Published in final edited form as:

J Mol Cell Cardiol. 2012 July ; 53(1): 125–133. doi:10.1016/j.yjmcc.2012.04.005.

Normalizing the metabolic phenotype after myocardial infarction; impact of subchronic high fat feeding

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Abstract

The normal heart relies primarily on the oxidation of fatty acids (FA) for ATP production, whereas during heart failure (HF) glucose utilization increases, implying pathological changes to cardiac energy metabolism. Despite the noted lipotoxic effects of elevating FA, our work has demonstrated a cardioprotective effect of a high fat diet (SAT) when fed after myocardial infarction (MI), as compared to normal chow (NC) fed cohorts. This data has suggested a mechanistic link to energy metabolism. The goal of this study was to determine the impact of SAT on the metabolic phenotype of the heart after MI. Male Wistar rats underwent coronary ligation surgery (MI) and were evaluated after 8 weeks of SAT. Induction of MI was verified by echocardiography. LV function assessed by *in vivo* hemodynamic measurements revealed improvements in the MI-SAT group as compared to MI-NC. Perfused working hearts revealed a decrease in cardiac work in MI-NC that was improved in MI-SAT. Glucose oxidation was increased and FA oxidation decreased in MI-NC compared to shams suggesting an alteration in the metabolic profile that was ameliorated by SAT. ³¹P NMR analysis of Langendorff perfused hearts revealed no differences in PCr:ATP indicating no overt energy deficit in MI groups. Phospho-PDH and PDK₄ were increased in MI-SAT, consistent with a shift towards fatty acid oxidation (FAO). Overall, these results support the hypothesis that SAT post-infarction promotes a normal metabolic phenotype that may serve a cardioprotective role in the injured heart.

Keywords

myocardial infarction; cardiac metabolism; metabolic substrate oxidation; high fat; pyruvate dehydrogenase; pyruvate dehydrogenase kinase 4; coronary ligation

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Disclosures: none declared

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1. Introduction

Myocardial infarction (MI) constitutes a major health concern in industrialized countries: nearly 1 million cases are reported annually in the US alone and MI is a significant clinical risk factor for progression to heart failure (HF) [1]. Injury to the heart, such as MI, has implications for energy metabolism as heart muscle must continuously perform contractile work at a substantial energetic cost. To meet its high energy demand the heart is capable of oxidizing a range of substrates including fatty acids (FA), glucose, lactate and amino acids [2–4]. Despite the flexibility in substrate utilization, the healthy heart normally satisfies its energetic needs with FA which constitute 60–80% of the total substrate oxidation [2, 5–9]. In contrast to the normal heart, the myocardium during the progression of HF and/or hypertrophy develops a greater reliance on glucose metabolism to support ATP production through increased glycolysis, oxidation, and anaplerotic carbon influx to the Krebs cycle [7, 10–13]. This observation of increased glucose utilization has led to therapeutic approaches to promote glucose metabolism in the failing heart with the assumption that this constitutes a positive adaptation to injury, particularly as glucose theoretically increases the efficiency of cardiac ATP generation under the hypoxic conditions of the infarct site [14]. This strategy has garnered additional support from transgenic models with enhanced cardiac lipid uptake that results in increased tissue lipids and lipotoxicity as evidenced by apoptosis, excessive lipid droplet accumulation, inflammatory mediator infiltration, and necrosis with associated remodeling processes that culminate in cardiac dysfunction [15–18]. Epidemiological data has shown that high circulating lipids are a risk factor in heart disease [19] and high fat feeding alone has been demonstrated to induce cardiac dysfunction independent of injury [20]. These observations have led to a consensus that glucose is the preferred substrate of the failing heart and enhancing cardiac lipids is likely to exacerbate dysfunction. However, promoting glucose metabolism (or inhibiting FA oxidation) in the infarcted heart may not be desirable in the viable, non-ischemic myocardium. The dynamic nature of myocardial metabolism during injury remains poorly understood and underscores the importance of defining how lipids, the preferred energy substrate, satisfy the energetic needs of the normal versus the injured heart.

