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Evaluation of indirect blood pressure measurement, plasma endothelin-1 and serum cortisol in clinically healthy horses

Utvärdering av indirekt blodtrycksmätning, plasmaendothelin-1 och serumkortisol på kliniskt friska hästar

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Abstract

Equine metabolic syndrome (EMS) it's a clinical condition that includes obesity, regional adiposity, insulin resistance (IR) and laminitis. A similar syndrome exists in human medicine, the metabolic syndrome (MS), were systemic hypertension also is a prominent feature. The systemic hypertension seen in humans with MS is partly due to a vascular endothelial dysfunction with an enhanced endothelin-1 (ET-1) mediated vasoconstriction. There are indications that systemic hypertension might be a feature in EMS as well but if ET-1 is involved in the development of the systemic hypertension in horses is not known. Increased circulating cortisol levels have also been found in humans with MS. To our knowledge, there are no studies that have measured serum cortisol concentrations in horses diagnosed with EMS, and the question remain whether horses with EMS differ in cortisol concentrations compared to controls. Before blood pressure, plasma ET-1 and serum cortisol can be evaluated in horses with EMS, it is necessary to test the day-to-day variation of these parameters on clinically healthy horses.

The aim of the present study was to evaluate the day-to-day variation of indirect oscillometric blood pressure measurements, plasma ET-1 and serum cortisol concentrations in healthy Standardbred and Icelandic horses, and to detect any potential breed differences in theses parameters. Additional aims were to study if transportation could influence the results obtained and to compare the two indirect blood pressure measurement devices, the Cardell® Veterinary Vital Signs Monitor (Cardell) Model 9402, and the HDO Memo Diagnostic (HDO) horse model 1.35.00.

Nine horses of each breed were included in the study. Blood pressure was measured and blood samples were collected between 6 and 9 am on two separate days. Eight of the horses (four Standardbred horses, four Icelandic horses) were transported to a new stable were they stayed overnight. On the morning after the transportation, the blood pressure measurements and the sampling procedure were repeated.

The results showed that indirect blood pressure measurements, plasma ET-1 and serum cortisol all had an acceptable day-to-day variation. There were no differences in mean blood pressure between the two breeds but the Icelandic horses had significantly lower mean serum cortisol and significantly higher mean plasma ET-1 concentrations compared to the Standardbred horses. Plasma ET-1 was significantly elevated after transportation, but the mean serum cortisol and mean blood pressure did not differ from the values obtained in the home environment.

The results indicate an acceptable day-to-day variation of the tested parameters, but the higher plasma ET-1 concentrations recorded after transportation, might have been caused by a stress response associated with the transportation and being housed in a new environment. The differences detected in plasma ET-1 and serum cortisol between the two breeds might be related to differences in genetic setup, training status as well as management conditions. The tested parameters are all potential candidates for further investigation in

horses clinically affected of EMS and a further evaluation of possible breed differences in the tested parameters is desirable.

Sammanfattning

Equint metabolt syndrom (EMS) innefattar kliniska symptom såsom regional och generell fetma, insulinresistens (IR) och fång. Ett liknande syndrom finns inom humanmedicinen, metabolt syndrom (MS), där även systemisk hypertension är en del i sjukdomsbilden. Den systemiska hypertensionen som ses hos människor beror delvis på vaskulär dysfunktion med en ökad endothelin-1-medierad (ET-1) vasokonstriktion. Det finns indikationer på att systemisk hypertension även kan vara en del i EMS, men om ET-1 medverkar till systemisk hypertension på häst är i dagsläget inte utrett. Ökade nivåer av kortisol i cirkulationen har hittats hos människor med MS. Vad vi vet finns det inga studier som har mätt serumkortisolkoncentrationen hos hästar diagnostiserade med EMS, så frågan kvarstår huruvida dessa hästar skiljer sig i kortisolkoncentration jämfört med kontroller. Innan blodtryck, plasma-ET-1 och serumkortisol kan mätas hos hästar med EMS är det nödvändigt att utvärdera dag-till-dagvariationen hos dessa parametrar hos kliniskt friska hästar.

Syftet med denna studie var att utvärdera dag-till-dagvariationen hos indirekt oscilometrisk blodtrycksmätning, plasma-ET-1 och serumkortisol hos friska varmblodstravare och islandshästar, samt att hitta eventuella rasvariationer i dessa parametrar. Ytterligare syften var att studera huruvida transport kan påverka resultaten av parametrarna samt att jämföra två indirekta blodtrycksmaskiner, Cardell® Veterinary Vital Signs Monitor (Cardell), modell 9402, och HDO Memo Diagnostic (HDO), hästmodell 1.35.00.

