td>
</tr>
<tr>
<td>Sinus block</td>
<td>0 (0–0.04)</td>
<td>0 (0–0.09)</td>
</tr>
</tbody>
</table>

See Table 1 for key.

Table 5—Values of variables indicative of HRV derived from continuous ECG recordings of the ponies in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All ponies</th>
<th>Shetland Ponies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group (n = 9)</td>
<td>Case group (n = 12)</td>
</tr>
<tr>
<td>HR* (beats/min)</td>
<td>41.1 ± 5.6</td>
<td>46.3 ± 8.5</td>
</tr>
<tr>
<td>SD of R-R intervals (ms)</td>
<td>202.1 (174.8–278.2)</td>
<td>194.5 (116.7–229.0)</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>73.1 (58.9–213.7)</td>
<td>102.6 (58.5–170.1)</td>
</tr>
<tr>
<td>Low-frequency power (%)</td>
<td>47.3 ± 11.0</td>
<td>46.1 ± 10.2</td>
</tr>
<tr>
<td>High-frequency power (%)</td>
<td>15.2 (8.6–32.6)</td>
<td>17.0 (12.3–27.0)</td>
</tr>
<tr>
<td>LF:HF</td>
<td>3.2 (1.4–6.4)</td>
<td>3.1 (1.7–3.9)</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>314.9 (151.1)</td>
<td>251.5 ± 107.6</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>314.9 ± 106.2</td>
<td>237.9 ± 122.1</td>
</tr>
</tbody>
</table>

Values for nonparametric data (SD of R-R intervals, RMSSD, high-frequency power, LF:HF, and SD1) are reported as median (IQR). Values of parametric data (HR, low-frequency power, and SD2) are reported as mean ± SD.

*Heart rate during a 4-hour interval was calculated by means of HRV software.

RMSSD = Root mean square of successive differences. SD1 = Standard deviation of the points in the Poincaré plot perpendicular to the line of identity. SD2 = Standard deviation of the points in the Poincaré plot along the line of identity. See Table 1 for remainder of key.
were excluded from HRV analysis because of insufficient data. No significant difference in median ultrasonographic splenic volume was identified between all ponies in the control (3.7 L [IQR, 2.3 to 5.2 L]) and case (3.6 L [range, 3.1 to 4.8 L]) groups (P = 0.54) or between Shetland Ponies in the control (3.2 L [range, 1.7 to 4.8 L]) and case (3.3 L [IQR, 2.8 to 4.0 L]) groups.

In the case group, significant correlations were identified between MWT and RWT and the plausible metabolic mechanisms involved in the increased MWT and RWT, post-OST serum insulin concentration, systolic and mean BP, and frequency domain HRV values. Mean wall thickness was positively correlated with systolic BP in all ponies (r = 0.54; P = 0.02) and in Shetland Ponies alone (p = 0.68; P = 0.02), mean BP in the Shetland Ponies alone (p = 0.63; P = 0.04), and LF:HF in all ponies (p = 0.66; P = 0.04) and negatively correlated with high-frequency power (p = –0.71; P = 0.02) in all ponies. Relative wall thickness was positively correlated with post-OST serum insulin concentration in all ponies (r = 0.71; P = 0.001) and in Shetland Ponies alone (r = 0.69; P = 0.01), systolic BP in Shetland Ponies alone (r = 0.66; P = 0.02), and mean BP in Shetland Ponies alone (r = 0.67; P = 0.02).

**Discussion**

The purpose of the present study was to explore the cardiovascular component of EMS in ponies to determine whether equine clinicians and researchers should consider this aspect of the syndrome. Ponies with EMS had significantly greater values than healthy control ponies for an echocardiographic variable associated with LVWT. Increases in left ventricular size can be separated in concentric remodeling, eccentric hypertrophy, or concentric hypertrophy, depending on RWT, LVM, or both being increased.32 Previous studies3,4,14 involving horses have revealed concentric hypertrophy associated with hypertensive cardiac disease or laminitis.

Ponies with EMS in the study reported here had a greater LVWT than healthy control ponies without an increase in LVM, fitting a definition of concentric remodeling. Interestingly, this finding corresponds with findings reported for humans with metabolic syndrome25 or humans with normotension or mild-to-moderate hypertension24 in which RWT, but not LVM, is correlated with serum or plasma insulin concentration. Activation of insulin-like growth factor 1 receptors by insulin promotes myocardial hypertrophy in rats,24 independent of sympathetic stimulation,36 and the correlation between markers of hypertrophy or remodeling and serum insulin concentration in ponies of the present study was interesting. Values for LVM need to be interpreted with caution because, to the authors’ knowledge, the applied formula has been validated only in Thoroughbreds.27

Hypertension and an increase in cardiac afterload represent the most intuitive mechanisms underlying left ventricular hypertrophy.28 The degree of hypertrophy is not correlated with a single blood pressure measurement in horses or humans with hypertensive cardiomyopathy41,29 but is proportionate to the area under the lifetime BP curve.30 In contrast, a single BP measurement was strongly correlated with variables associated with LVWT in ponies with EMS in the present study.

