Myocardial infarction in rats causes partial impairment in insulin response associated with reduced fatty acid oxidation and mitochondrial gene expression

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Objective: Myocardial infarction leads to contractile dysfunction. In patients with diabetes, impaired contractility has been associated with the loss of insulin effects and mitochondrial dysfunction. We assessed cardiac insulin sensitivity and mitochondrial and contractile function in rats after ligation of the left coronary artery.

Methods: At 2 weeks after left coronary artery ligation, we performed echocardiography in vivo and assessed the substrate use and insulin response in the isolated working heart and the regulation of insulin (Akt, glucose transporter type 4) and mitochondrial signaling (p38 mitogen-activated protein kinase, peroxisome proliferator-activated receptor-γ coactivator 1α, mitochondrial transcription factor A) using polymerase chain reaction and Western blotting.

Results: The infarcted hearts were dilated and had a reduced ejection fraction (ejection fraction < 50%). The basal glucose oxidation was preserved, but the fatty acid oxidation was significantly reduced. Insulin's effect on substrate oxidation was significantly impaired for both the decrease in fatty acid oxidation and the increase in glucose oxidation. However, insulin-stimulated glucose uptake was normal in the infarcted hearts, consistent with normal insulin-induced phosphorylation of Akt and unchanged mRNA expression of glucose transporter type 4. The impaired oxidative response to insulin was associated with reduced mRNA expression of the genes regulating fatty acid oxidation (long-chain-acyl-coenzyme A dehydrogenase, carnitine palmitoyltransferase 1, peroxisome proliferator-activated receptor-α) and mitochondrial biogenesis (mitochondrial transcription factor A). Although mRNA expression of the mitochondrial master regulator peroxisome proliferator-activated receptor-γ coactivator 1α was normal in the infarcted hearts, the protein expression of its post-transcriptional activator, p38 mitogen-activated protein kinase, was significantly reduced.

Conclusions: Myocardial infarction in rats caused partial insulin resistance at the level of substrate oxidation, which was associated with mitochondrial and cardiac contractile dysfunction. Mitochondrial dysfunction was characterized by a reduced capacity to oxidize fatty acids and might have resulted from impaired mitochondrial biogenesis through the lack of p38 mitogen-activated protein kinase. (J Thorac Cardiovasc Surg 2010;140:1160-7)

Myocardial infarction is the main cause of heart failure in the Western world.[1] The onset of heart failure after infarction is thought to occur because of a series of cellular changes leading to cardiac dysfunction, a process termed "remodeling."[2] Although this concept has been long known, the mechanisms involved in this setting are still unclear. Different mechanisms have been suggested as a cause of cardiac dysfunction. Several investigators have described "energy-starvation" concepts, implying the lack of adenosine triphosphate as a determinant of pump failure.[3,4] These concepts brought the focus to the adenosine triphosphate-generating machinery of the cell (ie, the mitochondria) and the components regulating its function.

Insulin, in addition to affecting substrate selection, can influence mitochondrial biogenesis and function. The link between the loss of insulin's effects and impaired heart function has received specific attention with the recognition of diabetic cardiomyopathy.[5] Studies in mouse models with a specific knockout of the cardiac insulin receptor (CIRKO mice) showed mitochondrial dysfunction and cardiomyopathy, suggesting cardiac insulin resistance as a potential mechanism for contractile dysfunction.[6] However, whether cardiac insulin resistance is involved in infarction-induced contractile dysfunction is unknown. Therefore, we assessed

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Abbreviations and Acronyms

CPT-1 = carnitine palmitoyltransferase 1
GLUT4 = glucose transporter type 4
MAPK = mitogen-activated protein kinase
LCAD = long-chain-acyl-coenzyme A dehydrogenase
PGC-1α = peroxisome proliferator-activated receptor-γ coactivator 1α
PPARα = peroxisome proliferator-activated receptor-α
Tfam = mitochondrial transcription factor A

the cardiac insulin response and cardiac contractile and mitochondrial function in a rat model of myocardial infarction.

MATERIALS AND METHODS

Materials

The chemicals were obtained from Sigma Aldrich (Deisenhofen, Germany), Merck (Darmstadt, Germany), Serva (Heidelberg, Germany), Essex (München, Germany), Bayer (Leverkusen, Germany), Narkodorm-n (Neumünster, Germany), Cell Signalling (Frankfurt am Main, Germany), and Bio-Rad Laboratories (München, Germany).

Animals and Surgical Interventions

Male Sprague-Dawley rats (weight 200–300 g) were obtained from Charles River Laboratories (Sulzfeld, Germany) or from the animal facility of the University of Leipzig and were fed ad libitum with free access to water at 21°C with a light cycle of 12 hours. The Animal Welfare Committee of the University of Leipzig (Leipzig, Germany) approved the use of the rats and the experimental protocols.