Nio hästar av varje ras deltog i studien. Blodtryck mättes och blodprov samlades in mellan klockan 6-9 på morgonen under två separata provtagningsdagar. Åtta av hästarna (fyra varmblodstravare och fyra islandshästar) blev även transporterade till och tillbringade natten i ett för dem nytt stall. På morgonen efter transporteringen mättes åter blodtrycket och blodprov samlades in.

Resultaten visade att indirekt blodtrycksmätning, plasma-ET-1- och serumkortisolkoncentration hade en acceptabel dag-till-dagvariation. Det var inga skillnader i medelblodtryck mellan raserna men islandshästarna hade signifikant lägre medelkortisolkoncentration och signifikant högre medelkoncentration av plasma-ET-1 jämför med varmblodstravarna. Plasma-ET-1 var signifikant höjt efter transportering medan medelserumkortisol och medelblodtryck inte skilde sig från värden uppmätta i hemmiljö.

Studien visar en acceptable dag-till-dagvariation av de testade parametrarna. Den förhöjda ET-1-koncentrationen efter transport kan ha orsakats av stress associerat med transporten och vistelsen i ny miljö. Skillnaderna som upptäcktes i plasma-ET-1- och serumkortisolkoncentration mellan de båda raserna kan vara relaterat till genetiska olikheter, olika träningsstatus eller vara skötselrelaterade. Testade parametrar är alla potentiella kandidater för vidare utvärdering hos hästar med EMS samt att vidare undersökningar rörande rasskillnader vore önskvärt.

Distribution

1 Introduction

Equine metabolic syndrome (EMS) was introduced in veterinary medicine in 2002. It's a clinical condition that includes certain parameters such as obesity, regional adiposity, insulin resistance (IR) and clinical or subclinical laminitis (Johnson, 2002; Frank *et al.* 2010). A similar syndrome exists in human medicine; the metabolic syndrome (MS) which is characterized by IR, abdominal obesity, lipid disorders, coagulopathies, hyperglycemia and systemic hypertension (Fulop *et al*. 2006). There are indications that the systemic hypertension might be a feature in EMS as well, as ponies with EMS and a previous history of laminitis have been shown to have elevated systemic blood pressure (Bailey *et al.* 2008). These ponies had a significantly elevated mean blood pressure during summer compared to controls. The systemic hypertension seen in obese MS patients with IR is partly due to vascular endothelial dysfunction (Reaven, 2005). In this group of patients the production of the vasodilator nitric oxide is decreased (Stuhlinger *et al*. 2002) and they have an enhanced endothelin (ET-1) mediated vasoconstriction (Cardillo *et al*. 2004). ET-1 is a potent vasoconstrictor (Levin, 1995; Katz *et al.* 2003) and has been proposed as a mediator involved in laminitis (Katwa *et al.* 1999). Elevated ET-1 concentrations have earlier been found in horses with clinical endotoxaemia (Menzies-Gow *et al*. 2005) and in horses with summer pasture-associated recurrent airway obstruction (SPA-RAO) (Costa *et al.* 2009). If ET-1 is involved in the development of the seasonal hypertension reported in ponies is not known, as there are no studies that describe plasma ET-1 levels in horses diagnosed with EMS. There are different blood pressure measurement devices available on the market for measurement of indirect systemic oscillometric blood pressure in horses and the type of device used also has an effect on the blood pressure values obtained (Mitchell *et al.* 2010).

The overlap between MS and Cushings´s disease in humans has raised the question if elevated cortisol levels are associated with the development MS (Walker 2006; Anagnostis *et al.* 2009). There are studies that have shown that patients with MS have higher circulating concentrations of cortisol, but also studies that have failed to find this relationship (Maggio *et al.* 2007). To our knowledge there are no studies that have measured serum cortisol concentrations in horses diagnosed with EMS, and the question remains whether horses with EMS differ in cortisol concentration compared to controls. Before further investigation of these parameters is indicated, the reproducibility of these parameters must be evaluated.

