Myocardial insulin resistance induced by high fat feeding in heart failure is associated with preserved contractile function

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Heart failure (HF) has been associated with a metabolic substrate shift from a primary fuel of fatty acids to one of glucose (3, 26). While a normal heart derives ~60–90% of its energy from fatty acids, the failing heart shifts its metabolic profile to one that recapitulates a fetal gene program with glucose, instead of fatty acids, being the primary energy substrate (45, 54). The long-standing assumption has been that changing fuel sources during HF is a protective mechanism, since carbohydrate oxidation uses less oxygen than fatty acid oxidation when calculated per molecule of ATP produced (21, 23). Indeed, recent evidence has suggested that inhibition of mitochondrial fatty acid uptake or β-oxidation (which is often associated with an increase in glucose oxidation) may be a valuable therapeutic strategy in the treatment of cardiovascular dysfunction associated with obesity, diabetes, and ischemic heart disease/myocardial infarction (26, 51). However, existing studies have shown that high fat feeding itself does not negatively impact cardiac function (6, 13) and may actually improve function under pathological conditions such as hypertrophy and HF (7, 11, 30, 33). This novel paradigm has been supported by previous studies performed in our laboratory (40, 42) that showed that a high-fat diet during mild to moderate HF preserves contractile function better than a standard diet.

Risk factors commonly associated with an increased incidence of HF include hypertension, coronary artery disease, left ventricular (LV) hypertrophy, valve disease, diabetes, obesity, and dyslipidemia. However, a number of clinical studies (18, 20, 52, 59, 60) have recently identified a strong positive relationship between insulin resistance and the subsequent development of HF and/or hypertrophy. A study (18) reported that insulin resistance predicts HF incidence independent of other established risk factors, including diabetes and obesity. In addition, the degree of insulin resistance correlates with functional class and subsequent mortality from HF (60). It should be noted, however, that many of these clinical studies actually evaluated the relationship between peripheral insulin resistance and HF, and this should not be misconstrued as implying the presence of myocardial insulin resistance (19, 57). Indeed, it remains to be determined whether insulin resistance (either peripheral or myocardial) is merely a marker of disease severity or whether it actually contributes to the progression of HF. Several potential mechanisms that have been proposed to link HF and insulin resistance include increased activation of the neurohumoral system (53, 61), increased inflammatory processes (58), increased signaling by adipokines (10), and possibly the pharmacological exacerbation of insulin resistance by conventional HF treatment (2). Furthermore, arterial substrate levels [e.g., glucose and free fatty acids (FFAs)] have a direct impact on cardiac metabolism and may affect regulatory pathways linking substrate utilization and myocardial function. However, a definitive mechanism linking myocardial insulin resistance and HF has yet to be identified and is the subject of current investigative research.

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Chronic elevations in circulating FFAs are associated with pathological conditions such as obesity, type 2 diabetes, and nutritional conditions involving excessive dietary fat intake. The subsequent accumulation of fatty acids and other fatty acid metabolites in nonadipose tissues (such as the heart and skeletal muscle) has been implicated as an important contributor to insulin resistance. Furthermore, elevated plasma FFAs correlate with increasing peripheral insulin resistance in a dose-dependent manner (43). However, a consensus regarding a link between myocardial insulin resistance resulting from increased exposure to FFAs and contractile dysfunction has yet to be clearly defined. Long-term feeding of a high-saturated fat diet has been reported to contribute to peripheral and myocardial insulin resistance but was not associated with any cardiac pathophysiology in a porcine model of diet-induced obesity (24). Alternatively, FFA depletions has been shown to decrease cardiac work and myocardial efficiency in patients with idio- pathic dilated cardiomyopathy, suggesting that fatty acids are required for optimal function in the failing heart (56). In contrast, Ouwens et al. (35) reported that high-fat diet-induced myocardial insulin resistance contributed to a hypertrophic-like cardiac phenotype characterized by LV remodeling and decreased fractional shortening and ejection fraction. Likewise, a short duration of high-fat diet-induced insulin resistance, accompanied by enhanced LV remodeling and contractile dysfunction, has been associated with pressure overload-induced HF (38). Thus, the relationship between elevations in FFAs, insulin resistance, and myocardial contractile function remains controversial.

