Rescue of cardiac leptin receptors in \(db/db\) mice prevents myocardial triglyceride accumulation

Michael E. Hall,1,2 Matthew W. Maready,1 John E. Hall,1 and David E. Stec1

1Department of Physiology and Biophysics, Center for Excellence in Cardiovascular-Renal Research, University of Mississippi Medical Center, Jackson, Mississippi; and 2Department of Medicine/Division of Cardiology, University of Mississippi Medical Center, Jackson, Mississippi

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Hall ME, Maready MW, Hall JE, Stec DE. Rescue of cardiac leptin receptors in \(db/db\) mice prevents myocardial triglyceride accumulation. Am J Physiol Endocrinol Metab 307: E316–E325, 2014. First published June 17, 2014; doi:10.1152/ajpendo.00005.2014.—Increased levels of leptin, a hormone derived from adipocytes, have been suggested to protect against myocardial triglyceride accumulation in obesity. Leptin not only regulates food consumption and metabolic rate through central nervous system (CNS) actions but also stimulates \(\beta\)-oxidation of fatty acids in peripheral tissues through its CNS actions as well as direct leptin receptor (LepR)-mediated effects on peripheral tissues (1). This increase in \(\beta\)-oxidation of fatty acids shunts them away from storage, thus reducing tissue lipid accumulation (43). Several studies have demonstrated that leptin administration reverses steatosis in the liver and pancreas (47). Leptin has also been reported to lower cardiac triglyceride levels and preserve systolic function in a mouse model of severe lipotoxic cardiomyopathy (28). This evidence suggests that leptin may play an important role in protecting the heart against lipotoxic cardiomyopathy. However, in these studies leptin administration also had important CNS actions that reduced body weight and overall adiposity, which could reduce cardiac lipids independent of direct effects of leptin on the heart (32). In addition, leptin administration could also elicit other CNS actions that increase tissue fatty acid oxidation. To our knowledge, there have been no studies that assessed the importance of leptin’s direct effects on the heart in attenuating obesity-induced cardiac dysfunction and lipid accumulation.

To determine directly the role of leptin in preventing cardiac lipid accumulation in obesity, we generated a murine model in which the LepR was expressed specifically in cardiomyocytes of obese \(db/db\) mice. Reexpression of cardiomyocyte leptin receptors in \(db/db\) mice did not cause LVH but reduced cardiac triglycerides and improved cardiac function. Compared with lean wild-type (WT) or \(db/db\)-cardiac LepR rescue mice, \(db/db\) mice exhibited significantly lower E/A ratio, a measurement of early to late diastolic filling, which averaged 1.5 \pm 0.07 in \(db/db\) vs. 1.9 \pm 0.08 and 1.8 \pm 0.11 in WT and \(db/db\)-cardiac LepR rescue mice, respectively. No differences in systolic function were observed. Although \(db/db\) and \(db/db\)-cardiac LepR rescue mice exhibited similar increases in plasma triglycerides, insulin, glucose, and body weight, cardiac triglycerides were significantly higher in \(db/db\) compared with WT and \(db/db\) cardiac LepR rescue mice, averaging 13.4 \pm 4.2 vs. 3.8 \pm 1.6 vs. 3.8 \pm 0.7 mg/g, respectively. These results demonstrate that despite significant obesity and increases in plasma glucose and triglycerides, \(db/db\) cardiac LepR rescue mice are protected against myocardial lipid accumulation. However, we found no evidence that leptin directly causes LVH.

