

## Effect of Pravastatin on Echocardiographic Circulation Parameters in Dogs

Shinji ARITA<sup>1,2)</sup>, Noboru ARITA<sup>2)</sup> and Yoshiaki HIKASA<sup>1)\*</sup>

<sup>1)</sup>Laboratory of Veterinary Internal Medicine, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan

<sup>2)</sup>Arita Sougo Animal Hospital, 1-14-6 Nishi, Hachihonmatsu, Higashihiroshima, Hiroshima 739-0147, Japan

(Received 11 October 2013/Accepted 22 November 2013/Published online in J-STAGE 6 December 2013)

**ABSTRACT.** The purpose of this study was to determine the effect of pravastatin (PS) on hemodynamic parameters in healthy dogs. Five beagle dogs were repeatedly used in each of the 4 groups. One group was not medicated (control). Dogs in other groups received 0.5, 1.0 or 2.0 mg/kg PS orally q24hr, for 4 weeks. Physical examination, blood biochemical tests, blood pressure measurements and Doppler echocardiography were performed before and 1, 2 and 4 weeks after PS administration in all dogs. PS significantly reduced the left atrial-to-aortic diameter ratio (LA/Ao), early diastolic transmitral flow (E) wave, E/early diastolic mitral annulus motion velocity (Em) ratio, left ventricular (LV) fractional shortening, LV ejection fraction, mid systolic myocardial velocity gradient, stroke volume (SV), cardiac output (CO), right and left ventricular Tei indices and elevated Em and early diastolic myocardial velocity gradient. Heart rate was not significantly altered during PS administration, but mean blood pressure decreased slightly. The hematological and blood biochemical values were within normal limits during PS administration. These results revealed that PS administration increases LV expansion capacity and decreases LV constriction and left atrial pressure. It has been suggested that PS may be effective in improving heart failures with LV diastolic dysfunction or elevated left atrial pressure in dogs.

**KEY WORDS:** canine, cardiac function, echocardiography, hemodynamics, pravastatin.

doi: 10.1292/jvms.13-0505; *J. Vet. Med. Sci.* 76(4): 481–489, 2014

Pravastatin (PS), the 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor, reduces blood cholesterol levels by inhibiting HMG-CoA reductase in the mevalonic acid pathway [27]. PS has also been reported to reduce the incidence and severity of myocardial infarction in humans [22, 34]. This cardioprotective efficacy may be caused by the pleiotropic effects and antihypercholesterolemic properties of PS [39]. For example, PS inhibits cardiac remodeling caused by pressure overload in mice [41] and reduces cardiac angiotensin (AT) expression level in spontaneously hypertensive rats [12]. In hypercholesterolemic males, statin derivatives reduce AT subtype 1 (AT1) receptor expression, inhibit AT-induced vasoconstriction and enhance the effects of AT receptor antagonists [24]. Moreover, statin derivatives exert anti-inflammatory responses in humans [2]. Long-term administration of PS reduces plasma concentrations of C-reactive protein, an established marker of inflammation, independent of a change in lipid profile in human patients with myocardial infarction [28]. In addition, statin derivatives, including PS, exert an antiarrhythmic effect in multiple species, including mice, rats and humans [1, 15, 16, 19, 37], as well as an antithrombotic effect in humans [6]. Furthermore, statins activate the PI3-kinase/Akt pathway through inhibi-

tion of the mevalonic acid pathway [27] and activates endothelial nitric oxide synthase followed by an increase in the nitric oxide (NO) production [7, 21, 29]. In addition, statins induce the differentiation of vascular endothelial progenitor cells through the PI3-kinase/Akt pathway [8]. The plethora of cardiovascular effects of statins has been revealed through *in vitro* and *in vivo* murine and human studies. Therefore, we hypothesized that statins, particularly PS, may be a powerful therapeutic agent in veterinary medicine for the treatment of cardiovascular diseases in dogs. Furthermore, canine models of heart failure are critical for the translation of new discoveries to humans. Therefore, it is critical to examine the cardiovascular effects of statins to better understand their mechanism of action. To the best of our knowledge, there are no reports in the literature on the cardiovascular effects of statins, including PS, in dogs. Therefore, the aim of the present study was to fully characterize the effects of PS on key hemodynamic and functional parameters in this species as well as to determine its potential safety and efficacy for veterinary applications.

### MATERIALS AND METHODS

**Animals:** Five healthy adult, neutered female beagle dogs aged 9–19 months (mean weight,  $7.9 \pm 0.3$  kg) were used in this study. Animals were fed a standard commercial dry food formulated for dogs and raised in an appropriate animal management facility. Examinations performed before the experiments confirmed that all dogs were healthy with physical examination and hematological values within reference limits. The study protocol was approved by the Animal Research Committee of Tottori University, Tottori, Japan.

\*CORRESPONDENCE TO: HIKASA, Y., Laboratory of Veterinary Internal Medicine, Joint Department of Veterinary Medicine, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan. e-mail: hikasa@muses.tottori-u.ac.jp

©2014 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

**Experimental design and drug administration:** The dogs were randomly assigned to one of 4 groups. One group was not medicated (control). Dogs in the other groups received 0.5, 1.0 or 2.0 mg/kg of pravastatin sodium (PS; pravastatin 10 mg tablet, Tanabe Mitsubishi, Osaka, Japan) orally q24 hr, for 4 weeks; hereafter, referred to as PS 0.5, PS 1 and PS 2. Physical examination, blood biochemical tests, blood pressure measurement and Doppler echocardiography were performed immediately before PS administration (time 0; baseline) and 1, 2 and 4 weeks after PS administration. There were at least 30 days between successive treatments for each dog. PS was given with breakfast, and venous blood sampling and each measurement were performed 2 hr later.