Cortisol concentrations are known to have a circadian rhythm in horses (Irvine *et al.* 1994) and transportation is known to alter the normal rhythm both during and up to approximately 11 hours after transportation (Forhead *et al.* 1995). The diagnostic test often used to detect horses suffering from EMS is a combined glucose and insulin tolerance test (CGIT-test) (Eiler *et al.* 2005; Frank *et al.* 2010) and this is usually performed in a clinical setting. The stress response that the transportation to the clinic and the new environment might cause, could affect not only cortisol concentrations, but also the results of all other tests used for diagnosing the disease.

1.1 The aims of the study

This prospective experimental study aims to:

- Evaluate the day-to-day variation of systemic indirect blood pressure measurements in standing Standardbred and Icelandic horses without clinical signs of disease and compare two indirect oscillometric blood pressure measurement devices.
- Evaluate the day-to-day variation of plasma ET-1 and serum cortisol concentrations in Standardbred and Icelandic horses without clinical signs of disease.
- Study if there exist any differences in systemic blood pressure, plasma ET-1 and serum cortisol concentrations between the two breeds.
- Study the possible influence of transportation and housing in a new environment on systemic blood pressure, plasma ET-1 and serum cortisol concentrations.

2 Material and Methods

2.1 Horses

None of the horses included in the study showed any signs of disease on the clinical examination that was performed before the start of the study. BCS (Henneke *et al*. 1983) and Cresty Neck Score (CNS) (Carter *et al*. 2009) were estimated for each horse. The study was sanctioned by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

2.1.1 Standardbred horses

Nine Standardbred horses, seven mares and two geldings, with a mean age of 13 ± 5 years (range 6-20) and with a mean body weight of 505 ± 48 kg (range 425-586), were included in the study. All horses were owned by the Department of Clinical Science and kept in box stalls with a daily stay in a paddock. All were sedentary horses and fed a standardized diet consisting of hay (three times/day) and concentrate (once daily) and provided water ad libitum.

2.1.2 Icelandic horses

Nine Icelandic horses, seven mares and two geldings, with a mean age of 14 ± 3 years (range 10-18) and with a mean body weight of 381 ± 35 kg (range 333-444), were included in the study. All horses were privately owned and were mainly kept in loose outside housing but were also kept in box stalls on a regular basis. Eight of the horses performed lighter exercise three to five times a week and one horse was sedentary. These horses were fed grass haylage (three times/day) and provided water ad libitum.

2.1.3 Horses in the transportation study

Four of the Standardbred horses (three mares and one gelding) and four of the Icelandic horses (two mares and two geldings) were included in the transportation study. The horses were not accustomed to transportation.

2.2 Blood pressure measurement devices

Cardell® Veterinary Vital Signs Monitors model 9402, and The Memo Diagnostic High Definition Oscillometry Monitor[1], horse model 1.35.00 were used in the study.

Both devices automatically inflate an occluding cuff and use the oscillometric measurement technique for determination of systolic, diastolic, mean arterial blood pressure and heart rate. The cuff width for the Cardell device was 11 cm and for the HDO 8 cm. The same cuff size accompanied each device through all measurements.

2.3 Experimental design

2.3.1 Day-to-day variation

The blood pressure measurements and the sample collection were performed between Mars and April 2011, before the horses had access to summer pasture.

The systemic blood pressure was measured between 6 and 9 am on two separate days (M1 and M2) with a maximum of one day between measurements. The cuffs were placed on the base of the horse's tail while the horse was standing in the box stall. Five consecutive determinations of systolic and diastolic blood pressure were performed on each horse, using both devices. On the first day of the study, the starting order in which of the two blood pressure measurement devices were used was randomized by raffle. The next day the starting orders of the devices were switched. In each horse, blood samples were drawn immediately after both blood pressure measurements had been completed.

2.3.2 Transportation study

The horses were transported in a two-horse trailer for one hour and the horses were then housed in a new environment overnight. The next morning, the blood

[1] MD; S + B medVetGmbH, Babenhausen, Germany

pressure was measured and blood samples collected (M3) in the same manner as previously described.

2.3.3 Blood collection and analysis

Blood samples for analysis of plasma ET-1 and serum cortisol were drawn using vacutainer technique and collected in evacuated NaEDTA[2] and Serum[3] glass tubes. The blood samples were drawn immediately after the blood pressure measurements had been completed on each horse. All samples were kept on ice and centrifuged (+4°C, 906g, 10 min) within 30 minutes after sampling. Plasma and serum were separated into Ependorff tubes and then stored in -80°C until analysis.

