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Prolonged Exposure to High Dietary Lipids is not Associated with Lipotoxicity in Heart Failure

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Abstract

Previous studies have reported that elevated myocardial lipids in a model of mild-to-moderate heart failure increased mitochondrial function, but did not alter left ventricular function. Whether more prolonged exposure to high dietary lipids would promote a lipotoxic phenotype in mitochondrial and myocardial contractile function has not been determined. We tested the hypothesis that prolonged exposure to high dietary lipids, following coronary artery ligation, would preserve myocardial and mitochondrial function in heart failure. Rats underwent ligation or sham surgery and were fed normal (10% kcal fat) (SHAM, HF) or high fat diet (60% kcal saturated fat) (SHAM+FAT, HF+FAT) for sixteen weeks. Although high dietary fat was accompanied by myocardial tissue triglyceride accumulation (SHAM 1.47±0.14; SHAM+FAT 2.32±0.14; HF 1.34±0.14; HF+FAT 2.21±0.20 μmol/gww), fractional shortening was increased 16% in SHAM+FAT and 28% in HF+FAT compared to SHAM and HF, respectively. Despite increased medium-chain acyl-CoA dehydrogenase (MCAD) activity in interfibrillar mitochondria (IFM) of both SHAM+FAT and HF+FAT, dietary lipids also were associated with decreased state 3 respiration using palmitoylcarnitine (SHAM 369±14; SHAM+FAT 307±23; HF 354±13; HF+FAT 366±18 nAO·min⁻¹·mg⁻¹) in SHAM+FAT compared to SHAM and HF+FAT. State 3 respiration in IFM also was decreased in SHAM+FAT relative to SHAM using succinate and DHQ. In conclusion, high dietary lipids promoted myocardial lipid accumulation, but were not accompanied by alterations in myocardial contractile function typically associated with lipotoxicity. In normal animals, high dietary fat decreased mitochondrial respiration, but also increased MCAD activity. These studies support the concept that high fat feeding can modify multiple cellular pathways that differentially affect mitochondrial function under normal and pathological conditions.

Keywords

lipotoxicity; mitochondria; heart failure; dietary fat; oxidative phosphorylation

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INTRODUCTION

Myocardial function is clearly compromised under pathophysiological conditions including obesity, insulin resistance, diabetes and heart failure. These conditions are characterized by increases in circulating plasma non-esterified fatty acids that can result in enhanced lipid accumulation in non-adipose tissues such as the heart. These metabolic abnormalities may play an important role in the progression of ventricular dysfunction (or disease progression) [1–3]. Increasing dietary fat intake also may impact myocardial structure and function through elevations in circulating fatty acids that exert direct effects on cardiac tissues. Likewise, conditions of high plasma fatty acids and lipid accumulation are associated with alterations in mitochondrial function [2,4–7]. Animal studies have shown that excess myocardial lipid uptake and accumulation is associated with increases in lipotoxic intermediates leading to contractile dysfunction, hypertrophy, and decreased survival [8–10]. However, clinical and epidemiological studies paradoxically have shown that lowering total dietary fat intake results in no significant differences in coronary artery disease rates [11] and that overweight or obese status compared with normal weight or cachexic leads to better survival rates among patients with a variety of cardiac conditions [12–16]. Nevertheless, until recently, the effects of high dietary fat on myocardial and mitochondrial function in the context of heart failure had not been systemically evaluated.

Recent studies have shown clearly that a high fat diet does not adversely affect myocardial contractile or mitochondrial function [17–21]. Eight weeks of high saturated fat feeding in a Wistar rat model of coronary artery ligation-induced heart failure resulted in no impairments in mitochondrial oxidative phosphorylation or electron transport chain (ETC) complex activities [18,19]. On the contrary, administration of a high fat diet in heart failure increased state 3 respiration as well as short- (SCAD), medium- (MCAD), and long-chain (LCAD) acyl-CoA dehydrogenase activities. Interestingly, high fat diet in this model did not alter the mRNA or protein expression of the acyl-CoA dehydrogenases, possibly reflecting a post-translational modification that alters enzyme function. Left ventricular (LV) contractile function and the progression of LV remodeling also were not exacerbated by high fat feeding in heart failure; instead, peak LV +dP/dt was improved [19]. Interestingly, these effects of high fat were not evident in normal animals, suggesting that the high fat effect on mitochondrial and myocardial contractile function may have been limited to mild-to-moderate heart failure/dysfunction [19]. Similar studies in a Dahl Salt-Sensitive rat model of hypertension-induced cardiomyopathy have shown that administration of a high saturated fat diet reduced LV hypertrophy, improved contractile function, and prevented LV dilation [22]. Chess *et al.* [21] also showed that despite greater plasma non-esterified fatty acid and leptin concentrations, high fat feeding did not exacerbate ventricular remodeling or dysfunction in a mouse model of pressure overload-induced cardiac dysfunction. These results would support the intriguing concept that a high fat diet may limit the extent of injury experienced by the heart under conditions of stress.

