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Asymmetric dimethylarginine—A potential cardiac biomarker in horses☆

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Abstract *Introduction/objectives:* Asymmetric dimethylarginine (ADMA) is a cardiac biomarker in humans, symmetric dimethylarginine (SDMA) a renal biomarker in humans, cats, and dogs. The purpose of this prospective study was to investigate if measuring serum ADMA and SDMA concentrations via ELISA allows detection of cardiac disease in horses in a routine laboratory setting. In this context, reference values in horses were established.
Animals, materials, and methods: Seventy-eight horses with no known medical history were compared to 23 horses with confirmed structural cardiac disease with/or without arrhythmias. Horses underwent physical examination, electrocardiography, echocardiography and venous blood sampling and were staged based on the severity of cardiac disease from 0 to II. Asymmetric dimethylarginine and SDMA were measured via ELISA and crosschecked using liquid chromatograph triple quadrupole mass spectrometry. Reference intervals with 90th percent confidence intervals were evaluated and standard software was used to test for significant differences in ADMA, SDMA, and the L-arginine/ADMA ratio between groups.
Results: The reference ranges were 1.7—3.8 μmol/L and 0.3—0.8 μmol/L for ADMA

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and SDMA, respectively. Serum ADMA was higher in horses with heart disease compared to healthy horses ($p < 0.01$) and highest in horses with stage II heart disease ($p = 0.02$). The L-Arginine/ADMA ratio was significantly higher in healthy animals than those with cardiac disease ($p = 0.001$).
Conclusions: Reference values for serum ADMA and SDMA using ELISA methods are presented in horses. This study confirms the association between heart disease and increased serum ADMA concentration as well as a decreased L-Arginine/ADMA ratio in horses.

Abbreviations

ADMA	asymmetric dimethylarginine
LC-MS/MS	liquid chromatograph triple quadrupole mass spectrometry
NO	nitric oxide
SDMA	symmetric dimethylarginine

Introduction

Asymmetric dimethylarginine (ADMA) is a naturally occurring modified amino acid that inhibits the production of nitric oxide (NO), a key regulator of vascular tone [1—3]. Nitric oxide is produced from L-arginine as the natural substrate of NO synthase [2—4]. However, NO synthesis can be reduced by at least two of three different derivatives of L-arginine: symmetric dimethylarginine (SDMA), ADMA, and possibly also by monomethyl-L-arginine [4—6]. All three are generated via intracellular methylation of arginine via protein arginine methyltransferases and released into plasma in exchange for arginine and other cationic amino acids [4—7].

An increasing number of studies have linked elevated concentrations of circulating ADMA to cardiovascular disease [3,8—14]. Furthermore, a growing number of studies showed a strong link between the L-arginine/ADMA ratio and the severity of chronic heart failure in humans [15—17], raising the question whether this ratio might also be useful for identifying cardiovascular risk in equines.

After intracellular uptake by major organs such as the liver, brain, or kidney over 80% of circulating ADMA is eliminated through enzymatic degradation by the enzyme dimethylarginine dimethylaminohydrolase [18—20]. Symmetric dimethylarginine is primarily eliminated through renal filtration and correlates with both measured and estimated glomerular filtration rate. The importance of SDMA as an endogenous marker of renal function in humans, cats, and dogs is rising [19—24]. In dogs and cats, SDMA is used both for early identification and monitoring of decreased renal function in kidney disease [21,22].

In contrast and despite promising potential, research on the topic of SDMA and ADMA in cardiac or renal disease in horses is currently lacking. This is unfortunate since due to the species-independent chemical structure of SDMA, ELISA techniques developed for humans have emerged as useful and cost-efficient screening tools in veterinary sciences under practical clinical conditions [21,22].

The purpose of this prospective study was to investigate if measuring serum SDMA and ADMA concentrations via ELISA in a routine laboratory setting allows detection of cardiac disease in horses. We hypothesized that ADMA serum concentration might be higher in horses with cardiac disease compared to healthy horses. In this context, reference values in horses were established.

Animals, materials, and methods

The study was performed in compliance with guidelines from the Ethical Committee and European Union legislation. Permission to conduct the study was attained from national authorities (license number 0206/18). Horses were recruited either at national or international horse competitions or by a public call for study participation.

