


Effect of a long-term high-energy diet on cardiovascular parameters in Shetland pony mares

Nicky M. M. D' Fonseca¹  | Martijn Beukers² | Inge D. Wijnberg¹ |
 Cristobal Navas de Solis³ | Marta de Ruijter-Villani¹ | David A. van Doorn^{1,4} |
 Tom A. E. Stout¹ | Ellen Roelfsema¹

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

²Division of Diagnostic Imaging, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

³Department of Clinical Studies, New Bolton Center, University of Pennsylvania, Philadelphia, PA, USA

⁴Division of Nutrition, Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Correspondence

Nicky M. M. D' Fonseca, Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 112, 3584 CM Utrecht, The Netherlands.
 Email: n.m.m.dfonseca@uu.nl

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Abstract

Background: Changes in cardiovascular parameters, including blood pressure (BP) and cardiac anatomical dimensions, are an inconsistent feature of the equine metabolic syndrome. The order in which these changes arise is unknown.

Objectives: Determine the order in which EMS-associated changes in cardiovascular parameters arise.

Animals: Twenty Shetland pony mares.

Methods: High-energy (HE) diet mares were fed 200% of net energy requirements for 1 (n = 3) or 2 (n = 7) consecutive diet-years, with 17 weeks of hay-only between years. Noninvasive BP measurements and echocardiograms were performed during both years. Resting 24-hour ECGs and measurements of autonomic tone (splenic volume and packed cell volume [PCV]) were performed at the end of diet-year 1. Results were compared to control mares receiving a maintenance diet for 1 (n = 7) or 2 (n = 3) consecutive years.

Results: In year 1, HE mares had significantly higher values than control mares for mean relative left ventricular wall thickness ($P = .001$). After 2 diet-years, mean systolic ($P = .003$), diastolic ($P < .001$) and mean arterial BP ($P = .001$), heart rate (HR; $P < .001$), and mean left ventricular wall thickness ($P = .001$) also were significantly increased in HE compared to control mares. No pathological arrhythmias or differences in splenic volume or PCV were detected.

Conclusions and Clinical Importance: Ingesting a HE diet first induced minor changes in BP, and progressed to left-sided cardiac hypertrophy in Shetland pony mares. These findings are of interest given the increasing incidence of obesity in horses.

KEYWORDS

arrhythmias, blood pressure, cardiac hypertrophy, obesity

Abbreviations: BCS, body condition score; DBP, diastolic blood pressure; HR, heart rate; LVM, left ventricular mass; MAP, mean arterial pressure; MWT, mean wall thickness; PCV, packed cell volume; RWT, relative wall thickness; SBP, systolic blood pressure

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1 | INTRODUCTION

Equine metabolic syndrome (EMS) is defined as a collection of clinicopathological changes predisposing to the development of endocrinopathic laminitis, with insulin dysregulation (ID) as the central feature.¹ Changes in cardiovascular parameters, such as blood pressure (BP), heart rate (HR), and cardiac anatomical dimensions are an inconsistent feature of EMS. In a case-control study, Shetland ponies with EMS had higher resting HR, higher systolic (SBP) and mean (MAP) arterial blood pressure, and increased left ventricular wall thickness, compared to healthy controls.² Another study showed that laminitis-prone ponies had significantly higher MAP during the summer period, compared to controls, when kept at pasture all year round.³ The chronological order in which the EMS-associated changes in cardiovascular parameters arise, and the relationship to the development of obesity and ID, however, are unknown.

Insulin has cardiovascular regulating properties and can act both as a vasodilator, by stimulating endothelium-derived nitric oxide, and as a vasoconstrictor by triggering release of endothelin-1.^{4,5} Moreover, insulin stimulates the renin-angiotensin-aldosterone system, inducing increased renal sodium reabsorption and blood volume expansion.^{6,7} There are indications that the vasodilator pathway is suppressed during insulin resistant (IR) states, whereas the vasoconstrictor pathway is intact or heightened,^{5,8-10} leading to diminished variability in the BP response because of limited vasodilatory capacity, as illustrated by the effect of insulin infusion on BP during an euglycemic hyperinsulinemic clamp (EHC) in IR horses.^{11,12} Systolic, diastolic (DBP), and mean arterial blood pressure of control horses decreased gradually during the EHC, whereas obese EMS-horses experienced no decrease¹¹ and normal weight IR horses experienced less decrease than did controls,¹² indicating a decreased vasodilatory response in IR horses.

Increased BP can lead to left ventricular hypertrophy (LVH) because of pressure overload.^{2,13} In people, LVH, whether defined by wall thickness or left ventricular mass (LVM), is associated with a higher frequency of, and more complex, ventricular arrhythmias in the absence of coronary artery disease.¹⁴ Horses with EMS-associated LVH therefore may have a higher risk of developing arrhythmias.