Since both HF and a high-fat diet are independently associated with insulin resistance, these two conditions may act synergistically and thereby impact both the existence and consequences of an insulin-resistant state. This is particularly important given that insulin resistance is generally considered to be maladaptive under most normal physiological conditions. The goal of this study was to examine the relationship between myocardial insulin resistance and contractile function with high fat feeding in mild to moderate HF. We hypothesized that the combination of HF and a high-fat diet would result in a myocardial insulin-resistant phenotype associated with altered insulin signaling, which serves a cardioprotective function in coronary artery ligation-induced LV dysfunction/HF.

MATERIALS AND METHODS

Chemicals. All chemicals were of research grade or higher and were purchased from Fisher Scientific or Sigma Aldrich unless otherwise noted.

Study design. All animal protocols were approved by and conducted according to guidelines set forth by the Institutional Animal Care and Use Committee of Case Western Reserve University. Animals were maintained in a reverse light-dark cycle (12-h intervals), and all experimental procedures were performed in the dark cycle. Infarct surgery was performed as previously described (27). Briefly, male Wistar rats weighing 300–350 g were anesthetized, intubated, and ventilated on room air with 1.5–2% isoflurane. The left main coronary artery was ligated for the induction of HF (HF groups), and a group of animals underwent a sham surgery (sham groups) in which surgery at 8 wk.

Five weeks after ligation surgery, the first group of rats (cohort 1; n = 10–14 rats/group) underwent a glucose tolerance test (GTT) at the Mouse Metabolic Phenotyping Core at Case Western Reserve University. Rats were fasted for 18 h before being tested. Animals were anesthetized, and a tail arterial catheter was placed as previously described (15) with a total surgery time of 10–15 min. Animals were allowed to recover in restrainers for 1 h after catheter placement before the GTT was performed. Animals remained conscious but restrained for the duration of the GTT. At time 0, an intraperitoneal injection of glucose was given (2 g/kg body wt) (34), and blood samples for measurements of plasma glucose and insulin were taken at 0, 15, 30, 60, 120, and 180 min. Blood glucose concentrations were determined immediately upon sampling via a glucometer (One Touch Ultra). Animals recovered and were returned to animal housing to await echocardiography at 7 wk and terminal surgery at 8 wk.

Echocardiography. At 7 wk, all animals underwent anesthetized echocardiography to assess ventricular function as previously described (29). Two-dimensional short-axis and long-axis views, M-mode measurements, and Doppler flow measurements were taken via a 15-MHz transducer attached to a Sequoia C256 system. Measurements were used to calculate end-diastolic and end systolic dimensions, areas, and volumes as well as stroke volume, fractional shortening, ejection fraction, cardiac output, cardiac index, and measures of aortic flow. The operator was blinded for all echocardiographic measurements.

PET. After 8 wk, a second group of rats (cohort 2; n = 6–7 rats/group) were fasted for 18 h for cardiac PET. Rats were anesthetized with 2%–2.5% isoflurane in oxygen and positioned with their hearts centered in the field of view of a micro-PET R4 scanner (Siemens) (22). After the induction of anesthesia and before an injection of [18F]2-fluoro-2-deoxy-D-glucose ([18F]FDG, 9.25 MBq, 250 µCi) via the tail vein. After the acquisition of the fasted state scan, a bolus of 0.5 g/kg body wt glucose with 1 U/kg body wt insulin (Novolog) was given, followed by a steady infusion of 0.5 g glucose with 0.6 units insulin per hour via the tail vein. Five minutes after the glucose-insulin bolus, the second emission scan was begun using ~37 MBq (1,000 µCi) [18F]FDG. The steady glucose-insulin infusion was continued over the duration of the insulin-stimulated scan, with blood glucose levels checked at 5, 15, 45, and 90 min by a glucometer (One Touch Ultra) from blood taken from the tail vein catheter. Glucose readings were kept between 200 and 600 mg/dl to maintain the glucose concentration in a standard fed range. If glucose readings exceeded 600 mg/dl, the glucose-insulin infusion was stopped, additional time points were taken, and the glucose-insulin infusion was restarted once measured blood glucose dropped below 600 mg/dl.

