Leptin Signaling and Obesity: Cardiovascular Consequences

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Abstract—Leptin, among the best known hormone markers for obesity, exerts pleiotropic actions on multiple organ systems. In this review, we summarize major leptin signaling pathways, namely Janus-activated kinase/signal transducers and activators of transcription and mitogen-activated protein kinase, including possible mechanisms of leptin resistance in obesity. The effects of leptin on the cardiovascular system are discussed in detail, including its contributions to hypertension, atherosclerosis, depressed myocardial contractile function, fatty acid metabolism, hypertrophic remodeling, and reduction of ischemic/reperfusion injury. The overall goal is to summarize current understanding of how altered leptin signaling in obesity contributes to obesity-related cardiovascular disease. (Circ Res. 2007;101:545-559.)

Key Words: leptin ■ obesity ■ cardiovascular disease

Over 60% of people in the United States are overweight or obese. Extensive evidence now supports the notion that maladaptation of the biological system for weight maintenance makes it extremely difficult for people to maintain weight loss.1 Several genes have been identified to disclose a physiological system that maintains body weight within a range of about twenty pounds.2 A key element of this system is leptin, the 16-kDa hormonal product of the obesity (ob) gene.3 Leptin is primarily secreted by adipocytes and is a classic member of the more than 50 identified adipocytokines that participate in adipose tissue hormonal signaling.4 Since its identification in 1994, leptin has attracted much attention as one of the most important central and peripheral signals for the maintenance of energy homeostasis.5–8 For example, a 9-year-old girl with extreme obesity was found to lack leptin.9 Leptin treatment reduced her weight to the normal range for her age, and the same effects were observed in her similarly affected cousin.10 Plasma leptin is generally proportional to adipose mass.11,12 The primary physiological role of leptin is to communicate to the central nervous system (CNS) the abundance of available energy stores and to restrain food intake and induce energy expenditure. The absence of leptin therefore leads to increased appetite and food intake that result in morbid obesity. Notably, only rare cases of severe early childhood obesity have been associated with leptin deficiency.9,13 The remainder of the obese population typically have elevated leptin levels.14 The failure of leptin to induce weight loss in these cases is thought to be the result of leptin resistance.

Hyperleptinemia, nearly universally observed in human obesity and animal models, is accompanied by a disruption of the usual activities of the hormone, possibly at different
stages in the circulatory transport and/or in the signaling cascade. Disruption of leptin signaling in the hypothalamus results in obesity and confirms the central role of this hormone in the maintenance of energy balance.7,15,16 Emerging evidence suggests that leptin resistance in the CNS may be selective, namely that the effects of leptin on central metabolic processes is disrupted, whereas its other effects, such as the sympathetic activation of blood pressure, is still retained.17 In addition to its actions in the CNS, leptin receptors (Ob-Rs) are found in multiple peripheral tissue types and affect many systemic processes, such as reproduction, immunity, and cardiovascular functions.18–20

Obesity is also a part of the metabolic syndrome, which is diagnosed by a set of criteria that include abdominal obesity, insulin resistance, dyslipidemia, and hypertension. This patient population faces increased risk for type 2 diabetes and cardiovascular diseases. The widely distributed Ob-Rs make the hormone an attractive candidate for a molecular link in the pathogenesis of obesity-related diseases. Although disruption of leptin signaling can lead to altered phenotypic expression and function of peripheral organs, the relative contributions of central versus peripheral signal disruption are still controversial. Increasing our understanding of how leptin and/or leptin resistance affects the heart and vasculature will be important for gaining comprehension of obesity-related threats to cardiovascular health.

**Leptin Signaling**

**Leptin and Leptin Receptor**

Leptin is primarily secreted by adipocytes and circulates at a level of 5 to 15 ng/mL in lean subjects.21 Its expression is increased by overfeeding, insulin, glucocorticoids, endotoxin, and cytokines and is decreased by fasting, testosterone, thyroid hormone, and exposure to cold temperature.22,23 In the heart, increased leptin expression is seen following reperfusion after ischemia,24,25 and leptin concentration in cardiomyocyte culture serum is increased with endothelin (ET)-1 and angiotensin (Ang) II treatment,26 suggesting the heart as a site of leptin production.

Six isoforms of the Ob-R (a to f) have been identified in the murine model, and they are closely related to the class I cytokine receptor family. Ob-Ra and Ob-Rb represent the dominant isoforms in the heart, whereas the others are expressed at low levels27 and are not well conserved among species.28,29 Ob-Re is the secreted form that binds circulating leptin and regulates the concentration of free leptin.30

**Janus-Activated Kinase/Signal Transducers and Activators of Transcription**

Ob-Rs have been shown to activate Janus-activated kinase (JAK), signal transducers and activators of transcription (STAT), insulin receptor substrate, and the mitogen-activated protein kinase (MAPK) pathways. The best-characterized pathway in leptin signaling is the JAK/STAT pathway (Figure 1).31 Ligand binding causes Ob-R to undergo homooligomerization32,33 and to bind to JAK2. Protein tyrosine phosphatase 1B (PTP1B) is also capable of inhibition of leptin signaling. JAK2 phosphorylation can lead to activation of MAPK and insulin receptor substrate/PI3K signaling pathways. See text. GRB2 indicates growth factor receptor–bound protein 2.