Horses without cardiac disease served as reference group. Inclusion criteria for the healthy group were no known history or clinical signs of cardiac disease or any other diseases. Cardiovascular

disease was excluded based on echocardiography and electrocardiography. Horses with a physiological atrioventricular block were included in the healthy group, provided that the block disappeared during physical activity. Horses with more than trivial (physiological) valve regurgitation on colou flow Doppler echocardiography or a pathological arrhythmia on electrocardiogram were excluded from the healthy group. Cardiovascular disease was further excluded based on venous blood sampling for cardiac biomarkers (cardiac troponin I, lactate dehydrogenase, α-hydoxybutyrate dehydrogenase). For exclusion of renal disease creatinine and urea were measured. For horses to be included within the reference group, cardiac troponin I, lactate dehydrogenase, α-hydoxybutyrate dehydrogenase, creatinine, and urea had to be within the reference ranges. Only horses with confirmed heart disease were included in the second group of horses with cardiac disease. Animals with the presence of any other disease were excluded from the study.

In addition to obtaining the medical history for each animal, all animals were subjected to a general physical examination, echocardiography, electrocardiography, and venous blood sampling for cardiac and renal biomarkers. Heart rate, respiratory rate, rectal temperature, pulse quality, mucous membranes, and heart sounds were assessed.

Echocardiography and electrocardiography

Transthoracic echocardiography was performed by means of a portable ultrasound unit[d] with a phased array transducer and simultaneous electrocardiogram recording. A single observer assessed cardiac structures, valvular competence, chamber dimensions and the diameters of the aorta, the pulmonary artery, and left ventricular systolic function by routine two-dimensional, motion mode, and color flow Doppler echocardiography[e].

Electrocardiographic recordings were obtained with a Holter recording system[f] with two channels and bipolar leads. Electrodes were secured with adhesive foam patches.

Grading of cardiac disease

Severity of cardiac disease was graded using a modification of the system proposed by Gehlen 2010 [25]. Horses in stage 0 were healthy and horses in stage I had mild to moderate valvular incompetence and cardiac dimensions within the reference range. Horses in stage II had increased cardiac dimensions due to severe valvular incompetence, in some cases with concomitant additional arrhythmia or increased cardiac troponin I and pericardial effusion. The valvular incompetence was graded according to the quantification methods defined by Gehlen et al. [25,26], Stadler et al. [27], Vahanian et al. [28] and Young et al. [29].

Laboratory analysis

Blood samples were obtained from the jugular vein via 18-gauge needles in all horses. Samples were collected in 6 mL EDTA tubes and 20 mL serum separator tubes. Serum was separated by centrifugation at a force of 1800×*g* for 10 min after clotting was completed. EDTA-plasma was also separated by centrifugation at a force of 1800×*g* for 10 min. Serum and EDTA-plasma were transferred into 2 mL cryotubes and frozen on dry ice until storage at −80°C. Serum and EDTA-plasma were sent on dry ice for analysis to different laboratories. Total storage time was 8 months at −80°C. Lactate dehydrogenase, α-hydoxybutyrate dehydrogenase, creatinine, and urea were measured with ELISA technology with conventional methods from serum samples[g]. Cardiac troponin I was determined in serum by an immunoassay system[h].

Symmetric dimethylarginine and asymmetric dimethylarginine were measured in serum samples in duplicate with commercial human ELISA

[d] Vivid I, GE Healthcare GmbH, Torgauer Str. 12—15, Berlin, Germany.
[e] EchoPAC, clinical workstation software, GE Healthcare GmbH, Torgauer Str. 12—15, Berlin, Germany.
[f] Televet-100 electrocardiogram device from Engel Engineering Service GmbH, Heusenstamm, Germany.
[g] Analyses of lactate dehydrogenase, α-hydoxybutyrate dehydrogenase, creatinine, and urea were run by Laboklin GmbH & CoKG with an analyzer from Bio Aim Scientific Inc., Steubenstraβe 4, 97,688 Bad Kissingen, Germany.
[h] Analysis of cardiac troponin I was run by Laboklin GmbH & CoKG with Immulite 2000XPi Siemens, Steubenstraβe 4, 97,688 Bad Kissingen, Germany.

technology[i]. The average of the duplicate determinations was used.

Ten values, six of the highest measured values from the group with cardiac disease and four values within the medium range measured in the control group, were crosschecked using liquid chromatograph triple quadrupole mass spectrometry (LC-MS/MS)[j].

For L-arginine analysis 400 μL EDTA plasma, 400 μL sample dilution buffer (including standard norleucine 100 nmol/mL), and 200 μL precipitation solution for the deproteinization were stored at 4°C for 20 min. The suspension was centrifuged at a force of 13,150×*g* for 5 min and the supernatant subsequently filtered with a membraspin filter at a force of 13,150×*g* for 5 min. L-arginine was analysed by the amino acid analyser Aracus[k]. Integration and calculation of the analysis were performed via aminoPeak software[k].

