Comparative Medicine
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Vol 68, No 2
April 2018
Pages 156–162

Original Research

Using the Oculocardiac Reflex to Characterize Autonomic Imbalance in a Naturally Occurring Canine Model of Valvular Insufficiency

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Congestive heart failure (CHF) is characterized by reduced heart rate variability. Although various methods to achieve parasympathetic withdrawal and sympathetic activation have been documented, stimulation of the vagal nerve (that is, vagal maneuvers) has not often been used to assess the autonomic impairment associated with disease progression in veterinary species. In this study, we investigated cardiac autonomic control in a naturally occurring canine model of mitral insufficiency by means of individual responses to oculocardiac reflex. Indices of heart rate variability were calculated from 5-min ECG tracings obtained before and after ocular compression. After compression, significant increases in the standard deviation of RR intervals, root mean square of the successive differences in RR intervals, and vasovagal tonus index were documented in healthy control animals. In addition, these indices were increased in asymptomatic dogs with remodeled hearts, but no variation occurred in symptomatic animals. Although only the percentage change in vasovagal tonus index differed significantly between controls and diseased dogs, all other parameters showed a tendency to behave the opposite way in the symptomatic group as in the control and asymptomatic groups. Our results document CHF-dependent autonomic dysfunction in a canine model of valve insufficiency.

Abbreviations: CHF, congestive heart failure; HR, heart rate; MRR, mean RR interval; NR, no remodeling; OCR, oculocardiac reflex; RA, remodeled and asymptomatic; RMSSD, root mean square of the successive differences in RR intervals; RS, remodeled and symptomatic; SDNN, standard deviation of the RR intervals; VVTI, vasovagal tonus index.

Heart rate (HR) is determined by the rate of sinoatrial nodal discharge and autonomic tone influence.[14] Therefore, parasympathetic and sympathetic nervous impulses are responsible for decreases and increases in HR, respectively.[23] When vagal centers in the medulla oblongata are stimulated, acetylcholine binds to receptor sites present in the sinoatrial node, decreasing the discharge rate. Similarly, the stimulation of sympathetic centers in the spinal cord produces norepinephrine, which binds to β1 receptors sites at the sinoatrial node, increasing HR.[14] The autonomic influence determines the adaptive circadian variations of HR, due to the prevailing balance of the autonomic impulses in the heart.[21]

The oculocardiac reflex (OCR) is the heart's physiologic response to traction applied to extraocular muscles or digital compression of the eyeballs.[2,8] This vagal maneuver is obtained through the indirect stimuli of the ophthalmic branch of the trigeminal nerve, causing negative chronotropic and inotropic responses in healthy subjects. The reflex, primarily described in human beings, has recently been characterized in conscious dogs and rabbits.[38]

Congestive heart failure (CHF) is a syndrome to which most cardiovascular disorders tend to evolve in advanced conditions. In CHF, as a result of structural, neural, and electrophysiologic remodeling, HR undergoes a sustained increase in response to autonomic imbalance.[37] Due to a slight fall in systemic arterial pressure because of a failing heart, vagal restraint on HR is reduced, from either decreased stretching or diminished function of arterial baroreceptors. In addition, levels of circulating cacthecolamines are elevated in heart failure syndrome, which in turn increase sympathetic activation.[1] This dysregulated autonomic tone leads to increases in resting HR, which is known to influence the prognosis of CHF.[11]

Sustained sympathetic activation and parasympathetic withdrawal have been characterized in numerous cardiovascular diseases, and the imbalance is considered an aggravating factor in circulatory failure.[7,12,25] Numerous measures of autonomic function have been proposed, especially in light of their value in determining prognosis in structural cardiac diseases and assessing safety in pharmacology studies.[5,30] Through surgical and pharmacologic means, many researchers have focused on documenting the precise mechanisms of impaired autonomic function in CHF.[9,15,26,33] Nevertheless, the exact role played by the autonomic imbalance in the progression of cardiac diseases—and that of disease progression in autonomic imbalance—remains unclear. Therefore, in the current investigation, we sought to evaluate the reliability of a novel way to assess the level of autonomic imbalance in CHF patients. Dogs diagnosed with naturally occurring degenerative mitral valve disease were selected, and their response to experimentally induced OCR was used as an alternative method to evaluate the integrity of autonomic cardiac control.

