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Preliminary assessment of left ventricular function by tissue Doppler imaging and two-dimensional speckle tracking echocardiography in horses with equine metabolic syndrome

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Summary: The equine metabolic syndrome (EMS) has many parallels to the human metabolic syndrome (HMS). The HMS predisposes to cardiovascular diseases. Standard echocardiographic exams showed few abnormalities in HMS and advanced echocardiographic techniques are needed to detect altered cardiac function. Few studies have investigated cardiac function in horses with EMS. As the pathomechanism of HMS and EMS are similar, the objective of the study was to determine whether horses with EMS have altered cardiac function assessed by tissue Doppler imaging (TDI) and two-dimensional speckle tracking (2DST) echocardiography. TDI and 2DST were performed in horses with confirmed EMS. The severity of the EMS was assessed with an EMS score and by the severity of insulin resistance (IR). Scores, age, bodyweight (BWT), height at the withers were compared among groups by parametric or non-parametric t-tests or ANOVAs with correction for multiple comparison. All 32 horses included showed a phenotype of EMS and IR. The study revealed no changes in the systolic left ventricular (LV) function. Concerning the diastolic LV function, late diastolic myocardial velocity ($p = 0.012$) and the early to late diastolic myocardial velocity ratio ($p = 0.001$) differed among age groups. However, no difference in the diastolic LV function could be shown between the EMS score or IR groups. In conclusion the diastolic LV function was reduced with age. However, the severity of clinical signs of EMS or the severity of IR do not seem to contribute to any variation in the LV systolic or diastolic function. However, larger studies will be needed to confirm our results.

Keywords: ultrasonography, heart, ponies, insulin resistance

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Introduction

The equine metabolic syndrome (EMS) has been described since 2002 as an endocrine disorder leading to multiple metabolic dysfunction and a predisposition for laminitis in young to middle-aged horses (*Johnson* 2002, *Frank* et al. 2010, *Durham* 2016, *Durham* et al. 2019). Obesity in association with chronic overfeeding of diets rich in nonstructured carbohydrates, reduced and a hereditary predisposition might contribute to the insulin dysregulation that is underlying EMS (*Kronfeld* et al. 2006, *Frank* 2009, *Durham* et al. 2019, *Carslake* et al. 2021). Further, obesity associated dysregulation of adipokines and inflammatory mediators play also an important role in the pathogenesis of EMS (*Durham* et al. 2019). The clinical diagnosis is based on dynamic tests that assess the ability to metabolize glucose. The insulin dysregulation is compensated if the glucose intolerance is characterized by a hypersecretion of insulin concentration in response to a glucose challenge that allows the maintenance of the basal glucose level in the upper reference range (*Frank* and *Tadros* 2014). An abnormal response to a glucose challenge is used to diagnose EMS. Furthermore, typical clinical features, such as local or general adiposity or a history of laminitis, are supportive of the diagnosis of EMS (*Johnson* 2002, *Frank* et al. 2010, *Durham* 2016, *Durham* et al. 2019).

The EMS has been compared with the human metabolic syndrome (HMS) (*Frank* et al. 2010, *Ragno* et al. 2019). The HMS is considered a predisposing factor for cardiovascular diseases (as atherosclerotic cardiovascular disease, hypertension or left ventricular dysfunction) as patients with HMS have a two to three times increased risk of developing severe cardiovascular disease. Although alteration in heart dimensions has been reported (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015), standard echocardiographic exams of patients with HMS has shown few abnormalities in the indices of systolic function derived from two-dimensional echocardiography, such as the ejection fraction or the fractional shortening (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015, *Wang* et al. 2015). However, tissue Doppler imaging (TDI) and particularly two-dimensional speckle tracing echocardiography (2DST) has allowed the earlier detection of abnormal systolic function (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015). This was particularly visible when differences in segmental or longitudinal deformation could be differentiated with 2DST (*Crendal* et al. 2013, *Wang* et al. 2015). The diastolic function seemed to be affected earlier in the disease process and translated into a reduced amplitude of the myocardial motion in the early diastole in combination with increased late diastolic motion, visible in both conventional imaging and TDI (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015). Furthermore, the severity of the cardiac dysfunction correlated with the number of diagnostic criteria for HMS (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015).

Concerning the EMS fewer studies are available. In one study, an increased mean and relative wall thickness could be measured in ponies with EMS in comparison to healthy controls, even when most of the dimensions were still within reference values (*Heliczer* et al. 2017). There was also a significant association with blood insulin values (*Heliczer* et al. 2017). However, cardiac function has not yet been assessed with the tissue Doppler technique in ponies with EMS or in horses that can as well develop EMS (*Durham* et al. 2019). As the mechanism of HMS and EMS are similar, we hypothesized that horses with EMS have altered cardiac function that would correspond to the severity of the EMS.

Materials and methods

Animals and inclusion criteria

The study sample was recruited among patients presented at the teaching herd of the equine hospital of the Freie University Berlin. Each horse had a full clinical examination (including assessment of the alertness, the respiratory, cardiovascular and gastrointestinal tract and musculoskeletal system) (*Hammond* 2008) and complete echocardiography performed based on average measurements obtained in three cardiac cycles (*Stadler* et al. 1988, *Long* 1992, *Long* et al. 1992, *Gehlen* 2010). Inclusion criteria were clinical suspicion of EMS (history of "easy keepers", high body score with regional fat deposits) and no evidence of either heart diseases (e.g. heart valve regurgitations or arrhythmias) or acute episodes of pain. The presence of a history of laminitis was recorded and included in the clinical criteria leading to a suspicion of EMS. However, a horse was excluded from the study if it showed clinical signs of acute laminitis. Furthermore, concomitant presence of pituitary pars intermedia dysfunction was diagnosed based on the basal adrenocorticotropic hormone dosage (solid-phase, two-site sequential chemiluminescent immunometric assay, Immulite 2000, Siemens run by Laboklin GmbH & Co. KG, Labor für klinische Diagnostik, Bad Kissingen, Germany, interpretation according to the seasonal reference ranges (*Copas* and *Durham* 2012), sensitivity: 5 pg/ml, intraassay coefficient of variation: 2.97 %, interassay coefficient of variation: 8.92 % as indicated by Laboklin) or after a thyrotropin-releasing hormone stimulation test, based on the protocol described elsewhere (*Beech* et al. 2011, *Durham* 2016) and based on laboratory intern cut of < 110 pg/ml for negative, 110–220 pg/ml intermediary, and > 200 pg/ml for positive for tests performed in mid-November to mid-July (*Beech* et al. 2011, *Durham* 2016). Horses with positive results were excluded from the study.