**Sample collection:** Blood 9.5 ml was collected from the jugular vein of each dog. A 0.5 ml aliquot was mixed with ethylene diamine tetraacetic acid (EDTA) for blood cell counts, and the another 1.0 ml was mixed with heparin for plasma biochemical measurements. In addition, a 3.0 ml aliquot was mixed with aprotinin-containing EDTA for atrial natriuretic peptide (ANP) measurement. The other 5 ml was transferred to a tube for serum collection to measure N-terminal pro-brain natriuretic peptide (NT-proBNP) and NO concentrations. After centrifugation, the plasma or serum was separated. The blood cell counts were measured using an automatic blood cell analyzer. Blood urea nitrogen, creatinine, total bilirubin, total cholesterol, triglyceride and inorganic phosphorus concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatine phosphokinase activities were measured using an automatic biochemistry analyzer (Fuji Dri Chem 3500V, Fuji Film Medical, Tokyo, Japan). Plasma sodium, potassium and chloride concentrations were measured using a clinical electrolyte analyzer (Fuji Dri Chem 800V, Fuji Film Medical). Serum NT-proBNP concentration was measured using an enzyme-linked immunosorbent assay at a reference laboratory (IDEXX Laboratories, Tokyo, Japan). Plasma ANP level was measured using a chemiluminescence enzyme immunoassay at another reference laboratory (Fukuyama Medical Laboratory, Hiroshima, Japan). The total NO was measured using a colorimetric method with nitrate reductase enzyme (Nitric Oxide total detection kit, Enzo Life Sciences Inc., Farmingdale, NY, U.S.A.) at our laboratory. Serum and plasma samples for NT-proBNP, ANP and NO measurements were stored at  $-40^{\circ}\text{C}$  for analysis. Samples for NT-proBNP and ANP measurements were sent to the reference laboratory within 1 day. The NO was measured within 1 month at our laboratory. Other blood samples were immediately analyzed after the sampling.

**Echocardiography and circulation parameter measurements:** Mean blood pressure (MBP) was measured by the oscillometric method using a noninvasive blood pressure monitor (Dinamap 8300, Critikon, Tampa, Florida, U.S.A.) attached to the tail ridge in the prone position. Transthoracic 2-dimensional; M-122 mode; pulsed continuous wave; and tissue Doppler echocardiography were performed with dogs in the right or left lateral recumbency using a digital ultrasonography system (Prosound  $\alpha 7$ , Hitachi Aloka Medical, Tokyo, Japan) with a 5 MHz probe. Heart rate (HR) was si-

multaneously calculated from the preceding R-to-R interval on the electrocardiogram. Using the M-mode method, the left atrium/aorta ratio (LA/Ao) was measured from the left ventricular (LV) outflow tract view in the long-axis plane under right lateral recumbency. LV fractional shortening (FS) [30], LV ejection fraction (EF) [30], end-diastolic interventricular septum wall-thickness (IVSd), end diastolic LV inner dimension (LVIDd), end-diastolic LV posterior wall thickness (LVPWd), end-systolic interventricular septum wall thickness (IVSs), end-systolic LV inner dimension (LVIDs) and end-systolic LV posterior wall thickness (LVPWs) were measured in the LV short-axis view. Using pulsed Doppler echocardiography in left lateral recumbency, the early diastolic transmitral flow (E) wave, late diastolic transmitral flow (A) wave, ratio of peak velocity of E to that of A (E/A) and deceleration time of the E wave (DecT) were recorded in the left apical 4-chamber view. Moreover, in the apical 5-chamber view, a pulsed-wave sample volume was placed just under the aortic valve, and thus, the cross-sectional area (CSA) of the left ventricular outflow tract, aortic ejection flow velocity (AEV) and time velocity integral (TVI) were measured, and stroke volume (SV) [18] and cardiac output (CO) were calculated. CO was calculated as  $\text{SV} \times \text{HR}$ . Furthermore, time (a) from the end of the left ventricular inflow to the initiation of re-inflow was measured from the left apical 4-chamber view. Time (b) from the onset to the end of the LV ejection flow was measured from the apical 5-chamber view. The LV Tei index was calculated as  $(a - b)/b$  [36]. Likewise, the right ventricular Tei index was determined from time (a) from the end of the right ventricular tricuspid inflow to the initiation of re-inflow in the apical 4-chamber view and time (b) from the onset to the end of the right ventricular ejection flow in the apical short-axis view [36]. Using tissue Doppler imaging, the mitral annulus motion velocity wave was recorded from the left apical 4-chamber. The early diastolic mitral annulus motion velocity ( $E_m$ ) and atrial systolic mitral annulus motion velocity ( $A_m$ ) were measured, and the ratio of  $E_m$  to  $A_m$  ( $E_m/A_m$ ) was calculated. The ratio of E wave to  $E_m$  ( $E/E_m$ ) was also calculated. Moreover, the endomyocardial ( $V_{\text{end}}$ ) and epimyocardial velocities ( $V_{\text{epi}}$ ) were measured at the posterior wall of the left ventricle in the short-axis view, and the myocardial velocity gradient (MVG) was calculated by dividing the difference ( $V_{\text{end}} - V_{\text{epi}}$ ) by a distance between the two points [38]. MVG was measured at mid systole (MVGs), early diastole (MVGe) and atrial systole (MVGa). The measurement of each echocardiographic parameter was repeatedly performed at least 3 times, and the averaged value was adopted as data. All measures and follow-up on each dog were performed by the same investigator.

**Statistical analysis:** All data obtained were analyzed with a commercially available software (StatMate3, ATMS, Tokyo, Japan). One-way analysis of variance was used to examine the time effect within each group for blood biochemical, echocardiographic and circulation variables. When a significant difference was detected, the Tukey test was used to compare the means. Student's *t*-test was used for comparison between the treatments at each time point.