Received: 03 Aug 2017. Revision requested: 10 Sep 2017. Accepted: 30 Oct 2017.
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Table 1. Descriptive statistics (either mean ± 1 SD or median [interquartile range]) of the recruited population categorized according to echocardiographic and clinical criteria in healthy controls and mitral insufficient dogs with no cardiac remodeling or symptoms (NR), remodeled hearts but no symptoms (RA), or remodeled heart and symptoms (RS)

	Controls (n = 16)	NR (n = 31)	RA (n = 10)	RS (n = 11)	P
Age (y)	2.2 (1.5–8.0)^{A}	11.0 (9.0–13.0)^{B}	11.0 (8.5–12.0)^{B}	13.0 (12.0–14.2)^{B}	<0.0001[a]
Sex (female/male)	10/6 (63% ± 34%)	18/13 (58% ± 42%)	7/3 (70% ± 30%)	5/6 (45% ± 55%)	—
Weight (kg)	9.0 (9.0–17.0)	9.7 (5.3-13.7)	8.6 (5.8–13.6)	7.1 (5.1–9.0)	0.1028
Left ventricular internal diameter during diastole relative to body surface area (mm/m^2)	62.5 ± 15.3^{A}	67.3 ± 17.2^{A}	75.7 ± 14.9^{A,B}	90.6 ± 17.0^{B}	0.0003
Left ventricular internal diameter during systole relative to body surface area (mm/m^2)	36.2 (24.7–42.6)	34.4 (29.3–39.8)	40.7 (34.6–46.3)	41.7 (37.0–49.9)	0.0906[a]
Wall stress index during diastole	3.8 ± 1.4	3.6 (0.9)	4.1 ± 0.9	4.1 ± 1.0	0.4128
Wall stress index during systole	1.3 ± 0.3	1.3 (0.4)	1.3 ± 0.7	1.3 ± 0.4	0.9102
Fractional shortening (%)	43.2 ± 5.9^{A}	46.9 ± 8.8^{A,B}	43.2 ± 10.0^{A,B}	51.9 ± 6.5^{B}	0.0327
Left atrium:aorta	1.2 (1.1–1.3)^{A}	1.1 (1.0–1.2)^{A}	1.6 (1.4–1.7)^{B}	2.1 (1.9–2.3)^{B}	<0.0001[a]
Mitral E wave (cm/s)	82.8 ± 19.1^{A}	66.4 ± 10.7^{B}	92.5 ± 18.6^{A}	151.9 ± 30.5^{C}	<0.0001
Mitral E wave:A wave	1.4 (1.0–1.6)^{AB}	0.9 (0.7–1.2)^{A}	1.1 (0.8–1.4)^{A}	2.3 (1.6–3.2)^{B}	<0.0001[a]
Isovolumic relaxation time (ms)	58.0 (40.2–56.2)	51.0 (43.0–60.5)	51.0 (41.5–51.2)	35.0 (17.5–49.5)	0.1514[a]
E wave/isovolumic relaxation time	1.5 (1.1–2.4)^{A}	1.3 (1.1–1.6)^{A}	1.7 (1.4–2.2)^{AB}	3.9 (2.5–9.6)^{B}	<0.0001[a]

Within each row, different uppercase letters indicate values that differ significantly ($P < 0.05$).
[a]Nonparametric analysis.

Materials and Methods

Domestic dogs (*Canis lupus familiaris*) included in this prospective transversal observational study were healthy client-owned pets, patients admitted for regular cardiac evaluation, and dogs under preoperative examination for elective surgery at a veterinary teaching facility. All animals were housed and kept under evaluation for no longer than 40 min, in a room appropriately equipped for cardiac examination and either returned to their owners or transferred to receive additional medical care. All procedures were previously approved by the IACUC and complied with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.[16]

To be included in the study, a diagnosis of naturally occurring mitral insufficiency was required, which was based on the echocardiographic criteria of impaired valvar anatomy and function.[6] Dogs with echocardiographic evidence of any congenital or acquired cardiac disease other than degenerative mitral valve disease were excluded from the study, as were patients with history of intrathoracic or abdominal tumors, those with ophthalmic disorders and brachycephalic dogs. Once selected, the dogs were further classified according to cardiac remodeling and clinical history regarding signs attributable to CHF, including coughing, exercise intolerance, and respiratory distress. The 3 groups were: NA, no cardiac remodeling and asymptomatic; RA, cardiac remodeling present but asymptomatic, and RS, cardiac remodeling and symptomatic. In addition, healthy animals lacking signs of valvular dysfunction were recruited as controls. All echocardiograms were performed by experienced veterinary cardiologists using an ultrasonography system (MyLab 30, Esaote, Genova, Italy) equipped with 5.0- and 7.5-MHz phased array transducers (models P240 and P023, Esaote).

Once enrolled in the study, dogs were maintained in right lateral recumbency by using gentle physical restraint to obtain ECG tracings. Toothless alligator electrodes were attached to the skin and wet with alcohol to improve electrical conduction. The left and right arm electrodes were placed at the elbows, and the left and right leg electrodes were placed on the stifles. ECG was performed continuously and uninterrupted for 5 min. During the last minute, manual ocular compression was applied by using the thumbs to exert continuous digital force over the superior eyelids of both eyes simultaneously for 1 min, until the 5-min ECG run was complete. The operator made a conscious attempt to always place the thumbs at the center of both superior eyelids. The amount of pressure applied was empirically estimated as the force sufficient to cause slight retrobulbar displacement of the eyes without induction of any physical signs of ocular or physical discomfort, such as changes in HR, vocalization, or body movement. No instruments, such as pressure gauges, were used to quantify the pressure exerted on the eyelids. To avoid interinvestigator discrepancy, the same investigator, who was blinded to patients' clinical conditions, performed all ocular compressions in all dogs. Poor-quality recordings and ECG tracings in which arrhythmias prevented a continuous run of 20 RR intervals of sinus rhythm were not used in the study.