Address for reprint requests and other correspondence: D. E. Stec, Dept. of Physiology & Biophysics, Univ. of Mississippi Medical Center Jackson, MS 39216 (e-mail: dstec@umc.edu).
were made after the mice were euthanized at 30 wk of age. Triglyceride analysis and reported as milligrams per deciliter. All samples were quantitated using the Quantikine leptin immunoassay (R & D Systems, Minneapolis, MN). Mouse Insulin ELISA Kit (Crystal Chem, Downers Grove, IL). Insulin levels were measured by ELISA with the Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Downers Grove, IL). Cardiac-specific LepR transgenic mice (Myh6-LepR) were generated on a mixed B6SJL genetic background and bred back to C57BL/6J mice for more than 10 generations. To create LepR mice were derived on a mixed B6SJL genetic background and mated with heterozygous transgenic Myh6-LepR, heterozygous Myh6-MerCreMer to create the transgenic construct (Myh6-LepR). The plasmid Myh6-LepR was then digested with the restriction enzyme NorI and then purified and injected into fertilized mouse embryos using established pronuclear injection techniques. Myh6-LepR mice were derived on a mixed B6SJL genetic background and bred back to C57BL/6J mice for more than 10 generations. To create cardiac-specific LepR rescue mice, heterozygous Myh6-LepR mice were crossed with heterozygous mice containing the LepRdb mutation, which were also on the C57BL/6J genetic background (Jackson Laboratories, Bar Harbor, ME). To generate db/db mice with and without the Myh6-LepR transgene, heterozygous leprDB mice were mated with heterozygous transgenic Myh6-LepR heterozygous leprDB mice. The offspring were then genotyped for the Myh6-LepR transgene using the primers 5’-GCTGTTAGGAAAGTCAG-3’ and 5’-GATAGGCCAGTATTAGTCAG-3’ as well as the lepRdb mutation using a restriction enzyme digestion (RsaI) of PCR reaction. db/db Mice that inherited the Myh6-LepR transgene are referred to as “rescue” mice. Experiments were also performed on age-matched db/db mice on the C57BL/6J background purchased from Jackson Laboratories.

Blood glucose measurements. Blood glucose levels were obtained from mice that were fasted for 8 h with access to water. Blood was obtained from lightly anesthetized mice via retroorbital bleeding. Blood glucose concentration was determined immediately from a 5-μl blood sample with the Accuchek Advantage glucometer (Roche, Madison, WI).

Plasma insulin, leptin, and triglyceride measurements. Plasma insulin levels were measured by ELISA with the Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Downers Grove, IL). Plasma leptin levels were also measured by ELISA with the mouse Quantikine leptin immunoassay (R & D Systems, Minneapolis, MN). Plasma triglycerides were measured using a triglyceride reagent (GPO Trinder; Sigma-Aldrich, St. Louis, MO) and read at an optical density of 500 nm, with standards ranging from 0 to 200 mg/dL. Triglyceride concentration of each sample was determined using linear regression analysis and reported as milligrams per deciliter. All samples were measured in triplicate for each of the measurements.

Cardiac triglyceride measurements. All triglyceride measurements were made after the mice were euthanized at 30 wk of age. Triglycerides were measured from 100 mg of heart tissue homogenized in 200 μl of homogenization buffer (18 mM Tris, pH 7.5, 300 mM mannitol, and 50 mM EGTA). After homogenization, 4 ml of 2:1 chloroform-methanol mixture was added to the samples, and the samples were incubated for 1 h at room temperature and mixed every 15 min. After this time, 800 μl of water was added to the samples, and the samples were mixed thoroughly and centrifuged for 5 min at 4,700 rpm at 4°C. The organic phase was then collected and dried under vacuum. After this time, samples were resuspended in 60 μl of T-butanol, followed by 40 μl of 2:1 Triton X-114-methanol mixture and sampled mixed for 10 s. To perform the triglyceride measurements, 10 μl of this mixture was added to 1 ml of triglyceride reagent (GPO Trinder) and incubated for 5 min at 37°C. Optical densities of the samples along with standards ranging from 0 to 200 mg/dl were measured at 500 nm. Triglyceride concentration of each sample was determined using linear regression analysis and reported as milligrams per deciliter. All samples and standards were measured in triplicate.

Echocardiography. Echocardiographic assessment of cardiac function was conducted using a Vevo 770 High Resolution In Vivo Imaging System (Visualsonics, Toronto, ON, Canada), as described previously (16, 17). Measurements were made with a 710B RMV scanhead with a center frequency of 25 MHz. Echocardiography was performed on each mouse beginning at 12 wk of age and subsequently at 6-wk intervals until 30 wk of age. All echocardiographic data were obtained within a 15-min session and were made according to the American Society of Echocardiography Guidelines using the leading edge technique (27). Mice were anesthetized with 1.5% isoflurane gas, and body temperature was maintained on a prewarmed pad. Heart rate was monitored via an EKG transducer pad throughout each echocardiography session. Two-dimensional B-Mode parasternal long-axis views were obtained first to visualize the atrial and mitral valves, and then the transducer was rotated clockwise 90° to obtain the parasternal short-axis view. At least three M-Mode images were obtained at the midpapillary muscle level from this view. Ejection fraction (EF%) and fractional shortening (FS%) were analyzed and calculated using the Visualsonics Advanced Cardiovascular Measurements Package, using the mean of 12–15 cardiac cycles derived from three separate M-Mode images during each session. Left ventricular (LV) wall dimensions were determined from the M-Mode images and included end-diasotic dimension, end-systolic dimension, diastolic left ventricular wall dimensions, and systolic left ventricular wall dimensions. An apical four-chamber view was then obtained for Doppler analysis of the mitral valve inflow. Mitral E wave (early ventricular filling wave) and A wave (late diastolic filling from atrial contraction) velocities and isovolumetric relaxation (IVRT) and E wave deceleration times (DT) were obtained by Doppler analysis from at least five representative waveforms.