It remains to be determined however, whether the effects of high fat feeding on mitochondrial and ventricular function eight weeks following the induction of mild-to-moderate heart failure/dysfunction would persist following a more prolonged period of high fat feeding or whether the more deleterious effects associated with lipotoxicity would become evident. Previous work from our laboratory demonstrated a significant increase in LV end-systolic area and a decrease in fractional area of shortening from eight to twenty weeks following ligation surgery, suggesting a progressive deterioration in LV systolic function in heart failure rats [17]. Furthermore, Wilson *et al.* [23] reported that cardiac function was maintained in Wistar rats fed a long-term (32–48 weeks) high fat diet. Therefore, the goal of this study was to examine the impact of prolonged exposure to high dietary lipids on myocardial and mitochondrial function in coronary artery ligation-induced heart failure rats. We hypothesized that myocardial

and mitochondrial function would be preserved in heart failure rats fed high fat. We tested our hypothesis in rats fed a high saturated fat diet for sixteen weeks following coronary artery ligation surgery to induce heart failure. The present study demonstrates that sixteen weeks of high fat feeding in mild-to-moderate heart failure/dysfunction prevents LV hypertrophy, preserves ventricular systolic function, and does not alter mitochondrial function. In normal animals, sixteen weeks of high fat feeding decreases mitochondrial respiration, but does not adversely affect LV function.

METHODS

Study Design and Induction of Myocardial Infarction

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and the Institutional Animal Care and Use Committee at Case Western Reserve University. Animals were maintained on a reverse 12h:12h light:dark cycle, and all procedures/tissue harvests were performed 2–4 hours into the dark cycle.

For the induction of heart failure, male Wistar rats were anesthetized with isoflurane (1.5–2.0%), intubated, and ventilated. The left main coronary artery was ligated as previously described [18,19,24]. Following ligation or sham surgery, rats were randomly assigned to either normal chow (SHAM n=8, HF n=9) (caloric content; 14% fat, 26% protein, 60% carbohydrate) or a high saturated fat chow (SHAM+FAT n=10, HF+FAT n=9), [Caloric content; 60% fat (25% palmitic, 33% stearic and 33% oleic acid), 20% protein, 20% complex carbohydrates] (Research Diets, Inc.) for sixteen weeks.

Echocardiography

Myocardial function was evaluated by echocardiography eight and sixteen weeks post ligation using a Sequoia C256 System (Siemens Medical) with a 15 MHz linear array transducer as previously described [24]. Briefly, rats were anesthetized with 1.5–2.0% isoflurane, the chest shaved, the animal situated in the supine position and ECG limb electrodes were placed. Two-dimensional (2D), 2D-guided M-mode, and Doppler echocardiographic studies of aortic flows were performed via parasternal and foreshortened apical windows. End-diastolic and end-systolic areas were measured using software resident on the ultrasonograph. Measures of cardiac index, ejection fraction, area of fractional shortening, and percent fractional shortening were calculated as previously described [24]. All data were analyzed in an investigator-blinded fashion.

Hemodynamic Measurements

LV pressure and contractile properties were assessed sixteen weeks following ligation surgery. Rats were anesthetized (1.5–2.0% isoflurane), intubated, and ventilated. A microtip pressure transducer catheter (3.5 Fr, Millar Instruments) was introduced via the right carotid artery. Measurements of heart rate, end-diastolic pressure, peak LV +dP/dt, and peak LV –dP/dt were recorded over a 30s period using a Digi-Med® Heart Performance Analyzer- τ^{TM} .

Preparation of Mitochondria

Following LV cannulation, blood samples were drawn from the inferior vena cava. Right ventricle, LV, and scar mass were obtained by gravimetric measurements. A myocardial tissue sample was harvested and quick-frozen. The scar and balance of the LV were placed in a buffer containing 100mM KCl, 50mM 3-[N-Morpholino] propanesulfonic acid (MOPS), internal salt, and 0.5mM EGTA (pH 7.4). Cardiac subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria were isolated according to Palmer *et al.* [25] except that a modified Chappell-

Perry buffer (containing 100mM KCl, 50mM MOPS, 1mM EGTA, 5mM MgSO₄·7H₂O, and 1mM ATP, pH 7.4, 4°C) was used for isolation of mitochondria. IFM were harvested following treatment of skinned fibers with 5mg/gww trypsin for 10 minutes at 4°C [26]. 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 (Strathkelvin) at 30°C [27]. Mitochondria were incubated in a solution consisting of 80mM KCl, 50mM MOPS, 1mM EGTA, 5mM KH₂PO₄, and 1mg/ml defatted bovine serum albumin (pH 7.4). The rate of oxidative phosphorylation and uncoupled respiration was measured using several substrates. Glutamate assesses complexes I, III, and IV. Succinate assesses complexes II, 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 cytochrome c, used in conjunction with ascorbate, assesses complex IV. Mitochondrial respiration was measured using lipid substrates octanoylcarnitine plus malate and palmitoylcarnitine plus malate [28]. State 3 (ADP-stimulated) respiration, state 4 (ADP-limited) respiration, respiratory control ratio (RCR) (state 3/state 4), and ADP/O ratio (ADP added per oxygen consumed) were determined as previously described [29,30].