Once the recording was over, the ECG tracing was used to calculate the mean RR interval (MRR), standard deviation of RR intervals (SDNN), root mean square of successive differences in RR intervals (RMSSD), and vasovagal tonus index (VVTI), which is an alternative indicator of HR variability and was obtained by using the natural logarithm of the variance of 20 consecutive RR intervals. These indices were calculated according to the following equations:[4,35]

$$\mathrm{MRR} = \bar{I} = \frac{1}{N-1}\sum_{n=2}^{N} I(n)$$

$$\mathrm{SDNN} = \sqrt{\frac{1}{N-1}\sum_{n=2}^{N}\left[I(n) - \bar{I}\right]^2}$$

$$\mathrm{RMSSD} = \sqrt{\frac{1}{N-2}\sum_{n=3}^{N}\left[I(n) - I(n-1)\right]^2}$$

$$\mathrm{VVTI} = \mathrm{NL}\left[\mathrm{VAR}\left(\mathrm{R\text{-}R1} - \mathrm{R\text{-}R20}\right)\right]$$

where N is the number of RR intervals, NL is the natural logarithm, and VAR is the variance. Each index was calculated twice for every tracing: the first one before the beginning of ocular compression and the other 20 s after the beginning of ocular

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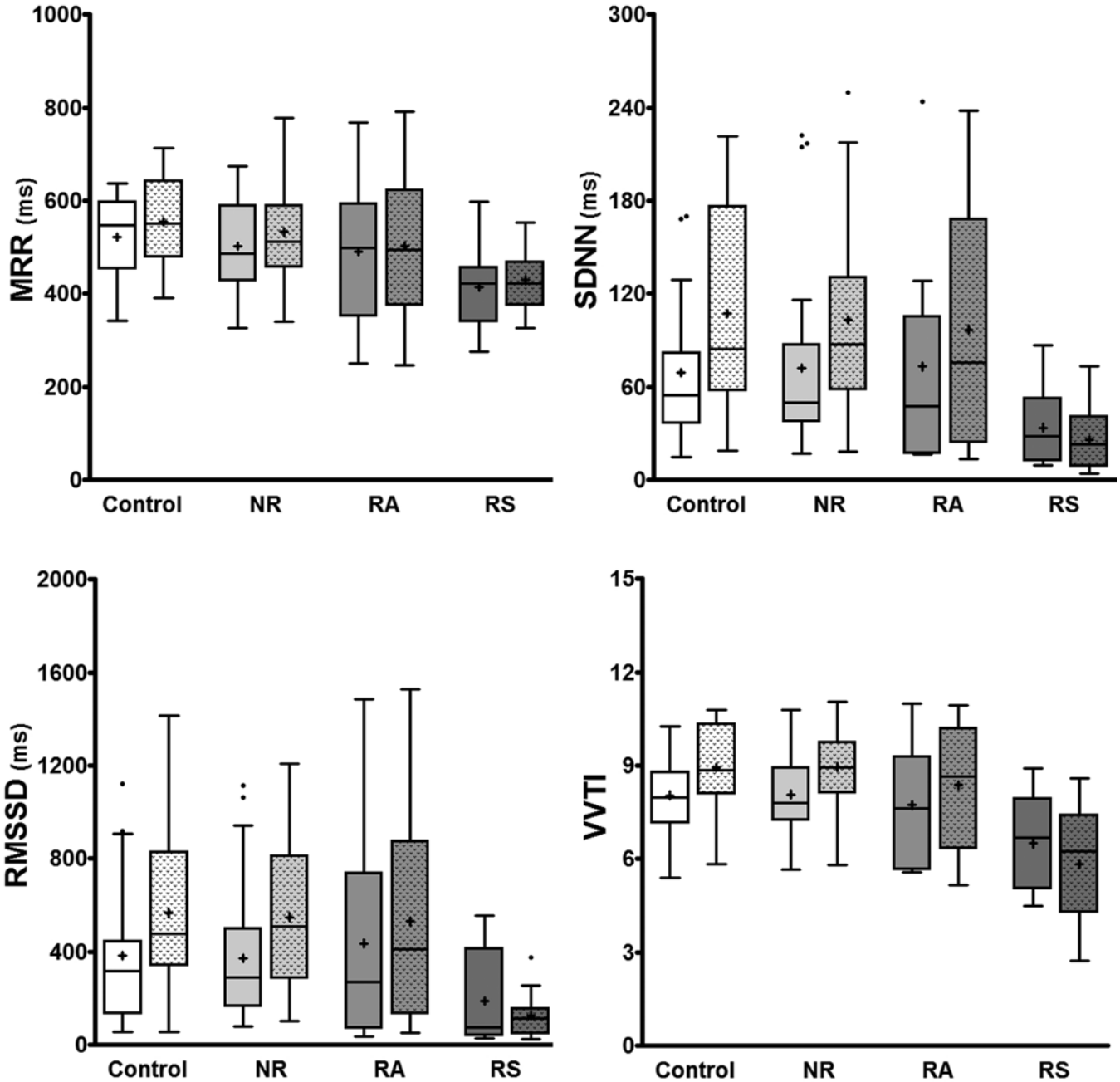

