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Moderate severity heart failure does not involve a downregulation of myocardial fatty acid oxidation

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Departments of [1]Physiology and Biophysics, [2]Biochemistry, [3]Medicine, [4]Nutrition, and [5]Pharmacology, Case Western Reserve University and [6]Medical Research Service, Louis Stokes Department of Veteran Affairs Medical Center, Cleveland, Ohio 44106; and [7]Henry Ford Heart and Vascular Institute, Detroit, Michigan 48202

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Chandler, Margaret P., Janos Kerner, Hazel Huang, Edwin Vazquez, Aneta Reszko, Wenjun Z. Martini, Charles L. Hoppel, Makoto Imai, Sharad Rastogi, Hani N. Sabbah, and William C. Stanley. Moderate severity heart failure does not involve a downregulation of myocardial fatty acid oxidation. *Am J Physiol Heart Circ Physiol* 287: H1538–H1543, 2004. First published June 10, 2004; 10.1152/ajpheart.00281.2004.—Recent human and animal studies have demonstrated that in severe end-stage heart failure (HF), the cardiac muscle switches to a more fetal metabolic phenotype, characterized by downregulation of free fatty acid (FFA) oxidation and an enhancement of glucose oxidation. The goal of this study was to examine myocardial substrate metabolism in a model of moderate coronary microembolization-induced HF. We hypothesized that during well-compensated HF, FFA oxidation would predominate as opposed to a more fetal metabolic phenotype of greater glucose oxidation. Cardiac substrate uptake and oxidation were measured in normal dogs ($n = 8$) and in dogs with microembolization-induced HF ($n = 18$, ejection fraction = 28%) by infusing three isotopic tracers ([9,10-^{3}H]oleate, [U-^{14}C]glucose, and [1-^{13}C]lactate) in anesthetized open-chest animals. There were no differences in myocardial substrate metabolism between the two groups. The total activity of pyruvate dehydrogenase, the key enzyme regulating myocardial pyruvate oxidation (and hence glucose and lactate oxidation) was not affected by HF. We did not observe any difference in the activity of carnitine palmitoyl transferase I (CPT-I) and its sensitivity to inhibition by malonyl-CoA between groups; however, malonyl-CoA content was decreased by 22% with HF, suggesting less in vivo inhibition of CPT-I activity. The differences in malonyl-CoA content cannot be explained by changes in the Michaelis-Menten constant and maximal velocity for malonyl-CoA decarboxylase because neither were affected by HF. These results support the concept that there is no decrease in fatty acid oxidation during compensated HF and that the downregulation of fatty acid oxidation enzymes and the switch to carbohydrate oxidation observed in end-stage HF is only a late-stage phenomenon.

cardiac; metabolism; malonyl CoA

HEART FAILURE (HF) is a progressive disorder characterized by profound depression of left ventricular (LV) systolic and diastolic function, compensatory hypertrophy, and dilation, reduced cardiac output, elevated LV filling pressure, and increased systemic vascular resistance. Abnormalities of energy metabolism have often been cited as key elements in the poor LV function that characterizes the HF state (8, 12, 40, 41, 43). Recently, studies (26, 32, 39) in patients with HF, in the dog pacing-induced HF model, and in the rat infarct model have suggested that the rates of myocardial fatty acid and carbohydrate oxidation in mild-to-moderate HF are normal, but there is a dramatic downregulation of fatty acid oxidation (FAO) in severe, end-stage HF. Sack et al. (39) and Razeghi et al. (32) reported a downregulation of mRNA for the FAO enzymes long-chain acyl-CoA dehydrogenase (LCAD) and medium chain acyl-CoA dehydrogenase (MCAD), as well as protein levels of MCAD in biopsies from New York Heart Association Class IV HF patients with severe LV dysfunction and those undergoing heart transplantation. A similar downregulation of FAO enzymes [MCAD and carnitine palmitoyl transferase I (CPT-I)] accompanied by enhanced glucose oxidation was also reported in dogs with advanced end-stage HF induced by rapid ventricular pacing (26). Thus severe end-stage HF appears to be characterized by a switch to a more fetal metabolic phenotype characterized by downregulation of FAO and enhancement of glucose oxidation.

