Metabolic and structural abnormalities in dogs with early left ventricular dysfunction induced by incessant tachycardia

Kathleen Mc Entee, DVM, PhD; Thierry Flandre, DVM; Cécile Dessy, DVM, PhD; Daniel Desmecht, DVM, PhD; Cécile Clercx, DVM, PhD; Marc Balligand, DVM, PhD; Charles Michaux, DVM, PhD; Eva Jonville, DVM; Nathalie Miserque, DVM; Marc Henroteaux, DVM; Bruce Keene, DVM, MSc

Objective—To assess morphologic and metabolic abnormalities in dogs with early left ventricular dysfunction (ELVD) induced by rapid right ventricular pacing (RRVP).

Animals—7 Beagles.

Procedure—Plasma carnitine concentrations were measured before and after development of ELVD induced by RRVP. At the same times, transvenous endomyocardial biopsy was performed, and specimens were submitted for determination of myocardial carnitine concentrations and histologic, morphometric, and ultrastructural examination.

Results—In 4 dogs in which baseline plasma total carnitine concentration was normal, RRVP induced a decrease in myocardial total and free carnitine concentrations and an increase in myocardial esterified carnitine concentration. In 3 dogs in which baseline plasma total carnitine concentration was low, plasma and myocardial carnitine concentrations were unchanged after pacing. Structural changes associated with pacing included perinuclear vacuolization in 3 dogs. Morphometric analyses indicated there was a decrease in myofiber cross-sectional diameter and area following pacing. Electron microscopy revealed changes in myofibrils and mitochondria following pacing.

Conclusions and Clinical Relevance—Results indicated that moderate to severe alterations in myocyte cytoarchitecture are present in dogs with ELVD induced by RRVP and that in dogs with normal plasma carnitine concentrations, myocardial carnitine deficiency may be a biochemical marker of ELVD. Results also indicated that transvenous endomyocardial biopsy can be used to evaluate biochemical and structural myocardial changes in dogs with cardiac disease. (*Am J Vet Res* 2001;62:889–894)

Dilated cardiomyopathy (DCM) is a chronic myocardial disease characterized by a progressive decrease in myocardial contractility and a progressive increase in chamber size.[1,a] Since the advent of echocardiography, clinical signs of congestive heart failure associated with low fractional shortening and dilatation of the cardiac chambers in the absence of other detectable heart diseases has been considered diagnostic of symptomatic DCM in dogs.[1] Dilated cardiomyopathy is sometimes detected at an early stage in dogs that do not have any clinical signs of heart failure but do have cardiac arrhythmias, gallop sounds, or echocardiographic abnormalities such as mild left ventricular systolic dysfunction and mild left ventricular dilatation. Diagnosis of this occult phase of DCM remains difficult, however, because of the overlap between echocardiographic results in healthy dogs and dogs with occult DCM.[2]

Rapid right ventricular pacing (RRVP) is an established method of inducing progressive cardiac dysfunction that mimics spontaneous DCM. The hemodynamic, neurohormonal, structural, and metabolic abnormalities associated with overt congestive heart failure induced by RRVP in pigs, dogs, and rabbits have been well described.[3-7,b] Recently, hemodynamic, renal, and neurohormonal abnormalities in dogs with **early left ventricular dysfunction (ELVD)** induced by RRVP have been reported.[8,9] However, little is known about structural and metabolic alterations in such dogs. The purpose of the study reported here, therefore, was to assess morphologic and metabolic abnormalities in dogs with ELVD induced by RRVP.

Materials and Methods

Dogs—Seven male purebred Beagles weighing 13 to 19 kg and between 4 and 5 years old were used in the study. Dogs were housed under standard conditions, fed maintenance dry dog food[c] once daily, and given free access to water. The experimental protocol was approved by the National Institutional Animal Care and Use Committee.

Experimental model—Dogs were anesthetized, and a bipolar pacemaker lead[d] was surgically inserted in the right jugular vein and advanced to the right ventricular apex under fluoroscopic guidance. The lead was connected to a mutiprogrammable pulse generator[e] that was inserted into a small subcutaneous pocket created in the cervical region. Dogs were allowed to recover for 14 to 21 days after pacemaker implantation.

