Effects of diet-induced obesity on inflammation and remodeling after myocardial infarction

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Epidemiological studies indicate that obesity, insulin resistance, and diabetes are important comorbidities of patients with ischemic heart disease and increase mortality and development of congestive heart failure after myocardial infarction. Although ob/ob and db/db mice are commonly used to study obesity with insulin resistance or diabetes, mutations in the leptin gene or its receptor are rarely the cause of obesity in humans, which is, instead, primarily a consequence of dietary and lifestyle factors. Therefore, we used a murine model of diet-induced obesity to examine the physiological effects of obesity and the inflammatory and healing response of diet-induced obese (DIO) mice after myocardial ischemia-reperfusion injury. DIO mice developed hyperinsulinemia and insulin resistance and hepatic steatosis, with significant ectopic lipid deposition in the heart and cardiac hypertrophy in the absence of significant changes in blood pressure. The mRNA levels of chemokines at 24 h and cytokines at 24 and 72 h of reperfusion were higher in DIO than in lean mice. In granulation tissue in 72 h of reperfusion, macrophage density was significantly increased, whereas neutrophil density was reduced, in DIO mice compared with lean mice. At 7 days of reperfusion, collagen deposition in the scar was significantly reduced and left ventricular (LV) dilation and cardiac hypertrophy were increased, indicative of adverse LV remodeling, in infarcted DIO mice. Characterization of a murine diet-induced model of obesity and insulin resistance that satisfies many aspects commonly observed in human obesity allows detailed examination of the adverse cardiovascular effects of diet-induced obesity at the molecular level.

insulin resistance; cardiac lipotoxicity; ischemia-reperfusion injury; left ventricular remodeling

The incidence of overweight and obesity in the Western world has risen dramatically; in the United States, two-thirds of the adult population is overweight and one-third is obese (27). Extreme obesity is known to impact the structure and function of the heart in terms of hemodynamic load, altered left ventricular (LV) remodeling, and impaired ventricular function leading to overt heart failure (30, 31). In at least four community-based, prospective studies, body mass index (BMI) incrementally predicted heart failure risk above and beyond known risk factors for heart failure. Obesity is known to independently increase the risk for hypertension, diabetes, and dyslipidemia, all of which increase the risk for myocardial infarction and subsequent heart failure. On the other hand, when associated with fewer comorbidities, obesity paradoxically has been found to be protective in the short term after a myocardial infarction (39). Nonetheless, the risks for recurrent myocardial infarction and adverse outcome in the long term are increased in patients with BMI >25 kg/m² (40).

In recent years, with the advances in gene targeting and trapping technologies, the mouse model is increasingly being used to model human diseases for studies at the mechanistic level. To take advantage of these technologies, we have developed a murine model of myocardial infarction (41) and used it extensively to examine the role of specific genes in the pathobiology of ischemic myocardial injury (5, 14, 23). The process of cardiac repair following myocardial infarction is initiated by an acute inflammatory response largely dictated by temporally and spatially coordinated expression of chemokines and cytokines, cell adhesion molecules, growth factors, and extracellular matrix proteins. In the early phase, the recruited leukocytes contribute to the clearing of necrotic cardiomyocytes. Subsequently, granulation tissue, consisting of macrophages, myofibroblasts, and neovessels, is formed. In the final maturation phase, infarct myofibroblasts deposit a network of collagen-based extracellular matrix protein, resulting in the formation of a scar. Repair of the infarct is accompanied by extensive geometric and structural changes of the ventricle, termed LV remodeling. Adverse remodeling of the infarcted ventricle ultimately leads to dysfunction and is an important predictor of mortality.

The mouse model offers an opportunity to study in detail the pathophysiology of myocardial infarction. The genetic mouse models of obesity and insulin resistance (ob/ob) or diabetes (db/db) are commonly used for obesity-related studies, including myocardial infarction (20, 26, 29). Although ob/ob and db/db are excellent mouse models for studies of some aspects of obesity-related physiology, mutations in the leptin gene (ob/ob) or its receptor (db/db), leading to overt obesity are rarely described in humans. In fact, much of the obesity epidemic is a result of energy imbalance caused by consumption of a high-calorie and high-fat diet combined with a sedentary lifestyle. Furthermore, the pathology in genetic models of obesity develops in combination with several other...
abnormalities (18). Leptin has profound effects on the immune system that are underscored by the early observation of thymus atrophy in db/db mice (17). Evidence from several studies supports a proinflammatory role for leptin (16). The inherent immune-modulating abnormalities presented by mouse models of leptin or leptin receptor deficiency make these models unattractive for studies that examine inflammatory changes related to cardiac repair after myocardial infarction in the context of obesity. Furthermore, severe hyperglycemia in mouse models of diabetes may also adversely affect the cardiovascular system. The present study describes a murine dietary model of obesity with an intermediary phenotype, in contrast to the morbid obesity displayed by ob/ob and db/db mice and examines the impact of diet-induced obesity on inflammation and cardiac repair after myocardial infarction. We hypothesized that the systemic and cardiac metabolic abnormalities associated with obesity induced by a “Western” diet would adversely influence the ability of the heart to heal effectively.