The infarct model in rats has been previously described in detail.[7] To induce myocardial infarction, the rats were anesthetized with fentanyl, midazolam, and medetomidin (0.005 mg fentanyl/kg, 2 mg midazolam hydrochloride/kg, and 0.15 mg medetomidin hydrochloride/kg intramuscularly), intubated with 16-gauge tubing, and ventilated with room air (1 mL/100 g, 96 breaths/min). Lateral thoracotomy was performed, and, after visualization of the heart, the pericardium was gently open. The left coronary artery was localized using the pulmonary artery and the left atrium as anatomic markers. A 6-0 Prolene suture line was then used to ligate the coronary. After the thorax and skin were closed, the rats were extubated and kept on warming pads for the recovery periods. Age-matched, sham-operated rats that had undergone the same procedure without coronary occlusion were used as the control group.

After 2 weeks, echocardiography was conducted, and the hearts were harvested and used for the isolated working heart perfusion experiment (set 1), insulin stimulation ex vivo (set 2), or polymerase chain reaction and Western blot assessment (set 3). For sets 2 and 3, the hearts were excised and weighed. The scar tissue was macroscopically resected and weighed before the hearts were snap-frozen in liquid nitrogen. The scar areas were determined as the percentage of scar weight in relation to the heart weight. The scar area was not used for additional analysis.

Echocardiography

The rats were anesthetized with fentanyl, midazolam, and medetomidin, their chests were shaved, and they were examined in the supine position with a 12-MHz phased array transducer (Agilent/Philipps, Hamburg, Germany). Two-dimensional, parasternal, short-axis echocardiograms were obtained from the left ventricle at the papillary muscle level. The fractional shortening was determined according to Teichholz and colleagues,[8] and the ejection fraction was calculated according to Simpson's rule. Echocardiographic examinations were performed in 9 rats from the infarct group and 10 rats from the control group.

Isolated Working Heart Perfusion

The preparation for the isolated working heart perfusion has been previously described in detail.[9,10] The rats were anesthetized with sodium pentobarbital (5 mg/100 g body weight intraperitoneally). After injection of heparin (200 IU) into the inferior vena cava, the heart was rapidly removed and placed in ice-cold Krebs-Henseleit bicarbonate buffer. The aorta was cannulated, and hearts were perfused as working hearts at 37°C, with recirculating Krebs-Henseleit buffer (200 mL) containing 1% bovine serum albumin, Cohn fraction V, fatty acid free (Celliance, Toronto, Ontario, Canada). The perfusate calcium concentration was 2.5 mM. The hearts were perfused with glucose (5 mM) and oleate (0.4 mM) as substrates. The perfusate was gassed with 95% oxygen and 5% carbon dioxide, and recirculated. All experiments were performed with a preload of 15 cm H_2O and an afterload of 100 cm H_2O. After stabilization, the hearts were perfused for 30 minutes, followed by an insulin addition (1 mU/mL), and another 30 minutes of perfusion. In this period, all samples were withdrawn and the measurements performed. The heart rate and systolic and diastolic aortic pressure were measured continuously using a Hugo Sachs transducer and recording system (TAM-A type 705/1; Hugo Sachs Elektronik, March-Hugstetten, Germany). The heart rate was measured as the beats per minute and the cardiac output as millimeters per minute. The cardiac power was determined as follows: [flow (mL/min) $\times$ pressure (100 cm H_2O)]/612 (correction factor).

Samples of the coronary effluent (2 mL) were withdrawn every 5 minutes for the assessment of glucose uptake and oxidation and fatty acid oxidation. The different rates were determined from the production of 14CO2 from [U-14C] glucose and 3H2O from [9,10-3H] oleate or [2-3H] glucose. These radioactive substrates allow the precise determination of glucose uptake ([2-3H] glucose), glucose oxidation ([U-14C] glucose) and fatty acid oxidation ([9,10-3H] oleate). Glucose and fatty acid oxidation were determined simultaneously during 10 heart perfusions for the control group and 9 for the infarct group. Glucose uptake was assessed in a separate set of perfusions (n = 5 in each group).

Quantitative Real-Time Polymerase Chain Reaction

Myocardial mRNA was isolated from frozen tissue samples using the Qiagen RNeasy mini kit. Synthesis of complementary DNA was performed with the cDNA synthesis kit from Fermentas (St. Leon-Rot, Germany), and TaqMan quantitative real-time reverse transcriptase-polymerase chain reaction was performed using AmpliTaq Gold (ABI; Applyed Bio Systems, Darmstadt, Germany), with the conditions suggested by the manufacturer, on the ABI 7900 HT, as previously described.[11] Forward and reverse primers were designed using the Universal Probe Library Assay Design Center. For each set of primers, a basic local alignment search tool search revealed that sequence homology was obtained only for the target gene. The reaction mixture consisted of 1 µL diluted template, 0.025 U/µL Taq Polymerase, 0.01 U/µL AmpErase, 5.5 mM $MgCl_2$, 200 µM dNTP mix, 1$\times$ TaqMan buffer A, 200 nM forward and reverse primers, and 100 nM probe. The results were normalized to S29 transcription as a housekeeping gene product, which was not different by experiment among all samples. The numbers of rats included ranged from 7 to 11 in each group.