It is unknown, however, whether these types of alterations in the metabolic phenotype occur during moderate severity compensated HF. The fall in FAO and rise in carbohydrate oxidation that was observed during advanced end-stage failure was not reported during a period of moderately severe, pacing-induced HF (33). This observation is supported by studies in New York Heart Association Class II–III patients that showed elevated rates of myocardial FAO and decreased carbohydrate oxidation, compared with healthy individuals (30). Similarly, by using positron emission tomography, Wallhaus et al. (47) observed a greater rate of myocardial uptake of a radiolabeled fatty acid analog and less deoxyglucose uptake in Class III HF patients compared with healthy subjects. The biochemical mechanisms responsible for this switch are not known. FAO is regulated by myocardial malonyl-CoA, which inhibits CPT-I and mitochondrial fatty acid uptake (9, 20). We recently observed that neither CPT-I nor MCAD activities were reduced in dogs with moderate compensated HF induced by coronary microembolization (LV ejection fraction 27%) (29); however, we did not measure the tissue content of malonyl-CoA or malonyl-CoA sensitivity of CPT-I. The results from these studies suggest that a "switch" in metabolic phenotype characterized by a downregulation of FAO may not occur in moderate HF but rather may be an end-stage phenomenon associated with cardiac decompensation.

The purpose of this study was to examine myocardial substrate uptake and oxidation in dogs with moderate well-compensated HF. We employed a well-established canine coronary microembolization model of HF that results in irreversible progressive LV dysfunction and remodeling (36, 38). We hypothesized that in dogs with moderate severity microembolization-induced HF, the myocardium would have normal rates of FAO and glucose oxidation compared with healthy animals. Substrate uptake and oxidation were measured by using simultaneously infused isotopically labeled oleate, glucose, and lactate. Myocardial tissue was analyzed for the activity of key metabolic enzymes (pyruvate dehydrogenase and CPT-I) and intermediates (malonyl-CoA) and for the malonyl-CoA sensitivity of CPT-I.

METHODS

Animal model. The dog model of intracoronary microembolization-induced HF was used. The protocol was approved by the Henry Ford Hospital Institutional Animal Care and Use Committee. Eighteen healthy mongrel dogs underwent multiple sequential intracoronary microembolizations to produce chronic LV dysfunction and failure as previously described (36, 38). Animals were sedated with intravenous oxymorphone hydrochloride (0.22 mg/kg), and diazepam and anesthesia were maintained with 1–2% isofluorane. With the dogs under general anesthesia, cardiac catheterizations were performed under sterile conditions with the chest closed. Intracoronary injections (4–6 injections) of polystyrene latex microspheres (70–102 μm in diameter) were administered 1–2 wk apart into the coronary arteries. Left ventriculograms were performed before each embolization to determine LV ejection fractions. Embolizations were discontinued when LV ejection fraction was <35%. Dogs were allowed to recover for a period of 2–3 wk to ensure that infarctions produced by the last microembolization were completely healed. Eight normal dogs were used as age-matched controls.

Surgical preparation. After an overnight fast, animals were sedated with sodium thiopental (2.5% iv) and intubated, and anesthesia was maintained with isofluorane (0.75–1.5%). The animals were ventilated to maintain blood gases in the normal range (P_{O_2} > 100 mmHg, P_{CO_2} 35–45 mmHg, and pH 7.35–7.45) (42). The heart was exposed via a left thoracotomy. A small polyethylene cannula was placed in the interventricular vein to collect venous blood samples from the LV free wall. Heparin was infused to prevent clotting and thrombus formation (200 U/kg bolus, followed by 100 U·kg^{-1}·h^{-1} iv). The left atrial appendage was cannulated for the infusion of stable-labeled microspheres to measure myocardial blood flow before and immediately after the collection of blood samples. A 7-Fr Milar Mikrotip dual-transducer catheter was used to assess LV pressure (LVP). Regional segment length was measured in the anterior free wall (LAD bed) using sonomicrometry as previously described (Triton Technologies; San Diego, CA) (13, 23). The crystal pair was positioned at approximately midwall depth. Regional myocardial contractile function was assessed from the LVP-segment length loop area in the anterior wall.