MATERIALS AND METHODS

All animals received humane care in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985), and the protocol was approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were bred in-house. The mice (3–5 per cage) were Mills, St. Louis, MO) after weaning until 6–8 wk of age. Obesity was induced by ad libitum access to a high-fat diet containing 10% (w/w) casein, 20% (w/w) bovine milk fat, and 70% (w/w) sucrose (55). The high-fat diet would adversely influence the ability of the heart to heal effectively.

Noninvasive cardiac indexes. Noninvasive cardiac Doppler measurements were made on mice anesthetized with isoflurane (1% in 100% oxygen) administered at a continuous flow rate (20 ml/min). A 10-MHz probe was used to obtain Doppler signals from the aortic root and the mitral inflow track (51). Doppler signals from each mouse were acquired and stored using a Doppler signal-processing workstation (Indus Instruments, Houston, TX). Noninvasive cardiac indexes of systolic and diastolic function were obtained off-line from stored signals.

Noninvasive blood pressure measurement. A tail pressure cuff (or tail cuff) and Doppler sphygmomanometer were used for noninvasive blood pressure measurement in mice (44). Briefly, the mouse was anesthetized with 1.5% isoflurane and placed supine on an ECG-heater board. The board temperature was adjusted to maintain the body temperature of the mouse at 37 ± 1°C. A tail cuff was placed close to the base of the tail, and a 20-MHz pulsed Doppler cuff probe was placed immediately distal to the tail cuff. The tail cuff was pressurized to suprasystolic levels to occlude the tail artery completely and then released gradually. The cuff pressure at which the tail blood flow reappeared was recorded as systolic pressure, and the pressure at which the tail blood flow became continuous was recorded as diastolic pressure. The tail blood flow velocity signal sensed by the Doppler probe and the tail-cuff pressure signal sensed by a pressure sensor (Meritrans MER100, Merit Medical Systems, South Jordan, UT) were acquired by a Doppler signal-processing workstation (Indus Instruments) and stored for off-line analysis.

Murine myocardial infarction protocol. Male and female lean and diet-induced obese (DIO) mice were anesthetized by an injection of pentobarbital sodium (10 µg/g ip). A closed-chest mouse model of reperfused myocardial infarction was used as previously described (13, 41) to avoid the confounding effects of surgical trauma and inflammation, which may influence the baseline levels of chemokines. The left anterior descending coronary artery was occluded for 1 h and then reperfused for 1, 3, and 7 days (n = 8 for each group). At the end of the experiment, the mice were killed with an overdose of pentobarbital sodium, and the hearts were frozen at −80°C for RNA extraction or fixed in Z-fix and embedded in paraffin for histological studies. Sham-operated animals were implanted with a suture around the left anterior descending coronary artery but were not subjected to coronary artery occlusion and reperfusion.

Perfusion fixation and quantitative morphometry. For assessment of cardiac remodeling after infarction, sham-operated and 7-day-reperfused hearts were perfusion fixed as described by Michael et al. (36). Briefly, cardioplegic solution was perfused through the jugular vein to promote relaxation, the heart was excised and rinsed in cold cardioplegic solution, the aorta was cannulated, and a PE-50 catheter was pushed through the mitral valve into the LV and secured in place. The hearts were fixed for 10 min by aortic perfusion of 10% zinc-buffered formalin at a constant pressure of ~16 cmH2O. The entire heart was cross-sectioned from base to apex at 250-µm intervals. Ten serial 5-µm sections were obtained for each interval. LV dimensions were assessed in terms of LV mass and end-diastolic volume by quantitative morphometry with ImagePro software by methods described in detail by Michael et al. (36) Scar size was expressed as percentage of LV volume.