Insulin Stimulation Ex Vivo

The hearts were harvested according to the protocol for the isolated working heart. After cannulation of the aorta, the hearts were retrogradely perfused with Krebs-Henseleit buffer at 37°C containing 5 mM glucose in the presence or absence of insulin (1 mU/mL) for

TABLE 1. Heart, lung, and body weights and fractional scar content of rats 2 weeks after left coronary artery ligation or sham operation

Variable	Sham group	Infarct group	*P* value
BW (g)	287 ± 5	308 ± 10	.05
HW (mg)	1513 ± 65	1639 ± 108	.31
HW/BW ratio	6.58 ± 0.07	6.23 ± 0.18	.77
LW/BW ratio	4.28 ± 0.10	4.22 ± 0.10	.68
Scar area (%)	NA	7.20 ± 0.74	NA

Data are presented as mean ± standard error of mean; n = 6–10/group. Scar area determined by macroscopically excising scar tissue and weighing it in relation to heart weight. *BW,* Body weight; *HW,* heart weight; *HW/BW,* heart weight/body weight; *LW/BW,* lung weight/body weight; *NA,* not applicable.

TABLE 2. Cardiac size and function 2 weeks after left coronary artery ligation

Variable	Sham group (n = 6–10)	Infarct group (n = 6–10)	*P* value
Heart rate (beats/min)	270 ± 6	283 ± 8	.21
LVEDD (mm)	7.33 ± 0.16	9.62 ± 0.17	< .001
LVPWD (mm)	1.86 ± 0.18	1.75 ± 0.17	.66
LVPWS (mm)	2.72 ± 0.14	2.36 ± 0.08	< .001
Ejection fraction (%)	68.0 ± 2.6	36.1 ± 3.1	< .001
Fractional shortening (%)	41.4 ± 1.4	18.5 ± 1.8	.04
Cardiac power ex vivo (mW/g dry)	31.6 ± 1.8	22.9 ± 3.8	.03

Data are presented as mean ± standard error of mean. *LV,* Left ventricle; *LVEDD,* LV end-diastolic diameter; *LVPWD,* LV posterior wall in diastole; *LVPWS,* LV posterior wall in systole.

5 minutes and then immediately snap-frozen for analysis by Western blotting.

Immunoblotting

Western blots were performed on a semidry Western blot apparatus. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the proteins were blotted to a polyvinyldine diflouride membrane and incubated with primary and secondary antibodies. The bands were visualized and semiquantified by chemiluminescence using AIDA software. At the end, the membrane was dyed with naphthol blue black solution to visualize the proteins and then used to normalize the band intensity. For Western blotting, the number of rats used was 8 for each group.

Statistical Analysis

The data are presented as the mean ± standard error of the mean. The data were analyzed using one-way analysis of variance or Student's *t* test, as appropriate. Post hoc comparisons among the groups were performed using the Holm-Sidak test.

RESULTS

Table 1 lists the heart, lung, and body weight of the infarcted and sham-operated groups. The rats with infarction weighed 7% more than the sham-operated rats. The heart weights were not significantly different between the 2 groups. The heart/body weight ratios were also normal in the infarct group, and no evidence was seen of pulmonary edema or significant pulmonary congestion, as demonstrated by the normal lung/body weight ratios. The macroscopically identified scar area caused by left coronary artery ligation was similar among the rats, with values of 5.6% to 9.8% of the total heart weight. The mortality after left coronary artery ligation was 17%. Of those that died, 12% had died perioperatively and the other 5% during the 2-week follow-up period.

Table 2 lists the echocardiographic parameters of the infarcted and sham-control groups. No difference was seen in the heart rate between the infarcted and control rats. Myocardial infarction led to significant dilation of the left ventricle, as demonstrated by a significant increase in the left ventricular end-diastolic diameter. The infarcted rat hearts had contractile dysfunction with a reduced left ventricular posterior wall at systole, ejection fraction, and fractional shortening (14%, 52%, and 45% less than that of the control rats, respectively). Ex vivo evaluation of cardiac function in the isolated working heart system also revealed impaired cardiac contraction (45% lower than in the sham-operated groups). No evidence of hypertrophy was seen 2 weeks after infarction, as demonstrated by the normal left ventricular posterior wall in diastole and heart/body weight ratios.