Progressive cardiac dysfunction was induced by means of RRVP. A heart rate of 180 beats/min was maintained for the first 10 days; a rate of 215 beats/min was then maintained for the next 7 to 15 days.

Received Dec 15, 1999.
Accepted Jul 6, 2000.
From the Departments of Small Animal Clinical Sciences (Mc Entee, Clercx, Balligand, Jonville, Miserque, Henroteaux), Histology of Domestic Animals (Dessy), Pathology of Domestic Animals (Flandre, Desmecht), and Genetics (Michaux), Faculty of Veterinary Medicine, University of Liège, 4000 Liège, Belgium; and the Department of Medical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 (Keene).
Supported by the Research Council of the University of Liège. Presented at the 9th Annual Congress of the European Society of Veterinary Internal Medicine, Perugia, Italy, October 1999.
The authors thank Pr. Dr. Albert Thiry, Mary Schmidt, Jean-François Bradfer, Michael Sarlet, and Youssef Khelil for technical assistance.

Clinical evaluations—The dogs were examined just prior to activation of the pacemaker and every 3 to 4 days afterward to assess the progression of experimentally induced heart disease. Evaluations consisted of a standard physical examination, ECG, thoracic radiography, and Doppler echocardiography. Pacemakers were turned off when echocardiographic examination revealed fractional shortening < 28% in association with a > 10% increase in left ventricular end diastolic diameter, compared with each dog's baseline values. Results of these evaluations are published elsewhere.[10]

Evaluation of plasma carnitine concentrations—Heparinized venous blood samples were obtained at the beginning (baseline) and end of the pacing period. Samples were immediately centrifuged for 15 minutes at 1,500 × g at 4 C. Plasma was harvested and stored at –20 C until analyzed for total and free carnitine concentrations, using a radioenzymatic method.[11] Dogs were assigned to 1 of 2 groups on the basis of baseline total plasma carnitine concentration. Group-1 dogs had baseline total plasma carnitine concentrations > 12 µmol/L, which is the lower limit of the published reference range for dogs.[12] Group-2 dogs had baseline total plasma carnitine concentrations < 12 µmol/L.

Endomyocardial biopsy—Myocardial samples for microscopic and biochemical analyses were obtained from the right interventricular septum by use of a modified transvenous endomyocardial biopsy technique, as described.[13] Specimens were taken from each dog twice: before implantation of the pacemaker and at the end of the pacing period, 1 hour after deactivation of the pacemaker. Disposable endomyocardial biopsy forceps[f] were used. During each procedure, 7 endomyocardial biopsy specimens, each weighing approximately 2 to 5 mg, were obtained.

Tissue specimens for evaluation of myocardial carnitine concentration were blotted dry and immediately frozen in liquid nitrogen. Myocardial carnitine concentration was measured by use of a radioenzymatic method specially adapted for endomyocardial biopsy specimens that quantitates total carnitine, free carnitine, and esterified carnitine concentrations, with reported values indexed to the amount of noncollagen protein in each sample.[13]

Endomyocardial biopsy specimens intended for light microscopy were preserved in buffered 10% formaldehyde, embedded in paraffin, and sectioned. Four-millimeter-thick sections were cut from the paraffin blocks, mounted on glass slides, and stained with H&E or periodic acid-Schiff stain. Qualitative and quantitative analyses of muscle fibers and nuclei were performed. Morphometric measurements were made with a computerized operator-assisted device[g] at 600× magnification by a single individual who did not know whether specimens had been obtained before or after pacing. Fiber diameter was measured as the shortest diameter of cross and oblique sections at the level of the nuclei. Fiber and nucleus areas were measured by use of computerized planimetry; transverse sections of the fibers at the level of the nuclei were selected.

