High-fat diet postinfarction enhances mitochondrial function and does not exacerbate left ventricular dysfunction

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Rennison JH, McElfresh TA, Okere IC, Vazquez EJ, Patel HV, Foster AB, Patel KK, Chen Q, Hoit BD, Tserng KY, Hassan MO, Hoppel CL, Chandler MP. High-fat diet postinfarction enhances mitochondrial function and does not exacerbate left ventricular dysfunction. Am J Physiol Heart Circ Physiol 292: H1498–H1506, 2007. First published November 17, 2006; doi:10.1152/ajpheart.01021.2006.—Lipid accumulation in nonadipose tissue due to enhanced circulating fatty acids may play a role in the pathophysiology of heart failure, obesity, and diabetes. Accumulation of myocardial lipids and related intermediates, e.g., ceramide, is associated with decreased contractile function, mitochondrial oxidative phosphorylation, and electron transport chain (ETC) complex activities. We tested the hypothesis that the progression of heart failure would be exacerbated by elevated myocardial lipids and an associated ceramide-inhibited induction of mitochondrial oxidative phosphorylation and ETC complex activities. Heart failure (HF) was induced by coronary artery ligation. Rats were then randomly assigned to either a normal (10% kcal from fat; HF, n = 8) or high saturated fat diet (60% kcal from saturated fat; HF+Sat, n = 7). Sham-operated animals (sham; n = 8) were fed a normal diet. Eight weeks postligation, left ventricular (LV) function was assessed by echocardiography and catheterization. Subsarcolemmal and interfilibrillar mitochondria were isolated from the LV. Heart failure resulted in impaired LV contractile function [decreased percent fractional shortening and peak rate of LV pressure rise and fall (±dp/dt)] and remodeling (increased end-diastolic and end-systolic dimensions) in HF compared with sham. No further progression of LV dysfunction was evident in HF + Sat. Mitochondrial state 3 respiration was increased in HF + Sat compared with HF despite elevated myocardial ceramide. Activities of ETC complexes II and IV were elevated in HF + Sat compared with HF and sham. High saturated fat feeding following coronary artery ligation was associated with increased oxidative phosphorylation and ETC complex activities and did not adversely affect LV contractile function or remodeling, despite elevations in myocardial ceramide.

oxidative phosphorylation; electron transport chain; ceramide; lipotoxicity

FATTY ACIDS (FA) are the dominant energy source for the adult mammalian heart and also are utilized for membrane biosynthesis, generation of lipid signaling molecules, posttranslational protein modification, and transcriptional regulation (43). Chronic exposure to FA can result in an imbalance between FA uptake and utilization that potentially can trigger cytotoxic mechanisms, leading to cell dysfunction or death, a phenomenon known as lipotoxicity. Extensive clinical and animal studies have shown that excess lipid accumulation in nonadipose tissue due to enhanced circulating FA may play an important role in pathophysiological conditions such as heart failure, obesity, insulin resistance, and diabetes (15, 43, 58).

A loss of synchronization between FA availability and utilization in cardiomyocytes, despite otherwise normal or upregulated β-oxidation capacity, can lead to an increase in the accumulation of tissue ceramide (24). Ceramide, a lipid signaling molecule, has been implicated in the formation of reactive oxygen species and peroxidation of membrane lipids (11), as well as apoptosis (15). The accumulation of ceramide from either the hydrolysis of membrane sphingomyelin or from de novo synthesis from palmitoyl-CoA and serine (54) has been implicated in the development of a “lipotoxic” cardiomyopathy. Severe myocardial contractile dysfunction has been observed in mice with increased cardiac FA import due to cardiac-specific overexpression of acyl-CoA synthetase, an enzyme that catalyzes esterification of long-chain FA resulting in lipid accumulation (6). In this model, lipid accumulation was associated with cardiac hypertrophy, followed by the development of left ventricular (LV) dysfunction, increased ceramide content, and premature death. Studies in Zucker diabetic fatty rats found that increased myocardial triglyceride (TG) stores were accompanied by marked elevations in tissue ceramide and a decrease in contractile function. These effects were prevented by a reduction in cardiac TG overaccumulation (58). Similarly, mice overexpressing peroxisome proliferator-activated receptor-α (PPAR-α) develop a severe cardiomyopathy characterized by marked lipid accumulation, enhanced ceramide production, and a decrease in fractional shortening, which is exacerbated when mice are fed a high-fat diet. Interestingly, the lipotoxic cardiomyopathy was reversed by discontinuing the high-fat diet (10). A potential mechanism by which ceramide could lead to myocardial dysfunction was proposed following studies in isolated rat heart mitochondria, in which a rapid decline in mitochondrial oxidative phosphorylation and inhibition of ubiquinol-ferricytochrome c oxidoreductase (complex III) occurred in the presence of ceramide (13). Direct inhibition by ceramide of mitochondrial electron transport chain (ETC) complex III activity, and the associated reductions in oxidative phosphorylation, could provide a novel mechanism of modulating mitochondrial function and thereby alter myocardial contractile function.