Figure 1 shows the substrate oxidation rates in the isolated working heart in the basal state and after insulin addition. Basal glucose oxidation was normal after infarction. However, fatty acid oxidation was significantly reduced at the basal levels. The insulin-induced stimulation of glucose oxidation and inhibition of fatty acid oxidation were significantly blunted at 2 weeks after infarction. The impairment in insulin response was evident from both the effect on the absolute values and the percentage of basal rates. In contrast to the oxidation rates, glucose uptake was normal both before and after insulin stimulation (Table 3). No evidence was seen for a lack of insulin response at this level, whether the insulin-induced increase was expressed in absolute values or as a percentage of the baseline values.

Insulin's effect on glucose uptake is mainly mediated through Akt-stimulated glucose transporter type 4 (GLUT4) translocation.[12] Akt is also a mediator of hypertrophy.[13] Akt can be activated through phosphorylation at serine 473 and/or threonine 308 in response to insulin or hypertrophic stimuli.[14] Figure 2 shows the total Akt expression and the phosphorylated fraction of Akt, with or without insulin stimulation, at both Akt phosphorylation sites. The total Akt level was normal in the infarcted hearts. Consistent with the lack of hypertrophy and the normal glucose uptake in the infarcted hearts, the basal and insulin-induced phosphorylation were the same in infarcted hearts and in those from the control group.

Figure 3 shows the mRNA expression of the genes involved in substrate use and the regulation of mitochondrial biogenesis. Although GLUT4 expression was normal

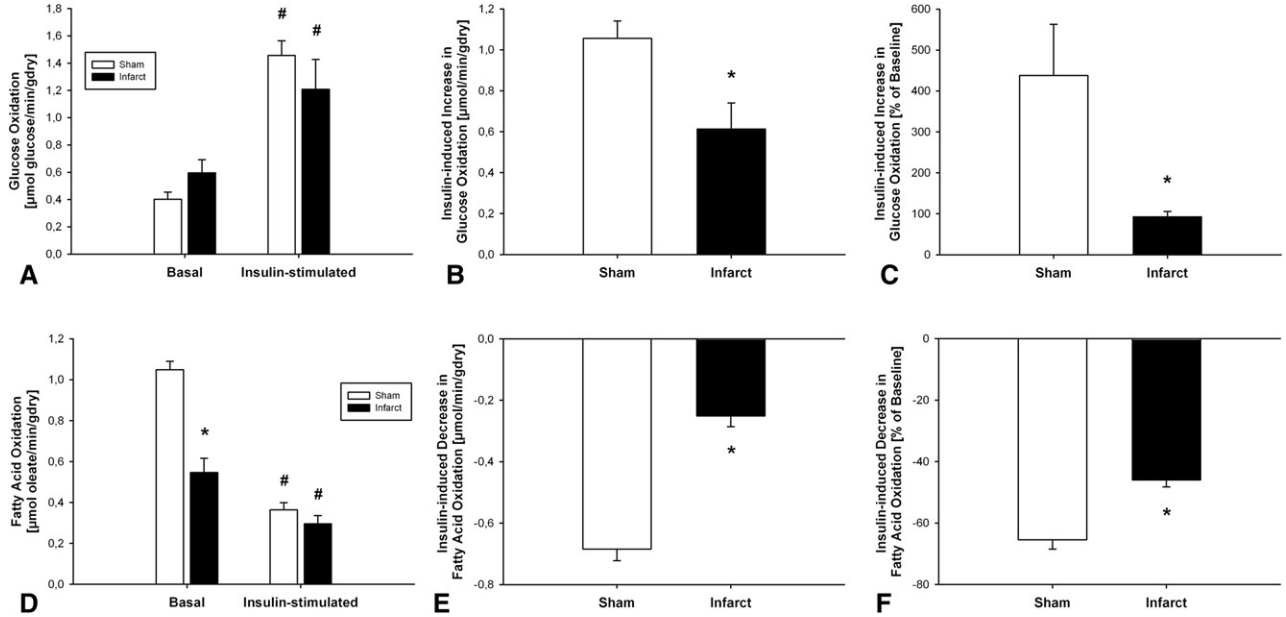

FIGURE 1. Substrate oxidation rates of isolated working rat hearts from rats that underwent left coronary artery ligation or a sham procedure and their response to insulin. A, Rates of basal and insulin-stimulated glucose oxidation. Insulin-stimulated increase in glucose oxidation as B, absolute values and C, percentage of basal rates. D, Rates of basal and insulin-stimulated fatty acid oxidation. Insulin-stimulated decrease in fatty acid oxidation as E, absolute values and F, percentage of baseline. *Open bars* indicate sham control group; *solid bars,* infarct group. Values presented as mean ± standard error of mean; n = 5 to 7 per group. **P* < .05 compared with control, #*P* < .05 compared with basal values of same group.