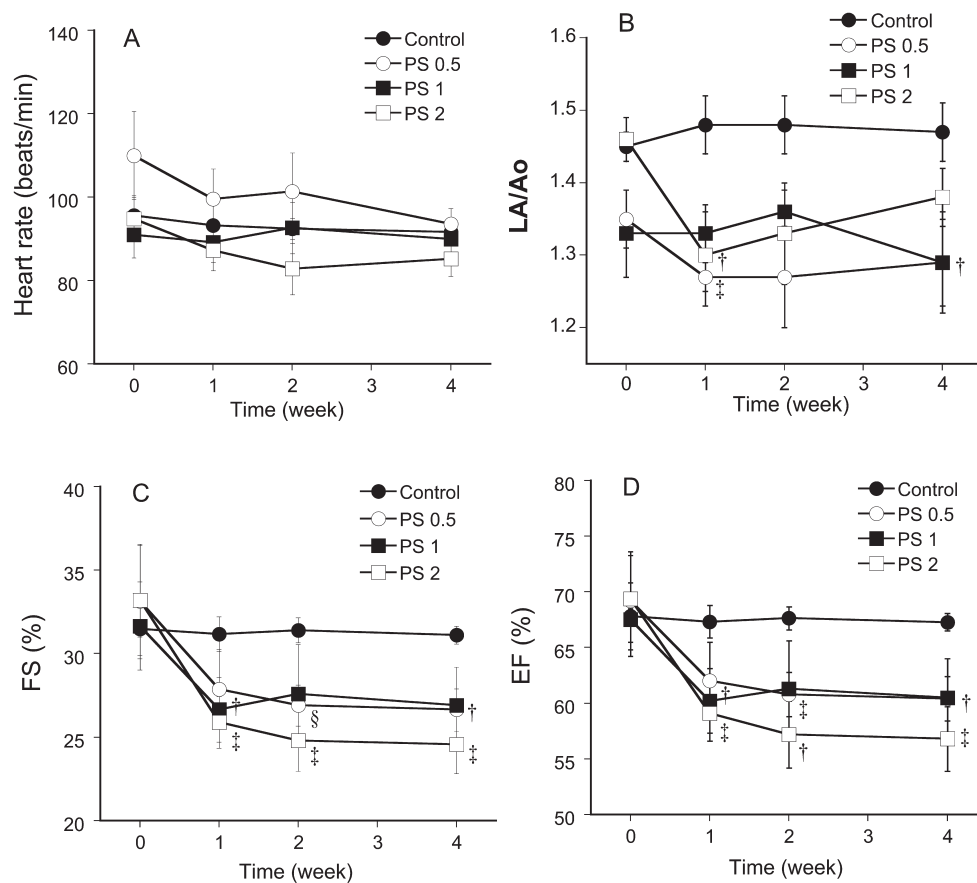


Fig. 1. Changes in heart rate (A), left atrial-to-aortic ratio (LA/Ao; B), left ventricular fractional shortening (FS; C) and left ventricular ejection fraction (EF; D) after pravastatin administration in five dogs. Data are shown as the mean  $\pm$  standard error. PS 0.5, 1 and 2 = pravastatin 0.5, 1 and 2 mg/kg, respectively. \* $P < 0.05$ , significantly different from the baseline (time) value. † $P < 0.05$ , ‡ $P < 0.01$  and § $P < 0.001$ , significantly different from the control group.

Results were expressed as mean  $\pm$  standard error. The level of significance in all tests was set at  $P < 0.05$ .

## RESULTS

**Echocardiographic and circulation variables:** Changes in HR, LA/Ao, FS and EF are shown in Fig. 1. There were no significant changes in HR after PS administration within or between the groups (Fig. 1A). LA/Ao significantly tended to decrease at 1 week in the PS 2 group compared with baseline, but not significantly. LA/Ao in PS 0.5, 1 and 2 groups was significantly lower than that in the control group at 1 week, 4 weeks and 1 week, respectively (Fig. 1B). Both FS and EF in all PS-treated groups tended to decrease after PS administration, but not significantly. Both FS and EF in PS 0.5 and 1 groups significantly decreased compared with those in the control group at 2–4 weeks and 1 week, respectively (Fig. 1C and 1D). Both FS and EF in PS 2 group significantly decreased at 1–4 weeks compared with those in the control group (Fig. 1C and 1D). The differences in

both parameters were greatest in the PS 2 group. There were no significant differences within or between the groups in other key echocardiographic measurements, including IVSd, LVIdD, LVPWd, IVSs, LVIdS and LVPWs.

Table 1 shows the changes in E wave, A wave, E/A, DecT and E/Em values after PS administration. E wave at 4 weeks after PS administration significantly decreased in the PS 2 group compared with baseline and was lower than the control group at the same time point. The A wave did not significantly change in response to any of the treatments. E/A at 2 weeks in the PS 1 group significantly decreased compared with baseline. E/A in the PS 2 group tended to decrease after PS administration, but the differences were not statistically significant. DecT in the PS 0.5 and 2 groups significantly increased in comparison with baseline at 1, 2 or 4 weeks after PS administration. In contrast, E/Em in the PS 2 group significantly decreased at 4 weeks compared with baseline, and E/Em in both PS 1 and 2 groups was significantly lower than that in the control at 4 weeks after PS administration.

As shown in Fig. 2, LV Tei index after PS administration

Table 1. Changes in E wave, A wave, E/A, DecT and E/Em values after pravastatin administration in five dogs

Variables	Group	Time after pravastatin administration (week)			
		0	1	2	4
E wave (cm/sec)	Control	65.3 ± 3.0	64.6 ± 3.7	65.4 ± 4.1	63.3 ± 3.0
	PS 0.5	68.0 ± 2.9	66.8 ± 2.9	62.2 ± 2.2	61.7 ± 2.6
	PS 1	67.3 ± 1.9	60.1 ± 2.7	62.1 ± 2.4	63.0 ± 2.2
	PS 2	73.0 ± 2.8	64.3 ± 2.5	64.5 ± 1.7	58.6 ± 3.2*†
A wave (cm/sec)	Control	40.5 ± 1.4	45.5 ± 3.7	40.0 ± 4.5	42.3 ± 2.6
	PS 0.5	39.9 ± 2.8	49.2 ± 2.8	38.3 ± 4.2	39.4 ± 3.3
	PS 1	32.8 ± 1.8	40.9 ± 4.2	44.3 ± 2.9	43.8 ± 5.7
	PS 2	37.5 ± 4.5	42.3 ± 3.3	44.9 ± 3.0	41.3 ± 3.4
E/A	Control	1.61 ± 0.05	1.44 ± 0.09	1.67 ± 0.10	1.50 ± 0.07
	PS 0.5	1.74 ± 0.13	1.37 ± 0.05	1.69 ± 0.16	1.60 ± 0.11
	PS 1	2.12 ± 0.18	1.53 ± 0.15	1.42 ± 0.09*	1.53 ± 0.17
	PS 2	2.09 ± 0.30	1.56 ± 0.14	1.46 ± 0.07	1.44 ± 0.12
DecT (msec)	Control	93.6 ± 4.5	90.0 ± 4.2	86.4 ± 6.2	90.0 ± 3.3
	PS 0.5	80.4 ± 5.6	98.4 ± 3.1*	97.2 ± 2.2*	91.2 ± 3.5
	PS 1	76.8 ± 5.8	88.8 ± 5.2	91.2 ± 3.5	96.0 ± 7.3
	PS 2	78.0 ± 4.4	92.4 ± 5.9	92.4 ± 4.1	104.4 ± 7.2*
E/Em	Control	8.1 ± 0.4	8.1 ± 0.5	8.2 ± 0.4	8.2 ± 0.3
	PS 0.5	8.3 ± 0.5	8.2 ± 0.4	7.5 ± 0.3	7.7 ± 0.4
	PS 1	9.1 ± 0.6	7.2 ± 0.5	7.6 ± 0.6	7.5 ± 0.2†
	PS 2	9.7 ± 0.4	8.3 ± 0.4	8.2 ± 0.3	7.5 ± 0.5*†

Data are shown as the mean ± SE. E=the early diastolic transmitral flow, A=late diastolic transmitral flow, E/A=ratio of peak velocity of early diastolic transmitral flow to peak velocity of late diastolic transmitral flow, DecT=deceleration time of early diastolic transmitral flow wave and E/Em=ratio of peak velocity of early diastolic transmitral flow to early diastolic mitral annulus motion velocity. PS 0.5, 1 and 2=pravastatin 0.5, 1 and 2 mg/kg, respectively. \*  $P<0.05$  and \*\*  $P<0.01$ , significantly different from the baseline (time 0) value. †  $P<0.05$ , significantly different from the control group.

was significantly lower in all PS-treated groups than the control. The right ventricular Tei index after PS administration was also significantly lower in PS 1 group than that in the control group.