Plasma ET-1 concentrations were measured in duplicates using a commercial ELISA-kit[4] that has previously been validated for use in horses[5] and serum cortisol concentrations (hydrocortisone Compound F) were measured in duplicates with a radioimmunoassay-kit[6] that also has been validated for use in horses[7].

2.4 Statistical analysis

In each horse, the lowest and highest recorded value of the five consecutive blood pressure determinations were excluded and the mean systolic and diastolic pressure was calculated from the three remaining determinations (triplicates). The statistical analysis was performed using SigmaPlot software version 11[8].

The results of repeated tests for indirect blood pressure measurement, plasma ET-1 and serum cortisol were compared by use of a paired t-test (M1 and M2) or one way repeated measures analyses of variance (M1, M2, and M3; transportation study). Differences between individual means for the three days were tested with a tukey test. Differences in the two breeds for measurement of indirect blood pressure measurement, plasma ET-1 and serum cortisol were compared by use of a t-test whereas differences between the blood pressure measurement devices were compared by use of a paired t-test. Measurement error, defined as the variation between measurement of the same quantity on the same animal, blood pressure

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[2] BD, Vacutainer, BD, Belliver, Industrial Estate, Plymouth, UK
[3] BD, Vacutainer, BD, Belliver, Industrial Estate, Plymouth, UK
[4] Endothelin (1-21) enzyme immunoassay CAT.NO.BI-20052 (12*8) Biomedica Medizinprodukte GmbH & Co KG, A-1210 Wien
[5] Biomedica Medizinprodukte GmbH & Co KG, A-1210 Wien
[6] Coat-A-Count® Cortisol, TKCO2, SIEMENS
[7] Department of clinical chemistry, Swedish University of Agricultural Sciences, Uppsala
[8] Systat Software UK Limited, London, UK

device and ELISA- and radioimmunoassay-kit, was expressed as the coefficient of variation (CV). The standard deviation for duplicate or triplicate measurements was calculated according to Bland (2000). The CV was calculated as the SD divided by the means of each set of two or three measurements and expressed in percentage.

Mean values were expressed as mean ± SD. The level of significance was set at $P < 0.05$.

3 Results

3.1 Body condition score and cresty neck score

The BCS ranged from 5 to 6 in the Standardbred horses, and from 5 to 7 in the Icelandic horses. The CNS ranged from 1 to 2 in the Standardbred horses and from 2 to 3 in the Icelandic horses.

3.2 Day-to-day variation

Regardless of which blood pressure measurement device that was used, there was no difference in mean systolic or diastolic blood pressure between M1 and M2. There was no significant difference between M1 and M2 in the mean concentration of plasma ET-1 and serum cortisol. One Icelandic horse had plasma ET-1 concentrations that were regarded as unreasonably high (16.41 and 14.59 pg/ml) and another Icelandic horse showed a great variation in plasma ET-1 concentrations between measurements (3.09 and 14.54 pg/ml). These values were therefore excluded from the calculations of plasma ET-1. One of these horses took part in the transportation study, and the ET-1 values of this horse were excluded because of concentrations above the detection limit. The intra-assay CV for plasma ET-1 was 6.7% and the intra-assay CV for serum cortisol was 5.7%. The intra-assay CV´s for the Cardell device were 5.4% in systole and 13.9% in diastole and the intra-assay CV´s for the HDO device were 9.5 % in systole and 12.1 % in diastole.

3.2.1 Comparison of blood pressure measurement devices

The mean systolic blood pressure differed significantly between the two devices (HDO, 114 ± 14 mmHg; Cardell, 104 ± 12 mmHg), but not the mean diastolic blood pressure (HDO, 71 ± 11 mmHg; Cardell, 65 ± 13 mmHg). The inter-assay CV was better in systole compared to in diastole for both devices. The Cardell showed a better inter-assay CV in systole than the HDO, but there was no difference between the two devices in diastole.