For analysis of each scan, dynamic image sequences were reconstructed with 12 frames × 5-s duration, 8 × 30 s, 5 × 60 s, and 16 × 5 min, using software provided by the scanner manufacturer (Siemens). Specifically, a Fourier rebinning and two-dimensional ordered subset expectation maximization algorithm (16 subsets and 8 iterations) were used to reconstruct dynamic image sequences of 128 × 128 × 63 voxels with a spacing of 0.85 × 0.85 × 1.2 mm. Images included correction for radioactive decay, attenuation, random coincidences, scatter, and dead time (14, 50).

PET data analysis. To assess insulin resistance, the metabolic rate of glucose (MRglu) under fasting (first scan) and insulin-glucose-administered (second scan) conditions was calculated. The standard FDG two-tissue compartment model (37) with four rate constants
Terminal hemodynamic measurements and plasma and tissue harvest. At 8 wk, cohort 1 animals were anesthetized, and the right carotid artery and right jugular vein were isolated via a median neck dissection. A 2.0-Fr Millar pressure-volume transducer (SPR-838, Millar Instruments, Houston, TX) was introduced via the right carotid artery into the LV as previously described (27). Heart rate, ventricular volume, peak LV systolic pressure, end-diastolic pressure, \(-\frac{dP}{dt}\), LV stroke work, and power were determined. Volume measures were calibrated via saline boluses through the jugular vein. Measurements were analyzed via PVAN/Chart 5 software (AD Instruments).

After hemodynamic measurements, animals were immediately killed in the fed state (cohort 1) and fed + insulin-stimulated state (cohort 2). A separate group of animals was killed in a fasted state (cohort 3; \(n = 5–6\) rats/group) for tissue harvest and protein expression. Briefly, the heart was exposed via a sternotomy, and venous blood samples were taken from the inferior vena cava. If the animal was insulin stimulated, an injection of 1 U/kg body wt normolog insulin (Henry Schein) was made into the inferior vena cava and allowed to circulate for 5 min before tissue removal. The right ventricle was trimmed from the heart before removal of the LV. Tissue was weighed and then immediately clamp frozen, placed in liquid nitrogen, and stored at \(-80^\circ\text{C}\) until analyzed further. If present, scar tissue was removed from the LV, weighed, and discarded before being clamp frozen.

Tissue and blood metabolic substrate analysis. Insulin levels were measured from serum samples taken from the arterial tail catheter during GTTs using a 96-well format spectrophotometric ELISA assay according to the manufacturer’s suggestion (Mercodia). Terminal blood metabolites including glucose, FFAs, and plasma triglycerides were analyzed as previously described (36). A heparinized glucose sample was also tested via a glucometer (One Touch Ultra) during terminal surgery. LV tissue triglyceride content was performed on extracted tissue homogenates and analyzed via an enzymatic assay (Wako).

Peripheral insulin resistance calculations. Insulin resistance was calculated using both the homeostasis model assessment of insulin resistance (HOMA-IR) and area under the curve (AUC) methods. HOMA-IR was calculated as follows: fasting glucose (in mg/dl) \(\times\) fasting insulin (in \(\mu U/l\))/405 (27). For AUC, GTT glucose concentrations were plotted versus time, and the AUC was calculated from the trapezoidal area fitting to represent total glucose/time (in g/dl \(\cdot\) min \(^{-1}\)).

