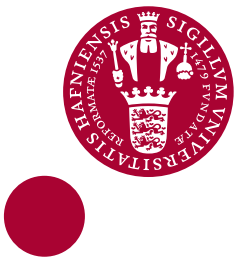

Decreased plasma concentration of nitric oxide metabolites in dogs with untreated mitral regurgitation

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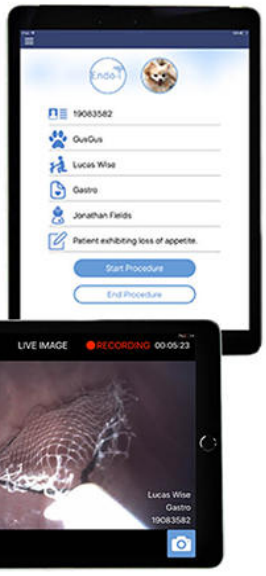

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Decreased Plasma Concentration of Nitric Oxide Metabolites in Dogs with Untreated Mitral Regurgitation

Henrik D. Pedersen, Trine Schütt, Rikke Søndergaard, Karen Qvortrup, Lisbeth H. Olsen, and Annemarie T. Kristensen

Endothelium-dependent (nitric oxide [NO]-mediated) vasodilation is impaired in humans with heart failure. This dysfunction is an important therapeutic target. The plasma concentration of the NO metabolites nitrate and nitrite (collectively referred to as NOx) is a measure of whole-body NO production, provided that the dietary intake of the ions is low. Fifty clinically healthy dogs older than 1 year (median 5.0 years; interquartile interval 2.6–8.2 years) were studied, including 9 controls of various breeds, 23 Cavalier King Charles Spaniels (CKCSs) with no or minimal mitral regurgitation (MR), 9 CKCSs with mild MR (regurgitant jet occupying 15–50% of the left atrial area), and 9 CKCS with moderate to severe MR (jet >50%) due to myxomatous valve disease. None of the dogs received medication. The dogs were given NOx-free water and a diet with a low concentration of NOx for 96 hours before blood sampling. Multiple linear regression analysis revealed that dog group, but not gender, age, serum creatinine concentration, and platelet count, was associated with NOx concentrations. Control dogs had the same NOx concentration (median 20.0 μM; interquartile interval 15.1–25.5 μM) as CKCSs without MR (median 18.7 μM; interquartile interval 15.5–25.9 μM). Compared to CKCSs without MR, the NOx concentration was lower in CKCSs with mild (median 12.9 μM; interquartile interval 11.0–13.5 μM; $P = .04$) and moderate to severe (median 11.2 μM; interquartile interval 6.9–17.1 μM; $P = .02$) MR. In conclusion, CKCSs with mild to severe, clinically silent MR have decreased plasma NOx concentrations, suggesting that endothelial dysfunction develops early in the course of developing MR in dogs.

Key words: Endothelial dysfunction; Heart failure; Mitral valve prolapse; Nitric oxide; Pathophysiology.

Nitric oxide (NO) plays a key role in the regulation of numerous processes in the body. Vasodilation in response to physiologic stimuli such as acetylcholine and increased blood flow is mainly mediated by NO produced in the endothelium.[1,2] Humans with heart failure and dogs with experimentally induced heart failure have impaired endothelium-dependent vasodilation.[2–4] Endothelial dysfunction is an integral part of the complex pathophysiology of the development of heart failure, and correction of the condition is an important therapeutic target.[5] In humans, endothelial dysfunction is present early in the course of developing heart failure, although the etiology of heart failure seems to be less important.[5–7] The presence of endothelial dysfunction in dogs with naturally occurring heart disease has not been documented.

Mitral regurgitation (MR) caused by myxomatous mitral valve disease is the most common cause of heart failure in dogs. Mitral valve prolapse plays an important role in the development of myxomatous mitral valve disease.[8–10] Mitral regurgitation has been studied in Cavalier King Charles Spaniels (CKCSs), which are predisposed to mitral valve prolapse. Approximately 90% of CKCSs have mitral valve prolapse at 3–4 years of age and approximately 90% have auscultatory evidence of MR at 10 years of age.[10,11] In addition to mitral valve prolapse, approximately one third of CKCSs have clinically inapparent, inherited thrombocytopenia.[12] Whether this condition is related in any way to mitral valve disease in these dogs presently is unclear.