(Figure 3, *A*), a significant reduction was seen in the mRNA expression of carnitine palmitoyltransferase 1 and long-chain-acyl-coenzyme A dehydrogenase (Figure 3, *B* and *C*), which regulate mitochondrial fatty acid uptake and oxidation, respectively. These alterations are consistent with the reduced fatty acid oxidation rates of the infarcted hearts when perfused as isolated working hearts (Figure 1). Impaired fatty acid oxidation was also supported by the reduced expression of peroxisome proliferator-activated receptor-α (PPARα) (Figure 3, *D*), the main transcription factor for all fatty acid oxidation genes (including long-chain-acyl-coenzyme A dehydrogenase). The expression of mitochondrial transcription factor A (Tfam) (Figure 3, *E*), the transcription factor regulating the expression of the mitochondrial genome (exclusively genes for the respiratory chain), was also significantly reduced. PPARα and Tfam are both targets of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), a metabolic co-activator and master mitochondrial regulator.[15] PGC-1α is regulated at both transcriptional and post-transcriptional levels. Figure 3, *F* shows that mRNA expression of PGC-1α was normal 2 weeks after infarction. However, Figure 4 reveals a significant decrease in protein expression of p38 mitogen-activated protein kinase (MAPK), a post-transcriptional activator of PGC-1α.[16]

TABLE 3. Glucose uptake of infarcted isolated working rat hearts 2 weeks after left coronary artery ligation with or without insulin stimulation

Glucose uptake	Sham group (n = 5)	Infarct group (n = 5)	*P* value
Basal (µmol/min/g dry)	1.62 ± 0.22	2.36 ± 0.70	.35
Insulin-stimulated (µmol/min/g dry)	3.36 ± 0.38	3.46 ± 0.93	.92
Change			
Absolute value (µmol/min/g dry)	1.73 ± 0.29	1.09 ± 0.48	.29
%	113 ± 24	68 ± 22	.21

Data are presented as mean ± standard error of mean.

DISCUSSION

We have demonstrated that myocardial infarction in rats causes partial insulin resistance at the level of substrate oxidation in the remote myocardium. This cardiac insulin resistance is associated with mitochondrial and contractile dysfunction. Mitochondrial dysfunction is characterized by reduced fatty acid oxidation capacity and might be due to impaired mitochondrial biogenesis through a lack of p38 MAPK. The results suggest a role for cardiac insulin resistance and mitochondrial defects in ventricular remodeling and contractile dysfunction after myocardial infarction. If true, treatment of cardiac insulin resistance might improve contractile function and thereby help improve cardiac surgery outcomes because the preoperative ejection fraction is inversely correlated with perioperative mortality.[17] Several aspects of our findings require additional discussion.

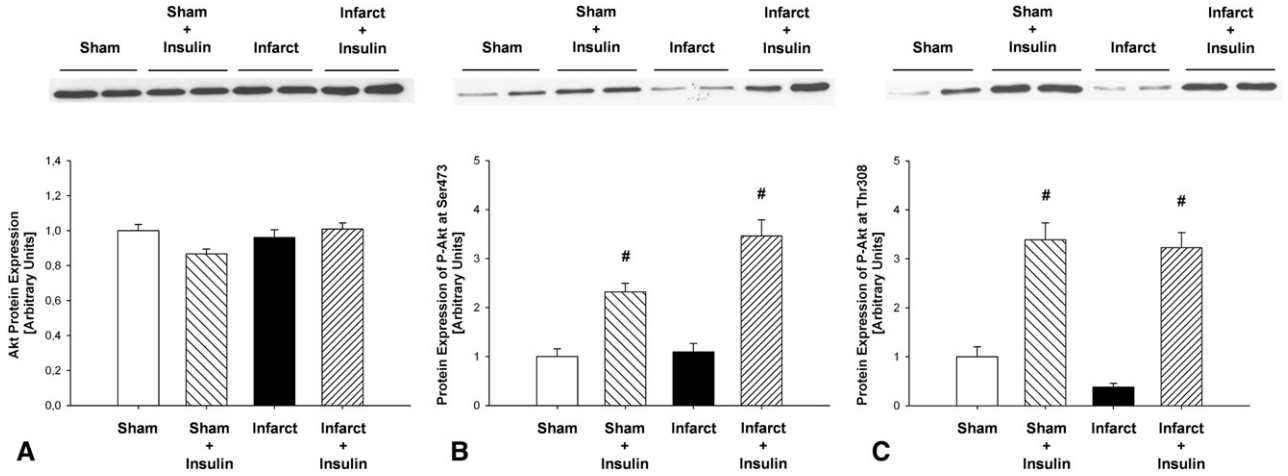

FIGURE 2. Basal and insulin-stimulated phosphorylation of Akt in infarcted and sham control hearts. A, Total protein expression of Akt. Level of phosphorylated Akt at B, Ser473 and C, Thr308 with *(hatched bars)* and without *(solid bars)* insulin stimulation in control and infarcted hearts. *White solid bars* indicate control group without insulin; *light-hatched bars,* control group with insulin; *black solid bars,* infarct group without insulin; and *dark-hatched bars,* infarct group with insulin. Representative Western blots shown. Values presented as mean ± standard error of mean; n = 4 per group. #$P < .05$ compared with levels without insulin from same group.