The following data were recorded for each horse: Age, height at the withers, bodyweight (BWT), breed and sex (stallion, gelding, mare). The breed was further characterized into three categories according to the guidelines of the Fédération Équestre Nationale (FN), (*Düe* et al. 1997): Warmblooded (WB), ponies and small breeds (Po), race horses and special breeds (Oth). The BWT was taken on a body scale (Bizerba SE & Co. KG, Balingen, Germany). The height was determined using a conventional measuring tape.

Further, overall adiposity was assessed and graded by the cresty neck score (CNS) (*Carter* et al. 2009) and by including the regional adipose tissue deposit on other locations than the neck. Such deposits have been described as being located close to the tail head, behind the shoulder, or in the preputial or mammary region (*Frank* et al. 2010) and are not included in the CNS. Each of the regions was accorded one point. A global EMS score (EMSs) was determined by utilizing this data. In this score, points were accorded to clinical signs suggestive of EMS (i.e. CNS, regional adiposity, presence of a history of laminitis (*Frank* et al. 2010), as shown in Table 1.

Determination of insulin resistance

After inclusion into the study, all horses were stabled for 24 h for acclimatization prior to any further analyses. The horses were fasted over 6 h and thereafter the presence of IR was confirmed by a combined glucose insulin test (CGIT) (*Eiler* et al. 2005). For the latter, an intravenous catheter (Braunüle MT® Luer Lock, B. Braun Melsungen AG, Melsungen, Germany) was placed into one of the jugular veins and blood samples collected in appropriate tubes. Heparinized blood samples (BD A-Line, Becton, Dickinson and Company, Plymouth, UK) were used for glucose determination and the samples analyzed immediately with a blood gas analyzer (Cobas® b 123 POC Analyser Roche Diagnostics Deutschland GmbH, Mannheim, Germany). Regarding insulin determination, 10 mL of serum was collected into plain tubes (Sarstedt AG & Co.KG, Nürnbrecht, Deutschland) and sent refrigerated to a

Table 1 Points accorded to define the equine metabolic syndrome (EMS) score (EMSs). | *Punkte wie sie zur Etablierung des equinen metabolischen Syndrom (EMS) Scores (EMSs) vergeben wurden.*

Variables	Graduation	Points accorded
Cresty neck score (CNS)	CNS < 3	0
	CNS = 3	2
	CNS > 3	3
Regional adiposity	Additional adipose tissue depots other than neck (1 point per region)	Min 1
		Max 3
History of laminitis	No	0
	Yes	2

commercial laboratory (Laboklin GmbH & Co. KG, Labor für klinische Diagnostik, Bad Kissingen, Germany). The insulin concentration was determined by a chemiluminescence immunoassay (ADVIA Centaur® XP, Siemens Healthcare GmbH, Erlangen, Germany, sensitivity: 0.5 mU/l, intraassay coefficient of variation: 1.42–4.33 %, interassay coefficient of variation: 5.2–5.79 % as indicated by Laboklin).

Basal values for glucose and insulin were determined at time point 0. Glucose was then given intravenously over 60 s at a concentration of 150 mg/kg of a 40 % glucose solution (Glucose-Lösung 40 Prozent ad us. vet., B. Braun Vet Care, Melsungen, Germany). Immediately after the glucose administration, porcine insulin zinc at a dosage of 0.1 IU/kg was given intravenously (Caninsulin®, 40 IU/ml, MSD Animal Health, Schwabenheim, Germany). Thereafter, blood samples were collected at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135 and 150 min to determine the glucose and at 45 min for a second insulin determination. The CGIT was considered positive for EMS if more than one of the following three conditions were present: 1) The glucose concentration did not reach the basal value within 45 min, 2) the insulin value at the baseline was above a reference value of 23.4 µU/ml (as established for horses and ponies living in Germany), or 3) the insulin value was higher than 100 µU/ml at 45 min (*Eiler* et al. 2005, *Ahlers* and *Schusser* 2010, *Frank* et al. 2010). The severity of the IR was further graded as low, medium or high according to Table 2. The EMSs and the IRs as well as the number of horses in each group is summarized in Table 4.

Tissue Doppler imaging and two-dimensional speckle tracking echocardiography

Echocardiography (two-dimensional, TDI and 2DST) was performed by one observer (SL) on right-side short and long axis views with a portable ultrasound system (Vivid I, Ge Medical) and a phased array transducer allowing a frequency form 1.7/3.4 MHz with activated octave harmonics, as described previously (*Gehlen* 2010, *Gehlen* and *Neukirch* 2013, *Gehlen* and *Neukirch* 2014, *Gehlen* and *Bildheim* 2018a, *Gehlen* and *Bildheim* 2018b). Surface electrocardiography was obtained simultaneously.

In brief, pulsed wave (pw)-TDI was recorded on a right-side parasternal short axis view (SAX) at the chordae level just below the mitral valve as described previously (*Gehlen* and *Neukirch* 2013, *Gehlen* and *Neukirch* 2014, *Gehlen* and *Bildheim* 2018a, *Gehlen* and *Bildheim* 2018b). The sector width and the imaging depth of the views were individually adapted to achieve a frame rate of 40–80 frames/s. Regarding pw-TDI recordings, the sampling gates were placed successively into the left free wall (LW) and into the intraventricular septum (IVS). The correct subendocardial positioning of the sample gate was done during diastole and cine loops of five cycles were recorded for each position. The time velocity curve had a scale ranging from -20–20 cm/s. The measures determined were peak myocardial velocity in systole (S, in m/s), early diastolic peak myocardial velocity (E, in m/s), late diastolic peak myocardial velocity (A, in m/s) and the E/A ratio. Supplementary 2D cine loops each of five cycle length of the same right-side short axis view (SAX) were stored for the 2DST, as described previously (*Gehlen* and *Neukirch* 2013, *Gehlen* and *Neukirch* 2014, *Gehlen* and *Bildheim* 2018a, *Gehlen* and *Bildheim* 2018b). During the offline analyses, the endocardium of the left ventricle was tracked at the end of systole and the software defined a circular region of interest in this image automatically. The software allowed to calculate the radial strain (SR, %), the circumferential strain (SC, %) and the strain rate (SRR) for maximal systolic (SRR-S, in s-1), early diastolic (SRR-E, in s-1) and late diastolic (SRR-A, in s-1) velocities, expressed as mean over all myocardial segments. In individual cases not every individual measurement could be evaluated in the off-line analysis.

An EchoPac PC Software (GE VINGMED ULTRASOUND AS, Horton, Norwegen, Version 110.1.1) was used for the offline analyses and three cycles were measured and averaged by one observer (SL).

