Disruption of Leptin Signaling Contributes to Cardiac Hypertrophy Independently of Body Weight in Mice

Lili A. Barouch, MD; Dan E. Berkowitz, MD; Robert W. Harrison, BS; Christopher P. O’Donnell, MD; Joshua M. Hare, MD

**Background**—Whether left ventricular hypertrophy (LVH) in obesity results from increased hemodynamic load or altered neurohormonal signaling remains controversial. Dysregulation of leptin, a neurohormone essential to energy homeostasis, is implicated in the pathogenesis of obesity. Because leptin has cardiovascular bioactivity, we hypothesized that disruption of leptin signaling mediates the development of obesity-associated LVH.

**Methods and Results**—We measured left ventricular (LV) wall thickness and LV mass with echocardiography in mice lacking leptin (ob/ob, n=15) or functional receptor (db/db, n=10) and controls at 2, 4, and 6 months of age. None of the mice had LVH at 2 months. Progressive obesity developed in ob/ob and db/db mice. At 6 months, LVH occurred in ob/ob and db/db compared with controls. We observed corresponding myocyte hypertrophy by light microscopy. To separate the direct contribution of leptin deficiency from mechanical effects of obesity, we induced weight loss in 6- to 8-month-old ob/ob mice either by leptin infusion or caloric restriction. Mice in both groups lost similar weight compared with placebo-treated controls. Leptin infusion completely reversed the increase in wall thickness with partial resolution of myocyte hypertrophy, whereas calorie-restricted mice had no decrease in wall thickness and a lesser change in myocyte size.

**Conclusions**—Together these data show that the effect of leptin on LV remodeling is not attributable to weight loss alone, indicating that leptin has antihypertrophic effects on the heart, either directly or through a leptin-regulated neurohumoral pathway. Disruption of leptin signaling may represent a novel mechanism in LVH and related cardiovascular disorders. **(Circulation. 2003;108:754-759.)**

**Key Words:** echocardiography ■ hypertrophy ■ leptin ■ obesity

There is growing evidence that obesity confers increased risk for structural heart disease independently of associated conditions such as hypertension and diabetes. For example, as with other heart failure risk factors, obesity frequently leads to left ventricular hypertrophy (LVH).1-4 Two general mechanisms have been implicated in the pathogenesis of LVH, namely, increased mechanical load and neuroendocrine signaling derangements.5 Although epidemiologic data support a direct pathophysiologic link, obesity-specific neuroendocrine mediators have not yet been identified.

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Leptin, the obesity gene (ob) product,6 is a hormone that regulates energy balance and adipose stores7 while also exerting cardiovascular activity.8 Leptin deficiency or insensitivity produces obesity and altered metabolic rate in humans and in animal models.9 Hyperleptinemia is associated with obesity-related hypertension, vascular endothelial dysfunction, and chronic congestive heart failure.10-12 Furthermore, leptin centrally activates the sympathetic nervous system13,14 but has direct negative inotropic effects on isolated cardiac myocytes.15,16

In this study we tested the hypothesis that leptin signaling disruption leads to obesity-associated LVH, implicating leptin as an essential factor for maintaining normal LV wall thickness and mass. We studied mice lacking either leptin (ob/ob) or functional leptin receptor (db/db) to demonstrate the development of LVH and associated cellular and hemodynamic changes with obesity. We then tested the prediction that weight loss induced by leptin repletion, but not by caloric restriction, would restore normal LV architecture in ob/ob mice with LVH.

**Methods**

**Animals**

We studied ob/ob and db/db mice with C57Bl/6J background. Controls were heterozygote littermates for echocardiography and hemodynamic studies and C57Bl/6J wild-type (WT) for morphometrics and histology. All animals were purchased from Jackson...
Heterozygote controls were phenotypically normal, with no differences compared with previously published WT measurements of weight, wall thickness, LV mass, or hemodynamic parameters. Mice were housed in an animal facility with a 12-hour light-dark cycle and allowed water and food ad libitum except during the period of caloric restriction for the pair-fed group. The Institutional Animal Care and Use Committee of The Johns Hopkins University School of Medicine approved all protocols and experimental procedures.