Western blot analysis of tissue. Protein expression was determined in powdered LV tissue from both insulin-stimulated and fasted animals. Powdered frozen LV tissue was resuspended in homogenization buffer containing 20 mM Tris (pH 7.8), 137 mM NaCl, 2.7 mM KCl, 1 mM MgCl2, 1% (wt/vol) Triton X-100, 10% (wt/vol) glycerol, 1 mM EDTA, 1 \(\mu\)g/ml leupeptin, 1 \(\mu\)g/ml aprotonin, 0.2 mM PMSF, 1 mM \(\beta\)-glycerophosphate, 50 mM NaF, and 1 mM sodium orthovanadate [adapted from Bulhak et al. (5)]. Tissue was homogenized using glass on glass homogenizers and allowed to sit on ice for 30 min. Each sample was then sonicated for 30 s and spun at 5,000 g for 20 min at 4°C. The supernatant was transferred to a clean tube and stored at \(-80^\circ\text{C}\). Protein concentration was determined via a micro-BCA protein assay (Pierce). Fifty milligrams of protein was suspended in standard Laemmli buffer and run on a 10% SDS-polyacrylamide gel (Bio-Rad) and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Protein transfer was verified using Ponceau staining. Membranes were blocked in 5% BSA in Tris-buffered saline with Tween 20 and treated with 1:1,000 dilution of primary antibody overnight. Protein targets included phosphorylated and total forms of Akt and glycosyn synthase kinase (GSK)-3\(\beta\). Phosphorylated protein was determined first, the membrane was stripped (Restore stripping buffer, Pierce) and reblocked, total protein levels were probed, and heat shock complement (HSC)70 (loading control) was then quantified from the same PVDF membrane. Anti-phospho-Akt (Ser473\%), anti-Akt, anti-pGSK-3\(\beta\) (Ser\%, and anti-GSK-3\(\beta\) primary antibodies were from Cell Signaling, and anti-HSC70 (used as a loading control) primary antibody was from Santa Cruz Biotechnology. Target bands were identified using chemiluminescent detection (Supersignal, Pierce). Protein bands on film were quantified using densitometry with the ImageJ program (NIH). For each target of interest, a single gel was run containing all of the samples so as to avoid artifactual differences in band intensities. The Western blot images shown in the figures contained one representative sample from each condition run on a single gel, although these images are provided for reference only and were not used for quantitation.

Statistical analysis. All statistical analyses for metabolic and functional assessments were performed with SigmaStat software. Statistical differences were calculated using two-way ANOVA followed by post hoc analysis for all data except GTTs. Since it is known that both HF and high fat feeding are associated with peripheral insulin resistance and thus affect GTTs (59), one way ANOVA was used to compare experimental groups (sham + SAT, HF + NC, and HF + SAT) versus the sham + NC control group. Data are expressed as group means \(\pm SE\). Significance was established at \(P < 0.05\).

RESULTS

Body and heart weights. Terminal body weights were not different between groups; however, the change in total body weight over 8 wk was significantly greater for both high-fat diet groups (sham + SAT and HF + SAT) compared with normal chow groups (sham + NC and HF + NC; Table 1). LV weights as well as LV weight-to-body weight ratios were significantly increased in both the HF + NC and HF + SAT groups. Scar tissue weights were not different between the HF groups (Table 1).

Cardiac function as assessed by echocardiography. Cardiac function and parameters of remodeling were assessed by echocardiography 7 wk after ligation or sham surgery. End-diastolic and end-systolic areas were both significantly increased in both HF groups, providing evidence of ventricular remodeling after ligation surgery. Areas of fractional shortening were significantly decreased in HF versus sham groups. High-fat diet alone had no effect on echocardiographic measures within the sham or HF groups (Table 1).

Tissue and plasma substrates. Plasma and LV tissue samples were taken from fed animals 8 wk after ligation or sham surgery. Plasma glucose concentrations were significantly lower in HF + SAT animals versus HF + NC animals. Plasma FFAs and triglycerides were significantly higher in both SAT groups compared with NC groups. Tissue triglycerides were elevated in HF + SAT animals compared with both their dietary (HF + NC) and surgical (sham + SAT) controls. LV triglycerides were also elevated by high fat feeding in sham + SAT animals compared with sham + NC animals (Table 2).
In vivo hemodynamic measurements. In vivo cardiac function was measured using a pressure-volume catheter at the time of terminal surgery, 8 wk after ligation or sham surgery. Systolic contractility, as assessed by +dP/dt, was depressed in HF + NC animals. In contrast, +dP/dt was significantly improved in the HF + SAT group compared with the HF + NC group (Fig. 1A), with no difference compared with the sham + SAT group. LV stroke work was significantly depressed in both HF groups compared with their sham controls, but HF + SAT animals had significantly improved LV stroke work compared with the HF + NC group (Fig. 1B). −dP/dt, a measure of diastolic function, was depressed with ligation surgery but was improved in the HF + SAT group compared with the HF + NC group (Fig. 1C). In addition, the time constant of relaxation (τ), a measure of diastolic relaxation time, was lengthened in HF + NC but not HF + SAT animals (Fig. 1D). Other hemodynamic measurements, including heart rate, arterial pressure, and end-diastolic pressure, were not different among groups (data not shown).