Statistical analyses

Commercially available software was used for the statistical analyses (Microsoft Excel 2013, Microsoft Corporation, Redmond, USA, SPSS® Statistics, Version 24, IBM®, GraphPad Prism®, version 5.01, GaphPad software, San Diego, CA USA). The total data series were checked for normal distribution with a Shapiro-Wilk test and by assessing the distribution of the histograms by kurtosis and skewness. Levene's test was used to test for equality of variance.

Normally distributed values were reported as mean ± standard deviation (SD). Comparison between two groups were done by using a Student's t-test. A one-way ANOVA with a Bonferroni correction for multiple comparison for five comparisons was used and p values adjusted for comparison of more than two groups (p 0.05/5 = 0.01). Data with non-normal distribution were re-

Table 2 Categorization of insulin resistance (IR) based on a combined glucose insulin test (CGIT). | *Kategorisierung des Insulinresistenz (IR) basierend auf dem kommbinierten Klukose Insulin Test.*

Degree of IR	CGIT	
IR0	No IR	Basal glucose level reached within 45 min/No hyperinsulinemia present
IR1	Low grade IR	Basal glucose level reached between 60 and 90 min/No hyperinsulinemia present
IR2	Medium grade IR	Basal glucose level reached between 60 and 90 min/Resting hyperinsulinemia present or Basal glucose level reached between 91 and 135 min/No hyperinsulinemia present
IR3	High grade IR	Basal glucose level reached between 91 and 135 min/Resting hyperinsulinemia present or Basal glucose level reached after 135 min with or without hyperinsulinemia

ported as median and range and the comparisons between groups were done with corresponding nonparametric tests (Wilcoxon signed-rank or Kruskal-Wallis test). Data that failed to show equal variance were compared with an Aspin-Welch test.

Results

Animals

The study sample was composed of 32 horses, including 20 mares and 12 geldings. The age, size, BWT were 12.5 year ± 5.5 years (mean ± SD, median 12.0 years, range 3–26 years), 153 ± 23.2 cm (mean ± SD, median158 cm, range 88–179 cm), 482 ± 157 kg (mean ± SD, median 535 kg, range 112–680 kg, n = 31), respectively. The horses included were categorized according the signalement shown in Table 3.

Small horses (< 155 cm) were nearly all ponies (10/11) and the largest horses (> 165 cm) were mostly WBs (9/10). Similarly, the group of light horses (< 455 kg) were mostly classified as ponies (9/10) and the horses with a BWT > 580 kg were mostly WBs (6/10).

Nineteen horses were presented for lameness or control examination for a known lameness, 10/19 had a history of laminitis. Further reasons for presentation were respiratory tract examination (3/32), referral for a CGIT due to suspected EMS (2/32), eye diseases (2/32), acute abdominal pain (1/32), a dermatological problem (1/32) and accompanying another horse to the hospital (1/32). Three horses from the research and teaching herd of the university were also included (3/32).

Table 3 Demographic data and distribution of horses according to signalment categories. | *Demographische Daten der Probanden und Verteilung in den Signalement Kategorien.*

Variables	Values
Mean age (years) ± SD	12.5 ± 5.5
Median age (years) (range)	12.0 (3–26)
Number of horses < 10 years old	11
Number of horses 10–14 years old	12
Number of horses > 14 years old	9
Mean size (cm) ± SD	153 ± 23.2
Median size (cm) (range)	158 (88–179)
Number of horses < 155 cm	11
Number of horses 155–165 cm	11
Number of horses > 165	10
Mean bodyweight (kg) ± SD	482 ± 157
Median bodyweight (kg) (range)	535 (112–680)
Number of horses < 455 kg	10
Number of horses 455–580 kg	12
Number of horses < 588 kg	10
Breeds	
Warmblood	9
Pony or small breeds	15
Other breeds	8

All horses included had a CNS of 3 or more. Regarding the overall EMSs, 17 horses reached an EMSs of 1 and 14 reached an EMSs of 2 (Table 4). Based on the CGIT test, 6/31 horses were classified as low-grade IR (IR1), 13/31 horses were classified as IR2 and the remaining 12/31 horses were classified as IR3 (there was no CGIT performed for one horse, Table 4).

Pw-TDI echocardiography

Most measurements were within the span of 2 standard deviations established for a similar group of horses and measured with the same equipment, same setting and same software version as in the present study (*Wittschorek* 2015). Standard two-dimensional echocardiographic measurements are reported in Table 5. Systolic function was assessed as LW peak systolic (S) myocardial velocity, or as IVS S myocardial velocity. The comparison for systolic function revealed no differences in the systolic function for the age groups, BWT and size groups. The categorization according to the scores established (EMSs, IR score) did not show a difference in the systolic function in the comparison among the groups (Figure 1).

Age had a significant influence on pw-TDI measurements of the diastolic function. Horses less than 10 years of age had slower LW A velocity than horses > 14 years old, and, subsequently, younger horses had larger LW E/A ratio than older horses (Figure 2).

The ratio E/A was smallest for the middle-sized horses in the LW. There was no statically significant difference between the groups of different BWT in the LW pw-TDI measurements. However, lighter horses (< 455 kg) had slower E velocities measured in the IVS than heavier horses with subsequently lower IVS E/A ratios in the group < 455 kg.

However, there was no statistically significant differences for the E and A velocity or the ratio E/A when comparing group

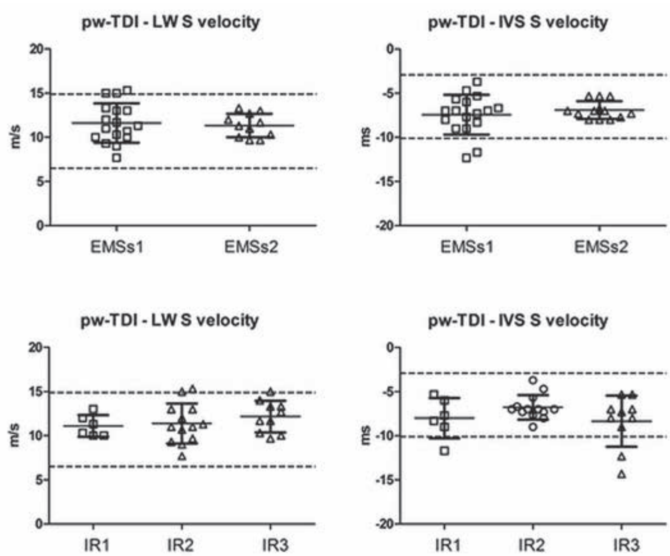
Table 4 Graduation to form the equine metabolic syndrome (EMS) score (EMSs) groups and the insulin resistance (IR) groups and number of horses per group. The definition regarding how points have been accorded in the EMSs or IR score is explained in Table 1 and 2. | *Gradierung die zur Bildung der equine metablische Syndrome (EMS) Score (EMSs) Gruppen und des IR Gruppen dienten, sowie Anzahl Probanden pro Gruppe. Die Definition des EMSs und des IR score sind in Tabelle 1 und 2 erklärt.*

Graduation of the score	Points obtained	Definition	Number of horses
EMSs1	0	No EMS	0
	1–3	Mild EMS	1
	4–7	Moderate EMS	16
EMSs2	8–11	Severe EMS	14
IR1		Low-grade	6
IR2		Medium grade	13
IR3		High grade	12

EMSs1 to group EMSs2 (Figure 3) or between groups IR1, 2 or 3 in pw-TDI (Figure 4).