Mitral valve prolapse in dogs, as well as in humans, is associated with a high renin and low aldosterone profile, an increased platelet reactivity, and a low plasma magnesium concentration.[8,9] In addition, affected dogs and humans have an exaggerated respiratory sinus arrhythmia, and mitral valve prolapse may be associated with autonomic dysfunction.[13] In the light of this and the central role played by NO in the circulatory regulation, we investigated endothelial NO production in dogs with mitral valve prolapse.

Nitric oxide is a gaseous free radical, and thus is a very unstable molecule. The NO radical binds with high affinity to a variety of other molecules and is rapidly oxidized into the stable metabolites nitrate and nitrite (collectively referred to as NOx).[14] It is difficult to measure NO directly and in addition to that, it is difficult to use such measurements to estimate the overall NO turnover in the body. For this purpose, determination of the stable metabolites can be used instead, and the plasma NOx concentration appears to be a good measure of the overall NO production, provided that the dietary intake of nitrate and nitrite is low and the patients are fasted.[15–20] In fasted healthy humans, as much as 90% of plasma NOx has been estimated to come from NO produced by nitric oxide synthase.[19]

The purpose of this study was to compare the plasma concentration of NOx in control dogs with that found in CKCSs with no or minimal MR, and to evaluate whether the findings in the latter group were influenced by the presence of thrombocytopenia and the degree of mitral valve prolapse. Furthermore, the plasma concentration of NOx in CKCSs with no or minimal MR was compared with that found in CKCSs with mild to severe, clinically silent MR. Whether the plasma NOx concentration in CKCSs was associated with various measures of the degree of MR also was tested.

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Table 1. Characteristics of the control group and the 41 clinically healthy Cavalier King Charles Spaniels (CKCSs).[a]

		CKCSs		
	Controls (n = 9)	Controls (n = 23)	Mild MR (n = 9)	Moderate to Severe MR (n = 9)
Gender (male/female)	5/4	13/10	6/3	2/7
Age (months)	39 (18–59)	50 (28–70)	92 (71–105)	123 (86–145)***
Weight (kg)	26.5 (18.8–31.5)***	8.0 (7.0–9.0)	9.6 (8.6–10.4)	8.8 (8.2–9.3)
Platelets ($\times 10^9$/L)	139 (130–150)	115 (77–171)	223 (107–238)	177 (163–362)*
Creatinine (mg/dL)	1.17 (1.10–1.20)**	0.78 (0.60–0.90)	0.62 (0.60–0.80)	0.71 (0.60–1.00)
BUN (mg/dL)	10.6 (10.1–12.9)	10.9 (7.0–12.3)	9.2 (7.6–9.5)	10.9 (9.2–11.2)
MVP (mm)	−0.2 (−0.7–0.0)***	0.8 (0.5–1.7)	1.8 (1.3–2.0)*	2.8 (2.3–3.3)***,§
LA/Ao ratio	1.16 (1.12–1.24)	1.18 (1.10–1.28)	1.28 (1.22–1.35)	1.38 (1.28–1.66)***,§
Plasma NOx (μM)	20.0 (15.1–25.5)	18.7 (15.5–25.9)	12.9 (11.0–13.5)*	11.2 (6.9–17.1)*

MR, mitral regurgitation; BUN, blood urea nitrogen; MVP, mitral valve prolapse; LA/Ao ratio, left atrial to aortic root ratio; NOx, nitrate + nitrite.

[a] Except for gender, data are shown as medians and interquartile intervals.

*, **, and *** indicate statistically different from the 23 CKCS controls ($P < .05$, $P < .01$, and $P < .001$, respectively).

§ Indicates statistically different from the 9 CKCSs with mild MR ($P < .05$).

