# Myocardial concentrations of fatty acids in dogs with dilated cardiomyopathy

Caren E. Smith, MS; Lisa M. Freeman, DVM, PhD; Mohsen Meydani, DVM, PhD; John E. Rush, DVM, MS

**Objective**—To compare myocardial concentrations of fatty acids in dogs with dilated cardiomyopathy (DCM) with concentrations in control dogs.

**Sample Population**—Myocardial tissues from 7 dogs with DCM and 16 control dogs.

**Procedure**—Myocardial tissues were homogenized, and total fatty acids were extracted and converted to methyl esters. Myocardial concentrations of fatty acids were analyzed by use of gas chromatography and reported as corrected percentages.

**Results**—The amount of docosatetraenoic acid (C22:4 n-6) was significantly higher in myocardial samples from dogs with DCM (range, 0.223% to 0.774%; median, 0.451%), compared with the amount in samples obtained from control dogs (range, 0.166% to 0.621%; median, 0.280%). There were no significant differences between DCM and control dogs for concentrations of any other myocardial fatty acids.

**Conclusions and Clinical Relevance**—Although concentrations of most myocardial fatty acids did not differ significantly between dogs with DCM and control dogs, the concentration of docosatetraenoic acid was significantly higher in dogs with DCM. Additional investigation in a larger population is warranted to determine whether this is a primary or secondary effect of the underlying disease and whether alterations in fatty acids may be a target for intervention in dogs with DCM. (*Am J Vet Res* 2005;66:1483–1486)

**Dilated cardiomyopathy (DCM)** is a common cause of cardiac arrhythmias and sudden death in dogs. Large- and giant-breed dogs, including Boxers, Doberman Pinschers, Great Danes, and Irish Wolfhounds, are most commonly affected.[1,2] In some breeds, such as the Doberman Pinscher, up to a third of dogs with DCM die suddenly because of cardiac arrhythmias.[3,4] In addition to the arrhythmias, DCM often progresses to **congestive heart failure (CHF)**, resulting in a poor prognosis. Mean survival for dogs with DCM and CHF for Doberman Pinschers was 62 days in 1 study.[4] In a study[5] of fatality for several breeds of dogs with DCM, median survival was 2.3 months.

Changes in plasma lipid concentrations have been observed in dogs with CHF. Dogs with naturally developing DCM have reduced plasma concentrations of 2 major omega-3 fatty acids, eicosapentaenoic acid (ie, C20:5 n-3) and docosahexaenoic acid (ie, C22:6 n-3), and the omega-6 fatty acid arachidonic acid (ie, C20:4 n-6).[6] There also are changes in the heart during CHF. Studies[7–11] of nonhuman animals and people revealed a shift from metabolism of fatty acids to metabolism of glucose in the myocardium during CHF. Increased use of glucose may result in improved energy efficiency in the myocardium, which could be a compensatory and cardioprotective response to CHF. Whether a long-term shift in energy substrates would be beneficial is unclear.

Additional myocardial alterations are associated with cardiomyopathy in several species. Turkeys with induced DCM have higher concentrations of docosatetraenoic acid and lower concentrations of α-linolenic acid in the phospholipid fraction of the myocardium, compared with concentrations in clinically normal turkeys.[12] Cardiomyopathic hamsters have a lower ratio of oleic acid to stearic acid and a higher ratio of arachidonic acid to docosahexaenoic acid, compared with ratios in clinically normal hamsters.[13] Finally, in people, altered myocardial lipids, including higher concentrations of stearic acid and eicosapentaenoic acid and lower concentrations of eicosatrienoic acid and docosapentaenoic acid, have been documented[14] in a specific form of infantile X-linked DCM that is associated with mitochondrial disease. However, it is unknown whether composition of myocardial fatty acids is altered in dogs with experimentally induced or naturally developing DCM.