**Figure 1.** Leptin receptor signaling. The binding of leptin to its receptor leads to formation of the Ob-R/JAK2 complex that results in cross-phosphorylation. Tyr1138 on Ob-Rb is crucial for STAT3 activation, which stimulates SOCS3 expression that negatively inhibits leptin signaling via Tyr985 and additional sites on JAK2. Protein tyrosine phosphatase 1B (PTP1B) is also capable of inhibition of leptin signaling. JAK2 phosphorylation can lead to activation of MAPK and insulin receptor substrate/PI3K signaling pathways. See text.
dimerize and translocate to the nucleus to activate transcription of target genes, which includes the gene for a member of the suppressors of the cytokine signaling family (SOCS3),16,36,39 SOCS3 binding to Tyr985 and other sites within the Ob-Rb/JAK2 complex mediates negative feedback on leptin signaling.40,41 JAK2 phosphorylation of Tyr985 also consequently leads to phosphorylation of the src homology 2 (SH2) domain of the tyrosine phosphatase SHP-2 (src homology 2-containing tyrosine phosphatase), which activates the extracellular signal-regulated kinase (ERK) signaling pathway.36 Overexpression of SHP-2 has been shown to blunt SOCS3-mediated inhibition, likely through competitive binding to Tyr985.40 JAK2 autophosphorylation is independent of tyrosine phosphorylation sites on Ob-Rb and has many subsequent effects. One of these effects is to phosphorylate insulin receptor substrate proteins, which recruit the phosphatidylinositol 3′-kinase (PI3K) to activate downstream signals.7,42 In the heart, the leptin-associated PI3K pathway, along with ERK cascades, seems to be important in cardiomyocyte proliferation and protecting cardiac tissue from ischemia/reperfusion injury.24,43

Mitogen-Activated Protein Kinase
Phosphorylation of Tyr985 on Ob-Rb leads to SHP-2 and Grb-2 activation of ERK1/2 of the MAPK family.36 ERK1/2 activation also occurs via a pathway independent of Tyr985. In this case, JAK2 associates with the SH2 domain-containing SHP-2.36,44 Therefore, both Ob-Ra and Ob-Rb can activate MAPK.35,36 Shc, another SH2–containing protein that is able to associate with Grb-2, has also been shown to phosphorylate tyrosine kinase after leptin treatment.45 Leptin-induced phosphorylation of STAT3 and ERK1/2 have been observed in isolated adult C57BL/6 mouse cardiomyocytes, with maximal activation at 15 minutes after treatment. Four-week leptin treatment also elevated STAT3 and ERK1/2 phosphorylation and abundance in cardiac tissue from leptin-deficient ob/ob mice but not from Ob-Rb–deficient db/db mice.46 Because Ob-Ra is intact in db/db mice and the distal portion of Ob-Rb is not essential for MAPK signaling,35 ERK1/2 phosphorylation would be expected to increase even if STAT3 phosphorylation were unaltered. Therefore, the results suggest that either Ob-Rb is the predominant signaling receptor in the mouse heart or that JAK2-induced ERK1/2 phosphorylation does not occur at a significant level in cardiomyocytes. ERK1/2 activation leads to the expression of target genes, such as c-fos and egr-1, which participate in cell proliferation and differentiation.

Phosphorylation of p38 MAPK in response to leptin has also been demonstrated. The α and β isoforms of p38 MAPK are broadly distributed, including at relatively high levels in the heart.47 Although the specific mechanism leading to leptin-induced p38 MAPK activation has not been elucidated, it is associated with the onset of hypertrophy and programmed cell death in both rat vascular smooth muscle cells (VSMCs) and cardiomyocytes.24,48,49

As with other cytokines, leptin has the ability to activate the stress-activated protein kinase c-Jun N-terminal kinase (JNK).50,51 However, we observed that phosphorylation of JNK was unaltered in cultured adult cardiomyocytes from C57BL/6, ob/ob, and db/db mouse strains with 15-minute leptin treatment.46 The effects of long-term leptin treatment on JNK activity in the heart remain to be investigated. Nuclear factor κB has been proposed as an attractive downstream target for p38 and JNK MAPK pathways because this transcription factor is essential in the transcriptional regulation of proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β.52 Additional leptin signaling pathways are difficult to consolidate because of tissue specificity. For a comprehensive review of leptin signaling capabilities, see reviews by Fruhbeck52 or Sweeney.53

Leptin Signaling in the CNS
Hypothalamic Leptin Decreases Food Intake
Most Ob-Rs in the CNS are located in the basomedial hypothalamus.54,55 In neurons that synthesize proopiomelanocortin and express Ob-Rb, leptin stimulates the synthesis of proopiomelanocortin, which is processed to produce α-melanocyte–stimulating hormone and activate downstream melanocortin-3 and -4 receptors to decrease appetite.56 Leptin also inhibits the appetite-stimulating hormone neuropeptide Y in the arcuate nucleus, and causes inhibition of agouti-related peptide that restrain melanocortin-3 and -4 receptor signaling.55,56

Ob-Rb–stimulated JAK2/STAT3 signaling is crucial in leptin control of feeding and energy expenditure. Although short forms of the leptin receptor may be capable of activating JAK, insulin receptor substrate, and MAPK proteins, only Ob-Rb is capable of activating STAT3.35,57,58 A homologous replacement of Ob-Rb by a receptor mutant for Tyr1138 established that STAT3 is indispensable for the regulation of expression of proopiomelanocortin and neuropeptide Y in the hypothalamus.16