Materials and Methods

Dogs and Feeding

Nine clinically healthy control dogs (2 Labrador Retrievers, 2 Pembroke Welsh Corgis, 1 Saint Bernard, 1 Wirehaired Dachshund, 1 Bearded Collie, 1 Siberian Husky, and 1 Golden Retriever) and 41 CKCSs were included. All dogs were privately owned, clinically healthy, without history of disease, receiving no medications, not pregnant or lactating, older than 1 year, and without abnormalities on routine serum biochemistry (Table 1). Additionally, control dogs were only included if they had no or minimal MR (regurgitant jets occupying ≤15% of the left atrial area). Fecal specimens from 3 consecutive days were examined by using the Baerman technique to ensure that all dogs were free of *Angiostrongylus vasorum* infection (which is found in approximately 3% of dogs in the northern Copenhagen area). All dogs were examined at the Department of Anatomy and Physiology at the Royal Veterinary and Agricultural University in Copenhagen, Denmark. The CKCSs were divided into 3 groups according to the size of their regurgitant jets (see later): a group with no or minimal MR (jets occupying ≤15% of the left atrial area), a group with mild MR (15% < jets ≤ 50%), and a group with moderate to severe MR (jets occupying >50% of the left atrial area). In the period from 96 to 24 hours before blood sampling, the dogs were fed exclusively a dry food[a] with a low, controlled content of nitrate and nitrite (40 and 10 mg/kg, respectively), together with NOx-free water.[b] In the last 24 hours before blood sampling, the dogs were given only the NOx-free water.[b] The energy content of the diet was 1,580 kJ/100 g, and the dogs were fed according to the manufacturer's recommendations (eg, 200 g/d to a 10-kg dog and 408 g/d to a 25-kg dog).

To assess whether the CKCSs with moderate to severe MR had been close to developing decompensated heart failure at the time of the study, the owners of the 9 dogs in that group were telephoned 9 months later, and questioned regarding signs of disease developed in the period after the study.

Blood Collection and Platelet Counts

Blood samples were taken for NOx analyses as well as for routine hematologic and biochemical analyses. The samples were taken during the initial examination, after the dogs had been given approximately 15–45 minutes to acclimatize, and with the owner present next to the head of the dogs. The samples were collected by jugular venipuncture with a syringe and needle,[c] with the dogs in a sitting position. Within 20 seconds, the blood was transferred into a tube[d] containing a clot activator and tubes[d] containing ethylenediaminetetraacetic acid (EDTA). All samples were centrifuged and plasma was separated within 30 minutes of collection. The plasma was kept frozen at −80°C until NOx analysis.

Within 4 hours from blood sampling, 20 μL of the EDTA anticoagulated blood was mixed with 1.98 mL of a reagent containing ammonium oxalate to hemolyze red blood cells.[e] Within 3 hours from this dilution, the platelets were counted manually with a Bürker Türk counting chamber.

Cardiac Examination

All cardiac examinations were performed by one of the authors (HDP). After the dogs had been given time to acclimatize, they were auscultated in a standing position. The murmur intensity was graded 1–6.[21] Only left apical systolic murmurs are reported here.

Echocardiography[f] was performed on all dogs and recorded on a super-VHS video recorder. The mitral valve was evaluated with a 5.0-MHz phased-array transducer using the right parasternal long axis 4-chamber view, with the dogs in right lateral recumbency. The transmitting frequency was optimized according to the size of the dog, to get the best images of the mitral valve, and attempts were made to scan all parts of the valve. The left ventricular diameter was assessed by using M-mode at the level of the chordae tendineae (guided by a 2-dimensional [2-D] short-axis view). To assess the left atrial and aortic root diameter, a 2-D short-axis view at the level of the aortic valve was used.[22] Color-flow mapping of the mitral valve area and left atrium was made with a 3.5-MHz phased-array transducer using the left caudal 4-chamber view with the dogs in left lateral position. The Doppler transmitting frequency was 2.2 MHz, and the pulse repetition frequency was adjusted to get an aliasing velocity of 0.8 m/s. The flow gain was adjusted to the maximal possible level without getting background noise.

Measurement of NOx

All materials used to store and analyze the plasma samples (pipettes, tubes, microtiter plates, and so on) were rinsed twice in NOx-free water[b] to remove any contamination with nitrate or nitrite.[23] Before performing the analyses, all samples were thawed and ultrafiltered twice through 10-kDa filters,[g] by centrifugation at 14,820 × *g*. The concentration of NOx was measured with a commercially available kit.[h] In brief, nitrate is 1st reduced to nitrite by using nitrate reductase. The nitrite in the samples is then converted into a purple compound, and the absorbance is measured at 540 nm. The procedures described

in the booklet supplied with the kit were followed, except that other microtiter plates[i] were used instead of those supplied with the kit. All analyses were done in duplicate; readings were accepted only if they differed by less than 10%. Only the average of the 2 readings is reported here.

