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Nutritional Alterations and the Effect of Fish Oil Supplementation in Dogs with Heart Failure

Lisa M. Freeman, John E. Rush, Joseph J. Kehayias, James N. Ross, Jr., Simin N. Meydani, Don J. Brown, Gregory G. Dolnikowski, Bonnie N. Marmor, Michael E. White, Charles A. Dinarello, and Ronenn Roubenoff

Alterations in body composition and nutritional status are common in humans with heart failure and are related, in part, to increases in cytokine concentrations. Cytokines have not been studied previously in dogs with naturally occurring cardiac disease nor has fish oil administration been used in this population to decrease cytokine production. The purposes of this study were to characterize nutritional and cytokine alterations in dogs with heart failure and to test the ability of fish oil to reduce cytokines and improve clinical outcome. Body composition, insulinlike growth factor-1, fatty acids, and cytokines were measured in 28 dogs with heart failure and in 5 healthy controls. Dogs with heart failure then were randomized to receive either fish oil or placebo for 8 weeks. All parameters were measured again at the end of the study period. At baseline, 54% of dogs with heart failure were cachectic and the severity of cachexia correlated with circulating tumor necrosis factor-α concentrations ($P = .05$). Cytokine concentrations at baseline, however, were not significantly increased in dogs with heart failure compared to controls. Baseline plasma arachidonic acid ($P = .02$), eicosapentaenoic acid ($P = .03$), and docosahexaenoic acid ($P = .004$) concentrations were lower in dogs with heart failure than in controls. Fish oil supplementation decreased interleukin-1β (IL-1) concentrations ($P = .02$) and improved cachexia ($P = .01$) compared to the placebo group. The mean caloric intake of the heart failure dogs as a group was below the maintenance energy requirement ($P < .001$), but no difference was found in food intake between the fish oil and placebo groups. Insulinlike growth factor-1 concentrations ($P = .01$) and reductions in circulating IL-1 concentrations over the study period ($P = .02$) correlated with survival. These data demonstrate that canine heart failure is associated with cachexia, alterations in fatty acids, and reduced caloric intake. Fish oil supplementation decreased IL-1 concentrations and improved cachexia. In addition, reductions in IL-1 predicted survival, suggesting that anticytokine strategies may benefit patients with heart failure.

Key words: Body composition; Cachexia; Cytokines; Dilated cardiomyopathy; Fatty acids; Insulinlike growth factor-1.

Wasting associated with heart failure, commonly known as cardiac cachexia, was 1st described by Hippocrates.[1] More recent nutritional surveys indicate that up to 68% of hospitalized human cardiac patients are malnourished.[2] The weight loss that occurs in cachexia is quite different from that seen in simple starvation. The majority of the weight lost in simple starvation is composed of fat mass, whereas lean tissue is relatively spared. Cachexia primarily involves depletion of lean body mass, with attendant declines in strength, performance capacity, and immune competence.[3] Changes in body composition are common in human patients with heart failure. Total body weight may be decreased compared to the premorbid weight, but cachexia may be masked and total body weight may increase in patients who accumulate large quantities of fluid. Studies have reported both increased and reduced body weight in human heart failure patients, but all agree that lean body mass, by a variety of measures, is reduced in these patients (up to 35% below normal in 1 study).[4,5]

Cachexia has important clinical implications. Human surgical patients with cardiac cachexia have higher post-cardiopulmonary bypass morbidity and mortality, require ventilation for a longer period of time, and incur greater hospital costs.[6,7] The detrimental effects on morbidity and mortality may stem from associated decreases in immune competence, or from the fact that a loss of greater than 40% of lean body mass is incompatible with life.[3] Additionally, although cachexia was once thought to affect skeletal muscle selectively and to spare vital organs such as the heart, loss of lean body mass is now known to affect the entire organism, including the heart.[8] Thus, the loss of cardiac muscle mass may contribute to myocardial dysfunction found in many patients with heart failure.

The pathogenesis of cardiac cachexia is multifactorial and contributing factors include inadequate dietary intake, increased energy requirements, excessive losses, or altered metabolism.[9] The cytokines tumor necrosis factor-α (TNF) and interleukin-1β (IL-1) may play a major role in the pathogenesis of cachexia through their ability to reduce energy intake and increase resting energy expenditure and protein turnover.[10–13] Over the last several years, these and other cytokines have been shown to be increased in some human patients with heart failure.[14,15] One study of cytokines in humans with heart failure also found that a higher percentage of cachectic heart failure patients had increased circulating TNF concentrations compared to noncachectic patients.[15] However, a recent study was unable to detect increased concentrations of TNF in elderly human patients with moderate heart failure.[16] Increased concentrations of cytokines were found in our preliminary studies of dogs with heart failure.[17]

From the Department of Clinical Sciences, Tufts University School of Veterinary Medicine, North Grafton, MA (Freeman, Rush, Ross, Brown); the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA (Freeman, Kehayias, Meydani, Dolnikowski, Marmor, Roubenoff); the Department of Animal Science, University of Minnesota, St. Paul, MN (White); and Tupper Research Institute, Department of Medicine, Tufts University School of Medicine, Boston, MA (Dinarello). *Presented in part at the 1995 and 1996 ACVIM Forums.*

Reprint requests: Lisa M. Freeman, DVM, PhD, Department of Clinical Sciences, Tufts University School of Veterinary Medicine, 200 Westboro Road, North Grafton, MA 01536; e-mail: lfreeman@infonet.tufts.edu.