Specimens for electron microscopy were fixed in Millouig 4% phosphate-buffered glutaraldehyde at pH 7 for 24 hours, postfixed for 1 hour in 1% aqueous osmium tetroxide, and embedded in resin. Sections were cut at a thickness of 1 µm, stained with toluidine blue, and examined at a magnification of 100× to select areas where myofibers were oriented in a longitudinal direction. The selected blocks were used to obtain thin sections for electron microscopy. Three grids each containing 3 thin sections were prepared from each block. Thin sections were stained with uranyl acetate and lead citrate and examined. Areas of the tissue blocks where myofibers were oriented in a longitudinal direction were selected. Representative sections of each block were photographed at 2 calibrated magnifications of 8,000× and 25,000× for analysis.

Statistical analyses—Data were expressed as mean ± SD. Baseline values of 32 Doppler- echocardiographic variables[10] and of histologic variables (fiber diameter and area, nucleus area, and nucleus-to-fiber area) were compared between group-1 and group-2 dogs by use of Student *t*-tests for unpaired comparisons. The duration of different stages of ELVD[10] was also compared between group-1 and group-2 dogs, using the Student *t*-test for unpaired comparisons.

Histologic differences obtained before and after pacing were compared by use of Student *t*-tests for paired comparisons. Plasma and myocardial carnitine concentrations were analyzed by means of repeated-measures ANOVA, using a 2 × 2 factorial design.[14] The first factor related to baseline total plasma carnitine concentration (group 1 or 2); the second factor related to plasma and myocardial total, free, and esterified carnitine concentrations before and after pacing. For all analyses, values of $P < 0.05$ were considered significant.

Results

Clinical evaluations—All dogs were clinically normal prior to the pacing period. Mean ± SD duration

Table 1—Plasma and myocardial carnitine concentrations in 7 dogs before and after induction of early left ventricular dysfunction by rapid right ventricular pacing

	Group 1		Group 2	
Variable	**Before**	**After**	**Before**	**After**
Plasma carnitine concentration (µmol/L)				
Total	**29.52 ± 10.28**	**24.85 ± 4.82**	**6.37 ± 0.75***	**6.87 ± 1.65**
Free	26.90 ± 7.68	21.95 ± 5.00	5.07 ± 0.61*	5.53 ± 1.85
Esterified	2.62 ± 2.65	2.90 ± 1.85	1.30 ± 0.26	1.33 ± 0.21
Esterified-to-free	0.08 ± 0.07	0.14 ± 0.10	0.27 ± 0.11†	0.26 ± 0.05
Myocardial carnitine concentration (nmol/mg of NCP)				
Total	**5.06 ± 1.30**	**3.50 ± 0.63‡**	**1.60 ± 0.49***	**1.40 ± 0.5**
Free	4.60 ± 1.39	2.49 ± 0.65§	1.14 ± 0.42*	0.81 ± 0.44
Esterified	0.48 ± 0.21	1.01 ± 0.11§	0.46 ± 0.07	0.58 ± 0.16
Esterified-to-free	0.09 ± 0.07	0.43 ± 0.13§	0.44 ± 0.13*	0.79 ± 0.28

Group 1 consisted of 4 dogs with normal plasma total carnitine concentrations before pacing. Group 2 consisted of 3 dogs with low plasma total carnitine concentrations before pacing.

*Significantly ($P < 0.01$) different from value for group-1 dogs. †Significantly ($P < 0.05$) different from value for group-1 dogs. ‡Significantly ($P < 0.05$) different from value obtained before pacing. §Significantly ($P < 0.01$) different from value obtained before pacing.

NCP = Noncollagen protein.

of pacing was 19 ± 2.7 days. Mean duration of pacing for the 4 group-1 dogs (ie, dogs for which baseline total plasma carnitine concentration was normal) was not significantly (P = 0.38) different from that for the 3 group-2 dogs (ie, dogs for which baseline total plasma carnitine concentration was low). During the pacing period, no signs of overt congestive heart failure such as lethargy, inappetence, increased body weight, ascites, or dyspnea were detected. No electrocardiographic or radiographic signs of cardiac enlargement were observed during the pacing period. At the end of the pacing period, a moderate systolic left apical murmur was heard in 1 dog, monomorphic isolated left ventricular premature beats at a mean frequency of 10 beats/min were detected in another dog, and a pulmonary interstitial pattern was observed in a third dog.