Echocardiographic Measurements

The video-recorded echocardiograms were later reviewed, with the observer (HDP) being unaware of the identity and clinical findings of the dogs. The degree of mitral valve prolapse (relative to the mitral annular plane) was assessed by measuring the maximal protrusion (in 0.5-mm increments) of the anterior leaflet, the posterior leaflet, and the coaption point (or the most protruding tip if the leaflets did not coapt).[8] Only the average of these 3 measurements is reported here. The regurgitant jet area was estimated by eye as the percentage of the left atrium that was occupied by the largest jet (to the nearest 5%). The left ventricular end diastolic diameter (LVEDD) was measured at the beginning of the QRS complex. The diameters of the left atrium and the aortic root were measured shortly after ventricular systole, when the left atrium was at its maximum diameter. A left atrial to aortic root ratio (LA/Ao ratio) was calculated. The LVEDDs and LA/Ao ratios reported here are averages of 5 consecutive heart cycles.

Statistical Analyses

All statistical calculations were performed with statistical software.[j] The level of significance was $P < .05$. To test whether the different disease-related variables (dog group [4 groups; see Table 1], jet size, LVEDD, LA/Ao ratio, and murmur intensity) influenced the plasma concentration of NOx, 5 multiple linear regression analyses were performed, in which the disease-related variable in question was included as an independent variable together with age, gender, serum creatinine concentration, and platelet count. Except for the analysis with dog group as independent variable, the analyses were performed only on the 41 CKCSs. To compare the disease-related variables jet size, LA/Ao ratio, murmur intensity, and LVEDD in pairs, 6 multiple linear regression analyses were performed in which 2 disease-related variables (and the interaction between them) were the independent variables (together with age, gender, serum creatinine concentration, and platelet count) and the plasma concentration of NOx was the response variable. In all analyses, dog group, murmur intensity, and gender were included as class variables, with the remaining explaining variables included as continuous ones. Murmurs graded 3, 4, and 5 were analyzed together, and LVEDD was divided by body weight$^{0.29}$, to normalize it to the size of the dog.[24] The models were gradually reduced (by manual, backward selection) until only variables with statistically significant effects remained. The residuals were tested for normality and homogeneity of variation by Shapiro-Wilks test and residual plot, respectively. A logarithmic transformation was used for NOx and a square root transformation was used for LVEDD, to make the distributions approximately normal.

Fisher's exact test was used to assess differences between dog groups with regard to gender distribution. To assess other differences between dog groups, 1-way analyses of variance were performed. In case of significant group effect, pairwise comparisons of the 4 groups were made by using *t*-tests. The resulting *P*-values were corrected for multiple testing by the Bonferroni method. In the group of 23 CKCSs with no or minimal MR, Student's *t*-test and simple linear regression, respectively, were used to test whether presence of thrombocytopenia (platelet count $< 100 \times 10^9$/L) and degree of mitral valve prolapse influenced the plasma NOx concentration. Because the majority of the data were not normally distributed, data are given as median values and interquartile intervals unless otherwise stated.

Results

The 9 control dogs all had either no regurgitant jet (n = 7) or one occupying ≤10% of the left atrial area, and they all had no heart murmur. Of the 23 CKCSs with no or minimal MR, 13 dogs had no heart murmur, 9 had a grade 1 murmur, and 1 had a grade 2 murmur. Of the 9 dogs with mild MR, 2 dogs had no heart murmur, 4 had a grade 2 murmur, and 3 had a grade 3 murmur. Of the 9 dogs with moderate to severe MR, 1 dog had a grade 1 heart murmur, 2 had a grade 2 murmur, 2 had a grade 3 murmur, 3 had a grade 4 murmur, and 1 had a grade 5 murmur. Eleven (27%) of the 41 CKCSs had thrombocytopenia ($<100 \times 10^9$ platelets/L).

At the 9-month follow-up, 7 of the 9 CKCSs with moderate to severe MR were clinically healthy. The remaining 2 dogs developed pulmonary hypertrophic osteoarthropathy (n = 1) and a cough likely related to heart disease (n = 1), 6 months after sampling. Except for the cough, the latter dog (which on the day of blood sampling had a grade 5 murmur and a LA/Ao ratio of 1.74) was well without cardiac medication at follow-up.

Compared with the 9 control dogs, the 23 CKCSs with no or minimal MR had significantly lower body weight, lower serum creatinine concentration, and higher degree of mitral valve prolapse (Table 1). No other differences were found between those 2 groups in the evaluated variables. The CKCSs with mild MR only differed significantly from the 23 CKCSs with no or minimal MR by having a higher degree of mitral valve prolapse and a lower plasma concentration of NOx (Table 1; Fig 1). However, the CKCSs with moderate to severe MR differed with regard to several variables: they had higher age, higher platelet counts, higher degree of mitral valve prolapse, higher LA/Ao ratio, and lower plasma NOx concentration (Table 1; Fig 1). A platelet count higher than 350×10^9/L was found in 3 (33%) of the CKCSs with moderate to severe MR. Only 1 of the remaining 41 dogs (a CKCS with mild MR) had such a high platelet count. The CKCSs with moderate to severe MR only differed significantly from the group of CKCSs with mild MR by having a higher degree of mitral valve prolapse and a higher LA/Ao ratio (Table 1).