Enzyme Activities

SCAD, MCAD, and LCAD activities were measured in isolated SSM and IFM as the phenazine ethosulfate stimulated reduction of cytochrome c using 2mM butyryl-CoA (a 4-carbon substrate), 0.2mM octanoyl-CoA (an 8-carbon substrate), or 0.05mM palmitoyl-CoA (a 16-carbon substrate) respectively, as described previously [31]. Citrate synthase was assessed as previously described [32].

Plasma and Tissue Metabolic Products

Non-fasted glucose, non-esterified fatty acids, and triglyceride concentrations in plasma were measured using an enzymatic spectrophotometric kit [33]. Tissue triglyceride content was measured in homogenate extracts using an enzymatic spectrophotometer method (Wako Chemicals). Insulin and leptin concentrations in plasma were assayed by ELISA (ALPCO Diagnostics). Adiponectin concentrations in serum were measured by ELISA (B-Bridge, San Diego CA).

Statistical Analysis

Differences among SHAM, SHAM+FAT, HF, and HF+FAT were determined using a two-way ANOVA followed by Bonferroni t-tests for multiple comparisons. Data are expressed as group means±SEM. Significance was established at $P<0.05$.

RESULTS

Body and Heart Mass

High fat feeding increased body weight in SHAM+FAT and HF+FAT (Table 1). LV weights and biventricular weight-to-tibia length were not different between groups, indicating that sixteen weeks of high fat feeding was not associated with ventricular hypertrophy relative to normal chow fed groups. However, despite significant differences in body weight, there was evidence of cardiac hypertrophy in HF relative to SHAM (i.e. increased biventricular weight-to-body weight) that was prevented by high fat feeding in HF+FAT (Table 1). Mean scar tissue weight did not differ between the two ligated groups (HF 184±27; HF+FAT 152±16 mg).

Cardiac Function and Echocardiographic Measures

The progression of myocardial systolic dysfunction and remodeling was assessed by echocardiography at sixteen weeks following coronary artery ligation. End-diastolic and end-systolic areas were increased and cardiac index and area of fractional shortening were decreased in HF compared to SHAM (Table 1). There was no evidence of ventricular systolic dysfunction or remodeling in SHAM+FAT (Table 1). Furthermore, LV dysfunction was not exacerbated in HF+FAT compared to HF.

Myocardial contractile function also was assessed by LV cannulation sixteen weeks following coronary artery ligation. High fat feeding in SHAM did not alter peak LV +dP/dt and -dP/dt (Figure 1A, B) or end-diastolic pressure (Table 1), but decreased heart rate (Table 1). Coronary artery ligation resulted in impaired LV function in HF compared to SHAM as assessed by increased end-diastolic pressure as well as decreased peak LV +dP/dt and -dP/dt, heart rate, fractional shortening, and ejection fraction (Figure 1). High fat feeding resulted in improved fractional shortening and ejection fraction in HF+FAT compared to HF. Thus, LV contractile function, systolic function, and the progression of LV remodeling were not exacerbated by prolonged exposure to dietary lipids in normal or ligated animals.

Metabolic Substrates and Humoral Factors

Sixteen weeks of high fat feeding was accompanied by elevated plasma non-esterified fatty acids and tissue triglycerides in SHAM+FAT and HF+FAT (Table 2). High fat feeding also was associated with an increase in plasma leptin and serum adiponectin (Table 2). Plasma glucose and insulin were not altered by heart failure or high fat (Table 2).

Mitochondrial Content and Enzyme Activities

Citrate synthase activity, an enzymatic measure of the mitochondrial content of myocardial tissue, was unaltered by high fat, but was decreased in HF (Table 3). Myocardial tissue MCAD activity was not different between groups.

Mitochondrial protein yield was increased in SSM of SHAM+FAT compared to SHAM and HF+FAT (Table 3). The same effect was evident in IFM (Table 3). In fact, total protein yield (SSM+IFM) in SHAM+FAT was 30% greater than SHAM and 75% greater than HF+FAT. At the same time, mitochondrial activities of citrate synthase, SCAD, MCAD, and LCAD (Table 3), measures expressed per milligram of protein, were not decreased, suggesting that the increase in protein yield was not due to contamination by non-mitochondrial protein.

In agreement with our findings at eight weeks [19], MCAD activity in SSM and IFM was unaltered by heart failure but was increased in HF+FAT compared to HF (Table 3). Additionally, though we have previously shown that mitochondrial MCAD activity was unaltered by eight weeks of high fat feeding in SHAM animals [19], MCAD activity in the IFM was elevated in SHAM+FAT compared to SHAM following sixteen weeks of high fat feeding. Neither SCAD nor LCAD was altered by heart failure or high fat in SSM or IFM.

Oxidative Phosphorylation

The effects of sixteen weeks of high fat feeding on mitochondrial function also were assessed. Mitochondrial respiration was measured using lipid substrates, octanoylcarnitine and palmitoylcarnitine, to assess the ability of the mitochondria to oxidize lipids of different chain lengths. In the SSM, state 3 respiration was not altered using either lipid substrate (Figure 2A). Using long-chain lipid substrate palmitoylcarnitine, state 3 respiration in IFM was not altered by HF, but was decreased by 17% in SHAM+FAT relative to SHAM (Figure 2B). Though state 3 respiration was increased by 16% in HF+FAT relative to SHAM+FAT it was not

different from HF. State 3 respiration in IFM using medium-chain length fatty acid octanoylcarnitine was not different.