Tabell 1. ***Day-to-day variation.*** *Mean (±SD) values for systemic, systolic and diastolic blood pressure (measured with the HDO and the Cardell device), plasma ET-1 and serum cortisol concentration measured on two separate days (M1 and M2). P < 0.05. No significant differences between M1 and M2 were detected for any of the variables.*

Variable	M1	M2	CV %
HDO			
BP_S (mmHg)	114 ± 14	113 ± 18	15
BP_D (mmHg)	71 ± 11	67 ± 15	21
Cardell			
BP_S (mmHg)	104 ± 12	102 ± 10	8
BP_D (mmHg)	65 ± 13	60 ± 10	19
ET-1 (pg/ml)	2.3 ± 1.4	2.7 ± 2.0	37
Cortisol (nmol/l)	129 ± 76	126 ± 81	18

BP_S -systolic blood pressure, BP_D –diastolic blood pressure, CV -Coefficient of variation. n=18, except for in M1 and M2 in the ET-1 measurement where n=16.

3.3 Breed comparison

Regardless of which blood pressure measurement device that was used, there was no difference in mean systolic or diastolic blood pressure between the two breeds.

Mean concentrations of plasma ET-1 and serum cortisol both differed significantly between the two breeds with the Icelandic horses having significantly higher plasma ET-1 concentrations and significantly lower serum cortisol concentrations compared to the Standardbred horses.

Tabell 2. ***Breed comparison.*** *Mean (±SD) values for systemic, systolic and diastolic blood pressure (measured with the HDO and the Cardell device), plasma ET-1 and serum cortisol concentration measured in two different breeds (Standardbred horses and Icelandic horses).* * *P < 0.05 versus Standardbred horses.*

Variable	Standardbred horses	Icelandic horses
HDO		
BP_S (mmHg)	118 ± 17	110 ± 11
BP_D (mmHg)	74 ± 11	67 ± 11
Cardell		
BP_S (mmHg)	103 ± 9	105 ± 14
BP_D (mmHg)	64 ± 7	66 ± 17
ET-1 (pg/ml)	1.5 ± 0.5	3.2 ± 1.7*
Cortisol (nmol/l)	188 ± 63	70 ± 22*

BP_S -systolic blood pressure, BP_D –diastolic blood pressure. n=9, except for in the Icelandic horses in the ET-1 measurement where n=7.

3.4 Influence of transportation

Transportation and housing in a new environment did not affect the mean systolic or diastolic blood pressure or mean serum cortisol concentration.

The mean plasma ET-1 concentration was significantly higher when measured after transportation compared to in the home environment.

Tabell 3. ***Influence of transportation.*** *Mean (±SD) values for systemic, systolic and diastolic blood pressure (measured with the HDO), plasma ET-1 and serum cortisol concentration measured on two separate days (M1 and M2) and after transportation (M3).* **P < 0.05 versus MI and M2.*

Variable	M1	M2	M3	CV %
HDO				
BP_S (mmHg)	114 ± 6	110 ± 11	119 ± 10	6
BP_D (mmHg)	71 ± 6	67 ± 9	75 ± 7	9
ET-1 (pg/ml)	1.7 ± 0.9	1.7 ± 0.9	2.6 ± 1.2*	31
Cortisol (nmol/l)	113 ± 60	115 ± 74	122 ± 35	33

BP_S -systolic blood pressure, BP_D –diastolic blood pressure, CV -Coefficient of variation. n=8, except for in M1, M2 and M3 in the ET-1 measurement where n=7.

4 Discussion

The results of the present study showed that all the parameters showed an acceptable day-to- day variation and are therefore of interest as markers to study the cardiovascular aspects of EMS. Systemic blood pressure did not differ between the two breeds and both blood pressure measurement devices gave values with an acceptable day-to-day variation.

The indirect oscillometric blood pressure technique has been found to overestimate the systolic blood pressure in dogs compared to the direct blood pressure technique (Geddes *et al.* 1982; Meyer *et al.* 2010). However, if the clinician is aware of the possible risk that indirect blood pressure measurement techniques might overestimate the blood pressure, the risk of falsely diagnosing hypertension in horses appears to be little. In the present study, the HDO device gave higher values in systole compared to the Cardell device. This could partly be related to the difference in cuff size between the two devices. The size of the cuff is known to influence the obtained blood pressure values were a cuff that is too wide underestimates the blood pressure whereas a cuff that is too narrow tends to overestimate the values. The optimum cuff width for horses is one-fifth of the tail circumference (Latshaw *et al*. 1979). It is therefore important that the cuff used is adjusted for each type of horse. Considering the results from the presents study, both devices can be used to measure systemic blood pressure in Standardbred as well as Icelandic horses. However, there are results that indicate that the HDO device has a better correlation with the direct blood pressure measurement technique compared to the Cardell device (Mitchell *et al.* 2010). A previous study in dogs also showed that although the blood pressure values obtained with the HDO device showed a greater variability than the values obtained with the direct blood pressure measurement technique, there was a good correlation between the two techniques (Meyer *et al.* 2010). Both devices can be run on battery, which makes it possible to use them during field conditions. The results from our study showed that the