Previous studies in our laboratory have demonstrated a protective effect of a high saturated fat diet (SAT) on contractile function in a rat model of coronary artery ligation-induced infarction [21, 22]. This preservation of myocardial contractility is accompanied by increased acyl CoA dehydrogenase activities (the initial reaction in FAO) and FAO intermediates along with increased rates of mitochondrial respiration using various substrates, including FA [21, 23, 24]. An enhancement of FA metabolism in MI with SAT is further implicated by microarray profiling which shows expression trends consistent with increased FAO [22]. In addition to a potential increase in FAO, we have demonstrated a cardiac-specific reduction in glucose uptake in the myocardium with SAT suggesting an impact on both glucose and FA metabolism [25]. These observations imply that increased lipids benefit the injured heart through a biochemical mechanism related to energy metabolism to promote FA metabolism with a reciprocal depression of glucose metabolism. The goal of the current study was to determine the metabolic substrate oxidation profile of the infarcted heart with SAT and the post-translational status of pyruvate dehydrogenase, a critical convergent point of glucose and FAO. The strong dependence of cardiac function on energy production may be the primary driving force for the cardioprotection we have reported with high dietary fat by increasing the availability of FA and decreasing glucose utilization commonly associated with HF. Insight into the ability of the post-infarcted heart to metabolize FA is important given that recent studies targeting metabolic modulation are conflicted as to whether increased FA are detrimental in the failing heart.

2. Materials & Methods

2.1 Chemicals

All chemicals used were of research grade or higher and obtained through Fisher (PA), unless otherwise noted. Chloral hydrate was dissolved at 0.1mg/ml in sterile-filtered water (Sigma, MO). Radioisotopically-labeled glucose and oleate were obtained from American Radiolabeled Chemicals (MO).

2.2 Animals, Diet & Surgical Procedures

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animal* (NIH Publication No. 85–23) as approved by the Institutional Animal Care and Use Committee (IACUC) at Case Western Reserve University. Male Wistar rats (300–350g) were kept on a reverse light-dark cycle and all procedures were performed 3–6 hours into the dark phase to minimize diurnal variations [26]. Rats were randomly assigned to a sham (SH) group or coronary ligation to induce myocardial infarction (MI) groups; surgical procedures were performed as previously described [27]. Following surgery, rats were fed 8 weeks on a normal chow (NC) or a high saturated fat diet (SAT) containing 60% kcal from lipid (25% palmitic, 33% stearic, & 33% oleic acid, Research Diets, NJ).

2.3 Echocardiography

Seven weeks post-surgery, left ventricular (LV) function was assessed in all groups to verify surgical assignments using data acquired with a Sequoia C512 system (Siemens Medical, PA) as previously described [27]. End-diastolic and end-systolic dimensions were calculated using software included with the ultrasonograph and fractional shortening (FS) was determined as described [27]. Validation of the coronary ligation procedure to create a MI sufficient to induce LV dysfunction/heart failure was performed; rats in the MI groups with an EF and FS above 75 and 0.40 respectively, were excluded from the study due to insufficient injury.

2.4 In vivo Hemodynamic Measures

Eight weeks following MI, rats were anesthetized (isoflurane, 2.0%) and a 2.0 F Millar pressure-volume transducer (Millar Instruments, TX) was introduced via the right carotid artery into the LV as previously described [22, 28]. Volume measures were calibrated using a saline bolus introduced through the jugular vein. Heart rate, ventricular volume, peak LV systolic pressure, end diastolic pressure, ejection fraction, $\pm$ dP/dt, LV stroke work and power were calculated from raw data analyzed with PVAN/Chart 5 software (ADInstruments, CA). After hemodynamic measurements, animals were immediately sacrificed and the heart was removed; venous blood was also sampled from the inferior vena cava. Excised LV tissue was immediately quick-frozen in liquid nitrogen and stored at -80°C until analyzed further.

2.5 Plasma & Tissue metabolite analysis: Triglycerides (TG), glucose & glycogen

Samples taken after hemodynamic measures were employed for metabolite analysis. Plasma glucose and TG were quantitated using a commercially available kit employing an enzyme-coupled spectrophotometric method according to manufacturer's suggestions (Wako Chemicals, VA). Tissue TG was determined using 30 mg frozen, powdered tissue homogenized in 5.0 ml of 2:1 (v:v) chloroform:methanol. Soluble extracts were evaporated to dryness prior to use in a commercially available assay (Wako Chemicals, VA). Glycogen content was determined using previously described methodology with minor modifications [29]. Briefly, 30 mg of frozen, powdered tissue was denatured and homogenized in 3M perchloric acid (1:3, w:v), then neutralized with 8 volumes of ddH₂O. Samples and

standards were incubated with 2 U amyloglucosidase to release glucosyl units (Sigma, IL) followed by spectrophotometric quantitation using a commercially available glucose kit (Wako, VA).