**In Vivo Hemodynamics**

We performed LV catheterization in 6-month-old 

**Echocardiography**

We studied 

**Histology**

Hearts were excised and perfused retrograde with 10% formalin at 1 mL/min for 10 minutes to fix in distention. We sliced hearts in cross-section for gross pathology and then preserved the tissue in paraffin blocks. Slides were prepared with H&E stain for structural analysis by light microscopy and with Masson’s trichrome stain to assess the presence and extent of fibrosis. Slides stained with methanamine silver to clearly delineate the basement membranes were used to measure myocyte size. We measured myocyte diameters in regions of myocardium with parallel myocyte fascicles in longitudinal sections. Eight representative high-powered fields distributed around the myocardium were used to measure 35 to 50 cells from each heart.

**Weight Loss by Administration of Exogenous Leptin and Pair Feeding**

We induced weight loss in 6- to 8-month-old 

**Leptin and Insulin Assays**

Plasma leptin and insulin levels were measured in duplicate by radioimmunoassay (Linco Diagnostic Services).

### TABLE 1. Ventricular Parameters in 2-Month-Old Mice

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<thead>
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<th>Metric</th>
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<td>ob Control</td>
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<td>db/db</td>
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<tr>
<td>Body weight, g</td>
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<td>Heart rate, bpm</td>
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<td>Wall thickness, mm</td>
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<td>EDD, mm</td>
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<td>ESD, mm</td>
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<tr>
<td>LV mass, mg</td>
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<tr>
<td>Normalized LV mass, mg/g</td>
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<td>FS, %</td>
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*P < 0.05 vs ob control; †P < 0.01 vs db control.

### TABLE 2. Ventricular Parameters in 6-Month-Old Mice

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*P < 0.0001 vs ob control; †P < 0.01 vs db control.
Data Analysis
Data are presented as mean±SEM. Statistical significance (P<0.05) was determined by 2-tailed t test or ANOVA with Student Newman-Keuls post hoc analysis.

Results

Development of LVH With Obesity
There were no differences in LV wall thickness among the 4 groups at 2 months. There were slightly smaller chamber dimensions and lower LV mass in ob/ob and somewhat greater systolic dimension with corresponding lower fractional shortening in db/db (Table 1). LV mass normalized to body weight was lower in the obese mice, primarily because of higher weight rather than lower LV mass.

By 6 months, ob/ob and db/db mice were morbidly obese, having nearly doubled in weight (Table 2), and had substantially increased LV wall thickness (Figure 1A) and LV mass (Figure 1B) compared with littermate controls. Even though ob/ob LV mass was lower at 2 months, it was still greater than littermate controls at 6 months. Heart rates remained unchanged. Interestingly, variations in LV chamber dimensions and fractional shortening that were present in the young animals were no longer present at 6 months (Table 2). Because of massively increased body weight, normalized LV mass remained lower in the obese mice even though absolute LV mass increased.

Hemodynamic Changes With Obesity in ob/ob Mice
To determine whether hemodynamic load, ie, blood pressure, contributed to increased LV mass and wall thickness, we performed LV catheterization on 6-month-old ob/ob mice (n=12) and compared them to age-matched littermate controls (n=11) (Table 3). Heart rate, systolic blood pressure, and LV end-diastolic pressure did not differ between ob/ob mice and littermate controls, indicating that hypertension did not contribute to the observed changes in LV mass or wall thickness.

Cellular Changes With Obesity in ob/ob Mice
We performed microscopic histology to evaluate the cellular changes in ob/ob mice. In concordance with our echocardiographic findings, marked cellular hypertrophy was present in ob/ob hearts compared with WT (Figure 2). Myocyte diameter was increased in ob/ob mice (22.4±0.5 μm, P<0.001) compared with WT (14.3±0.4 μm), and significantly distorted nuclear architecture in ob/ob mice was evident throughout. Similar changes were seen in the db/db mice (data not shown). We did not observe any significant interstitial fibrosis, adipose infiltration of the myocardium, or metabolic inclusions in the cytoplasm.