Table 2—Morphometric histologic data for 5 dogs before and after induction of early left ventricular dysfunction by rapid right ventricular pacing

Variable	Before	After
Fiber diameter (μm)	20.8 ± 2.1	17.7 ± 1.9*
Fiber area (μ^2m)	57.3 ± 8.1	42.8 ± 12.6*
Nucleus area (μ^2m)	3.4 ± 0.3	2.9 ± 0.3†
Nucleus-to-fiber area	0.064 ± 0.01	0.073 ± 0.01

*Significantly ($P < 0.01$) different from value obtained before pacing. †Significantly ($P < 0.05$) different from value obtained before pacing.

Doppler echocardiographic evaluations—Baseline preejection period of the left ventricle was significantly ($P < 0.01$) shorter in group-1 (52 ± 3 ms)

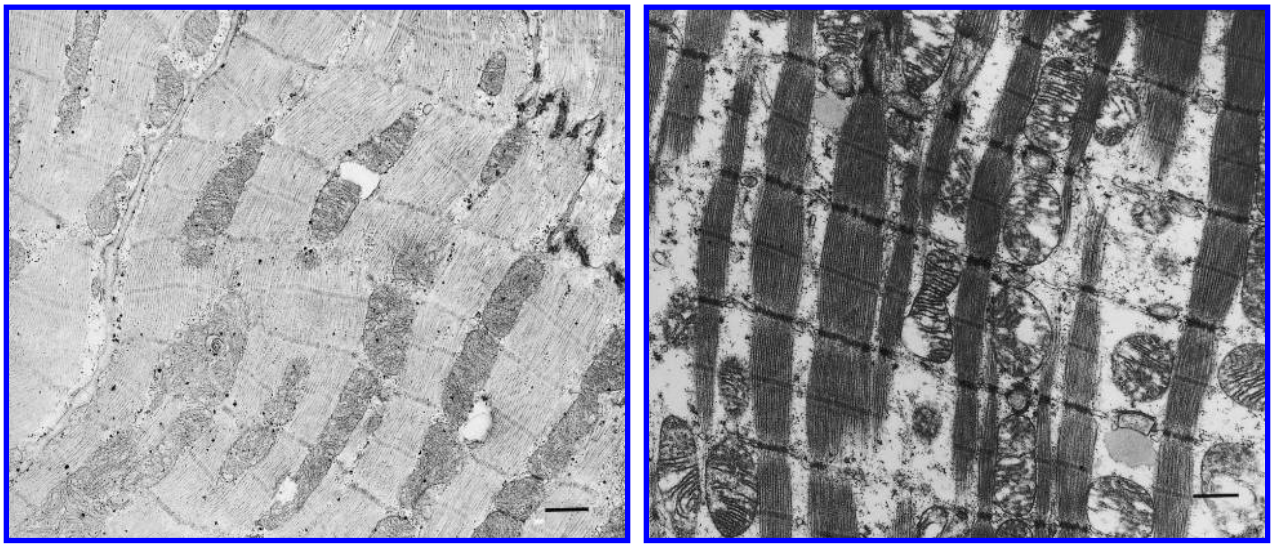

Figure 1—Transmission electron photomicrographs of longitudinal sections of myocardial cells from a dog before (left) and after (right) induction of early left ventricular dysfunction by rapid right ventricular pacing. Notice that before pacing, myofibrillar bundles occupied much of the myocyte volume and mitochondria filled spaces between myofibrils, whereas after pacing, a decrease in myofibrillar content was observed, as well as vacuolization of mitochondria. Bar = 1.25 μm.