Statistics

Reference intervals were determined from 78 healthy horses for SDMA and ADMA. Data were evaluated according to Principles of Quality Assurance and Standards for Veterinary Clinical Pathology guidelines and calculated using the program Reference Value Advisor [30]. Except for SDMA, data were not normally distributed (Anderson-Darling test). The instructions of the International Federation of Clinical Chemistry—Clinical and Laboratory Standards Institute C28-A3 guidelines were followed in order to calculate nonparametric 90% reference intervals using bootstrap methods [31]. The 95% reference intervals were calculated by nonparametric methods, and then, the 90% confidence intervals about the lower and upper limits of the reference interval were calculated.

Statistical Package for the Social Sciences version 25 was used for all statistical analyses described in the following. Differences between horses with and without cardiac disease regarding ADMA, SDMA, and L-arginine/ADMA ratio were investigated using the t-test (for SDMA) and the Mann-Whitney U test (two groups, healthy (stage 0) and horses in stage I and II) or Kruskal-Wallis test (comparison of healthy horses (stage 0), horses stage I to II), as appropriate. p-values < 0.05 were regarded as statistically significant.

Lin's Concordance Coefficient was calculated to assess agreement between ten laboratory ADMA and SDMA values estimated with the ELISA[i] and the reference method LC-MS/MS[j]. Since some ADMA samples ran far out of the human reference range for the LC-MS/MS[j] method, samples were confirmed once again after dilution.

Results

The study population involved 78 healthy horses and 23 horses with confirmed cardiac disease.

The study population of healthy horses consisted of animals without known clinical disease, aged between 4 and 21 years (average: 11.9 years, median: 12 years). Animals weighed between 285 and 575 kg (average: 417 kg, median 410 kg).

All horses in the group exercised regularly and most horses were used for endurance sport (n = 66). Horses originated from diverse locations (Germany, Switzerland, Belgium, Italy, Austria, Sweden, Norway, Portugal, Croatia, the Netherlands, United Kingdom, Algeria, Australia, Chile, Uruguay, Columbia, Brazil, Ecuador, USA, Malaysia, Thailand, South Africa, and Bahrain). Breeds included Arabian Thoroughbred (n = 34), Arabian Partbred (n = 5), Shagya Arabian (n = 4), Anglo-Arabian (n = 7), German Warmblood (n = 3), Standardbred (n = 5), German Riding Pony (n = 4), Haflinger crossbreed (n = 1), Pinto (n = 1), Appaloosa (n = 1), Arabian Akhal-Teke crossbreed (n = 1), Karbarda (n = 1), and unknown (n = 11). There were 43 geldings, one stallion, and 34 mares.

Horses in the group with cardiac disease (n = 23) were between 2 and 29 years of age (average: 15.8 years, median: 15 years) and weighed between 240 and 750 kg (average 536 kg, median 580 kg). All horses came from Germany. Breeds consisted of German Warmblood (n = 14), Standardbred (n = 2), German Riding Pony (n = 2), Arabian Thoroughbred (n = 1) Appaloosa (n = 1), American Quarter Horse (n = 1), Pinto (n = 1), Trakehner (n = 1). The group included 13 geldings, three stallions, and seven mares. One horse that initially presented as healthy was excluded from the study, since it had a heart murmur 3/6 and more than trivial aortic valve regurgitation on color flow Doppler echocardiography.

[i] Analyses of ADMA and SDMA via Fast ELISA were run by DLD Gesellschaft für Diagnostika und medizinische Geräte mbH, Adlerhorst 15, 22,459 Hamburg, Germany.

[j] Analyses of ADMA and SDMA via LC - MS/MS were run by Medizinisches Labor Bremen GmbH, Haferwende 12, 28,357 Bremen, Germany.

[k] Analysis of L-arginine was run by MembraPure, Gesellschaft für Membrantechnik mbH, Wolfgang Küntscher Str. 14, 16,761 Hennigsdorf/Berlin, Germany.

Table 1 Reference range (90th percent confidence interval) in μmol/L for asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) in healthy animals. Horses with cardiac disease in stage I and II, with median and average ADMA (μmol/L) concentration. An asterisk (*) indicates data are normally distributed and a section sign (§) indicates data are not normally distributed. Values with different superscripts (a, b) are significantly different ($p < 0.005$). ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine.

Stage	Reference range SDMA (μmol/L)	Reference range ADMA (μmol/L)	SDMA (μmol/L) * Mean ± SD	ADMA (μmol/L)§ Median (25 -percentile, 75 -percentile)	Number of horses
0	0.3—0.8	1.7—3.8	0.525 ± 0.114[a]	2.53 (2.16, 2.80)[a]	78
90% CI for lower limit	0.3—0.3	1.5—1.8			
90% CI for upper limit	0.7—0.9	3.5—3.8			
I			0.518 ± 0.092[a]	2.73 (2.50, 3.19)[a,b]	15
II			0.584 ± 0.055[a]	3.19 (3.00, 3.57)[b]	7

Reference values for ADMA and SDMA were determined in 78 healthy horses (Table 1). Lin's Concordance Coefficient revealed an excellent test agreement between the ELISA[i] and the reference method (LC-MS/MS[j]) with 0.91 for ADMA and 0.94 for SDMA.