Mechanisms of Central Leptin Resistance
One theory of leptin resistance involves intracellular signaling disruption. STAT3 and protein tyrosine phosphatase 1B are 2 molecules known to attenuate leptin signaling.40,59–63 Hypothalamic protein tyrosine phosphatase 1B levels are not known to be altered in obesity, although protein tyrosine phosphatase 1B–null animals develop increased insulin sensitivity and have a lean phenotype.64 As previously discussed, leptin can induce its own negative feedback through STAT3-induced SOCS3 accumulation during prolonged Ob-Rb stimulation,16,40,61 which can occur via Tyr985 or additional sites in the Ob-Rb/JAK2 complex.40,41 At low concentrations of leptin, incremental changes in leptin would be almost fully translated into increased Ob-Rb signaling, whereas, at high levels, accumulated SOCS3 would counter most of the increase in Ob-Rb signaling.39,65

Another possible mechanism of central leptin resistance stems from the dependency of leptin on saturable transport across the vascular barrier and, probably to a lesser extent, across the choroid plexus to reach the arcuate nucleus.66 The activity of this blood–brain barrier transport system seems to decrease in diet-induced obese (DIO) rodents,67 resulting in failure of circulating leptin to reach its targets in the brain.
Increasing evidence suggests that leptin signaling is preferentially reduced in the arcuate nucleus of the hypothalamus but not in other regions, such as the ventromedial, dorsomedial, and/or premamillary nucleus of the hypothalamus that also express Ob-Rs.68

Leptin Increases Sympathetic Outflow
In addition to reducing appetite and controlling weight gain, leptin centrally activates the sympathetic nervous system. It significantly increases plasma norepinephrine and epinephrine concentrations via the ventromedial hypothalamus.69 Whereas chronic leptin overstimulation in the hypothalamus decreases the ability of leptin to regulate appetite, its sympathetic excitatory effects are maintained as increased arterial pressure and renal sympathetic nerve activity are presented in obesity (Figure 2).70 This observation suggests that central leptin resistance is selective.

The concept of selective resistance is suggested by a comparison between ob/ob and Agouti yellow obese mice. Lower arterial pressure is observed in ob/ob mice, which is increased by leptin reconstitution despite the accompanying weight loss.71 In contrast, Agouti yellow obese mice develop obesity resulting from ubiquitous overexpression of agouti protein, which blocks MC4R rather than directly affecting leptin. They have elevated arterial pressure similarly to DIO mice, despite the fact that they have milder obesity than ob/ob mice.71,72 This preservation of sympathoactivation effects of leptin despite disruption of its weight control effects has been confirmed in DIO mice, which is considered a more physiologic model of human obesity. In C57BL/6J mice fed a 10-week high-fat diet, intraperitoneal leptin administration failed to decrease appetite and body weight, but increased renal sympathetic nerve activity. Sympathetic nerve activity in brown adipose tissue and in hindlimb did not increase on leptin administration, indicating region-specific preservation of leptin sympathoactivation that serves to protect its circulatory effects.73

The sympathoactivation effect is completely abolished by selective destruction of the arcuate nucleus.74 As the arcuate nucleus is required for both metabolic and sympathetic effects of leptin, these results seem to suggest that leptin resistance occurs mainly through intracellular signaling disruption. Under resting conditions, it is estimated that only 5% to 25% of Ob-R isoforms are located at the cell surface.75 The ligand–receptor complex internalizes, and studies have shown that leptin internalization was greater for the Ob-Rb isoform.75,76 Preferential downregulation of Ob-Rb in response to prolonged leptin exposure may be important in regulating different tissue sensitivity to leptin and another cause for selective leptin resistance.

Leptin stimulation of adrenergic overdrive can lead to numerous adverse effects on the cardiovascular system. Both in vitro and in vivo studies have demonstrated adrenergic influences on the growth of cardiomyocytes.77,78 Patients with the metabolic syndrome have increased sympathetic activity, hypertension, and higher occurrences of left ventricular hypertrophy (LVH).79,80 Sympathetic influences also modulate the elastic properties of large and medium-size arteries.

Figure 2. Systemic leptin function. Chronic hyperleptinemia impairs the centrally mediated metabolic actions of the hormone, although its activation of sympathooutflow is preserved. Selective central leptin resistance results in obesity and adverse effects on the cardiovascular system including hypertension, atherosclerosis, and LVH. Although leptin can protect against ectopic lipid deposition in nonadipose tissue, whether this effect is abolished because of (selective) peripheral leptin resistance requires further examination.
and promote endothelial dysfunction, contributing to the development of vascular structural alterations and the occurrence of atherosclerotic lesions.\textsuperscript{81}

\textbf{Leptin and the Cardiovascular System}

Hyperleptinemia is associated with obesity-related hypertension and chronic congestive heart failure (HF) in humans, and vascular endothelial and myocardial dysfunction in animal models.\textsuperscript{82–85} The role of leptin and leptin resistance in the pathogenesis of LVH and HF in obesity remains controversial. The Table provides a summary of the leptin signaling pathways involved in the cardiovascular system.