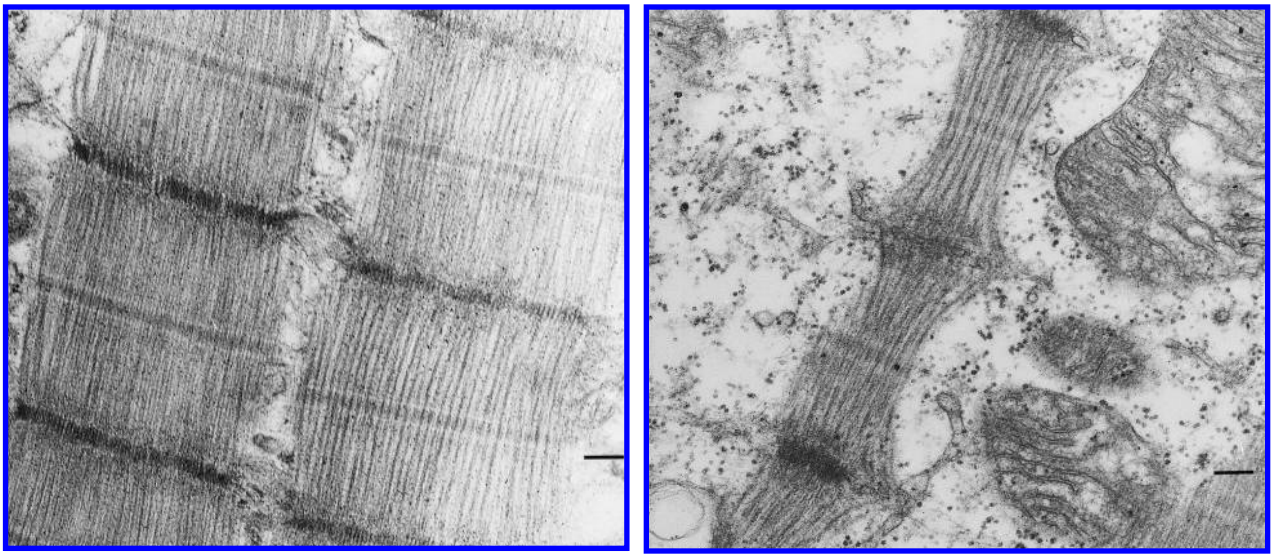

Figure 2—Transmission electron photomicrograph of longitudinal sections of myocardial cells from a dog before (left) and after (right) induction of early left ventricular dysfunction by rapid right ventricular pacing. Notice that before pacing, a regular array of thick and thin myofilaments and Z-lines was apparent, whereas after pacing, thickening and irregularities of the Z-lines, sarcomere disruption, and loss of the myofibrils' lateral register were observed. Bar = 0.4 μm.

than in group-2 (63 ± 3 ms) dogs. Baseline peak velocity of the mitral A wave was significantly ($P < 0.05$) higher in group-1 (0.49 ± 0.02 m/s) than in group-2 (0.35 ± 0.04 m/s) dogs. For the 27 other echocardiographic variables that were measured, significant differences were not found between groups.

Carnitine metabolism—In group-1 dogs, RRVP induced significant decreases in myocardial total and free carnitine concentrations and significant increases in myocardial esterified carnitine concentration and in the esterified-to-free carnitine concentration ratio. However, plasma total, free, and esterified carnitine concentrations were unchanged (**Table 1**). In group-2 dogs, RRVP did not induce any significant changes in plasma and myocardial carnitine concentrations. A significant interaction between the effect of group and the effect of RRVP was observed for myocardial total (P = 0.05), free (P = 0.03), and esterified (P = 0.01) carnitine concentrations.

Light microscopic findings—Before the pacing period, no relationship between fiber diameter, fiber area, nucleus area, or nucleus-to-fiber area and groups of dogs was found. Because of the small size of some specimens, light microscopy was performed on specimens from only 5 of the 7 dogs. Heterogeneity of myofiber size, atrophic myofibers, angular myofibers, and hypercontraction bands were sometimes observed in specimens obtained before or after pacing. Perinuclear vacuolization was observed in 3 dogs after pacing.

For determination of the shortest myofiber diameter, a mean of 50 (SD, 6) cells was analyzed. For determination of myofiber and nuclear areas, a mean of 25 (SD, 6) cells was analyzed. Baseline shortest myofiber diameter, myofiber area, and nuclear area were similar in the 2 groups (P = 0.66, 0.83, and 0.12, respectively). Pacing induced significant decreases in mean shortest myofiber diameter, myofiber area, and nuclear area, but the ratio of nuclear area to myofiber area was unchanged (**Table 2**).

