# Impaired Long-Chain Fatty Acid Oxidation and Contractile Dysfunction in the Obese Zucker Rat Heart

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**We investigated whether decreased responsiveness of the heart to physiological increases in fatty acid availability results in lipid accumulation and lipotoxic heart disease. Lean and obese Zucker rats were either fed ad libitum or fasted overnight. Fasting increased plasma nonesterified fatty acid levels in both lean and obese rats, although levels were greatest in obese rats regardless of nutritional status. Despite increased fatty acid availability, the mRNA transcript levels of peroxisome proliferator–activated receptor (PPAR)-α–regulated genes were similar in fed lean and fed obese rat hearts. Fasting increased expression of all PPAR-α–regulated genes in lean Zucker rat hearts, whereas, in obese Zucker rat hearts, muscle carnitine palmitoyltransferase and medium-chain acyl-CoA dehydrogenase were unaltered with fasting. Rates of oleate oxidation were similar for hearts from fed rats. However, fasting increased rates of oleate oxidation only in hearts from lean rats. Dramatic lipid deposition occurred within cardiomyocytes of obese, but not lean, Zucker rats upon fasting. Cardiac output was significantly depressed in hearts isolated from obese rats compared with lean rats, regardless of nutritional status. Fasting increased cardiac output in hearts of lean rats only. Thus, the heart's inability to increase fatty acid oxidation in proportion to increased fatty acid availability is associated with lipid accumulation and contractile dysfunction of the obese Zucker rat. *Diabetes* 51:2587–2595, 2002**

Obesity, insulin resistance, and diabetes are now major worldwide epidemics (1–3). Although much evidence exists suggesting a genetic component to these disorders, the dramatic surge in their incidence during recent years most likely reflects rapid alterations in lifestyle. The latter includes increased food availability and intake as well as a trend toward a sedentary lifestyle. Like these metabolic disorders, heart failure also continues to be on the rise in Western societies (4). Obesity, insulin resistance, and diabetes are the largest comorbidities of patients with heart failure and adversely affect outcomes of cardiovascular disease (5). It is therefore important to understand the relationship between cardiomyopathy and these metabolic disorders.

One common feature of obesity, insulin resistance, and diabetes is the propensity for increased circulating fatty acids. The role of fatty acids extend far beyond that of providing energy for the cell. Fatty acids mediate cellular signaling (6), affect gene expression (7), and are integral components in biological membranes (8). It is therefore not surprising that the myocyte has developed mechanisms to balance fatty acid availability with fatty acid utilization, thereby maintaining constant levels of fatty acids (and their derivatives) in the cell. One such mechanism is mediated by the nuclear receptor peroxisome proliferator–activated receptor (PPAR)-α (9,10). Upon activation of PPAR-α by the direct binding of fatty acids, this transcription factor increases the expression of various genes, including those involved in fatty acid metabolism (11,12). The latter results in an increased capacity of the cell to fully oxidize fatty acids in the face of increased fatty acid availability. If this system becomes impaired, a loss of synchronization between fatty acid availability and fatty acid oxidation would result in accumulation of fatty acid derivatives within the cardiomyocyte and subsequent lipotoxicity. This result is exemplified by the PPAR-α knockout mouse, in which loss of a functional PPAR-α gene results in lipid accumulation and contractile dysfunction of the heart upon fasting (a situation when circulating fatty acids increase) (10).

Zhou et al. (13) showed that the hearts of an animal model of type 2 diabetes (Zucker diabetic fatty [ZDF] rat) possess elevated intracellular lipid deposition and contractile dysfunction, potentially because of impairment of the PPAR-α system. However, neither this study nor the study in the PPAR-α knockout mice investigated whether this lipid accumulation and subsequent contractile dysfunction was due to a loss of synchronization between fatty acid availability and fatty acid oxidation. The purpose of the present study was therefore to investigate whether an impairment in fatty acid oxidation in response to increased fatty acid availability is the cause of contractile dysfunction in the obese Zucker rat, an animal model of obesity and insulin resistance (but not type 2 diabetes). The results show an inability of the obese Zucker rat heart

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iNOS, inducible nitric oxide synthase; MCAD, medium chain acyl-CoA dehydrogenase; MCD, malonyl-CoA decarboxylase; mCPTI, muscle carnitine palmitoyltransferase I; MHC, myosin heavy chain; $MVO_2$, myocardial oxygen consumption; NEFA, nonesterified fatty acid; PDK4, pyruvate dehydrogenase kinase 4; PPAR, peroxisome proliferator–activated receptor; SERCA2a, sarcoendoplasmic reticulum $Ca^{2+}$-ATPase 2a; TNF, tumor necrosis factor; UCP, uncoupling protein.

to adequately increase fatty acid oxidation in response to increased fatty acid availability (induced by fasting), resulting in lipid deposition within the cardiomyocytes. Impairment in the PPAR-α system may be the cause of this loss of synchronization because fasting failed to increase the expression of several PPAR-α–regulated genes to the same extent in the obese Zucker rat heart compared with the lean Zucker rat heart. Although the precise mechanism of lipotoxicity is unknown, it does not appear to be due to induction of either inducible nitric oxide synthase (iNOS) or tumor necrosis factor (TNF)-α.

## RESEARCH DESIGN AND METHODS

**Animals.** Male lean (Fa/?, 324 ± 6 g) and obese (*fa/fa*, 506 ± 9 g) Zucker rats were obtained from Harlan (Indianapolis, Indiana), were subsequently kept in the Animal Care Center of the University of Texas–Houston Medical School under controlled conditions (23 ± 1°C; 12-h light/12-h dark cycle), and received standard laboratory food and water ad libitum. For those rats in the fasted group, food was withdrawn at 1600 the day before experimentation.