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Enhanced myocardial lipid accumulation appears to be associated with impaired myocardial contractile function. However, the effect of enhanced lipid accumulation and increased ceramide production on mitochondrial function and their concomitant effects on ventricular function under pathophysiological conditions such as heart failure have not been systematically evaluated. This study was designed to test the hypothesis that the progression of heart failure would be exacerbated by elevated myocardial lipids and an associated ceramide-induced inhibition of mitochondrial oxidative phosphorylation (specifically through inhibition of complex III activity). We tested our hypothesis in rats fed a high saturated fat diet following coronary artery ligation surgery to induce heart failure.

**METHODS**

**Study design and induction of myocardial infarction.** This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and was approved by the Institutional Animal Care and Use Committee at Case Western Reserve University. Animals were maintained on a reverse 12-h:12-h light-dark cycle (i.e., lights off at 6:00 AM), and all procedures and tissue harvests were performed in the fasted state between 3 and 6 h into the dark phase of the cycle.

In the initial design of this study, rats were fed normal chow (10% calories from fat, 20% calories from protein, and 70% calories from carbohydrate) or a high saturated fat diet [60% calories from fat (25% palmitic, 33% stearic, and 33% oleic acid), 20% calories from protein, and 20% calories from carbohydrate; Research Diets] for 2 wk. For the induction of heart failure, rats were anesthetized with isoflurane before coronary artery ligation and 8 wk following coronary artery ligation surgery to induce heart failure.

**Cardiac subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria.** Following LV cannulation, blood samples were drawn from the inferior vena cava. LV, right ventricle, and scar mass were obtained by gravimetric measurements. A myocardial tissue sample (~100 mg) was harvested and immediately quick frozen. The scar and the balance of the LV were placed in KME (100 mM KCl, 50 mM MOPS, internal salt, and 0.5 mM EGTA). Cardiac subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria were isolated using the procedure of Palmer et al. (38) except that a modified Chappell-Perry buffer (containing 100 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM MgSO4·7H2O, and 1 mM ATP, pH 7.4 at 4°C) was used for isolation of both mitochondrial populations. The IFM were harvested following treatment of skinned fibers with 5 mg/g wet weight tropsin for 10 min at 4°C (35). Mitochondrial protein concentration was determined by the Lowry method using bovine serum albumin as a standard.

**Mitochondrial oxidative phosphorylation.** Oxygen consumption in SSM and IFM was measured using a Clark-type oxygen electrode at 30°C (50). Mitochondria were incubated in a solution consisting of 80 mM KCl, 50 mM MOPS, 1 mM EGTA, 5 mM KH2PO4, and 1 mg/ml bovine serum albumin at pH 7.4. The rate of oxidative phosphorylation and uncoupled respiration was measured using several substrates. Glutamate assesses complexes I, III, and IV. Durohydroquinone (DHQ), an analog of coenzyme Q, assesses complexes III and IV. N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD), an electron carrier that reduces complex IV, was used in conjunction with ascorbate to assess complex IV. Mitochondrial respiration also was measured using FA substrates carnitine-dependent palmitoyl-CoA plus malate and palmitoyl carnitine plus malate (23). Palmitoyl-CoA is converted to palmitoyl carnitine in the mitochondrial intermembrane space by carnitine palmitoyltransferase I (CPT-I) and can then be transported into the mitochondrial matrix via the carnitine-acylcarnitine transporter. Palmitoyl carnitine is able to bypass CPT-I and enter the matrix directly. These two substrates assess whether an effect on FA metabolism is due to CPT-I activity or the oxidation of the FA. State 3 (ADP stimulated) respiration, state 4 (ADP limited) respiration, respiratory control ratio (RCR) (state 3/state 4), and ADP/O ratio (ratio of oxygen consumed following addition of known amount of ADP) were determined as previously described (3,9).

**Mitochondrial ETC complex activity.** Samples of SSM and IFM (10 mg cholate/1 mg mitochondrial protein) were mixed in 1 ml buffer [75 mM mannitol, 220 mM sucrose, 2 mM EDTA, and 5 mM MOPS (pH 7.4)] with mammalian protease inhibitor cocktail (1 µl/1 ml buffer) and were kept on ice. Assays were completed on the day of preparation.