Experimental protocol. After the instrumentation was completed, a bolus infusion of 100 mg [1-^{13}C]lactate and 20 μCi of [U-^{14}C]glucose was given followed by a continuous infusion of [U-^{14}C]glucose (0.3 μCi/min) and [9,10-^{3}H]oleate (0.6 μCi/min), and [1-^{13}C]lactate (2.0 mg/min) that was introduced into the femoral vein at a rate of 4.5 ml/h. After 60 min of tracer infusion, three pairs of arterial and interventricular venous samples were drawn 5 min apart (60, 65, and 70 min after tracer infusion) from the femoral artery and from a vein on the anterior LV free wall. Cardiovascular measurements were recorded immediately before each arterial and venous blood sample collection. Heart rate, LVP, peak positive and negative first derivative of pressure (dP/dt), and segment length were continuously recorded using a commercial on-line data-acquisition system (Crystal Biotech

model CBI8000 with Biopaq software). After the last blood sample was taken, small myocardial biopsies (10–20 mg) were taken with a 14-gauge biopsy needle, followed by a large (~3 g) punch biopsy from the anterior LV free wall. These biopsy samples were immediately freeze-clamped (3–5 s) on aluminum blocks precooled in liquid nitrogen and stored at −80°C for subsequent analysis. Tissue ATP and lactate were assayed in the needle biopsy samples, and the punch biopsy was assayed for the concentrations of tissue glycogen and malonyl CoA and for the activity of pyruvate dehydrogenase (PDH).

Immediately after the punch biopsy, a portion of the anterior LV free wall was harvested for the isolation of fresh mitochondria, both subsarcolemmal and interfibrillar.

Analytic methods. Detailed analytic methods have been previously cited in the literature (3). Arterial and venous pH, P_{CO_2}, and P_{O_2} were determined on a blood gas analyzer (NOVA Profile Stat 3, NOVA Biomedical Waltham), and hemoglobin concentration and saturation were measured on a hemoximeter (Avoximeter; San Antonio, TX). Blood samples for glucose, lactate, and [^{14}C]glucose were deproteinized in ice-cold 1 M perchloric acid (1:2 vol/vol) and analyzed for glucose and lactate using enzymatic spectrophotometric assays on a 96-well plate reader as previously described. Blood samples for [^{14}C]glucose and [^{14}C]lactate measurements were neutralized with K_2CO_3, and the neutral eluate ran through ion-exchange resin columns (Bio-Rad AG 50W-X8 Resin and Bio-Rad AG1-X8 Formate Resin) to separate [^{14}C]glucose as previously described. Total glucose concentration and ^{14}C activity were then measured in the eluate to calculate [^{14}C]glucose specific activity. Plasma [^{3}H]oleate concentration was measured by extracting the fatty acids from 0.5 ml of plasma in 3 ml of heptane-isopropanol (3:7) and counting the organic phase as previously described. ^3H_2O concentration was measured by distilling 0.5 ml of plasma in custom-made, modified Hickman stills (Kontes Glass, Custom Shop). Blood $^{14}CO_2$ concentration was measured by expelling $^{14}CO_2$ with the addition of concentrated lactic acid and trapping it in hyamine hydroxide as previously described. Plasma free fatty acids (FFA) were measured by using a commercially available enzymatic spectrophotometric kit (Wako Chemicals; Richmond, VA).