Electron microscopic findings—Changes in the myofibrils and mitochondria were the most consistent effects of RRVP. Before pacing, myofibril bundles occupied much of the myocyte volume, and mitochondria filled spaces between myofibrils (**Fig 1**). The mitochondria were dense with evenly distributed cristae. After pacing, myofibril content was decreased, and areas of cytoplasm free of myofibrils and mitochondria were observed. The mitochondria appeared abnormal; cristae were disrupted, leading to intramitochondrial vacuoles. Similarly, before pacing, a regular array of thick and thin myofilaments and Z-lines were apparent (**Fig 2**). After pacing, frequent thickenings and irregularities of the Z-lines, sarcomere disruption, and loss of the myofibrils' lateral register were observed. Many, but not all, cardiac muscle cells also had an enlarged perinuclear region containing giant lipid droplets, lysosomes, or cytoplasm with polyribosomes, rough sarcoplasmic reticulum, and Golgi apparatus. Moderate vacuolization of the smooth sarcoplasmic reticulum was sometimes observed.

Discussion

In the present study, RRVP induced a decrease in left ventricular fractional shortening associated with left ventricular dilatation. These alterations mimic echocardiographic changes observed in dogs with spontaneous DCM.[1] The method used in this study did not induce any relevant clinical signs of overt congestive heart failure. Therefore, this protocol of RRVP induced an early stage of cardiac dysfunction that mimics the occult stage of spontaneous DCM in dogs.

In the past, alterations in myocardial metabolism and structure have most often been investigated in patients with end-stage heart failure. Since the advent of techniques for transvenous endomyocardial biopsy[13] and of micromethods for biochemical analyses,[11] biochemical and structural changes can now be investigated during early stages of cardiac dysfunction as well as serially during the course of the disease.

In the present study, metabolic and structural alterations of ELVD induced by RRVP were evaluated by examining transvenous endomyocardial biopsy specimens from the right interventricular septum. The advantage of this technique is that sampling can be repeated several times on the same dog. Disadvantages are that biopsy specimens can be obtained from only a single site and that the specimens that are obtained are small. Results of this study, however, indicate that despite these drawbacks, transvenous endomyocardial biopsy can be used to evaluate biochemical and structural myocardial changes, with results comparable to those reported in previous studies, in which more invasive techniques such as transmural biopsy or dissection of the whole heart were used.[5-7,b]

Surprisingly, in the present study, baseline plasma and myocardial total carnitine concentrations were less than the lower limit of the reference range in 3 of the 7 dogs.[12] This systemic carnitine deficiency was not associated with obvious clinical, functional, structural, or ultrastructural signs of cardiac dysfunction. Moreover, only 2 of 32 echocardiographic variables were significantly different between the 2 groups of dogs. The cause of this systemic carnitine deficiency was not investigated. However, a genetic predisposition cannot be excluded, because 2 of the 3 dogs were brothers. All dogs were fed with the same relatively carnitine-poor dog food before and during the study.[h]

In the 3 dogs in the present study with systemic carnitine deficiency, RRVP did not induce any significant changes in plasma or myocardial carnitine concentrations. In the 4 dogs in which baseline plasma and myocardial carnitine concentrations were normal, however, RRVP induced significant decreases in myocardial total and free carnitine concentrations and significant increases in myocardial esterified carnitine concentration. As a result, the myocardial total carnitine concentration was less than the lower limit of the reference range, and the myocardial esterified-to-free carnitine concentration ratio was > 0.15. Decreased myocardial carnitine concentrations have previously been reported in conjunction with congestive heart failure induced by the same method,[7] making it now clear that carnitine deficiency occurs early in the

course of pacing-induced cardiac dysfunction, rather than simply as a result of end-stage heart failure.