\textbf{Vascular Effects of Leptin}

\textit{Development of Hypertension}

Both intravenous infusion and intracerebroventricular administration of leptin lead to increased arterial pressure and heart rate in rodents.\textsuperscript{86,87} Blockade of the adrenergic system inhibits the pressor response to leptin.\textsuperscript{88} In addition to increased sympathetic activity in hyperleptinemia, other mechanisms may also contribute to the development of obesity-related hypertension. Leptin has been shown to increase the generation of reactive oxygen species (ROS) in endothelial cells\textsuperscript{50,89} and to stimulate secretion of proinflammatory cytokines such as TNF-\textalpha and IL-6, both of which are promoters of hypertension and atherosclerosis.\textsuperscript{90}

While stimulating blood flow via increased sympathetic output, leptin has also been shown to have direct vasodilatory effects. In both intact rodents and in endothelial cells, leptin induces NO production.\textsuperscript{83,91} Leptin administration in anesthetized rats causes a dose-dependent increase in NO metabolite concentrations, and inhibition of NO synthesis increases arterial pressure.\textsuperscript{91} In vitro studies have shown that leptin evokes an endothelium-dependent relaxation of arterial rings.\textsuperscript{92,93} In endothelial cells, leptin activates a PI3K-independent Akt-endothelial NO synthase (eNOS) phosphorylation pathway to increase NO production, which can be abolished by erbstatin A, a Ca\textsuperscript{2+}-independent tyrosine kinase inhibitor.\textsuperscript{94} However, the vasodilator action of leptin was not found in conscious rats and the NOS inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester did not unmask any pressor effect.\textsuperscript{95} At a concentration sufficient to increase sympathetic nerve outflow, leptin did not change arterial pressure or blood flow in mesenteric, lower aortic, and renal arteries.\textsuperscript{96} Blockade of NO synthesis increased heart rate and renal vascular and glomerular response but did not substantially augment the pressor response to leptin,\textsuperscript{97} indicating a negligible role of leptin-stimulated NO production on blood pressure in vivo. Leptin has been shown to increases the release of ET-1, a vasoconstrictr secreted primarily by endothelial cells but also by macrophages, fibroblasts, and cardiomyocytes, possibly countering the effects of NO.\textsuperscript{98}

\textit{Development of Atherosclerosis}

The proatherogenic action of leptin is likely attributable to a combination of its effects on various cell types. In endothelial cells, leptin induces oxidative stress, increases production of monocyte chemoattractant protein-1 and ET-1 and potentiates proliferation.\textsuperscript{50,89,98,99} In VSMCs, leptin promotes migration, proliferation, and hypertrophy.\textsuperscript{100–102} Leptin also promotes calcification of cells of the vascular wall and facilitates thrombosis by increasing platelet aggregation.\textsuperscript{103,104}

Hyperleptinemia has been associated with coronary atherosclerosis in type 2 diabetes, and this association has been shown to be independent of insulin resistance.\textsuperscript{105} In apolipoprotein E−deficient (apoE\textsuperscript{-/-}) mice that are atherosclerosis prone, recombinant leptin treatment further promoted atherosclerosis and thrombosis, despite its metabolic benefits.\textsuperscript{106} This result indicates that elevation of leptin concentration in obesity increases the risk for atherosclerotic damage.

Paradoxically, leptin resistance is also proatherogenic. ApoE\textsuperscript{-/-} mice lacking Ob-Rb (apoE\textsuperscript{-/-} db/db) are characterized by a 5-fold higher area of spontaneous atherosclerotic lesions in the aorta than apoE\textsuperscript{-/-} with intact Ob-R.\textsuperscript{107} Unlike apoE\textsuperscript{-/-} mice, apoE\textsuperscript{-/-} db/db mice are also obese and insulin resistant. Taken together, these data suggest either that a supraphysiological level of exogenous leptin self-induces resistance that is harmful or that the atherogenic effect seen in apoE\textsuperscript{-/-} db/db is an effect more relevant to lipid profile change, which is absent in apoE\textsuperscript{-/-} mice.

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\textsuperscript{54,55}
Leptin Attenuates Cardiomyocyte Contractility

**Possible Mechanisms Leading to Increased NO Production**

Similar to its effects in endothelial cells, acute leptin infusion in isolated rat ventricular myocytes increases NO activity, which leads to attenuated cardiac contractility (Figure 3).[108] Intracellular Ca²⁺ transients were lowered and NO production were increased with leptin. These effects were blocked by the NO inhibitor N⁶-nitro-L-arginine methyl ester[108] and by JAK2 or p38 MAPK inhibitors AG-490 and SB203580.[109]

The intermediate steps by which leptin signaling leads to increase in NO production and the specific NOS isoforms that mediate the effects of leptin have not been fully elucidated. In rat VSMCs, leptin inhibits the contractile response induced by Ang II through increased NO production. The upregulation of inducible NO synthase through mechanisms involving JAK2/STAT3 and PI3K/Akt pathways is responsible for the increase of NO bioavailability in VSMCs.[110]

Elucidation of the NOS isoform(s) responsible for the actions of leptin in the heart would lead to a better understanding of its role in myocardial contractility and hypertrophy responses. We have shown that spatial confinement of different constitutive NOS isoforms within separate subcellular compartments of the cardiac myocyte allows NO signals to have independent, and even opposite, effects on cardiac phenotype and contractile response.[111] Overexpression of eNOS inhibits hypertrophy in the remote myocardium and preserved cardiac function after myocardial infarction, possibly through attenuation of β-adrenergic–stimulated compensatory hypertrophy.[112] Neuronal NO synthase and eNOS independently contribute to the development of cardiac hypertrophy, leading to marked age-related concentric hypertrophic remodeling in double-knockout mice lacking both neuronal NO synthase and eNOS.[113,114] Understanding the leptin crosstalk with the β-adrenergic signaling system in cardiomyocytes would provide significant insight into the understanding of myocardial dysfunction in obesity.

Whereas leptin-induced NO increase directly depresses cardiomyocyte contractility, systemic actions such as increased sympathetic modulation may indirectly stimulate contractility. Just as leptin-stimulated NO production in endothelial cells may have a negligible role in blood pressure in vivo, cardiac contractile depression may not manifest under normal physiologic conditions. However, the depressant effect may become important when considered in conjunction with alterations occurring in obese states.