Assessment of peripheral insulin resistance. GTTs were performed on restrained, conscious animals (cohort 1) 5 wk after infarction or sham surgery. As HF and high dietary fat are associated with peripheral insulin resistance (19, 24, 34, 38, 57), the sham + NC group served as the primary control group to which all groups were statistically compared. As shown in Fig. 2A, HF + SAT animals had elevated blood glucose levels at 30, 60, and 120 min versus sham + NC animals. AUCs were also calculated for all groups, and sham + SAT, HF + NC, and HF + SAT animals had increased AUCs compared with sham + NC animals (Fig. 2B).

Fasting and peak insulin blood samples were taken at 0 and 30 min of the GTT, respectively. A trend of increased insulin concentration with high fat feeding was seen at 30 min (Fig. 2C), but it was not significant. The HOMA-IR score, which takes into account fasting glucose and fasting insulin concentrations, again suggested a trend of increased insulin resistance with high fat feeding, but there were no significant differences between sham and HF groups (Fig. 2D).

Assessment of myocardial insulin resistance. A separate cohort of animals (cohort 2) underwent PET with [18F]FDG 8 wk after sham or ligation surgery. PET was performed to assess cardiac glucose uptake at a baseline (fasting) time point, followed by insulin and glucose stimulation, to monitor cardiac glucose uptake. No glucose uptake was noted in scar tissue (as to be expected in nonviable tissue); thus, the volume of the scar was not included in the analysis. Average glucose uptake traces are shown for basal (Fig. 3A) and insulin-stimulated (Fig. 3C) conditions. Because FDG is phosphorylated and trapped in the intracellular compartment, its uptake is cumulative, and the final time point (90 min) specific uptake value (SUV) for each scan was used to calculate total glucose uptake in each animal. Ninety-minute SUVs are shown for basal (Fig. 3B) and insulin-stimulated (Fig. 3D) animals, and no statistical differences between SUVs were seen. Representative emission scan images compiled after insulin and glucose stimulation in sham + NC, sham + SAT, HF + NC, and HF + SAT animals are shown in Fig. 4.

In addition to SUVs, myocardial insulin responsiveness was gauged by determining the ratio of the insulin-stimulated SUV to basal SUV, as shown in Fig. 5A. SUV ratios in HF + SAT animals were decreased compared with HF + NC animals, suggesting decreased insulin-responsive glucose uptake in HF + SAT animals (Fig. 5A). As detailed in MATERIALS AND METHODS, the standard FDG kinetic model was used to estimate MR_{glu} values. The ratio of MR_{glu} (insulin/MR_{glu} (basal)) was used to assess the ability of the animal to increase MR_{glu} when
challenged with an insulin stimulus (Fig. 5B). HF + SAT animals had a decrease in the MR$_{glu}$ ratio compared with sham + SAT animals, but HF itself did not affect MR$_{glu}$ (Fig. 5B).

Protein expression and phosphorylation of insulin signaling targets. Expression levels of proteins important in the insulin signaling pathway were analyzed by Western blot analysis in both basal (fasted; cohort 3) and insulin-stimulated (cohort 2) LV tissues. Total protein levels were normalized to HSC70, which was chosen as a loading control since both actin and GAPDH are known to be altered in HF and by diet (28, 44). Akt is a key signaling molecule in the insulin signaling pathway. At baseline in the fasted state, the ratio of phospho-Akt to total Akt was decreased in sham + SAT and HF + NC versus sham + NC animals (Fig. 6B). The basal phospho-Akt-to-Akt
ratio in HF + SAT animals was not significantly different than sham + SAT or HF + NC animals (Fig. 6B). Total Akt protein levels were elevated in sham + SAT and HF + NC animals compared with sham + NC animals at baseline, with no difference seen in HF + SAT animals (Fig. 6B). In insulin-stimulated animals, there were no significant differences in either the phospho-Akt-to-total Akt ratio or total Akt protein (Fig. 6C), although there was a trend for a decreased phospho-Akt-to-total Akt ratio in insulin-stimulated HF + SAT animals (Fig. 6C). Representative Western blot results are shown in Fig. 6A.