**Heart perfusions.** Isolated hearts were perfused using the working heart preparation as described previously (14). Hearts were initially perfused in the Langendorff mode with Krebs-Henseleit buffer containing 5 mmol/l D-glucose, followed by a 30-min nonrecirculating perfusion in the working mode with Krebs-Henseleit buffer containing 5 mmol/l D-glucose (plus 20 μCi/l [U-$^{14}$C]-glucose), 0.5 mmol/l sodium L-lactate (plus 2 μCi/l [U-$^{14}$C]-lactate), 0.05 mmol/l sodium L-pyruvate, 0.4 mmol/l sodium oleate (plus 30 μCi/l [9,10-$^{3}$H]-oleate) bound to 1% BSA (fraction V, fatty acid free; Intergen, Purchase, NY), and 40 μU/ml insulin (Lilly). After 30 min of perfusion (15 cm $H_2O$ preload/100 cm $H_2O$ after load), hearts were freeze-clamped and stored in liquid nitrogen before dry weight was determined. Rates of oxygen consumption, carbohydrate oxidation (combination of exogenous glucose and lactate oxidation), oleate oxidation, and cardiac power and efficiency were determined as described previously (15).

**Plasma nonesterified fatty acid levels.** Immediately before heart isolation from the rats, 1 ml blood was withdrawn from the inferior vena cava. The sample was placed on ice and centrifuged for 10 min at full speed in a desktop microfuge. The supernatant was retained and stored at −80°C before nonesterified fatty acid (NEFA) level was determined. Plasma NEFA levels were measured spectrophotometrically with a commercially available kit (Wako Chemicals, Richmond, VA). Specimen blanks were prepared for all samples to correct for possible hemolysis.

**Lipid deposition.** Semiquantification of lipid deposition within the cardiomyocytes was performed by oil red O staining. In a separate group of rats that were not used in the heart perfusion studies, transverse sections were made through the heart immediately after isolation and rapidly frozen in optimal cutting temperature solution. The remainder of the heart was rapidly freeze-clamped and used for gene expression studies. Oil red O staining was performed on heart sections by a histology core facility at the University of Texas–Houston Health Science Center using standard procedures. Photomicrographs of oil red O–stained sections were taken on a Zeiss Axiophot microscope at 20× magnification using a Leaf Microlumina digital camera. A total of five images were analyzed with Image Pro Plus software using color cube–based selection criteria to ensure that only oil red O–stained droplets were counted and area of staining of all droplets from five fields per photomicrograph were averaged with background subtraction (nontissue regions).

**RNA extraction and quantitative RT-PCR.** RNA extraction and quantitative RT-PCR of samples was performed using previously described methods (16,17). Specific quantitative assays for the genes of interest were designed from the rat sequences available in GenBank. Primers and probes were designed around specific splice junctions, preventing the recognition of any contaminating genomic DNA. The sequences of these primers and probes have been published previously (18–21). The correlation between the number of PCR cycles required for the fluorescent signal to reach a detection threshold and the amount of standard was linear over a 5-log range of RNA for all assays (data not shown). The level of transcripts for the constitutive housekeeping gene product cyclophilin was quantitatively measured in each sample to control for sample-to-sample differences in RNA concentration. Expression is reported as the number of transcripts per number of cyclophilin molecules.

**Statistical analysis.** Data are presented as the mean ± SE. Statistically significant differences between groups were calculated by the Student's *t* test. A value of $P < 0.05$ was considered significant.

## RESULTS

**Oxygen consumption and substrate selection.** Figure 1 illustrates the rates of myocardial oxygen consumption ($MVO_2$), exogenous carbohydrate oxidation, and exogenous oleate oxidation for the four groups of hearts investigated. Fasting had no effect on $MVO_2$ for hearts isolated from either lean or obese rats (Fig. 1*A*). $MVO_2$ tended to be greater for hearts isolated from lean rats compared with obese rats (Fig. 1*A*). The rate of exogenous carbohydrate oxidation tended to decrease with fasting in both the lean and obese Zucker rats, although this decrease did not reach statistical significance (Fig. 1*B*). When hearts isolated from lean and obese rats were compared, the rate of exogenous carbohydrate oxidation was significantly less in obese Zucker rat hearts, regardless of the nutritional status (Fig. 1*B*). Fasting significantly increased the rate of exogenous oleate oxidation in hearts isolated from lean Zucker rats only; no effect of fasting was observed in the obese Zucker rat hearts (Fig. 1*C*). The rate of exogenous oleate oxidation tended to be greater for hearts isolated from lean rats compared with obese rats; this increase reached statistical significance when fasted animals were compared (Fig. 1*C*).

To determine substrate selection of the perfused hearts, the contribution of carbohydrate and oleate oxidation to total oxygen consumption was calculated. This calculation enables substrate selection to be determined, taking into account any changes in oxygen consumption (which is affected by both cardiac efficiency and cardiac work/output). Under our perfusion conditions, hearts isolated from fed lean Zucker rats have a 13% reliance on exogenous carbohydrate oxidation and a 45% reliance on exogenous oleate as a substrate, whereas the remaining 42% is presumably due to endogenous substrate oxidation (intracellular glycogen, fatty acids, and lipids). Although the reliance on exogenous oleate oxidation was not different for hearts isolated from lean and obese Zucker rats, the reliance of exogenous carbohydrate as a fuel tended to be lower in the obese Zucker rat heart (Fig. 2). Fasting had no significant effects on the reliance on carbohydrate oxidation in either lean or obese Zucker rats (Fig. 2*A*). In contrast, fasting significantly increased reliance on oleate oxidation in hearts isolated from lean Zucker rats only (Fig. 2*B*).

**Cardiac power and efficiency.** Cardiac power was higher for hearts isolated from lean Zucker rats compared with those from obese Zucker rats, regardless of nutritional status (Fig. 3*A*). Fasting significantly increased cardiac power in the lean Zucker rat heart only (Fig. 3*A*). Cardiac efficiency tended to be lower for hearts isolated from lean Zucker rats compared with those from obese Zucker rats (fed state; Fig. 3*B*). Fasting had no significant effects on cardiac efficiency (Fig. 3*B*).