Two-dimensional speckle tracking echocardiography

The variables assessed for systolic function were SR, SC, SRR-S averaged over all segments (Table 6 and 7). There were no statistically significant differences detected for the three age groups (SC p = 0.799, SR p = 0.109, SRR-S p = 0.617), the three size groups (SC p = 0.751, SR p = 0.752, SRR-S p = 0.353) or the three BWT groups (SC p = 0.769, SR p = 0.822, SRR-S p = 0.918). Similarly, there was no statically significant difference between the two EMSs groups (SC p = 0.174, SR p = 0.655, SRR-S p = 0.474) or the three IR groups (SC p = 0.755, SR p = 0.739, SRR-S p = 0.256).



	Comparison EMSs1 to EMSs2	
	P values	Mean difference (95% CI)
LW S velocity	0.769	0.3 (-1.3 to 1.8)
IVS S velocity	0.850	0.3 (-1.4 to 2.1)

	For comparisons						
	Overall	IR1 to IR2		IR2 to IR3		IR1 to IR3	
	P values	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)
LW S velocity	0.621	-	-0.3 (-2.7 to 2.2)	-	-0.8 (-2.9 to 1.3)	-	-1.1 (-3.6 to 1.5)
IVS S velocity	0.475	-	-1.2 (-4.0 to 1.5)	-	1.6 (-0.8 to 3.9)	-	0.3 (-2.5 to 3.2)

Fig. 1 Left ventricular systolic function assessed by pulsed wave (pw) tissue Doppler imaging (TDI) and reported as scatter dot blots with mean and standard deviation according equine metabolic syndrome score (EMSs) and insulin resistance (IR) groups (b). CI, confidence interval; IVS, intraventricular septum; LW, left ventricular free wall; y, years. The dashed line represents the reference range for horses (Wittschorek 2015). | *Linksventrikuläre systolische Funktion gemessen mittels Spektral-Gewebedopplerechokardiographie und dargestellt als Punktdiagram mit Mittelwert und Standardabweichung in dem equinen metabolischem Syndrom (EMS) Score (EMSs) Gruppen und den Insulinresistenz (IR) Gruppen. Die unterbrochene Linie entspricht den Referenzwerten für Pferde (Wittschorek 2015).*

Table 5 Basic 2D echocardiographic measurements of horses included in the study. There were no statistically significant differences between the horses classified as equine metabolic syndrome (EMS) score 1 (EMSs1) and EMSs2. | *Standard 2D echokardiographische Messungen der Probanden der Studie. Es konnte kein statistisch signifikanter Unterschied dargestellt werden zwischen den Pferden in den Gruppen equines metabolisches Syndrome (EMS) Score 1 (EMSs1) und EMSs2.*

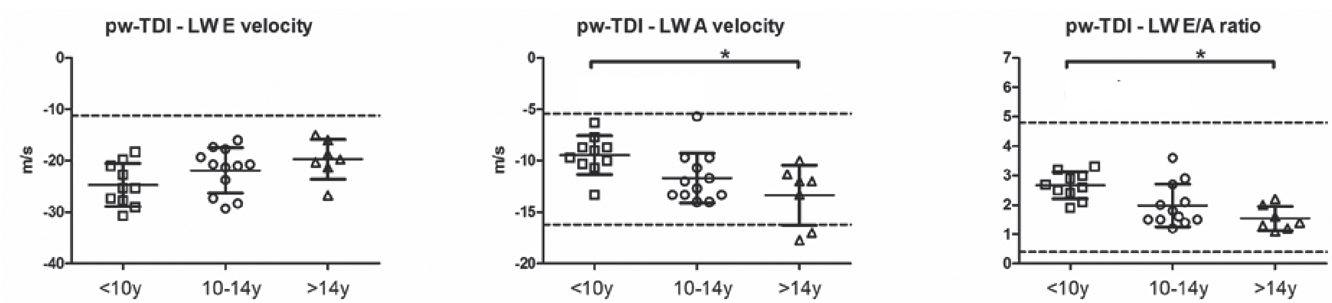
		All horses	EMSs1	EMSs2	P value for comparison EMSs1 vs EMSs2	Reference values (*Wittschorek* 2015)
		Mean ± SD	Mean ± SD	Mean ± SD		
Left atrial maximal diameter	cm	8.3 ± 1.8	8.2 ± 1.9	8.6 ± 1.8	0.593	9.5 ± 1.2
Left ventricular internal diameter at end-diastole	cm	10.3 ± 1.7	10.1 ± 1.9	10.5 ± 1.5	0.611	10.1 ± 1.5
Left ventricular free wall at end-diastole	cm	2.1 ± 0.5	2.0 ± 0.5	2.3 ± 0.4	0.082	2.2 ± 0.5
Interventricular septal thickness at end-diastole	cm	2.9 ± 0.7	2.8 ± 0.7	3.2 ± 0.6	0.160	2.4 ± 0.6
Mean left ventricular wall thickness at end-diastole	cm	2.5 ± 0.6	2.4 ± 0.6	2.8 ± 0.5	0.099	
Relative left ventricular wall thickness at end-diastole		0.49 ± 0.1	0.5 ± 0.1	0.53 ± 0.1	0.050	
Left ventricular fractional shortening	%	41.9 ± 4.7	41.3 ± 5.2	42.9 ± 3.7	0.384	45.9 ± 4.1
Aortic diameter (at sinus Valsalva level)	cm	6.7 ± 1.3	6.6 ± 1.3	6.9 ± 1.3	0.546	6.9 ± 0.8
Pulmonary artery diameter (at pulmonary sinus level)		5.4 ± 1.1	5.3 ± 1.3	5.6 ± 0.9	0.517	5.2 ± 0.5