**Leptin Deficiency Leads to Decreased Responsiveness to β-Adrenergic Stimulation**

In 10 week-old ob/ob isolated myocytes, attenuated sarcoplasmic shortening and calcium transients and depressed sarcoplasmic reticulum Ca²⁺ stores were seen in response to isoproterenol stimulation of the β-adrenergic receptor or to
post–receptor level stimulation with forskolin and dibutylryl-cAMP. Leptin replenishment in ob/ob mice restored each of these abnormalities toward normal without affecting gross (wall thickness) or microscopic (cell size) measures of cardiac architecture. Decreased Gαs (52 kDa), increased sarcoplasmic reticulum Ca2+-ATPase, and depressed phosphorylated phospholamban abundance were detected in ob/ob mice. In addition, protein kinase A activity in ob/ob mice was depressed at baseline and corrected toward wild-type (WT) level with leptin repletion.46 In the H9c2 cardiac cell line, 30-minute leptin treatment increased basal and catecholamine-stimulated adenylate cyclase activity, whereas 18-hour treatment was associated with a reduced adenylate cyclase activity and a different responsiveness to isoproterenol and norepinephrine stimulation, likely attributable to differential activation of Gαs. Adenylate cyclase, Gαs (52 kDa), Goα, p21-ras, and phosphorylated ERK1/2 expressions were increased with short-term leptin treatment and decreased at 18 hours, whereas Gαs (45 kDa) continued to increase at 18 hours. Receptor level leptin resistance is conceivable in myocytes, as Ob-R expression is seen to decrease at 18-hour leptin treatment.115 Taken together, leptin deficiency or resistance leads to decreased β-adrenergic response, whereas moderate leptin stimulation can improve the contractile response.

Mechanisms Involving ROS
Mitochondrial formation of ROS is enhanced in obesity. Xanthine oxidoreductase and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase are 2 main sources of superoxide (O2−) production in the heart. O2− is capable of generating a large family of ROS by interacting with other molecular compounds. In ob/ob hearts, impaired cardiac contractile function is accompanied by elevated oxidative stress, lipid peroxidation, protein carbonyl formation, redistribution of myosin heavy chain isoforms from myosin heavy chain-α to -β, and oxidative modification of SERCA2a.116 Neuronal NO synthase constrains xanthine oxidoreductase activity.117,118 Reduced neuronal NO synthase expression is observed in 2- to 6-month-old ob/ob mice, which leads to increased xanthine oxidoreductase production of O2−, thereby causing an imbalance between the production of ROS and reactive nitrogen species.119 This nitroso–redox imbalance may be partially responsible for the myocardial dysfunction seen in ob/ob mice. Activation of NADPH oxidase is also seen in the ob/ob heart.116 Treatment with apocynin, a NADPH oxidase inhibitor, reversed cardiac contractile dysfunction in ob/ob myocytes but failed to reserve SERCA2a oxidative modification.116 8-Bromo-cGMP, a membrane-permeable cGMP analog, induced a greater negative effect in ob/ob than lean C57BL/6J mice. However, the effect of adding a NO donor was similar in the obese and lean models, indicating that some cGMP-independent effect of NO prevents the enhanced negative cGMP effects in ob/ob cardiomyocytes.120 NAPDH-mediated reduction in NO bioavailability could explain the failure of NO donor to elicit further negative inotropic response in obese models.121,122 Additionally, the interaction between NO and ROS produces peroxynitrite, which can nitrosylate proteins and exert positive inotropic effects, thereby offsetting the cGMP-dependent reduction in contractility.120 Peroxynitrite has shown both negative and positive inotropy in isolated cardiomyocytes,123,124 although it is generally accepted to trigger apoptosis in cardiomyocytes in vitro and in vivo, possibly through a pathway involving caspase-3 activation and the cleavage of poly(ADP-ribose) polymerase.125

Another recent study proposes that ET-1 is upstream of NADPH oxidase in leptin-induced myocardial contractile response.126 There are at least 2 cardiac ET-1 receptors, ETα and ETβ. Both are known to mediate cardiomyocyte inotropic response, and ETα receptors also affect hypertrophy.127 Leptin administration to rat neonatal cardiomyocytes induced intracellular O2− generation and upregulated protein expression of p67phox and p47phox subunits of NAPDH, the effect of which is attenuated by ETα and ETβ receptor antagonists and apocynin, suggesting that the ET-1 receptors are likely upstream of NADPH oxidase in leptin-induced cardiac contractile response.126

Additional Mechanisms Affecting Contractility
Obesity is a lipotoxic disease featuring overtly elevated ceramide levels (see section below, Leptin Shifts Myocardial Metabolism Toward Fatty Acid Utilization). The de novo ceramide pathway has been postulated to be key to the lipoapoptosis of pancreatic β cells and cardiomyocytes in obese individuals.128,129 The ability of ceramide to amplify leptin-induced depression of contractility in adult rat left ventricular myocytes was recently demonstrated.130 Although ceramide alone did not elicit any effect on cell mechanics and intracellular Ca2+ transients, it sensitized leptin-induced effects on myocyte shortening and intracellular Ca2+ transients. In vivo obese concentrations of plasma leptin lie in the low nanomolar range, which is seemingly disconnected from the high in vitro leptin concentration (≥10 nmol/L) needed to affect cardiac contractile function. The observation that ceramide may augment the cardiac depressive effect of leptin provides an additional explanation for hyperleptinemia-associated cardiac dysfunction in obesity.