State 3 respiration also was assessed using ETC complex specific substrates. In SSM, state 3 respiration was unaltered using glutamate, succinate, and DHQ (Figure 2A). Similar to our data using lipid substrates, state 3 respiration using succinate, and DHQ were each decreased by 20% in IFM of SHAM+FAT relative to SHAM (Figure 2B). Though state 3 respiration using succinate and DHQ were each increased by 28% in HF+FAT relative to SHAM+FAT, they were not different from HF. The differential effects observed in SSM and IFM are not surprising as they have previously been shown to be distinct populations that respond differently under pathological conditions [34].

In SSM, state 4 respiration was elevated 15% using succinate and 20% using DHQ in HF+FAT compared to SHAM+FAT (Figure 3A). In IFM, state 4 respiration was elevated by 15% in HF compared to SHAM using succinate only, and was decreased 21% using palmitoylcarnitine in SHAM+FAT compared to SHAM (Figure 3B). Additionally, though state 4 respiration in IFM was increased in HF+FAT compared to SHAM+FAT using octanoylcarnitine (27%), glutamate (13%), succinate (22%), and DHQ (19%), it was not different when compared to HF.

RCR, a measure of the coupling of oxidation to phosphorylation, was not altered in SSM by heart failure or high fat (Table 4). In IFM, RCR was decreased in HF+SAT compared to SHAM +SAT using octanoylcarnitine, and in SHAM+FAT and HF compared to SHAM using succinate.

ADP/O, a measure of mitochondrial efficiency, was decreased using succinate in SSM of HF +FAT compared to SHAM+FAT and HF and using DHQ in HF+FAT compared to SHAM +FAT (Table 5). In IFM, ADP/O using succinate was decreased in HF+FAT compared to SHAM+FAT and HF. ADP/O using DHQ in IFM was increased in HF and SHAM+FAT compared to SHAM (Table 5).

DISCUSSION

The present study showed that sixteen weeks of high fat feeding in mild-to-moderate heart failure/dysfunction improved ventricular systolic function and increased mitochondrial MCAD activity, but did not alter mitochondrial respiration, despite elevations in myocardial triglycerides and the progression of ventricular remodeling (Table 6). In normal animals, sixteen weeks of high fat feeding did not adversely affect LV contractile or systolic function and increased mitochondrial MCAD activity even though mitochondrial respiration was decreased. These findings suggest that high fat feeding impacted multiple cellular processes that differentially altered mitochondrial function in normal and diseased conditions. In heart failure animals fed normal diet, myocardial contractile and systolic dysfunction was not accompanied by evidence of mitochondrial alterations or lipotoxicity, suggesting that in a model of mild-to-moderate heart failure, ventricular systolic dysfunction was not directly associated with alterations in mitochondrial respiration.

Consistent with our previous investigation [19], some effects of high fat feeding in normal rats differed from those in heart failure rats. A unique finding of the current study is that the deleterious effects of high fat were more pronounced in normal animals than under pathological conditions. There was no evidence of mitochondrial dysfunction in SHAM+FAT following eight weeks of high fat feeding [19], but state 3 respiration in IFM was decreased in this group following sixteen weeks of high fat feeding (Table 6). In HF+FAT, eight weeks of high fat feeding increased state 3 respiration in SSM with no change in IFM [19]; however, following sixteen weeks of high fat feeding, state 3 respiration in SSM was no longer elevated (Table 6).

Although lipotoxicity was not evident in the current study, our data suggests that a more prolonged period of high saturated fat feeding could promote further decreases in state 3 respiration and that decreased mitochondrial respiration would be more pronounced in normal animals than in mild-to-moderate heart failure. Our data also suggests that alterations in mitochondrial dysfunction would be evident in the IFM prior to the SSM. Mitochondrial abnormalities that include matrix dilution and cristolysis have been reported in a model of excess lipids such as the Zucker Diabetic Fatty rat [35] and following seven weeks of high fat in normal rats [36]. Several studies also have shown that excess myocardial lipid accumulation in the heart is accompanied by the generation of toxic lipid intermediates and contractile dysfunction [8,9,37]. In contrast, previous studies have shown no deleterious effects on ventricular function following eight weeks [20] and 32–48 weeks [23] of high fat feeding in normal rats, as well as following sixteen weeks of high fat feeding in mice [21]; however, isolated mitochondrial function was not assessed in these studies. Together, these studies indicate that the effects of high fat feeding on mitochondrial and myocardial function have not been fully elucidated. It is important to consider that many studies have assessed cardiac lipotoxicity in genetically-manipulated animal models, whereas the current study assessed the impact of prolonged exposure to dietary lipids in normal animals. Whether the high fat induced alterations in mitochondrial function following greater than sixteen weeks of high fat feeding would be associated with contractile dysfunction is unknown.