The diastolic function was assessed as the early diastolic strain rate (SRR-E) and the late diastolic strain rate (SRR-A), averaged over all segments (Table 6 and 7). There was no statistically significant difference among the three age groups (SRR-E p = 0.320, SRR-A p = 0.098), three BWT groups (SRR-E p = 0.576, SRR-A p = 0.819) or three size groups (SRR-E p = 0.600, SRR-A p = 0.926). Categorization in two EMSs groups did not show any significant difference (SRR-E p = 0.115, SRR-A p = 0.625). A significant difference for SRR-E among the groups was found for the IR score (p = 0.009). However, this difference was not statistically significant anymore when comparing the 3 groups among them (IR 1 vs.IR 2 p = 0.016; IR1 vs IR 3 p = 0.016; IR 2 vs, IR 3 p = 1.0). There were no statically significant differences in mean SRR-A among the IR groups (p = 0.59)

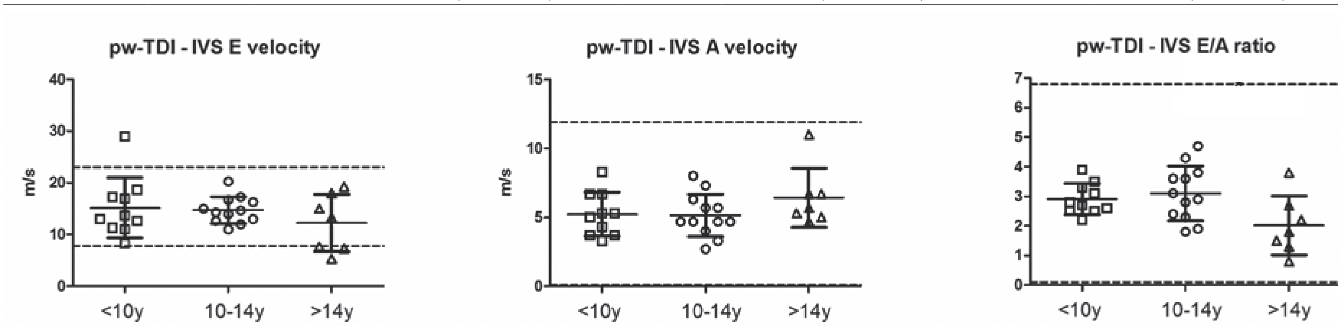
Discussion

Our results showed that the left ventricular function assessed as pw-TDI and 2DST measurements was influenced mainly by age but not by the presence of EMS, assessed as EMSs, or IR, assessed as IR score. Furthermore, the differences between the groups seen in our study were small and mostly within the span of 2 standard deviations previously determined for a similar group of healthy horses (*Wittschorek* 2015). Therefore, based on the included horses' groups, EMS affecting the left ventricular function as described in humans (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015) could not be confirmed. However, due to the small size of groups, it cannot be excluded for the whole equine population.

The diagnosis of IR was based on the CGIT (*Eiler* et al. 2005). However, due to German legislation, we used a porcine insulin zinc, labelled for animal use, instead of the human recombinant insulin used in the original study (*Eiler* et al. 2005). Therefore, the kinetic of the glucose concentration could have been slightly affected. However, it is unlikely that the glucose response to insulin would have been extended over 90 min in healthy horses. The classification in the IR score was based on both the basal insulin level and the response to the CGIT and,



				For comparisons			
	Overall	< 10y to 10–14y		10–14y to > 14y		< 10y to > 14y	
	P values	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)
LW E velocity	0.063	-	-2.8 (-7.4 to 1.8)	-	-2.2 (-7.3 to 2.9)	-	-5.0 (-10.3 to 0.2)
LW A velocity	0.012	0.020	2.2 (-0.4 to 4.8)	0.450	1.7 (0.9 to 6.9)	0.006	3.9 (-1.2 to 4.5)
LW E/A ratio	0.001	0.027	0.7 (0.1 to 1.3)	0.225	0.4 (-0.3 to 1.1)	0.001	1.1 (0.4 to 1.8)



				For comparisons			
	Overall	< 10y to 10–14y		10–14y to > 14y		< 10y to > 14y	
	P values	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)
IVS E velocity	0.708	-	0.4 (-4.7 to 5.5)	-	2.5 (-3.1 to 8.2)	-	2.9 (-2.9 to 8.8)
IVS A velocity	0.305	-	0.1 (-1.8 to 2.0)	-	-1.3 (-3.4 to 0.8)	-	-1.2 (-3.4 to 1.9)
IVS E/A ratio	0.031	1	-0.2 (-1.1 to 0.7)	0.033	1.1 (0.1 to 2.1)	0.113	0.9 (-0.1 to 1.9)

Fig. 2 Left ventricular diastolic function assessed by pulsed wave (pw) tissue Doppler imaging (TDI) and reported as dot blots with mean and standard deviation according to age groups. CI, confidence interval; IVS, intraventricular septum; LW, left ventricular free wall; y, years. The dashed line represents the interval between 2 standard deviations obtained in a similar population of healthy horses (*Wittschorek* 2015). * statistically significant, as reported in the table. | *Linksventrikuläre diastolische Funktion gemessen mittels Spektral-Gewebedopplerechokardiographie und dargestellt als Punktdiagram mit Mittelwert und Standardabweichung in den jeweiligen Alterskategorien. Die unterbrochene Linie entspricht den Referenzwerten für Pferde (Wittschorek 2015). * statistisch signifikant.*

Table 6 Measures obtained with 2D Speckle tracking echocardiography in all horses and in the groups equine metabolic syndrome score1 (EMSs1) or 2 (EMSs2). The measures represent means obtained over all myocardial segments. | *Messungen des "D und in allen Probanden in den Gruppen equines metabolisches Syndrome (EMS) Score 1 (EMSs1) und EMSs2. Die Messungen sind Mittelwerte etabliert über alle Wandsegmente.*

	2D Speckle tracking echocardiography														
	All horses					EMSs1					EMSs2				
	Radial strain	Circum-ferential strain	SR S velocity	SR E velocity	SR A velocity	Radial strain	Circum-ferential strain	SR S velocity	SR E velocity	SR A velocity	Radial strain	Circum-ferential strain	SR S velocity	SR E velocity	SR A velocity
Number of horses	29/31	29/31	29/31	29/31	29/31	17/17	17/17	17/17	17/17	17/17	12/14	12/14	12/14	12/14	12/14
Mean ± SD	**54.0** ± 17.1	**-19.9** ± 4.6	**1.5** ± 0.3	**-1.6** ± 0.5	**-0.9** ± 0.3	**54.6** ± 17.1	**-20.8** ± 5.3	**1.4** ± 0.2	**-1.5** ± 0.5	**-0.9** ± 0.3	**53.2** ± 17.8	**-18.6** ± 3.2	**1.5** ± 0.4	**-1.8** ± 0.6	**-0.9** ± 0.3
Minimum	23.3	-28.2	0.9	-3.2	-1.6	24.5	-28.2	1.2	-2.2	-1.6	23.3	-23.9	0.9	-3.2	-1.5
25% Percentile	41.5	-23.1	1.3	-1.9	-1.1	41.5	-25.1	1.3	-1.9	-1.0	40.7	-21.8	1.2	-2.2	-1.1
Median	**51.9**	**-20.0**	**1.4**	**-1.5**	**-0.8**	**47.9**	**-21.3**	**1.4**	**-1.5**	**-0.8**	**53.5**	**-17.8**	**1.5**	**-1.5**	**-0.9**
75% Percentile	69.6	-17.1	1.7	-1.3	-0.7	69.6	-18.2	1.6	-1.1	-0.7	69.6	-16.6	1.7	-1.4	-0.8
Maximum	81.9	-10.2	2.4	-0.7	-0.5	81.9	-10.2	1.8	-0.7	-0.6	77.1	-13.2	2.4	-1.3	-0.5
P value (for comparison between the two EMSs groups)						0.174	0.655	0.474	0.115	0.625					