Tissue samples were pulverized in a stainless-steel tissue pulverizer cooled in liquid nitrogen. Tissue concentrations of ATP and ADP were measured by using the ATP Bioluminescent Assay Kit (Sigma-Aldrich). Tissue lactate concentrations were measured by using an enzymatic spectrofluorometric assay. Tissue glycogen was assayed on perchloric acid extracts by using the amyloglucosidase method as described by Passoneau and Lauderdale (31). Tissue malonyl-CoA was assayed by using gas chromatography-mass spectrometry as described previously (34).

Actual and total PDH activity were determined by using a newly developed radiochemical assay (45). The assay is based on the production of [1-^{14}C]acetyl-CoA from [2-^{14}C]pyruvate, which is converted to [1-^{14}C]acetylcarnitine in the presence of excess L-carnitine and carnitine acetyltransferase. The positively charged product [1-^{14}C]acetylcarnitine is then separated from the negatively charged radiolabeled substrate by exclusion chromatography. Malonyl-CoA decarboxylase activity was determined as previously described by Kerner et al. (18). Briefly, [1-^{14}C]acetyl-CoA from [2-^{14}C]malonyl-CoA is converted to [1-^{14}C] acetylcarnitine, which is then separated from the negatively charged radiolabeled substrate by exclusion chromatography as described above.

The two populations of mitochondria, the subsarcolemmal and interfibrillar, were isolated from hearts of normal and HF dogs using the procedure of Palmer et al. (27), except that a modified Chappel-Perry buffer (myofibril relaxing buffer including magnesium and ATP) was used (10) and the protease nagarse was replaced by trypsin (25).

CPT-I activity of isolated mitochondria was determined using the radiochemical forward assay as previously described (15) and is defined as the fraction of CPT activity inhibited by 200 μM malonyl-CoA. The IC$_{50}$ values of malonyl-CoA for CPT-I on isolated dog heart mitochondria was determined at a fixed concentration of palmi-

Table 1. *Summary of hemodynamic data in normal dogs and dogs with microembolization-induced heart failure*

	Normal	Heart Failure
n	8	18
LVP, mmHg	117±6	93±3*
Heart rate, beats/min	126±4	109±3*
Mean myocardial blood flow, ml·min^{-1}·g wet wt^{-1}	1.03±0.10	0.87±0.07
Subendocardial blood flow, ml·min^{-1}·g wet wt^{-1}	1.02±0.08	0.89±0.07
MV̇O$_2$, μmol·min^{-1}·g wet wt^{-1}	3.19±0.36	2.46±0.27

Values are means ± SE; *n,* number of dogs. LVP, left ventricular pressure; MV̇O$_2$, myocardial oxygen consumption. *$P < 0.05$ heart failure vs. normals.

toyl-CoA and increasing concentrations of malonyl-CoA as specified in the appropriate figure legends.

Frozen tissue samples were also analyzed for the concentration of citric acid cycle intermediates by gas chromatography-mass spectrom-

Table 2. *Summary of metabolic data in normal dogs and dogs with microembolization-induced heart failure*

	Normals	Heart Failure
Rate of glucose uptake, μmol·min^{-1}·g wet wt^{-1}	0.287±0.076	0.190±0.068
Rate of glucose oxidation, μmol·min^{-1}·g wet wt^{-1}	0.184±0.035	0.135±0.018
Glucose oxidation as % MV̇O$_2$	36.7±4.6	26.7±2.4
Rate of FFA tracer uptake, μmol·min^{-1}·g wet wt^{-1}	0.056±0.014	0.059±0.009
Rate of FFA oxidation, μmol·min^{-1}·g wet wt^{-1}	0.040±0.007	0.045±0.007
FFA Oxidation as % MV̇O$_2$	36.1±6.9	47.5±5.9
Rate of lactate tracer uptake, μmol·min^{-1}·g wet wt^{-1}	0.82±0.18	1.05±0.21
Rate of lactate release, μmol·min^{-1}·g wet wt^{-1}	0.077±0.032	0.145±0.059

Values are means ± SE. FFA, free fatty acid.

etry (GC-MS) (6, 28) by using [^{2}H$_6$]succinate, [^{2}H$_4$]fumarate, and [2,2,4,4-^{2}H$_4$]citrate as internal standards.