Quantitative histology and immunohistochemistry. After identification of the area below the suture, serial sections were stained with picrosirius red for identification of collagen fibers (22). The picrosirius-stained slides were scanned using a Zeiss Axioskop microscope, and quantitative evaluation was performed using ImagePro software (23). Percent collagen staining was assessed in infarcts as well as the peri-infarct area after 7 days of perfusion. Immunohistochemistry was performed for identification of neutrophils with rat anti-mouse neutrophil antibody (Serotec, Oxford, UK), macrophages with rat anti-mouse Mac-2 antibody (Cederlane Laboratories, Burlington, NC), and myofibroblasts with monoclonal α-smooth muscle actin antibody (Sigma). For staining, a peroxidase-
based technique with the Vectastain Elite rat kit (Vector Laboratories, Burlingame, CA) was used. Sections were counterstained with eosin, and the density of labeled cells in the infarcted area was determined after 3 days of reperfusion. Macrophase, neutrophil, and myofibroblast density is expressed as cells per square millimeter.

RNA extraction, RNase protection assay, and quantitative real-time PCR. RNA was isolated from hearts using TRIzol reagent according to the manufacturer’s instructions. mRNA expression levels of the chemokines macrophage inflammatory protein (MIP)-1α, MIP-1β, MIP-2, monocyte chemoattractant protein-1 (MCP-1), and interferon-γ-inducible protein-10 (IP-10) and the cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-10, macrophage colony-stimulating factor, transforming growth factor (TGF)-β1 (TGF-β1), TGF-β2, TGF-β3, and osteopontin were determined by RNase protection assay (RiboQuant, Pharmingen) according to the manufacturer’s protocol. Phosphorimaging was performed (Storm 860, Molecular Dynamics), and the signals were quantified using ImageQuant software. Expression was normalized to ribosomal protein L32 mRNA levels.

For real-time PCR studies, genomic contamination was removed by treatment of total RNA with DNase, as described by the manufacturer (DNase-free, Ambion). First-strand cDNA was synthesized using SuperScript II RT and random hexamer primers as described in the manufacturer’s protocol (Invitrogen, Carlsbad, CA). The relative standard curve method was used to measure the expression levels of hypoxia-inducible factor-1α (HIF-1α) using murine TaqMan primers and probes (Applied Biosystems, Foster City, CA) on a sequence detection system (Prism 7000, Applied Biosystems). Target gene expression was normalized to an internal control, cyclophilin B, which was measured using SYBR green chemistry and published primer sequences (53).

Statistical analysis. Statistical differences between groups were analyzed by unpaired Student’s t-test, and differences between multiple groups were assessed by ANOVA with Bonferroni’s correction. Values are means ± SE. Kaplan-Meier survival statistics were performed with SPSS software. P ≤ 0.05 was considered significant.

RESULTS

Physical and metabolic features of DIO mice. The lean and metabolic characteristics of lean and DIO male and female mice are summarized in Table 1. After they were fed a high-fat diet for 24 wk, male and female DIO mice were significantly heavier than their lean counterparts, with an average weight gain of ~1.6- to 1.7-fold. Diet-induced obesity also caused hepatic steatosis in male and female mice; obese male mice had markedly larger fatty livers than obese female mice (4.22 ± 0.80 vs. 1.75 ± 1.0 g, P < 0.05), largely because of the accumulation of neutral lipids in the liver (data not shown). On the other hand, the average fat pad mass was significantly greater in obese female than in obese male mice: 4.41 ± 0.46 vs. 2.46 ± 0.09 g (P < 0.01). Heart weights were modestly, but significantly, higher in obese male and female mice than in their respective lean controls (P < 0.01). Fasting plasma triglyceride levels were significantly elevated in obese male and female mice compared with their respective lean controls. In the nonfasted state, circulating NEFAs were significantly (P < 0.05) higher in male and female DIO mice than in their respective lean controls; however, fasting NEFA levels were not significantly different between the groups.

To monitor hemodynamic changes resulting from obesity, we measured cardiac indexes and arterial blood pressure noninvasively in the mice and found no significant differences between lean and obese mice in early peak flow velocity (80.4 ± 3.4 vs. 74.6 ± 0.9 cm/s), atrial peak flow velocity (52.6 ± 2.5 vs. 55.5 ± 7.0 cm/s), and peak aortic flow velocity (98.7 ± 0.9 vs. 100.0 ± 5.5 cm/s). Also, no significant differences were observed in systolic blood pressure (93.0 ± 4.8 vs. 99.0 ± 4.0 mmHg for males and 89.0 ± 1.8 vs. 85 ± 5.9 mmHg for females) or diastolic blood pressure (75.0 ± 4.8 vs. 78.0 ± 5.8 mmHg for males and 72.0 ± 5.8 vs. 72.0 ± 4.3 mmHg for females) measured by tail cuff-Doppler sphygmomanometry.