2.6 Isolated Working Heart Perfusions

Rat hearts were perfused as described previously with minor modifications [30, 31]. Briefly, 8 weeks subsequent to MI rats were anesthetized with a graded, lethal dose of chloral hydrate i.p. (500mg/kg, Sigma, MO). Heparin (100 μ U) was given in the first injection. Upon deep anesthesia, a thoracotomy was performed to quickly excise the heart and dissect out the aorta in ice-cold Krebs-Henseleit buffer (KB) containing (molar): 0.025 NaHCO₃, 0.1185 NaCl, 0.00474 KCl, 0.00118 KH₂PO₄, 0.00118 MgSO₄, 0.025 CaCl₂, and 0.005 glucose. Upon aortic cannulation, hearts were perfused in a retrograde fashion (Langendorff model) with KB equilibrated with 95/5% O₂/CO₂ at 37 °C for 2–4 minutes while left atrial cannulation was performed. Hearts were perfused in a semi-recirculating manner (aortic outflow was recirculated) using a 5.0 μ m inline filter with equilibrated KB buffer containing 1% (w/v) bovine serum albumin (BSA; SeraCare, MA) bound to 0.4 mM oleate with 60 μ Ci/L [9,10-³H] oleate, 80 μ Ci/L [U-¹⁴C] glucose and 10 μ U/ml insulin. Oleate-BSA conjugates were dialyzed overnight to balance [Ca²⁺]_{free} prior to use. The perfusion protocol consisted of 20 minutes at a workload of 100 cm H₂O (74 mmHg) with a preload of 15 cm H₂O (11 mmHg). Functional and metabolic parameters were assessed every 5 minutes. The 15 and 20 minute time points were averaged to represent steady state conditions. Aortic and coronary flows were measured with inline volumetric glassware (expressed as ml/min/g dry heart wt.); 10 ml of coronary effluent was collected for metabolic measurements. Cardiac LV (hydraulic) work was calculated as previously defined and expressed in mWa [31]. Oxygen consumption (MVO₂) was determined using inline Clarke-type electrodes (YSI, CO) calibrated to air-saturated water at 37°C (values expressed as μ mols/min/g dry heart wt). Heart rate (HR) and pressure (P) was monitored continuously in the aortic line with a pressure transducer (Millar Instruments, Tx) and data was recorded and analyzed with Chart (ADInstruments, CO). Perfusions in which the HR was less than 180 bpm or bouts of arrhythmia occurred were excluded from analysis. At the end of the perfusion, hearts were quickly frozen in liquid nitrogen and stored at –80°C until dry weight analysis.

2.7 Glucose and Fatty Acid Oxidation Rates

Rates of exogenous glucose and oleate oxidation were determined in coronary effluents of perfused hearts based on previously published methodology [31, 32]. Briefly, glucose oxidation was measured as ¹⁴CO₂ liberated from 0.6 ml perfusion buffer acidified with a ½ volume of 6 M HCl in sealed vials. Hyamine hydroxide (0.75 ml) was used as a trapping agent for the ¹⁴CO₂ in a separate reservoir and sealed samples degassed overnight at 25°C. Ten volumes of Ultima Gold scintillation cocktail (Perkin Elmer, MA) were added to the ¹⁴CO₂ saturated hyamine hydroxide for scintillation analysis. Oleate oxidation was measured as ³H₂O separated from 0.5 ml of coronary effluent on a column (2.0 ml bed volume) of AG 1×8 anion exchange resin converted to the hydroxyl form according to manufacturer's suggestion (Biorad, CA). Tritiated water was eluted in 10 volumes of water and added to 10 ml of scintillation fluid. Blanks, along with total activity samples, were generated for both substrate oxidation assays and all processed samples were counted in a LS 6500 liquid scintillation counter (Beckman Coulter, CA). Corrected counts were multiplied by coronary output and expressed as μ mols/min/g dry wt. Exogenous substrate oxidation rates were also converted to percent MVO₂ by calculating the reduction of ½O₂ per reducing equivalent generated for each respective substrate; 1 mol glucose = 6 mol O₂ and 1 mol oleate = 25.5 mol O₂.