Statistical analysis. The following variables were analyzed using a *t*-statistic for two means: *1*) baseline hemodynamic and metabolic data in normal and HF animals, and *2*) tissue concentrations and enzyme activities in normal and HF animals. All significance tests were performed at the 0.05 level. All mean values are reported means ± SE.

RESULTS

Hemodynamic and angiographic findings. The induction of microembolization-induced HF resulted in a mean LV ejection fraction of 28 ± 1%, which was accompanied by significant decreases in heart rate and peak LVP compared with normal dogs (Table 1). The dogs in HF were also characterized by significant decreases in maximum and minimum dP/d*t*, rate pressure product, and LV power index (Fig. 1). The decrease in LV function with HF was not accompanied by any difference in myocardial blood flow, subendocardial blood flow, or myocardial O$_2$ consumption (MV̇O$_2$) between the two groups of dogs (Table 1). An index of mechanical efficiency was calculated as the ratio of LV power to MV̇O$_2$, and no significant difference was found between the normal and HF dogs (704 ± 85 vs. 715 ± 100 mmHg·mm·μmol^{-1}·g^{-1}).
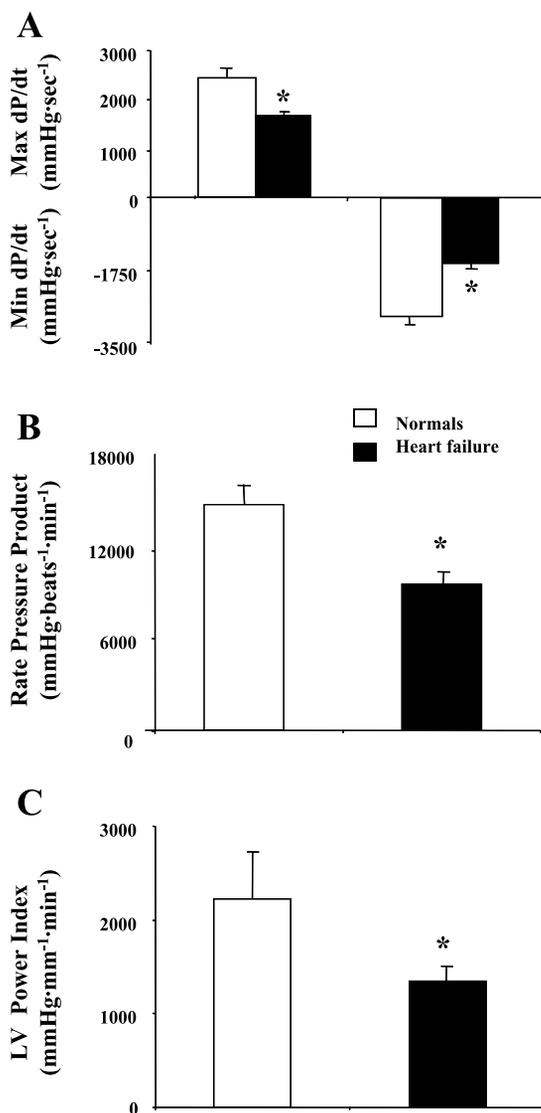

Fig. 1. Hemodynamic parameters in normal (*n* = 8) and heart failure dogs (*n* = 18). There were significant differences between groups as assessed by maximum and minimum first derivative of left ventricular (LV) pressure with time (*A,* max and min dP/d*t*), rate pressure product (*B,* mmHg·beats^{-1}·min^{-1}), and power index (*C,* mmHg·mm^{-1}·min^{-1}) calculated as the percent fractional shortening times the rate pressure product. Values are means ± SE. *$P < 0.05$.
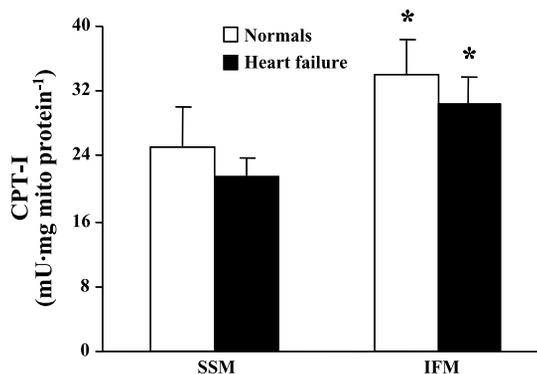