To determine whether diet-induced obesity resulted in development of insulin resistance, we measured plasma insulin and glucose levels after an overnight fast. Male and female DIO mice exhibited hyperinsulinemia compared with their respective lean controls; the severity of hyperinsulinemia was more pronounced in obese male mice (Fig. 1A). In addition, fasting glucose levels were significantly elevated in obese male mice compared with lean mice: 168.4 ± 10.3 vs. 126.2 ± 13.3 mg/dl (Fig. 1B). HOMA-IR indicated that male DIO mice were severely insulin resistant (P = 0.001; Fig. 1C).

Cardiac triglyceride levels in lean and DIO mice. Insulin resistance is frequently associated with lipid deposition in nonadipose tissue. As noted earlier, obese mice had significantly larger fatty livers than lean mice; we also found lipid accumulation in skeletal muscle of obese mice (data not shown). We extracted total lipids from the hearts of male lean and obese mice to determine changes in the composition of cardiac lipids. As shown by TLC in Fig. 2A, cardiac triglyceride deposition was increased in obese mice compared with lean mice. Biochemical analysis indicated that cardiac triglyceride levels were 34% higher in obese male mice than in lean controls (P = 0.02; Fig. 2B).

Myocardial infarction in lean and DIO mice. An established myocardial infarction model of closed-chest ischemia-reperfusion injury was employed to study the myocardial inflammatory and healing response in DIO mice. Fifty-nine DIO animals underwent an occlusion-reperfusion procedure or a sham surgery, and 40 survived until the end of the experiment, representing a survival rate of 67.8%. Sixty-three lean mice were subjected to an occlusion-reperfusion procedure or a sham surgery, and 50 animals survived the procedure, representing a 79.3% survival rate. The difference in survival rate between the two groups was not significant. Mice were subjected to 1 h of coronary artery occlusion and 24 h, 72 h, and

Table 1. Physical and metabolic variables of lean and obese mice

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<tr>
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<th>Males</th>
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<tr>
<td></td>
<td>Lean</td>
<td>Obese</td>
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<tr>
<td>Body wt, g</td>
<td>27.45±1.26</td>
<td>46.41±2.42*</td>
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<tr>
<td>Liver wt, g</td>
<td>1.35±0.06</td>
<td>4.22±0.80*</td>
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<td>Heart wt, g</td>
<td>0.13±0.003</td>
<td>0.15±0.006*</td>
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<tr>
<td>Fat pad wt, g</td>
<td>1.79±0.14</td>
<td>2.46±0.09*</td>
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<tr>
<td>TG, mg/dl</td>
<td></td>
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<tr>
<td>Nonfasting</td>
<td>73.01±4.8</td>
<td>61.99±2.1</td>
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<tr>
<td>Fasting</td>
<td>51.13±3.05</td>
<td>67.19±2.52*</td>
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<tr>
<td>NEFA, mM</td>
<td></td>
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</tr>
<tr>
<td>Nonfasting</td>
<td>0.75±0.01</td>
<td>0.92±0.04†</td>
</tr>
<tr>
<td>Fasting</td>
<td>0.41±0.07</td>
<td>0.50±0.04</td>
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Values are means ± SE; n > 5 for body, liver, heart, and fat pad weights and fasting triglycerides (TG) and nonesterified fatty acids (NEFA); n = 3 for nonfasting TG and NEFA. *P < 0.01. †P < 0.05.
7 days of reperfusion for studies on inflammatory and healing responses. The mean scar size determined by planimetry (percentage of LV wall mass) was 4.2 ± 1.4% in the lean infarcted group (n = 11) and 9.5 ± 3.6% in the obese group (n = 9, P = 0.133).

Chemokine and cytokine expression after myocardial infarction. We examined the chemokine mRNA expression profile in sham-operated and infarcted animals at 24 h of reperfusion by RNase protection assay. In sham-operated lean and obese animals, the baseline expression of MIP-1α, MIP-1β, MIP-2, and MCP-1 was minimal and similar. The magnitude of induction of all of the chemokines was significantly higher in the infarcted than in the sham-operated animals; however, the magnitude of induction of MIP-1α, MIP-1β, MIP-2, MCP-1, and IP-10 was significantly higher in the infarcted hearts of obese than lean mice (Fig. 3).