Figure 1. Box plots depicting the effect of the oculocardiac reflex (OCR) on the (A) mean RR interval (MRR), (B) standard deviation of the RR intervals (SDNN), (C) root mean square of successive differences in RR intervals (RMSSD), and (D) vasovagal tonus index (VVTI), which were used as surrogates for autonomic control in healthy control dogs and mitral insufficient dogs with no cardiac remodeling (NR), cardiac remodeling but no symptoms (RA), or cardiac remodeling and symptoms (RS). Hatched boxes represent the parameters recorded after OCR. Outliers are shown; + indicates the mean.

compression. In addition, echocardiographic indices of congestion and cardiac function were documented for posterior correlation and included the left atrium:aorta ratio, body surface area-indexed left ventricular internal diameter during diastole and systole, wall stress index in diastole and systole, fractional shortening, mitral E wave velocity, mitral E-to-A ratio, isovolumic relaxation time, and mitral E-to-isovolumic relaxation time ratio.[6]

All data underwent Shapiro–Wilk testing to check for normal distribution. To investigate differences between groups, ANOVA followed by Tukey multiple-comparison tests were applied to MRR and VVTI data, and Kruskal–Wallis testing followed by the Dunn test was used for SDNN and RMSSD data. Later, Mann–Whitney and *t* tests were used to compare nonparametric and parametric pre- and postOCR data, respectively, for each index in every group. The percentage change in each index was analyzed, by using the variation between pre- and postOCR values, and Kruskal–Wallis and Dunn tests were used to compare these data among groups. Finally, either the Pearson or Spearman test was used to assess whether correlations existed between the percentage change in each index and the echocardiographic parameters of congestion and cardiac function. All analyses were performed by using GraphPad Prism (version 5.0, San Diego, CA) with default settings. For all analyses, the level of significance was defined as a P value less than 0.05.

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Results

We recruited 68 client-owned dogs for this study. Several breeds were represented, and the dogs varied from 1 to 16 y in age and from 2.5 to 32 kg in weight. Beagles (n = 8), Dachshunds, (n = 6), and Miniature Pinschers (n = 5) were overrepresented in the study population. Body weight did not differ between groups (P = 0.3943), but age differed between the control and RS groups (P = 0.0074). Descriptive statistics of the studied population are summarized in Table 1.

We assigned 16 healthy dogs as controls, and 31, 10, and 11 dogs were assigned to the NR, RA, and RS groups, respectively. The box plot graphs in Figure 1 demonstrate how the OCR interfered with indicators of autonomic balance in all dogs regardless of cardiac condition. In addition, a significant difference between groups emerged when the values of all 4 parameters obtained during ocular compression were compared: for every index, the symptomatic group presented significantly lower values than control and NR groups. In the NR group, ocular compression significantly increased SDNN (from 49.7 to 87.3, P = 0.0002), RMSSD (from 289.7 to 506.4, P = 0.0003) and VVTI (from 8.1 to 8.9, P < 0.0001), whereas healthy controls demonstrated an increase in VVTI (from 8.1 to 8.9, P = 0.0278) only. Remodeled groups showed no significant variation in any of these parameters after compression.

When we calculated the percentage change between values before and after OCR, only VVTI in the RS group yielded a significant difference (P = 0.0419). Despite the absence of significant differences, plotting the data revealed numerically higher absolute values for SDNN and RMSSD in the control, NR, and RA dogs than for the RS group. In addition, in the RS group, these parameters also tended to increase after OCR (that is, negative value) rather than decrease (that is positive change value) in the control and asymptomatic groups (Figure 2).

Regarding the echocardiographic indices of congestion and function, weak negative correlations were found between SDNN and the wall stress index during systole (R = –0.2508), left atrium:aorta ratio (R = –0.3558), and mitral E (R = –0.2714), as well as between RMSSD and body surface area-indexed left ventricular internal diameter during systole (R = –0.2695), wall stress index during systole (R= –0.3188), and left atrium:aorta ratio (R = –0.2810). In addition, VVTI percentage change was correlated with various echo indices (Figure 3) In contrast, no correlation existed between MRR and the collected echocardiographic data.