Fig. 2. Carnitine palmitoyl transferase I (CPT-I) specific activity of cardiac subsarcolemmal (SSM) and interfibrillar mitochondria (IFM) isolated from normal and microembolization-induced heart failure dogs. Values are means ± SE of nine separate mitochondrial preparations in each group. *$P < 0.05$ SSM vs. IFM in both groups of animals.
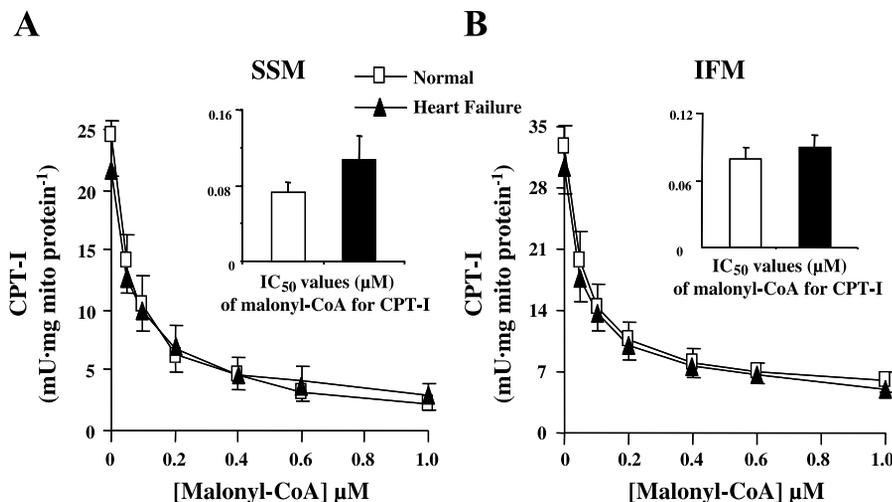
A

B

Fig. 3. Determination of IC_{50} values of malonyl-CoA for CPT-I on SSM (*A*) and IFM (*B*) isolated from normal and heart failure dogs. Inset in each panel represents the mean IC_{50} values (in μM) of malonyl-CoA for CPT-I in SSM and IFM mitochondria in normal and heart failure dogs. Values are means $\pm$ SE of nine separate mitochondrial preparations in each group.

Metabolic findings. The arterial concentrations of glucose, lactate, and FFA were not different between normal and HF dogs. Table 2 presents the rates of FFA, glucose and lactate uptakes, rates of exogenous FFA and glucose oxidation, and lactate release. There were no significant differences in the rates of myocardial uptake or oxidation for FFA, glucose, or lactate between normal dogs and dogs with HF. The contribution of both glucose and FFA oxidation as a percentage of $M\dot{V}O_2$ were also not different in HF dogs compared with normal dogs (Table 2). Thus no differences were observed in myocardial substrate metabolism between the two groups.

Total PDH activity (1.62 ± 0.07 vs. 1.63 ± 0.14 μmol$\cdot$min$^{-1}\cdot$g wet wt^{-1}), active PDH (0.86 ± 0.09 vs. 0.73 ± 0.09 μmol$\cdot$min$^{-1}\cdot$g wet wt^{-1}), and the percentage of total PDH activity that was in the active form (55 ± 7 vs. $45 \pm 4\%$) were not different between the normal and HF dogs.