**Plasma NEFA levels.** Plasma NEFA levels were 1.9-fold higher in obese fed rats than lean fed rats (Fig. 4). Fasting increased plasma NEFA levels in both lean and obese rats. Obese fasted rats had the greatest levels of plasma NEFAs.

**Lipid deposition.** Figure 5*A* shows representative staining of hearts from each of the four groups. After semiquantification of staining, hearts isolated from obese Zucker rats possessed increased lipid deposition compared with lean Zucker rats, regardless of nutritional status (Fig. 5*B*).

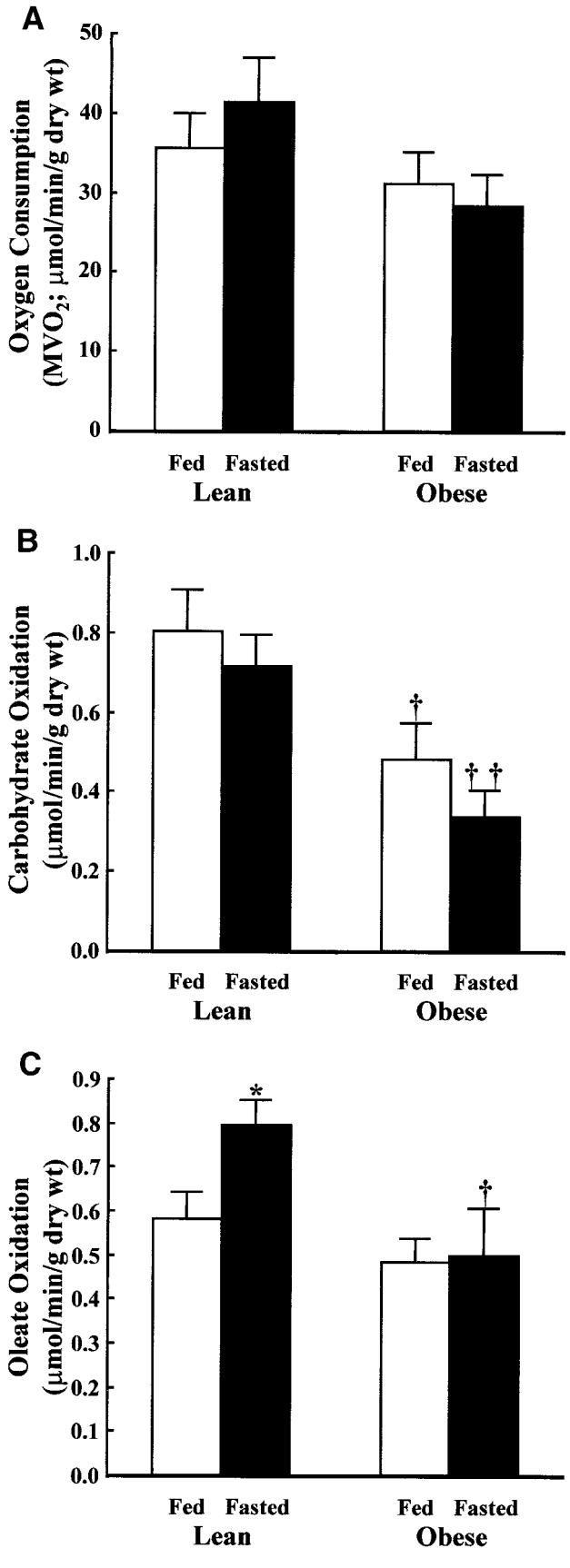


**FIG. 1. Myocardial oxygen consumption (*A*), carbohydrate oxidation (*B*), and oleate oxidation (*C*) in perfused lean and obese Zucker rat hearts. Data are means ± SE for between five and nine independent observations. **P* < 0.05 for lean fed vs. lean fasted; †*P* < 0.05 and ††*P* < 0.01 for lean vs. obese at the same nutritional status.**

Fasting increased lipid deposition in hearts of obese Zucker rats (Fig. 5*B*). In contrast, fasting decreased staining in hearts of lean Zucker rats.

**Expression of PPAR-α and PPAR-α–regulated genes.** The expression of PPAR-α was the same in hearts isolated from lean and obese Zucker rats in the fed state (Fig. 6*A*). Fasting significantly decreased PPAR-α expression in hearts from both lean and obese Zucker rats (Fig. 6*A*). However, fasting lowered PPAR-α expression to the greatest extent in the obese Zucker rat heart (Fig. 6*A*).

In the fed state, expression of all of the PPAR-α–regulated genes investigated (pyruvate dehydrogenase kinase 4 [PDK4], uncoupling protein [UCP]-3, malonyl-CoA decarboxylase [MCD], medium chain acyl-CoA dehydrogenase [MCAD], and muscle carnitine palmitoyltransferase I [mCPTI]) was the same in hearts from both lean and obese Zucker rats (Fig. 6*B–F*). For the lean Zucker rat heart, fasting increased the expression of all of these PPAR-α–regulated genes (Fig. 6*B–F*). In contrast, fasting of the obese Zucker rat increased cardiac expression of PDK4, UCP3, and MCD only (Fig. 6*B–D*).

**Expression of potential modulators of contractile function.** To investigate further the potential mechanisms by which contractile function is depressed in the obese Zucker rat heart, the expression of genes encoding for modulators of mitochondrial function (UCP2), calcium homeostasis (sarcoendoplasmic reticulum $Ca^{2+}$-ATPase 2a [SERCA2a]), contractile proteins (myosin heavy chain [MHC]-α and MHC-β), and inducers of contractile dysfunction (iNOS and TNFα) were measured.

Not unexpectedly, the expression of UCP2 was not different between hearts isolated from lean and obese Zucker rats nor was expression affected by fasting (Fig. 7*A*) (19,22). Similar results were obtained for SERCA2a and MHC-α expression (Fig. 7*B* and *C*). In contrast, MHC-β expression was 2.2-fold greater in hearts isolated from fed obese Zucker rats compared with fed lean Zucker rats (Fig. 7*D*). Fasting significantly increased MHC-β expression in the lean Zucker rat (Fig. 7*D*). Both iNOS and TNF-α expression was lower in hearts isolated from obese Zucker rats compared with lean Zucker rats (Fig. 7*E* and *F*). Fasting tended to decrease the expression of these modulators of cardiac function (Fig. 7*E* and *F*).