All ETC complex activities were measured as specific donor-acceptor oxidoreductase activities using a diode array spectrophotometer (17, 25, 26). Donors and acceptors were chosen to span specific regions of the complete ETC. NADH Q reductase (complex I) was measured as the rotenone-sensitive reductase and assesses complex I. NADH ferricyanide reductase is a measure of the proximal portion of complex I. Ubiniquinol-cytochrome c oxidoreductase was measured as the antimycin-sensitive decyubiquinol-cytochrome c oxidoreductase to assess complex III. NADH-cytochrome c oxidoreductase was measured as the rotenone-sensitive reductase, assessing complexes I and III. Succinate-cytochrome c oxidoreductase was measured as the antimycin-sensitive reductase to assess complexes II and III. Succinate dehydrogenase measures the proximal portion of complex II. Succinate dehydrogenase + Q measures complexes II and coenzyme Q. Cytochrome-c oxidase assay measures complex IV activity and is expressed as the first-order rate constant (55). Citrate synthase, a mitochondrial marker enzyme, was measured as previously described (47). Aconitase, a mitochondrial matrix enzyme used as a marker of oxidative stress, was measured as the rate of isomerization of citrate from the substrate isocitrate.

**Detection of H2O2 production.** The rate of H2O2 production in mitochondria was determined using the oxidation of the fluorescent indicator, Amplex Red, in the presence of horseradish peroxidase (34). The concentrations of horseradish peroxidase and Amplex Red in the incubation mixture were 0.1 U/ml and 50 µM, respectively.
Fluorescence was recorded in a microplate reader (1420 Victor2, PerkinElmer Life Sciences) with 530-nm excitation and 590-nm emission wavelengths. Standard curves obtained by adding known amounts of H2O2 to assay medium in the presence of the reactants (Amplex Red and horseradish peroxidase) were linear up to 2 μM. Background fluorescence was measured in the absence of mitochondria and presented as fluorescence minus background (in pmol⋅mg protein⁻¹⋅30 min⁻¹). Mitochondria were incubated at 0.1 mg of protein/ml at 30°C. H2O2 production was initiated using succinate (5 mM). Rotenone (2.4 μM) was used to inhibit the reverse flow of electrons to complex I. Maximal radical production at complex III was induced by inhibition of the Qi (the inner binding site for coenzyme Q). Reduced H2O2 generation. Catalase (643 U/ml) was used to dissipate H2O2 in the incubation system.

**RESULTS**

**Coronary artery ligation mortality rates.** Surgical mortality for the sham animals was 0% (0/8). Administration of high saturated fat diet for 2 wk before coronary artery ligation resulted in an increased surgical mortality rate in saturated-fat-fed (30/45, 67%) compared with normal chow-fed rats (9/24, 38%) ($P = 0.0007$ by χ²-analysis). Given the significantly higher mortality rate in the animals fed saturated fat diet before coronary artery ligation compared with those fed normal diet, the group fed a high saturated fat diet before coronary artery ligation was subsequently removed from the study due to a potential “survivor bias” in this group of animals.

**Body and heart mass.** High saturated fat feeding initiated immediately following coronary artery ligation increased body mass compared with sham and HF (Table 1). Total LV mass (LV mass + scar tissue mass) was increased in HF + Sat compared with sham; however, when normalized to body weight (total LV mass/body mass), there were no differences between groups. Mean scar tissue mass did not differ between the two ligated groups (HF and HF + Sat).

**Cardiac function and LV remodeling.** LV function before coronary artery ligation did not differ between groups (data not shown). Heart failure induced by coronary artery ligation resulted in impaired LV contractile function 8 wk postligation as assessed by decreased peak LV ±dP/dt and increased myocardial performance index in HF compared with sham but not in the HF + Sat group (Fig. 1). Percent fractional shortening was decreased in HF and HF + Sat compared with sham. LV remodeling also was evident from increased end-diastolic and end-systolic dimensions in HF compared with sham (Fig. 1). Peak LV systolic pressure and heart rate were unchanged following coronary artery ligation (data not shown). High saturated fat feeding immediately following coronary artery ligation (HF + Sat) caused no further decrease in LV contractile function or progression of LV remodeling compared with HF.

**Metabolic substrates: free fatty acids, TG, ceramide, leptin, and insulin.** Plasma free fatty acids were not altered by heart failure or high-fat feeding (Table 2). Plasma TG (Table 2) and myocardial tissue TG (Fig. 2) were increased in HF + Sat compared with sham and HF. Similarly, myocardial tissue C16-ceramide content was elevated in HF + Sat compared with sham and HF (Fig. 2). C16-ceramide content also was measured in the SSM and IFM; however, in 33% of SSM samples and 84% of IFM samples, the ceramide content was below the lower limit of detection by gas chromatography. Plasma leptin was elevated in HF + Sat compared with sham and HF (Table 2). Insulin levels in the plasma were not different (Table 2).