Our aim was to determine the chronological order in which EMS-associated changes in cardiovascular parameters, specifically BP, HR, cardiac dimensions, and heart rhythm, arise using long-term overfeeding. Ultrasonographic splenic volume and packed cell volume (PCV) were measured as markers of increased sympathetic tone,² which seems to play a role in the pathophysiology of metabolic syndrome in humans.¹⁵ It was hypothesized that, by providing a high-energy (HE) diet that would gradually induce ID and obesity, an increase in BP would first be induced, followed by LVH and eventually development of cardiac arrhythmias. Splenic volume was expected to decrease and PCV to increase as a result of relative sympathetic dominance.

2 | MATERIALS AND METHODS

2.1 | Horses and husbandry

The study was performed between February 2014 and October 2016. Twenty Shetland pony mares (aged 3-9 years; body condition score [BCS] of 4-7/9¹⁶; starting weight of 167 ± 23 kg) were monitored over a 3-year period. This breed was chosen because of its genetic predisposition to ID.¹⁷ Mares were assigned to either a control (n = 10) or HE (n = 10) group. Only mares were included in the study, because the study was part of a larger study examining the effects of HE diet provision on the epigenetic signature of embryos. Over the 3 study years, mares participated during 1 or 2 consecutive diet years, either starting in study year 2014 or 2015 (see Table 1). The experimental diet was fed for 24 weeks in 2014, 36 weeks in 2015, and 34 weeks in 2016 (Figure 1). General health of all mares was assessed daily by monitoring HR, rectal temperature, and gait. The experimental protocol was approved by the Committee on Animal Welfare of Utrecht University, the Netherlands (ethical committee approval No. 2014.III.02.021).

The study was initiated in 2014 as a pilot study. During this first year, BP and echocardiographic measurements, 24-hour ECGs, and measurements of splenic volume and PCV were performed. On the basis of results during 2014, only BP and echocardiographic measurements were continued in study years 2015 and 2016, during which the composition of the control and HE groups changed because of the removal or addition of ponies. Because the changes seen followed repeatable patterns, the results of the 3 years were combined and compared, based on the number of weeks that the diet was fed. The composition of the control and HE groups is described in detail, per study parameter, in the sections below.

The ID status was assessed by periodic oral glucose tolerance testing (OGTT) and, for 7 of the 10 HE mares, was reported in a previous study.¹⁸ In 2014, insulin concentrations were measured using a commercial radioimmunoassay (RIA). Because the RIA kit was no longer available in 2015 and 2016, insulin concentrations instead were determined using a chemiluminescence immunometric assay (CLIA), as reported previously.¹⁸ Therefore, absolute values are not directly comparable between study years 2014 and 2015-2016. The HE mares became ID after 10-12 weeks of overfeeding in study year 2014 (OGTT peak plasma insulin concentration range of 10-84 mU/L at the start, and 10-332 mU/L at the end of the HE diet period; significantly higher area under the curve [AUC] for insulin in the HE compared to the control group). The HE mares reversed their ID during the winter of 2014-2015, but became ID again after 9-13 diet weeks in study year 2015 (peak insulin concentrations of 18.6-61.3 mU/L at the start and 51.6-262 mU/L at the end of the diet year; significantly higher AUC for insulin). The 3 remaining HE mares, introduced in 2015, were not described in the previous study. The OGTT peak plasma insulin concentrations of these 3 mares ranged from 2 to 10.8 mU/L at the start and 7.7 to 9.3 mU/L at the end of study year 2015. These 3 HE mares were

TABLE 1 The time periods that individual ponies (A-T) were present divided over different study years (filled box). Empty boxes indicate absence in the corresponding period.

Group	Pony	2014					2015					2016							
		P0	P1	P2	P3	P4	P5	P0	P1	P2	P3	P4	P5	P0	P1	P2	P3	P4	P5
C	ABC				✓	✓	✓	✓	✓	✓	✓	✓	✓						
	D				✓	✓	✓												
	EFG							✓	✓	✓	✓	✓	✓						
	HIJ													✓	✓	✓	✓		
HE	KLMN				✓	✓	✓	✓	✓	✓	✓	✓	✓						
	OPQ				✓	✓	✓												
	RST							✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	

Note: Blood pressure measurements that were performed in the control and high-energy (HE) groups during study years 2014-2016 in five consecutive periods (P0-5) are indicated by a check mark.

Abbreviations: P0, diet week 0; P1, diet weeks 1-5; P2, diet weeks 6-10; P3, diet weeks 11-15; P4, diet weeks 16-20; P5, diet weeks 21-25.

**FIGURE 1** Study years 2014-2016 with the corresponding number of weeks that the diet was fed, and the corresponding months (starting in May 2014) below the figure

followed up in study year 2016, during which their OGTT peak plasma insulin concentrations ranged from 2 to 108 mU/L at the start and 9.9 to 256 mU/L at the end of the diet year (ie, 1 HE mare became ID during study year 2016¹⁹).