## DISCUSSION

We have found that isolated working hearts from the obese Zucker rat show depressed contractile function compared with the lean Zucker rat heart when perfused under identical conditions. Previous studies have already suggested that, in normal rats, fasting overnight improves cardiac performance (23,24). Consistent with these reports, we have found that fasting in lean Zucker rats increases cardiac output. However, the beneficial effect of fasting on cardiac performance is lost in the obese Zucker rat.

We hypothesized that the above differences in contractile function between the lean and obese Zucker rat heart may be due to one or more of the following: *1*) alterations in substrate use, *2*) alterations in UCP expression, *3*) alterations in calcium handling proteins in the heart, *4*) alterations in contractile protein composition of the heart, and *5*) lipotoxicity.

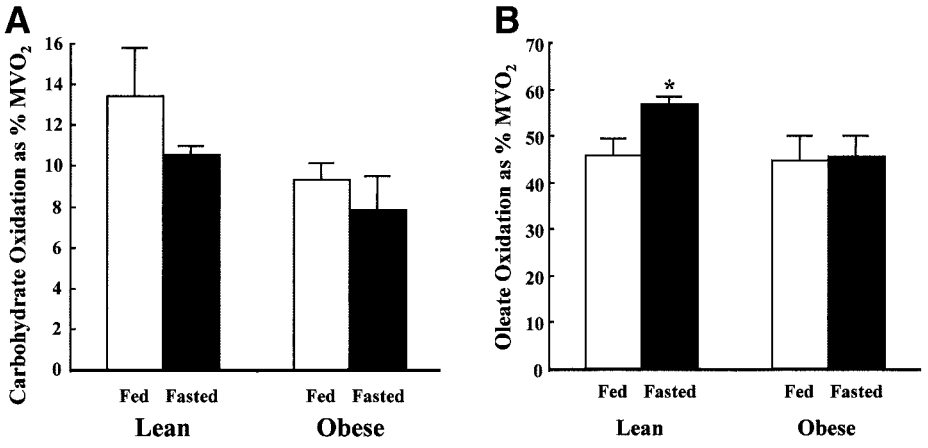


**FIG. 2. Reliance on carbohydrate (*A*) and oleate (*B*) oxidation as an energy source for perfused lean and obese Zucker rat hearts. Data are means ± SE for between five and seven independent observations. **P* < 0.05 for lean fed vs. lean fasted.**

**Substrate selection.** Both total carbohydrate oxidation and the reliance on carbohydrate as an energy source were lower in hearts isolated from obese Zucker rats compared with those from lean Zucker rats. Fasting tended to decrease these parameters, presumably because of inhibition of pyruvate dehydrogenase (at the levels of increased PDK4 expression and increased fatty acid oxidation). More ATP is derived from carbohydrate oxidation than fatty acid oxidation per molecule of $O_2$ used (i.e., glucose and lactate are more efficient fuels in terms of ATP generation per $O_2$ used). The decreased reliance of the obese Zucker rat heart on carbohydrate oxidation may therefore contribute to both the decrease in cardiac power and the decrease in cardiac efficiency observed during the perfusions. However, carbohydrate oxidation does not explain why cardiac power increases in hearts isolated from fasted lean rats (nor the normalization of cardiac efficiency for hearts isolated from obese rats upon fasting).

**UCPs.** UCP1 uncouples oxidative phosphorylation from ATP synthesis, thereby decreasing the ratio of ATP synthesis to $O_2$ use (25,26). The heart expresses two homologues of UCP1—namely UCP2 and UCP3 (19,22). The levels of expression of UCP2 and UCP3 were similar between hearts of lean and obese Zucker rats. Consistent with recent studies published from our laboratory (19), UCP2 expression was unaffected in the heart by fasting. However, the expression of UCP3 increases dramatically in hearts from both lean and obese animals with fasting. Therefore, altered UCP expression is not consistent with the decreased cardiac power and efficiency observed in hearts isolated from obese Zucker rats or with the apparent increase in cardiac power (lean rats) and efficiency (obese rats) with fasting. The possibility remains that UCP2 and/or UCP3 are differentially regulated at the posttranscriptional level in the hearts of lean and obese rats (e.g., UCP activity is known to be affected directly by fatty acids and nucleotides) (25,26). However, it should be noted that the roles of UCP2 and UCP3 may extend beyond that of stereotypical uncoupling of oxidative phosphorylation and may include antioxidant functions and modulation of fatty acid oxidation efficiency (27,28).

**Calcium cycling.** Abnormal calcium cycling has been linked to the contractile dysfunction associated with various cardiomyopathies, including ischemic, dilated, and diabetic (29–31). Multiple ion channels and effector proteins are involved in the complex processes involved in calcium cycling during each and every excitation-relaxation sequence (32). Of these, SERCA2a is one of the most extensively investigated, for which expression is diminished in animal models of human cardiomyopathies. For example, both pressure overload–induced hypertrophy and streptozotocin-induced diabetes are associated with