It also is interesting to note that following eight weeks of high fat feeding MCAD activity was not altered in SHAM+FAT [19], but was increased in IFM following sixteen weeks of high fat feeding (Table 6). In HF+FAT, MCAD activity was increased in SSM at eight weeks [19], and not only did it remain elevated in SSM, but it also was elevated in IFM at sixteen weeks (Table 6). These data suggest that if the duration of high fat feeding were extended, mitochondrial MCAD activity would remain elevated. The increase in MCAD activity in both normal and heart failure animals fed high fat represents a pathway that is activated by dietary lipids in both models. Therefore, high fat diet induced both beneficial and detrimental effects on mitochondrial function- an increase in MCAD activity and a decrease in mitochondrial respiration. The cellular mechanism that would account for these observations is not entirely clear; however, our data does provide some insight. Following sixteen weeks of high fat feeding, state 3 respiration was decreased in SHAM+FAT using palmitoylcarnitine (a long-chain fatty acid) as a respiratory substrate; however, LCAD activity was unchanged (Table 6). Similarly, state 3 respiration using octanoylcarnitine (a medium-chain fatty acid) was unaltered and MCAD activity was increased in IFM of SHAM+FAT. Together, these data indicate that state 3 respiration was not limited by acyl-CoA dehydrogenase activity and that the decreased state 3 respiration was due to high fat induced mitochondrial alteration(s) downstream of β -oxidation. Whether the detrimental effects of high fat would predominate following a longer duration of high fat feeding is unknown.

Although mitochondrial dysfunction has been described in several animal models of heart failure, the finding that mitochondrial oxidative phosphorylation was not altered in heart failure animals fed normal diet is in agreement with our previous findings at eight weeks [18,19]. Defects in mitochondrial structure and function are dependent on both the model used to induce heart failure and the severity of LV dysfunction. A recent study by Murray *et al.* [38], using the coronary artery ligation-induced model of heart failure, also reported no reductions in mitochondrial state 3 respiration, but decreased ADP/O in failing rats compared to controls. However, the degree of ventricular systolic dysfunction (approximately 50% reduction in ejection fraction in heart failure relative to controls) indicated a more advanced stage in the progression of failure compared to our studies that are characterized as more mild-to-moderate dysfunction [20–35% in heart failure relative to sham (Figure 1D) [18,19]]. In contrast, Rosca *et al.* [39] recently reported marked decreases in state 3 respiration in coronary microembolization-induced heart failure, a canine model of moderate-to-severe heart failure.

Alterations in gene expression also are dependent on both the model and the degree of severity of LV dysfunction. For example, Heather *et al.* [40] showed that MCAD activity was reduced by 33% at six months post coronary artery ligation, a time point at which ejection fraction was reduced by more than 50% relative to sham. In the present study, myocardial MCAD activity was not decreased in HF; however, as the level of LV dysfunction would be considered mild-to-moderate our findings may reflect an earlier stage in disease progression. This concept is supported by a study by Sack *et al.* [41] which showed that LV hypertrophy was associated with decreased mRNA expression of *mcad*, with no change in protein or enzyme activity. In contrast, when LV hypertrophy progressed to heart failure, decreased *mcad* was accompanied by decreased protein and enzyme activity. These data support the existence of a temporal pattern for metabolic changes that are contingent on the stage or progression of myocardial dysfunction/failure. In fact, results from this study and our previous investigations, suggest that alterations in ventricular systolic function were not a direct result of, or necessarily associated with, alterations in mitochondrial function, at least in the context of high fat feeding under conditions of myocardial injury. Our results that support this novel concept include the findings that, 1) there were marked decreases in ventricular function in HF at sixteen weeks despite no alterations in mitochondrial respiration; 2) ventricular function in SHAM+FAT was either unaltered or improved, despite a decrease in mitochondrial respiration; and 3) improvements in ventricular systolic function in HF+FAT were associated with increases in mitochondrial respiration above that of HF and SHAM. These data point to a possible “disconnect” between mitochondrial and ventricular function that may be model dependent. That is to say, that in this model of mild-to-moderate heart failure/dysfunction, alterations in myocardial ventricular function do not directly reflect changes in mitochondrial function. However, as ventricular dysfunction progresses to more severe or decompensated heart failure, mitochondrial alterations would be expected to contribute to, or be associated with, ventricular dysfunction.

The nutritional composition of the high fat diet must also be considered in light of our findings. An inevitable result of producing a diet high in fat is that the carbohydrate content of the diet is decreased (saturated fat diet caloric content; 60% fat, 20% protein, 20% complex carbohydrates; normal diet caloric content; 14% fat, 26% protein, 60% carbohydrate). Recent studies have shown a high fructose [42,43] or sucrose diet [44], but not complex carbohydrate diet, exacerbated LV dysfunction and increased mortality, suggesting that the protective effects of high fat may actually be a result of a diet low in simple sugars. Alternatively, high fat diets also have been shown to promote insulin resistance, a condition that was not apparent in our study following sixteen weeks of high fat feeding, but should be examined more thoroughly in future investigations. The effects of a high carbohydrate (either complex or simple sugars) diet on mitochondrial oxidative phosphorylation and ETC activities have not been examined and should be the target of future investigations. The composition of specific fatty acids in the diet also may play a critical role in determining the impact of a nutritional intervention on myocardial function. A recent study by Chicco *et al.* [45] demonstrated that despite having similar effects on cardiac morphology and function, the composition of the high fat diet differentially affected mortality with high linoleic acid significantly improving survival rates relative to a high fat lard based diet. Similarly, Okere *et al.* [46] showed that saturated and unsaturated fatty acids elicit different effects on PPAR α -mediated gene expression, fat distribution, and cardiomyocyte apoptosis. Additional studies are needed to fully elucidate the effects of dietary composition on ventricular and mitochondrial function.