2.2 | Husbandry

During 2014, control ponies were housed as a group but fed individually, and HE ponies were housed and fed individually. During 2015 and 2016, all ponies were housed and fed individually. All groups were bedded on wood shavings. In the winter between the experimental diet provision periods of 2014-2015 and 2015-2016, measurements ceased because of the underlying (principally reproductive) aims of the study (mares were in anestrus). During these winter periods, all mares were housed together as a single group on straw and fed only hay ad libitum. Groups were allowed access to a sand paddock every other day to enable social contact and limited exercise throughout the study.

2.3 | Diet

The composition of the diet has been previously described.²⁰ In brief, the diet of both control and HE mares consisted of a concentrate feed (36% sugar and starch, 13% fat), grass hay (9% sugar, 2% fat), and 30 g of a feed supplement to ensure adequate provision of minerals,

trace elements, and vitamins (Pavo Vital Complete; Pavo, Boxmeer, the Netherlands). Hay and concentrate were fed in multiple meals per day at 0800, 1300, and 1700 hours. Control mares were fed 100% of their daily net energy (NE) requirements (85% of NE intake as hay and 15% as concentrate) set by the Centraal Veevoederbureau,²¹ approximately $0.348 \text{ megajoule NE} \times \text{BW}^{0.75}$ to maintain moderate body condition throughout the study. High-energy mares were fed 200% of NE requirements (42.5% of NE intake as hay and 57.7% as concentrate) to induce weight gain, as previously described²² but with minor modifications. Energy intake was adapted to weight gain throughout the study to maintain a 200% NE intake. During the winter periods, all mares were fed a hay-only diet ad libitum. All mares had free access to water and a salt lick (KNZ; Hengelo, the Netherlands).

2.4 | Body weight and body condition score

The BCS was evaluated for all ponies at the start and end of each study year, using a 9-point scale.¹⁶ All ponies were weighed weekly using a calibrated weighing scale (Epelsa BCN100M: Grupo Epelsa, Madrid, Spain).

2.5 | Blood pressure measurements

Blood pressure measurements were performed once a week on Thursdays between 1300 and 1500 hours, and are presented as

means of 5-week periods (P). The first and second diet years of mares are indicated by the subscripts _I (first diet year) and _{II} (second diet year). Measurements were performed during the acclimatization week (PO; ie, 1 week in which the HE diet was increased incrementally to the full ration) and in 5 periods of 5 weeks each (P1-5; Table 1). Blood pressure measurements were performed, using an oscillometric monitor (Cardell Veterinary Vital Signs Monitor Model 9402, Midmark Corporation, Versailles, Ohio, US) with a cuff placed on the tail (coccygeal artery), measuring SBP and DBP. To decrease stress, BP in all ponies was measured in their own stables while they were eating hay and concentrate. For each pony, tail girth was calculated using the thickness (diameter) of the tail base (tail girth = $2\pi r$). Depending on the width of the tail, a matching large (SV10: bladder width, 10.2 cm) or small (SV8: bladder width, 8 cm) cuff was applied, using a bladder width/tail girth (BW/TG) ratio of 0.4 to 0.6 in accordance with the instructions of the monitor manufacturer. Each BP recording was repeated 5 times per pony, with 1 minute in between to allow restoration of blood flow. The highest and lowest values of the 5 measurements were left out of the analysis. Results only were accepted if the difference among the 3 remaining SBP results was <5 mm Hg. The 3 remaining results were converted to a closer approximation of the true arterial pressure by first adjusting results to the bladder width/tail girth ratio (CUBTAV) using the following formulas according to a previously described method²³:

$$\text{Systolic pressure CUBTAV} = \frac{\text{Systolic pressure}}{\left(0.703 + \frac{0.101}{\left[\frac{\text{BW}}{\text{TG}}\right]}\right)},$$

$$\text{Diastolic pressure CUBTAV} = \frac{\text{Diastolic pressure}}{\left(0.916 + \frac{0.083}{\left[\frac{\text{BW}}{\text{TG}}\right]}\right)}.$$

Next, results were corrected to heart level (CCBTAV), using the vertical distance from the level of the ventral surface of the tail base to the notch of the left humeral tuberosity (CorSh) and olecranon (CorE) using the following formula:

$$\text{CCBTAV} = \text{CUBTAV} + \left(\frac{\text{CorSh} + \text{CorE}}{2}\right) \times 0.77.$$

The MAP was calculated using the SBP and DBP results corrected to heart level using the following formula:

$$\text{MAP} = \text{DBP} + \frac{1}{3}(\text{SBP} - \text{DBP}).$$

Heart rate was obtained from the oscillometric monitor. The mean of 3 results was calculated per week for each parameter, including 5 separate measurements per 5-week period in the final analysis.

The periods in which measurements were performed are shown in Table 1. Measurements for diet weeks 0-10 of study year 2014 are missing because the oscillometric monitor was not yet available.

Measurements for diet weeks 16-25 of study year 2016 are missing because of an outbreak of strangles.