2.8 ³¹P NMR analysis of Phosphocreatine to ATP ratios

Animals were anesthetized, hearts were excised, then cannulated via the aorta as described under “Isolated Working Perfused Hearts” above. Freely beating hearts were perfused with KB equilibrated with 95/5% O₂/CO₂ in Langendorff mode at 37°C and a constant pressure of 80 mmHg. To monitor HR and P, a pressure balloon catheter was inserted via the atrial appendage into the LV and inflated with water to an LV end-diastolic pressure reading of < 10 mmHg. Functional data from the pressure transducer was recorded continuously and subsequently analyzed using Chart (ADInstruments, CO). Cannulated hearts were placed in a 20 mm glass NMR tube for insertion into the probe. ³¹P NMR scans for phosphate peaks associated with phosphocreatine (PCr) and ATP were acquired on a Bruker AVANCE-400 9.4T vertical-bore spectrometer with a 20 mm broadband NMR probe tuned to ³¹P. Field homogeneity was adjusted by shimming on the proton signal to a line width of 30–50 Hz. ³¹P spectra were acquired in 64 scans using a 161-MHz, 45° excitation pulse with a 1.8 second repetition time. Post-processing of NMR data included 20-Hz line broadening, Fourier transformation, and phase correction using the NMR dedicated software, MesRec (Mestrelab Research SL, Spain). Peak assignments were made with reference to the well-established resonance signals of PCr and ATP [33, 34]. The area under the curve was determined for each peak of interest by employing Lorentzian curve fitting and these values were used to calculate the ratio of PCr to the β phosphate group of ATP. Experiments in which HR was lower than 180 bpm or function deteriorated over the duration of the perfusion were excluded from analysis.

2.9 Immunoblotting

Non-perfused LV tissue harvested subsequent to hemodynamic measures was used in Western blotting. Frozen LV tissue was powdered and added to isolation buffer containing 20 mM Tris (pH 7.8), 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1% (w/v) Triton X-100, 10% (w/v) glycerol, 1 mM EDTA, with protease (78425, ThermoFisher, USA) and phosphatase inhibitor cocktails (5726, P0044, Sigma Aldrich, MO) added as per manufacturer's suggestions. Samples were processed in texturized glass-on-glass homogenizers and incubated at 4°C for 30 minutes prior to centrifugation at 5,000 × *g* for 20 min (4°C). The resulting supernatant was stored at –80°C until analyzed. Protein concentration was determined via a BCA protein assay kit (Pierce, IL). Twenty micrograms of protein in standard Laemmli buffer was run on a 10% SDS-polyacrylamide gel (Bio-Rad, CA), then transferred to polyvinylidene difluoride (PVDF) (Millipore, MA). Protein transfer was verified by Ponceau staining. Membranes were blocked with 5% BSA in Tris-buffered saline with Tween 20 (0.01%) and were incubated overnight with primary antibodies. Antibodies were as follows: PDH, 1:2,000 dilution (Cell Signaling, MA), phospho-PDH^{ser293} (pPDH), 1:1,000 dilution (Calbiochem, NJ), PDK₄, 1:2,000 dilution (gift from R. Harris) and HSC70 (Santa Cruz, CA) used as a loading control (1:5,000). Chemiluminescent detection was achieved with the Supersignal West Pico system (Pierce, IL) on standard autoradiography film (Denville, NJ). Developed films were scanned on an Epson P4490 (transmittance mode) at 720 dpi and unaltered images were used for densitometry measures in Image J software in the histogram analysis mode (NIH, MD). Densitometric ratios were derived from sequential probing of membranes stripped between incubations with Restore® Western blot stripping buffer (Pierce, IL). Protein band intensities across gels were normalized to a sample run on all the gels.

2.10 Statistical Analysis

Statistical differences were determined by two-way ANOVA with Bonferroni post-hoc analysis and *p* < 0.05 as the criterion for significance. All values are presented as the mean ± standard error of the mean (SEM).

3 Results

3.1 Gravimetric, Echocardiographic, & Hemodynamic Data Following MI with High Fat Feeding

After 8 weeks of high fat (SAT) feeding following MI, there were no differences in body or heart weight (determined as the dry weight of perfused hearts) between groups (Table 1). To verify the presence of MI-induced cardiac dysfunction, echocardiography was performed at 7 weeks. Moderate LV remodeling and dysfunction was evident in both MI groups as demonstrated by an increase in end diastolic and end systolic area, in addition to a significant depression of fractional shortening. Eight weeks following MI and SAT, hemodynamic data was acquired to evaluate changes in pressure-volume parameters. Myocardial contractile function assessed by ejection fraction (%), stroke work, and $\pm$ dP/dt, was significantly depressed in MI-NC compared to SH-NC, but significantly improved in MI-SAT compared to MI-NC (Figure 1). There was no impact of SAT alone (SH-SAT) on any of these measures of contractile function (Figure 1).