Acute leptin treatment. Mice were treated with an intraperitoneal injection of leptin (100 μg in a 0.1-ml volume). After 1 h, mice were euthanized and hearts collected and stored at −80°C until use for assessment of phosphorylation of STAT3 (p-STAT3) protein.

Western blots. Western blots were performed on lysates prepared from whole hearts collected at the end of the experimental protocol (30 wk). Samples of 50 μg of protein were boiled in Laemmli sample buffer (Bio-Rad, Hercules, CA) for 5 min and electrophoresed on 10% SDS-polyacrylamide gels and blotted onto a nitrocellulose membrane. Membranes were blocked with Odyssey blocking buffer (LI-COR, Lincoln, NE) for 2 h at room temperature and then incubated with primary antibodies overnight at 4°C. Membranes were incubated with either Alexa (fluor) 680 (Molecular Probes) or IRDye 800 (Rockland, Gilbertsville, PA) secondary antibodies for 1 h at room temperature. Membranes were visualized using an Odyssey infrared imager (LI-COR), which allows for simultaneous detection of two proteins. Densitometry analysis was performed using Odyssey software (LI-COR).

Antibodies for Western blots were as follows: p-STAT3-Tyr705 (no. 9145, 1:1,000 dilution; Cell Signaling Technology, Danvers, MA), STAT3 (no. 9139, 1:1,000; Cell Signaling Technology), phospho-ERK1/2-Thr202/Tyr204 (no. 4370, 1:1,000; Cell Signaling Technology), ERK1/2 antibodies (no. 4695, 1:1,000; Cell Signaling Technology), p-Akt-Ser473 (no. 9271, 1:1,000; Cell Signaling Technology), p-Akt-Ser473 (no. 9275 1:1,000; Cell Signaling Technology), p-Akt-Thr308 (no. 9275 1:1,000; Cell Signaling Technology), p-Akt-Ser473 (no. 9145, 1:1,000; Cell Signaling Technology), p-Akt-Thr308 (no. 9139, 1:1,000; Cell Signaling Technology), anti-glucose transporter 4 (GLUT4; ab645, 1:2,500; Abcam, Cambridge, MA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; ab8245, 1:10,000; Abcam), anti-tubulin and ex-
Molecular Research Center, Cincinnati, OH). Following DNase treatment, RNA (1 µl) was used in a reverse transcription reaction using an oligo-dT primer with AMV reverse transcriptase in a 20-µl final volume. Reverse transcriptase PCR was performed on 1 µl of the reverse transcription reaction in a 25-µl volume using standard PCR reaction conditions. The PCR reactions for leptin receptor and GAPDH were cycled as follows: 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min for a total of 35 cycles. Primers used to detect the leptin receptor were as follows: LepR+, 5'-AGTGATATTTGGTCTCTTTCT-3'; LepR-, 5'-GAGTGGTCAAAGAAGTAGGAG-3'. The primers amplified a 400-bp fragment. The primers used to detect GAPDH were as follows: GAPDH+, 5'-AAGAAGGTTGGTGAAAGCAGCAT-3'; GAPDH-, 5'-GATGTTATGCAAGAGAGTAGGGA. The primers amplified a 405-bp fragment. Real-time PCR was performed on 1 µl of reverse transcription reaction prepared as above using a SYBR Green-containing PCR mix (Bio-Rad, Hercules, CA). All samples were normalized to levels of endogenous 18s rRNA. Data are presented as the difference in threshold cycles between the leptin receptor and 18s within each group compared with the difference in threshold cycle of the control mice. Real-time PCR was performed three separate times with RNA from each of the four groups. The primers for the leptin receptor were identical to those listed above. The primers for the 18s were as follows: 18s, 5'-TAAGTCCCTTTGTACACA; 18s-, 5'-GATCAGGGGCTCTACAAAAC-3'.