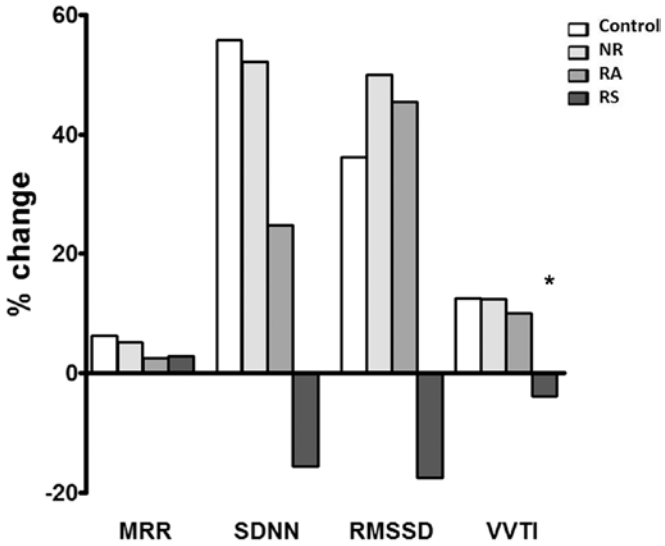

Figure 2. Median percentage change (before oculocardiac reflex – after oculocardiac reflex) of the surrogates used to assess autonomic balance in healthy control dogs and mitral insufficient dogs with no cardiac remodeling (NR), cardiac remodeling but no symptoms (RA), or cardiac remodeling and symptoms (RS) undergoing manual ocular compression to induce the oculocardiac reflex. *, P < 0.05.

Discussion

CHF is characterized by autonomic imbalance, which results in sustained elevation and diminished variability of HR.[34] In the current study, we characterized this autonomic imbalance by means of a reduced response to a vagal maneuver in a naturally occurring canine model of mitral valve insufficiency. First described in people,[2,8] the OCR is a peripheral subtype of trigeminal cardiac reflexes and an important cause of profound bradycardia during ocular surgery, especially in pediatric procedures.[10] Moreover, manual compression of the eyeballs, either together or individually, significantly decreases HR in dogs.[38]

Experimental evidence of increased sympathetic and reduced vagal tone in CHF has encouraged the development of quantitative markers of autonomic activity.[36] HR variability is an important measure of autonomic tone and is defined as the fluctuation in time between normal sinus beats (RR intervals).[39] Although assessing the autonomic nervous system is not simple, HR variability indices have proved to be clinically useful, because analyses of beat-to-beat changes in HR provide sensitive and early information regarding impaired cardiac function, even before clinical signs develop.[36] These indices typically are obtained by linear methods of either time or frequency domain analysis.[5,39] The time–domain analysis consists of statistical calculations based on normal RR intervals. In the current study, we used MRR, SDNN, RMSSD, and VVTI—all of which have been used to assess autonomic dysfunction in both dogs[4,22,28,38] and humans.[20,35,36,39] However, this current investigation is the first to assess how vagal stimulus through digital ocular compression interferes with these parameters. Although SDNN is influenced by short-term high-frequency variations as well as low-frequency components, reflecting total autonomic activity, only high-frequency variations, which usually are related to parasympathetic influence, are thought to play a role in the other 3 parameters.[5,36]

In our study, the symptomatic group responded differently during digital ocular compression than every other group. The lack of response to OCR in RS dogs, as demonstrated by the absence of significant changes in all 4 parameters, is in accordance with the parasympathetic withdrawal in human patients with CHF.[9] This information has since been supported by many studies that have assessed autonomic function during CHF in various species.[11,13,21,31] More importantly, this finding illustrates not the lack of vagal activity but rather a diminished response to parasympathetic stimuli in these patients. In addition, the lack of significant difference between the RS and RA groups for MRR and VVTI (Figure 1) supports the theory that some grade of autonomic imbalance precedes clinical evidence of cardiovascular disorders.[36]

In the before-after analyses within each group, the parameters in the RS group not only appeared to respond less to OCR but also behaved opposite to other groups, showing a clear tendency to increase with this classic vagal maneuver. This interesting finding might be attributable to the previously mentioned parasympathetic withdrawal together with subtle sympathetic activation due to discomfort related to eyeball compression, which is likely disguised in dogs in which the vagal response is preserved. However, the other and more intriguing explanation