The activity of CPT-I, the key enzyme-regulating fatty acid transport into the mitochondria, was not different between the normal and HF dogs in either the subsarcolemmal or the interfibrillar mitochondria; however, CPT-I activity in both groups was different between the two subpopulations of mitochondria (Fig. 2). Interfibrillar mitochondria had higher CPT-I activity for both normal and HF dogs compared with subsarcolemmal mitochondria. Furthermore, the IC_{50} values of malonyl-CoA for CPT-I were not different either between the subpopulations of mitochondria or between normal and HF dogs (Fig. 3).

Although no differences in activity and malonyl-CoA sensitivity of CPT-I between the normal dogs and dogs with HF were found, tissue malonyl-CoA levels were 22% lower in the HF dogs compared with the normal dogs (Table 3). These results suggest there would be less in vivo inhibition of CPT-I activity in the HF dogs due to the lower malonyl-CoA concentrations. The lower malonyl-CoA levels cannot be explained by changes in the Michaelis-Menten constant (K_m, 0.051 ± 0.003 vs. 0.075 ± 0.026 μM) or maximal velocity (V_{max}, 0.65 ± 0.09 vs. 0.73 ± 0.15 U/g wet wt) for malonyl-CoA decarboxylase because neither were affected by HF. Myocardial tissue levels of ATP, lactate, and glycogen were not different between the two groups (Table 3).

The tissue contents of citrate, succinate, and fumarate were not different between normal and HF dogs (Table 4), suggesting that the citric acid cycle is not depleted of intermediates in moderate severity HF.

DISCUSSION

Previous studies in patients (7, 39) and animal models (16, 21, 26, 33) found that in advanced HF, there is a switch in myocardial metabolic phenotype away from fatty acid toward glucose oxidation. On the other hand, indirect measurements of substrate oxidation in patients with moderate severity HF showed increased FAO and impaired glucose oxidation (30). Our results demonstrate that in dogs with moderate severity coronary microembolization-induced HF there are normal rates of myocardial FFA and glucose oxidation compared with normal healthy animals. We also observed a decrease in malonyl-CoA content with HF, suggesting less in vivo inhibition of CPT-I activity, without, however, an increase in FAO. These results support our hypothesis that moderate severity HF is accompanied by normal rates of FFA and glucose oxidation compared with healthy animals.

Our results contrast sharply with those reported in severe end-stage HF, where human and animal studies have reported

Table 3. *Tissue concentrations for malonyl CoA, ATP, lactate, and glycogen in normal dogs and dogs with microembolization-induced heart failure*

	Normal	Heart Failure
Malonyl CoA, nmol/g wet wt	1.05 ± 0.06	$0.81 \pm 0.05*$
Tissue ATP, μmol/g wet wt	8.6 ± 0.8	7.2 ± 0.6
Tissue lactate, μmol/g wet wt	3.7 ± 0.4	4.8 ± 1.3
Tissue glycogen, μmol/g wet wt	39.9 ± 3.3	35.1 ± 2.6

Values are means $\pm$ SE. *$P < 0.05$ heart failure vs. normals.

Table 4. *Tissue concentrations of citric acid cycle intermediates in normal dogs and dogs with microembolization-induced heart failure*

	Normal	Heart Failure
Citrate, μmol/g wet wt	0.211 ± 0.014	0.236 ± 0.018
Succinate, μmol/g wet wt	0.236 ± 0.023	0.233 ± 0.036
Fumarate, μmol/g wet wt	0.053 ± 0.004	0.047 ± 0.009

Values are means $\pm$ SE.