The downstream signaling target GSK-3β was also analyzed in LV tissue from basal and insulin-stimulated animals. At baseline in the fasted state, there were no differences in the ratio of phospho-GSK-3β to total GSK-3β (Fig. 6B), but there were significantly higher levels of total GSK-3β protein seen in HF animals compared with sham control animals (Fig. 6B). There was also a significant increase in total GSK-3β protein in basal HF + SAT animals versus HF + NC animals (Fig. 6B). Similar to the basal state, the insulin-stimulated state produced no changes in the ratio of phospho-GSK-3β to total GSK-3β (Fig. 6C), but an increase in total GSK-3β was seen in HF animals compared with sham animals and in insulin-stimulated HF + SAT animals versus HF + NC animals (Fig. 6C). Representative samples are shown in Fig. 6A (note that the images are representative only; phospho-GSK-3β was present in basal samples used for quantitation, but a lesser exposure is shown here to clearly demonstrate insulin-stimulated results).

DISCUSSION

The novel finding of this study is that preservation of LV systolic and diastolic function in response to high saturated fat feeding in HF is associated with myocardial insulin resistance and alterations in insulin signaling. Although numerous studies have proposed a link between peripheral insulin resistance and myocardial dysfunction, our data refute this assertion given that diminished peripheral glucose uptake under conditions of high fat feeding (sham + SAT), HF (HF + NC), and the combination of both (HF + SAT) were not correlated with myocardial contractile function. Alterations in cardiac insulin signaling in the HF + SAT group, specifically, diminished activation of Akt and increased total GSK-3β, may contribute to the myocardial insulin resistance that is associated with improvements in contractile function. These results suggest that myocardial insulin resistance may serve a cardioprotective role relative to contractile function with high fat feeding in mild to moderate HF.

Individually, HF and a high-fat diet have been shown to result in peripheral insulin resistance, but the relationship between peripheral and myocardial insulin resistance remains undefined. While some models manifest both peripheral and myocardial insulin resistance (24, 34, 38), a synchronization of these two conditions cannot be assumed (19, 57). Studies (1, 9, 18, 55) have shown that the degree of peripheral insulin resistance is predictive of HF functional class, although a cause-and-effect relationship has not been established. While other studies have described insulin resistance in either a high-fat diet or cardiac dysfunction, no study to date has examined the impact of high fat feeding and HF in combination on peripheral and myocardial insulin resistance.

Peripheral insulin resistance has previously been reported in high-fat diet and HF independently (19, 24, 34, 38, 57), and, based on these findings, we anticipated a synergistic effect of combined high-fat diet and HF. GTTs demonstrated the expected decrease in glucose clearance with HF and high-fat diet individually. However, the HF + SAT group demonstrated an...
equivalent reduction in glucose clearance compared with sham + SAT and HF + NC groups. This suggests that HF and high-fat diet individually induce peripheral insulin resistance, with no additive or synergistic effect of HF and high-fat diet combined. While GTTs are commonly considered to be a good clinical indicator of peripheral insulin resistance, more rigorous tests, such as 2-deoxyglucose uptake in peripheral tissue, glucose-clamp experiments, and pancreatic islet evaluations, are considered the gold standard for assessing peripheral insulin resistance. However, since no differences were seen with dietary intervention between the two HF groups, a decrease in peripheral glucose clearance cannot account for the preserved contractile function we observed in the HF + SAT group. Therefore, for the purposes of this study, we did not pursue further evaluations of peripheral insulin resistance.