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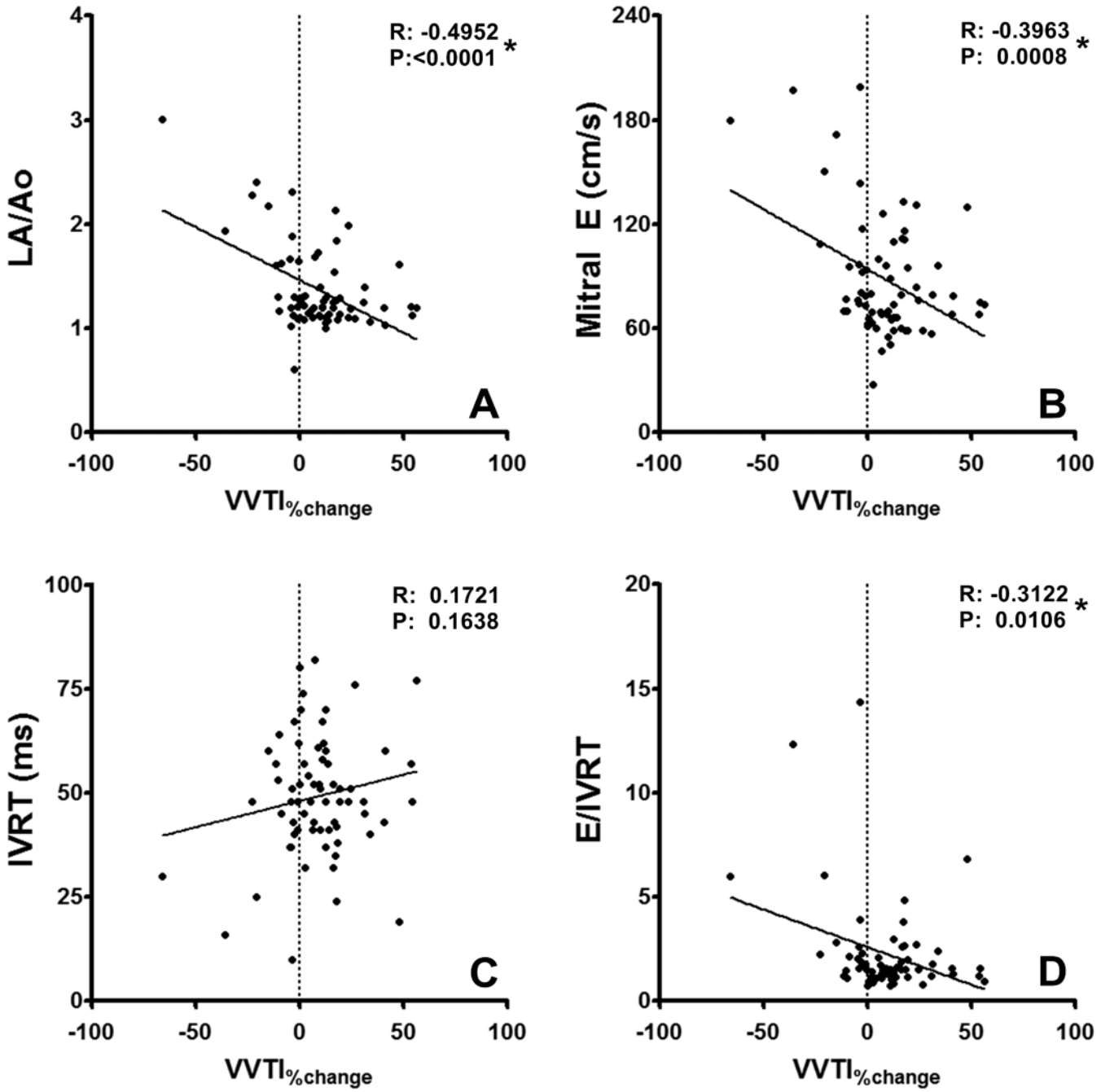

Figure 3. Scatter plots depicting the correlations between the percentage change in VVTI and the (A) left atrium-to-aorta ratio, (B) mitral E wave, (C) isovolumic relaxation time, and (D) mitral E wave-to-isovolumic relaxation time ratio. *, $P < 0.05$.

is that, in addition to parasympathetic withdrawal, patients in CHF have increased sensitivity to sympathetic activation.[11,17,31] This hypothesis becomes stronger when the percentage variation of the surrogates obtained for each group in our study are considered. The opposite behavior of the RS group is most likely a result of sustained sympathetic tone rather than transitional sympathetic activation. In addition, the clear similarity between the NR and RA groups and the control, otherwise healthy group, as compared with the RS dogs, suggests that, unlike parasympathetic withdrawal, which appears to be present at initial stages, sustained sympathetic activation is more evident in overtly affected patients. Similar findings have been previously documented in people with CHF.[3,7]

The significant correlation between the before-after percentage variation of the surrogates of cardiac autonomic regulation and the echocardiographic indices of congestion and function supports the theory that individual response to OCR diminishes with the progression of cardiac disease. In severe cases of mitral valve regurgitation, volume overload leads to left atrial and ventricular remodeling, both of which are associated with clinical onset.[29] Many echocardiographic indices tend to become altered when an asymptomatic mitral insufficient dog progresses into symptomatic CHF.[6] Therefore, the significant correlations that we found here likely support the decreased parasympathetic role as CHF becomes overt. This finding is in accordance with data previously documented in people,[3,9] dogs,[21] and rats.[24]

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An important limitation of this research is the impossibility of controlling external factors that potentially interfere with autonomic tone, including stress and fear. Most dogs enrolled in this study were calm and quiet during the procedure and appeared to tolerate the ocular compressions. However, some animals were more resistant to physical restraint and more uncomfortable by the end of the maneuver, likely resulting in intensification of sympathetic tone. In addition, the significant age difference between the control group (average, 2.2 y) andRS group (average, 13 y) may represent a confounding factor, leading to questions regarding whether advanced age plays a role in autonomic imbalance, regardless of cardiac condition. However, the vast existing data supporting parasympathetic withdrawal during CHF in many different species and the fact that our study used dogs with naturally occurring mitral insufficiency, in which prevalence and progression increase markedly with age, strongly validate the obtained results.