Table 2 shows the PS-induced changes in tissue Doppler echocardiographic metrics, including Em, Am, Em/Am, MVGs, MVGe and MVGa. Em measured 4 weeks after PS administration was significantly higher in the PS 1 group than in the control group at the same time point. Both Am and Em/Am did not significantly change after PS administration in any group. MVGs in all PS-treated groups significantly decreased at 1 or 4 weeks compared with baseline. MVGs in the PS 1 group were significantly lower than those in the control group at 4 weeks after PS administration. MVGe in all PS-treated groups tended to increase after PS administration, but not significantly, and MVGe values were significantly higher in all PS-treated groups than those in control group at 1, 2 or 4 weeks after PS administration. The MVGa levels were not significantly altered after PS administration.

Changes in AEV, VTI, CO, SV and MBP values are shown in Table 3. Both AEV and VTI tended to decrease after PS administration in PS-treated groups with significant decreases of AEV at 2 weeks in the PS 0.5 group and VTI at 1–4 weeks in the PS 1 group compared with baseline. Both AEV and VTI in all PS-treated groups were significantly lower than the control group at 1, 2 or 4 weeks after PS administration. Both CO and SV were also significantly

lower in all PS-treated groups than the control group at 1, 2 or 4 weeks after PS administration. MBP tended to decrease slightly after PS administration in PS-treated groups, but the decrease was not significant. MBP in PS 1 group was slightly and significantly lower than the control group at 2 weeks after PS administration.

*Hematological and blood biochemical variables:* Packed cell volume, white blood cell and platelet counts did not significantly change in any group. NT-proBNP and ANP concentrations did not significantly change after PS administration in any group (Table 4). NO concentration in PS-treated groups tended to increase after PS administration, although this increase was not significant because of a large variance. Total cholesterol tended to decrease after PS administration in the PS 2 group, but did not significantly change in any group. Plasma triglyceride, creatinine, total bilirubin, inorganic phosphorus, blood urea nitrogen, sodium, potassium and chloride concentrations and aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and creatine phosphokinase activities did not significantly change after PS administration in any group. No other behavioral adverse effects were observed in the groups.

## DISCUSSION

Statins are classified as water-soluble and fat-soluble agents. It is known that in myocardial infarction, even if the



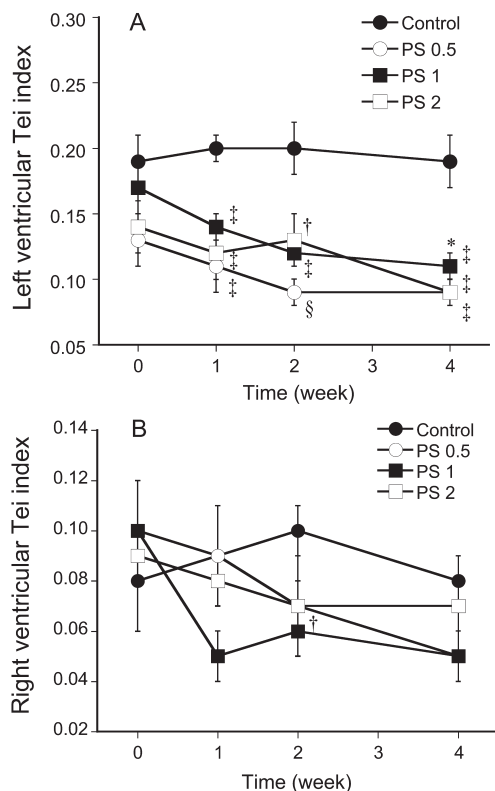


Fig. 2. Changes in the right (A) and left (B) ventricular Tei indices after pravastatin administration in five dogs. Data are shown as the mean  $\pm$  standard error. PS 0.5, 1 and 2 =pravastatin 0.5, 1 and 2 mg/kg, respectively. \* $P$ <0.05, significantly different from the baseline (time 0) value. † $P$ <0.05, ‡ $P$ <0.01 and § $P$ <0.001, significantly different from the control group.

ischemia is repaired, recovery of the myocardial contraction may be delayed because of the stunning phenomenon [4]. A previous study has reported that a fat-soluble simvastatin in comparison with a water-soluble PS administered 2 mg/kg orally q24hr for 3 weeks delayed the recovery of the myocardial contraction after reperfusion in the myocardial ischemic dogs produced by the ligation of the left anterior descending coronary artery experimentally [14]. With regard to these reports, a water-soluble statin, PS, was selected, and its dosages were determined as 0.5–2 mg/kg/day in the present study. In addition, the period of medication with PS in our study was determined as 4 weeks in reference to a previous study that simvastatin treatment was performed for 4 weeks in clinical trials of human patients with chronic heart failure [17].

The results of this study revealed that PS administration reduced LA/Ao, E wave and E/Em and elevated Em and MVGe in healthy dogs. Em is an index of the ability of early diastolic LV relaxation. It has been reported that the reduction of Em correlates with the decrease in LV dilation in humans [25] and veterinary medicine [13]. It has been also reported that E/Em is an index to estimate both the left

atrial pressure and LV filling pressure [26, 33]. Therefore, the reduction of E/Em and elevation of both Em and MVGe induced by PS treatment in the present study indicate an enhancement of the LV expansionability. Our results in dogs were in agreement with a previous finding that PS improved the LV expansionability in AT II-induced hypertensive mice [40]. This may be due to the relaxation of the cardiac muscle caused by the NO derived from eNOS [5]. However, in the present study, plasma NO levels did not significantly change after PS treatment, although the level of NO production may be high at the cellular level. On the other hand, statins including PS can attenuate the myocardial remodeling by suppressing the activity of small GTP-binding proteins, such as Ras, Rho and Rac, through inhibition of the mevalonic acid pathway [27, 35]. This effect may be also responsible for the relaxation of the cardiac muscle in the present study.