Of the horses with cardiac disease, 14 were in stage I and 9 in stage II (Table 1). The values of ADMA and cardiac troponin I as well as the clinical symptoms (stage II) are given in Table A (available in Supplemental Material online).

Apart from one horse with pericardial effusion (horse stage II), cardiac troponin I was within the reference range (<0.03 ng/mL) (Table A, available in Supplemental Material online).

The values of (normally distributed) SDMA data did not differ significantly between healthy horses and horses with cardiac disease ($p = 0.6$), but the values of ADMA differed significantly, with horses suffering from cardiac disease having higher values than healthy animals ($p = 0.003$) (Fig. 1). Likewise, no significant differences could be detected between animals in stage 0 to II ($p = 0.2$) for SDMA, but ADMA showed significant differences between the groups ($p = 0.007$) with significantly higher values in stage II horses compared to healthy ones (adjusted $p = 0.02$) (Table 1, Fig. 2). In addition,

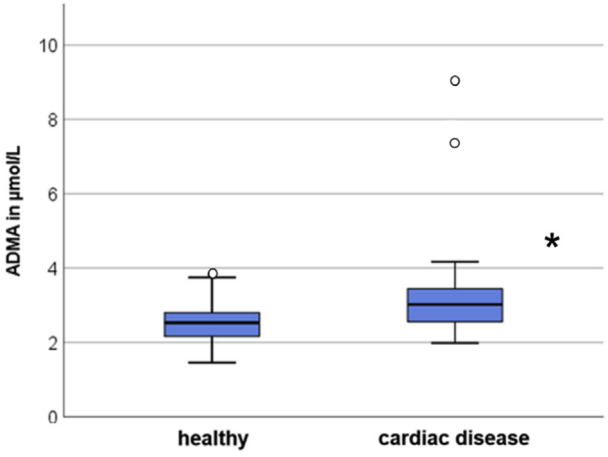

Fig. 1 Boxplot showing serum asymmetric dimethylarginine (ADMA) concentration in μmol/L in healthy horses and horses with cardiac disease. Asymmetric dimethylarginine (μmol/L) showed significantly higher values in horses with cardiac disease (stage II) compared to healthy animals. The circles (∘) represent outliers between 1.5 and 3.0 times the interquartile range (IQR). The asterisk (*) indicates $p = 0.003$. ADMA: asymmetric dimethylarginine.

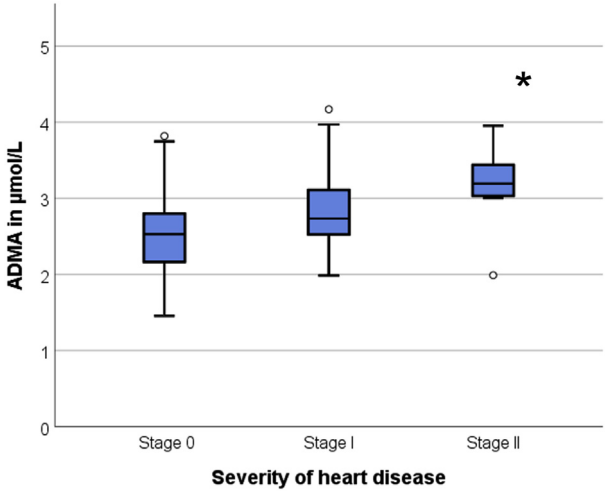

Fig. 2 Boxplot showing serum asymmetric dimethylarginine (ADMA) concentration in μmol/L in healthy horses (stage 0) and horses with cardiac disease in stage I and II. The circles (∘) represent outliers. The asterisk (*) indicates a significant difference between stage 0 and stage II horses, $p = 0.007$. ADMA: asymmetric dimethylarginine.

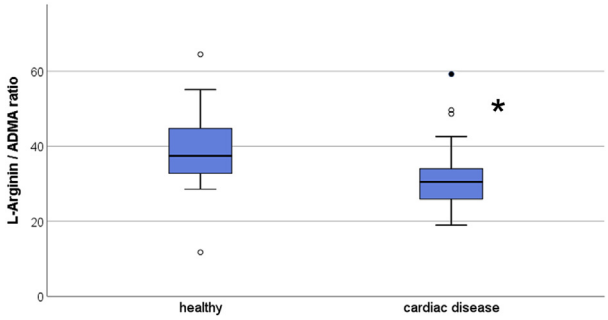

Fig. 3 Boxplot showing the distribution of the ratio between L-arginine (μmol/L) and asymmetric dimethylarginine (ADMA) (μmol/L) measured in healthy horses and in horses with cardiac disease, showing a clear shift of the distribution toward higher values in healthy animals. Differences between healthy horses and horses with cardiac disease were statistically significant ($p = 0.001$, Mann-Whitney U test). The circles (∘) represent outliers between 1.5 and 3.0 times the interquartile range (IQR), the filled circle (•) indicates outliers beyond 3.0 times IQR. The asterisk (*) indicates $p = 0.001$. ADMA: asymmetric dimethylarginine.