Understanding the metabolic changes associated with DCM could lead to alternative treatments, such as nutritional interventions that may decrease the morbidity and mortality rates of dogs with DCM. The purpose of the study reported here was to determine whether composition of myocardial lipids is altered in dogs with DCM, compared with composition in healthy control dogs.

## Materials and Methods

**Sample population**—Samples of myocardial tissues were obtained from 7 dogs with DCM and 16 healthy control dogs. All dogs with DCM were client owned; we obtained written permission from each owner for collection of samples and use of tissues.

A diagnosis of DCM was made on the basis of left atrial and left ventricular dilatation detected during 2-dimensional and M-mode echocardiography and a fractional shortening < 25% on M-mode echocardiography. Control dogs were judged to be free of cardiac disease on the basis of medical history and results of physical examination; these dogs were euthanized for reasons other than heart disease. In addition, the heart of each control dog had to appear normal on gross inspection of the pericardium, myocardium, and car-

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From the Department of Clinical Sciences, Cummings School of Veterinary Medicine at Tufts University, North Grafton, MA 01536 (Smith, Freeman, Rush); and the Vascular Biology Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111 (Meydani).
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Address correspondence to Dr. Freeman.

diac valves. None of the control dogs were receiving any medications.

All dogs were euthanized by IV injection of a combination of pentobarbital and phenytoin.[a] Samples were obtained immediately after the dogs were euthanized. Within 10 minutes after a dog was euthanized, a sample of myocardial tissue was collected from the free wall of the left ventricle near the apex of the heart, rinsed with saline (0.9% NaCl) solution, and frozen at –80°C.

**Analysis of fatty acids**—A pattern of fatty acids was determined for each myocardial sample by use of gas chromatography. All samples and solvents were kept on ice during sample preparation. Samples (0.10 to 0.15 g) were homogenized for 30 seconds in a mixture of chloroform:methanol[b] (1:1 [vol:vol]). Lipids were extracted from each sample by shaking in the chloroform:methanol mixture for 1 hour. Samples were centrifuged at 4°C, and the supernatant layer was then removed. Following addition of chloroform and high-performance liquid chromatography–grade water, samples were centrifuged again at 4°C. The lower phase (lipid extract) of each sample was then dried under nitrogen gas, and the residue was resuspended in 1.0 mL of benzene. Fatty acids were methylated by incubation with 5% methanolic hydrogen chloride for 2 hours at 70°C. Following the addition of 7% NaCl and 0.5 mL of hexane, the upper phase was dried under nitrogen gas. The dried residue was reconstituted in 500 µL of hexane. Extracts were analyzed within 7 days after preparation.

One microliter of each sample was injected into a gas chromatograph.[c] Fatty acid methyl esters of the eluted peaks were identified by comparison with chromatograms of known mixtures of fatty acid methyl esters generated by use of the same conditions for the gas chromatograph. Peaks were integrated by use of commercial software.[d] Myocardial concentrations of fatty acids were reported as corrected percentages.

**Statistical analyses**—Fatty acid data were reported as the median and range. Echocardiographic data and body weights were reported as mean ± SD. When possible, data that were not normally distributed were logarithmically transformed prior to analysis. Comparison of specific fatty acids between dogs with DCM and control dogs was performed by use of independent *t* tests (normally distributed data) or Mann-Whitney *U* tests (skewed data), with Bonferroni correction for multiple comparisons. Correlations were calculated by use of the Spearman correlation coefficient with Bonferroni correction. Commercial statistical software[e] was used for analysis. Values of $P < 0.05$ were considered significant.