Statistics. All results are presented as means ± SE. One-way analysis of variance (ANOVA) was performed on heart weight/tibia length ratios. One-way ANOVA was performed on all echocardiographic measurements with post hoc testing using Tukey’s Studentized Range Test. Unpaired Student’s t-test was used for fasting glucose levels, plasma triglyceride levels, myocardial triglyceride and ATP levels, and protein expression. All statistics were performed using GraphPad Prism 5 (La Jolla, CA). Results were considered statistically significant at a level of P < 0.05.

RESULTS

Cardiomyocyte-specific expression of the rat LepR was achieved in transgenic mice using the α-myosin heavy chain promoter (Fig. 1A). Analysis of LepR expression by reverse-transcriptase PCR in Myh6-LepR mice indicated higher levels of the LepR mRNA compared with nontransgenic mice (Fig. 1B). The Myh6-LepR transgenic mice were then bred onto the db/db background to create mice that lack functional LepRs throughout the body, with the exception of the heart (db/db cardiac LepR rescue mice). LepR expression in the heart was determined in Myh6-LepR transgenic mice, db/db mice, and db/db LepR rescue and compared with control and wild-type (WT) mice by real-time PCR. Levels of LepR were increased significantly in transgenic and db/db LepR cardiac rescue mice and decreased in db/db compared with WT control mice (Fig. 1B). The functionality of the cardiac LepR levels was determined in WT and Myh6-LepR mice by examining the levels of

![Image](http://ajpendo.physiology.org.org/)}
phosphorylated STAT3 in the heart following acute treatment with leptin. Acute leptin treatment resulted in greater levels of p-STAT3 in both male and female Myh6-LepR mice compared with WT mice (Fig. 1C).

Rescue of cardiac LepR protects against myocardial triglyceride accumulation despite obesity, hyperglycemia, and high plasma triglyceride levels. At 30 wk of age, body weight, plasma insulin, plasma triglyceride, and plasma leptin levels were measured in lean WT control, db/db, and db/db-cardiac LepR rescue mice. db/db and db/db-cardiac LepR rescue mice were significantly obese compared with control mice (body weights: 62.2 ± 4.4, 58.3 ± 7.7, and 29.8 ± 2.3 g, respectively; Fig. 2A). Blood insulin levels in the db/db and the db/db-cardiac lepR rescue mice were similar and significantly higher than in controls (Fig. 2B; P < 0.05). Fasting plasma triglyceride levels were also similar between db/db and db/db-cardiac LepR rescue mice and were significantly elevated (P < 0.05) compared with control mice (Fig. 2C). Plasma leptin levels were significantly higher (P < 0.05) in both db/db and db/db-cardiac LepR rescue mice compared with controls (Fig. 2D). Fasting blood glucose levels measured every 6 wk starting from week 12 were significantly higher in db/db and rescue mice compared with lean mice (Fig. 3). There was no significant difference in fasting blood glucose levels observed between db/db and rescue mice at any time point measured (Fig. 3). db/db Mice exhibited significantly higher myocardial triglyceride levels compared with control mice (13.5 ± 4.5 vs. 3.9 ± 1.6, P < 0.05); however, myocardial triglyceride levels in db/db-cardiac LepR rescue mice were completely normalized and were not significantly different than in lean controls [3.8 ± 0.7, P = not significant (NS)] (Fig. 4).

Effect of cardiomyocyte overexpression of leptin on cardiac hypertrophy. Heart weight/tibial length (HW/TL) ratios were measured after euthanization at age 30 wk in each mouse (Fig. 2E). db/db Mice and db/db-cardiac LepR rescue mice had higher HW/TL ratios compared with lean controls (125.8 ± 4.4 and 115.8 ± 3.6 mg/cm compared with 94.8 ± 3.6 mg/cm respectively, P < 0.05; Fig. 2E), although this effect was attenuated by nearly 10% in db/db-cardiac LepR rescue mice compared with db/db mice (P = NS). Additionally, diastolic posterior wall thickness (PWTd) was reduced in db/db-cardiac LepR rescue mice compared with db/db mice, although it was not statistically significant.