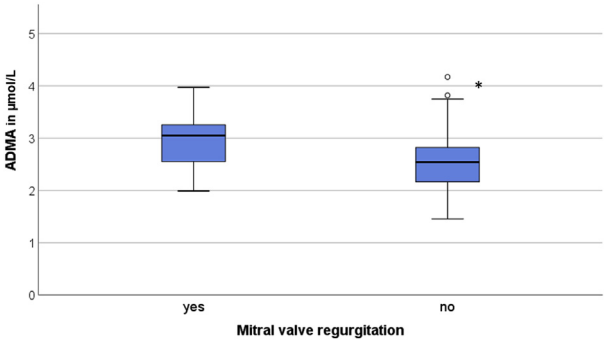

Fig. 4 Boxplot showing asymmetric dimethylarginine (ADMA) serum concentration in μmol/L in horses with mitral valve regurgitation (n = 14, 8 in stage I and 6 in stage II) and in healthy horses (without valve regurgitation) (n = 78). Horses with mitral valve regurgitation had significantly higher serum ADMA (μmol/L) concentrations than healthy horses. The circles (∘) represent outliers. The asterisk (*) indicates $p = 0.007$. ADMA: asymmetric dimethylarginine.

the ratio between L-arginine and ADMA was significantly higher in healthy horses than in horses with cardiac disease ($p = 0.001$) (Fig. 3).

Six horses suffered from atrial fibrillation, three with and three without increased left atrial diameter and area. Furthermore, one horse was diagnosed with premature atrial complexes.

Horses with arrhythmia had an increased ADMA serum concentration. Unfortunately, the data structure did not make it possible to apply a multivariable analysis, which would have given further information concerning other influence factors.

Horses with arrhythmia ($p = 0.01$), mitral valve regurgitation ($p = 0.007$) (Fig. 4), and regurgitation of more than two valves ($p = 0.02$) were associated with a significantly higher ADMA.

Discussion

Immunoassays have become the method of choice for measurement of diagnostic markers in biouids such as serum. In the present study, ADMA differed significantly between healthy horses and horses with cardiac disease. Animals with cardiac disease had higher values than healthy animals. In contrast to humans, the L-arginine/ADMA ratio in horses showed no advantage compared to ADMA alone. However, the finding that the L-arginine/ADMA ratio is significantly higher in healthy horses than in horses suffering from cardiac disease is an important finding. Since ADMA competes with L-arginine for NO synthase, the bioavailability of NO depends on the balance between the two. The L-arginine/ADMA ratio is an indicator of NO bioavailability and is therefore emerging as a potential marker of cardiovascular diseases in human medicine [32]. In what way the L-arginine/ADMA ratio affects horses and if it is a cause or the result of the associated cardiac disease cannot be clarified in the present study.

Despite our finding that ADMA differed significantly between healthy animals and animals with cardiac disease, the latter group rarely exceeded the upper reference range of 3.8 μmol/L (Table A, available in Supplemental Material online). This is most likely a result of the limited number of horses and the wide range of data included in the study, leading to a high reference range for the 90th percent confidence interval and calling for the necessity of follow-up studies with a greater number of animals.

In humans, ADMA measurement can be useful for assessment of endothelial dysfunction as a predictor of cardiovascular disease risk [3,6,8]. However, horses do not suffer from "typical" human cardiovascular diseases, such as hypertension, atherosclerosis, carotid artery intima-media thickness, angina pectoris, and heart attacks. However, atrial fibrillation is a disease seen in both humans and horses.

A study conducted by Horowitz and coworkers has shown that ADMA is elevated in human patients with permanent atrial fibrillation [33]. Notably, under conditions of NO synthase substrate (arginine) depletion, ADMA leads to an "uncoupling" of

NO synthase so that electron transfer is shifted from L-arginine to molecular oxygen, yielding the radical superoxide anion. The role of endogenous oxygen radicals in targeting DNA, proteins, lipids, and other components of the cell has long been known. Interestingly, studies on knockout mice that lacked an enzyme responsible for mitochondrial radical superoxide anion removal exhibited perinatal lethality due to cardiac dysfunction and congestive heart failure [34]. However, it appears likely that elevated levels of ADMA are the consequence and not the cause of cardiac disease in horses.