**Electron microscopy.** Neither heart failure nor high saturated fat feeding had an effect on the morphology of the SSM or IFM, as noted by electron microscopy (Fig. 3).

**Oxidative phosphorylation.** Protein yield in the SSM and IFM was not altered by heart failure or high-fat feeding (Table 3). Mitochondrial recovery during the isolation protocol also did not differ between groups. State 3 respiration was not altered by heart failure using glutamate, DHQ, or TMPD-ascorbate as respiratory substrates. However, with the use of glutamate and DHQ, it was elevated in both mitochondrial populations of HF + Sat (Table 3).

State 4 respiration was not altered by heart failure (Table 3). It was not different using glutamate; however, with the use of DHQ, it was elevated in both populations of HF + Sat. RCR was not altered by heart failure or high-fat feeding in the SSM (sham: glutamate, 5.26 ± 0.42; DHQ, 3.81 ± 0.64) or IFM (sham: glutamate, 7.79 ± 1.51; DHQ, 3.99 ± 0.37). ADP/O also did not differ with heart failure or high-fat feeding in the SSM (sham: glutamate, 2.35 ± 0.09; DHQ, 1.47 ± 0.11) or IFM (sham: glutamate, 2.44 ± 0.07; DHQ, 1.54 ± 0.13). Mitochondrial respiration was measured using FA substrates carnitine-dependent palmitoyl-CoA + palmitoyl-carnitine + malate to assess their ability to oxidize FA. State 3 respiration was not altered by heart failure using either FA substrate (Table 3). However, it was elevated in the SSM and IFM of HF + Sat using both FA substrates.

Heart failure did not alter state 4 respiration using either FA substrate (Table 3). It was elevated in the SSM of HF + Sat.

**Table 1. Body and heart mass in sham, HF, and HF + Sat 8 wk following coronary artery ligation surgery**

<table>
<thead>
<tr>
<th>Body mass, g</th>
<th>Sham</th>
<th>HF</th>
<th>HF + Sat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>458 ± 9</td>
<td>466 ± 11</td>
<td>540 ± 15†</td>
</tr>
<tr>
<td>Total LV mass, mg</td>
<td>929 ± 32</td>
<td>956 ± 41</td>
<td>1,067 ± 41*</td>
</tr>
<tr>
<td>Total LV mass/body mass, mg/g</td>
<td>2.03 ± 0.06</td>
<td>2.05 ± 0.06</td>
<td>1.98 ± 0.07</td>
</tr>
<tr>
<td>Scar, mg</td>
<td>104 ± 16</td>
<td>133 ± 44</td>
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</tbody>
</table>

Values are means ± SE. Sham, sham-operated animals (n = 8); HF, heart failure group fed a normal diet (n = 8); HF + Sat, heart failure group fed a high saturated fat diet (n = 7); LV, left ventricular. *P < 0.05 vs. sham; †P < 0.05 vs. HF.

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using carnitine-dependent palmitoyl-CoA + malate and in both populations of HF + Sat using palmitoylcarnitine + malate. RCR was not altered by heart failure or high-fat feeding in the SSM (sham: palmitoyl-CoA + malate, 3.26 ± 0.27; palmitoylcarnitine + malate, 3.26 ± 0.29) or IFM (sham: palmitoyl-CoA + malate, 4.13 ± 0.45; palmitoylcarnitine + malate, 3.83 ± 0.44). ADP/O also did not differ with heart failure or high-fat feeding in the SSM (sham: palmitoyl-CoA + malate, 2.37 ± 0.16; palmitoylcarnitine + malate, 2.24 ± 0.20) or IFM (sham: palmitoyl-CoA + malate, 2.54 ± 0.13; palmitoylcarnitine + malate, 2.31 ± 0.12).

Mitochondrial ETC and enzyme activity. Complex III activity was not inhibited in either HF or HF Sat compared with sham (Table 4). Activities of complex II in the SSM and complex IV in the SSM and IFM were elevated in HF + Sat compared with sham and HF. Complex I + III, complex I, NADH-dehydrogenase, complex II + III, complex II + Q, and succinate dehydrogenase activities in the SSM and IFM were unchanged in both HF and HF + Sat when compared with sham.

Citrate synthase activity was elevated in the SSM in HF Sat compared with HF (Table 4). The increased activity was not due to an increase in mitochondrial protein content and did not result in greater increases in state 3 respiration (relative to the IFM) but may simply reflect differential responses of the SSM and IFM in diseased states as previously reported (30).