Direct Effect of Leptin Repletion on LV Wall Thickness
Next we predicted that these changes were not solely attributable to mechanical effects of obesity but rather that leptin repletion would have an antihypertrophic effect. We induced weight loss in 6- to 8-month-old ob/ob mice (weight, 72.1±1.0 g; wall thickness, 0.80±0.02 mm; LV mass, 93.1±3.1 mg). After 4 to 6 weeks, leptin-infused mice (37.1±3.5 g, P<0.0001; n=8) and pair-fed mice (37.4±1.9 g, P<0.0001 versus baseline; n=7) lost substantial weight, whereas control mice remained obese (76.2±2.0 g, P=NS versus baseline; n=4). We confirmed adequate leptin reple-

| TABLE 3. Baseline Hemodynamics in 6-Month-Old ob/ob Mice |
| --- | --- | --- |
| ob/ob | ob Control |
| N | 12 | 11 |
| Heart rate, bpm | 598±17 | 581±16 |
| Systolic blood pressure, mm Hg | 115±4 | 120±4 |
| LVEDP, mm Hg | 9.5±0.8 | 7.7±0.7 |
| P=NS for all. |  |  |
tion in a subset of leptin-infused (19.2±1.2 ng/mL; n=2) compared with pair-fed mice (2.7±1.0 ng/mL, P<0.002; n=3). Interestingly, there was no difference in insulin levels after weight loss (2.0±1.1 versus 2.5±0.9 ng/mL; leptin versus pair-fed, P=NS).

Although leptin and pair-fed mice lost similar weight, there were dramatic differences in LV mass and wall thickness. The leptin group had complete reversal of gross hypertrophy, with return of LV wall thickness to normal. However, both the pair-fed group and controls had no change in LV wall thickness (Figure 3A). Similarly, the leptin group had substantial reduction in LV mass, with no changes in either pair-fed mice or controls (Figure 3B). Heart rate, LV dimensions, and fractional shortening remained unchanged (data not shown).

Cellular Changes With Weight Loss in ob/ob Mice

To correlate changes in cellular structure with the echocardiographic changes, we performed histologic analysis on ob/ob mice from both leptin-infused and pair-fed groups. Cellular hypertrophy decreased by ~25% in leptin-infused mice (16.3±0.3 μm, P<0.001 versus obese ob/ob). Myocyte diameter also decreased in pair-fed animals, but to a lesser degree than in mice that received leptin (18.7±0.3 μm, P<0.001 versus obese ob/ob, and P<0.001 versus leptin-infused). Nuclear hypertrophy and distortion remained prominent in both groups (data not shown).

Discussion

The major new finding of our study is that leptin contributes to the maintenance of normal LV wall thickness and myocyte cellular structure independently of overall body weight or hemodynamic load. Based on our observation that leptin repletion restored gross LV hypertrophy toward normal in ob/ob mice but caloric restriction did not, we conclude that either leptin itself or a component of its downstream signaling pathway has a direct antihypertrophic effect on the heart. Furthermore, both leptin repletion and calorie restriction reduced myocyte hypertrophy, although leptin did so to a much greater degree, indicating that at a cellular level, there is an additive effect of these 2 interventions. Thus, we propose that alterations in leptin signaling represent a novel mechanism in the modulation of obesity-associated LVH.