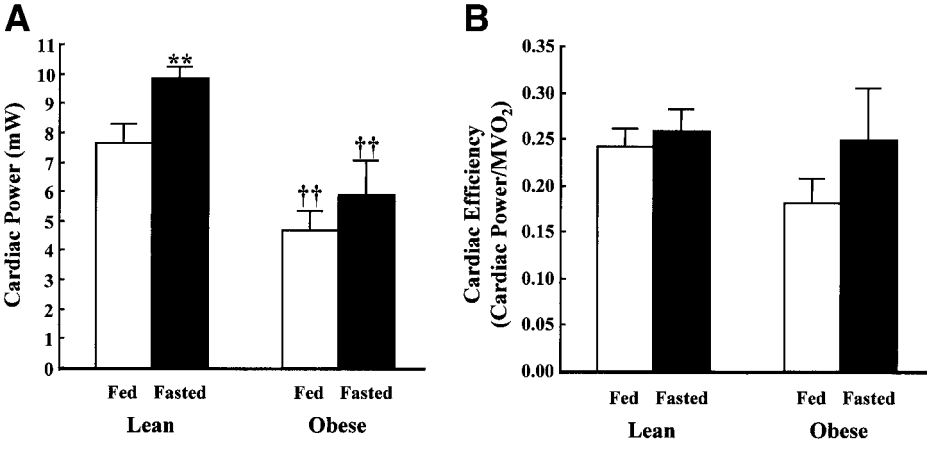


**FIG. 3. Cardiac power (*A*) and efficiency (*B*) by perfused lean and obese Zucker rat hearts. Data are means ± SE for between six and nine independent observations. ***P* < 0.01 for lean fed vs. lean fasted; ††*P* < 0.01 for lean vs. obese at the same nutritional status.**

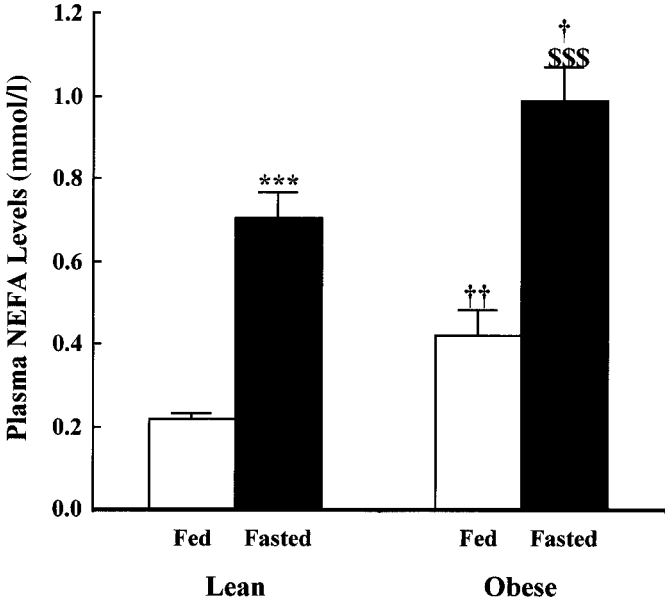


**FIG. 4. Plasma NEFA levels in lean and obese Zucker rat hearts. Data are means ± SE for six independent observations. ***$P < 0.001$ for lean fed vs. lean fasted; \$\$\$$P < 0.001$ for obese fed vs. obese fasted; †$P < 0.05$ and ††$P < 0.01$ for lean vs. obese at the same nutritional status.**

decreased expression of SERCA2a in the heart (29,33,34). It is therefore conceivable that the contractile dysfunction observed in the present study was due to decreased SERCA2a expression. However, the level of SERCA2a mRNA was not different between hearts isolated from lean and obese Zucker rats. Although these observations suggest that contractile dysfunction in the obese Zucker rat heart is not due to decreased SERCA2a expression, abnormal calcium handling due to altered expression of other ion channels (e.g., $Na^+/Ca^{2+}$ exchanger) and effectors (e.g., phospholamban) or due to posttranscriptional effects (e.g., fatty acyl-CoAs directly affecting the ryanodine receptor) cannot be excluded.

**Contractile proteins.** During the adaptation of the heart to the environment within which it finds itself, the expression of contractile protein isoforms (e.g., the MHC isoforms) often change (35–37). These contractile protein isoforms have differing efficiencies (i.e., the amount of force generated per ATP used). MHC-α is the fast, less efficient isoform, whereas MHC-β is the slow, more efficient isoform (36). The present study demonstrates that MHC-β expression is higher in hearts isolated from obese Zucker rats compared with lean Zucker rats. In addition, fasting increased MHC-β expression in hearts isolated from lean rats. MHC-α expression was not significantly different between the four groups. Because MHC-β is associated with a lower velocity of contraction, increased expression may play a role in the decreased cardiac power observed in the obese Zucker rat heart. However, given that MHC-α (the predominant MHC isoform in the adult rat heart) was not different between the lean and obese Zucker rat hearts and that cardiac efficiency was lower in the obese Zucker rat heart despite the higher level of expression of the more efficient MHC-β isoform, it is unlikely that altered MHC isoform expression is the main cause of altered cardiac function.

**Lipotoxicity.** If fatty acid availability exceeds the rate of fatty acid oxidation, then fatty acyl-CoAs accumulate within the cell. A significant proportion of excess fatty acyl-CoAs become esterified to form lipid droplets within the cytosol, whereas the remainder can perturb normal cellular signaling mechanisms, resulting in lipotoxicity (38,39). The cell overcomes this problem by increasing fatty acid oxidation capacity in the face of increased fatty acid availability. This feed-forward mechanism is regulated in part by PPAR-α (9,10). Binding of fatty acids to PPAR-α induces the expression of various genes involved in fatty acid metabolism (e.g., MCD, mCPTI, and MCAD), carbohydrate metabolism (e.g., PDK4), and mitochondrial function (e.g., UCP3) (18,19,40–42). If this PPAR-α regulatory system were to become impaired, then lipotoxicity would result.