Aconitase was elevated in HF Sat compared with sham and HF in the SSM and in HF + Sat when compared with HF in the IFM (Table 4). A loss or decrease in mitochondrial aconitase activity is an intracellular indicator of oxidative damage. Thus the increased aconitase activity in our SSM and IFM indicates an increased antioxidant capacity that is consistent with the lack of increase in H₂O₂ production (see below).

H₂O₂ production. H₂O₂ production in the presence of rotenone did not change in the SSM (673 ± 98, 677 ± 118, and 817 ± 76 pmol·mg protein⁻¹·30 min⁻¹) or IFM (658 ± 102, 719 ± 190, and 735 ± 55) of sham, HF and HF + Sat respectively. Maximal H₂O₂ production in the presence of antimycin A was unaltered by heart failure or high saturated fat

Table 2. Plasma free fatty acids, triglycerides, leptin, and insulin in sham, HF, and HF + Sat 8 wk following coronary artery ligation surgery

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>HF</th>
<th>HF + Sat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids, μmol/ml</td>
<td>0.39±0.09</td>
<td>0.32±0.04</td>
<td>0.55±0.12</td>
</tr>
<tr>
<td>Triglycerides, mg/ml</td>
<td>0.15±0.03</td>
<td>0.18±0.05</td>
<td>0.37±0.08†</td>
</tr>
<tr>
<td>Leptin, pg/ml</td>
<td>518±140</td>
<td>656±183</td>
<td>1,527±243*†</td>
</tr>
<tr>
<td>Insulin, pmol/ml</td>
<td>94.1±24.7</td>
<td>139±56</td>
<td>200±55</td>
</tr>
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</table>

Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. HF.
feeding. Inhibition of maximal H$_2$O$_2$ production by stigmatellin was similar in all groups.

**DISCUSSION**

The principal finding of this study is that administration of a high saturated fat diet in a model of coronary artery ligation-induced heart failure increased mitochondrial oxidative phosphorylation and ETC complex activities. Additionally, administration of a high saturated fat diet did not adversely affect LV contractile function or the progression of LV remodeling. Despite elevations in myocardial ceramide, there was no evidence of a lipotoxic effect on either contractile or mitochondrial function.

Numerous studies have shown that elevations in myocardial TG and/or ceramide content are associated with cardiac contractile dysfunction and remodeling and that this loss of contractile function can be prevented and/or reversed by the reduction of these toxic lipid intermediates (5, 6, 56, 58). In this study, we hypothesized that the progression of heart failure would be exacerbated by elevated myocardial lipids and an associated ceramide-induced inhibition of mitochondrial respiration and ETC complex activities. Coronary artery ligation resulted in a predictable increase in LV contractile dysfunction and remodeling that is considered to be characteristic of mild to moderate heart failure. However, high saturated fat feeding elevated myocardial ceramide and TG without any further progression of heart failure or evidence of a lipotoxic effect. In point of fact, contractile function (peak ±dP/dt and myocardial performance index; see Fig. 1) in the HF + Sat group was not significantly decreased relative to the sham group. These results are consistent with recently published data from our laboratory in a model of hypertension-induced cardiomyopathy, where administration of a high saturated fat diet reduced LV hypertrophy, improved contractile function, and prevented LV dilation despite elevated plasma FA and myocardial TG (37). Furthermore, Listenberger et al. (32) have suggested that, in response to an acute lipid overload, the ability to synthesize cardiac TG could play a critical role in protection from “lipotoxicity” by diverting excess FA from more cytotoxic pathways. The absence of a lipotoxic effect of ceramide has been demonstrated by Relling et al. (40), where acute ceramide exposure to ventricular myocytes directly enhanced peak cardiomyocyte shortening. However, longer durations of exposure caused a reversal of the positive peak shortening response to ceramide. Therefore, despite previous suggestions of a direct link between lipotoxic intermediates and LV contractile dysfunction, our results clearly demonstrate that high saturated fat feeding following coronary artery ligation is associated with accumulation of myocardial TG and ceramide but does not exacerbate LV dysfunction and dilatation.