In each of the 5 multiple regression analyses performed, the only independent variables with significant influence on the plasma concentration of NOx were the disease-related variables. Significant associations were found between plasma NOx and dog group (n = 50, $R^2 = 0.24$, $P = .006$), jet size (n = 41, $R^2 = 0.23$, $P = .002$), LA/Ao ratio (n = 41, $R^2 = 0.19$, $P = .005$), murmur intensity (n = 41, $R^2 = 0.17$, $P = .01$), and LVEDD (n = 41, $R^2 = 0.21$, $P = .003$). When the 4 disease-related variables jet size, LA/Ao ratio, murmur intensity, and LVEDD were analyzed in pairs, jet size outweighed the effects of the other 3 variables. The LVEDD outweighed the effects of LA/Ao ratio and murmur intensity, and the LA/Ao ratio outweighed the effects of murmur intensity. None of the interactions had significant influence. Thus, jet size apparently had a stronger influence on the plasma concentration of NOx than did the other disease measures, including LA/Ao ratio (Figs 1 and 2a versus 2b).

Within the group of CKCSs with no or minimal MR, 8

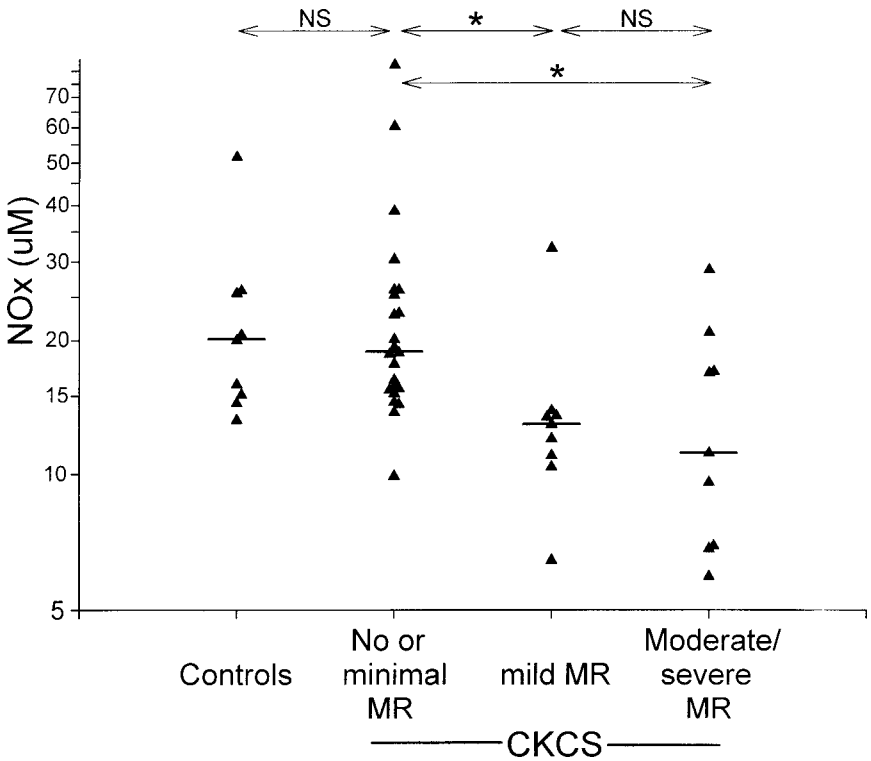

Fig 1. Plasma concentration of nitrate and nitrite (NOx) in control dogs and clinically healthy Cavalier King Charles Spaniels (CKCSs) with different degrees of mitral regurgitation (MR). Dogs with mild and moderate to severe MR had regurgitant jets occupying 15–50% and >50% of the left atrial area, respectively. NS, not significantly different; * $P < .05$.

dogs with thrombocytopenia had the same plasma concentration of NOx (22.9 μM; 16.5–25.6 μM) as 15 dogs without thrombocytopenia (17.7 μM; 15.5–25.9 μM) ($P = .95$). Furthermore, the mitral valve prolapse degree was not associated with the plasma NOx concentration ($R^2 = 0.0007$; $P = .91$).