2.6 | Echocardiographic examination

Echocardiographic measurements were performed after 0-4, 16, and 22-27 weeks of diet provision during the first year that mares were included in the study, which was either 2014 or 2015 (Table 2). Measurements were repeated in part of the group of mares that started the diet in 2014 after an additional 22-24 diet weeks in 2015 (Table 2). Echocardiographic examinations were performed by a single board-certified radiologist on ponies at rest while stabled, using an ultrasound machine (HD11 XE, Philips, Eindhoven, the Netherlands) equipped with a 1-3 MHz phased array transducer. Standard transthoracic 2-dimensional, M-mode, and color Doppler were used to assess cardiac anatomical morphology, valvular competence, heart chamber dimensions, and left ventricular systolic function using standard right and left parasternal long- and short-axis views. Three nonconsecutive cardiac cycles were recorded, from which the mean of 3 measurements was calculated. To determine changes in cardiac dimensions and monitor development of cardiac hypertrophy, mean wall thickness (MWT), relative left ventricular wall thickness (RWT), and LVM were compared between control and HE mares, and within the control and HE groups over time. Left ventricular internal diameter in systole (LVIDs) and diastole (LVIDd), interventricular septum thickness in systole (IVSs) and diastole (IVSd), and LV free wall thickness in systole (LVFWs) and diastole (LVFWd) were measured by ultrasound, using M-mode measurements from a short-axis view of the ventricles at the level of the chordal attachments to calculate the MWT, RWT, and LVM using the following previously reported^{24,25} formulas:

TABLE 2 Time points at which echocardiographic measurements were performed in the control and high-energy (HE) groups during their first and second diet years

Dietary year	Group	Pony	First				Second
			Echo 0 Wk 0-4	Echo 1 Wk 16	Echo 2 Wk 22-27	Echo 3 Wk 22-24	
C		ABC	✓	✓	✓	✓	
		D	✓	✓	✓		
		EF	✓		✓		
		G			✓		
		Total	6	4	7	3	
HE		KLM	✓	✓	✓	✓	
		NO PQ	✓	✓	✓		
		RST	✓		✓		
		Total	10	7	10	3	

Note: Individuals are indicated by letters. A filled-in box with a check mark indicates that the echocardiographic measurement in that column was performed on the pony in the corresponding row (ie, empty boxes indicate absent data points).

$$\text{MWT (cm)} = \frac{\text{LVFWd} + \text{IVSd}}{2},$$

$$\text{RWT} = \frac{\text{LVFWd} + \text{IVSd}}{\text{LVIDd}},$$

$$\text{LVM (g)} = 1.04 \times \left([\text{LVIDd} + \text{LVFWd} + \text{IVSd}]^3 - \text{LVIDd}^3 \right) - 13.6.$$

2.7 | Twenty-four-hour ECGs

Resting 24-hour ECGs were performed at the end of study year 2014 (4 control mares [A-D] and 7 HE mares [K-Q]), immediately after concentrate feeding had stopped. Measurements were recorded continuously using a digital Holter monitor (Televet 100, Engel Engineering Services GmbH, Heusenstamm, Germany) manually assisted with auto-analysis, 8% deviation, and a 50-Hz filter. Mares were clipped over 2 areas on the left side of the thorax (caudal to the scapula ventral to the thoracic vertebrae and caudal to the olecranon) and 2 electrodes were placed on each clipped area, fixed using Snögg Animal Polster (upper 2 electrodes) or BioDerm Adhesive Mounts (lower 2 electrodes) and attached to the digital Holter monitor. A saddle blanket and girth were placed over the fixed electrodes, with the device attached to the girth. The ECGs were analyzed for the presence of physiological (sinus arrhythmia, sinoatrial [SA] block, atrioventricular [AV] block grades 1 and 2) and presence and frequency of occurrence of pathological (supraventricular extrasystole [SVES], ventricular extrasystole [VES], supraventricular tachycardia, ventricular tachycardia, atrial fibrillation or atrial flutter, and AV block grade 3) arrhythmias.

2.8 | Splenic volume and PCV

Splenic volume and PCV were measured at the end of study year 2014 (4 control mares [A-D] and 7 HE mares [K-Q]). Ultrasonographic splenic volumes were calculated using measurements obtained with a 3-7 MHz large convex array transducer on an ultrasound machine as described previously.²⁶ Splenic volume was calculated using trans-abdominal and transthoracic measurements as described previously.²⁶ Ultrasonographic measurements of the spleen included: maximal length (*L*), width (*W*), and maximal thickness (T_{max}) at the flank and at each intercostal space. The mean thickness (T_{mean}) was calculated as the sum of all of the T_{max} measurements divided by the number of measurements. The length from the cranioventral apex to the most dorsal aspect of the spleen (*D*) and twice the maximal distance from the line drawn to measure “*D*” to the silhouette of the spleen (*d*) were recorded. The following formula was used to calculate splenic volume:

$$\text{Splenic volume} = (0.524 \times W \times T_{\text{mean}} \times L) - (0.524 \times D \times d \times T_{\text{mean}}).$$

Blood samples were collected in 4-mL EDTA tubes and, per pony, 2 sodium heparinized micro-hematocrit tubes (Assistant, Glaswarenfabrik Karl Hecht GmbH & Co KG, Germany) were filled

and centrifuged using a microcentrifuge. The PCV was measured in duplicate using a micro-hematocrit reader (Hawksley, Sussex, UK).