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The cytokines may offer a target for modulation of body composition and nutritional status in heart failure. One method of decreasing cytokine production is through nutritional modulation. The administration of fish oil, which is high in the *n*-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been shown to decrease TNF and IL-1 production in studies of human patients.[18,19] In addition, fish oil decreases weight loss in some animal models of cachexia.[20] The mechanism of action may be competitive inhibition of arachidonic acid metabolites or direct inhibitory effects on cytokine gene transcription.[21]

The purpose of this study was to characterize the body composition, nutritional status, and cytokine production in dogs with heart failure compared to normal controls. In addition to baseline comparisons, the ability of fish oil supplementation to stabilize or improve these parameters and, subsequently, improve clinical outcome was tested in the dogs with heart failure.

Materials and Methods

Subjects

Twenty-eight dogs with stable chronic heart failure (modified New York Heart Association [NYHA] functional class II–IV where class I = no limitation, physical activity, including normal exercise, does not cause symptoms; class II = slight limitation of physical activity, ordinary physical activity results in symptoms; class III = marked limitation of physical activity, less than ordinary activity leads to symptoms; class IV = inability to carry on any activity without symptoms, symptoms present at rest[22]) secondary to idiopathic dilated cardiomyopathy (DCM) were studied. Dogs with concurrent diseases known to cause cachexia or to affect cytokines such as cancer, diabetes mellitus, and hepatic failure were excluded from the study. If medication changes were judged to be necessary at the time of enrollment, enrollment was postponed for 7–10 days so that stabilization on the new medication could be achieved (1 dog in the fish oil group and 2 dogs in the placebo group). Medication adjustments were not restricted during the study period if they were deemed necessary by clinicians. The study was approved by the Tufts University Animal Care and Use Committee, and owners signed a consent form before enrolling their dogs in the study.

Body composition measurements of heart failure patients were compared to those of healthy control dogs. The control dogs were owned by hospital employees and students, and were deemed eligible on the basis of a normal history, physical examination, biochemistry profile, and echocardiogram.

Study Design

Heart failure and control dogs were admitted to the hospital after an overnight fast for the purposes of the study. A baseline echocardiogram was performed, measuring chamber dimensions and fractional shortening. Heparinized plasma was collected for determinations of IL-1, TNF, and prostaglandin E_2 (PGE_2), and plasma preserved with ethylenediaminetetraacetic acid was collected for fatty acid measurements. Serum was collected for a biochemistry profile and insulinlike growth factor-1 (IGF-1) concentrations. All blood was centrifuged and separated within 30 minutes, and samples were frozen immediately at −80°C until analysis. Body composition was measured by the 3 techniques listed below.

After baseline measurements, dogs with heart failure were randomized to either the fish oil or placebo group. Dogs in the fish oil group (n = 14) were given fish oil ethyl esters in capsules (Fish Oil Test Materials Program, National Institutes of Health, Bethesda, MD) to provide approximately 27 mg/kg/day EPA and 18 mg/kg/day DHA. Dogs in the placebo group (n = 14) were given corn oil ethyl esters in identical capsules (Fish Oil Test Materials Program, National Institutes of Health) as placebo. Placebo and fish oil capsules were isocaloric. Each 1-g fish oil capsule contained 1.5 mg alpha-tocopherol and 2.1 mg gamma-tocopherol. Each 1-g placebo capsule contained 1.1 mg alpha-tocopherol and 2.6 mg gamma tocopherol. Investigators and owners were blinded to the capsule formulation provided to each dog until the end of the study period.

Owners were provided with and encouraged to feed a dry, sodium-restricted commercial diet (h/d, Hill's Prescription Diets, Topeka, KS). So that this commercial diet was not a limitation to food intake, dogs were not restricted to this diet if they were anorectic. Owners were instructed to keep a 3-day food record during weeks 2, 4, and 6 of the study so that total dietary intake could be estimated. Each 3-day food record was recorded on 1 weekend day and 2 weekdays. Diet records were analyzed for total caloric intake, as well as protein, fat, carbohydrate, and fatty acid intake (Minnesota Nutrition Data System software, Nutrition Coordination Center, University of Minnesota, Minneapolis, MN [food database version 10a; nutrient database version 25, 1995]). Nutrient composition of dog foods was based on information provided by the manufacturers of individual products. Maintenance energy requirements (MERs) were calculated based on the formula: MER = [132 × (body weight in kg)$^{0.75}$].[23] Owners were called at biweekly intervals for progress reports on the dogs' condition, appetite, and diet.

Dogs with heart failure were reassessed after 8 weeks of fish oil or placebo administration. At that time, an echocardiogram was performed, and blood was collected for cytokine, PGE_2, and fatty acid analyses. Total body water, cachexia score, and ultrasonic measurement of subcutaneous fat thickness were performed after an overnight fast. Compliance with the capsule administration was assessed by capsule counts and changes in plasma fatty acid composition.

Body Composition Measurements

Body composition was measured after an overnight fast by 3 methods, as detailed below.