In the fed state, the obese Zucker rat has 1.9-fold higher circulating levels of NEFAs than the lean Zucker rat. This increase in fatty acid availability would be expected to result in increased expression of PPAR-α–regulated genes and therefore increased fatty acid oxidation. However, the expression of PPAR-α–regulated genes and the reliance on fatty acids as an energy source were the same in hearts isolated from lean and obese Zucker rats. Consistent with this dissociation between fatty acid availability and fatty acid oxidation capacity, the obese Zucker rat heart has greater lipid deposition than hearts isolated from lean Zucker rats. PPAR-α expression was not different in hearts isolated from lean and obese rats in the fed state, suggesting that the abnormal responsiveness of this system to fatty acids in the obese heart is independent of PPAR-α transcription and instead may be due to altered phosphorylation of PPAR-α or alterations in the dimerization partner (retinoid X receptor), cofactors (e.g., PPAR-γ coactivator-1), and/or repressors (e.g., COUP-TF [chicken ovalbumin upstream promoter–transcription factor]). The possibility exists that altered responsiveness to fatty acids may be through a PPAR-α–independent mechanism, as some PPAR-α–regulated genes (MCD, PDK4, and UCP3) remain inducible in the obese Zucker rat heart in response to fasting.

Fasting of lean Zucker rats increased the expression of PPAR-α–regulated genes and increased reliance on fatty acid oxidation as a source of energy. Although fasting of the obese Zucker rat increased circulating NEFAs to levels greater than those observed in the lean fasted rat, fasting did not increase the expression of all of the PPAR-α–regulated genes investigated and failed to increase fatty acid oxidation. This loss of synchronization between fatty acid availability and fatty acid oxidation was accompanied by a dramatic accumulation of lipid within the cardiomyocyte of the obese Zucker rat. Such lipid deposition was not observed in the lean Zucker rat heart upon fasting, in which this synchronization was maintained. Instead, intramyocardial lipids decreased in lean Zucker rats upon fasting. This decrease in intracellular lipid was associated with increased cardiac function in fasted lean Zucker rats. Although fasting decreased the expression of PPAR-α in the hearts of both lean and obese Zucker rats (consistent with our previously published work in the Wistar rat) (19), this decrease was more severe in the obese. The latter may play a role in subsequent lipotoxicity.

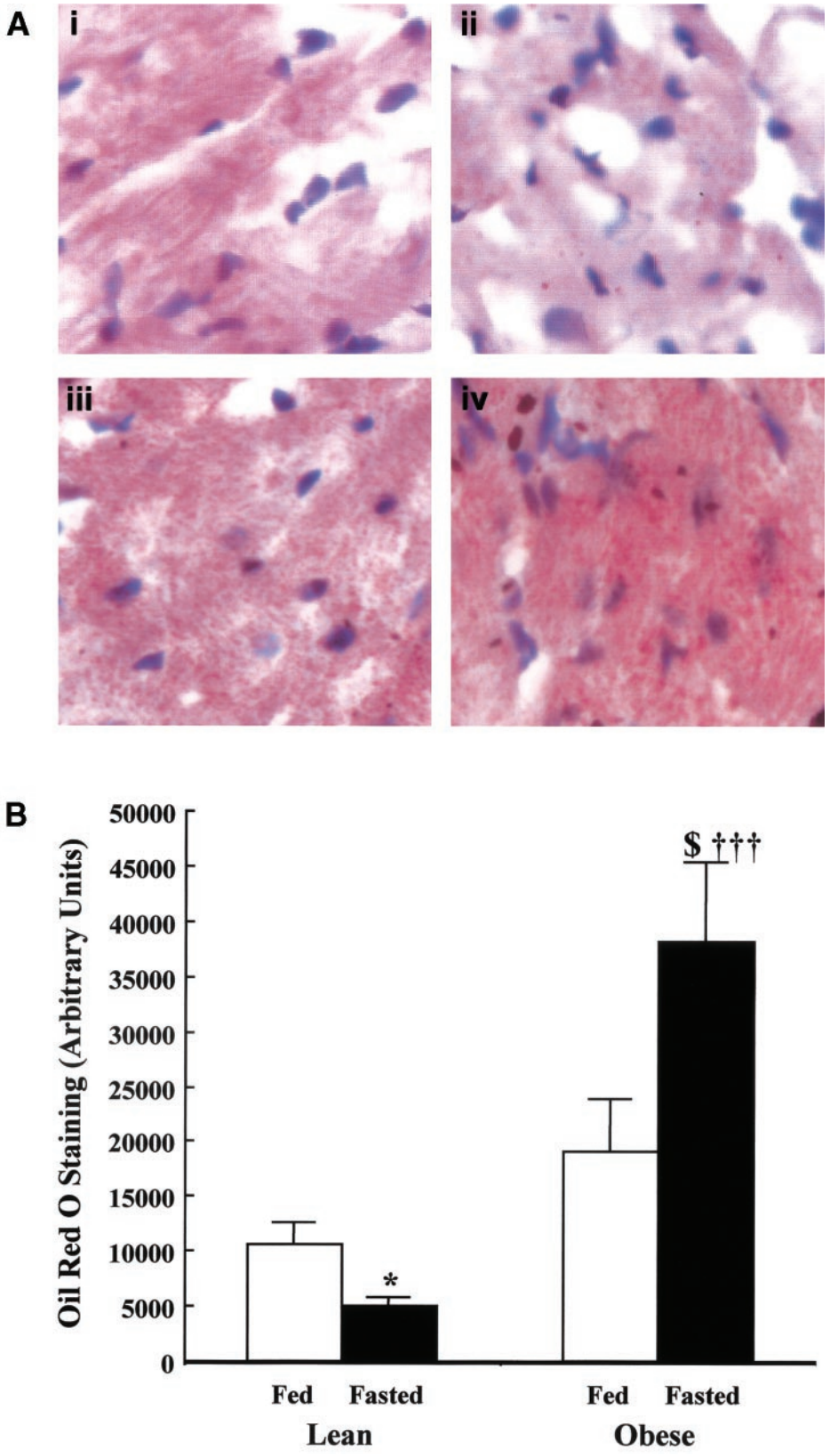


**FIG. 5. Lipid deposition in lean and obese Zucker rat hearts. *A*: Representative oil red O staining in sections isolated from a fed lean rat (*i*), a fasted lean rat (*ii*), a fed obese rat (*iii*), and a fasted obese rat (*iv*). *B*: Data (means ± SE) for six independent observations. **P* < 0.05 for lean fed vs. lean fasted; $*P* < 0.05 for obese fed vs. obese fasted; †††*P* < 0.001 for lean vs. obese at the same nutritional status.**