2.9 | Statistical analysis

All data are grouped by the number of years that mares consumed the diet (year 1 of consuming the diet, year 2 of consuming the diet), regardless of their starting year (2014, 2015, or 2016). For BP and HR data, the mean of 3 results was calculated per week for each parameter, including 5 separate measurements per 5-week period in the final analysis. For all data, normal distribution of the residuals was confirmed by visual inspection of the Q-Q plot. All normally distributed data were presented as mean \pm SD. A natural log transformation was applied to non-normally distributed data to meet the assumption of normality (SBP and MAP measured during first and second diet year of mares, DBP measured during second diet year of mares), and data were presented as median (range) values. Statistical analysis for SBP, DBP, MAP, HR, RWT, MWT, and LVM was based on a linear mixed model with post hoc Bonferroni testing, using IBM SPSS Statistics 26. Fixed effects in this model were group, period, and their interaction (group \times period), with pony as a random effect, including a random intercept per pony to account for the dependence of outcomes from the same pony. Pairwise comparisons were performed to assess differences between groups, and a post hoc Bonferroni correction was applied by dividing the alpha of $P < .05$ by the number of pairwise comparisons. After Bonferroni correction, $P < .008$ was used to indicate a statistical difference between groups for the BP measurements. For echocardiographic measurements, $P < .01$ was used to indicate a statistical difference between groups, and $P < .006$ to indicate a statistical difference within groups. An independent samples *t* test was used to compare the ultrasonographically measured splenic volume and PCV between control and HE mares. For splenic volume and PCV measurements, values of $P < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Body weight and body condition score

Increases in BCS and body weight (BW) were calculated over the periods in which measurements were performed, although the periods of measurement differed in duration per year (year 2014, 23 weeks; year 2015, 25 weeks; year 2016, 15 weeks). Median (range) BCS of HE group ponies increased by 2 units from BCS 6 (4-7) to 8 (5-9) during their first diet year, and by 1 unit from BCS 8 (6-8) to 9 (8-9) during their second diet year. Mean BW of the HE mares increased by 27% from 164 ± 23 to 209 ± 31 kg during their first diet year, and by 19% from 207 ± 27 to 247 ± 32 kg during their second diet year. Median (range) BCS of the control group did

not change during their first diet year (BCS 5 [range, 3-7]) and decreased by 1 unit from BCS 6 (4-6) to 5 (4-5) during their second diet year. Mean BW of the control mares increased by 1% from 171 ± 23 to 173 ± 21 kg during their first diet year and decreased by 3% from 161 ± 23 to 157 ± 20 kg during their second diet year.

3.2 | Blood pressure measurements

3.2.1 | Group 1—1 diet year

Mean ± SD for SBP, DBP, MAP, and HR for the control and HE group are presented in Table 3. No significant group or group × period effects were found for SBP (respectively, $P = .17$; $P = .27$). For DBP and MAP, a significant group effect (respectively, $P = .004$; $P = .02$), but no significant group × period interaction (respectively, $P = .25$; $P = .16$) were found. For HR, significant group ($P = .02$) and group × period ($P = .003$) effects were found.

3.2.2 | Group 2—2 diet years

Mean ± SD for SBP, DBP, MAP, and HR for the control and HE group are presented in Table 4. Significant group and group × period effects were found for SBP (respectively, $P = .009$; $P = .003$), DBP ($P = .001$; $P = .01$), MAP ($P = .001$; $P = .001$), and HR ($P = .005$; $P < .001$), with higher values for HE than control mares in specific periods (Table 4).

3.3 | Echocardiographic examination

Minor clinically irrelevant valvular insufficiencies were found in both groups (aortic insufficiency, 1 control and 1 HE mare; mitral insufficiency, 2 control mares; pulmonary insufficiency, 1 control mare; tricuspid insufficiency, 2 HE mares). Mean values for LVFWd, IVSd, and LVIDD were within reference limits determined for small ponies (Welsh Mountain ponies²⁷ for both groups. Mean ± SD for MWT, RWT, and LVM for the control and HE groups are presented in Table 5. For MWT and RWT, significant group (respectively, $P = .05$;

TABLE 3 Median ± range systolic and mean arterial blood pressure and mean ± SD diastolic blood pressure and heart rate values for the 10 control (maintenance diet) and 10 high-energy (HE) mares (diet equating to 200% of net energy requirements) measured during the acclimatization week (P0_i) and in periods of 5 consecutive weeks (P1-5_i) during their first diet year (i)