3.2 Function & Metabolism in Working Perfused Hearts

Ex vivo functional measures were obtained using the isolated working heart model and are presented in Table 2. Heart rate and rate-pressure product (RPP) was not altered across the groups. Coronary flow was significantly lower in the MI-NC group compared to the surgical control (SH-NC) resulting in a decreased cardiac output. Coronary flow rate was decreased in the MI-SAT group, though no significant change in aortic or total cardiac output with respect to SH-SAT was found. Cardiac work (mWa) was decreased in MINC compared to the surgical control (SH-NC) but was improved in MI-SAT compared to the dietary control (MI-NC). In addition to assessing functional parameters, substrate oxidation and oxygen consumption was evaluated in working perfused hearts. Glucose oxidation in MI-NC hearts was significantly increased with respect to both the SH-NC and MI-SAT group (Figure 2). Conversely, oleate oxidation was decreased in the MI-NC group compared to SH-NC and MI-SAT (Figure 2). In addition, MVO_2 was decreased in both MI groups with respect to surgical controls (Figure 3). To better gauge differences in the metabolic substrate pool, glucose and FA were converted to their molar oxygen equivalents (glucose = 6, oleate = 25.5) and the percent (%) contribution to MVO_2 was calculated (Figure 3). Overall, total exogenous substrate oxidation was not different amongst the groups. However, the contribution of glucose to exogenous substrate oxidation was significantly ($p < 0.05$) increased in the MI-NC group (statistics not noted on figure) compared to both SH-NC and MI-SAT (Figure 2). No differences between surgical controls (SH-SAT vs SH-NC) were noted for any of the measured perfusion parameters.

3.3 Plasma and Tissue Substrate Concentrations

Plasma and LV tissue samples were assessed for several substrates as presented in Table 3. Plasma glucose and tissue glycogen was not altered across the groups. Tissue triglycerides were elevated in both the SH-SAT and HF-SAT with respect to their dietary controls.

3.4 Phosphocreatine-to-ATP ratios of Langendorff Perfused Hearts

In a separate set of experiments, Langendorff perfused hearts were analysed by ^{31}P NMR to determine PCr to ATP ratios. Representative ^{31}P NMR spectra are shown; PCr:ATP ratios were not altered across groups (Figure 4).

3.5 PDH Phosphorylation and PDK₄ content in Cardiac Tissue

Relative phosphorylation of PDH, along with PDK₄ content was assessed by Western blot in a separate set of non-perfused hearts and representative bands are shown in Figure 5A and

D. The relative content of PDH and pPDH were determined, along with the ratio. The pPDH content in the MI-SAT group was significantly higher compared to both the surgical (SH-SAT) and dietary (MI-NC) control groups (Figure 5B); the relative content of PDH (normalized to HSC70) was not significantly altered amongst the groups (Figure 5C). PDK₄ protein content was also determined and was significantly increased in the MI-SAT group with respect to both the surgical (SH-SAT) and dietary (MI-NC) control groups (Figure 5E). Diet alone did not result in a significant increase in pPDH or PDK₄ (SH-SAT vs SH-NC).

4 Discussion

4.1 Summary

This study was designed to determine whether a high fat diet fed to rats for 8 weeks post-infarction invokes changes in the metabolic phenotype of the heart. The coronary ligation model of cardiac injury induces moderate, stable LV dysfunction that does not progress to severe decompensating HF [27]. This model of cardiac injury reproduces clinical features of MI and allows analysis of metabolism under conditions of stable LV dysfunction. As previously reported, myocardial contractile function (maximum $\pm$ dP/dt, stroke work and ejection fraction) is significantly improved in post-MI hearts following 8 weeks of a high fat diet [21–25]. The working heart model used in this study does exhibit functional changes in agreement with our *in vivo* hemodynamic measures as MI-NC hearts exhibit decreased cardiac output whereas the MI-SAT group does not.

Though the cause and effect relationship between HF and metabolic derangements has not been clearly established, this work demonstrates the presence of a metabolic alteration in remote myocardial tissue 8 weeks following MI. Our previous work has implicated energy metabolism in the infarcted heart as a mechanistic component in the cardioprotective effects of high dietary fat fed up to 16 weeks subsequent to MI [24]. In the current study using the working heart perfusion model, the substrate oxidation profile that accompanies the cardioprotection with high dietary fat was defined. The substrate oxidation data clearly demonstrate that elevated dietary fat modulates the metabolic phenotype of the heart to resemble the normal heart, with greater FAO and decreased glucose oxidation. It should be noted that the perfusion conditions employed here were at a normal physiological concentration of fatty acid (0.4 mM) to allow comparisons across all groups. This may underestimate the *in vivo* oxidation rates of FA in the high fat diet groups as exogenous lipid concentration heavily influences its metabolism both *in vivo* and in the perfused heart [6, 35]. In addition, tissue TG were elevated with the high fat diet and this may further alter endogenous rates of FAO. However, tissue TG were not different between MI-NC vs SH-NC suggesting the altered substrate oxidation profile (i.e. reduced FAO in MI-NC) is not the result of altered endogenous lipid stores with respect to cardiac injury alone. With respect to glucose metabolism, the current study did not evaluate alternative pathways (ie anaerobic glycolysis or anaplerotic carbon influx into the Krebs cycle) however, taking this into account would likely further inflate the observed glucose oxidation rates, particularly in the injured heart [13]. Additional work evaluating function and metabolism under conditions representing higher circulating FA concentrations and assessing flux through endogenous substrate stores along with alternative pathways for glucose metabolism may be necessary to observe the full impact of high fat on the injured heart.