Total Body Water. Deuterium oxide (99.9%, Cambridge Isotope Laboratories, Woburn, MA) was administered orally at a dosage of 0.1 g/kg body weight. Six hours after the dose was given, venous blood was collected and separated, and serum frozen at −80°C until analysis. The ratio of $^2H:^1H$ in samples was measured by isotope ratio mass spectrometry (IRMS). Serum samples for this study initially were prepared utilizing the standard zinc method.[24] In this method, zinc reduces the water in the serum samples to hydrogen gas at 500°C. The isotope ratio data from samples prepared using this technique were irreproducible because of carmelization of the serum upon heating. Therefore, an alternative method was developed that avoided the heating process inherent in the zinc technique. This method, based on gaseous hydrogen–water equilibrium in the presence of a platinum catalyst subsequently was used to prepare the samples.[25,26] Briefly, tubes (Pyrex, 0.96-cm outer diameter, 10.26 cm long), each containing sample, platinum catalyst, and hydrogen gas, were attached to the manifold ports of the IRMS (SIRA 10, Micromass, Dearborn, MI) by means of connectors (Cajon Ultra-Torr, Cambridge Valve and Fitting, Inc, Billerica, MA). Serum samples, an external standard (see below), and an internal reference (tap water) were analyzed concurrently. Air was 1st evacuated from the lines. The tubes then were placed in a liquid nitrogen bath to freeze the sample and each port was opened individually to the vacuum pumps to remove excess air from the sample tubes. Once the tubes were evacuated, hydrogen gas (5 psi) was admitted into the sample tubes for several seconds. The sample tubes then were allowed to equilibrate in a temperature-controlled air bath (maintained at 30.00 ± 0.05°C) for 1 hour. After the samples equilibrated, the hydrogen gas from the sample tube containing tap water was released into the reference side of the IRMS. This gas was used

as the internal reference for all subsequent sample analyses. Tantalum disks (Pacific American Technologies Corporation, Bellefonte, PA) with a 0.077-mm-diameter hole had been installed in the steel tubing attaching the connectors to the inlet valves of the IRMS to prevent droplets of serum from being carried into the vacuum lines. A liquid nitrogen trap also was set up to remove any residual moisture in the hydrogen gas. An external standard of deuterium oxide was mixed in tap water in the concentration that would be expected in each dog. Serum samples and external standards were analyzed for the $^2H:^1H$ isotope ratio concurrently with the internal reference. Total body water was calculated as follows:

$$\text{total body water (L)} = \frac{D \times APE_{dose} \times MW_{water}}{MW_{dose} \times 100 \times (\delta_r/\delta_p) \times R_{ref}},$$

where D = dose of deuterium (g), APE_{dose} = atom percent excess of dose (99.9%), MW_{water} = molecular weight of water (18.02), MW_{dose} = molecular weight of deuterium oxide (20.02), δ_r = deuterium enrichment in the external standard, δ_p = deuterium enrichment in the serum sample, and R_{ref} = ratio $^2H:^1H$ in the internal reference.

Dogs with significant ascites (n = 2) were excluded from the total body water analysis and thoracentesis was performed before deuterium administration in 1 dog that had pleural effusion at the time of enrollment. Dogs that spit out the deuterium or salivated excessively immediately after the dose (2 control dogs and 8 dogs with heart failure) were excluded from the analyses. Lean body mass was estimated from the total body water measurement, based on the assumption that water occupies 73% of the fat-free mass:

$$\text{lean body mass (kg)} = \text{total body water}/0.73.$$

Subcutaneous Fat Thickness. The mid-lumbar subcutaneous fat thickness was estimated by ultrasonography, based on the technique of Wilkinson and McEwan.[27] Three measurements were taken between the 3rd and 5th lumbar vertebrae, 2–3 cm lateral from the midline using a 7.5-MHz probe. The subcutaneous fat layer thickness in millimeters was calculated as the mean of the 3 measurements.

Cachexia Score. While blinded to treatment status of the dogs, 1 of the investigators (LMF) subjectively assessed the degree of cachexia in each dog at baseline and at the end of the 8-week experimental period. The degree of cachexia was judged subjectively on a scale of 0–4, based on visual examination and palpation of various muscle groups, and an overall cachexia score was assigned where 0 = no cachexia, 1 = mild cachexia, 2 = moderate cachexia, 3 = marked cachexia, and 4 = severe cachexia.

Assessment of Nutritional Status

Serum IGF-1 concentration was used as an indicator of baseline nutritional status in the dogs with heart failure and in the healthy controls. Analysis of IGF-1 concentrations was performed using a radioimmunoassay, as previously described.[28] The effective detection range of the radioimmunoassay was 5–640 pg IGF-1.

Fatty Acid Analysis

A fatty acid profile was determined on each dog by gas chromatography.[29] Briefly, 200 μL lipid was extracted from each plasma sample by chloroform:methanol (1:1, v:v). The extract was dried under N_2, and the residue was resuspended in 1.0 mL benzene. Fatty acids were methylated with 5% methanolic HCl at 70°C for 2 hours.[30] One microliter of fatty acid methyl ester was injected into the gas chromatograph (Model 5890, Hewlett Packard, Wilmington, DE) fitted with a 30-m × 0.25-mm-inner diameter capillary column (Model AT-WAX, Alltech, Deerfield, IL) with 0.25-μm film thickness. Eluted peaks were detected with a flame ionization detector. Peaks were identified and validated by chromatography of a mixture of authentic fatty acid methyl esters. Plasma fatty acid concentrations were reported as the percent normalized concentrations.

Cytokines and PGE_2 Measurements

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll–Hypaque density centrifugation, and were washed 3 times in sterile, pyrogen-free saline. Cells were cultured in RPMI-1640 growth media with 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. For cytokine assays, cells were cultured at a concentration of 5×10^6 cells/mL in 24-well flat-bottomed plates for 24 hours at 37°C and 5% CO_2 with either RPMI-1640 media or *Escherichia coli* endotoxin (Sigma Chemical Company, St Louis, MO) at concentrations of 0, 1, 10, 100, and 1,000 ng/mL. After incubation, cells were subjected to 3 freeze–thaw cycles to release cytoplasmic contents, and the final supernatant was analyzed for cytokine activities using the bioassays described below. Bioassays were used because no immunoassays were available for canine TNF and IL-1. For PGE_2 analysis, PBMC were cultured at a concentration of 1×10^6 cells/mL with either RPMI-1640 media or 100 ng/mL of phytohemagglutinin for 48 hours at 37°C and 5% CO_2. The cultures were centrifuged and supernatants were collected and frozen immediately at −80°C until analysis.