The mechanism(s) by which excessive fatty acid availability cause(s) contractile dysfunction (i.e., lipotoxicity) is not fully known. Long-chain fatty acyl-CoAs accumulate when fatty acid uptake by the cell exceeds β-oxidation. These CoA derivatives can be used in the synthesis of ceramide, diacylglycerol, and triacylglycerol (lipid), in

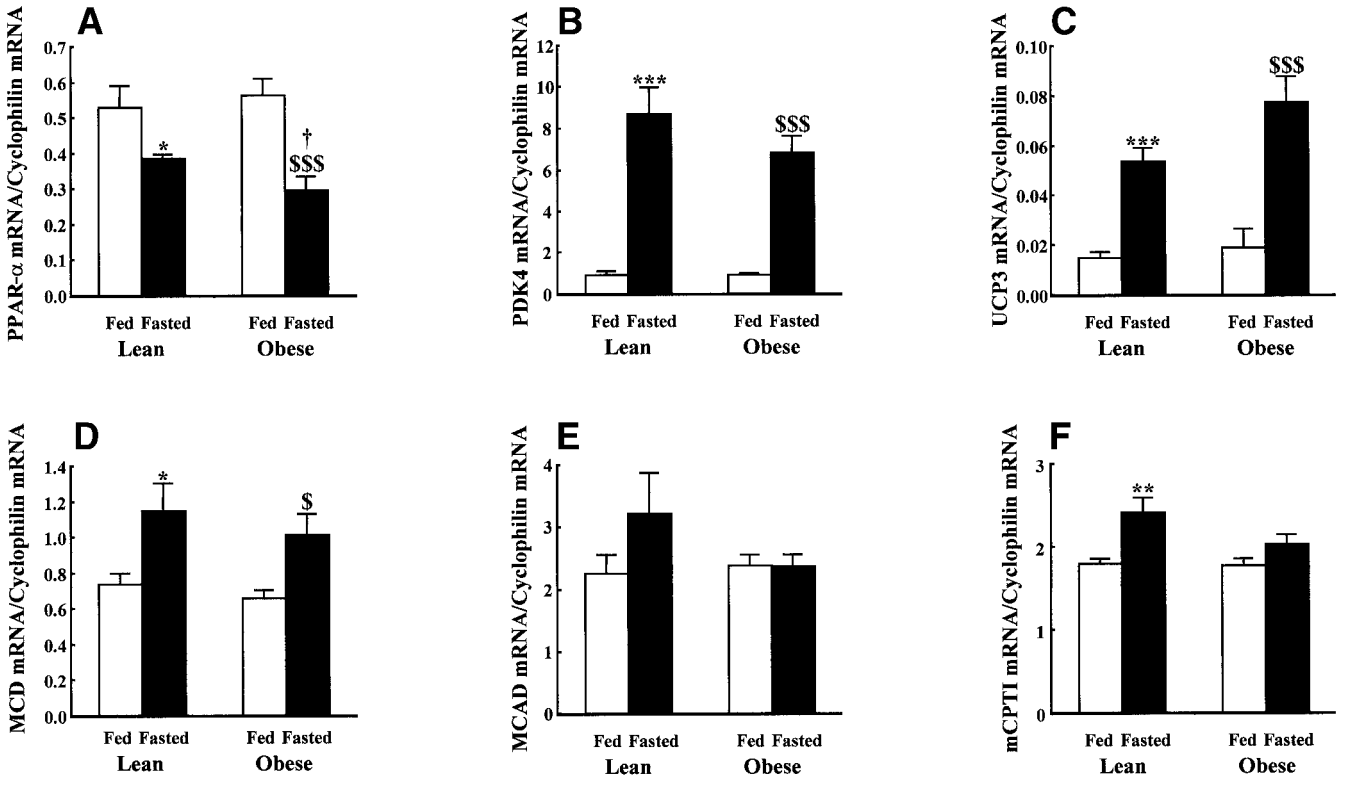


**FIG. 6. Expression of PPAR-α and PPAR-α–regulated genes in lean and obese Zucker rat hearts. Transcripts encoding for PPAR-α (*A*), PDK4 (*B*), UCP3 (*C*), MCD (*D*), mCPTI (*E*), and MCAD (*F*) were measured. All data are normalized to the housekeeping gene cyclophilin. Data are means ± SE for six independent observations. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 for lean fed vs. lean fasted; $*P* < 0.05 and $$$*P* < 0.001 for obese fed vs. obese fasted; †*P* < 0.05 for lean vs. obese at the same nutritional status.**

addition to directly affecting the activity of various proteins involved in metabolism (e.g., hexokinase), cellular signaling (e.g., specific protein kinase C isoforms), and calcium handling (e.g., ryanodine receptor) (39,43,44). It has been suggested that ceramide might cause contractile dysfunction in the ZDF rat through increased iNOS expression and induction of apoptosis (13). Contrary to expectation, iNOS expression was lower in hearts isolated from obese Zucker rats in both the fed and fasted states (as was TNF-α, another postulated inducer of contractile dysfunction). These results are consistent with recent reports from Listenberger et al. (45) that lipotoxicity occurs independently of ceramide in the heart.

**ZDF versus obese Zucker rat.** The ZDF rat is an animal model of type 2 diabetes because of β-cell insufficiency. In contrast, the obese Zucker rat is a model of obesity and insulin resistance (not type 2 diabetes because of adequate β-cell function). One possible mechanism by which β-cells decompensate in the ZDF rat is lipotoxicity. Lipid accumulation within the β-cells of these animals is associated with increased ceramide, iNOS, and apoptosis, as reported for the heart from these animals (46). In contrast, this lipotoxicity of the β-cell does not appear to be as severe in the obese Zucker rat. The same is true for the heart, in which the contractile dysfunction in the isolated heart of the obese Zucker rat is modest. The ability of these two animal models to cope with lipotoxicity at different levels may be the reason for both the β-cell and cardiac dysfunction. In the fed ZDF rat heart, there is decreased expression of both PPAR-α and PPAR-α–regulated genes, despite chronic exposure to elevated fatty acid levels (13). In contrast, the present study demonstrates that the obese Zucker rat heart possesses the same level of expression of PPAR-α and PPAR-α–regulated genes in the fed state compared with the hearts isolated from lean animals. The ability of the obese Zucker rat heart to prevent fatty acid oxidation capacity from falling below the basal level may be the mechanism by which these hearts (and perhaps the β-cells) minimize lipotoxicity.