The concept of a connection between insulin resistance and contractile dysfunction has been developed from the recognition that systemic insulin resistance predicts the heart failure incidence independently of diabetes and other established risk factors for heart failure.[18] Although strong clinical evidence exists for this relationship, less is known about the underlying mechanisms. The term ''systemic insulin resistance'' previously mentioned refers to impairment in insulin-stimulated glucose use, mainly in the skeletal muscle and adipose tissue. The heart is an insulin-sensitive organ; however, whether cardiac insulin resistance needs to be present when systemic insulin resistance is present is still unclear. In vivo studies of patients with diabetes using fluorodeoxyglucose-positron emission

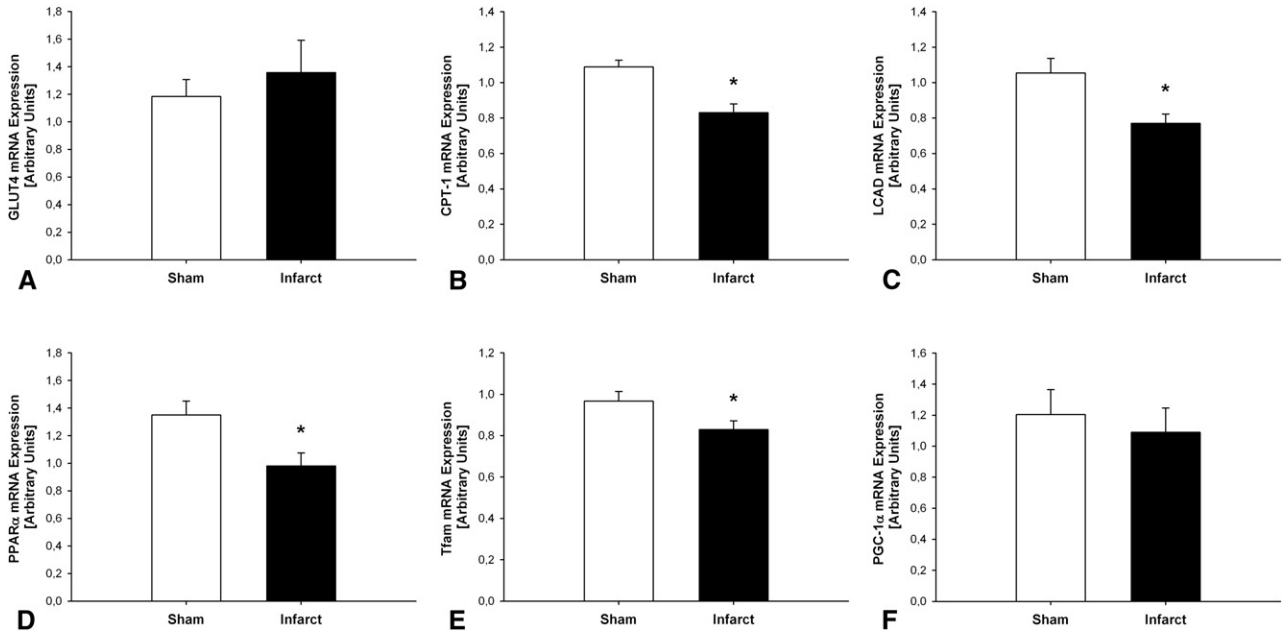

FIGURE 3. mRNA expression of genes involved in substrate use and mitochondrial biogenesis in infarcted and sham-control hearts. mRNA expression of A, glucose transporter type 4 *(GLUT4);* B, carnitine palmitoyltransferase 1 *(CPT-1);* C, long-chain-acyl-coenzyme A dehydrogenase *(LCAD);* D, peroxisome proliferator-activated receptor-α *(PPARα);* E, mitochondrial transcription factor A *(Tfam);* and F, peroxisome proliferator-activated receptor-γ coactivator 1α *(PGC-1α).* Values are presented as mean ± standard error of mean, n = 8 to 10 per group. *$P < .05$ compared with control group.