Seasonal arterial hypertension has been identified during the summer in ponies predisposed to laminitis.4 The present study was performed during the fall and winter, and a higher BP was observed in Shetland Ponies with EMS than in healthy Shetland Ponies. This effect may have been detected in only the Shetland Ponies because of a different BP response or different interaction with other cardiovascular, neural, or endocrine factors in this breed versus others.31 Alternatively, it may have reflected the fact that less variable data in a more homogeneous population allowed the detection of these differences. Small changes in BP may be difficult to detect because of the limited accuracy and precision of noninvasive BP monitors in standing horses8 or may be missed because of the lower sensitivity and specificity of such devices, compared with the sensitivity and specificity of ambulatory blood pressure monitors used in human medicine.32

Left ventricular hypertrophy is associated with an increase in sympathetic activity in humans33 and normotensive dogs.34 The ANS has a constant influence on instantaneous HR, and HRV analysis in the frequency domain allows differentiation between the distributions of sympathetic and parasympathetic activity and has been used in horses.35,36 High-frequency power is commonly accepted as a marker for parasympathetic activity, and LF:HF is suggested to be a marker of sympathetic tone in humans, although some controversy exists.37 The negative correlation of MWT with high-frequency power and positive correlation with LF:HF in the study reported here suggested an association between an increased sympathetic and decreased parasympathetic tone with the thickening of the left ventricular wall in ponies with EMS similar to that reported for humans with the condition.

Heart rate increases in humans with metabolic syndrome12 and is a marker of sympathetic activity58 and predictor of future hypertension59 in humans. In the present study, HR was higher in ponies with EMS than in healthy ponies, and a large proportion of ponies with EMS had sinus tachycardia. The reason for the discrepancy between the results of the HR obtained during BP measurements and the HR obtained during continuous ECG recordings remains unclear. The raw data obtained with both methods is similar and parallel, and perhaps the smaller sample size of ECG recordings prevented the detection of differences.

Autonomic nervous system imbalances, sympathetic hyperactivity, and parasympathetic underactivity play a major role in the development and maintenance of hypertension in humans.11 Chronic pain and an increase in sympathetic tone have been proposed as the main factors causing hypertension in hypertensive, chronically laminitic horses41. The release of
potent vasoconstrictive factors such as serotonin and endothelin-1 has been demonstrated in horses with laminitis, and the role of insulin as an important mediator in the orchestration of vasoactive proteins, the association of insulin dysregulation with endothelial dysfunction, and a potential increase in peripheral vascular resistance have been proposed. 

The relative importance of pain, autonomic dysregulation, or cardiovascular changes associated with insulin dysregulation in the modulation of BP in ponies with EMS cannot be determined with the methods used in the present study. Caloric restriction suppresses sympathetic tone in rodents, and exercise and diet improve autonomic balance in humans. In addition, research is ongoing in the field of sympathomodulatory pharmaceuticals, with promising results. The effects of different therapeutic interventions in horses and the reversibility of the cardiovascular changes in the ponies of the present study would be interesting research topics.

The correlations between the echocardiographic values, BP, serum insulin concentration, and HRV in the present study provided plausible explanations for the left ventricular hypertrophy and remodeling in ponies with EMS. However, the interactions of BP, insulin resistance, ANS activity, and cardiac hypertrophy in humans with metabolic syndrome are complex, and this is likely the case for EMS. Association and causality are not synonymous, and additional studies are needed to elucidate the interrelationships between these and other factors and their causative and temporary role in the initiation, progression, and consequences of cardiac morphological changes in horses with EMS.

Reported sequelae of increased LVWT and hypertensive cardiomyopathy in humans include hypertension, atrial fibrillation, ventricular arrhythmias, diastolic and systolic dysfunction, thrombotic and ischemic episodes, coronary and small vessel disease, and sudden cardiac death. Little information exists regarding the consequences of hypertensive cardiomyopathy in horses, and to the authors’ knowledge, no information exists regarding the long-term effects. It may be interesting to determine whether the long-term consequences reported for humans apply to horses as well. The proarrhythmic effects of fibrosis or increase in myocardial oxygen demand in these horses may be particularly relevant in this species because of the common use of horse as athletes.