Elevated adipose mass in obesity also increases the secretion of other proinflammatory factors, including TNF-α, IL-6, and Ang II. TNF-α and leptin both depress contractility in adult rat ventricular myocytes, although no additive response by the 2 proinflammatory factors was observed. Inhibitory effects were abolished by N4-monomethyl-L-arginine in both cases and in the case of combined exposure.131 Thus, the inhibitory effect on cardiac contraction by TNF-α and leptin may mask each other and share a common mechanism dependent on NO.131

It is interesting, however, that hypertension seems to attenuate leptin-induced cardiomyocyte contractile depression. Isolated rat ventricular myocytes from spontaneously hypertensive rats (SHR) displayed decreased leptin-induced depression of myocyte shortening and intracellular Ca2+ transients, as well as blunted leptin-induced NOS activity compared with the normotensive control mice. Additionally, treatment of SHR myocytes with JAK or p38 inhibitor led to further inhibition of myocyte shortening by leptin instead of abolishing such effects.109 The altered signal transduction of
JAK/STAT and p38 pathways to attenuate leptin-induced cardiomyocyte dysfunction possibly serves as a compensatory mechanism to prevent further impairment of ventricular function in sustained hypertension or hyperleptinemia.\textsuperscript{109}

**Leptin Shifts Myocardial Metabolism Toward Fatty Acid Utilization**

Accumulating evidence suggests that leptin regulates energy homeostasis through direct actions on peripheral lipid and glucose metabolism.\textsuperscript{132} Fatty acid (FA) oxidation produces the major source of ATP to sustain contractile function of the heart. AMP-activated protein kinase (AMPK) has a key role as a fuel gauge in the heart and regulates cardiac FA oxidation by phosphorylation and inhibition of acetyl–coenzyme A (CoA) carboxylase, which then lowers malonyl-CoA levels and stimulates carnitine palmitoyltransferase-1–induced FA oxidation.\textsuperscript{133} AMPK activation is also known to reduce FA incorporation into triacylglycerol.\textsuperscript{134}

Examination of leptin effects on cardiac FA oxidation confirmed that leptin infusion increases FA oxidation and triacylglycerol lipolysis in isolated working rat hearts, although this effect was independent of changes in the AMPK/acetyl-CoA carboxylase/malonyl-CoA axis. Neither did leptin affect glucose oxidation rates.\textsuperscript{135} Myocardial oxygen consumption was increased, possibly because of increased mitochondrial uncoupling protein activity.\textsuperscript{135} In DIO mice, plasma leptin concentration increase was associated with an induction of uncoupling protein 2 and an increase in phosphorylation of AMPK that led to increased FA oxidation.\textsuperscript{136} At this stage, leptin signaling in the heart as assessed by STAT3 phosphorylation remained unaltered from WT, suggesting that the shift to FA oxidation is at least mediated, in part, by leptin. Increased FA oxidation may confer antisteatosis protection to the myocardium during hyperleptinemia in early stages of obesity. This is consistent with the observation that DIO rodents exhibit minimal rise in myocardial triacylglycerol content, whereas ob/ob, db/db, and fa/fa animals show significantly greater accumulation.\textsuperscript{137} Leptin deficiency perturbs liporegulation in peripheral organs and results in lipotoxicity, lipoapoptosis, and generalized steatosis, manifesting in clinical conditions such as nonalcoholic steatohepatitis, type 2 diabetes, and lipotoxic cardiomyopathy.\textsuperscript{128} Lipotoxic cardiomyopathy has been identified in fa/fa rats and has been transgenically induced in acyl–CoA synthase transgenic mice.\textsuperscript{138} Overexpression of acyl–CoA synthase, which actives and esterifies FAs, led to severe cardiomyopathy that was attenuated by injection of recombinant adenovirus containing the leptin cDNA.\textsuperscript{138}

Increased de novo lipogenesis and decreased compensatory oxidation of FAs are two possible mechanisms for intracellular accumulation of lipids in nonadipose tissue. Enzymes involved in FA oxidation such as acyl-CoA oxidase and carnitine palmitoyltransferase-1 are observed at attenuated levels in fa/fa rats. Hydrolysis of the increased stores of triacylglycerol to fatty acyl-CoA in fa/fa rats also increased the substrate for de novo ceramide synthesis and activated the expression of inducible NO synthase in the myocardium, which led to acceleration of apoptosis.\textsuperscript{129} Ceramide also activated protein kinase C isoforms, which can induce gene transcription through MAPK and nuclear factor κB and are implicated in the development of cardiomyocyte hypertrophy.\textsuperscript{139} It seems paradoxical that enhanced expression of β oxidation enzymes such as long-chain acyl-CoA dehydrogenase and carnitine palmitoyltransferase-1 are seen in ob/ob mice,\textsuperscript{140} as leptin-deficient hearts are also characterized by increased FA utilization.\textsuperscript{141} This is likely attributable to long-term adaptation to the changes in the lipid profile in these animals. Genes for lipoprotein lipase, which generates free FAs, and FA transport proteins are seen at an greatly elevated level in ob/ob myocytes, which causes accumulation of free FAs that promote ceramide, inducible NO synthase, and NO production in the cardiomyocyte.\textsuperscript{140} When increased FA delivery and hyperinsulinemia are imposed in ob/ob hearts, myocardial function is maintained, albeit with decreased efficiency, whereas WT hearts are unable to adapt acutely.\textsuperscript{141}

Increased FA uptake accompanied by decreased oxidation leads to lipotoxicity in long-term leptin-treated cardiomyocytes. The antisteatotic effects of leptin in early obesity might be lost with the progression of leptin resistance occurring in late stage obesity, leading to lipotoxicity that promotes cardiomyocyte contractile dysfunction and apoptosis\textsuperscript{129} or acts in a maladaptive fashion because of increased ceramide synthesis.