Left ventricular systolic function is normal in 30-wk-old obese db/db mice. Echocardiographic parameters of left ventricular (LV) systolic function, including EF, FS, cardiac output, and stroke volume, were obtained every 6 wk from 12 wk of age until 30 wk of age. There were no significant differences in any measurements of LV systolic performance among the three groups (Table 1). The mean EF of db/db-cardiac LepR

Fig. 2. Metabolic profile of lean (n = 10), db/db (n = 9), and db/db-cardiac LepR rescue mice (n = 8). A: body weight. B: plasma insulin. C: plasma triglyceride. D: plasma leptin. E: heart weight to tibia length. *P < 0.05 compared with lean mice.

Fig. 3. Time course of plasma glucose measurements in lean (n = 10), db/db (n = 9), and db/db-cardiac LepR rescue mice (n = 8). Plasma samples were measured in fasted mice every 6 wk starting at 12 wk of age. *P < 0.05 vs. lean mice.
rescue mice was 63.1 ± 2.1 vs. 65.7 ± 1.7% in db/db mice and 62.5 ± 1.5% in lean controls (P = NS). Cardiac dimensions were similar among the three groups. There were no significant differences in chamber dimensions.

Rescue of cardiac LepR prevents diastolic dysfunction in obese db/db mice. Doppler analysis of the mitral valve inflow pattern was obtained to evaluate LV diastolic function. The mitral early-to-late (E/A) diastolic filling ratios were measured every 6 wk starting at 12 wk of age. E/A ratios were significantly lower in the db/db mice compared with lean control mice at 12, 24, and 30 wk, suggesting impaired relaxation (Fig. 5). Diastolic function was normalized in db/db rescue mice compared with db/db mice at weeks 24 and 30 (Fig. 5). There were no significant differences in IVRT or DT among the three groups (Table 1).

Rescue of LepR normalizes cardiac p-STAT3 and p-ERK levels and increases GAPDH, insulin signaling, and GLUT4 levels in db/db rescue mice. To determine the effect of rescue of the LepR on cardiac cell signaling, we performed Western blot analysis on proteins of the STAT and ERK pathways. db/db Mice exhibited a significant decrease in the levels of p-STAT3, which was normalized by rescue of the cardiac LepR (Fig. 6, A, B, and D). Cardiac total STAT3 protein was increased in db/db-cardiac LepR rescue mice compared with control and db/db mice (Fig. 6, A and C). db/db Mice exhibited significant decreases in p-ERK1/2, which was normalized in db/db-cardiac LepR rescue mice (Fig. 6, A, E, and G); however, there were no significant differences in total cardiac ERK1/2 in the three groups of mice (Fig. 6, A and F). Levels of GAPDH were increased in db/db-cardiac LepR rescue mice compared with control and db/db mice (Fig. 6, A and H).

The effect of rescue of the cardiac LepR on insulin signaling and glucose uptake was also examined by Western blot analysis. Rescue of the cardiac LepR was associated with increases in the levels of p-Akt. Both p-Akt-Ser473 and -Thr308 levels were significantly elevated in the hearts of the rescue mice compared with both db/db and control mice (Fig. 7, B and C). Next, the effect of rescue of the cardiac LepR on glucose uptake was examined by measuring the levels of GLUT4 from cardiac protein lysates. Rescue mice exhibited a significant increase in cardiac GLUT4 levels compared with both db/db and control mice (Fig. 7D).

Rescue of LepR does not alter cardiac inflammation or oxidative stress markers in db/db mice. To determine the effect of rescue of the LepR on cardiac inflammation and oxidative stress, we performed Western blot analysis on proteins associated with cardiac inflammation and oxidative stress. Levels of nuclear factor-κ-light-chain enhancer of activated B cells (NF-κB) were measured as an index of cardiac inflammation in control, db/db, and rescue mice. No significant differences in NF-κB levels were detected between any of the groups (Fig. 8B). To determine whether alterations in myocardial fatty acid oxidation resulted in increased