Parameter	Period	Control group	HE group	P value
Systolic blood pressure (mm Hg; median [range])	P0 _i	119 (114-132)	124 (112-125)	-
	P1 _i	120 (102-156)	126 (115-142)	-
	P2 _i	120 (108-143)	124 (113-138)	-
	P3 _i	119 (101-144)	126 (103-153)	-
	P4 _i	118 (101-146)	124 (107-143)	-
	P5 _i	114 (101-145)	124 (105-143)	-
Diastolic blood pressure (mm Hg; mean ± SD)	P0 _i	63 ± 2	72 ± 9	-
	P1 _i	63 ± 8	71 ± 8	-
	P2 _i	61 ± 8	72 ± 7	-
	P3 _i	63 ± 8	69 ± 5	-
	P4 _i	62 ± 7	67 ± 4	-
	P5 _i	60 ± 7	68 ± 4	-
Mean arterial pressure (mm Hg; median [range])	P0 _i	84 (78-87)	92 (79-94)	-
	P1 _i	80 (68-112)	90 (78-98)	-
	P2 _i	79 (69-97)	89 (79-103)	-
	P3 _i	81 (67-103)	88 (73-108)	-
	P4 _i	81 (68-99)	86 (76-97)	-
	P5 _i	78 (66-98)	87 (75-96)	-
Heart rate (beats/min; mean ± SD)	P0 _i	42 ± 4	46 ± 6	.51
	P1 _i	42 ± 7	49 ± 7	.03
	P2 _i	42 ± 7	48 ± 4	.09
	P3 _i	44 ± 6	53 ± 6	.001*
	P4 _i	48 ± 8	53 ± 4	.04
	P5 _i	43 ± 8	51 ± 5	.01

*Indicates a significant difference between groups ($P < .008$).

TABLE 4 Median \pm range systolic, diastolic, and mean arterial blood pressure and mean \pm SD heart rate values for the 3 control (maintenance diet) and 7 high-energy (HE; diet equating to 200% of net energy requirements) mares measured during the acclimatization week (P0_{II}) and in periods of 5 consecutive weeks (P1-5_{II}) during their second diet year (II)

Parameter	Period	Control group	HE group	P value
Systolic blood pressure (mm Hg; median [range])	P0 _{II}	120 (119-137)	131 (120-143)	.38
	P1 _{II}	113 (101-127)	127 (109-154)	.01
	P2 _{II}	113 (104-127)	125 (113-141)	.05
	P3 _{II}	110 (100-124)	131 (106-164)	.003*
	P4 _{II}	106 (99-120)	134 (121-161)	.001*
	P5 _{II}	108 (96-129)	134 (116-146)	.003*
Diastolic blood pressure (mm Hg; median [range])	P0 _{II}	65 (64-70)	72 (64-84)	.12
	P1 _{II}	58 (51-65)	69 (58-80)	<.001*
	P2 _{II}	58 (52-66)	68 (60-78)	.004*
	P3 _{II}	59 (54-62)	70 (59-94)	<.001*
	P4 _{II}	55 (49-63)	70 (63-93)	<.001*
	P5 _{II}	55 (47-61)	70 (56-80)	<.001*
Mean arterial pressure (mm Hg; median [range])	P0 _{II}	86 (83-89)	91 (84-103)	.17
	P1 _{II}	76 (69-86)	89 (75-102)	.001*
	P2 _{II}	76 (69-84)	89 (78-98)	.007*
	P3 _{II}	77 (70-81)	91 (76-117)	<.001*
	P4 _{II}	72 (66-82)	91 (85-115)	<.001*
	P5 _{II}	73 (65-83)	90 (76-102)	<.001*
Heart rate (beats/min; mean \pm SD)	P0 _{II}	47 \pm 7	43 \pm 6	.21
	P1 _{II}	44 \pm 3	49 \pm 6	.03
	P2 _{II}	41 \pm 6	51 \pm 5	<.001*
	P3 _{II}	45 \pm 5	52 \pm 4	.003*
	P4 _{II}	40 \pm 5	52 \pm 3	<.001*
	P5 _{II}	43 \pm 7	51 \pm 5	.001*

*Indicates a significant difference between groups ($P < .008$).**TABLE 5** Mean \pm SD values for the mean (MWT) and relative (RWT) left ventricular wall thickness and left ventricular mass (LVM) for the control (maintenance diet) and high-energy (HE; diet equating to 200% net energy requirements) mares measured after 0-4 (Echo 0), 16 (Echo 1), and 22-27 (Echo 2) diet weeks during the first diet year, and after 22-24 (Echo 3) weeks during the second diet year