Horowitz and coworkers [33] mentioned that the precise mechanism(s) whereby ADMA concentrations modulate outcomes in patients with chronic atrial fibrillation remains somewhat putative. Dimethylarginine dimethylaminohydrolase, the enzyme that plays a primary role in ADMA metabolism is inhibited by oxidative stress [35] and there is evidence that myeloperoxidase, an enzyme of leukocyte origin that has been implicated in the pathogenesis of atrial fibrillation, may deactivate dimethylarginine dimethylaminohydrolase [36]. Determination of concentrations and activity of both dimethylarginine dimethylaminohydrolase and myeloperoxidase might have helped to further delineate the precise mechanism(s) [36].

The immunoassay used in the current study has been evaluated for SDMA in humans, cats, and dogs and for ADMA in humans [21,22,37–40]. The immunoassay showed adequate precision with intra-and inter assay coecients of variation lower than 15% [21,22,38,39]. Similar studies in horses have not been published so far. The reference values for equine SDMA determined in the present study ranged between 0.3 and 0.8 μmol/L. Reference values for SDMA in humans, cats, and dogs cited by DLD Diagnostika GmbH[j] are quite similar (humans and cats: 0.30–0.75 μmol/L, dogs: 0.30–0.65 μmol/L) [39]. The equine reference values for ADMA established in this study appear to be noticeably higher than those found in humans (0.40–0.75 μmol/L) [37,40]. This raised the question why values in horses are so much higher or whether possibly, these values were falsely high. For this reason, we crosschecked six outliers and four controls from the medium range using mass spectrometry. In total, ten ADMA and SDMA values were crosschecked using LC-MS/MS[j] and both methods agreed very well. Notably, the ADMA ELISA test[i] used in the present study has already been assessed in humans and compared to the LC-MS/MS[j] method [37,40], also showing good agreement. Since ADMA and SDMA are well-defined, small compounds with a structure that is identical in humans and equines, our finding that both methods correlate well in horses is hardly surprising.

Therefore, there appears to be other reasons for the higher ADMA levels in horses. Notably, the uptake of ADMA into the cytosol for degradation occurs via an exchanger from the anion/cation transporter family that requires intracellular presence of dibasic amino acids such as arginine, lysine, and ornithine as a substrate [41]. As a herbivore, horses may well have a different amino acid profile than carnivorous species [42–44], possibly leading to a different profile.

The major limitation of the study is the small sample size of 23 horses with cardiac disease that lowered statistical power and might have impacted the ability to detect differences between the stages of cardiac disease, especially in stage II. Furthermore, there is the possibility of a breed effect on ADMA and SDMA concentration. Therefore, the reference intervals might not be relevant to other breeds of horses. Although samples from the entire range were selected for comparison of the ELISA[i] and LC-MS/MS[j] methods, sampling was not random since six of the highest measured values were included. Strictly speaking, it is thus not possible to generalize the results, although they are in good agreement with previous studies [37,39,40]. Furthermore, storage time of the samples was 8 months and therefore values could have decreased over time. No data on storage time for ADMA or SDMA has been published to date. The laboratory that carried out the analysis[i] has real time data on storage time for at least 5 years from 32 quality control samples (human serum) that were frozen at −23°C. Over the past 5 years, these quality control samples were measured seven times and showed almost identical results. At −80°C ADMA and SDMA should be at least as stable.

Conclusions

In the present study, horses with cardiac disease had significantly higher serum ADMA concentration and a decreased L-arginine/ADMA ratio compared to healthy horses. Although the physiological reasons for higher serum ADMA concentration and a decreased L-arginine/ADMA ratio in horses with cardiac disease remain elusive, this study demonstrates that ADMA is a promising biomarker not just in humans, but also shows diagnostic potential in identifying cardiac disease in horses under routine conditions.

The manuscript is original. No part of the manuscript has been published before, nor is any part of it under consideration for publication in another journal. There are no conflicts of interest to disclose.

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Supplementary data

Supplementary data to this article can be found online at .