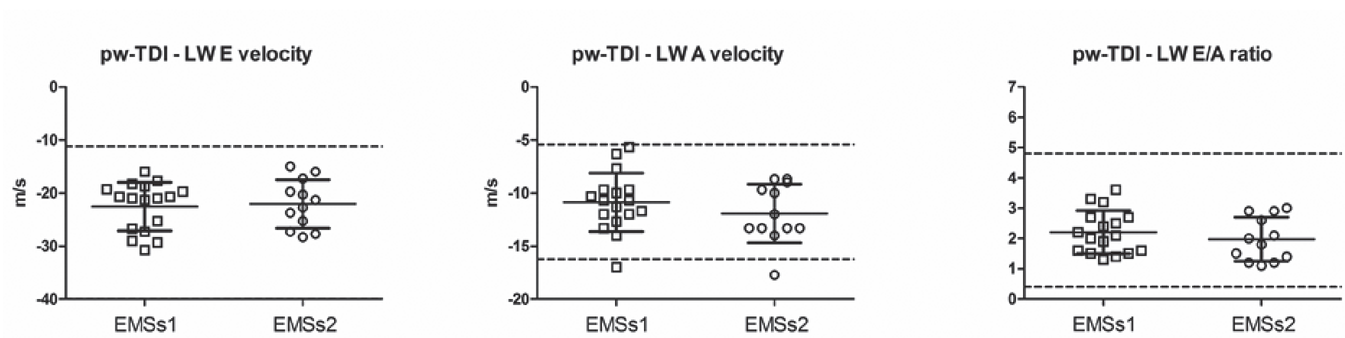
Table 7 Measures obtained with 2D Speckle tracking echocardiography in the insulin resistance group 1 (IR1), 2 (IR2) and 3 (IR3). The measures represent means obtained overall all myocardial segments. | *Messungen des "D und in allen Probanden in den Gruppen Insulinresistenz 1 (IR1), 2 (IR2), und 3 (IR3). Die Messungen sind Mittelwerte etabliert über alle Wandsegmente.*

	2D Speckle tracking echocardiography														
	IR1					IR2					IR3				
	Radial strain	Circum-ferential strain	SR S velocity	SR E velocity	SR A velocity	Radial strain	Circum-ferential strain	SR S velocity	SR E velocity	SR A velocity	Radial strain	Circum-ferential strain	SR S velocity	SR E velocity	SR A velocity
Number of horses	6/6	6/6	6/6	6/6	6/6	13/13	13/13	13/13	13/13	13/13	10/13	10/12	10/12	10/12	10/12
Mean ± SD	**52.04** ± 23.4	**-21.1** ± 6.0	**1.5** ± 0.2	**-1.2** ± 0.5	**-0.8** ± 0.2	**56.1** ± 14.0	**-19.3** ± 4.1	**1.4** ± 0.1	**-1.7** ± 0.4	**-0.9** ± 0.3	**52.5** ± 18.3	**-19.9** ± 4.7	**1.6** ± 0.4	**-1.8** ± 0.6	**-0.9** ± 0.3
Minimum	24.5	-28.2	1.2	-2.2	-1.2	38.9	24.5	1.1	-2.8	-1.6	23.3	-26.5	0.9	-3.2	-1.5
25% Percentile	30.8	-24.8	1.3	-1.6	-1.1	42.7	30.8	1.3	-1.9	-0.9	37.2	-23.8	1.3	-2.2	-1.2
Median	**47.5**	**-22.3**	**1.4**	**-1.0**	**-0.9**	**55.1**	**47.5**	**1.4**	**-1.7**	**-0.8**	**54.4**	**-19.6**	**1.7**	**-1.5**	**-1.0**
75% Percentile	78.9	-17.3	1.7	-0.7	-0.6	69.6	78.9	1.5	-1.5	-0.6	69.2	-15.9	1.8	-1.4	-0.8
Maximum	81.9	-10.2	1.7	-0.7	-0.6	77.1	81.9	1.6	-1.1	-0.6	77.1	-13.2	2.4	-1.3	-0.5
p^1	0.739	0.755	0.256	0.009	0.59										
p^2									0.016					0.016	
p^3														1.0	

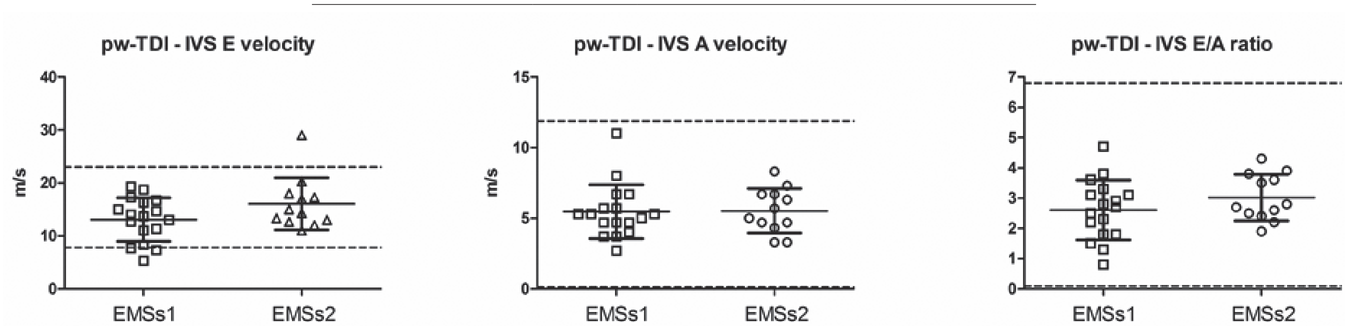
P^1 = P value (for comparison between the three IR groups) / P^2 = P values compared to IR1 / P^3 = P values compared to IR2 therefore, misclassification is unlikely. In addition, we assessed the severity of overall adiposity. The CNS (*Carter* et al. 2009) relies on assessing the adipose tissue in the neck region for which an endocrine activity has been supposed (*Burns* et al. 2010). This score does not assess the overall adiposity of the horses. Therefore, we included several other regions predisposed to fat deposits. This allowed us to assess the overall adiposity more precisely. Furthermore, several predisposing factors leading to EMS (laminitis, regional adiposity and CNS) were summarized in the EMSs. This score allows summarizing the clinical sings quantitatively. However, the score is not yet validated and this should be done in futures studies. None of the horses had concomitant pituitary pars intermedia dysfunction that was excluded based on the adrenocorticotropic hormone dosage or a thyrotropin-releasing hormone stimulation test. Therefore, the sample of horses studied represents a population with confirmed EMS.