Coronary artery ligation-induced heart failure in this study was not associated with decreased oxidative phosphorylation or ETC complex activities in the SSM or IFM. Abnormalities in mitochondrial oxidative phosphorylation have been reported to occur in the rat model of coronary artery ligation-induced heart failure (22, 42), the cardiomyopathic Syrian hamster model (18), the canine microembolization-induced heart failure model (44), and in the myocardium of failed explanted human hearts due to ischemic or idiopathic dilated cardiomyopathy (45). There also is evidence of ETC abnormalities. For example, abnormalities in mitochondrial morphology (31, 41) and in complexes I, III, IV, and V of the ETC have been reported to occur in animal models of heart failure (20, 33) and human patients with heart failure (2, 21). However, defects in mitochondrial function may be dependent on the method by which heart failure is induced. For example, the microembolization model of heart failure results in global ischemia in the heart, whereas the coronary artery ligation model of heart failure used in this study results in scar tissue formation that develops in the infarcted area of the LV. Although the scar was included with the LV in the mitochondrial preparation, it can be assumed that the scar is fibrous tissue and contains no viable mitochondria. Therefore, the isolated mitochondria are from viable LV tissue and should be functioning. Defects in mitochondrial function also may depend on the severity of LV dysfunction. Javadov et al. (22) demonstrated significant reductions in mitochondrial respiration 12 and 18 wk following ligation surgery, but they reported no alterations in mitochondrial function 6 wk following the induction of heart failure. Our study examines mitochondrial function 8 wk postligation surgery and therefore reflects early stages of heart failure. Mitochondrial dysfunction may be evident only when the progression of LV dysfunction and remodeling results in a more severe or decompensated stage of heart failure rather than the more mild to moderate dysfunction evident in our study.

We hypothesized that high saturated fat feeding in a model of coronary artery ligation-induced heart failure would decrease mitochondrial respiration and inhibit ETC complex III. Our results have shown that high saturated fat feeding in this
model of heart failure resulted in no impairments in mitochondrial oxidative phosphorylation or ETC complex activities. Instead, state 3 respiration (using glutamate, DHQ, palmitoylcarnitine + malate, and carnitine dependent palmitoyl-CoA + malate as respiratory substrates) and activities of ETC complexes II and IV were elevated with high saturated fat feeding. One possible explanation for these observations may be a FA-induced activation of PPAR-α (a nuclear transcription factor that activates the expression of genes encoding enzymes involved in FA uptake and metabolism) and its coactivator PPAR-γ coactivator-1α (PGC-1α) (19, 29, 48). PPAR-α and PGC-1α have been reported to be downregulated in hypertrophied and failing hearts and have been shown to stimulate cardiac gene expression of the FA metabolic pathways and regulate oxidative phosphorylation and mitochondrial biogenesis (12, 19, 29). Administration of a high-fat diet provides ligand for the activation of PPAR-α and PGC-1α and subsequently increases the expression of the FA metabolic enzymes involved in FA uptake and metabolism.

Table 3. Protein yield and mitochondrial oxidative phosphorylation rates in SSM and IFM of Sham, HF, and HF + Sat 8 wk following coronary artery ligation surgery

<table>
<thead>
<tr>
<th></th>
<th>SSM</th>
<th>IFM</th>
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<tr>
<td></td>
<td>Sham</td>
<td>HF</td>
</tr>
<tr>
<td>Protein yield, mg/g wet wt</td>
<td>8.7±1.1</td>
<td>10.1±1.3</td>
</tr>
<tr>
<td>State 3 respiration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>187±13</td>
<td>155±11</td>
</tr>
<tr>
<td>DHQ</td>
<td>407±39</td>
<td>368±47</td>
</tr>
<tr>
<td>TMPD-ascorbate</td>
<td>844±85</td>
<td>747±64</td>
</tr>
<tr>
<td>Palmitoyl-CoA + carnitine</td>
<td>141±10</td>
<td>126±10</td>
</tr>
<tr>
<td>Palmitoylcarnitine</td>
<td>173±11</td>
<td>142±11</td>
</tr>
<tr>
<td>State 4 respiration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>36.8±3.0</td>
<td>33.7±4.0</td>
</tr>
<tr>
<td>DHQ</td>
<td>125±18</td>
<td>104±25</td>
</tr>
<tr>
<td>Palmitoyl-CoA + carnitine</td>
<td>44.6±5.6</td>
<td>35.4±3.3</td>
</tr>
<tr>
<td>Palmitoylcarnitine</td>
<td>54.3±5.9†</td>
<td>40.0±1.9</td>
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</table>

Values are means ± SE. Respiratory rates are expressed as nA·min⁻¹·mg⁻¹. SSM and IFM, subsarcolemmal and interfibrillar mitochondria, respectively; DHQ, durohydroquinone; TMPD, N,N,N',N'-tetramethyl-p-phenylenediamine. *P < 0.05 vs. sham; †P < 0.05 vs. HF.
contractile function in PPAR-
mechanism contrasts with other studies that reported impaired enzymes could account for an increased ability of the mito-
uncoupling protein 3, and mitochondrial thioesterase 1) (19, 20).