TNF. The WEHI 164 subclone 13 cytotoxicity bioassay was used to measure TNF-like activity as previously described (WEHI 164 subclone 13 cells were kindly provided by Dr M.J. Kluger, The Lovelace Institute, Albuquerque, NM).[31] Results were read from a standard curve using recombinant human TNF tested in the same assay. The detection limit for this assay was 16 pg/mL.

IL-1. The D10S cell proliferation assay was used to measure IL-1-like activity (D10S cells courtesy of Dr R.V. House, IIT Research Institute, Chicago, IL).[32] Results were read from a standard curve using recombinant human IL-1 tested in the same assay. The detection limit for this assay was 20 pg/mL.

PGE_2. PGE_2 production by PBMC was determined by competitive radioimmunoassay. The radioimmunoassay method has been previously described, and employs a double-antibody procedure using rabbit antibodies to PGE_2 and sheep anti-rabbit α-globulin.[33,34]

Statistical Analysis

Unless otherwise noted, all reported values are mean ± standard deviation. The distributions of data were examined graphically. Data that were not normally distributed were transformed using logarithmic transformation. Baseline comparisons between groups employed 1-way analysis of variance with Tukey's honestly significant difference test. Comparisons between fish oil and placebo groups were made for body composition measurements, cytokine concentrations, IGF-1 concentrations, fatty acids, and food intake using independent *t*-tests, based on the differences between baseline and 8-week measurements. Ordinal data (class and cachexia score) were compared with the Mann-Whitney *U* test. Statistical analysis was performed using a commercial statistical software program (SYSTAT statistical software, Chicago, IL).

Results

Baseline Comparison of Dogs with Heart Failure and Control Dogs

Twenty-eight dogs with heart failure and 5 healthy control dogs were enrolled in the study. Baseline characteristics are compared in Table 1. At baseline, dogs with heart failure had a greater mean cachexia score than control dogs ($P = .01$). Fifteen dogs with heart failure (54%) had some degree of cachexia (cachexia score ≥ 1) compared to none of the controls ($P = .05$). Body weight, subcutaneous fat thickness, total body water, and lean body mass were not significantly different between dogs with heart failure and healthy controls. As expected, dogs with heart failure had lower mean fractional shortening ($P < .001$) and larger mean left ventricular internal

Table 1. Baseline comparison of healthy controls, all dogs with heart failure, and dogs with heart failure that survived through the 8-week study period (mean ± standard deviation).

	Healthy Controls (n = 5)	All Heart Failure Dogs (n = 28)	Surviving Heart Failure Dogs (n = 21)
Age (years)	7.4 ± 3.2	8.0 ± 2.8	8.2 ± 2.7
Body weight (kg)	43.7 ± 7.8	41.2 ± 18.1	44.7 ± 19.6
Cachexia score (0–4)	0.0 ± 0.0	1.7 ± 1.4[a]	1.0 ± 1.3[b]
Total body water (L)	29.6 ± 5.4	25.4 ± 9.3	26.2 ± 10.3
Lean body mass (kg)	40.5 ± 7.3	34.7 ± 12.7	35.8 ± 14.1
Subcutaneous fat (mm)	4.4 ± 0.7	3.8 ± 1.5	3.8 ± 1.5
Heart failure class (I–IV)	—	2.8 ± 0.6	2.7 ± 0.6
Fractional shortening (%)	33.8 ± 0.8	16.1 ± 7.9[a]	16.6 ± 6.7[a]
LVIDD (cm)	4.4 ± 0.5	6.0 ± 1.1[a]	6.1 ± 1.1[a]
LVIDS (cm)	2.9 ± 0.3	5.0 ± 1.1[a]	5.1 ± 0.9[a]
Circulating TNF (ng/mL)	0.10 ± 0.18	0.50 ± 0.82	0.38 ± 0.57
TNF (ng/mL)			
Unstimulated	0.35 ± 0.38	0.49 ± 0.83	0.55 ± 0.94
1 ng/mL endotoxin	2.47 ± 1.61	1.65 ± 1.41	1.44 ± 1.39
10 ng/mL endotoxin	3.68 ± 2.69	2.03 ± 2.67	1.43 ± 1.03
100 ng/mL endotoxin	8.81 ± 4.71	5.70 ± 5.00	4.83 ± 4.03
1,000 ng/mL endotoxin	12.76 ± 8.82	15.98 ± 13.98	14.82 ± 14.91
Circulating IL-1 (ng/mL)	0.69 ± 0.58	1.59 ± 2.77	1.10 ± 1.39
IL-1 (ng/mL)			
Unstimulated	0.77 ± 0.32	0.67 ± 0.43	0.70 ± 0.47
1 ng/mL endotoxin	1.99 ± 0.94	0.96 ± 0.63	1.00 ± 0.71
10 ng/mL endotoxin	1.44 ± 1.25	1.21 ± 0.67	1.29 ± 0.71
100 ng/mL endotoxin	1.76 ± 0.99	3.08 ± 2.57	3.04 ± 2.65
1,000 ng/mL endotoxin	9.47 ± 11.13	9.37 ± 11.54	10.02 ± 13.14
Unstimulated PGE_2 (ng/mL)	0.72 ± 0.69	9.65 ± 23.42	12.38 ± 26.60
Stimulated PGE_2 (ng/mL)	5.35 ± 4.43	26.08 ± 55.93	26.14 ± 61.21

LVIDD, left ventricular internal dimension in diastole; LVIDS, left ventricular internal dimension in systole; TNF, tumor necrosis factor; IL-1, Interleukin-1β; PGE_2, prostaglandin E_2.