PET imaging provided a noninvasive evaluation of in vivo cardiac glucose uptake in real time under basal and insulin-stimulated states. Both SUV ratios and MR_{glu} data from PET imaging demonstrated that HF + SAT animals were less sensitive to insulin stimulation compared with sham + NC, sham + SAT, or HF + NC animals. Interestingly, although both sham + SAT and HF + SAT animals had elevated circulating lipids, only HF + SAT animals showed myocardial insulin resistance, suggesting that the decreased cardiac glu-

![Fig. 4. Representative reconstructed images of cardiac PET imaging from sham + NC (A), sham + SAT (B), HF + NC (C), and HF + SAT (D) animals under insulin + glucose-stimulated conditions. Notice the lack of enhancement over the area of the LV scar in HF + NC and HF + SAT animals.]

![Fig. 5. Insulin sensitivity measurements calculated from PET experiments. A: ratio of 90-min insulin-stimulated SUV divided by 90-min basal SUV. B: glucose metabolic rate ratio calculated from blood glucose concentrations and kinetic constants as described in MATERIALS AND METHODS. *P < 0.05 vs. the dietary control group; †P < 0.05 vs. the surgical control group.]

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cose uptake seen in HF + SAT animals cannot be attributed to the Randle phenomenon (39). Myocardial insulin resistance in HF + SAT animals provides a potentially novel explanation for the preserved contractile function observed with high fat feeding in HF. Several mechanisms could account for the decreased insulin-stimulated cardiac glucose uptake seen with PET in HF + SAT animals. Impaired glucose transport and metabolic substrate inhibition could act as mediators of decreased glucose uptake and oxidation; however, both of these potential mechanisms are regulated upstream by the insulin signaling cascade. Therefore, several key protein targets of insulin signaling were further evaluated.

Phosphorylated and total protein populations of Akt and GSK-3β were used to evaluate the insulin signaling cascade. Akt controls the action of a number of targets involved in cell survival, growth, cytokine, and integrin-linked signaling pathways, but its activation by kinases downstream of the insulin receptor is the main signal for the role of phospho-Akt in regulating cellular metabolism. The metabolic signaling targets of phospho-Akt inhibit gluconeogenesis and activate glucose uptake as well as glycogen, lipid, and protein synthesis. In this study, signaling through Akt is altered in the basal (fasted) state of the heart. The phospho-Akt-to-total Akt ratio in the basal state was decreased with both high-fat diet and HF, although there was no additive effect seen in HF + SAT animals. This suggests less of the Akt pool is active in the presence of high-fat diet and HF, implying decreased downstream insulin signaling under basal conditions. Other studies have shown similar decreases in phospho-Akt with high-fat diet (34) and HF (31), but this study is the first to show a derangement in phospho-Akt to total Akt ratio, which would agree with the in vivo PET data, suggesting that HF +
SAT animals cannot respond to insulin stimulation as well as the other groups. GSK-3β was also chosen as a protein target of interest, as phosphorylation by Akt prevents GSK-3β inhibition of glycogen synthase. Thus, decreased Akt signaling (such as occurs with insulin resistance) decreases glycogen synthesis. GSK-3β can also phosphorylate insulin receptor substrate-1, blocking the ability of insulin receptor signal and exacerbating insulin resistance (12). The critical role of this regulatory protein has made it an attractive clinical target for the treatment of insulin resistance (17). Despite the ratio of phospho-GSK-3β to total GSK-3β in both basal and insulin-stimulated conditions being unchanged, total GSK-3β was increased with HF, and HF + SAT animals had significantly more GSK-3β than HF + NC animals. An increase in GSK-3β is seen with a peripheral insulin-resistant phenotype (32), and our results are consistent with insulin resistance in the LV tissue of HF + SAT animals.

One of the traditional therapeutic strategies for the treatment of patients suffering from acute myocardial infarction involves insulin stimulation to improve the delivery and utilization of glucose in myocardial tissue. The clinical practice of infusing glucose, insulin, and potassium after myocardial infarction (GIK therapy) was initially thought to be beneficial to long-term patient survival (49), but a meta-analysis of clinical studies has shown that prophylactic GIK therapy can actually be detrimental (or at best neutral) in terms of clinical outcomes (26). Similarly, considerable attention has recently been focused on clinical studies reporting that insulin-sensitizing drugs (e.g., thiazolidinediones, which are commonly prescribed for type II diabetic patients) have been linked to increases in cardiovascular events and mortality (16). Studies in a variety of animal models have also reported similar findings in that excessive cardiac insulin signaling exacerbates systolic dysfunction (48) and promotes pathological hypertrophy (46). Taken together, these clinical and animal studies suggest that increasing the heart’s exposure and sensitivity to insulin in the face of cardiac pathology may be detrimental to long-term morbidity and mortality. Our results go one step further and suggest that cardiac insulin resistance may be beneficial in mild to moderate HF in the presence of elevated dietary lipids.