Despite the limitations to the methodology employed, our observations do not suggest high fat after MI is detrimental, rather we have observed an improvement in LV contractile function and a substrate oxidation profile resembling the normal heart. Our findings support the hypothesis that high dietary fat subsequent to MI injury leads to improved myocardial function by enhancing fatty acid metabolism.

4.2 Metabolic changes accompany improved function following MI with high fat

As the heart is exquisitely tuned to maintain energy balance to ensure rapid delivery of ATP to contractile elements, we also investigated the PCr:ATP to determine whether the diminished oxidation rates of the MINC affect this reserve pool of ATP and if SAT impacts these potential changes. Since PCr serves as a shuttle for ATP to contractile elements, a decrease could explain differences in contractile function between MI groups. Studies have shown that declining PCr:ATP correlates with progressive loss of LV function in end-stage heart failure as the metabolic machinery fails to meet basal energetic needs which compromises function, though this decrease is modest [36–38]. As decompensation does not occur in our model [24, 27] it might be expected that PCr:ATP would be unaltered, though quantitative analysis of individual peaks was not possible to evaluate small changes in each phosphate species. Additionally, as these experiments assessed global changes of PCr:ATP, we cannot exclude the possibility of localized changes in the creatine kinase system that could influence ATP availability for cardiomyocyte function. However, despite the lack of a robust change in PCr:ATP, the substrate oxidation data implies a metabolic alteration in the MI-NC group that is not present with SAT supporting a role for energy metabolism in the protective effects of high dietary lipid.

Severe LV dysfunction is characterized by both a decrease in overall energy production and an increased reliance on glucose utilization [7, 39]. Increasing lipids in the face of this metabolic switch has been presumed to exacerbate pathology as studies in the uninjured heart show that high FA concentrations induce lipotoxicity and decrease cardiac function [16, 18]. However, clinical studies demonstrate that lowering FA diminishes cardiac efficiency, indicating a critical role of lipids in determining the metabolic phenotype of the injured heart [40]. In the present study, MVO_2 was not different between MI groups suggesting similar rates of oxidative phosphorylation supporting the lack of an overt energetic deficit. However, the balance between energy substrate use was altered as glucose oxidation was enhanced in MI-NC hearts accompanied by a decrease in FA oxidation, similar to the metabolic profile of more severe LV dysfunction [41]. With high fat feeding (MI-SAT) substrate oxidation rates did not differ from control values demonstrating an improvement over the MI-NC group. Increased circulating lipids stimulate greater uptake and subsequent rates of oxidation, thus an increase in FAO with the high fat diet would be anticipated. However, the SH-SAT group did not possess a greater rate of FA oxidation than the SH-NC controls, whereas MI-SAT was enhanced over the MI-NC group. Additionally, the perfusions were performed at a fixed FA concentration implying the differences in FAO must include intracellular molecular changes related to energy metabolism that are more evident in the MI-SAT group.

4.3 Regulatory proteins are involved in the metabolic reconditioning resulting from high fat after MI

Increased FAO can occur via acute increases in substrate flux, but post-translational alterations to proteins controlling metabolic pathways balance metabolism to long term changes in substrate concentrations. The primary metabolic substrates of the heart, FAs and glucose, converge at the Krebs cycle to undergo full oxidation. Fine-tuning of substrate entry at this point is accomplished in part, by inhibitory phosphorylation of PDH to decrease glucose-derived pyruvate entry into the Krebs cycle. Our results show that the content of pPDH is greater in MI-SAT as compared to MI-NC, implying a decrease in glucose oxidation that is consistent with the substrate oxidation data. PDH phosphorylation was not significantly enhanced in the SH-SAT group suggesting that this post-translational modification is perhaps controlled in a manner unique to the injured heart with high fat. Phosphorylation of PDH is mediated by PDH kinase 4 (PDK₄), a gene product that is transcriptionally upregulated in our model of high fat feeding and others [21, 42, 43]. The