In contrast, the reduction of LA/Ao, E wave and E/Em observed in the present study suggests the decrease in the left atrial pressure. This event may be because of the decrease in both cardiac preload and afterload that are caused by dilation of blood vessels through the action of NO derived from eNOS [31] and PS-induced reduction of cardiac AT expression [12]. In addition, as statins are partial inhibitors of Rho-kinase, the decrease in the left atrial pressure after PS treatment in the present study may be caused by PS-induced inhibition of vasoconstrictor effect of Rho-kinase through activation of myosin phosphatase [35]. Furthermore, the present study revealed that PS administration reduced FS, EF, MVGs and CO in healthy dogs. These results may also be caused by negative inotropic action of NO derived from eNOS [23]. The reduction of CO after PS administration may primarily be caused by the decrease in SV observed in the present study. It has been reported that PS administration reduces HR in spontaneously hypertensive rats [12]. In this study, however, we observed no evidence for significant changes in HR after PS administration. These results show that PS administration does not enhance sympathetic activity in dogs.

Elevations of both LA/Ao and E wave are useful for the evaluation of prognosis and severity of heart failure and the presence of mitral regurgitation in dogs [3]. Restoration of elevated left atrial pressures back to baseline is an important strategy for treating dogs with mitral regurgitation. Therefore, the present study suggests that PS-induced reduction of LA/Ao, E wave and E/Em, elevation of Em, and reduction of left atrial pressure may be useful for therapy in dogs with mitral regurgitation. On the other hand, the reduction of FS, EF and CO in this study may be felt uneasy about exacerbation of the heart failure. However, an elevation of the left ventricular afterload will be induced by the activation of the renin-angiotensin system in dogs with significant heart failure. In these conditions, PS may be effective in improving the heart failure through the reduction of the cardiac afterload due to arterial dilation. In addition, the use of PS in dogs with chronic heart failure may be recommended for a moderate severity stage rather than a seriously advanced terminal stage. With regard to the dosages of PS required to elicit the observed cardiovascular effects, our findings indicate that

Table 2. Changes in tissue Doppler echocardiographic variables after pravastatin administration in five dogs

Variables	Group	Time after pravastatin administration (week)			
		0	1	2	4
Em (cm/sec)	Control	8.1 ± 0.2	7.9 ± 0.2	7.9 ± 0.1	7.7 ± 0.2
	PS 0.5	8.3 ± 0.3	8.2 ± 0.4	8.3 ± 0.4	8.1 ± 0.4
	PS 1	7.5 ± 0.4	8.4 ± 0.5	8.3 ± 0.4	8.4 ± 0.3 <sup>†</sup>
	PS 2	7.5 ± 0.4	7.8 ± 0.3	7.9 ± 0.3	7.8 ± 0.2
Am (cm/sec)	Control	5.5 ± 0.3	5.5 ± 0.3	5.5 ± 0.3	5.5 ± 0.2
	PS 0.5	5.2 ± 0.5	5.4 ± 0.5	5.6 ± 0.4	5.4 ± 0.4
	PS 1	4.3 ± 0.5	5.5 ± 0.6	5.6 ± 0.2	6.0 ± 0.6
	PS 2	4.5 ± 0.6	5.5 ± 0.4	5.3 ± 0.7	5.2 ± 0.5
Em/Am	Control	1.49 ± 0.07	1.43 ± 0.8	1.44 ± 0.07	1.41 ± 0.07
	PS 0.5	1.64 ± 0.13	1.58 ± 0.18	1.52 ± 0.14	1.50 ± 0.07
	PS 1	1.80 ± 0.15	1.58 ± 0.14	1.48 ± 0.02	1.43 ± 0.09
	PS 2	1.75 ± 0.15	1.44 ± 0.09	1.57 ± 0.15	1.54 ± 1.13
MVGs (sec)	Control	1.76 ± 0.13	1.74 ± 0.13	1.78 ± 0.15	1.80 ± 0.10
	PS 0.5	2.30 ± 0.19	1.51 ± 0.26*	1.59 ± 0.19	1.52 ± 0.16*
	PS 1	2.09 ± 0.26	1.73 ± 0.06	1.46 ± 0.19	1.22 ± 0.18* <sup>†</sup>
	PS 2	2.19 ± 0.15	1.78 ± 0.11	1.70 ± 0.06	1.66 ± 0.14*
MVGe (sec)	Control	-1.73 ± 0.10	-1.61 ± 0.12	-1.68 ± 0.19	-1.68 ± 0.15
	PS 0.5	-1.51 ± 0.19	-1.99 ± 0.10 <sup>†</sup>	-2.06 ± 0.23	-2.19 ± 0.27
	PS 1	-1.35 ± 0.20	-2.22 ± 0.23 <sup>†</sup>	-2.36 ± 0.52	-2.62 ± 0.23 <sup>‡</sup>
	PS 2	-1.69 ± 0.29	-2.37 ± 0.24 <sup>†</sup>	-2.69 ± 0.30 <sup>†</sup>	-2.33 ± 0.20 <sup>†</sup>
MVGa (sec)	Control	-1.03 ± 0.17	-0.97 ± 0.16	-1.11 ± 0.08	-1.18 ± 0.23
	PS 0.5	-0.64 ± 0.52	-0.40 ± 0.85	-1.08 ± 0.16	-0.92 ± 0.20
	PS 1	-1.15 ± 0.12	-1.44 ± 0.29	-1.19 ± 0.10	-0.69 ± 0.44
	PS 2	-1.14 ± 0.44	-1.89 ± 0.28	-1.48 ± 0.37	-1.30 ± 0.49

Data are shown as the mean ± SE. Em=early diastolic mitral annulus motion velocity, Am=atrial systolic mitral annulus motion velocity, Em/Am=ratio of early diastolic mitral annulus motion velocity to atrial systolic mitral annulus motion velocity, MVGs=mid systolic myocardial velocity gradient, MVGe=early diastolic myocardial velocity gradient and MVGa=atrial systolic myocardial velocity gradient. PS 0.5, 1 and 2=pravastatin 0.5, 1 and 2 mg/kg, respectively. \* $P<0.05$ , significantly different from the baseline value. <sup>†</sup> $P<0.05$  and <sup>‡</sup> $P<0.01$ , significantly different from the control group.

a dose  $\geq 1$  mg/kg is required to produce marked reduction of E/Em and LA/Ao and elevation of Em, implicating a decrease in left atrial pressure. Although at lower doses (i.e., 0.5 mg/kg), PS can reduce LA/Ao, other parameters are not significantly altered. In addition, both right and LV Tei indices, reflecting changes in global cardiac function, decreased after PS administration, suggesting that PS may be effective in improving both left and right ventricular failure in dogs. Furthermore, hyperadrenocorticism or hypothyroidism that is a typical disease showing hypercholesterolemia in dogs may increase a risk of the heart failure, such as hypertension, myocardial hypertrophy or infarction [11, 20]. PS may be also effective in reducing these cardiac complications.