## Results

Tissue samples obtained from 7 dogs with DCM and 16 control dogs were studied. The DCM group included 4 Doberman Pinschers, 1 American Bulldog, 1 Boxer, and 1 Labrador Retriever. The control group included 3 Redbone Coonhounds, 1 Portuguese Water Dog, 1 Siberian Husky, and 11 mixed-breed dogs. There was a significant ($P = 0.001$) difference in the sex distribution between the DCM group (4 males [1 castrated] and 3 females [2 spayed]) and control group (16 females [2 spayed]). Median age was higher in the DCM group (7.1 years; range, 0.4 to 11.5 years), compared with median age for the control group (2.4 years; range, 0.3 to 14.6 years), but the values did not differ significantly ($P = 0.07$). Mean ± SD weight for the 7 dogs with DCM was 30.82 ± 8.83 kg. Diets were known for 22 of 23 dogs; none were enriched with omega-3 fatty acids.

Table 1—Mean ± SD echocardiographic measurements for 6 of 7 dogs with dilated cardiomyopathy (DCM).

| Variable | Mean ± SD |
|---|---|
| Aorta (cm) | 2.71 ± 0.61 |
| Left atrial diameter (cm) | 3.47 ± 0.77 |
| Left ventricle | |
| Internal dimension in diastole (cm) | 5.93 ± 0.60 |
| Internal dimension in systole (cm) | 4.98 ± 0.52 |
| Thickness of free wall in diastole (cm) | 0.93 ± 0.42 |
| Thickness of free wall in systole (cm) | 1.15 ± 0.49 |
| Interventricular septum in diastole (cm) | 0.84 ± 0.15 |
| Interventricular septum in systole (cm) | 1.11 ± 0.24 |
| Fractional shortening (%) | 16 ± 6 |
| End-diastolic volume index (mL/m$^2$) | 237 ± 117 |
| End-systolic volume index (mL/m$^2$) | 134 ± 43 |

Table 2—Mean (range) myocardial concentrations of fatty acids* in 7 dogs with DCM and 16 healthy control dogs.

| Fatty acid | DCM | Control |
|---|---|---|
| Myristic (C14:0) | 0.408 (0.201–1.113) | 0.657 (0.380–1.360) |
| Palmitic (C16:0) | 15.647 (10.262–22.147) | 16.247 (11.110–20.346) |
| Stearic (C18:0) | 15.242 (9.567–20.560) | 13.138 (9.866–17.932) |
| Oleic (C18:1 n-9) | 25.056 (14.754–41.392) | 28.136 (18.432–37.924) |
| γ-Linolenic (C18:3 n-3) | 0 (0–0.082) | 0 (0–0.104) |
| Eicosenoic (C20:1 n-9) | 0.199 (0.135–0.411) | 0.261 (0.163–0.315) |
| Eicosatrienoic (C20:3 n-6) | 0.435 (0.274–0.843) | 0.382 (0.208–0.685) |
| Arachidonic (C20:4 n-6) | 13.264 (5.690–27.461) | 13.191 (6.984–23.157) |
| Eicosapentaenoic (C20:5 n-3) | 0.088 (0–0.283) | 0.174 (0–0.256) |
| Docosatrienoic (C22:3 n-3) | 0.038 (0–0.061) | 0.042 (0.019–0.187) |
| Docosatetraenoic (C22:4 n-6) | 0.451[a] (0.223–0.774) | 0.280[b] (0.166–0.621) |
| Docosapentanoic C22:5 n-3) | 0.436 (0.114–0.742) | 0.388 (0.170–0.641) |
| Docosahexanoic (C22:6 n-3) | 0.303 (0.078–0.543) | 0.192 (0.077–0.517) |
| Total saturated | 30.431 (29.247–34.457) | 30.077 (28.507–32.870) |
| Total monounsaturated | 25.255 (14.922–41.765) | 28.353 (18.747–38.196) |
| Total polyunsaturated | 44.315 (25.408–54.054) | 40.887 (31.045–49.082) |
| Total omega-3 | 0.799 (0.308–1.433) | 0.806 (0.433–1.274) |
| Total omega-6 | 43.339 (24.825–51.778) | 39.582 (30.177–47.685) |
| Ratio of omega-6 to omega-3 | 46.104 (36.134–80.604) | 47.249 (36.918–71.864) |

*Values are reported as corrected percentages.
[a,b]Values with different superscript letters differ significantly ($P < 0.05$).