A potential limitation of the current study is that we assessed specifically the impact of high fat feeding on the heart, but did not assess the effects of high fat on extra-myocardial tissues. Obesity and elevated blood lipid levels are associated with disease conditions that include hepatic steatosis, type 2 diabetes, and coronary artery disease [47,48], all of which when left untreated are associated with increased rates of morbidity and mortality. Additionally, a high

fat diet is associated with increased coronary artery ligation-induced sudden death [18], indicating prolonged exposure to high dietary fat may have important consequences on cardiovascular health that were not assessed in the current study.

In summary, our results clearly show that high fat feeding impacts a variety of cellular processes that differentially alter mitochondrial function under normal and pathological conditions. High fat induced alterations in mitochondrial function; however, we have shown that the effects of high fat in normal animals were not necessarily evident under pathological conditions, such as mild-to-moderate heart failure/dysfunction. Mitochondrial alterations and lipotoxic conditions might be expected to contribute to ventricular dysfunction as ventricular dysfunction progresses to more severe or decompensated heart failure.

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Abbreviations

DHQ	Durohydroquinone
ETC	Electron Transport Chain
IFM	Interfibrillar Mitochondria
LCAD	Long-chain Acyl-CoA Dehydrogenase
LV	Left Ventricle
MCAD	Medium-chain Acyl-CoA Dehydrogenase
MOPS	3-[N-Morpholino] propanesulfonic Acid
RCR	Respiratory Control Ratio
SCAD	Short-chain Acyl-CoA Dehydrogenase
SSM	Subsarcolemmal Mitochondria
TMPD	<i>N,N,N',N'</i> -tetramethyl- <i>p</i> -phenylenediamine

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Figure 1.

Measurements of LV function. (A) peak LV +dP/dt and (B) peak LV -dP/dt obtained by LV cannulation as well as (C) fractional shortening and (D) ejection fraction obtained by echocardiography in SHAM, SHAM+FAT, HF, and HF+FAT sixteen weeks following coronary artery ligation surgery. Values are expressed as mean $\pm$ SEM. * $p < 0.05$ vs SHAM; † $p < 0.05$ vs SHAM+FAT; ‡ $p < 0.05$ vs HF.

Figure 2.

State 3 respiration in (A) SSM and (B) IFM using PC, OC, glutamate, succinate, and DHQ as respiratory substrates in SHAM, SHAM+FAT, HF, and HF+FAT sixteen weeks following coronary artery ligation surgery. Values are mean $\pm$ SEM. *P<0.05 compared to SHAM; †P<0.05 compared to SHAM+FAT; ‡P<0.05 compared to HF. Abbreviations- SSM, subsarcolemmal mitochondria; IFM, interfibrillar mitochondria; PC, palmitoylcarnitine; OC, octanoylcarnitine; DHQ, durohydroquinone; nAO, nanoatoms oxygen.

Figure 3.

State 4 respiration in (A) SSM and (B) IFM using PC, OC, glutamate, succinate, and DHQ as respiratory substrates in SHAM, SHAM+FAT, HF, and HF+FAT sixteen weeks following coronary artery ligation surgery. Values are mean ± SEM. *P < 0.05 compared to SHAM; †P < 0.05 compared to SHAM+FAT; ‡P < 0.05 compared to HF. Abbreviations- SSM, subsarcolemmal mitochondria; IFM, interfibrillar mitochondria; PC, palmitoylcarnitine; OC, octanoylcarnitine; DHQ, durohydroquinone; nAO, nanoatoms oxygen.

Table 1

LV function as assessed by echocardiography in SHAM, SHAM+FAT, HF, and HF+FAT sixteen weeks post coronary artery ligation surgery.

	SHAM		HF	
	Normal	Fat	Normal	Fat
Body Weight (g)	576± 25	663 ± 27	570 ± 16	642 ± 28
BV/BW (mg/g)	2.32 ± 0.06	2.14 ± 0.07	2.56 ± 0.07	2.34 ± 0.07
Heart Rate (bpm)	375±14	313±12	321±21	339±18
End diastolic pressure (mmHg)	6.57 ± 0.9	6.73 ± 1.2	10.5 ± 2.0	10.6 ± 1.8
End-Diastolic Area (cm²)	1.17 ± 0.05	1.12 ± 0.03	1.32 ± 0.05 [*]	1.37 ± 0.04 [†]
End-Systolic Area (cm²)	0.70 ± 0.04	0.67 ± 0.04	0.91 ± 0.05 [*]	0.89 ± 0.06 [†]
Cardiac Index(ml·min⁻¹·kg⁻¹)	156 ± 11	177 ± 15	127 ± 9 [*]	133 ± 15 [†]
Area Fractional Shortening (cm)	0.40 ± 0.03	0.41 ± 0.03	0.32 ± 0.02 [*]	0.35 ± 0.03

BV/BW represents biventricular weight to body weight. Values are expressed as mean ± SEM.