It is well known that plasma concentrations of ANP and NT-proBNP are markedly increased in dogs with heart failure [9, 10]. In the present study, neither parameter was altered after PS administration, suggesting absence of an adverse response to our treatment regimen and further underscoring its safety profile.

The development of rhabdomyolysis has been reported as an adverse effect of statin therapy in clinical medicine [32]. However, in our study, we saw no evidence of behavioral abnormalities suggestive of pain or elevation of

plasma creatine phosphokinase levels after PS administration. Furthermore, in the present study, no hematological and plasma biochemical abnormalities that suggest renal and liver pathology were observed during PS administration for 4 weeks. This indicates that 0.5–2 mg/kg PS could be safely used without any apparent adverse effect in dogs.

In conclusion, the present study revealed the major effects of PS in dogs that are consistent with increased LV expansion capacity and decreased LV constriction and left atrial pressure. As such, our present findings suggest that PS may be effective in improving the heart failure status of dogs with LV diastolic dysfunction or elevated left atrial pressure. To the best of our knowledge, this is the first study to systematically report the effect of PS on key echocardiographic and hemodynamic parameters in dogs.

**ACKNOWLEDGMENTS.** This study was supported in part by a Grant-in-Aid for Exploratory Research from the Japan Society for the Promotion of Science (to Y. Hikasa).

Table 3. Changes in AEV, VTI, CO, SV and MBP values after pravastatin administration in five dogs

Variables	Group	Time after pravastatin administration (week)			
		0	1	2	4
AEV (cm/sec)	Control	81.8 ± 5.6	74.3 ± 4.2	81.5 ± 2.2	76.9 ± 3.8
	PS 0.5	85.5 ± 4.4	72.3 ± 2.8	67.5 ± 3.6*†	69.8 ± 4.9
	PS 1	77.4 ± 1.5	67.1 ± 1.8	70.8 ± 2.8†	68.3 ± 3.8†
	PS 2	77.9 ± 6.3	67.1 ± 5.3	66.6 ± 3.2‡	66.9 ± 3.7†
VTI (cm)	Control	9.8 ± 0.5	9.8 ± 0.3	10.6 ± 0.3	9.7 ± 0.4
	PS 0.5	9.3 ± 0.7	8.3 ± 0.7†	8.5 ± 0.4‡	8.7 ± 0.6
	PS 1	8.9 ± 0.2	7.7 ± 0.3*‡	7.7 ± 0.3*§	7.6 ± 0.2*‡
	PS 2	9.1 ± 0.5	8.1 ± 0.5†	7.9 ± 0.6‡	7.9 ± 0.5†
CO (l/min)	Control	0.90 ± 0.07	0.85 ± 0.06	0.85 ± 0.06	0.86 ± 0.04
	PS 0.5	0.92 ± 0.10	0.76 ± 0.08	0.73 ± 0.10	0.74 ± 0.05†
	PS 1	0.74 ± 0.12	0.62 ± 0.06†	0.66 ± 0.08†	0.62 ± 0.04§
	PS 2	0.80 ± 0.12	0.70 ± 0.08	0.65 ± 0.07†	0.65 ± 0.09†
SV (ml)	Control	11.4 ± 0.5	11.2 ± 0.5	12.4 ± 0.4	11.2 ± 0.6
	PS 0.5	9.6 ± 0.7	8.2 ± 0.7§	8.7 ± 0.8‡	9.0 ± 0.7‡
	PS 1	9.4 ± 0.6	8.0 ± 0.6§	8.0 ± 0.4§	7.7 ± 0.2§
	PS 2	9.5 ± 0.8	8.3 ± 0.7†	8.3 ± 1.0‡	7.8 ± 0.9†
MBP (mmHg)	Control	80 ± 2	82 ± 2	85 ± 3	82 ± 4
	PS 0.5	103 ± 12	95 ± 4	85 ± 3	84 ± 5
	PS 1	92 ± 5	78 ± 3	78 ± 3†	81 ± 3
	PS 2	81 ± 3	75 ± 4	80 ± 4	81 ± 5

Data are shown as the mean ± SE. AEV=aortic ejection flow velocity, VTI=time velocity integral, CO=cardiac output, SV=stroke volume and MBP=mean blood pressure. PS 0.5, 1 and 2=pravastatin 0.5, 1 and 2 mg/kg, respectively. \* $P<0.05$ , significantly different from the baseline value. † $P<0.05$ , ‡ $P<0.01$  and § $P<0.001$ , significantly different from the control group.

Table 4. Changes in blood biochemical variables after pravastatin administration in five dogs

Variables	Group	Time after pravastatin administration (week)			
		0	1	2	4
NT-pro BNP (pmol/l)	Control	367 ± 60	343 ± 76	352 ± 64	450 ± 76
	PS 0.5	218 ± 30	247 ± 27	280 ± 32	250 ± 50
	PS 1	259 ± 18	179 ± 50	280 ± 38	311 ± 44
	PS 2	260 ± 18	340 ± 52	249 ± 38	238 ± 45
ANP (pg/ml)	Control	19.2 ± 3.4	18.1 ± 3.0	17.2 ± 2.9	19.9 ± 3.3
	PS 0.5	36.0 ± 10.1	28.3 ± 3.9	39.9 ± 8.0	40.1 ± 10.8
	PS 1	29.4 ± 5.0	39.0 ± 11.8	41.6 ± 10.7	30.8 ± 5.5
	PS 2	26.0 ± 5.5	24.1 ± 3.0	32.5 ± 3.4	20.9 ± 3.0
NO (μmol/l)	Control	29.3 ± 5.0	33.3 ± 4.3	39.4 ± 3.9	37.0 ± 6.4
	PS 0.5	36.3 ± 4.3	29.6 ± 5.0	45.1 ± 11.4	29.9 ± 5.8
	PS 1	29.6 ± 3.9	28.7 ± 7.2	26.8 ± 2.9	67.8 ± 41.3
	PS 2	31.6 ± 4.5	48.5 ± 12.1	28.6 ± 4.3	47.2 ± 7.7
TCHO (mg/dl)	Control	232 ± 25	223 ± 23	213 ± 22	238 ± 19
	PS 0.5	229 ± 22	228 ± 18	243 ± 18	248 ± 22
	PS 1	251 ± 26	239 ± 25	237 ± 22	247 ± 25
	PS 2	232 ± 26	220 ± 34	210 ± 25	219 ± 30

Data are shown as the mean ± SE. NT-pro BNP=N-terminal pro-brain natriuretic peptide, ANP=atrial natriuretic peptide, NO=nitric oxide and TCHO=total cholesterol. PS 0.5, 1 and 2=pravastatin 0.5, 1 and 2 mg/kg, respectively.