Cardell device showed a less day-to-day variation in systole compared to the HDO device. However, the Cardell device is large and impractical to handle in a field setting whereas the HDO device is smaller in size, lighter and can be handled using only one hand. The previously reported high sensitivity and good correlation with the direct blood pressure measurement technique for the HDO device is also advantageous.

As previously stated, the day-to-day variation for plasma ET-1 and serum cortisol was acceptable with no statistical significance detected between measurements in the home environment, although both parameters showed quite high inter-assay CV´s. The latter could be explained by the normal individual day-to-day fluctuations that have earlier been reported for cortisol, triglycerides, thyroxine, proteins and enzymes in serum of healthy humans (Statland *et al*. 1976), as well as for glucose and insulin in plasma of clinically healthy horses (Bröjer *et al.* unpublished data). The high SD´s seen in the day-to-day variation in serum cortisol and plasma ET-1 are likely related to the differences in mean values between the two breeds, individual fluctuations within the different breeds and the merged calculation of those in the day-to-day comparison.

The mean concentration of plasma ET-1 measured in the present study was higher for both breeds compared to the concentrations reported earlier in healthy Thoroughbred and Standardbred horses (McKeever *et al*. 1999; Menzies-Gow *et al.* 2004) but similar range to those reported in horses with SPA-RAO (Costa *et al.* 2009) and horses with clinical endotoxaemia (Menzies-Gow *et al.* 2005). It cannot be ruled out that some of the horses in the present study could have suffered from a disease causing an elevation in plasma ET-1. This is however considered unlikely as none of the horses included in the study showed any signs of disease on the clinical examination that was performed before the start of the study. A more likely explanation for this discrepancy in results between studies are differences in type of analyzing technique (ELISA) for quantification of ET-1 as well as genetic differences between breeds.

An interesting finding was the difference in plasma ET-1 and serum cortisol concentrations between the two breeds. The results of the present study showed that the Icelandic horses had significantly higher plasma ET-1 concentrations than the Standardbred horses. There was a considerable individual variation in the concentrations of plasma ET-1 in both breeds, which is in agreement with previous studies in both healthy horses and horses affected of SPA-RAO (Costa *et al.* 2009).

However, the individual variation was greater in among the Icelandic horses which is verified by the higher SD value seen in this group. The reason for this difference between breeds is not known, but might be related to both genetic differences as well differences in management.

Previous studies in ponies and Icelandic horses have reported that they are partially insulin resistant (Rijnen *et al.* 2003; Jeffcott *et al.* 1986; Bröjer *et al*. unpublished data). In a healthy individual, insulin stimulates the production of nitric oxide as well as ET-1, resulting in vasodilation. In an individual with IR or hyperinsulinemia the production of nitric oxide is decreased and the insulin-mediated vasodilation is therefore impaired (Pantelis *et al.* 2007). This could also explain the higher ET-1 values seen in the Icelandic horses compared to the Standardbred horses, as elevated insulin levels could cause an increase in ET-1 concentration (Piatti *et al.*1996). In the present study, the higher ET-1 levels in the Icelandic horses did not reflect any elevation in the systemic blood pressure compared to the Standardbred horses.

In horses, cortisol production has a circadian rhythm with peak levels in the early morning and a nadir at night (Stull *et al.* 1988; Irvine *et al.* 1995). Because of the circadian rhythm sampling results are also influenced by sampling time (Irvine *et al*. 1994). Investigation of cortisol in untrained Standardbred horses in home environment showed a circadian rhythm with a peak between 6 and 9 am (Irvine *et al.* 1994), and according to these results, the horses in the present study were sampled at the time of peak cortisol concentration. The circadian rhythm can easily be abolished by any disturbances like fasting and removal from the accustomed environment (Irvine *et al*. 1994; Forhead *et al.* 1995).