Table 1. Echocardiographic parameters in lean, db/db, and db/db rescue mice at 30 wk of age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean (n = 10)</th>
<th>db/db (n = 9)</th>
<th>db/db-Cardiac Rescue (n = 8)</th>
<th>P Value (Lean vs. Others)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection fraction, %</td>
<td>62.5 ± 1.5</td>
<td>65.7 ± 1.7</td>
<td>63.1 ± 2.1</td>
<td>0.397</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>33 ± 1</td>
<td>36 ± 1</td>
<td>34.6 ± 1.7</td>
<td>0.344</td>
</tr>
<tr>
<td>Cardiac output, μl/min</td>
<td>226 ± 1.3</td>
<td>277 ± 1.5</td>
<td>25.8 ± 1.6</td>
<td>0.092</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>48.5 ± 2.4</td>
<td>57.2 ± 2.8</td>
<td>56.9 ± 2.7</td>
<td>0.078</td>
</tr>
<tr>
<td>LVAWd, mm</td>
<td>0.87 ± 0.03</td>
<td>0.94 ± 0.04</td>
<td>0.98 ± 0.06</td>
<td>0.431</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>0.79 ± 0.04</td>
<td>0.88 ± 0.03</td>
<td>0.85 ± 0.02</td>
<td>0.218</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>4.13 ± 0.11</td>
<td>4.33 ± 0.1</td>
<td>4.22 ± 0.14</td>
<td>0.527</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>2.98 ± 0.1</td>
<td>2.94 ± 0.1</td>
<td>2.96 ± 0.12</td>
<td>0.975</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>18.1 ± 0.9</td>
<td>17.5 ± 0.4</td>
<td>17.5 ± 0.6</td>
<td>0.899</td>
</tr>
<tr>
<td>DT, ms</td>
<td>17.5 ± 1.6</td>
<td>23.8 ± 2</td>
<td>23.2 ± 2.4</td>
<td>0.134</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVAWd, left ventricular anterior wall diastole; LVPWd, left ventricular posterior wall diastole; LVIDd, left ventricular internal dimension diastole; LVIDs, left ventricular internal dimension systole; IVRT, isovolumic relaxation time; DT, deceleration time.

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oxidative stress, we measured the levels of two markers of oxidative stress, Cu/Zn superoxide dismutase (SOD) and HO-1. No differences in Cu/Zn (Fig. 8C) or HO-1 (Fig. 8D) levels were found between any of the groups.

DISCUSSION

Our results demonstrate that transgenic “rescue” of the cardiac LepR in obese db/db mice prevents myocardial lipid accumulation and subsequent cardiac diastolic dysfunction. These results support the hypothesis that intact leptin signaling in the heart may attenuate myocardial steatosis in obesity independent of central leptin signaling and changes in body weight or adiposity. Our results also provide no evidence that leptin has a prohypertrophic effect, as suggested previously (35).

db/db Mice in our study exhibited many of the metabolic derangements observed in human “metabolic syndrome,” including morbid obesity, severe hyperglycemia, high circulating plasma triglycerides, and hyperleptinemia. However, the hearts of db/db mice in which the LepR was selectively rescued in cardiomyocytes were protected from triglyceride accumulation. Although db/db mice did not develop systolic dysfunction in the time frame in which they were studied, our findings are in agreement with previous studies indicating that obese db/db mice exhibit cardiac diastolic dysfunction compared with lean controls (5, 12, 39, 53). However, the db/db-cardiac LepR rescue mice with intact cardiac leptin signaling were protected from myocardial triglyceride accumulation and associated left ventricular diastolic dysfunction. In fact, db/db-cardiac LepR rescue mice actually had slightly higher (albeit not statistically significant) E/A ratios compared with lean controls. This finding suggests that restoration of leptin receptors in the hearts of db/db mice normalized cardiac function as well as myocardial lipids despite the continued presence of severe obesity.

Diastolic dysfunction in the db/db mouse has been associated previously with increased diastolic sarcoplasmic reticulum calcium leak and decreased sarcoplasmic reticulum activity (5, 42). Thus, it is possible that rescue of the cardiac LepR could result in improvement of intracellular calcium handling due to activation of the calcium ATPase, sarcoendoplasmic reticulum Ca2+-ATPase, or alterations of contractile proteins such as phospholamban. The relationship between restoration of leptin signaling and intracellular calcium handling and its effects on contractile proteins needs to be examined further, as this could represent a novel pathway by which restoration of leptin signaling improves diastolic function in db/db cardiac LepR rescue mice.