Concerning the diastolic function, we could demonstrate an increase of the A velocity in the pw-TDI LW measurements with increasing age. Although the changes in the E velocity did not reach statistical significance in the study sample, the compensatory increase in A velocity would be in accordance with studies in humans showing a decreased diastolic function with aging (*Kloch-Badelek* et al. 2012, *Kuznetsova* et al. 2015). Similarly, decreased diastolic function with age has been shown in horses (*Gehlen* and *Bildheim* 2018b, *Gehlen* and *Bildheim* 2018a), even if some other studies could not report the same results (*Koenig* et al. 2017).

We could not show any difference in the left ventricular diastolic function among the EMSs groups and/or the IR groups. This result is in contradiction to findings in human medicine where a reduced early and increased late diastolic motion has been shown in conventional echocardiography and TDI (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015). A reduced diastolic compliance due to an increased rigidity of the extracellular matrix (*Katz* and *Zile* 2006) is considered one of the mechanism that could explain diastolic dysfunction seen in HMS. However, a reduced E velocity, leading to a compensatory increased A velocity, has also been explained by a reduced relaxation of the ventricle myocardium due to reduced elasticity, fibrosis in the



	Comparison EMSs1 to EMSs2	
	P values	Mean difference (95% CI)
LW E velocity	0.890	-0.46 (-4.0 to 3.1)
LW A velocity	0.266	1.1 (-1.1 to 3.2)
LW E/A ratio	0.429	0.2 (-0.3 to 0.8)



	Comparison EMSs1 to EMSs2	
	P values	Mean difference (95% CI)
IVS E velocity	0.074	-3.0 (-6.5 to 0.5)
IVS A velocity	0.822	-0.1 (-1.4 to 1.3)
IVS E/A ratio	0.209	-0.4 (-1.1 to 0.3)

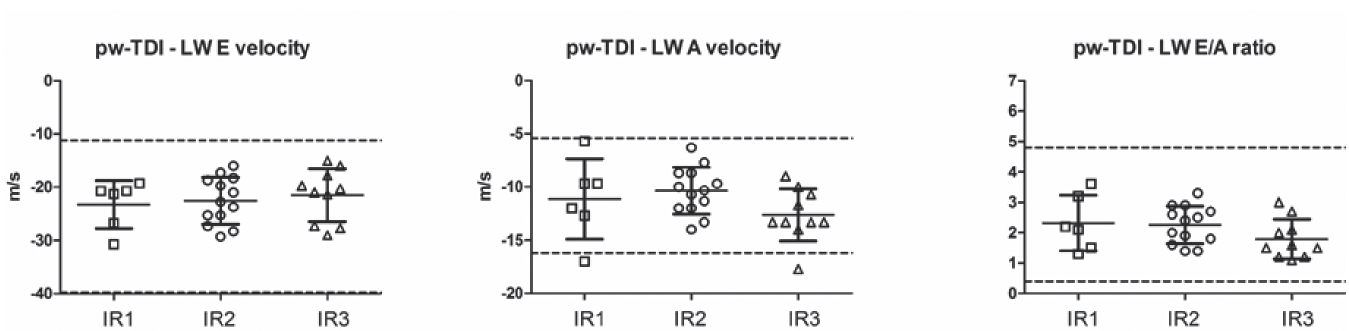
Fig. 3 Left ventricular diastolic function assessed by pulsed wave (pw) tissue Doppler imaging (TDI) and reported as dot blots with mean and standard deviation according to the equine metabolic syndrome score (EMSs) groups. CI, confidence interval; IVS, intraventricular septum; LW, left ventricular free wall; y, years. The dashed line represents the interval between 2 standard deviations obtained in a similar population of healthy horses (*Wittschorek* 2015). | *Linksventrikuläre diastolische Funktion gemessen mittels Spektral-Gewebedopplerechokardiographie und dargestellt als Punktdiagram mit Mittelwert und Standardabweichung in den equines metabolisches Syndrome Score (EMSs) Gruppen. Die unterbrochene Linie entspricht den Referenzwerte für Pferde (Wittschorek 2015).*

myocardium (*Shan* et al. 2000, *Chetboul* et al. 2004), or fat or amyloid deposits (*Klein* et al. 1989, *Schefer* et al. 2011).

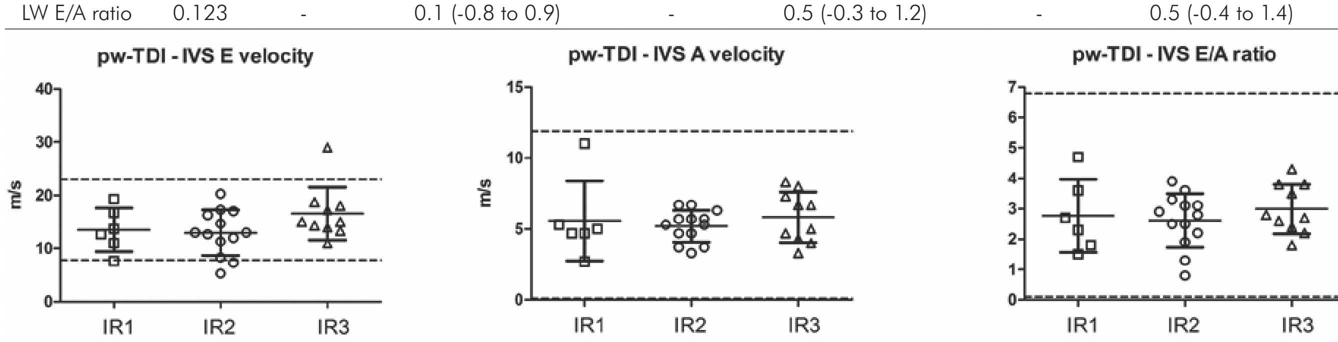
Similarly, no difference in the systolic left ventricular function among groups at different stages of disease could be shown in the study sample. Effects of HMS on the systolic function have been discussed in men but are not consistently reported (*Wong* and *Marwick* 2007b, *Wong* and *Marwick* 2007a, *Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015, *Wang* et al. 2015).