Table 4. Electron transport chain complex and mitochondrial enzyme activities in SSM and IFM of sham, HF, and HF + Sat 8 wk following coronary artery ligation surgery

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Sham</th>
<th>HF</th>
<th>HF + Sat</th>
</tr>
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<tbody>
<tr>
<td>Complexes I and III</td>
<td>2.897±308</td>
<td>2.474±403</td>
<td>2.439±125</td>
</tr>
<tr>
<td>Complex I</td>
<td>330±44</td>
<td>327±31</td>
<td>319±15</td>
</tr>
<tr>
<td>NADH-dehydrogenase</td>
<td>2.725±202</td>
<td>2.428±165</td>
<td>2.622±221</td>
</tr>
<tr>
<td>Complex III</td>
<td>4,083±664</td>
<td>3,471±612</td>
<td>5,171±964</td>
</tr>
<tr>
<td>Complexes II and III</td>
<td>295±24</td>
<td>225±39</td>
<td>322±59</td>
</tr>
<tr>
<td>Complex II</td>
<td>31.5±9.2</td>
<td>31.5±5.8</td>
<td>57.2±8.5*†</td>
</tr>
<tr>
<td>Complex II + Q</td>
<td>56.7±7.2</td>
<td>61.6±6.9</td>
<td>81.5±9.9</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>212±23</td>
<td>191±21</td>
<td>258±28</td>
</tr>
<tr>
<td>Complex IV (×10^4)</td>
<td>63.1±5.7</td>
<td>54.0±5.6</td>
<td>81.5±4.5*†</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>2,102±155</td>
<td>2,048±132</td>
<td>2,644±194†</td>
</tr>
<tr>
<td>Aconitase</td>
<td>740±79</td>
<td>628±45</td>
<td>1,018±75†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Electron transport chain complexes and enzyme activities are expressed as nmol-min⁻¹-mg mitochondrial protein⁻¹, with the exception of complex IV, which is expressed as the first-order rate constant (k, 1-min⁻¹-mg protein⁻¹). Q, coenzyme Q. *P < 0.05 vs. sham; †P < 0.05 vs. HF; ‡P = 0.058 vs. sham and HF.

An increase in the expression of these FA metabolic enzymes could account for an increased ability of the mitochondria to utilize FA and, consequently, for the increased state 3 respiration rates seen with FA substrates. This proposed mechanism contrasts with other studies that reported impaired contractile function in PPAR-α overexpressing mice (10) and in rats fed a PPAR-α agonist (57). However, we believe that, in the presence of elevated lipids (as occurs with high fat feeding), an induction of the PPAR-α regulated genes would prevent an imbalance between substrate supply and substrate utilization and, as a result, prevent further deterioration in contractile function. Additionally, PGC-1α increases mitochondrial number, upregulates the expression of mitochondrial enzymes, and increases rates of FA oxidation and coupled respiration (19), which also might account for the increased respiration and ETC complex activities reported in this study. Therefore, it will be important in future studies to elucidate the potential role of PPAR-α and PGC-1α in the enhanced mitochondrial function observed in heart failure animals fed a high-fat diet.

Another potential explanation for the absence of lipotoxicity in this investigation is the antilipolytic action of leptin. It has been suggested that the primary physiological role of leptin is to prevent FA accumulation in nonadipose tissues, such as skeletal muscle, liver, pancreas, and heart (52). In mice with severe lipotoxic cardiomyopathy, induced transgenically by cardiomyocyte-specific overexpression of the acyl-CoA synthase, elevations in plasma leptin levels completely prevented the dilated cardiomyopathy, elevations in myocardial TG stores, and cardiomyocyte hypertrophy (27). Similarly, during dietary-induced obesity, hyperleptinemia protected nonadipocytes from steatosis and lipotoxicity (28). It is important to note, however, that the plasma leptin levels reported in these studies far exceed the levels reported in our HF + Sat group. Thus the absence of lipotoxicity in our heart failure group fed a high saturated fat diet may be partially accounted for by elevations in plasma leptin that may be acting in a cardioprotective manner.