Discussion

This study shows that the plasma concentration of NOx is lower in CKCSs with mild to severe, clinically silent MR. The study also shows that control dogs and CKCSs with no or minimal MR have similar plasma concentrations of NOx, and that neither the degree of mitral valve prolapse nor the presence of clinically inapparent, inherited thrombocytopenia influenced NOx concentrations in the latter group. The findings suggest that endothelial dysfunction develops early in the course of developing MR in dogs.

Evidence supporting our finding of lower plasma NOx concentrations in CKCSs with MR is persuasive. First, only little a overlap in plasma NOx concentrations occurred between CKCSs with mild to severe MR and CKCSs with no or minimal MR. Second, the plasma concentration of NOx was significantly associated with all disease variables evaluated (albeit the R^2 was low for all variables). Third, the study conditions were highly standardized. The study was based on dogs from 1 breed, with 1 disease, that received no medication and had no renal problems. In addition, all samples were taken after the dogs had been fed exclusively NOx-free water and a diet with a low nitrate and nitrite content for 4 days (only water for the last 24 hours)—a protocol that should be more than sufficient to ensure that the dietary intake of nitrate and nitrite had a low and constant influence on the plasma NOx concentrations.[15,17,19,20] Finally, the differences in potential confounders (eg, age, gender, creatinine, blood urea nitrogen [BUN], and platelet count) among the 3 groups of CKCSs were small and generally not significant (although CKCSs with moderate to severe MR were older and had higher platelet counts than the CKCS control group). Furthermore, because of the observation that the potential confounders had no influence in any of the multiple regression analyses performed, the minor differences between the groups likely played no role for the main conclusions of the study.

The fact that the majority of the dogs in this study were active in the period leading up to the blood sampling (being transported to the clinic and received and acclimatized there) probably was a major factor that allowed the finding of differences among the groups. The activity of the dogs likely provided a long-lasting and widespread vasodilatory stimulus—the kind of stimulus necessary for endothelial dysfunction to be detected as a change in the NOx concentration in the circulation.[18,20] The stimulus needs to be widespread because of the volume of distribution, and long-lasting to allow nitrate to accumulate (the majority of the NOx found in blood is nitrate, which has a half-life in the circulation of approximately 5–8 hours in humans[14] and 4 hours in dogs[18]). The long half-life of nitrate implies that the duration of the acclimatization period before blood sampling only have had minor influence on the findings.