[a] Significantly different from healthy controls ($P < .01$).

[b] Significantly different from healthy controls ($P < .05$).

dimension in systole ($P = .001$) and diastole ($P < .001$) than healthy controls. Mean serum IGF-1 concentrations were not different between the heart failure (141.0 ± 61.3 ng/mL) and control (156.0 ± 52.7 ng/mL) groups ($P = .59$). Plasma fatty acid analysis, however, disclosed higher mean oleic acid (C18:1) concentration in dogs with heart failure (14.24 ± 4.08%) than in healthy controls (10.40 ± 0.57%; $P < .001$). Dogs with heart failure had lower mean concentrations of arachidonic acid (C20:4*n*-6), EPA (C20:5*n*-3), and DHA (C22:6*n*-3) than did healthy controls (arachidonic acid: 18.95 ± 3.61% for heart failure dogs versus 22.81 ± 2.56% for controls, $P = .02$; EPA: 0.15 ± 0.18% for heart failure dogs versus 0.54 ± 0.89% for controls, $P = .03$; DHA: 0.85 ± 0.42% for heart failure dogs versus 1.72 ± 1.18% for controls, $P = .004$). No significant differences between groups were detected for IL-1, TNF, or PGE_2. The same results were obtained when only dogs that completed the study were compared to the controls (Table 1).

Comparison of Fish Oil- and Placebo-Treated Dogs with Heart Failure

Twenty-eight dogs were randomized to either the fish oil (n = 14) or placebo (n = 14) groups. At the beginning of the study, the majority of dogs with heart failure were receiving furosemide (12 in each group), digoxin (12 in the fish oil group versus 10 in the placebo group), and an angiotensin-converting enzyme (ACE) inhibitor (ACEI) (13 in the fish oil group versus 14 in the placebo group). Other medications included diltiazem (2 in each group), hydrochlorothiazide-spironolactone (1 in the fish oil group versus 3 in the placebo group), and a β-adrenergic antagonist (3 in the fish oil group versus 6 in the placebo group). Neither the number of dogs receiving each medication nor the mean dosage of medications (on a mg/kg basis) was significantly different between the 2 groups of dogs with heart failure at baseline.

At baseline, the fish oil (n = 14) and placebo (n = 14) groups were not different in age, sex, body weight, total body water, lean body mass, subcutaneous fat, or severity of cardiac disease. Dogs in the fish oil group had a greater baseline cachexia score (mean cachexia score = 1.7 ± 1.4) than the placebo group (0.7 ± 1.1; $P = .05$). Mean concentrations of circulating IL-1 and PBMC production of IL-1 (stimulated by 10, 100, and 1,000 ng/mL endotoxin) were similar in the 2 groups, but constitutive production of IL-1 by unstimulated PBMC (0 ng/mL endotoxin) was higher in the fish oil group (0.86 ± 0.46) than in the placebo group (0.47 ± 0.31; $P = .02$). Baseline measurements of TNF and PGE_2 were not different between the groups. However, baseline concentrations of circulating TNF ($r = 0.37$; $P =$

Table 2. Mean changes over the study period for dogs in the fish oil group and dogs in the placebo group that completed the study (mean ± SD).

	Fish Oil Group (n = 9)	Placebo Group (n = 12)	*P* Value
Change in body weight (kg)	−0.3 ± 2.1	−0.4 ± 1.6	.87
Change in cachexia score (0–4)[a]	−0.6 ± 0.7	0.5 ± 0.7	.01
Change in total body water (L)	1.2 ± 2.1	−1.5 ± 2.9	.11
Change in lean body mass (kg)	1.7 ± 2.8	−2.1 ± 4.0	.11
Change in subcutaneous fat thickness (mm)	−0.7 ± 0.8	−0.8 ± 1.3	.74
Change in fractional shortening (%)	1.4 ± 7.3	−0.3 ± 4.5	.55
Change in LVIDD (cm)	0.03 ± 0.37	−0.31 ± 0.54	.11
Change in LVIDS (cm)	0.04 ± 0.60	−0.22 ± 0.58	.34
Change in circulating TNF (ng/mL)	−0.10 ± 0.90	0.00 ± 0.42	.75
Change in TNF (ng/mL)			
Unstimulated	0.18 ± 1.03	0.17 ± 0.32	.98
1 ng/mL endotoxin	−0.49 ± 1.19	−0.14 ± 1.75	.59
10 ng/mL endotoxin	0.77 ± 1.81	0.80 ± 2.16	.98
100 ng/mL endotoxin	−2.41 ± 3.03	3.48 ± 10.82	.13
1,000 ng/mL endotoxin	−2.06 ± 23.33	3.42 ± 8.76	.53
Change in circulating IL-1 (ng/mL)	−0.43 ± 0.91	0.18 ± 0.90	.15
Change in IL-1 (ng/mL)			
Unstimulated	−0.27 ± 0.64	0.63 ± 0.92	.02
1 ng/mL endotoxin	−0.45 ± 0.84	0.51 ± 0.92	.02
10 ng/mL endotoxin	−0.19 ± 0.76	0.44 ± 0.95	.11
100 ng/mL endotoxin	−1.23 ± 2.86	0.92 ± 1.63	.07
1,000 ng/mL endotoxin	−1.18 ± 5.06	2.22 ± 3.72	.14
Change in unstimulated PGE_2 (ng/mL)	−9.81 ± 24.02	5.37 ± 28.18	.21
Change in stimulated PGE_2 (ng/mL)	−39.73 ± 89.28	26.54 ± 48.37	.07

LVIDD, left ventricular internal dimension in diastole; LVIDS, left ventricular internal dimension in systole; TNF, tumor necrosis factor; IL-1, Interleukin-1β; PGE_2, prostaglandin E_2.