Parameter	Echo	Control group (mean \pm SD)	HE group (mean \pm SD)	P value (between groups)
MWT (cm)	0	1.66 \pm 0.16	1.64 \pm 0.17 ^a	.81
	1	1.60 \pm 0.22	1.64 \pm 0.09 ^a	.78
	2	1.52 \pm 0.16	1.68 \pm 0.14	.03
	3	1.51 \pm 0.16	1.94 \pm 0.15 ^b	.001*
RWT	0	0.49 \pm 0.046	0.50 \pm 0.049 ^c	.81
	1	0.47 \pm 0.027	0.51 \pm 0.030 ^c	.21
	2	0.45 \pm 0.04	0.54 \pm 0.056	<.001*
	3	0.47 \pm 0.018	0.62 \pm 0.030 ^d	<.001*
LVM (g)	0	734 \pm 150	694 \pm 151	-
	1	700 \pm 222	663 \pm 129	-
	2	644 \pm 123	674 \pm 152	-
	3	600 \pm 153	821 \pm 148	-

*Indicates a significant difference between groups ($P < .01$). Significant difference within groups ($P < .006$): ^{a,b} $P = .005$; ^{c,d} $P < .001$.

$P = .002$) and group \times period ($P = .002$; $P < .001$) effects were found, with higher values for HE than control mares in specific periods (Table 5). For LVM, no significant group ($P = .66$) or group \times period ($P = .07$) effects were found.

3.4 | Twenty-four-hour ECGs

No pathological arrhythmias were detected for control or HE group ponies. The majority of individuals showed physiological arrhythmias, with sinus arrhythmia being most common. Three mares showed some form of physiological block (AV block grade 2, 2 mares; SA block, 1 mare). Solitary SVES (1-9 times per 24 hours) were found in 3 of 7 HE mares vs 1 control mare but were not categorized as pathological arrhythmias based on frequency and timing.

3.5 | Splenic volume and PCV

For mean \pm SD splenic volume measured by ultrasonography, no significant difference ($P = .07$) was found between control (2.7 ± 1.3 L) and HE (4.8 ± 1.7 L) mares. Mean PCVs for both groups were within the range (0.24-0.48 g/dL) reported for clinically healthy Shetland ponies measured in summer.²⁸ No significant difference ($P = .27$) in mean \pm SD PCV was found between control (0.26 ± 0.014 g/dL) and HE (0.28 ± 0.037 g/dL) mares.

4 | DISCUSSION

Our aim was to determine whether EMS-associated changes in cardiovascular parameters arise in a predictable order in Shetland pony mares during a chronic overfeeding study, these included changes in BP, cardiac dimensions, and rhythm. We found that an increase in RWT, followed by an increase in BP, was induced first, without the development of clinical hypertension. Eventually, LVH developed within 2 years of overfeeding. No pathological arrhythmias at rest or differences in splenic volume or PCV were detected after 1 year of diet consumption.

Neither did 2 years of HE diet provision lead to the development of clinical hypertension in our study, because mean BP for the HE mares did not exceed upper reference limits reported for horses (clinically normal standing horse ranges: SBP, 86-159 mm Hg; DBP, 45-97 mm Hg; marginal hypertension: SBP, 147-159; DBP, 88-97 mm Hg).²³ Over time however mean SBP, DBP, and MAP became significantly higher in HE compared to control mares, indicating a diet-induced difference in BP between the groups. Our findings are similar to those of a previous study that suggested an inability of ID horses to undergo insulin-induced vasodilatation.¹¹ Inability to dilate blood vessels in response to insulin is assumed to be the result of a decrease in the vasodilatory (phosphatidylinositol 3-kinase) pathway during ID states, whereas the vasoconstrictor (mitogen-activated protein kinase) pathway is intact or enhanced,

leading to decreased endothelium-dependent vasodilatation.^{5,8-10} The intact or enhanced vasoconstrictor pathway can lead to an insulin-induced increase in peripheral resistance, potentially leading to an increase in BP.^{11,29} Seven of 10 HE mares in our study were ID and hyperinsulinemic after 10-12 weeks of overfeeding, as reported previously.¹⁸ Because mean and median SBP and DBP of the HE mares were approximately constant over time, whereas results for the control mares decreased during their second diet year by, respectively, 12 mm Hg (from median [range] 120 [119-137] to 108 [96-129] mm Hg) and 10 mm Hg (from 65 [64-70] to 55 [47-61] mm Hg), it appears that the control mares responded to the experimental conditions by a decrease in BP, whereas the HE mares did not. This observation may indicate that the blood vessels of ID mares are insensitive to stimuli that would normally lead to vasodilatation and, instead, a constant state of moderate vasoconstriction persists and maintains BP at high-normal levels. Similar findings were reported in another study, which documented vascular dysfunction in laminae vessels and facial skin arteries of horses with endocrinopathic laminitis associated with EMS or pituitary pars intermedia dysfunction.³⁰