Enhancement of inflammation and increases in oxidative stress are also potential pathways for altered diastolic function in db/db mice. Several studies have linked increases in inflam-
mation to development of cardiac dysfunction in obesity (51, 52). The role of leptin in contributing to cardiac inflammation in obesity is controversial, with studies from leptin-deficient rodents suggesting that leptin may be proinflammatory (29). However, studies from obese human patients did not find any effect of recombinant leptin treatment on levels of inflammatory markers (23). In the present study, we found no significant differences in cardiac NF-κB protein, an index of cardiac inflammation, in any of the groups. Likewise, we examined two important proteins that are markers for increases in oxidative stress: SOD and HO-1. We failed to detect any differences in the cardiac levels of these two proteins in any of the groups. These findings would argue against alterations in inflammation or oxidative stress as potential mechanisms for the effect of rescuing cardiac LepR to improve diastolic function or myocardial lipid accumulation in the present study.

Previous studies have also reported that lipid accumulation in the heart is associated with cardiac dysfunction (34, 37, 54). High myocardial triglyceride levels are associated with impaired biventricular strain in diabetic humans (33). Further-
more, caloric restriction in obese diabetic patients has decreased myocardial triglyceride content and improved myocardial diastolic function (18, 19, 46). Short-term high-fat diet in mice has resulted in increased myocardial lipid accumulation and reductions in left ventricular strain (20). Our results demonstrate that restoration of cardiac leptin signaling in obese db/db mice prevents myocardial lipid accumulation and improves diastolic function. These results are consistent with the hypothesis that the cardiac LepR signaling plays an important role in attenuating myocardial lipid accumulation independent of CNS and actions of leptin, changes in body weight, or blood levels of triglycerides and glucose, which has been studied extensively by Unger (43).

The mechanisms by which myocardial lipid accumulation induces cardiac dysfunction are poorly understood. One mechanism that has received increased attention is an imbalance in delivery and oxidation of fatty acids to the heart resulting in “lipotoxic” injury (43). The normal heart utilizes a combination of predominantly fatty acids and glucose for ATP generation and contractile work. In obesity, or states of an overabundance of fatty acids, there may be increased fatty acid oxidation if the heart is programmed to preferentially utilize these energy sources (7, 30, 50). As fatty acid delivery to the heart exceeds oxidative capabilities, lipid accumulation or myocardial steatosis develops. Myocardial steatosis may lead to the accumulation of toxic intermediates, such as ceramide or diacylglycerol, that contribute to oxidative stress, mitochondrial dysfunction, tissue fibrosis, and apoptosis (8, 34, 48). Alternatively, myocardial steatosis or triglyceride accumulation could serve as a storage reservoir for, and therefore protect against, the adverse effects of fatty acid metabolites such as ceramides and diacylglycerols on the heart (7). In the current study, we did not evaluate these lipid intermediates in the heart, nor did we examine whether the hearts of the rescue mice exhibited significant increases in enzymes associated with fatty acid oxidation or transport of fatty acids in the heart. This is a limitation of the present study and will require further investigation.

Leptin signaling in the heart occurs through three main pathways, including Jak2/IRS/Pi3K, Shp2/MAP kinase, and STAT3 (15). Disruption of these leptin signaling pathways causes dilated cardiomyopathies (21, 24, 26). Previous studies from our laboratory and others have also demonstrated the protective role of leptin against the development of heart failure, and this appears to be mediated at least in part by leptin’s role in attenuating myocardial lipid accumulation independent of CNS and actions of leptin, changes in body weight, or blood levels of triglycerides and glucose, which has been studied extensively by Unger (43). We also found evidence for enhanced signaling through the insulin pathway in the db/db-cardiac LepR rescue mice. These mice exhibited significant elevations in the levels of p-Akt compared with both db/db and lean controls. The increased Akt activity along with increased levels of GLUT4 transporters in the hearts of the db/db cardiac LepR rescue mice suggest that restoration of cardiac leptin signaling may also improve glucose uptake and utilization in these hearts. However, more direct measurements of glucose metabolism are needed to establish this mechanism.