[a] Negative sign indicates an improvement in cachexia score.

.05) and stimulated PGE_2 production (r = .37; P = .05) correlated with the cachexia score. No correlations were detected between IL-1, TNF, or PGE_2 and severity of heart failure (by class, fractional shortening, left ventricular size, or furosemide dose required).

Nine dogs in the fish oil group and 12 dogs in the placebo group completed the study (P = .19). Compliance, based on pill count, was not different between groups (mean compliance for all 21 dogs that completed the study = 94%; range = 74–100%).

Changes after Supplementation

Cardiac Parameters. Four dogs (1 in the fish oil group and 3 in the placebo group) required additional medication during the study period, although medications were not significantly different between groups at the time of the follow-up evaluation. Changes in cardiac size and contractility over the study period were not significantly different between groups (Table 2).

Plasma Fatty Acids. Plasma fatty acid composition was analyzed in the 21 dogs that completed the study. Data on changes in plasma fatty acids were reported as the percentage change from baseline values. A smaller increase in linoleic acid (C18:2) occurred in the fish oil group (1.01 ± 7.75%) than in the placebo group (18.88 ± 28.58%; P = .06). The decrease in plasma arachidonic acid in the fish oil group over the 8-week study period (−20.82 ± 7.09%) was significantly different from the change in the placebo group (−0.57 ± 21.11%; P = .008). Dogs in the fish oil group also had greater changes in EPA and DHA over the study period than did the placebo group (EPA: 1,773.01 ± 1,418.53% versus −31.74 ± 42.70%; P < .03; DHA: 229.04 ± 120.91% versus −11.43 ± 20.77%; P < .001). Dogs did not have a fishy odor to their breath, and owners of dogs in both groups, as well as the examining clinicians, were unaware of the type of capsule the dogs had been receiving.

Body Composition. The mean changes in body composition for each group over the 8-week study period are compared in Table 2. Dogs in the fish oil group had a greater mean improvement in cachexia score (−0.6 ± 0.7) than did dogs in the placebo group (0.5 ± 0.7; P = .01; Table 2). This difference between groups was independent of the higher baseline cachexia score in the fish oil group (P = .02 after adjustment for baseline differences). Total body water (P = .13) and lean body mass (P = .13) also increased in the fish oil group compared to the placebo group but these changes were not statistically significant. The changes in body weight and subcutaneous fat thickness were not different between the 2 groups.

Food Intake. Mean total caloric intake for the group as a whole was significantly below the MER at week 2 (P < .001), week 4 (P < .001), and week 6 (P < .001). The mean caloric intake was 84% of the MER at week 2, 72% of the MER at week 4, and 72% of the MER at week 6. No correlation was detected between IL-1, TNF, or PGE_2 concentrations and food intake. The changes in the mean total caloric intake (−167 ± 555 kcal for the fish oil group

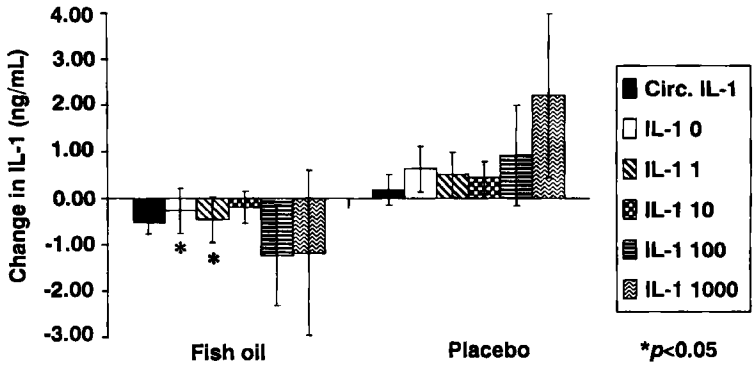

Fig 1. Changes in circulating and peripheral blood mononuclear cell (PBMC) production of interleukin-1β (IL-1) concentrations in dogs with heart failure supplemented with either fish oil or placebo (mean ± standard error of the mean). Numbers following IL-1 indicate the concentration of endotoxin used (in ng/mL) to stimulate PBMCs. An asterisk indicates that the change is significantly different from placebo group ($P \leq .05$).

versus −208 ± 611 kcal for the placebo group; $P = .88$), as well as the intakes of protein (−38 ± 78 kcal for the fish oil group versus −56 ± 83 kcal for the placebo group; $P = .62$), fat (−61 ± 120 kcal for the fish oil group versus −69 ± 111 kcal for the placebo group; $P = .88$), and carbohydrate (−88 ± 275 kcal for the fish oil group versus −202 ± 261 kcal for the placebo group; $P = .35$) over the 8-week study period did not differ significantly between groups (Table 2).

Cytokines and PGE$_2$. Over the 8-week study period, production of IL-1 by unstimulated PBMC ($P = .02$) and PBMC after stimulation with 1 ng/mL endotoxin ($P = .02$) decreased more in the fish oil group than in the placebo group for both assays (Fig 1). Changes in circulating IL-1 ($P = .15$) and IL-1 production by PBMC stimulated with 10 ($P = .11$), 100 ($P = .07$), and 1,000 ($P = .14$) ng/mL endotoxin also were consistent with this finding but did not reach statistical significance (Fig 1). The changes over the study period in circulating TNF and PBMC production of TNF did not differ between groups. Stimulated PGE_2 production decreased more in the fish oil group than in the placebo group ($P = .07$) over the study period.