*
p<0.05 vs SHAM;

†
p<0.05 vs SHAM+FAT;

‡
p<0.05 vs HF

Table 2

Plasma non-esterified fatty acids, glucose, insulin, and leptin, serum adiponectin, and myocardial tissue triglycerides in SHAM, SHAM+FAT, HF, and HF+FAT sixteen weeks following coronary artery ligation surgery.

	SHAM	SHAM+FAT	HF	HF+FAT
Fatty Acids ($\mu\text{mol/mL}$)	0.173 ± 0.016	$0.258 \pm 0.018^*$	0.160 ± 0.031	$0.290 \pm 0.028^{\ddagger}$
Tissue Triglycerides ($\mu\text{mol/g ww}$)	1.47 ± 0.14	$2.32 \pm 0.14^*$	1.34 ± 0.14	$2.21 \pm 0.20^{\ddagger}$
Glucose (mmol/L)	5.67 ± 0.25	5.83 ± 0.25	6.02 ± 0.27	5.74 ± 0.26
Insulin (pM)	164 ± 44	286 ± 29	234 ± 39	180 ± 35
Leptin (pg/mL)	1486 ± 248	$3018 \pm 296^*$	1229 ± 120	$2826 \pm 437^{\ddagger}$
Adiponectin ($\mu\text{g/ml}$)	6.92 ± 0.67	$11.43 \pm 0.73^*$	7.37 ± 0.66	$13.06 \pm 0.69^{\ddagger}$

Values are expressed as mean $\pm$ SEM.

*
p<0.05 vs SHAM;

$\ddagger$
p<0.05 vs SHAM+FAT;

$\#$
p<0.05 vs HF

Table 3
Mitochondrial marker enzyme activities and protein yields in SHAM, SHAM +FAT, HF, and HF+FAT sixteen weeks following coronary artery ligation surgery.

	SHAM	SHAM+FAT	HF	HF+FAT
LV Tissue				
Citrate Synthase ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{gww}^{-1}$)	141 $\pm$ 4	142 $\pm$ 3	130 $\pm$ 4 *	138 $\pm$ 4
MCAD ($\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{gww}^{-1}$)	12.0 $\pm$ 0.8	11.1 $\pm$ 0.3	10.5 $\pm$ 0.4	10.9 $\pm$ 0.6
SSM				
Protein Yield (mg/gww)	12.2 $\pm$ 0.8	15.4 $\pm$ 0.8 *	11.0 $\pm$ 1.2	9.0 $\pm$ 1.0 †
Citrate Synthase (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	2628 $\pm$ 207	2164 $\pm$ 112	2101 $\pm$ 86 *	2367 $\pm$ 268
SCAD (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	203 $\pm$ 8	197 $\pm$ 14	202 $\pm$ 5	224 $\pm$ 14
MCAD (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	82 $\pm$ 3	88 $\pm$ 3	81 $\pm$ 2	97 $\pm$ 5 ‡
LCAD (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	168 $\pm$ 7	169 $\pm$ 5	161 $\pm$ 5	170 $\pm$ 10
IFM				
Protein Yield (mg/gww)	9.9 $\pm$ 0.6	13.2 $\pm$ 1.3 *	8.8 $\pm$ 0.7	7.7 $\pm$ 0.8 †
Citrate Synthase (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	2911 $\pm$ 299	2645 $\pm$ 66	2722 $\pm$ 64	2764 $\pm$ 259
SCAD (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	237 $\pm$ 16	247 $\pm$ 5	229 $\pm$ 7	249 $\pm$ 24
MCAD (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	95 $\pm$ 4	106 $\pm$ 1 *	93 $\pm$ 2	104 $\pm$ 8 ‡
LCAD (nmoles $\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$)	187 $\pm$ 9	184 $\pm$ 4	174 $\pm$ 5	174 $\pm$ 10

SSM, subsarcolemmal mitochondria; IFM, interfibrillar mitochondria; SCAD, short-chain acyl-CoA dehydrogenase; MCAD, medium-chain acyl-CoA dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase. Values are expressed as mean $\pm$ SEM.

* p<0.05 vs SHAM;

† p<0.05 vs SHAM+FAT;

‡ p<0.05 vs HF

Table 4

Respiratory control ratio using palmitoylcarnitine, octanoylcarnitine, glutamate, succinate, and DHQ as respiratory substrates in (A) SSM and (B) IFM of SHAM, SHAM+FAT, HF, and HF+FAT.