In humans, the degree of endothelial dysfunction has

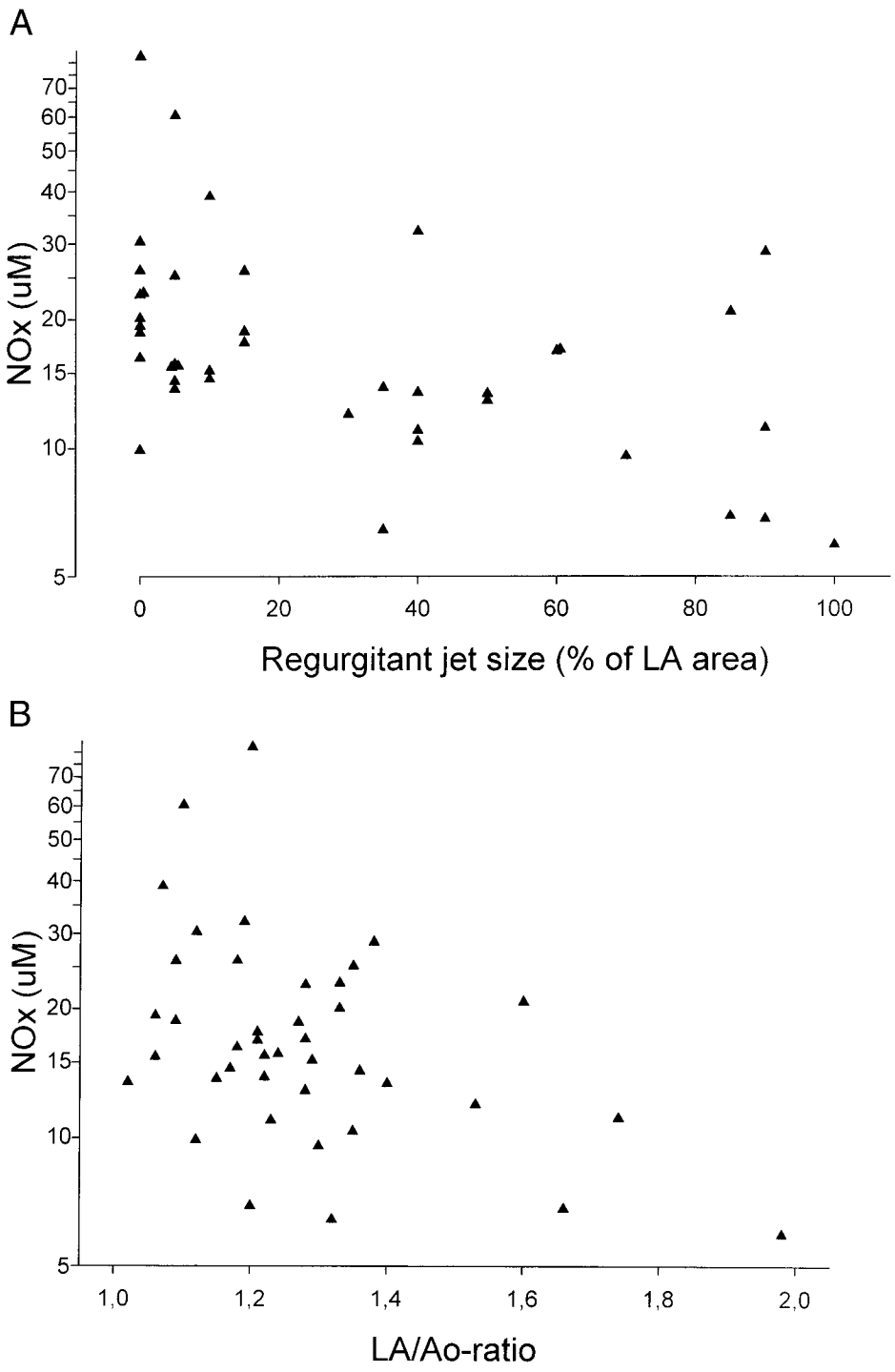

Fig 2. The plasma concentration of nitrate and nitrite (NOx) shown as a function of **(A)** the regurgitant jet size and **(B)** the left atrial to aortic root ratio (LA/Ao ratio) in 41 clinically healthy Cavalier King Charles Spaniels (R^2 = 0.23 and 0.19, respectively). LA, left atrium.

been found to increase with increasing degree of heart failure.[6,7] In accordance with that, it appears from Figure 1 that most of the dogs with a very low plasma NOx concentration (eg, $<$10 μM) had severe MR. However, as a group, the 9 dogs with moderate to severe MR were not significantly different from the dogs with mild MR. Conclusions are difficult to make from small groups, but a reason for this could be that a few of the old, more diseased dogs had other, undetected disorders that caused NOx accumulation. However, note that most of the dogs with moderate to severe MR still were clinically healthy at the 9-month follow-up, and that the 2 dogs that did develop clinical problems did so 6 months after the study. Another interesting finding in this study with regard to the association between degree of disease and plasma NOx concentration is that the NOx concentration appeared to relate better to regurgitant jet size

than to left atrial or left ventricular size. This likely reflects that the degree of MR in most of the dogs was so mild that the diameter of the left atrium and left ventricle were not markedly changed yet.

Dogs with pacing-induced heart failure as well as humans with heart failure caused by different spontaneous heart diseases have long been known to have endothelial dysfunction.[3,4] Recent studies in humans with different heart diseases (including valvular ones) have shown that endothelium-dependent vasodilation apparently is impaired early in the course of developing heart failure.[6,7] The findings presented here show that the same is true for dogs with MR. However, the argument could be made that CKCSs might not be representative of all dogs with MR. On the other hand, CKCSs without MR were found to have plasma NOx concentrations similar to the concentrations found in dogs from other breeds.

Nitrite and nitrate are excreted by the kidneys, and reduced renal function therefore leads to NOx accumulation in the blood. Consequently, it is important that renal function is normal if the plasma concentration of NOx is used to assess the NO turnover in the body. The importance of this notion is underscored by the fact that dogs with pacing-induced heart failure often have an increased plasma NOx concentration because of this phenomenon.[25] In the present study, the majority of the dogs were young or middle-aged and only clinically healthy dogs (ie, without signs of decompensated heart failure) were included. Furthermore, none of the included dogs had high serum concentrations of BUN and creatinine. The finding that control dogs had higher serum creatinine concentrations than the CKCSs merely reflects that the serum creatinine concentration is higher in larger dogs than in small dogs.[26]