Although db/db mice did not exhibit systolic dysfunction, the left ventricular diastolic functional derangements observed may be similar to those found in obese patients with heart failure and preserved ejection fraction (49). Obesity-induced cardiomyopathy is an increasingly recognized cause of non-ischemic cardiac dysfunction thought to be related to impaired myocardial metabolism (2, 22). The db/db mouse displays a cardiac phenotype similar to this cardiomyopathy that may predispose patients to adverse cardiac outcomes. Obesity is also strongly associated with risk factors for coronary artery disease and heart failure such as hypertension, diabetes, and dyslipidemia (9, 25). Therefore, our transgenic mouse model provides important insights into mechanisms by which obesity-mediated adverse cardiac outcomes may be prevented. However, further studies evaluating leptin signaling in the myocardium, particularly in relation to myocardial metabolism, are warranted.

Although our results suggest a protective role of leptin against obesity-mediated cardiac dysfunction, other studies have associated high leptin levels with cardiovascular diseases such as coronary atherosclerosis and congestive heart failure (6, 38). It is possible that high or low leptin levels may have detrimental effects on the heart (36). Although our observations imply a direct anti-steatotic effect of leptin on cardiomyocytes, it is conceivable that restoration of leptin signaling in the heart could affect neurohumoral or metabolic regulatory systems that may alter cardiac function and lipid content (1).

The present study also provides insight into the potential role of leptin in cardiac hypertrophy. There have been discrepant results in previous studies related to leptin’s effect on LVH. Studies in isolated rat cardiomyocytes and human pediatric ventricular myocytes suggest a prohypertrophic effect of leptin (35). This is in contrast with studies in ob/ob mice, which have demonstrated that administration of leptin was associated with a decrease in LVH independent of changes in body weight (3). We observed a nearly 10% reduction in HW/TL ratio in db/db cardiac LepR rescue mice compared with db/db mice in addition to reduced diastolic posterior wall thickness (PWTd); however, this change was not statistically significant. The observation that db/db LepR rescue mice, despite intact cardiac LepR signaling and high circulating leptin levels, exhibited reduced wall thicknesses and lower normalized heart weights compared with db/db mice of similar weights provides no evidence that leptin directly causes cardiomyocyte hypertrophy. Our observations suggest that cardiac hypertrophy associated with obesity is unlikely to be related directly to increased levels of leptin; instead, other factors associated with obesity, such as hypertension and increased preload due to
increased venous return, appear to be more important. It should be noted, however, that we did not directly examine cardiomyocyte diameters in the present study.

In summary, we developed and characterized a novel transgenic model in which cardiac LepRs were specifically rescued in obese lepR-resistant db/db mice. These cardiac LepR rescue mice are protected from myocardial triglyceride accumulation and left ventricular diastolic dysfunction associated with obesity. Despite systemic metabolic derangements, these mice have normal cardiac diastolic function compared with lean WT mice and improved cardiac function compared with obese db/db mice with an identical metabolic profile of morbid obesity, hyperglycemia, hypertriglyceridemia, and hyperleptinemia. Furthermore, our results suggest that leptin does not directly cause cardiac hypertrophy.

**Perspectives and significance.** Obesity causes an increase in fatty acid delivery and oxidation in the heart, which can have deleterious effects on cardiac function. Adipokines such as leptin appear to regulate cardiac energy metabolism and may have effects on cardiac structure and function. The role of the cardiac leptin signaling pathway in preventing lipid accumulation, lipotoxicity, and its effects on cardiac function is not fully understood. In the present study, we characterized a novel mouse model in which cardiac leptin receptors (LepR) were “rescued” specifically in the hearts of obese db/db mice with defective LepR. Despite severe obesity and major metabolic abnormalities, these db/db cardiac LepR rescue mice were protected against cardiac triglyceride accumulation and diastolic dysfunction observed in db/db mice. These results directly demonstrate that cardiac LepR signaling plays an important role in protecting against excessive cardiac lipid accumulation and possibly myocardial diastolic dysfunction in obesity independent of body weight or circulating lipid levels. Additionally, although leptin has been suggested to have both pro- and antihypertrophic effects on the heart, our results indicate that expression of cardiac leptin receptors in obese db/db mice with high levels of leptin does not cause cardiac hypertrophy. Further investigations should evaluate the mechanisms by which leptin protects the heart against lipid accumulation in obesity.

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**DISCLOSURES**

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


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