Survival. Nine of 14 dogs in the fish oil group and 12 of 14 dogs in the placebo group completed the study ($P = .19$). One dog in the fish oil group died suddenly, presumably from ventricular arrhythmias. The other 4 dogs in the fish oil group and both of the remaining dogs in the placebo group were euthanized for progressive heart failure. Survival was calculated from the time of diagnosis of DCM to death or censorship, and ranged from 2 weeks to 36 months. Except for 2 dogs in the placebo group that switched to fish oil after the study ended, dogs remained on their respective supplement after the completion of the study. The median survival was 10.5 months in the fish oil group and 11.9 months in the placebo group ($P = .51$). The median survival for both groups was 11.5 months.

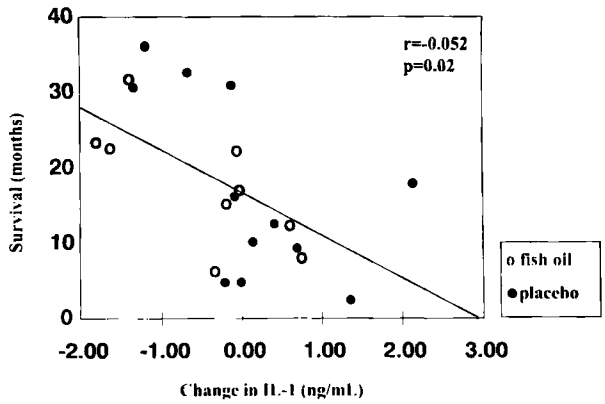

Fig 2. Comparison of changes in circulating interleukin-1β (IL-1) concentrations and survival in 21 dogs with heart failure that completed the study.

Reductions in circulating IL-1 over the study period correlated with survival ($r = .52$; $P = .02$; Fig 2). A significant positive correlation also occurred between baseline IGF-1 concentrations and survival ($r = .47$; $P = .01$). Multivariate linear regression analysis showed that only the reduction in IL-1 remained a significant predictor of survival ($P = .04$) after adjustment for age, cardiac dimensions and contractility, class, and β-adrenergic antagonist use. No correlation between TNF or PGE_2 and survival was detected.

Discussion

Although *n*-3 fatty acids have been tested in several studies of humans and animals, they have not previously been studied in dogs with heart failure. The current study demonstrates that administration of fish oil at a dosage of 27 mg/kg/day EPA and 18 mg/kg/day DHA altered plasma fatty acids and improved cachexia score in dogs with heart failure secondary to DCM. In addition, fish oil reduced IL-1 production. Although these short-term changes are important, reductions in IL-1 also may have long-term benefits in this population of dogs because a reduction in IL-1 correlated with improved survival. Several mechanisms might explain this association. One is that the reduction in IL-1 caused improved survival. Cytokines can act as negative inotropes, and reductions in cytokine concentrations have been hypothesized to improve cardiac contractility.[35-37] Although no significant changes in cardiac dimensions or fractional shortening were noted in this study, local alterations in contractility or cellular energetics may occur that affect survival. Another possible mechanism for a beneficial response of IL-1 reduction is through changes in muscle mass. IL-1 and TNF increase skeletal muscle protein turn-

over and decrease cardiac myocyte protein synthesis, resulting in muscle loss that can contribute to increased morbidity and mortality.[6,7,13,38,39] An alternate possibility for the correlation between reductions in IL-1 and survival is that the decreased IL-1 concentrations were the result of longer survival (ie, dogs that survived longer had improvements in cardiac function or other parameters that resulted in reduced IL-1).

Despite the relationship between reduction in IL-1 and improved survival, we found no effect of fish oil on overall mortality. Nine dogs in the fish oil group and 12 dogs in the placebo group survived to complete the study. Although not significant, these numbers raise the issue of potential detrimental effects of fish oil administration (5 deaths in the fish oil group versus 2 in the placebo group). However, only 1 dog in the fish oil group died of natural causes (presumably from ventricular arrhythmias). The other 4 dogs in the fish oil group and both dogs in the placebo group were euthanized at the owners' request. Although all 6 of these dogs were euthanized for worsening heart failure, the decision by the owners was made at a different stage of heart failure and for different reasons in each case. Therefore, judging differences in the absolute numbers of deaths between the 2 groups may be misleading. Another concern is that corn oil may not have been a true placebo and actually may have had adverse effects, in that dogs in the placebo group had changes in plasma fatty acid concentrations and cytokine production. An argument against this interpretation is that the natural course of the disease in the placebo group was not worse than in other studies of dogs with DCM. In fact, the median survival of our subjects was 11.5 months, whereas a recent study found a median survival of 2.2 months in dogs with DCM.[40]

The positive correlation between IGF-1 concentrations and survival in the dogs with heart failure underscores the clinical importance of alterations in nutritional status. Although IGF-1 concentrations correlated with measures of body composition (body weight, total body water, and lean body mass) and food intake (protein and total calories), none of these other measurements alone was related to survival. IGF-1 (formerly known as somatomedin-C) has been used in the past as a dynamic indicator of nutritional status and as a means to monitor the response to nutritional support.[41,42] The association between IGF-1 and survival suggests that IGF-1 may be useful as a clinically relevant indicator of nutritional status in veterinary patients and as a potential means of identifying dogs with heart failure that have a poor prognosis.