We examined the expression of IL-1β, IL-6, TNF-α, IL-10, osteopontin, and TGF-β isoforms at 24 and 72 h of reperfusion. In sham-operated lean and obese animals, we did not detect a difference in the baseline expression levels of these cytokines. The mRNA levels of some, but not all, of the cytokines were altered in the infarcted hearts of obese compared with lean mice. Increased expression of IL-6, IL-10, osteopontin, TGF-β1, and TGF-β3 was noted in the infarcted hearts of obese mice at 24 and 72 h of reperfusion (Fig. 4). Although relative expression of TGF-β2 was very low in the heart, it was also significantly increased in the infarcted hearts of obese compared with lean mice at 24 and 72 h (P < 0.05; data not shown). Expression of IL-1β and TNF-α was similar in the infarcted hearts of lean and obese mice (data not shown).

Furthermore, we determined the mRNA expression of HIF-1α, a transcription factor regulating angiogenesis, in the infarcted hearts of lean and obese mice at 24 h of reperfusion by real-time PCR. The baseline expression of HIF-1α was not significantly different between lean and obese sham-operated animals. At 24 h of reperfusion, HIF-1α was significantly upregulated in lean infarcted animals (P < 0.05) compared with lean sham-operated controls, whereas obese infarcted animals exhibited a trend toward increased HIF-1α expression compared with obese sham-operated controls (P = 0.16). The levels were not significantly different between lean and obese infarcted animals (data not shown).

Inflammatory infiltrate in granulation tissue. Immunohistochemistry was used to monitor macrophages and neutrophils in the infarcts of lean and obese mice at 72 h of reperfusion (Fig. 5). Macrophage density was significantly increased in obese compared with lean mice (P = 0.036), whereas neutrophil density was reduced in obese compared with lean mice (P = 0.056). The density of α-smooth muscle actin-positive fibroblasts, myofibroblasts, was similar in the infarcts of lean and obese mice (205 ± 32 and 229 ± 29 myofibroblasts/mm²).

Cardiac hypertrophy. Data for LV wall mass and interventricular septal mass are presented in Fig. 6. LV wall mass was significantly greater in obese sham-operated animals than in lean sham-operated controls: 59.03 ± 1.8 vs. 46.09 ± 0.75 mg (P = 0.001). Also, the interventricular septal mass was increased in obese sham-operated animals compared with lean sham-operated controls: 18.85 ± 0.54 vs. 14.98 ± 0.8 mg (P < 0.002). At 7 days of reperfusion, the LV wall mass in obese infarcted animals was significantly increased compared with that in obese sham-operated controls: 73.4 ± 4.6 vs. 59.03 ±

Fig. 1. Diet-induced obese (DIO) mice were insulin resistant. A and B: overnight fasting plasma insulin and glucose levels in male (M) and female (F) lean and obese mice. Glucose and insulin levels were significantly elevated in obese male mice compared with lean controls, and insulin levels were significantly elevated in obese female mice compared with lean controls. C: homeostasis model assessment for insulin resistance (HOMA-IR), calculated by a formula described in MATERIALS AND METHODS, indicated that obese male mice were severely insulin resistant. *P < 0.01 vs. lean males. *P < 0.05 vs. respective lean controls.
1.8 mg (P = 0.015). In the lean infarcted group, the change in wall mass was marginal and not significant: 51.43 ± 2.35 vs. 47.4 ± 2.05 mg in lean sham-operated animals. In addition, there was a similar trend toward an increase in interventricular septal mass in obese infarcted animals compared with obese sham-operated controls (21.7 ± 1.3 vs. 18.9 ± 0.54, P = 0.067), but no such trend was observed in the lean infarcted animals compared with the lean sham-operated group.

**Collagen accumulation within the scar.** We assessed collagen deposition in the infarct, as well as the peri-infarct area in lean and obese mice at 7 days of reperfusion. The mean collagen content of the remote septum in lean and obese mice was not significantly different (4.88 vs. 5.09%, P = not significant). Also, the amount of fibrosis in the peri-infarct area in the infarcted hearts of lean and obese mice was similar (24.28 and 24.47%, respectively); however, we noted that the scar was densely packed with replacement collagen fibers in lean mice, whereas replacement collagen was significantly reduced in the scar in obese mice (Fig. 7). The mean percent collagen in the scar of lean and obese mice was 46.6 and 26.7%, respectively (P < 0.05).