Under conditions of myocardial insulin resistance, a decrease in glucose uptake and oxidation would be associated with an increased oxidation of fatty acids, as suggested by the Randle hypothesis (39). Thus, HF animals fed a high-fat diet would be forced to use the most abundant fuel (fatty acids), and this may direct intracellular fatty acids to productive pathways such as β-oxidation and triglyceride synthesis instead of the formation of lipotoxic intermediates. Essentially, these results argue against the hypothesis that switching myocardial substrate metabolism from fatty acids to glucose would be beneficial to the failing heart (26, 45). In fact, another study (36) has suggested that depriving the failing heart of fats can be detrimental in terms of cardiac work, and increased fatty acid oxidation has been associated with preserved contractile function (8). Directing an excess supply of lipids toward triglyceride synthesis and storage has also been shown to limit cellular toxicity (25), which supports the idea that increased tissue triglyceride levels reported in HF + SAT animals may be a protective mechanism. Taken together, these results suggest that myocardial insulin resistance may inhibit glucose utilization and force the heart to channel excess FFAs to productive metabolic pathways that include β-oxidation and triglyceride synthesis.

Overall, our findings suggest a role for myocardial insulin resistance in the preservation of cardiac contractile function seen in HF animals fed a high-fat diet. In our model, we report that diminished activation of key insulin signaling targets is associated with preserved contractile function. However, it is important to acknowledge that in cardiomyocyte-specific insulin receptor knockout mice (a model lacking functional cardiac insulin receptors), ventricular function was impaired both at steady state (4) and after myocardial infarction (47) compared with wild-type controls. While cardiac insulin receptors are functionally absent in these knockout animals, basal rates of glucose uptake and oxidation are increased, whereas fatty acid oxidation is decreased (4), suggesting that noninsulin-dependent mechanisms of glucose regulation may be operational in this model.

It is important to recognize that HF is a complex condition, and myocardial insulin resistance is potentially only one of many mechanisms that may play a role in our model of high fat feeding in HF. Future studies will examine changes in Ca^{2+} handling and isolated myocyte/myofibrillar contractile properties at the cellular level and O_2 consumption and glucose/fatty acid oxidation at the ex vivo organ level. While our rodent coronary artery ligation model of injury mimics human myocardial infarction-based HF, there are distinct differences between these two models of HF. For example, use of coronary ligation to induce HF results in a localized area of ischemic tissue that eventually becomes nonmetabolizing scar tissue. However, the remaining ventricle is not subject to ongoing ischemia and is therefore not O_2 limited. Thus, an increased requirement for O_2 necessitated by increased fatty acid oxidation is likely not a limiting factor in our model. However, other models (e.g., microemobilization-induced HF) may be subject to limitations in O_2 supply, and, under these conditions, high fat feeding could possibly prove to be antiprotective due to alterations in myocardial efficiency.

The present study is the first to suggest that myocardial insulin resistance may play a role in cardioprotection during mild to moderate HF in the presence of a high-saturated fat diet. Interestingly, our data does not support a link between peripheral and myocardial insulin resistance, nor does it show a correlation between peripheral insulin resistance and cardiac function. These associations had been reported in previous studies that focused on either HF or high dietary fat, whereas in this study the combination of the two variables has been shown to differentially influence pathological outcomes. Future studies will examine whether the insulin resistance present in HF + SAT animals results in increased fatty acid oxidation at the expense of glucose oxidation and whether it is this increase in metabolic flexibility that spares the HF + SAT heart from the depressed cardiac function reported in the HF + NC heart. In summary, the present study illustrates that myocardial insulin resistance is associated with preserved cardiac contractile function in HF animals fed high saturated fat, and the alterations seen in the myocardial insulin signaling cascade provide a potential mechanism for metabolic flexibility in the failing heart.
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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