Although no differences were identified between groups in the frequency of resting or exercising arrhythmias were detected between healthy ponies and ponies with EMS in the present study, the findings need to be interpreted with caution because of the small sample size. Interestingly, the frequency of premature complexes in both groups of horses was higher than previously reported, with 10 of 31 (32%) ponies (case and control ponies) having ≥1 SVPC or VPC/h during Holter ECG monitoring. Four ponies (3 case ponies and 1 control pony) had ectopy detected during exercise tests. Although the presence of complex ventricular arrhythmias in 1 pony and frequent SVPCs in another pony during exercise could have been coincidental, additional studies with larger sample sizes would be necessary to draw conclusions about arrhythmogenesis in ponies with EMS.

In horses, the spleen stores a large volume of RBCs that can be mobilized into the bloodstream on contraction of the splenic capsule. The tone of the splenic capsule has been proposed to reflect the tone of the capacitance vasculature, which makes splenic size an interesting noninvasive target for assessment of autonomic input. No difference in ultrasonographic splenic volume was identified between groups in the present study, and this potential marker of sympathetic tone was not useful in the evaluation of the ANS in ponies with EMS.

The preliminary study reported here had several limitations, including the variability and implications of using privately owned ponies with naturally occurring disease and the limited sample size. The intensity of the exercise tests was rather mild and heterogeneous, given that the tests needed to be adapted to training level or behavior of the ponies, presence of lameness, and weather, housing, and management conditions, and these factors could have affected the results. However, exercise tests were modified in a similar number of ponies in each group. Included ponies had different housing conditions, diets, degrees of pasture or paddock access, and other exposures, which introduced variability and confounding factors into the analysis of continuous ECG recordings and HRV. Altering the routines of the ponies could have affected results, so we chose instead to record ECGs in routine housing conditions for each pony.

No differences were identified between groups in the proportion of ponies of different sexes, and a larger number of ponies of both sexes would be necessary to investigate associated differences in HRV, which have been demonstrated in humans with metabolic syndrome. High interindividual variability, particularly in HRV variables, resulted in limited statistical power to detect some differences between groups. As an example, for SD of R-R intervals, taking into account the observed variability, a sample size of 83 ponies/group would have been needed to achieve a power of 80% (α = 0.05; 2-sided test). The variability for many variables was larger than anticipated, and recruitment of additional ponies was not possible. The results should therefore be interpreted within the aforementioned limitations and considered preliminary; however, they support the supposition that the cardiovascular system is involved in EMS in ponies. Interestingly, body condition score did not differ significantly between pony groups, and this might have been attributable to breed predisposition to obesity and matching of management and housing conditions whenever possible.

Ponies with EMS in the present study had been suspected to have EMS or had a diagnosis of this condition for variable periods. This and the aforementioned management and housing conditions could
have affected progression of the EMS and many of the measured variables. Effort was made to exclude ponies with severe or recently developed laminitis, but nevertheless, the severity of the laminitis and presence of previous or current signs of pain could have affected the results independently of the effects of EMS. This effort indirectly led to a study sample that had overall mild clinical signs of EMS. The results do not allow conclusions regarding whether cardiovascular disease should be a concern for clinicians evaluating ponies with EMS. Studies with more controlled and homogeneous groups of horses with different stages of EMS and receiving different treatments may be helpful to improve our understanding of the cardiovascular component of EMS and to better understand the comparative aspects of this syndrome between horses and humans.

Regardless of any limitations, the study reported here provided evidence of subclinical cardiovascular changes in ponies with EMS, including an increase in HR and echocardiographic left ventricular hypertrophy or remodeling in all ponies and an increase in BP in Shetland ponies only. Variables associated with left ventricular hypertrophy were correlated with insulin response to an OST, degree of sympathetic and lower parasympathetic tone, and BP. No evidence of the severe cardiovascular changes that are commonly identified in humans with metabolic syndrome was detected in the study ponies.

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Footnotes


b. Contour, Bayer Inc, Mississauga, ON, Canada.


d. Siemens Healthcare, Gwynedd, Wales.

e. IRMA No. IM2050, Beckman Coulter, Nyon, Switzerland.

f. Logiq e GE Ultraschall, Solingen, Germany.

g. Cardell Veterinary Monitor 9402, CAS Medical Systems, Branford, Conn.

h. Televet 100, KRUUSE A/S, Marslev, Denmark.

i. M2202A radio translucent foam monitoring electrodes, Philips Medizin Systeme, Boeblingen, Germany.

j. Televet 100, version 6.0.0, Engel Engineering Services GmbH, Heusenstamm, Germany.

k. Kubios HRV software, version 2.2, Biosignal Analysis and Medical Imaging Group, University of Kuopio, Joensuu, Finland.


m. NCSS, version 9, NCSS Statistical Software, Kaysville, Utah.


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