Another limitation of this study was the absence of a mechanism to estimate the exact amount of compression applied over the eyelids, to increase the precision and repeatability of the response. Nevertheless, this somewhat empirical methodology has been used in several other investigations with humans and animals[18,19,27,32] and therefore is considered a standard model, to some extent. We are currently investigating a modified electronic von Frey anesthesiometer to elicit OCR by using a controlled amount of pressure applied on the eyelids.

In this study, dogs with naturally occurring mitral insufficiency were used as an animal model of heart disease leading to CHF, with the purpose of investigating autonomic imbalance. The vagal maneuver, represented by manual compression of the eyeballs, was a reliable, simple, and quick technique to demonstrate the deterioration of cardiac autonomic regulation as mitral insufficiency progresses toward CHF. Dogs with CHF demonstrated signs of parasympathetic withdrawal and increased sustained sympathetic activation when exposed to OCR. These findings contrasted with those from both the asymptomatic and control dogs, in which lack of parasympathetic response to ocular compression was not observed. Although many questions still remain regarding the exact influence of the autonomic nervous system in cardiovascular disorders, we believe our finding regarding the response to a simple vagal maneuver helps to shed light on how the autonomic nervous system behaves during CHF and may aid in defining prognosis.

References

1. **Amorim DS, Dargie HJ, Heer K, Brown M, Jenner D, Olsen EG, Richardson P, Goodwin JF.** 1981. Is there autonomic impairment in congestive (dilated) cardiomyopathy? Lancet **1:**525–527.
2. **Aschner B.** 1908. Ober einen bisher noch nicht beschriebenen reflex vom auge auf kreislauf und atmung. Wien Klin Wochenschr **21:**1529–1530. [In German].
3. **Binkley PF, Nunziata E, Haas GJ, Nelson SD, Cody RJ.** 1991. Parasympathetic withdrawal is an integral component of autonomic imbalance in congestive heart failure: Demonstration in human subjects and verification in a paced canine model of ventricular failure. J Am Coll Cardiol **18:**464–472.
4. **Calvert CA.** 1998. Heart rate variability. Vet Clin North Am. Small An Pract **28:**1409–1427.
5. **Champeroux P, Martel E, Jude S, Laigot C, Laveissière A, Weyn-Marotte AA, Fowler JS, Maurin A, Richard S, Babuty D.** 2013. Power spectral analysis of heart rate variability in cynomolgus monkeys in safety pharmacology studies: comparative study with beagle dogs. J Pharmacol Toxicol Methods **68:**166–174.
6. **Chetboul V, Tissier R.** 2012. Echocardiographic assessment of canine degenerative mitral valve disease. J Vet Cardiol **14:**127–148.
7. **Cohn JN, Levine TB, Olivari MT, Garberg V, Lura D, Francis GS, Simon AB, Rector T.** 1984. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. N Engl J Med **311:**819–823.
8. **Dagnini G.** 1908. Interne ad un riflesso provocato in aleuni emiplegici colin stimolo della cornea e celia pressione sul bulbo oculare. Boll Sci Med **8:**380–381. [Article in Italian].
9. **Eckberg DL, Drabinsky M, Braunwald E.** 1971. Defective cardiac parasympathetic control in patients with heart disease. N Engl J Med **285:**877–883.
10. **Espahbodi E, Sanatkar M, Sadrossadat H, Darabi Vafsi ME, Azarshahin M, Shoroughi M.** 2015. Ketamine or atropine: which one better prevents oculocardiac reflex during eye surgery? A prospective randomized clinical trial. Acta Med Iran **53:**158–161.
11. **Floras JS.** 2009. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. J Am Coll Cardiol **54:**375–385.
12. **Florea VG, Cohn JN.** 2014. The autonomic nervous system and heart failure. Circ Res **114:**1815–1826.
13. **Häggström J, Hamlin RL, Hansson K, Kvart C.** 1996. Heart rate variability in relation to severity of mitral regurgitation in Cavalier King Charles spaniels. J Small Anim Pract **37:**69–75.
14. **Hamlin R.** 1999. Normal cardiovascular physiology, p 25–37. In: Fox PR, Sisson DD, Moïse NS, editors. Textbook of canine and feline cardiology. Principles and practice, 2nd ed. Philadelphia (PA): WB Saunders.
15. **He X, Zhao M, Bi X, Sun L, Yu X, Zhao M, Zang W**. 2017. Novel strategies and underlying protective mechanisms of modulation of vagal activity in cardiovascular diseases. Br J Pharmacol **172:** 5489–5500.
16. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
17. **Ishise H, Asanoi H, Ishizaka S, Joho S, Kameyama T, Umeno K, Inoue H.** 1998. Time course of sympathovagal imbalance and left ventricular dysfunction in conscious dogs with heart failure. J Appl Physiol (1985) **84:**1234–1241.
18. **Joffe WS, Gay AJ.** 1966. The oculorespiratory cardiac reflex in the dog. Invest Ophthalmol **5:**550–554.
19. **Koerbel A, Gharabaghi A, Samii A, Gerganov V, von Gösseln H, Tatagiba M, Samii M.** 2005. Trigeminocardiac reflex during skull base surgery: mechanism and management. Acta Neurochir (Vienna) **147:**727–732.
20. **Lahiri MK, Kannankeril PJ, Goldberger JJ.** 2008. Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. J Am Coll Cardiol **51:**1725–1733.
21. **Little CJ, Julu PO, Hansen S, Reid SWJ.** 2005. Noninvasive real-time measurements of cardiac vagal tone in dogs with cardiac disease. Vet Rec **156:**101–105.
22. **López-Alvarez J, Boswood A, Moonarmart W, Hezzell MJ, Lotter N, Elliott J.** 2014. Longitudinal Electrocardiographic evaluation of dogs with degenerative mitral valve disease. J Vet Intern Med **28:**393–400.
23. **Montano N, Cogliati C, Dias da Silva VJ, Gnecchi-Ruscone T, Malliani A.** 2001. Sympathetic rhythms and cardiovascular oscillations. Autonom Neurosci **90:**29–34.
24. **Nihei M, Lee JK, Honjo H, Yasui K, Uzzaman M, Kamiya K, Opthof T, Kodama I.** 2005. Decreased vagal control over heart rate in rats with right-sided congestive heart failure: downregulation of neuronal nitric oxide synthase. Circ J **69:**493–499.
25. **Nolan J, Batin PD, Andrews R, Lindsay SJ, Brooksby P, Mullen M, Baig W, Flapan AD, Cowley A, Prescott RJ, Neilson JM, Fox KA.** 1998. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). Circulation **98:**1510–1516.
26. **Ogawa M, Zhou S, Tan AY, Song J, Gholmieh G, Fishbein MC, Luo H, Siegel RJ, Karagueuzian HS, Chen LS, Lin SF, Chen PS.** 2007. Left stellate ganglion and vagal nerve activity and cardiac arrhythmias in ambulatory dogs with pacing-induced congestive heart failure. J Am Coll Cardiol **50:**335–343.
27. **Oh AY, Yun MJ, Kim MJ, Kim HJ, Kim HS.** 2007. Comparison of desflurane with sevoflurane for the incidence of oculocardiac reflex in children undergoing strabismus surgery. Br J Anaesth **99:**262–265.