a metabolic switch to a more fetal phenotype characterized by a downregulation of FAO and enhancement of glucose oxidation (21, 26, 32, 39). Similarly, numerous studies in a variety of mammalian species have also observed an early shift from greater FAO to an increased reliance on glucose in pressure overload-induced hypertrophy (5, 22, 46). This change is mirrored by changes in the expression of genes encoding metabolic enzymes in the heart, including citrate synthase, CPT-I, and MCAD (32, 39). Whether the switch in myocardial substrate oxidation away from fatty acids toward glucose oxidation in advanced HF is a positive compensatory adaptation or a pathological maladaptation is a matter of considerable debate, but it has been proposed that this switch may ameliorate many of the hemodynamic and biochemical alterations associated with HF, and thereby alter the progression of the disease. Although there is a significant 50% increase in cardiomyocyte cross-sectional area in our canine microembolization model (37) indicative of a pathological hypertrophic response, myocardial substrate metabolism was unchanged. This implies that the basic pattern of substrate utilization may function on a continuum; i.e., the compensatory switch seen to occur in end-stage HF is an attempt to "rescue" the myocardium only when function has become severely compromised. The present results suggest that conditions that trigger the switch in myocardial energy metabolism have not yet occurred at this relatively early stage in this model. To fully address this issue, future studies need to assess myocardial metabolic phenotype over the progression from moderate to advanced end-stage HF in this model.

Data from HF patients and animal models suggest that high rates of FAO and impaired carbohydrate oxidation contributes to the mechanical dysfunction in HF. Impaired carbohydrate oxidation may contribute to mechanical dysfunction in the failing heart, as suggested by improved contractile function and efficiency in HF patients when carbohydrate oxidation is acutely stimulated with dichloroacetate (2), or intracoronary pyruvate (14). We observed that in dogs with moderate severity microembolization-induced HF, acute treatment with ranolazine, a direct inhibitor of fatty acid β-oxidation, significantly increased stroke volume, peak positive and negative dP/dt, LV ejection fraction, and cardiac output without effecting $M\dot{V}O_2$ and thus resulted in a greater LV mechanical efficiency in dogs with HF (4, 35). Moreover, these effects were not observed in normal dogs treated with ranolazine, suggesting that FAO contributes to contractile dysfunction in moderate severity HF (4, 35). Thus the maintained reliance on FAO reported during moderate severity HF may contribute to contractile dysfunction; however, the precise mechanism(s) by which FAO might impair function is not clear.

We observed a 22% reduction in total tissue malonyl-CoA content in the dogs with HF, but there was no significant reduction in flux of fatty acids through CPT-I and fatty acid oxidation. CPT-I resides in the outer mitochondrial membrane and catalyzes the formation of long-chain acylcarnitine from long-chain acyl-CoA in the compartment between the inner and outer mitochondrial membranes (17). Malonyl-CoA binds CPT-I on the cytosolic side of the enzyme (11, 19, 24) and has an IC_{50} on CPT-I activity in the heart of ~30 nM (19, 20). The concentration of malonyl-CoA was ~1 μM, which is well above the IC_{50} for inhibition of CPT-I, thus suggesting that the malonyl-CoA concentration at the site of inhibition on CPT-I is

much lower that whole tissue measurements. The concentrations of malonyl-CoA in the mitochondria and cytosol are not known. Clearly, extensive work is needed before the role of malonyl-CoA in the regulation of FAO in HF can be understood.

Myocardial ischemia of moderate severity dramatically alters fuel metabolism, decreasing the rate of oxygen consumption and ATP production, resulting in high rates of glycolysis and a switch to net lactate production (44). The present results demonstrate normal transmural and subendocardial myocardial blood flow (Table 1) and no evidence for accelerated glycolysis or lactate production (Table 2) in the dogs with HF. Thus we found no evidence for gross myocardial ischemia during this stage of the model. This is in contrast to the results of Andersson et al. (1) who reported low levels of lactate uptake ($n = 14$) or net lactate production ($n = 4$) in a group of patients with ischemic or dilated cardiomyopathies at rest. Thus our results would suggest that the microembolization HF model is in fact not characterized by marked ischemia, as evidenced by the absence of any increased lactate production at rest.

In summary, we have demonstrated normal rates of myocardial fatty acid and glucose oxidation in dogs with moderate severity microembolization-induced HF when compared with normal healthy animals. Our results support the concept that there is no decrease in FAO during compensated HF, and that the downregulation of FAO enzymes and switch to carbohydrate oxidation observed in decompensated end-stage HF is only an end-stage phenomemon.

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