To our knowledge, this is the first study to assess the relationship between myocardial tissue ceramide and integrated function in isolated mitochondria in an in vivo model of heart failure. Elevations in myocardial ceramide were not associated with inhibition of mitochondrial respiration and ETC complex activities or the production of H2O2 in isolated mitochondria of heart failure animals fed a high saturated fat diet. Although ceramide is also known to act as an intracellular signal to trigger apoptosis (15) and increase the peroxidation of membrane lipids (11), these effects were not assessed in the current study. One potential explanation that could account for the lack of a lipotoxic effect of ceramide on mitochondrial function is that the magnitude of increase in whole tissue ceramide reported in this study was insufficient to inhibit mitochondrial respiration or ETC complex activities or the production of H2O2 in isolated mitochondria of heart failure animals fed a high saturated fat diet. Although ceramide is also known to act as an intracellular signal to trigger apoptosis (15) and increase the peroxidation of membrane lipids (11), these effects were not assessed in the current study. One potential explanation that could account for the lack of a lipotoxic effect of ceramide on mitochondrial function is that the magnitude of increase in whole tissue ceramide reported in this study was insufficient to inhibit mitochondrial function. C2-ceramide (a membrane-permeable form) has been shown to reduce the activity of respiratory chain complex III in isolated mitochondria with a half-maximum effect at 5–7 μM (13). How whole tissue ceramide content reported here compares with that of a cell-permeable ceramide in isolated mitochon-
dria is unknown. However, the biochemical and biophysical action of an exogenous ceramide may not mimic the behavior of endogenous ceramide. Exogenous C2-ceramide is amphiphilic and can translocate from the plasma membrane to other cellular membranes, whereas endogenous ceramide remains tightly bound to the membrane where it is produced.

The absence of a lipotoxic effect of ceramide could also be explained by its origin. Inhibition of complex III by ceramide could be initiated at either the plasma membrane or the mitochondrial membrane. We have shown that elevated myocardial tissue ceramide (which would reflect ceramide located on the plasma membrane as well as the mitochondrial membranes) did not correspond to alterations in mitochondrial oxidative phosphorylation or ETC activities. However, the ceramide on the plasma membrane is lost during the isolation of mitochondria. Although the mitochondrial pool of ceramide was measured in both the SSM and IFM, the ceramide content was below the level of detection by gas chromatography. It, therefore, is unlikely that the mitochondrial pool of ceramide was sufficient to result in inhibition of complex III. (4, 53). It is important to acknowledge, though, that the absence of a sig-

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including CPT-I, medium chain acyl-CoA dehydrogenase, uncoupling protein 3, and mitochondrial thioesterase 1) (19, 49). An increase in the expression of these FA metabolic enzymes could account for an increased ability of the mitochondria to utilize FA and, consequently, for the increased state 3 respiration rates seen with FA substrates. This proposed mechanism contrasts with other studies that reported impaired contractile function in PPAR-α overexpressing mice (10) and in rats fed a PPAR-α agonist (57). However, we believe that, in the presence of elevated lipids (as occurs with high fat feeding), an induction of the PPAR-α regulated genes would prevent an imbalance between substrate supply and substrate utilization and, as a result, prevent further deterioration in contractile function. Additionally, PGC-1α increases mitochondrial number, upregulates the expression of mitochondrial enzymes, and increases rates of FA oxidation and coupled respiration (19), which also might account for the increased respiration and ETC complex activities reported in this study. Therefore, it will be important in future studies to elucidate the potential role of PPAR-α and PGC-1α in the enhanced mitochondrial function observed in heart failure animals fed a high-fat diet.

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significant lipotoxic effect of ceramide on mitochondrial function 8 wk following the induction of heart failure may simply reflect the impact of elevations in myocardial ceramide during the early stages of disease progression. It remains to be established what effect continued high-fat feeding and increases in ceramide might have at later stages in the progression of heart failure.

The composition of the FA may also play a role in mitochondrial function due to the differential effects of saturated versus unsaturated fats. Previous studies performed in vitro have clearly shown that long-chain saturated FA are involved in lipotoxic pathways leading to cardiomyopathy, whereas mono- and polyunsaturated FA are not (32, 46). In these studies, the long-chain saturated FA palmitate has been shown to induce apoptosis, cytochrome c release, caspase activation, and DNA laddering, all markers of cell death. However, saturated FA also stimulate mitochondrial oxidation in rats, whereas monounsaturated FA had no effect (14). Similarly, unsaturated FA are associated with greater lipoperoxidation (8), mitochondrial uncoupling, and reactive oxygen species production (7) when compared with saturated FA. Thus further studies are required to elucidate the mechanisms responsible for these differential effects of saturated and unsaturated FA, particularly in respect to their effect on mitochondrial respiration and ETC complex activities.

In summary, despite previous suggestions of a direct link between lipotoxic intermediates and LV contractile dysfunction, our results clearly show that high saturated fat feeding following coronary artery ligation did not exacerbate LV dysfunction and remodeling, at the time point examined. Furthermore, administration of a high saturated fat diet in a model of ligation-induced heart failure increased mitochondrial oxidative phosphorylation and ETC complex activities. The absence of a lipotoxic effect coincident with elevations in myocardial ceramide was an unexpected outcome of this study that warrants further investigation. Future studies should also examine whether the effects of high saturated fat feeding on myocardial contractile and mitochondrial function are observed under control conditions and not unique to injury models such as ligature-induced heart failure or at later stages in the progression of heart failure.

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