Diastolic blood pressure of HE mares was significantly higher than that of controls after 1-5 weeks of consuming the HE diet during their second diet year and increased earlier than did systolic BP. This finding is in contrast to a previous study that reported a difference in SBP but no difference in DBP between control and overweight but not obese EMS Shetland ponies (BCS 7/9).² Potentially, the difference in BP between the control and HE mares in our study was not related to ID alone, and, in this respect, obesity could be a second influencing factor.¹¹ Indeed, a recent study reported that diet-induced obesity led to increased BP in healthy Standardbred mares, without affecting insulin sensitivity.³¹ Leptin, an adipocyte-derived hormone, has been proposed as the key factor in the relationship between obesity and hypertension, by increasing sympathetic nerve and renal activity leading to increased sodium retention and blood volume expansion.³²⁻³⁴ Another factor that has been proposed to contribute to the relationship between obesity and hypertension is angiotensinogen, which also is produced by adipocytes.³⁵ It is therefore possible that the difference in BP between the control and HE mares in our study was related to obesity alone or the combination of ID and obesity.

Resting HR was significantly higher in HE than control mares in our study, from approximately 6 to 10 weeks of overfeeding during the second diet year (P_{2,II}), which is comparable to previous findings.^{2,11} The significant difference in HR between groups at P₃ of the first diet year might indicate that HE mares already were undergoing an increase in HR during the first year of diet consumption. Mean HR results for the HE group however did not exceed upper limits reported for clinically healthy Shetland ponies in the summer (range, 30-54 beats/min).²⁸ The increase in HR for the HE mares therefore is assumed to be a response to an increase in sympathetic tone.³⁶

With respect to cardiac dimensions, mean RWT and MWT were significantly higher for HE than control mares, and significantly increased within the HE group over time, which is consistent with a previous study examining Shetland ponies.² The MWT and RWT in

the previous study were significantly higher for EMS than control ponies, whereas no difference in LVM was found between the groups; these findings were proposed to fit the definition of concentric remodeling.¹³ We therefore conclude that the HE mares in our study developed concentric hypertrophy, as evidenced by an increase in left ventricular wall thickness without dilatation of the ventricle. Values for LVIDd, IVSd, and LVFWd did not, however, exceed upper reference limits (range, 5.1-9.0 cm, 1.3-2.3 cm, and 1.3-2.6 cm, respectively) for small ponies,²⁷ which may indicate that the changes in cardiac dynamics were still within the range of physiological adaptations. However, because no reference values were available specifically for Shetland ponies, care must be taken when interpreting the absolute cardiac measurements in our study. If and when these EMS-associated changes in cardiac dynamics might become pathological is not yet clear.

No pathological arrhythmias were detected in the HE mares in our study, which is consistent with the absence of pathological cardiac adaptations, and consistent with previous findings.² Two years of HE diet provision therefore seems not to be related to cardiac pathology in our Shetland pony mare study group. No exercising ECGs were performed because of inability of the untrained ponies to be worked in a repeatable and consistent fashion, which made it impossible to draw conclusions about the function of the heart during exercise.

The equine spleen can store large volumes of red blood cells that can be released by splenic contraction in response to increased activation of the sympathetic nervous system, which seems to play a role in the pathophysiology of the metabolic syndrome in humans.¹⁵ In our study, no significant difference in ultrasonographic splenic volume was found between control and HE mares after 1 year of diet consumption, which might indicate that measurement of splenic volume is not a useful way of evaluating the autonomic nervous system in ponies with EMS.² However, measurements were only performed once, and thus no definitive conclusions can be drawn. The PCV did not differ between HE and control mares, which is in agreement with the absence of a difference in splenic size, because a strong correlation between PCV and splenic volume has been described previously.²⁶

Our study had some limitations. The number of ponies examined during the second diet year was small. Only mares were included because of the wider scope of the study, which made it impossible to exclude a sex effect. The noninvasive technique that was used for BP measurements is less reliable than invasive techniques and gives more heterogeneous results, which could have influenced our data.¹² In addition, BP measurements for diet week 0-10 of study year 2014 were missing because the oscillometric monitor was not yet available, and measurements for diet week 16-25 of study year 2016 were missing because of an outbreak of strangles, which could have influenced our data. During the second diet year, only 1 echocardiographic examination was performed after 22-24 diet weeks. Changes in MWT therefore could have developed earlier during that year, and gone undetected. Total plasma protein concentrations were not measured in our study, which may have affected accurate interpretation of changes in PCV.

In conclusion, 2 years of HE diet provision to Shetland pony mares did not lead to measurable circulatory pathology. It is suggested that the minor EMS-associated changes in BP were related to decreased endothelium-dependent vasodilatation and to the effect of excessive fat accumulation in obese ID ponies, which in turn led to the development of nonpathological LVH. Whether these findings are of clinical relevance and would develop into circulatory pathology if the period of overfeeding had been continued should be determined in future studies.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Committee on Animal Welfare of Utrecht University, the Netherlands (ethical committee approval No. 2014.III.02.021).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Nicky M. M. D' Fonseca  <https://orcid.org/0000-0001-7490-414X>

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