References

[1] Dias RG, Negrao CE, Krieger MH. Nitric oxide and the cardiovascular system: cell activation, vascular reactivity and genetic variant. Arq Bras Cardiol 2011;96:68—75.
[2] Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol 2012;10:4—18.
[3] Ardigo D, Stüehlinger M, Franzini L, Valtueña S, Piatti PM, Pachinger O, Reaven GM, Zavaroni I. ADMA is independently related to flow-mediated vasodilation in subjects at low cardiovascular risk. Eur J Clin Invest 2007;37:263—9.
[4] Morris SM. Arginine metabolism: boundaries of our knowledge. J Nutr 2007;137:1602—9.
[5] Böger GI, Rudolph TK, Maas R, Schwedhelm E, Dumbadze E, Bierend A, Benndorf RA, Böger RH. Asymmetric dimethylarginine determines the improvement of endothelium-dependent vasodilation by simvastatin: effect of combination with oral L-arginine. J Am Coll Cardiol 2007;49:2274—82.
[6] Böger RH, Bode-Böger SM, Kienke S, Stan AC, Nafe R, Frölich JC. Dietary L-arginine decreases myointimal cell proliferation and vascular monocyte accumulation in cholesterol-fed rabbits. Atherosclerosis 1998;136:67.
[7] Tain YL, Hsu CN. Toxic dimethylarginines: asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA). Toxins 2017;9:92.
[8] Bai Y, Sun L, Du L, Zhang T, Xin W, Lan X, Du G. Association of circulating levels of asymmetric dimethylarginine (ADMA) with carotid intima-media thickness: evidence from 6168 participants. Ageing Res Rev 2013;12:699—707.
[9] Böger RH, Bode-Böger SM, Tsao PS, Lin PS, Chan JR, Cooke JP. An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. J Am Coll Cardiol 2000;36:2287—95.
[10] Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA 1991;88:4651—5.
[11] Tsao PS, Buitrago R, Chan JR, Cooke JP. Fluid flow inhibits endothelial adhesiveness. Nitric oxide and transcriptional regulation of VCAM-1. Circulation 1996;94:1682—9.
[12] Jiang DJ, Jia SJ, Dai Z, Li YJ. Asymmetric dimethylarginine induces apoptosis via p38 MAPK/caspase-3-dependent signalling pathway in endothelial cells. J Mol Cell Cardiol 2006;40:529—39.
[13] Böger RH, Bode-Böger SM, Mügge A, Kienke S, Brandes R, Dwenger A, Frölich JC. Supplementation of hypercholesterolaemic rabbits with L-arginine reduces the vascular release of superoxide anions and restores NO production. Atherosclerosis 1995;117:273—84.
[14] Wolf A, Zalpour C, Theilmeier G, Wang BY, Ma A, Anderson B, Tsao PS, Cooke JP. Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. J Am Coll Cardiol 1997;29:479—85.
[15] Seljeflot I, Nilsson BB, Westheim AS, Bratseth V, Arnesen H. The L-arginine-asymmetric dimethylarginine ratio is strongly related to the severity of chronic heart failure. No effects of exercise training. J Card Fail 2011;17:135—42.
[16] Anderssohn M, Rosenberg M, Schwedhelm E, Zugck C, Lutz M, Lüneburg N, Frey N, Böger RH. The L-arginine-asymmetric dimethylarginine ratio is an independent predictor of mortality in dilated cardiomyopathy. J Card Fail 2012;18:904—11.
[17] Ekeloef S, Oreskov JO, Falkenberg A, Burcharth J, Schou-Pedersen AMV, Lykkesfeldt J, Gögenur I. Endothelial dysfunction and myocardial injury after major emergency abdominal surgery: a prospective cohort study. BMC Anesthesiol 2020;20:67.
[18] Kielstein JT, Böger RH, Bode-Böger SM, Martens-Lobenhoffer J, Lonnemann G, Frölich JC, Haller H, Fliser D. Low dialysance of asymmetric dimethylarginine (ADMA) in vivo and in vitro evidence of significant protein binding. Clin Nephrol 2004;62:295—300.
[19] Kielstein JT, Salpeter SR, Bode-Boeger SM, Cooke JP, Fliser D. Symmetric dimethylarginine (SDMA) as endogenous marker of renal function—a meta-analysis. Nephrol Dial Transplant 2006;21:2446—51.
[20] Schwedhelm E, Böger RH. The role of asymmetric and symmetric dimethylarginines in renal disease. Nat Rev Nephrol 2011;7:275—85.
[21] Nabity MB, Lees GE, Boggess MM, Yerramilli M, Obare E, Yerramilli M, Rakitin A, Aguiar J, Relford R. Symmetric dimethylarginine assay validation, stability, and evaluation as a marker for the early detection of chronic kidney disease in dogs. J Vet Intern Med 2015;29:1036—44.
[22] Hall JA, Yerramilli M, Obare E, Yerramillie M, Jewell DE. Comparison of serum concentrations of symmetric dimethylarginine and creatinine as kidney function biomarkers in cats with chronic kidney disease. J Vet Intern Med 2014;28:1676—83.