**Leptin Effects on Cardiac Hypertrophy and Remodeling**

Postmortem clinical studies of obesity-associated hypertrophy have demonstrated excessive heart weight but low heart/body weight ratios.\textsuperscript{142} Fatty infiltration of the myocardium, presence of metabolic inclusions, excessive fibrosis, or increases in extracellular matrix do not appear to play a direct role in augmenting wall thickness in obesity-related hypertrophy.\textsuperscript{143} The findings on endomyocardial biopsy in the majority of obese patients with cardiomyopathy appear to be primarily myocyte hypertrophy.\textsuperscript{144} Hyperleptinemia is observed in patients with LVH.\textsuperscript{145} Leptin effects on cardiomyocyte hypertrophy is not entirely clear. We had previously shown leptin repletion to be antihypertrophic in ob/ob hearts. Four- to 6-week leptin infusion reduced weight and reversed LVH, whereas caloric restriction reduced weight but did not effectively reduce wall thickness and myocyte size.\textsuperscript{113} Reconstitution to normal levels may provide the antisteatotic effect of leptin that can reverse lipotoxic effects of FA deposition in the myocardium. On the other hand, leptin has been shown to induce hypertrophy in a concentration-dependent manner in cultured neonatal rat ventricular myocytes.\textsuperscript{48,146} Fasting plasma leptin levels are associated with increased myocardial wall thickness in hypertensive humans, although this may be related to leptin resistance.\textsuperscript{147} The different types of cardiomyocytes used in experiments could also contribute to the observed paradoxical effects of leptin on cardiomyocyte hypertrophy. Direct effects of leptin on cardiomyocyte hypertrophy, apoptosis, proliferation, and extracellular matrix remodeling could all contribute to maladaptive hypertrophy and HF in obesity.
Cardiomyocyte Hypertrophy

Leptin-induced neonatal cardiomyocyte hypertrophy occurs through a mechanism involving ET-1 and ROS generation. ET-1 has been shown to increase cardiomyocyte surface area without increasing cell proliferation. In this study, blocking the ET<sub>a</sub> receptor with the selective inhibitor ABT-627 partially but significantly reduced leptin-induced hypertrophy. An interdependence of ET-1 and leptin signaling has been proposed in the progression of myocardial dysfunction and hypertrophy, suggesting that leptin may cause chronic oxidative stress and inflammation in the myocardium, similar to other agents such as TNF-α, norepinephrine, and Ang II, all of which induce hypertrophy via ROS upregulation.

Mitosis and Proliferation

Leptin treatment at a level similar to plasma concentration in obese individuals increased proliferation of both HL-1 cardiac muscle cells and human pediatric ventricular myocytes. The proliferation was accompanied by increased DNA synthesis associated with increased ERK1/2 phosphorylation and increased association of the p85 regulatory subunit of PI3K with phosphotyrosine immunoprecipitates. ERK1/2 inhibition significantly attenuated the leptin-induced proliferative activity and DNA replication in HL-1 and pediatric human cardiomyocytes but failed to decrease [3H]-leucine incorporation in neonatal rat cardiomyocytes treated with leptin.

Other pathways likely involved in leptin-induced hypertrophy include the activation of adenylyl cyclase, peroxisome proliferator-activated receptor-α, and the JAK/STAT pathway associated with increased ERK1/2 phosphorylation and increased association of the p85 regulatory subunit of PI3K with phosphotyrosine immunoprecipitates.

Protection in Ischemia/Reperfusion Injury

Timely reperfusion is necessary to salvage myocardium from acute infarction, but reperfusion usually induces additional injury. A recent report shows that exogenous leptin given at early reperfusion in an isolated mouse heart model reduces infarct size. This cardioprotective action of leptin is associated with activation of the reperfusion injury salvage kinase pathway that includes PI3K/Akt and ERK1/2, ultimately leading to the inhibition of mitochondrial permeability transition pore opening. Infarct size in C57BL/6J mice fed a high-fat diet, serum and myocardial ET-1, myocardial leptin, and Ob-R mRNA are all elevated, whereas in ob/ob mice, both serum and myocardial ET-1 levels are not higher than WT mice, confirming a direct role of leptin in mediating increased myocardial ET-1 signaling. Simvastatin, a cholesterol-lowering drug decreases leptin-induced ROS-mediated hypertrophy in rat neonatal cardiomyocytes.
of Akt or its downstream targets such as eNOS. Additionally, there was increased phosphorylation of p38 MAPK and reduced abundance and phosphorylation of STAT3 and AMPK. 24 Leptin-stimulated ROS production and NO synthesis has been shown also to protect against ischemia reperfusion injury in the gut and kidney. 162,163 Clinically, it is interesting to note that patients with a higher body mass index have better outcomes following an acute coronary syndrome or percutaneous coronary intervention. 164,165