**LV end-diastolic volume.** For quantitative assessment of ventricular dimensions, the hearts were fixed in end diastole, and the data are shown in Fig. 8. We observed no significant difference in the end-diastolic volume of the LV in lean and DIO mice in the absence of infarction. After 7 days of reperfusion, end-diastolic volume was significantly larger in hearts of obese infarcted than obese sham-operated mice: 43.22 ± 4.1 (n = 8) vs. 31.20 ± 1.0 (n = 7) mm (P = 0.023). In the lean group, the increase was not significant.

**DISCUSSION**

A relation has been established for BMI and the risk for development of heart failure in the Framingham Heart Study (30). Obesity-related disorders are linked to the undesirable outcomes of ischemic heart diseases, although the mechanisms responsible are poorly defined. A major limitation of the genetic models of obesity, ob/ob and db/db mice, for studying inflammation and subsequent remodeling after infarction is the altered immune system of these animals, which may potentially influence the remodeling process in the setting of myocardial ischemia-reperfusion injury. In light of the increased clinical importance of obesity for the development of cardiovascular diseases in humans and the critical need to study the cardiac effects of obesity in an animal with an intact immune system, we report here the characterization of a murine model of diet-induced obesity that we have used to evaluate the effects of diet-induced obesity on inflammation and healing of the myocardium after infarction. We show that DIO mice developed hyperinsulinemia and insulin resistance. Importantly, we show here for the first time that diet-induced obesity in mice was associated with ectopic cardiac triglyceride deposition along with concentric hypertrophy. After a myocardial infarction, DIO mice exhibited an enhanced and prolonged inflammatory reaction that was associated with adverse remodeling, as evidenced by reduced collagen deposition in the scar and greater increases in LV mass and LV end-diastolic volume.

In C57BL/6J mice fed a high-fat (21% milk fat), high-sucrose diet for 6 mo, body weight and fat pad mass were increased; the average weight gain of DIO mice in our study was ~68%. In contrast, at 8 mo of age, the average body weight of ob/ob mice is ~2.3 times that of wild-type controls (data not shown), making it an extreme model of morbid obesity. The diet-induced obesity model in the mouse thus presents a closer approximation of the severity of obesity commonly observed in humans with myocardial infarction. In atherothrombotic patients in an international registry, 26.6% were obese (BMI ≥30 kg/m²) and 3.6% were morbidly obese (BMI ≥40 kg/m²); respective prevalences in North America were 36.5 and 5.8%, which were statistically significantly higher than in other regions (4). As in the genetic models of more severe obesity, DIO mice had increased liver mass, indicative of hepatic steatosis. Diet-induced obesity was accompanied by hyperinsulinemia in male and female mice.
Moreover, HOMA-IR indicated gender differences, with male obese mice developing severe insulin resistance compared with female obese mice. Recently, in an examination of the temporal patterns of insulin action and glucose metabolism after feeding a high fat-diet consisting of 55% of kilocalories from fat, Park et al. (43) reported that insulin-stimulated cardiac glucose metabolism was reduced by as early as 1.5 wk of high-fat feeding, along with blunted Akt-mediated insulin signaling and GLUT4 levels. Long-term (20 wk) feeding was associated with LV systolic dysfunction and mild hyperglycemia, hyperleptinemia, and reduced circulating adiponectin levels (43). The diet used in our study, in which 42% of kilocalories were from fat, is sometimes referred to as a Western diet, inasmuch as its fat composition closely resembles a high-fat diet of humans in North America and Europe.

Insulin resistance and diabetes are frequently associated with lipid deposition in tissues such as liver and skeletal muscle (34, 47, 52). Lipotoxicity, defined as tissue dysfunction induced by lipid deposition in nonadipose tissues, is widely believed to be a cause of development of insulin resistance. Furthermore, in animal models of diabetes and obesity, perturbations in cardiac fatty acid metabolism lead to ectopic cardiac lipid accumulation. We hypothesized that insulin resistance and elevated circulating NEFAs in diet-induced obesity would also result in lipid accumulation in the heart. Indeed, we show here for the first time a significant elevation of cardiac triglyceride content in severely insulin-resistant male DIO mice. Because the heart relies on fatty acid metabolism to a large extent for its much-needed energy requirements, the balance between uptake and utilization of fatty acids is tightly regulated. Our findings have
important implications in hearts of animals individuals with diet-induced obesity, given that lipid species are potentially capable of generating toxic metabolites that may be harmful to cardiomyocytes, compromising cardiac function, especially after an injury. Zhou et al. (56) demonstrated that cardiac lipid accumulation was associated with contractile dysfunction in obese Zucker diabetic fatty rats. To delineate the contributions of global metabolic defects from those of cardiac lipid accumulation in the development of cardiomyopathies, several investigators in recent years have created transgenic mice, in which fatty acid uptake is driven in excess of cardiac fatty acid utilization. Cardiac-specific overexpression of acyl-CoA syn-