Significant alterations in plasma fatty acids were found between dogs with heart failure and healthy controls, with a lower percentage of oleic acid and higher percentages of arachidonic acid, EPA, and DHA in dogs with heart failure. Although alterations in fatty acids have not been reported in human patients with heart failure, human breast cancer patients were shown in 1 study to have lower concentrations of EPA and DHA than controls.[43] Whether these differences are due to the underlying disease, drug therapy, or changes in appetite is not known, but they suggest that alterations in lipid metabolism may be present in patients with heart failure, and may be amenable to dietary therapy.

Although food intake did not differ between the fish oil and placebo groups, mean caloric intake for dogs with heart failure was below the predicted intake at all time points during the study period. This is, to our knowledge, the 1st study that reports a quantitative assessment of food intake in dogs with heart failure, and supports the general impression of insufficient food intake in this population. Nonetheless, this finding raises the question of how cachexia improved in the fish oil group despite the fact that the dogs ate fewer calories than expected based on the MER. Several explanations are possible. First, fish oil may have altered metabolism such that dietary energy was used more efficiently for lean tissue synthesis. Another possibility is that the techniques used in this study were not accurate enough for longitudinal determination of small changes in body composition. The expected MER calculation also may be inaccurate; the MER in dogs with heart failure may actually be lower than expected due to exercise restriction or metabolic changes associated with heart failure. Finally, dietary intake was measured by 3-day food records, and underreporting of food intake is possible, as has been shown in human studies.[44]

Similar to human heart failure patients, some alterations in body composition were found in these dogs with heart failure. Based on a subjective assessment, 54% of dogs with heart failure had some degree of cachexia. Future studies should employ more optimal techniques for the estimation of lean body mass such as the measurement of body cell mass by total body potassium, but the results of this study suggest that the subjective assessment of cachexia is a clinically useful method for the characterization of lean body mass.

Several potential reasons exist for the lack of differences in the total body water measurement in dogs with heart failure compared to healthy controls. First, a substantial number of dogs had to be excluded from the total body water analysis because they did not retain the entire oral dose of deuterium, thus giving an inaccurate measurement of total body water (2 control dogs, 4 dogs in the fish oil group, and 4 dogs in the placebo group). This limited our sample size and, thus, statistical power for this outcome (power = 75%). The problem with the oral deuterium dose could be circumvented in future studies by IV administration of the deuterium. Another concern is that dogs with heart failure may have had undetected shifts in intra- or extracellular water that were unaccounted for in the calculation of total body water. Finally, the assumption of constant hydration of lean tissue may be invalid in dogs with heart failure. Nonetheless, because the measurement of total body water and energy expenditure by IRMS in dogs is becoming more common, development of accurate and precise preparation techniques is essential. Sample preparation by the standard zinc method resulted in irreproducible data, which led us to develop the platinum technique for sample preparation. The results of this study demonstrate that for assaying canine serum samples by IRMS, the platinum technique is superior to the standard zinc method and offers high precision (mean coefficient of variation = 0.470%; range = 0.002–2.662%). To our knowledge, this is the 1st report of the use of this preparation technique in any species.

An unexpected finding was that dogs with heart failure

did not have significantly increased concentrations of cytokines compared to healthy controls. This may have been due to the unexpectedly high variability found in cytokine concentrations in these small groups, which may have masked an underlying difference due to low statistical power (power for cytokine determinations ranged from 41 to 94%). Another possibility is that ACEIs may have suppressed cytokine concentrations. ACEIs have been shown to suppress the in vitro production of IL-1 and TNF by PBMC.[45] In the current study, 27 of 28 dogs with heart failure were receiving ACEIs at the time of enrollment in the study. In contrast to studies in humans, we did not find a correlation between cytokine production and severity of heart failure (as measured by modified NYHA class).[46,47]

The dosage of fish oil selected for use in this study corresponded to the low dosage of fish oil used in a variety of published studies in humans and laboratory animals. However, other studies used dosages up to 55 mg/kg/day for EPA and 40 mg/kg/day for DHA. Although fish oil decreased IL-1 production in the current study, the reduction was not as pronounced as that reported previously. For example, Meydani et al,[19] using dosages similar to those of the current study, found between 48 and 90% reduction in IL-1 concentrations after 3 months of fish oil supplementation in healthy humans. The decrease in IL-1 in the current study using comparable amounts of endotoxin stimulation was only 36%. Therefore, although the dosage used in this study was well tolerated and resulted in reductions in IL-1 and some clinical improvements, higher dosages may provide additional benefits in dogs with heart failure.

The lack of change in both circulating and PBMC production of TNF suggests that, in this model, TNF was not influenced by the dosage of fish oil supplementation used. In contrast, studies in healthy humans using dosages of fish oil similar to those used in the current study showed reductions in TNF production.[18,19] A recent study in human heart failure patients given a fish oil supplement found a decrease of 59% in TNF, as measured by immunoassay.[48] However, mice fed a diet rich in fish oil had increased TNF production in comparison to mice fed diets high in corn or coconut oil.[49] These discrepancies may be the result of differences in study design or of small changes in method of sample collection and type of assay, which can have important effects on results.

In summary, heart failure secondary to DCM was associated with cachexia, the severity of which correlated with TNF concentrations. Fish oil supplementation for 8 weeks was well tolerated, decreased IL-1 and PGE_2 production, and reduced cachexia. In addition, the results of this study also demonstrated that baseline IGF-1 concentrations and reductions in circulating IL-1 concentrations correlate with survival in dogs with heart failure. Finally, although fish oil at this dosage provided modest clinical benefits, higher dosages may prove to have more potent effects on cytokine concentrations, which may translate into greater improvements in clinical outcome.

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