The CKCSs with moderate to severe MR had significantly higher platelet counts than the CKCSs with no or minimal MR. An explanation for this could be that this group by chance included few dogs with inherited macrothrombocytopenia. However, this is likely not the only explanation. It seems that something must have increased the platelet count in the dogs in this group inasmuch as 3 of the dogs (33%) had more than 350×10^9 platelets/L, a level that is known from this and previous studies rarely to be reached in CKCSs with no or mild MR.[12,27] In addition, in 2 of these 3 dogs, lower platelet counts (20 and 29% lower) have been found previously by manual counting, that is, at an earlier disease stage (data not shown). We speculate that an explanation for the high platelet counts could be platelet fragmentation caused by shear stress acting on the regurgitating blood.[28]

From this study, no relationship is apparent between the degree of mitral valve prolapse per se and the plasma NOx concentration in CKCSs. In accordance with that, CKCSs without MR (but usually with mitral valve prolapse) had the same concentration of NOx in plasma as control dogs without mitral valve prolapse. Therefore, findings such as a high renin and low aldosterone profile and an increased degree of respiratory sinus arrhythmia in dogs with mitral valve prolapse likely do not reflect changes in the endothelial NO production. In addition, the plasma concentration of NOx appears to be unaffected by the presence or absence of clinically inapparent, inherited thrombocytopenia. Therefore, the recent finding of increased platelet reactivity in CKCSs without thrombocytopenia (as compared with control dogs and CKCSs with thrombocytopenia)[27] apparently does not reflect changes in endothelial NO production.

With a decreased ability to produce NO, the endothelium will have a reduced ability to limit platelet activation, adhesion, and aggregation.[29] This is probably a major reason why a relationship is apparent between endothelial dysfunction and conditions such as atherosclerosis, thrombosis, and intimal hyperplasia in humans.[1,29,30] In that context, it is interesting that CKCS have been found to develop intimal hyperplasia in the femoral arteries as well as the main pulmonary artery[31,32] and that these vessel changes, as discussed elsewhere,[8,9] in many ways resemble the changes seen in myxomatous valve leaflets.

In patients with heart failure, an impaired endothelium-dependent vasodilation will reduce exercise capacity and increase the total peripheral resistance and thereby the left ventricular afterload. Therefore, and because endothelium-derived NO is known to be vasoprotective in many ways, correction of endothelial dysfunction is considered a major therapeutic goal.[5] Although much research is still needed, many methods to improve endothelial dysfunction have shown promising results in humans, including physical training, supplemental oral L-arginine, inhibition of angiotensin-converting enzyme, use of antioxidants such as vitamin C, growth hormone treatment, and endothelin-A receptor blockade.[33–39] Study of the effects of these different treatments, both on the plasma concentration of NOx and on the long-term prognosis, in dogs with MR would be interesting. Study of the effects of endothelin-receptor blockade perhaps would be especially interesting, because endothelin may be an important pathogenetic factor not only in the general circulation, but also locally in the valve leaflets.[40]

Based on this study it seems that it is relatively easy to get an assessment of the endothelial-dependent vasodilation in dogs. By using a simple standardized approach, it was possible to detect statistically significant changes without including a large number of patients. Therefore, the approach used in this study can be used for further studies of the pathophysiology of different heart diseases in dogs. In addition, the method can be used to assess the effects of cardiac medication on early stages of different heart diseases—before expensive and time-consuming double-blind, placebo-controlled trials are initiated. The only major weakness of the method appears to be its sensitivity to impaired renal function.

Footnotes

[a] GLP diet, batch 000113390, Leo Pharmaceuticals, Ballerup, Denmark
[b] Milli-Q Gradient system, Millipore Corporation, Bedford, MA
[c] Venofix 21G (0.8 × 20 mm) 30 cm, B. Braun, Melsungen, Germany
[d] Vacuette, Greiner Bio-One, Kremsmünster, Austria
[e] Unopette Microcollection System, Becton-Dickinson, Rutherford, NJ
[f] Vivid 3 echocardiograph, GE Medical, Milwaukee, WI
[g] Microcon YM-10 Microfilters, Millipore Corporation, Bedford, MA
[h] Nitrate/nitrite colorimetric assay kit, Cayman Chemical Company, San Diego, CA
[i] Polypropylen microtiter plates, In Vitro as, Fredensborg, Denmark
[j] SAS Statistical Software, Version 8, SAS Institute, Cary, NC

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