The diagnosis of DCM was made by use of echocardiography in all 7 dogs in the DCM group, but echocardiographic measurements were available for only 6 of the 7 dogs (**Table 1**). Measurements were typical for dogs with DCM. All dogs with DCM also had CHF, as evidenced by pulmonary edema, pleural effusion, or ascites. The most commonly used medications in the DCM group included furosemide (n = 7 dogs), angiotensin-converting enzyme inhibitors (6), digoxin (6), and β-adrenoceptor blockers (3). Other medications included hydrochlorothiazide and spironolactone (n = 2), procainamide (2), diltiazem (2), and spironolactone alone (1). Six dogs received furosemide orally and 1 received furosemide IV (mean daily dosage, 7.1 ± 3.2 mg/kg).

Dogs with DCM had significantly ($P$ = 0.02) higher myocardial concentrations of docosatetraenoic acid (ie, C22:4 n-6), compared with concentrations for the control group (**Table 2**). We did not detect significant differences for any other myocardial fatty acids between the DCM and control groups. We did not detect a correlation between age and fatty acid concentrations, nor was there a difference in fatty acid concentrations between male and female dogs with DCM.

## Discussion

Although the percentages of fatty acids in the left ventricle of healthy dogs have been described,[15] to our knowledge, analysis of fatty acid concentrations in the hearts of dogs with DCM has not been reported. Results for DCM and control dogs in the study reported here were similar to those in another study.[15] For both groups in the current study, relative concentrations of major fatty acids were as follows: oleic (18:1 n-9) > linoleic (18:2 n-6) > palmitic (16:0) > stearic (18:0) > arachidonic (20:4 n-6) > myristic (14:0). For healthy dogs in that other study,[15] concentrations were as follows: oleic > linoleic > palmitic > arachidonic > stearic > myristic. Therefore, control dogs in both studies and DCM dogs in the study reported here had the same relative concentrations for the 3 most common major fatty acids (eg, oleic acid, linoleic acid, and palmitic acid) and for the least common major fatty acid (eg, myristic acid).

Our finding that there were higher concentrations of docosatetraenoic acid in dogs with DCM is similar to that described[12] for the phospholipid fraction of the myocardium obtained from turkeys with furazolidone-induced DCM. In contrast, naturally developing DCM in turkeys is associated with lower concentrations of docosatetraenoic acid and 8 other fatty acids with altered concentrations.[12] Altered myocardial concentrations of fatty acids in animals with DCM could reflect altered lipid storage in myocytes, changes in membrane composition, or a switch in energy substrate from fatty acids to glucose. Alternatively, changes in fatty acid concentrations could be related to loss of myocytes and replacement with fatty or fibrous tissue that have been described in some forms of DCM in dogs.[16]

The mechanisms by which docosatetraenoic acid would be higher in the myocardium of dogs with DCM, compared with concentrations in control dogs, are unknown but could include increased uptake from plasma to the myocardium, decreased use, or increased myocardial biosynthesis from other myocardial fatty acids. Arachidonic acid can be elongated to form docosatetraenoic acid.[17] Although plasma concentrations of docosatetraenoic acid were not measured in another study[6] of dogs with DCM, lower plasma concentrations of arachidonic acid were documented in dogs with DCM in that study. The increased myocardial concentration of docosatetraenoic acid in the study reported here could have reflected an increased uptake of arachidonic acid from the plasma accompanied by an increase in the conversion of arachidonic acid to docosatetraenoic acid in the myocardium.