**Limitations of the study.** The present study has determined exogenous substrate use in lean and obese Zucker rat hearts, as measured ex vivo. However, endogenous substrate selection has not been addressed. It is possible that the observed impairment in the activation of exogenous oleate oxidation in the obese Zucker rat heart during fasting is due to dilution of the radiolabeled tracer in a larger pool of fatty acyl derivatives in the cardiomyocyte of the obese Zucker rat. Whether such dilution effects occur, the results show that fatty acid availability is clearly beyond the capacity for oxidation. In addition, the present study has not determined the molecular mechanism(s) by which fatty acid/lipid accumulation results in contractile dysfunction (although iNOS and TNF-α have been excluded as potential mediators). Future studies are required to address this important question.

**Conclusion.** We have shown that a loss of synchronization between fatty acid availability and fatty acid oxidation capacity in the heart of an animal model of obesity and insulin resistance (the obese Zucker rat) is associated with lipid deposition within the cardiomyocyte and contractile dysfunction. These observations give rise to the speculation that an impairment in myocardial fatty acid oxidation

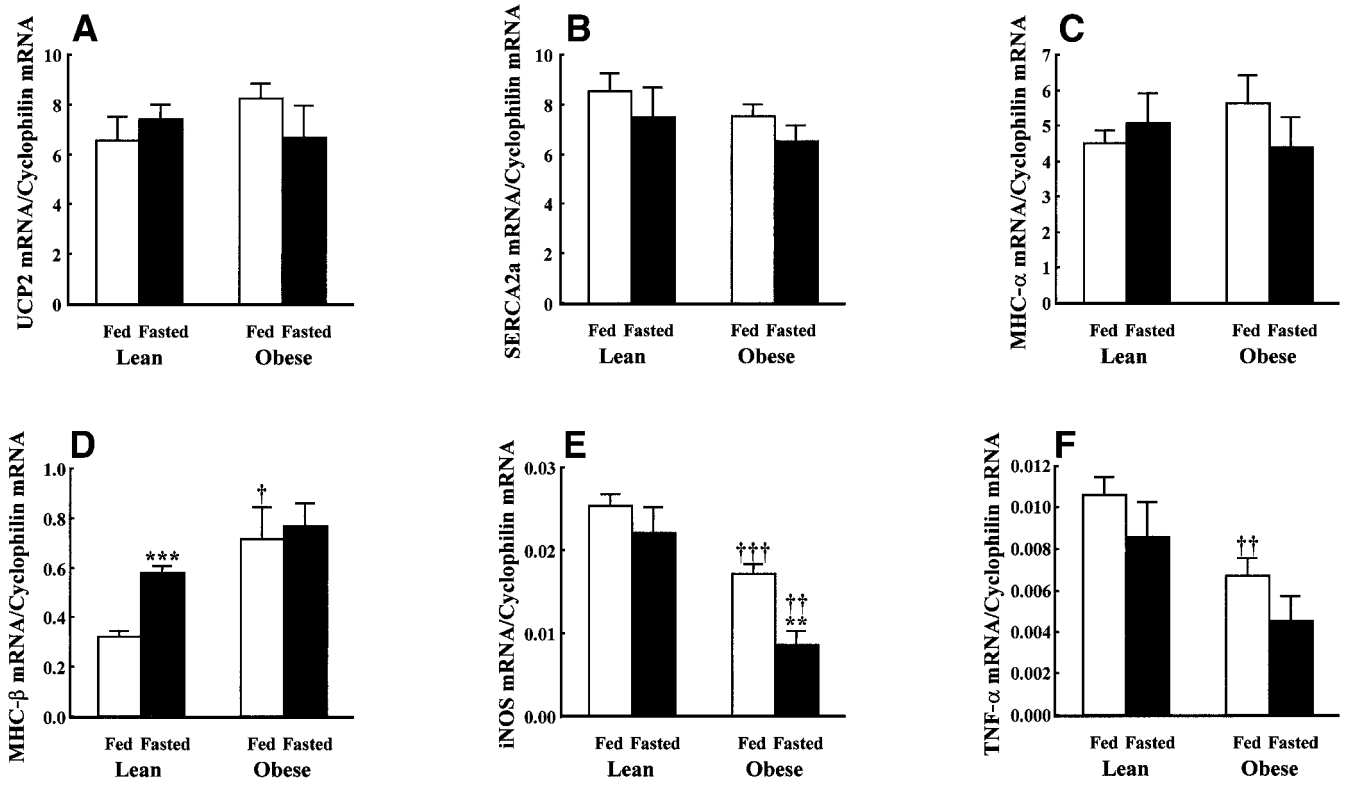


**FIG. 7. Expression of potential modulators of contractile function in lean and obese Zucker rat hearts. Transcripts encoding for UCP2 (*A*), SERCA2a (*B*), MHC-α (*C*), MHC-β (*D*), iNOS (*E*), and TNF-α (*F*) were measured. All data are normalized to the housekeeping gene cyclophilin. Data are means ± SE for six independent observations. ***P* < 0.01 and ****P* < 0.001 for lean fed vs. lean fasted; †*P* < 0.05, ††*P* < 0.01, and †††*P* < 0.001 for lean vs. obese at the same nutritional status.**

during obesity, insulin resistance, and/or diabetes may accelerate contractile dysfunction development associated with these metabolic disorders.


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