An increase in myocardial concentrations of carnitine esters has already been observed in animals with experimentally induced myocardial ischemia, in human patients with spontaneous myocardial ischemia, and in human patients with chronic heart failure undergoing valve replacement.[15-17] On the other hand, no significant change in the myocardial free-to-total carnitine ratio was observed in patients with heart failure of different origins,[18,19] and similar decreases in total, free, and esterified myocardial carnitine concentrations were found in dogs with congestive heart failure induced by RRVP.[7] Therefore, it seems that the metabolic response to cardiac dysfunction may depend on the origin or severity of cardiac disease. The accumulation of carnitine esters observed in the present study could be explained by an imbalance between the high energetic requirements of the rapidly paced myocardium and the limited capabilities of lipid β oxidation.[16,17]

No change in plasma carnitine concentration was observed in the present study. This is different from results reported by other authors. In previous studies of heart failure, myocardial carnitine depletion was often associated with an increase in plasma carnitine concentration, suggesting altered membrane transport or integrity.[8,20,21] Therefore, it seems that changes in plasma carnitine concentrations may be variable, depending on species, origin of the disease, and severity of heart failure. It is also possible that plasma carnitine concentration increases as renal clearance decreases when heart failure worsens.

The most striking histologic finding in the present study was perinuclear vacuolization observed in 3 dogs after pacing. Electron microscopy confirmed the presence of an enlarged perinuclear area free of myofibrils. Accumulation of lipid droplets observed in this region is the consequence of an alteration in lipid oxidation.[22] The lysosomes could be autophagic vacuoles eliminating altered myofibrils, and the dilated protein synthesis organelles could be the result of synthesis of new myofibrils or of hormonal factors. The decrease in myocyte cross-sectional diameter and area were consistent with the absence of myocardial hypertrophic response already described with this method of inducing heart failure.[4,6,b]

Our observations clearly demonstrated that moderate to severe alterations in myocyte cytoarchitecture are present in dogs with ELVD induced by RRVP. Similar findings have been described for dogs with overt congestive heart failure induced by RRVP.[4,6,b] Proposed explanations for these changes include stretching of myocytes because of the increase in preload, leading to breakdown of interfibrillar cytoskeletal filaments, and abnormalities in contractile protein synthesis rate.[4,5] In the present study, increased preload was suggested by the increase in left ventricular end diastolic diameter. Ultrastructural alterations are also observed in spontaneous cardiac diseases such as dilated and hypertrophic cardiomyopathy, ischemic cardiomyopathy, toxic cardiomyopathy, and cardiomyopathy secondary to nutritional deficiencies.[23] Therefore, the myofibrillar scarcity and disarray and mitochondrial degeneration observed in this study represent nonspecific ultrastructural markers of ELVD induced by RRVP.

[a]Ettinger S, Bolton G, Lord P. Idiopathic cardiomyopathy in the dog (abstr). *J Am Vet Med Assoc* 1970;156:1225.

[b]Bishop SP, Powell PC, Brissie N, et al. Myocardial cellular remodeling in canine pacing induced heart failure (abstr). *Circulation* 1992;86(Suppl I):I-754.

[c]Science diet maintenance, Hill's Pet Products (Benelux) SA/NV, Brussels, Belgium.

[d]Thin Line EZ, Model 438-10, Intermedics, Brussels, Belgium.

[e]Dart, Model 292-05, Intermedics, Brussels, Belgium.

[f]502-302, Cordis, Miami, Fla.

[g]Image-Pro Plus, version 3.0 for Power Macintosh, Media Cybernetics, Leyden, Netherlands.

[h]Keene BW, Mier HC, Meurs KM. Dietary carnitine deficiency in canine commercial diets (abstr). *J Vet Intern Med* 1992;5:112.

References

1. Kittleson MD. Primary myocardial disease leading to chronic myocardial failure (dilated cardiomyopathy and related diseases). In: Kittleson MD, Kienle RD, ed. *Small animal cardiovascular medicine*. St Louis: Mosby Inc, 1998;319–346.

2. Calvert CA. Update. Canine dilated cardiomyopathy. In: Kirk RW, Bonagura JD, eds. *Current veterinary therapy XI*. Philadelphia: WB Saunders Co, 1992;773–779.