## REFERENCES

- Adam, O., Frost, G., Custodis, F., Sussman, M. A., Schafers, H. J., Bohm, M. and Laufs, U. 2007. Role of Rac1 GTPase activation in atrial fibrillation. *J. Am. Coll. Cardiol.* **50**: 359–367. [Medline] [CrossRef]
- Albert, M. A., Danielson, E., Rifai, N. and Ridker, P. M. 2001. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *J. A. M. A.* **286**: 64–70. [Medline] [CrossRef]
- Borgarelli, M., Savarino, P., Crosara, S., Santilli, R. A., Chia-vegato, D., Poggi, N., Bellino, C., La Rosa, G., Zanatta, R., Haggstrom, J. and Tarducci, A. 2008. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. *J. Vet. Intern. Med.* **22**: 120–128. [Medline] [CrossRef]
- Braunwald, E. and Kloner, R. A. 1982. The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation* **66**: 1146–1149. [Medline] [CrossRef]
- Brunner, F., Maier, R., Andrew, P., Wolkart, G., Zechner, R. and Mayer, B. 2003. Attenuation of myocardial ischemia/reperfusion injury in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. *Cardiovasc. Res.* **57**: 55–62. [Medline] [CrossRef]
- Casani, L., Sanchez-Gomez, S., Vilahur, G. and Badimon, L. 2005. Pravastatin reduces thrombogenicity by mechanisms beyond plasma cholesterol lowering. *Thromb. Haemost.* **94**: 1035–1041. [Medline]
- Datar, R., Keasemeyer, W. H., Chandra, S., Fulton, D. J. and Caldwell, R. W. 2010. Acute activation of eNOS by statins involves scavenger receptor-B1, G protein subunit Gi, phospholipase C and calcium influx. *Br. J. Pharmacol.* **160**: 1765–1772. [Medline] [CrossRef]
- Dimmeler, S., Aicher, A., Vasa, M., Mildner-Rihm, C., Adler, K., Tiemann, M., Rutten, H., Fichtlscherer, S., Martin, H. and Zeiher, A. M. 2001. HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI3-kinase/Akt pathway. *J. Clin. Invest.* **108**: 391–397. [Medline]
- Fine, D. M., DeClue, A. E. and Reinero, C. R. 2008. Evaluation of circulating amino terminal-pro-B-type natriuretic peptide concentration in dogs with respiratory distress attributable to congestive heart failure or primary pulmonary disease. *J. Am. Vet. Med. Assoc.* **232**: 1674–1679. [Medline] [CrossRef]
- Greco, D. S., Biller, B. and Van Liew, C. H. 2003. Measurement of plasma atrial natriuretic peptide as an indicator of prognosis in dogs with cardiac disease. *Can. Vet. J.* **44**: 293–297. [Medline]
- Henik, R. A. 1997. Systemic hypertension and its management. *Vet. Clin. North Am. Small Anim. Pract.* **27**: 1355–1372. [Medline]
- Herring, N., Lee, C. W., Sunderland, N., Wright, K. and Paterson, D. J. 2011. Pravastatin normalises peripheral cardiac sympathetic hyperactivity in the spontaneously hypertensive rat. *J. Mol. Cell. Cardiol.* **50**: 99–106. [Medline] [CrossRef]
- Hori, Y., Kunihiro, S., Kanai, K., Hoshi, F., Itoh, N. and Higuchi, S. 2009. The relationship between invasive hemodynamic measurements and tissue Doppler-derived myocardial velocity and acceleration during isovolumic relaxation in healthy dogs. *J. Vet. Med. Sci.* **71**: 1419–1425. [Medline] [CrossRef]
- Ichihara, K., Satou, K. and Abiko, Y. 1993. Influences of pravastatin and simvastatin, HMG-CoA reductase inhibitors, on myocardial stunning in dogs. *J. Cardiovasc. Pharmacol.* **22**: 852–856. [Medline] [CrossRef]
- Issac, T. T., Dokainish, H. and Lakkis, N. M. 2007. Role of inflammation in initiation and perpetuation of atrial fibrillation: a systematic review of the published data. *J. Am. Coll. Cardiol.* **50**: 2021–2028. [Medline] [CrossRef]
- Komatsu, T., Tachibana, H., Sato, Y., Ozawa, M., Kunugida, F., Orii, M. and Nakamura, M. 2009. Long-term efficacy of upstream therapy using angiotensin-converting enzyme inhibitors and statins in combination with antiarrhythmic agents for the treatment of paroxysmal atrial fibrillation. *Int. Heart. J.* **50**: 465–476. [Medline] [CrossRef]
- Landmesser, U., Bahlmann, F., Mueller, M., Spiekermann, S., Kirchhoff, N., Schulz, S., Manes, C., Fischer, D., de Groot, K., Fliser, D., Fauler, G., Marz, W. and Drexler, H. 2005. Simvastatin versus ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. *Circulation* **111**: 2356–2363. [Medline] [CrossRef]
- Lewis, J. F., Kuo, L. C., Nelson, J. G., Limacher, M. C. and Quinones, M. A. 1984. Pulsed Doppler echocardiographic determination of stroke volume and cardiac output: clinical validation of two new methods using the apical window. *Circulation* **70**: 425–431. [Medline] [CrossRef]
- Li, L., Yao, Y., Wang, H., Ren, Y., Ma, L., Yan, J. and Gao, C. 2010. Pravastatin attenuates cardiac dysfunction induced by lysophosphatidylcholine in isolated rat hearts. *Eur. J. Pharmacol.* **640**: 139–142. [Medline] [CrossRef]
- Manning, P. J. 1979. Thyroid gland and arterial lesions of Beagles with familial hypothyroidism and hyperlipoproteinemia. *Am. J. Vet. Res.* **40**: 820–828. [Medline]
- Martinez-Gonzalez, J. and Badimon, L. 2007. Influence of statin use on endothelial function: from bench to clinics. *Curr. Pharm. Des.* **13**: 1771–1786. [Medline] [CrossRef]
- Nakamura, H., Arakawa, K., Itakura, H., Kitabatake, A., Goto, Y., Toyota, T., Nakaya, N., Nishimoto, S., Muranaka, M., Yamamoto, A., Mizuno, K. and Ohashi, Y. 2006. Primary prevention of cardiovascular disease with pravastatin in Japan (MEGA Study): a prospective randomized controlled trial. *Lancet* **368**: 1155–1163. [Medline] [CrossRef]
- Napp, A., Brixius, K., Pott, C., Ziskoven, C., Boelck, B., Mehlhorn, U., Schwinger, R. H. and Block, W. 2009. Effects of the beta3-adrenergic agonist BRL 37344 on endothelial nitric oxide synthase phosphorylation and force of contraction in human failing myocardium. *J. Card. Fail.* **15**: 57–67. [Medline] [CrossRef]
- Nickenig, G., Baumer, A. T., Temur, Y., Kebben, D., Jochen-hovel, F. and Bohm, M. 1999. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation* **100**: 2131–2134. [Medline] [CrossRef]
- Oki, T., Tabata, T., Yamada, H., Wakatsuki, T., Shinohara, H., Nishikado, A., Iuchi, A., Fukuda, N. and Ito, S. 1997. Clinical application of pulsed Doppler tissue imaging for assessing abnormal left ventricular relaxation. *Am. J. Cardiol.* **79**: 921–928. [Medline] [CrossRef]
- Ommen, S. R., Nishimura, R. A., Appleton, C. P., Miller, F. A., Oh, J. K., Redfield, M. M. and Tajik, A. J. 2000. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: a comparative simultaneous Doppler-catheterization study. *Circulation* **102**: 1788–1794. [Medline] [CrossRef]
- Ramasubbu, K., Estep, J., White, D. L., Deswal, A. and Mann, D. L. 2008. Experimental and clinical basis for the use of statins in patients with ischemic and nonischemic cardiomyopathy. *J. Am. Coll. Cardiol.* **51**: 415–426. [Medline] [CrossRef]
- Ridker, P. M., Rifai, N., Pfeffer, M. A., Sacks, F. and Braunwald, E. 1999. Long-term effects of pravastatin on plasma concentra-