The Icelandic horses in the present study had significantly lower concentrations of plasma cortisol than the Standardbred horses. The concentrations measured in the Icelandic horses, are similar to the concentrations measured in Icelandic horses in an earlier study (Ragnarsson *et al.* 2011). In that study, no differences in the concentrations of serum cortisol could be detected between Standardbred and Icelandic horses feed the same diet (Ragnarsson *et al.* 2011). Our groups of horses were not feed the same diet, but previous research have failed to prove any impact of different diets on cortisol concentrations in horses (Stull *et al.* 1988). The mean concentration of cortisol of the sedentary Standardbred horses in the present study is in good agreement with the mean concentration previously reported in well trained cross-bred trekking horses (Medica *et al.* 2010) indicating that the differences in cortisol concentrations must be influenced by other factors than fitness status. Chronic inflammation is known to depress cortisol concentrations in horses (Mills *et al.* 1997) and systemic inflammation is an important component of EMS (Vick *et al*. 2007). The lower mean cortisol concentrations shown in the Icelandic horses in the present study could be related to breed differences, partial IR and/or possible subclinical systemic inflammation or be management dependent.

After transportation and exposure to a new environment, plasma ET-1 concentrations differed significantly compared to the concentrations measured in the home environment. This could be related to a prolonged stress reaction caused by the transportation in combination with spending the night in a new environment. This is supported by the fact that a previous study in horses has shown that ET-1 concentrations increase post-exercise, but return to baseline after 10 minutes of recovery (McKeever *et al*. 2002). No significant difference was detected in the mean cortisol concentrations in the transportation study compared to the measurement in the home environment. This is in contrast to findings from a previous study, were higher serum cortisol levels were found in horses that had been transported compared to a group of non-transported horses (Medica *et al.* 2010). In a study on donkeys it was noted that transportation altered the normal rhythm of resting cortisol to an increased rate of continuous secretion in both fed and fasted animals. The cortisol concentration decreased after transportation and a circadian rhythm was reestablished 8.5-10.5 hours later (Forhead *et al.* 1995). The reason for this discrepancy in results could be related to differences in sampling time. The horses in the present study spent a minimum of 12 hours in the new box stalls before the sample collection took place which might have been long enough for the pulsatile secretion of cortisol to reestablish (Forhead *et al.* 1995; Schmidt *et al.* 2010). In the study by Medica *et al.* (2010), the sample collection was carried out the same day as the transportation and the time interval between transportation and sampling might have been too short for a reestablishment of the pulsatile secretion. It is also possible that the results would have been different if we had measured a metabolite of cortisol in feces post-transportation as a previous study has shown significantly elevated levels of this metabolite one day post-transport (Schmidt *et al*. 2010). However, the results of the present study indicate that serum cortisol and indirect blood pressure can be measured with reliable results if samples are collected at least 12 hours post-transportation.

The significant difference in the concentrations of ET-1 and cortisol between the two breeds was not reflected in any differences in blood pressure. The previously reported systemic hypertension in laminitis-prone ponies was detected in the summer (Bailey *et al.* 2008). The blood pressure measurements in the present study were recorded during the winter. It would therefore be of interest to measure blood pressure as well as plasma ET-1 and serum cortisol on Icelandic- and Standardbred horses over the whole year to be able to detect potential seasonal variations and breed related differences.

Previous studies have found that laminitis prone ponies have a different metabolic profile compared to non-laminitic ponies with significantly higher BCS, plasma insulin concentrations as well as decreased cortisol concentrations (Kibby *et al.* 2006; Bailey *et al.* 2008). The metabolic profile and the predisposition for laminitis also seem to be inherited (Kibby *et al.* 2006). It is possible that the differences reported in plasma ET-1 and serum cortisol between breeds also are inherited and part of a metabolic profile associated with EMS. However, this must be further investigated on a larger material of horses of different breeds.

In conclusion, indirect blood pressure measurement, plasma ET-1 and serum cortisol all showed an acceptable day-to-day variation and are candidates for further investigation in horses with Equine metabolic syndrome. Both the Cardell and the HDO device can be used for measurement of indirect blood pressure in horses, but the HDO device was most practical to handle, especially in a field setting. There seem to exist a breed difference in the concentrations of plasma ET-1 and serum cortisol, and that has to be considered if one intends to use these parameters in clinical settings. There are indications that test results can be influences by transportation and housing in a new environment, as was the case with ET-1 in the present study. Future studies that investigate the possible seasonal variation in blood pressure, ET-1 and cortisol on healthy horses of different breeds and of horses clinically affected of EMS are desirable.

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