The results of the diastolic function for the classification in size groups or BWT groups is more difficult to interpret. Most small or light horses were ponies and showed statistically significantly lower E velocities for some of the measurements. A reduced diastolic function in this group could be explained by a more severe level of adiposity and/or EMS in ponies (*Treiber* et al. 2006, *Frank* et al. 2010) that are known to be predisposed to this disease. However, further studies in a larger group of ponies is necessary.

The main limitation is the low number of horses included and the absence of an age-matched control group. The low number of horses did not allow us to perform a multivariate analysis and, therefore, no analyses of interaction (e.g. between BWT and EMSs or increased age and EMSs) could be done. Similarly, the multiple comparison performed reduced the limit for significant p values and choosing another statistical approach would possibly have given different results. However, such questions could be addressed in future studies including a larger number of horses. Comparing our groups to healthy controls of the same age would have allowed us to discriminate better between the groups. However, such a matching population was not available and, therefore, we compared the results whenever possible to values published for a similar population (*Wittschorek* 2015). This comparison revealed that the differences observed were small and all within reference ranges. This finding contradicts our hypothesis and studies in human medicine (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015) but is in



	For comparisons						
	Overall	IR1 to IR2		IR2 to IR3		IR1 to IR3	
	P values	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)
LW E velocity	0.820	-	-0.7 (-6.5 to 5.1)	-	-1.0 (-6.0 to 4.0)	-	-1.7 (-7.8 to 4.3)
LW A velocity	0.101	-	-0.8 (-4.1 to 2.6)	-	2.3 (-0.6 to 5.1)	-	1.5 (-2.0 to 5.0)
LW E/A ratio	0.123	-	0.1 (-0.8 to 0.9)	-	0.5 (-0.3 to 1.2)	-	0.5 (-0.4 to 1.4)



	For comparisons						
	Overall	IR1 to IR2		IR2 to IR3		IR1 to IR3	
	P values	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)	P values	Mean difference (95% CI)
IVS E velocity	0.146	-	0.5 (-5.1 to 6.2)	-	-3.6 (-8.4 to 1.2)	-	-3.0 (-9.0 to 2.9)
IVS A velocity	0.0718	-	0.4 (-1.9 to 2.6)	-	-0.6 (-2.6 to 1.3)	-	-0.3 (-2.6 to 2.1)
IVS E/A ratio	0.598	-	0.2 (-1.0 to 1.3)	-	-0.4 (-1.4 to 0.6)	-	-0.2 (-1.4 to 1.0)

Fig. 4 Left ventricular diastolic function assessed by pulsed wave (pw) tissue Doppler imaging (TDI) and reported as dot blots with mean and standard deviation according to insulin resistance (IR) groups. CI, confidence interval; IVS, intraventricular septum; LW, left ventricular free wall; y, years. The dashed line represents the interval between 2 standard deviations obtained in a similar population of healthy horses (*Wittschorek* 2015). | *Linksventrikuläre diastolische Funktion gemessen mittels Spektral-Gewebedopplerechokardiographie und dargestellt als Punktdiagramm mit Mittelwert und Standardabweichung in den Insulinresistenz (IR) Gruppen. Die unterbrochene Linie entspricht den Referenzwerten für Pferde (Wittschorek 2015).*

accordance with one other study in ponies with EMS where minimal changes in 2D echocardiographic measurements have been reported (*Heliczer* et al. 2017). Analyzing ponies and other breeds separately would have allowed us to show the altered function in predisposed breeds better (*Treiber* et al. 2006, *Frank* et al. 2010) in comparison to less predisposed breeds.

Concerning the echocardiographic measurements, the image quality could have affected our results. Indeed, all horses were obese and presented a CNS ≥ 3 and 2 or more additional adipose tissue deposits. Therefore, the quality of the ultrasonographic images were affected. However, the quality of the right-side short axis (SAX) view was judged satisfactory in all cases to be used for further measurements.

The width and depth of the echocardiographic images was chosen to have a frame rate between 54.1 and 86.3 frames/s for pw-TDI, and between 44.1 and 79.4 frames/s for 2DST. These frame rates were at the lower end for high-quality measurements for pw-TDI and 2DST (*Schwarzwald* et al. 2009a, *Schwarzwald* et al. 2009b, *Decloedt* et al. 2013a, *Decloedt* et al. 2013b) and should be optimized in future studies. Furthermore, the myocardial movements with rapid velocities, particularly the isovolumetric contraction or the isovolumetric relaxation for the diastole, are difficult to measure in horses (*Schwarzwald* et al. 2009b, *Decloedt* et al. 2013a, *Koenig* et al. 2017) and could not be included in the present study. This technical limitations might have affected the overall value of the optained LV measurements.

The longitudinal axis for TDI is not technically available in horses (*Gehlen* et al. 2009, *Schwarzwald* et al. 2009b, *Decloedt* et al. 2013a). Therefore, only a small part of the complex myocardial motion can be assessed in horses with this technique. The 2DST uses another technical approach and it is not angle-dependent. It has been used to assess the cardiac function in horses (*Schwarzwald* et al. 2009a, *Schefer* et al. 2011, *Decloedt* et al. 2013b, *Gehlen* and *Nagel* 2014) based on the short axis view that allows the assessment of the circumferential and radial strain. The influence of HMS on cardiac function in humans is particularly visible in the longitudinal view (*Crendal* et al. 2013, *Tadic* et al. 2014, *Erturk* et al. 2015). Therefore, longitudinal measurement, as described recently (*Decloedt* et al. 2011, *Decloedt* et al. 2012, *Decloedt* et al. 2020), should be included in further studies.

Conclusions

In conclusion, we could show that age could affect cardiac left ventricular function assessed by pw-TDI and 2DST. However, EMS and IR had no effect on left ventricular function in our study sample. However, further larger studies are required to address this topic and investigate the difference to findings in human medicine further.

Conflict of interest statement

The authors declare no conflict of interest.

Animal health and owner's informed consent statement

All diagnostic procedures performed, and all blood samples taken were part of the clinical work-up of the patients during the first examination or a clinically indicated follow-up examination and were performed according to standard protocols at the equine hospital of the Freie University Berlin. Therefore, the sampling of horses included in the study was not classified as an animal experiment by the State Office of Health and Social Affairs Berlin (LaGeSo). Owners' verbal consent to involve their horses in the study was obtained during the admission process at the hospital.

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