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28. **Oliveira MS, Muzzi RA, Araújo RB, Muzzi LA, Ferreira DF, Nogueira R, Silva EF.** 2012. Heart rate variability parameters of myxomatous mitral valve disease in dogs with and without heart failure obtained using 24-hour Holter electrocardiography. Vet Rec **170:**622–622.
29. **Reynolds CA, Brown DC, Rush JE, Fox PR, Nguyenba TP, Lehmkuhl LB, Gordon SG, Kellihan HB, Stepien RL, Lefbom BK, Meier CK, Oyama MA.** 2012. Prediction of first onset of congestive heart failure in dogs with degenerative mitral valve disease: the PREDICT cohort study. J Vet Cardiol **14:**193–202.
30. **Saul JP, Arai Y, Berger RD, Lilly LS, Colucci WS, Cohen RJ.** 1988. Assessment of autonomic regulation in chronic congestive heart failure by heart rate spectral analysis. Am J Cardiol 61:1292–1299.
31. **Schwartz PJ, Ferrari GM.** 2010. Sympathetic–parasympathetic interaction in health and disease: abnormalities and relevance in heart failure. Heart Fail Rev **16:**101–107.
32. **Singh J, Roy S, Mukherjee P, Konar D, Konar A, Hazra S.** 2010. Influence of topical anesthetics on oculocardiac reflex and corneal healing in rabbits. Int J Ophthalmol **3:**14–18.
33. **Sosunov EA, Anyukhovsky EP, Gainullin RZ, Plotnikov A, Danilo P Jr, Rosen MR.** 2001. Long-term electrophysiological effects of regional cardiac sympathetic denervation of the neonatal dog. Cardiovasc Res **51:**659–669.
34. **Stein PK, Bosner MS, Kleiger RE, Conger BM**. 1994. Heart rate variability: a measure of cardiac autonomic tone. Am Heart J **127:**1376–1381.
35. **Takase B.** 2010. Role of heart rate variability in noninvasive electrophysiology: prognostic markers of cardiovascular disease. J Arrhythm **26:**227**–**237.
36. **Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.** 1996. Heart rate variability: standards of measurements, physiological interpretation, and clinical use. Circulation **93:**1043–1065.
37. **Tomaselli GF, Marban E.** 1999. Electrophysiological remodeling in hypertrophy and heart failure. Cardiovasc Res **42:**270–283.
38. **Turner Giannico A, de Sampaio MO, Lima L, Corona Ponczek C, De Lara F, Montiani-Ferreira F.** 2013. Characterization of the oculocardiac reflex during compression of the globe in Beagle dogs and rabbits. Vet Ophthalmol **17:**321–327.
39. **Vanderlei LCM, Pastre CM, Hoshi RA, Carvalho TD, Godoy MF.** 2009. Basic notions of heart rate variability and its clinical applicability. Rev Bras Cir Cardiovasc **24:** 205–217.

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