Fig. 4. Increased and prolonged cytokine expression in DIO mice after myocardial infarction at 24 and 72 h of reperfusion. IL-6 was significantly elevated in infarcted hearts of obese mice at 24 h of reperfusion and IL-10, osteopontin, and transforming growth factor (TGF)-β3 were markedly elevated at 72 h of reperfusion compared with lean mice. TGF-β1 was upregulated substantially at 24 and 72 h of reperfusion. Values are means ± SE (n = 8 in each infarcted group, n = 3 in each sham group). *P < 0.05 vs. lean at 24 h. *P < 0.05 vs. lean at 72 h.

Fig. 5. Leukocyte infiltration in infarcts of lean and obese mice 72 h after myocardial infarction. Macrophages and neutrophils were evaluated by immunohistochemistry in the infarcted region of the left ventricle (LV). Values are means ± SE (n = 6). Number of macrophages was significantly greater in infarcts of obese than lean mice; neutrophil density tended to be lower in infarcts of obese than lean mice.
thetase-1 (10), peroxisome proliferator-activated receptor-α, or glycosylphosphatidylinositol-anchored lipoprotein lipase leads to lipid accumulation in the myocardium, which is associated with systolic ventricular dysfunction. Transgenic mice with cardiac-specific overexpression of fatty acid transport protein-1 also import free fatty acids in excess of their capacity to use them and exhibit diastolic ventricular dysfunction (9, 10, 19, 55). Whether intramyocardial lipid deposition is a feature of human heart failure is not entirely clear. Estimates of myocardial lipid by magnetic resonance spectroscopy suggest that obese (BMI > 30 kg/m²) individuals with evidence of impaired contractile function have abnormally high levels of triglycerides in the heart (50). Lipid deposition as determined by oil red O staining was detected in sections of the LV free wall in a group of obese (BMI > 30 kg/m²) diabetic patients with nonischemic heart disease (46).

Long-standing obesity in humans is associated with eccentric hypertrophy and diastolic dysfunction (2, 3). However, a few studies also report concentric remodeling of the heart in obese subjects in the absence of comorbid conditions such as...
hypertension. Accordingly, we noted cardiac hypertrophy in DIO mice. Increased wall and septal mass was indicative of concentric hypertrophy in DIO mice. The wet heart weight of obese male and female mice was marginally but significantly higher than that of lean controls. To assess whether the mice were hypertensive, a factor that has been identified to induce cardiac hypertrophy, we measured the tail-cuff blood pressure in these mice and found no significant difference between the groups. Furthermore, the cardiac output was not altered.

In the Framingham Heart Study, the risk for heart failure increased by 5% in men and 7% in women for each 1-kg/m² increment in BMI (30). Loss of functional myocardium subsequent to an acute myocardial infarction is a frequent cause of cardiac remodeling and chronic heart failure. We used a model of reperfused myocardial infarction to examine the inflammatory reaction and LV remodeling in DIO mice. Myocardial repair after a myocardial infarction is a dynamic process, involving an inflammatory process, the clearing of necrotic tissue, and the replacement of collagen, ultimately leading to a healed scar.