3. Wilson JR, Douglas P, Hickey WF, et al. Experimental congestive heart failure produced by rapid ventricular pacing in the dog: cardiac effects. *Circulation* 1987;75:857–867.

4. Spinale FG, Fulbright BM, Mukherjee R, et al. Relation between ventricular and myocyte function with tachycardia-induced cardiomyopathy. *Circ Res* 1992;71:174–187.

5. Travill CM, Williams TDM, Pate P, et al. Haemodynamic and neurohumoral response in heart failure produced by rapid ventricular pacing. *Cardiovasc Res* 1992;26:783–790.

6. Komamura K, Shannon P, Ihara T, et al. Exhaustion of Franck-Starling mechanism in conscious dogs with heart failure. *Am J Physiol* 1993;265:H1119–H1131.

7. Pierpont MEM, Foker JE, Pierpont GL. Myocardial carnitine metabolism in congestive heart failure induced by incessant tachycardia. *Basic Res Cardiol* 1993;88:362–370.

8. Redfield MM, Aarhus LL, Wright RS, et al. Cardiorenal and neurohumoral function in a canine model of early left ventricular dysfunction. *Circulation* 1993;87:2016–2022.

9. Stevens TL, Burnett JC, Kinoshita M, et al. A functional role for endogenous atrial natriuretic peptide in a canine model of early left ventricular dysfunction. *J Clin Invest* 1995;95:1101–1108.

10. Mc Entee K, Clercx C, Soyeur D, et al. Usefulness of tests for detection of cardiac abnormalities in dogs with experimentally induced early left ventricular dysfunction. *Am J Vet Res* 2001;62: 448–455.

11. Parvin R, Pande SV. Microdetermination of carnitine and carnitine acyltransferase activity. *Anal Biochem* 1977;79:190–201.

12. Keene BW. L-Carnitine deficiency in canine dilated cardiomyopathy. In: Kirk RW, ed. *Current veterinary therapy XI*. Philadelphia: WB Saunders Co, 1992;780–783.

13. Keene BW, Kittleson ME, Atkins CE, et al. Modified transvenous endomyocardial biopsy technique in dogs. *Am J Vet Res* 1990; 51:1769–1772.

14. *SAS user's guide: version 6 edition*. 4th ed. Vol 2. Cary, NC: SAS Institute Inc, 1989.

15. Masumura Y, Kobayashi A, Yamazakin. Myocardial free carnitine and fatty acylcarnitine levels in patients with chronic heart failure. *Jpn Circ J* 1990;54:1471–1476.

16. Pepine CJ. The therapeutic potential of carnitine in cardiovascular disorders. *Clin Ther* 1991;13:2–21.

17. Shug AL, Thomsen JH, Folts JD, et al. Changes in tissue levels of carnitine and other metabolites during myocardial ischemia and anoxia. *Arch Biochem Biophys* 1978;187:25–33.

18. Regitz V, Bossaller C, Strasser R, et al. Metabolic alterations in end-stage and less severe heart failure: myocardial carnitine decrease. *J Clin Chem Clin Biochem* 1990;28:611–617.

19. Regitz V, Shug AL, Fleck E. Defective myocardial carnitine metabolism in congestive heart failure secondary to dilated cardiomyopathy and to coronary, hypertensive and valvular heart disease. *Am J Cardiol* 1990;65:755–760.

20. Pierpont MEM, Judd D, Goldenberg IF, et al. Myocardial carnitine in end stage heart failure. *Am J Cardiol* 1989;64:56–60.

21. Nakamura T, Sugihara H, Kinoshita N, et al. Serum carnitine concentrations in patients with idiopathic hypertrophic cardiomyopathy: relationship with impaired myocardial fatty acid metabolism. *Clin Sci* 1999;97:493–501.

22. Miyagawa J, Kuwajima M, Hanafusa T, et al. Mitochondrial abnormalities of muscle tissue in mice with juvenile visceral steatosis associated with systemic carnitine deficiency. *Virchows Arch* 1995;426:271–279.

23. Van Vleet JF, Ferrans VJ. Myocardial diseases of animals. *Am J Pathol* 1986;124:99–178.