protein content of PDK₄ was increased in the MI-SAT group further supporting the altered regulation of PDH in the context of injury with high fat. Studies in the uninjured heart have demonstrated PDK₄ over-expression alone results in increased FAO, but also limits substrate flexibility [44, 45]. In the context of MI, increased PDK₄ expression has not been characterized with regards to its impact on metabolism, but has been shown to be induced in the early post-MI phase [46]. Our data, taken 8 weeks following MI with SAT, suggests that increased PDK₄ activity is beneficial to the heart as substrate oxidation rates parallel those of the controls. PDK₄ expression has been shown to be modulated by several transcription factors, including FOXO1 which is not fully characterized, but acts as a downstream effector in insulin signaling [47]. In conjunction with our previous data showing decreased glucose uptake in MI-SAT hearts [25], PDK₄ may represent a control point in a larger regulatory network that adjusts the metabolic phenotype to balance energy metabolism under a range of physiological conditions.

Further work to define the upstream molecular events responsible for improving the metabolic machinery in MI is necessary, but several factors are known to influence substrate use in the heart. Increased circulating FA promote FAO under normal physiological conditions [6, 48, 49] and increased FA transport could be an upstream stimulus by which high dietary fat subsequent to injury is cardioprotective. High fat feeding has been shown to result in long term translocation of FA transporters to the cell surface [20] that could be the underlying mechanism for promoting FAO after MI, though the in the normal heart a high fat diet can also lead to dysfunction. The aforementioned decrease in glucose uptake also hints at a cardiac-specific change in the metabolic regulatory response to high fat that includes altered glucose metabolism in addition to FAO [25]. This perhaps represents a coordinated regulation of substrate entry dictated by an overlap in metabolic and pathologically-stimulated regulatory pathways. The cardiac-specific insulin resistance we have published previously [25] and the altered regulation of substrate entry through PDH both exist 8 weeks subsequent to injury and diet. Defining the primary molecular or biochemical stimulus driving the increased reliance on FA in the MI-SAT group requires additional investigation at earlier time points to delineate these temporal changes, a subject of ongoing studies in our lab. The current work suggests the injured heart possesses a complicated (and poorly understood) altered energetic state that rather than being intolerant of lipid, appears to be responsive to metabolic re-conditioning by high fat with a positive impact on function.

4.4 Conclusions

High fat feeding alters the metabolic phenotype of the injured heart. This alteration may contribute to the protective effects on cardiac function as metabolic capacity and the heart's ability to sustain contraction are closely related. It is interesting to note that the responses of the heart after high fat feeding in the current study seem divergent with respect to MI. The MI-SAT group appears more responsive to high fat in terms of the regulatory elements associated with the entry of pyruvate into downstream metabolism. This study serves to highlight the necessity for future studies evaluating the heart in terms of metabolic regulatory pathways in the context of injury. It is also noteworthy that, in addition to the vast pharmacologic interventions aimed at controlling the progression of heart failure, FAs could hold promise for the therapeutic management of the post-infarcted heart.

Acknowledgments

The authors would like to express their gratitude to Drs. Robert Harris and Pengfei Wu for kindly donating the PDK₄ antibody. In addition, we also thank Drs. Ming Lu and Ya Chen for their technical expertise with the ³¹P NMR experiments. This work was supported by a grant from the National Heart, Lung, and Blood Institute of the NIH (MPC; HL081857).

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Highlights

- Previous work has shown high dietary fat (SAT) is cardioprotective post-infarction.
- Energy metabolism changes were evaluated as the potential cardioprotective mechanism.
- SAT increases fatty acid oxidation and reduces glucose oxidation post-MI.
- Pyruvate dehydrogenase regulation is also affected, distinct from SAT or MI alone.
- We conclude SAT after MI improves lipid metabolism to confer cardioprotection.

Figure 1.

In vivo hemodynamic parameters from anesthetized rats after 8 weeks of high fat feeding post-MI. Values represent the mean $\pm$ SEM ($n = 5-6$). Statistical differences were defined by a two-way ANOVA using Bonferroni post-hoc analysis ($p < 0.05$). *Denotes statistical difference from the respective surgical control (SH). †Denotes statistical difference with respect to the dietary control group (NC).

Figure 2.

Glucose and oleate oxidation rates obtained from working perfused hearts. Values represent the mean $\pm$ SEM ($n = 13-16$). Statistical differences were defined by a two-way ANOVA using Bonferroni post-hoc analysis ($p < 0.05$). *Denotes statistical difference from the respective surgical control (SH). †Denotes statistical difference with respect to the dietary control group (NC).

Figure 3.

Myocardial oxygen consumption and exogenous substrate oxidation rates presented as percent (%) of oxygen consumption in working perfused hearts (see materials and methods for additional information). Values represent the mean $\pm$ SEM ($n = 13-16$). *Denotes statistical difference from the respective surgical control (SH).