To isolate the direct effects of leptin from those of hemodynamic load, it was essential that we select a model of obesity that is normotensive, such as the ob/ob mouse. In this regard, previous animal models of obesity with LVH were associated with elevated blood pressure, making it difficult to separate the relative contributions of these 2 factors. For example, mice with transgenic brown fat ablation develop a similar degree of LVH to ob/ob mice but are hypertensive.19 Our present results strongly support a direct antihypertrophic role for leptin in preventing LVH. Leptin signaling in human obesity can be regarded as analogous to diabetes mellitus, manifesting in 2 forms but with similar clinical features, insulin deficiency (type 1) and insulin resistance (type 2).20 We cannot be certain that our leptin-deficient model, the equivalent of type 1, exactly replicates the pathophysiology operative in human obesity, a state of leptin excess and resistance21 and therefore equivalent to type 2. Nevertheless, we have established an important physiological role for leptin in prevention of hypertrophic LV remodeling. To the extent that there is relative leptin deficiency downstream of the receptor in both the deficient and resistant states, it is likely that the signaling abnormalities in the ob/ob
mouse are clinically relevant to the development of obesity-associated LVH. It is also important to note that there are cases of leptin-deficient, obese humans (ie, type 1 equivalent), although specific cardiovascular changes were not reported.

**Potential Mechanisms**

The precise cardiac signaling mechanism activated by leptin remains to be established, although it is well-recognized that leptin receptors are present on cardiac myocytes. There are several neurohumoral antihypertrophic signaling pathway that should be considered. For instance, leptin activation may stimulate cardiac nitric oxide signaling, which we have shown to exert antihypertrophic effects. Leptin signaling may also activate the more traditional pathways for the development of hypertrophy, such as protein kinase C, mitogen-activated protein kinase, or phosphatidylinositol-3-kinase–mediated signaling pathways. Involvement of G/G signal transduction pathways or adrenergic modulation could be involved as well.

Another potential contributor to LVH may be changes in insulin sensitivity. As with clinical obesity, both ob/ob and db/db mice are hyperglycemic and hyperinsulinemic, having the equivalent of type 2 diabetes. It is well known that the insulin and leptin hormonal signaling axes are closely related, which is not surprising given the prevalence of diabetes in obesity. However, because insulin levels did not differ with leptin repletion and pair feeding, it is unlikely that altered insulin sensitivity accounts for reversal of LV hypertrophy.

The leptin hormonal axis also is closely tied with the sympathetic nervous system. The importance of β-adrenergic signaling in protection from obesity has recently been demonstrated using a transgenic knockout of all 3 β-adrenergic receptors. Furthermore, acute leptin-associated hypertensive effects can be disrupted by α and β blockade. Thus, sympathetic overactivity is likely to play a major role in physiological alterations when leptin signaling is disrupted, particularly in hyperleptinemic states.

**Implications for Clinical Obesity**

We have demonstrated that both ob/ob and db/db mice undergo cardiac remodeling similar to that observed in postmortem clinical studies of obesity-associated hypertrophy, with excessive heart weight but low heart to body weight ratios. Fatty infiltration of the myocardium did not seem to play a role in increased wall thickness, nor did the presence of metabolic inclusions, excessive fibrosis, or increases in extracellular matrix. This, too, is concordant with clinical data showing myocyte hypertrophy by endomyocardial biopsy in most obese patients with cardiomyopathy.

**Limitations**

Although leptin-repleted and pair-fed mice lost similar weight, body fat and lean body mass are different because of the catabolic state induced by leptin repletion. Cardiovascular studies with weight gain and loss in other obese mouse models, such as New Zealand obese and agouti mice, will help clarify the effects of total weight versus lean body mass and are planned in our laboratory for the near future. In this study, we have primarily characterized the cardiovascular changes associated with weight gain and loss in the ob/ob mouse, while including the db/db for purposes of comparison. A full characterization of the db/db as well as the biochemical mechanisms underlying the process of hypertrophy in ob/ob is underway in our laboratory.

**Conclusions**

The present results demonstrate a specific role for relative leptin deficiency—either absolute deficiency or deficiency in downstream signaling—in LVH, independent of body weight per se. Indeed, we have shown that leptin exerts a direct antihypertrophic effect on the heart, and abnormalities in leptin signaling may represent a novel mechanism for the development of LVH. These findings have important potential clinical implications for obesity-associated heart disease.

**Acknowledgments**

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**References**

35. Warnes CA, Roberts WC. The heart in massive (more than 300 pounds or 136 kilograms) obesity: analysis of 12 patients studied at necropsy. *Am J Cardiol*. 1984;54:1087–1091.
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