A.				
	SSM			
	SHAM	SHAM+FAT	HF	HF+FAT
Palmitoylcarnitine	4.05 ± 0.19	3.79 ± 0.37	3.63 ± 0.20	3.76 ± 0.25
Octanoylcarnitine	3.85 ± 0.22	3.81 ± 0.17	3.40 ± 0.28	3.40 ± 0.14
Glutamate	5.83 ± 0.50	4.70 ± 0.41	4.89 ± 0.37	5.09 ± 0.38
Succinate	3.11 ± 0.16	2.71 ± 0.06	2.78 ± 0.12	2.80 ± 0.11
DHQ	2.76 ± 0.14	2.85 ± 0.11	2.53 ± 0.14	2.68 ± 0.15
B.				
	IFM			
	SHAM	SHAM+FAT	HF	HF+FAT
Palmitoylcarnitine	3.93 ± 0.18	4.09 ± 0.19	3.63 ± 0.21	3.68 ± 0.08
Octanoylcarnitine	3.89 ± 0.23	3.88 ± 0.17	3.49 ± 0.22	3.37 ± 0.08 [†]
Glutamate	6.58 ± 0.47	5.44 ± 0.51	6.06 ± 0.42	6.04 ± 0.37
Succinate	3.39 ± 0.25	2.84 ± 0.09 [*]	2.82 ± 0.14 [*]	3.14 ± 0.17
DHQ	3.25 ± 0.18	3.05 ± 0.16	2.88 ± 0.09	3.06 ± 0.16

Values are expressed as mean ± SEM.

^{*} p<0.05 vs SHAM;

[†] p<0.05 vs SHAM+FAT;

[‡] p<0.05 vs HF

Table 5

ADP/O using palmitoylcarnitine, octanoylcarnitine, glutamate, succinate, and DHQ as respiratory substrates in (A) SSM and (B) IFM of SHAM, SHAM+FAT, HF, and HF+FAT.

A.				
	SSM			
	SHAM	SHAM+FAT	HF	HF+FAT
Palmitoylcarnitine	2.67 ± 0.12	2.74 ± 0.12	2.68 ± 0.08	2.61 ± 0.08
Octanoylcarnitine	2.63 ± 0.12	2.80 ± 0.13	2.63 ± 0.09	2.58 ± 0.09
Glutamate	2.68 ± 0.12	2.83 ± 0.10	2.82 ± 0.07	2.73 ± 0.10
Succinate	1.76 ± 0.07	1.91 ± 0.09	1.91 ± 0.09	1.58 ± 0.06 ^{†‡}
DHQ	1.47 ± 0.09	1.70 ± 0.08	1.55 ± 0.11	1.47 ± 0.08 [†]
B.				
	IFM			
	SHAM	SHAM+FAT	HF	HF+FAT
Palmitoylcarnitine	2.71 ± 0.21	2.88 ± 0.11	2.76 ± 0.06	2.69 ± 0.06
Octanoylcarnitine	2.70 ± 0.12	2.86 ± 0.11	2.67 ± 0.07	2.75 ± 0.13
Glutamate	2.86 ± 0.15	2.94 ± 0.08	2.99 ± 0.06	2.94 ± 0.08
Succinate	1.83 ± 0.11	2.00 ± 0.07	2.03 ± 0.06	1.76 ± 0.05 ^{†‡}
DHQ	1.61 ± 0.07	1.86 ± 0.12 [*]	1.83 ± 0.06 [*]	1.70 ± 0.07

Values are expressed as mean ± SEM.

* p<0.05 vs SHAM;

[†] p<0.05 vs SHAM+FAT;

[‡] p<0.05 vs HF

Table 6

Summary of changes induced by high fat or heart failure.

	SHAM+FAT			HF			HF+FAT		
	8	16		8	16		8	16	
LV Functional Measurements									
Area Fractional Shortening	↔	↔		↓	↓		↔	↔	
Ejection Fraction	nr	↔		nr	↓		nr	↑	
Peak -dP/dt	↔	↔		↓	↓		↑	↔	
Peak +dP/dt	↔	↔		↓	↓		↔	↔	
Mitochondrial Parameters (SSM)									
State 3 Respiration									
Palmitoylcarnitine	↔	↓		↔	↔		↑	↔	
Octanoylcarnitine	↔	↔		↔	↔		↑	↔	
Glutamate	↔	↔		↔	↔		↑	↑	
MCAD Activity	↔	↑		↔	↔		↔	↔	
Protein Yield	↔	↑		↔	↔		↔	↔	
Mitochondrial Parameters (IFM)									
State 3 Respiration									
Palmitoylcarnitine	↔	↓		↔	↔		↔	↔	
Octanoylcarnitine	↔	↔		↔	↔		↔	↔	
Glutamate	↔	↔		↔	↔		↔	↔	
MCAD Activity	↔	↑		↔	↔		↔	↑	
Protein Yield	↔	↑		↓	↔		↔	↔	

Summary of changes induced by heart failure or high fat at 16 weeks (current study) relative to previously published changes at 8 weeks (Rennison JH *et al. Cardiovasc Res.* 79(2):331–40, 2008). Depicted are changes in SHAM+FAT or HF relative to SHAM and in HF+FAT relative to HF. ↑ increase; ↓ decrease; ↔ no change; nr, not reported.