- tion of C-reactive protein. The Cholesterol and Recurrent Events (CARE) Investigators. *Circulation* **100**: 230–235. [[Medline](#)] [[CrossRef](#)]
29. Rikitake, Y. and Liao, J. K. 2005. Rho GTPases, statins, and nitric oxide. *Circ. Res.* **97**: 1232–1235. [[Medline](#)] [[CrossRef](#)]
  30. Sahn, D. J., DeMaria, A., Kisslo, J. and Weyman, A. 1978. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* **58**: 1072–1083. [[Medline](#)] [[CrossRef](#)]
  31. Sase, K. and Michel, T. 1997. Expression and regulation of endothelial nitric oxide synthase. *Trends Cardiovasc. Med.* **7**: 28–37. [[Medline](#)] [[CrossRef](#)]
  32. Schindler, C., Thorns, M., Matschke, K., Tugtekin, S. M. and Kirch, W. 2007. Asymptomatic statin-induced rhabdomyolysis after long-term therapy with the hydrophilic drug pravastatin. *Clin. Ther.* **29**: 172–176. [[Medline](#)] [[CrossRef](#)]
  33. Schober, K. E., Bonagura, J. D., Scansen, B. A., Stern, J. A. and Ponzio, N. M. 2008. Estimation of left ventricular filling pressure by use of Doppler echocardiography in healthy anesthetized dogs subjected to acute volume loading. *Am. J. Vet. Res.* **69**: 1034–1049. [[Medline](#)] [[CrossRef](#)]
  34. Shepherd, J., Cobbe, S. M., Ford, I., Isles, C. G., Lorimer, A. R., MacFarlane, P. W., McKillop, J. H. and Packard, C. J. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *New Engl. J. Med.* **333**: 1301–1307. [[Medline](#)] [[CrossRef](#)]
  35. Shimokawa, H. and Takeshita, A. 2005. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler. Thromb. Vasc. Biol.* **25**: 1767–1775. [[Medline](#)] [[CrossRef](#)]
  36. Tei, C. 1995. New non-invasive index for combined systolic and diastolic ventricular function. *J. Cardiol.* **26**: 135–136. [[Medline](#)]
  37. Thuc, L. C., Teshima, Y., Takahashi, N., Nishio, S., Fukui, A., Kume, O., Ezaki, K., Miyazaki, H., Yufu, K., Hara, M., Nakagawa, M. and Saikawa, T. 2011. Cardioprotective effects of pravastatin against lethal ventricular arrhythmias induced by reperfusion in the rat heart. *Circ. J.* **75**: 1601–1608. [[Medline](#)] [[CrossRef](#)]
  38. Uematsu, M., Miyatake, K., Tanaka, N., Matsuda, H., Sano, A., Yamazaki, N., Hiram, M. and Yamagishi, M. 1995. Myocardial velocity gradient as a new indicator of regional left ventricular contraction: detection by a two-dimensional tissue Doppler imaging technique. *J. Am. Coll. Cardiol.* **26**: 217–223. [[Medline](#)] [[CrossRef](#)]
  39. Vaughan, C. J., Murphy, M. B. and Buckley, B. M. 1996. Statins do more than just lower cholesterol. *Lancet* **348**: 1079–1082. [[Medline](#)] [[CrossRef](#)]
  40. Xu, Z., Okamoto, H., Akino, M., Onozuka, H., Matsui, Y. and Tsutsui, H. 2008. Pravastatin attenuates left ventricular remodeling and diastolic dysfunction in angiotensin II-induced hypertensive mice. *J. Cardiovasc. Pharmacol.* **51**: 62–70. [[Medline](#)] [[CrossRef](#)]
  41. Zhao, H., Liao, Y., Minamino, T., Asano, Y., Asakura, M., Kim, J., Asanuma, H., Takashima, S., Hori, M. and Kitakaze, M. 2008. Inhibition of cardiac remodeling by pravastatin is associated with amelioration of endoplasmic reticulum stress. *Hypertens. Res.* **31**: 1977–1987. [[Medline](#)] [[CrossRef](#)]