Myocardial remodeling is dependent on the initial acute inflammatory reaction after reperfusion of the infarcted myocardium and is associated with upregulation of chemokines, cytokines, adhesion molecules, and growth factors (24). In a time-course study of reperfused infarcted myocardium in wild-type C57BL/6J mice, we previously showed that the chemokine response peaks 3–6 h after reperfusion and is rapidly downregulated after 24–72 h of reperfusion (13). At 24 h of reperfusion, we found that the expression of chemokines in the infarcts of lean mice had declined almost to sham levels, whereas obese mice displayed an inappropriate response in terms of elevated and prolonged expression of chemokines. The profile of cytokine expression in infarcted hearts of obese mice was also altered; we noted sustained and prolonged expression of IL-6, an inflammatory cytokine, and IL-10, an anti-inflammatory cytokine. Many of the inflammatory cytokines contain NF-κB response elements. Ischemia-reperfusion strongly induces NF-κB, thereby upregulating κB-responsive genes such as IL-6, IL-1β, and TNF-α (6, 7, 24, 35). HIF-1α, a transcription factor, is acutely activated during ischemia-reperfusion in animal models and is a regulator of angiogenesis. There is evidence that HIF-1α also regulates expression of chemokines indirectly via heme oxygenase, which is expressed under hypoxic conditions (42). In addition, it has been reported that cytokines such as TNF-α activate HIF-1α under normoxic conditions (45). The alteration in chemokine expression in lean and obese infarcted mice in our study cannot be explained entirely by HIF-1α expression, because mRNA expression of HIF-1α was not significantly different between lean and obese animals at 24 h of reperfusion. Osteopontin, a multifactorial protein associated with remodeling and matrix organization and a marker of monocyte-to-macrophage differentiation, was significantly upregulated in the infarcts of obese mice (25, 32, 33). Recruited inflammatory cells, such as macrophages and mast cells, have been documented to elaborate TGF-β isoforms, which are crucial for containing the inflammation, as well as regulating collagen deposition (15, 38). We observed increased expression of all three TGF-β isoforms in the infarcts of obese mice. We expected the sustained and prolonged chemokine and cytokine expression in obese mice to influence the leukocyte infiltrate. Indeed, at 72 h of reperfusion, macrophage density was significantly higher in the infarcts of obese than lean mice. Although the neutrophil chemotactants MIP-1α and MIP-2 were elevated in the infarcts of obese mice, to our surprise, at 72 h of reperfusion, obese mice exhibited a trend toward reduced neutrophil density. In contrast to our observation, Jones et al. (29) observed increased leukocyte infiltration at 2 h of reperfusion after a 30-min coronary artery occlusion in a model of obesity and diabetes (db/db) compared with wild-type animals, leading to greater myocardial necrosis. Enhanced phagocytosis of neutrophils by macrophages may possibly be a reason for the reduced neutrophil density we have noted in our study.

Obesity is associated with altered production of proinflammatory cytokines by adipose tissue; these cytokines have been implicated in the metabolic complications of obesity. In addition to triglyceride deposition in the heart, DIO mice in our study had increased levels of free fatty acids, specifically saturated fatty acids and arachidonic acid (data not shown), which are proapoptotic and proinflammatory. Elevated levels of fatty acids are not only causally related to development of insulin resistance but are also closely linked to inflammatory pathways by activation of κB-responsive genes (1, 28).

Macrophages constitute an important component of the inflammatory infiltrate. They are not only responsible for clearing the neutrophils to limit the expansion of the infarct, but they also secrete growth factors, such as TGF-β1, which regulate the activity of fibroblasts. Phenotypically modified fibroblasts, myofibroblasts, play a central role in progression of fibrosis on the basis of their ability to produce procollagens (12, 49). Collagen deposition within the scar is critical for maintaining the tensile strength of the myocardium and preventing rupture (48). The density of myofibroblasts was not significantly different in the infarcts of lean and obese mice at 72 h of reperfusion. However, at 7 days of reperfusion, obese mice had significantly reduced replacement collagen in the scar, which may contribute to adverse LV remodeling. Indeed,
we noted adverse remodeling at 7 days of reperfusion in the obese mice. Although the scar size tended to be larger in the obese group, the difference was not statistically significant ($P = 0.133$). We found that obese mice responded with enhanced hypertrophic remodeling to myocardial infarction with reperfusion. At the same time, the LV chamber size was significantly increased in infarcted hearts of obese compared with lean mice. In a recent study, Greer et al. (26) reported increased short-term mortality after coronary artery occlusion and reperfusion in obese diabetic $db/db$ mice compared with wild-type controls. In addition, they observed significant adverse remodeling in terms of LV dilation, cardiac hypertrophy, and contractile dysfunction in these genetically obese and diabetic mice. Infarct size was not examined in their study.

In summary, we have shown that diet-induced insulin resistance with significant cardiac lipid deposition and concentric cardiac hypertrophy. After a myocardial injury, DIO mice exhibited an altered inflammatory response and healing process. The adverse remodeling observed in obese mice in response to myocardial infarction and reperfusion may be detrimental to the heart, leading to heart failure.

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