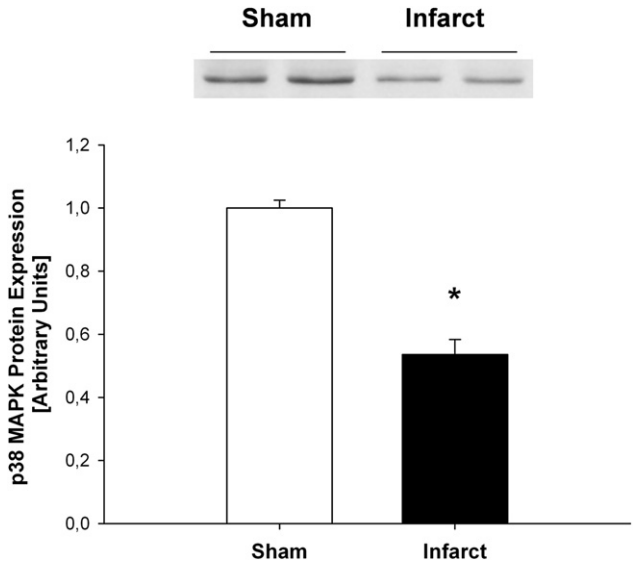

FIGURE 4. Protein expression of p38 mitogen-activated protein kinase *(MAPK)* in sham-operated control and infarcted hearts. Representative Western blots shown. Values presented as mean ± standard error of mean, n = 4 per group. *$P < .05$ compared with control group.

tomography techniques have shown normal insulin-stimulated myocardial glucose uptake[19]; however, the results are questionable owing to methodologic limitations.[20,21] In contrast, animal models of systemic insulin resistance develop myocardial insulin resistance.[22] Cardiac insulin resistance induced by specific knockout of the insulin receptor in the heart resulted in mitochondrial and contractile dysfunction.[6] Thus, cardiac insulin resistance might represent a potential mechanism for heart failure by causing mitochondrial dysfunction.

We have demonstrated that myocardial infarction resulted in an impaired insulin response at the level of substrate oxidation but normal insulin-stimulated glucose uptake, supported by the unchanged insulin-induced Akt phosphorylation and normal GLUT4 gene expression. Although insulin-stimulated glucose uptake has been widely used as a marker for insulin sensitivity, insulin exerts many more effects on the substrate metabolism. For instance, insulin can boost glycolysis and glucose oxidation independently of glucose uptake.[23] Insulin can also activate glycogen synthesis and, importantly, insulin resistance in those with type 2 diabetes mellitus has been shown to be characterized by impaired glucose oxidation and glycogen synthesis, although glucose uptake is normal.[24] Other than its acute effects on the glucose metabolism, insulin regulates the expression of genes involved in cell growth and mitochondrial biogenesis.[25] Therefore, impairment of insulin's effects can develop at different levels, leading to partial insulin resistance. Thus, assessing insulin-stimulated glucose uptake and Akt phosphorylation alone might not fully reflect insulin sensitivity.

We had opted to perform our investigations at 2 weeks after inducing infarction, because it was our goal to assess the early changes in cardiac metabolism and insulin sensitivity after infarction. One other study has investigated cardiac insulin sensitivity of the heart 10 weeks after myocardial infarction.[26] The investigators found reduced insulin-stimulated glucose uptake and reduced GLUT4 expression. Although they did not assess insulin's effect on substrate oxidation, it is tempting to speculate that our observation of a reduced insulin effect on substrate oxidation and normal uptake response 2 weeks after infarction reflects the beginning of insulin resistance that could get worse during continued postinfarct remodeling.

The diminished insulin response at the level of substrate oxidation was associated with mitochondrial dysfunction. A standard method for the characterization of mitochondrial function is the measurement of the maximal respiratory capacity in isolated mitochondria. In the present study, we did not evaluate the mitochondrial respiration; thus, one could question the presence of mitochondrial dysfunction. However, we showed an overall decrease in fatty acid oxidation, which could be explained by the downregulation of carnitine palmitoyltransferase 1 and long-chain-acyl-coenzyme A dehydrogenase, as well as their transcriptional activator, the nuclear receptor, PPARα. These alterations reflect true impairment in mitochondrial oxidative function in vivo, a situation that justifies the use of the term ''mitochondrial dysfunction.'' In addition to oxidative dysfunction, mitochondrial biogenesis could also be impaired. This is characterized by reduced gene expression of Tfam, which plays a critical role in the construction of the respiratory chain complexes.[27] Both PPARα and Tfam are downstream targets of PGC-1α, the master metabolic co-activator governing substrate use and mitochondrial biogenesis.[15] We found no change in PGC-1α mRNA levels. However, the regulation of PGC-1α is highly complex. Its activity is not only controlled by the levels of gene or protein expression but also by post-translational modification. The phosphorylation and acetylation status affects the activity of PGC-1α significantly, but inhibition of PGC-1α through its repressor is also an important mechanism. The binding of the repressor to PGC-1α can be counteracted by p38 MAPK.[16] Furthermore, exercise has been shown to activate PGC-1α downstream targets in skeletal muscle through p38 MAPK, without increasing PGC-1α protein levels.[28] In the present study, we showed decreased protein expression of p38 MAPK in association with downregulation of the PGC-1α downstream targets (PPARα, Tfam). These data would support the reduction in PGC-1α signaling at the post-translational level, leading to impaired mitochondrial biogenesis and oxidative function.