Figure 4.

³¹P NMR analysis of PCr:ATP ratio in Langendorff perfused hearts. **A.** Representative ³¹P NMR spectra. **B.** Quantitative representation of PCr:ATP ratios from ³¹P NMR. Values represent the mean ± SEM (n = 7–8). No statistical differences were observed.

Figure 5.

Western blot analysis of phosphorylated pyruvate dehydrogenase (pPDH) and PDH kinase 4 (PDK₄) protein content in LV tissue. **A.** Representative western blots for pPDH, PDH, and HSC70 (loading control). **B.** Relative content of pPDH. **C.** Total content of PDH. **D.** Representative blots for PDK₄ and HSC70. **E.** Total content of PDK₄. Values represent the mean $\pm$ SEM (n = 4–5). Statistical differences were defined by two-way ANOVA with Bonferroni post-hoc analysis (p < 0.05). *Denotes statistical difference from the respective surgical control (SH). †Denotes statistical difference vs. respective dietary control (NC).

Table 1

Gravimetric & echocardiographic parameters from rats 8 weeks post-MI with high fat feeding.

	SH-NC	SH-SAT	MI-NC	MI-SAT
<i>Gravimetric</i>				
Body weight (g)	501 ± 9	518 ± 7	489 ± 8	506 ± 12
Heart weight (g, dry)	0.286±0.038	0.297±0.031	0.275±0.041	0.287±0.037
<i>Echocardiography</i>				
End-diastolic area (cm²)	1.05 ± 0.03	1.02 ± 0.02	1.19 ± 0.04 *	1.16 ± 0.03 *
End-systolic area (cm²)	0.48 ± 0.02	0.43 ± 0.03	0.78 ± 0.05 *	0.71 ± 0.05 *
Fractional Shortening (%)	0.53 ± 0.01	0.54 ± 0.02	0.25 ± 0.02 *	0.25 ± 0.02 *

Values represent the mean ± S.E.M. (n = 13–16). Statistical significance was determined by 2-way ANOVA followed by the Bonferroni *post hoc* analysis method (p < 0.05).

* Denotes a statistical difference from the respective surgical control group.

Table 2

Perfusion parameters from isolated working heart perfusions 8 weeks post-MI with high fat feeding.

	SH-NC	SH-SAT	MI-NC	MI-SAT
Heart Rate (HR, bpm)	221 ± 8	223 ± 9	199 ± 10	223 ± 14
RPP (bpm*mmHg*10³)	21.0 ± 0.5	21.1 ± 0.7	18.5 ± 0.9	21.2 ± 1.6
Aortic (ml·min⁻¹·g dry wt⁻¹)	168 ± 9	154 ± 7	149 ± 10	153 ± 8
Coronary (ml·min⁻¹·g dry wt⁻¹)	101 ± 4	102 ± 3	86 ± 4 *	90 ± 4 *
Output_{total} (ml·min⁻¹·g dry wt⁻¹)	269 ± 10	256 ± 9	235 ± 12 *	243 ± 10
Cardiac work (mWa)	12.3 ± 0.3	12.3 ± 0.2	10.5 ± 0.4 *	11.3 ± 0.5

Values represent the mean ± S.E.M. (n = 13–16). Statistical significance was determined by 2-way ANOVA followed by the Bonferroni *post hoc* analysis method (p < 0.05).

* Denotes a statistical difference from the respective surgical control group. RPP = “rate pressure product” (HR × P_{max}).

Table 3

Plasma and tissue metabolite concentrations in rat hearts 8 weeks post-MI with high fat feeding.

	SH-NC	SH-SAT	MI-NC	MI-SAT
<i>Plasma</i>				
Glucose (mM)	9.28 ± 0.08	9.11 ± 0.50	8.71 ± 0.98	10.2 ± 0.84
Triglycerides (mg/ml)	0.41 ± 0.10	0.39 ± 0.14	0.51 ± 0.13	0.46 ± 0.12
<i>Tissue</i>				
Glycogen (mg/g wet wt)	3.35 ± 0.41	2.73 ± 0.55	3.20 ± 0.44	3.10 ± 0.29
Triglycerides (mg/g wet wt)	2.71 ± 0.35	4.24 ± 0.58 *	2.65 ± 0.46	4.36 ± 0.64 †

Values represent the mean ± S.E.M. (n = 4–5). Statistical significance was determined by 2-way ANOVA followed by the Bonferroni *post hoc* analysis method (p < 0.05).

* Denotes a statistical difference from the respective surgical control group.

† Denotes statistical difference from respective dietary control group