Heart failure is associated with altered lipid metabolism, including decreased β-oxidation of myocardial fatty acids and a shift to metabolism of glucose, but this shift does not explain the selective increase for only 1 specific fatty acid.[7–11] Docosatetraenoic acid is readily oxidized and could conceivably accumulate when oxidation of fatty acids is decreased.[18] However, with the exception of a higher, but not significantly ($P$ = 0.08) different, concentration of stearic acid in the myocardium of dogs with DCM, compared with the concentration in control dogs, higher concentrations of other oxidizable fatty acids (eg, palmitic acid, oleic acid, linoleic acid, and arachidonic acid) were not observed in our study.

Whether the altered concentration of docosatetraenoic acid in dogs with DCM was a primary aspect of DCM or a secondary effect of the underlying syndrome of CHF is unknown, but the change is most likely to be secondary. Altered myocardial concentrations of fatty acids in 1- to 2-day-old turkeys with DCM and in children who died of infantile DCM between 24 and 48 months of age suggest that changes in fatty acids in these species could develop relatively early in the disease.[12,14] Evaluating concentrations of fatty acids prior to the onset of CHF in dogs with DCM would help establish whether alterations in fatty acids are an early change or are associated with CHF, typically a late-stage consequence of DCM.

The study reported here had a number of limitations, one of which was the inclusion of 4 breeds of dogs in the DCM group. Although DCM typically results in progressive ventricular dilatation and eventual CHF in dogs that do not succumb to sudden death, breed-related differences in the pathologic changes of the myocardium in dogs with DCM have been described.[16,19] Two histopathologic forms of DCM, 1 characterized by myocardial fibrous-fatty lesions and the other by myocardial attenuated wavy fibers, may reflect differing mechanisms of pathologic changes that may be seen more frequently in some breeds than in others.[16,19] Restricting future studies to dogs of a single breed would reduce possible breed-related variations.

A second limitation was the fact that echocardiography was not performed on all control dogs. Although these dogs had typical results for physical examinations and the heart of each control dog appeared grossly normal at the time of sample collection, these dogs could have had subclinical heart disease.

A third possible limitation concerns differences between the DCM and control groups with respect to age and sex. Dogs in the DCM group were older, but not significantly so, and no correlations were found between fatty acid concentrations and age. Sex distributions differed significantly between the DCM and control groups, but there were no significant differences in myocardial concentrations of fatty acids between males and females in the DCM group. Better matching on the basis of age and sex for DCM and control groups would be desirable in future studies; however, we were limited by the number and types of dogs that were clinically free of heart disease from which we could obtain samples.

Dietary intake of dogs also can affect myocardial composition of fatty acids, and feeding dogs an identical diet and then obtaining samples of myocardial tissue would be useful to confirm the findings reported here. Myocardial samples in our study were collected from the apex of the left ventricle. Therefore, the results are specific only for this area of the heart. Samples obtained from other areas of the left ventricle or other chambers of the heart could yield disparate results. Studies in which concentrations of fatty acids in myocardial, plasma, and noncardiac tissues are compared would also be useful to improve our understanding of fatty acid metabolism during health and disease.

Finally, logistics involved with obtaining suitable myocardial tissue from affected dogs limited the number of dogs with DCM and reduced the statistical power of the study. Future studies that include a larger number of dogs with DCM may allow other differences to be detected for the myocardial fatty acids of dogs with DCM.

Higher concentrations of docosatetraenoic acid were detected in the myocardium of dogs with DCM, compared with concentrations in the control dogs. This is supportive of altered lipid metabolism in the hearts of dogs with DCM. Whether this change is a positive adaptation in response to CHF or a pathologic consequence that could be a target for treatment remains to be determined.

a. Beuthanasia-D Special, Schering-Plough Animal Health, Union, NJ.
b. PowerGen homogenizer 125, Fisher Scientific, Fair Lawn, NJ.
c. Model 5890 with HP-5 column, Hewlett-Packard, Palo Alto, Calif.
d. Chemstation, Hewlett-Packard, Palo Alto, Calif.
e. Systat, version 10.0, SPSS Inc, Chicago, Ill.

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