In contrast to these interpretations, the coexistence of a reduced cardiac insulin response at the level of substrate oxidation and mitochondrial dysfunction could also be

interpreted differently. It could be used to question the presence of cardiac insulin resistance in our model. It is possible that a defect in the mitochondrial oxidative machinery results in a reduced ability to increase glucose oxidation after insulin stimulation. This would mean that the attenuated cardiac insulin response would only be an expression of impaired oxidative capacity but not of insulin resistance. However, 2 considerations argue against this interpretation. First, the response to insulin of both glucose and fatty acid oxidation were diminished. Thus, by definition, insulin resistance is present. Second, the basal rates of glucose oxidation in our model were normal, indicating competent glucose oxidative capacity and not impairment. Therefore, we believe that cardiac insulin resistance exists independent of mitochondrial oxidative defects.

The final issue requiring discussion is the relationship between cardiac insulin resistance and mitochondrial dysfunction. As mentioned, cardiac insulin resistance might cause mitochondrial and contractile dysfunction. Furthermore, it has recently been shown that impaired cardiac insulin signaling in CIRKO mice accelerated the development of mitochondrial dysfunction and worsened survival after myocardial infarction despite an equivalent infarct size.[29] However, whether partial cardiac insulin resistance 2 weeks after infarction causes mitochondrial dysfunction and contributes to ventricular remodeling remains unclear and warrants additional studies. Although cardiac insulin resistance could also be considered an epiphenomenon in this case, it can be seen as a marker for mitochondrial dysfunction and the ventricular remodeling process and could may thus serve as a risk factor for infarction-induced cardiac dysfunction.

CONCLUSIONS

Myocardial infarction in rats causes partial insulin resistance at the level of substrate oxidation, associated with mitochondrial and cardiac dysfunction. Mitochondrial dysfunction is characterized by a reduced capacity to oxidize fatty acids possibly because of impaired mitochondrial biogenesis through decreased p38 MAPK signaling.

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Discussion

Dr Todd Rosengart *(Stony Brook, NY).* I would like to congratulate the authors on a nice presentation. This is obviously an important clinical problem in the context of the prevalence of diabetes in the western world and increasing heart failure as a potential complication of this incidence.

My question goes to a little bit of the ''cart–before-the-horse'' dilemma. You created a model where you clearly demonstrate insulin resistance and then subsequent heart failure, but I am not quite sure that you have demonstrated a causal effect as opposed to an intermediate step in your described pathophysiologic process. So, I was wondering if you have thought about challenging your system with antihyperglycemic rescue agents that can reverse insulin resistance to show whether you can prevent heart failure in your model system as a means of validating your hypothesis.

Dr Amorim. That is a very good point. Actually, there is a work from Rong Tian in *Circulation* last year in which they used metformin in infarcted rats and they found an improvement in survival, although they only focused on AMPK activation. And, actually, that is work in progress for us now; we are trying to see if metformin or pioglitazone can improve cardiac function.

Dr Rosengart. Similarly, you mentioned that you began to look at levels of Akt. You can similarly either downregulate Akt with wortmannin or even potentially upregulate this system with gene transfer of AKT as an alternative way of preventing your CHF phenotype, so that may also help further prove that the insulin resistance is causal rather than maybe just an intermediate step. Have you considered manipulating AKT in your models?

Dr Amorim. Well, the causal role is actually the focus of our work now. But the point is that, even if cardiac insulin resistance predisposes or is caused by contractile dysfunction, the focus is that it is present in contractile dysfunction and it could be used as a marker of heart failure.

Dr Rosengart. And, similarly, have you considered looking at diabetic models as opposed to physiologic injury for your stimulator of injury, in OB mice or the like?

Dr Amorim. Yes. But it is difficult to separate the vascular commitment from the muscular.

Dr Rosengart. My last question is: In your abstract, you mention that you measured dyspnea in the rats as a parameter. So, I just needed to know how you assessed dyspnea in a rat; I was fascinated by that.

Dr Amorim. Well, I can show some photos where you can see that they are really blue and they are really short of breath and it is easy to recognize, actually, if you see one.

Dr Pedro J. del Nido *(Boston, Mass).* One brief question. Mitochondria seems to be a common theme here in a lot of the chronic heart failure and also even in the ischemia-reperfusion models. Insulin obviously is a very important growth factor and also for mitochondrial biogenesis. Have you looked at the mitochondrial component of your heart failure model?

Dr Amorim. Yes, we are working on that now.