[23] Young LE, Rogers K, Wood JL. Heart murmurs and valvular regurgitation in Thoroughbred racehorses: epidemiology and associations with athletic performance. J Vet Intern Med 2008;22:418—26.
[24] Younes M, Robert C, Barrey E, Cottin F. Effects of age, exercise duration, and test conditions on heart rate variability in young endurance horses. Front Physiol 2016;7:155.
[25] Gehlen H. Pferdekardiologie. Hannover: Schültersche Verlagsgesellschaft mbH & Co. KG; 2010. p. 165.
[26] Gehlen H, Stadler P, Deegen E. Grading of equine mitral valve insufficiency by colour flow Doppler echocardiography. Pferdeheilkunde 1998;14:303—14.
[27] Stadler P, Höch M, Frühauf B, Deegen E. Echocardiography in horses with and without heart murmurs in aortic regurgitation. Pferdeheilkunde 1995;11:373—83.
[28] Vahanian A, Baumgartener H, Bax J, Butchart E, Dion R, Filippatos G, Flachskampf F, Hall R, Lung B, Kasprzak J, Nataf P, Tornos P, Torracca L, Wenink A. Guidelines on the management of valvular heart disease: the task force on the management of valvular heart disease of the European society of cardiology. Eur Heart J 2007;28:230—68.
[29] Young LE, Rogers K, Wood JL. Left ventricular size and systolic function in Thoroughbred racehorses and their relationships to race performance. J Appl Physiol 2005;99:1278—85.
[30] Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton RM, Barnhart KF, Blanco-Chavez J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol 2012;41:441—53.
[31] CLSI and IFCC. C28-A3 document; Defining, establishing and verifying reference intervals in the clinical laboratory: approved guideline. 3rd ed.vol. 28; 2008. p. 1—76.
[32] Bode-Boger SM, Scalera F, Ignarro LJ. The L-arginine paradox: importance of the L-arginine/asymmetrical dimethylarginine ratio. Pharmacol Ther 2007;114:295—306.
[33] Horowitz JD, De Caterina R, Heresztyn T, Alexander JH, Andersson U, Lopes RD, Steg PG, Hylek EM, Mohan P, Hanna M, Jansky P, Granger CB, Wallentin L. Asymmetric and symmetric dimethylarginine predict outcomes in patients with atrial fibrillation: an Aristotle Substudy. J Am Coll Cardiol 2018;72:721—33.
[34] Sugamura K, Keaney JF. Reactive oxygen species in cardiovascular disease. J Free Radic Biol Med 2011;51:978—92.
[35] Frombaum M, Therond P, Djelidi R, Beaudeux JL, Bonnefont-Rousselot D, Borderie D. Piceatannol is more effective than resveratrol in restoring endothelial cell dimethylarginine dimethylaminohydrolase expression and activity after high-glucose oxidative stress. Free Radic Res 2011;45:293—302.
[36] von Leitner EC, Klinke A, Atzler D, Slocum JL, Kielstein JT, Maas R, Schmidt-Haupt R, Pekarova M, Hellwinkel O, Tsikas D, D'Alecy LG, Lau D, Willems S, Kubala L, Ehmke H, Meinertz T, Blankenberg S, Schwedhelm E, Gadegbeku CA, Böger RH, Baldus S, Sydow K. Pathogenic cycle between the endogenous nitric oxide synthase inhibitor asymmetrical dimethylarginine and the leukocyte-derived hemoprotein myeloperoxidase. Circulation 2011;124:2735—45.
[37] Schulze F, Wesemann R, Schwedhelm E, Sydow K, Albsmeier J, Cooke JP, Böger RH. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. Clin Chem Lab Med 2004;42:1377—83.
[38] Kuleš J, Bilić P, Beer Ljubić B, Gotić J, Crnogaj M, Brkljačić M, Mrljak V. Glomerular and tubular kidney damage markers in canine babesiosis caused by Babesia canis. Ticks Tick Borne Dis 2018;9:1508—17.
[39] https://www.dld-diagnostika.de/uploads/uploads/SDMA-D-85d2.pdf.
[40] https://www.dld-diagnostika.de/product/84.
[41] Teerlink T, Luo Z, Palm F, Wilcox CS. Cellular ADMA: regulation and action. Pharmacol Res 2009;60:448—60.
[42] DeBoer ML, Martinson KL, Kuhle KJ, Sheaffer CC, Hathaway MR. Plasma amino acid concentrations of horses grazing alfalfa, cool-season perennial grasses, and teff. J Equine Vet Sci 2019;72:72—8.
[43] Dor C, Adamany JL, Kisielewicz C, de Brot S, Erles K, Dhumeaux MP. Acquired urea cycle amino acid deficiency and hyperammonaemic encephalopathy in a cat with inflammatory bowel disease and chronic kidney disease. JFMS Open Rep 2018;4. 2055116918786750.
[44] Tamura Y, Ohta H, Kagawa Y, Osuga T, Morishita K, Sasaki N, Takiguchi M. Plasma amino acid profiles in dogs with inflammatory bowel disease. J Vet Intern Med 2019;33:1602—7.