**Leptin Resistance in the Cardiovascular System?**

The relative contributions of central and peripheral leptin effects to disease pathogenesis are difficult to decipher. Central leptin resistance disrupts hypothalamic control of energy homeostasis, which results in obesity and increased lipid production. This in turn may lead to ectopic lipid deposition and lipotoxicity in peripheral organs. The attempt to separate the effects of this pathological process from the physiological effects of leptin in the cardiovascular system has proven to be challenging, complicated by different isoform signaling capabilities and possible resistance in the periphery. The question remains whether peripheral leptin resistance occurs in the myocardium itself in obesity. Even though chronic leptin stimulation has been shown to decrease Ob-R expression in various studies, 75,115 DIO mice show increased Ob-R mRNA expression. 151 On the other hand, Ob-Rb expression in left ventricular homogenate is lower than WT. 126 However, mRNA expression does not necessarily correlate with receptor density at the membrane; therefore, these results are not conclusive in determining whether leptin resistance can occur at the myocyte receptor level. One recent study suggests that leptin resistance does not occur in the myocardium in a model of early central resistance. Eight-week DIO C57BL/6 mice showed attenuated leptin phosphorylation of STAT3 in hypothalamic tissue, whereas no such attenuation was shown in whole-heart homogenate. 136

Paradoxical results have been reported in almost all leptin-related effects on the myocardium; that is, excessive exogenous leptin and leptin deficiency often lead to the same end. Whether these effects occur through entirely different mechanisms, are mediated through differential regulation of Ob-R isoforms, or are attributable to peripheral resistance requires further investigation. If peripheral myocardial resistance does occur, these differences could be resolved if we consider leptin deficiency and leptin resistance both to be states of dysfunctional downstream signaling.

Interestingly, obesity-induced leptin resistance, although not reported in the myocardium, has been shown to extend to affect platelets and the vascular wall. 166 Obese concentrations of leptin significantly attenuate coronary vasodilation to intracoronarily administered acetylcholine and significantly attenuate relaxation in left circumflex coronary rings in control dogs. These effects were not seen when the same concentrations of leptin were administered to dogs fed a high-fat diet, suggesting that leptin resistance does occur in the vasculature. This resistance is not attributable to altered coronary dilation, increased endothelium-derived hyperpolarizing factor, nor changes in coronary Ob-R mRNA levels. 166

A recent hypothesis relevant to both central and peripheral leptin resistance involves leptin interaction with circulating factors in the blood. 167 Five serum leptin–interacting proteins have been isolated, one of which is C-reactive protein. It directly inhibits the binding of leptin to Ob-Rs and blocks its ability to signal in cultured cells. Infusion of human C-reactive protein into ob/ob mice blocked leptin treatment effects on satiety and weight reduction. Physiological concentrations of leptin stimulate expression of C-reactive protein in human primary hepatocytes, 167 and human C-reactive protein has been correlated with increased adiposity and plasma leptin, 168 suggesting an systemic self-induced negative feedback that may cause leptin resistance in the obese state. 167

**Leptin Antagonists**

Leptin antagonists used in animal models have been shown to block central leptin effects and increase appetite and food intake. 169,170 Three approaches have been employed to antagonize leptin activity: (1) binding free leptin in the circulation, (2) competitive Ob-R binding by mutants that do not cause signaling activation, and (3) specific anti–Ob-R monoclonal antibodies. An example of the first approach is a recombinant human and mouse Ob-R/Fc chimeric glycoprotein. 171,172 Only the latter 2 approaches have been employed in cardiac-related research. In neonatal rat ventricular cardiomyocytes, rat L39A/D40A/F41A leptin mutein blocked hypertrophic effects and abolished increases in Ob-R gene expression elicited by leptin, Ang II, or ET-1. 26 The hypertrophic effects of leptin are also prevented by antibodies to Ob-Ra and Ob-Rb. 26 Cardiac dysfunction did not develop in rats treated with Ob-R antibodies after coronary artery ligation compared with sham, indicating that blocking Ob-R can improve postinfarction HF in rats. 173 The recent proposal of nanobodies (a unique form of antibodies that is characterized by a single antigen-binding domain and generally does not cross the blood–brain barrier) may lead to an antagonist that could selectively inhibit peripheral activities of leptin. 174 This form of leptin antagonist might be clinically useful, as they can target peripheral adverse effect of leptin without inducing central weight gain.

**Summary**

Obesity leads to cardiac hypertrophy, ventricular dysfunction, reduced diastolic compliance, and hypertension, as well as type 2 diabetes and hyperlipidemia. 175 The high risk of developing cardiovascular diseases in obesity has drawn much effort to study the neurohormone effects of leptin on cardiac function and remodeling. Hyperleptinemia, central leptin resistance, and leptin deficiency are all associated with impaired postreceptor leptin signaling and contractile response. Short-term administration of leptin seems to have beneficial effects on the myocardium, including antisteatotic actions, protection against ischemia/reperfusion injury, and participation in compensatory myocyte hypertrophy. Interaction with enhanced ROS production pathways in obesity, on the other hand, can cause lipotoxicity and deleterious myo-
cardiac effects such as cell death and maladaptive hypertrophy. Perturbations of leptin signaling and other signal transduction pathways regulated by leptin in cardiomyocytes likely underlie the pathology of cardiomyocyte hypertrophy in obesity. In particular, alterations in JAK/STAT, MAPK, NO, and β-adrenergic pathways have been implicated in the negative inotropic and hypertrophic responses. Additional studies investigating the integrated effects of leptin on cardiomyocytes via SOCS3, PI3K/Akt, protein kinase C, and other signaling pathways could provide a more comprehensive understanding of leptin action on the cardiovascular system.

The unresolved debate about selective preservation of peripheral leptin signaling in the setting of hyperleptinemia and central resistance complicates the interpretation of experimental results involving the myocardium. Despite such challenges, a picture is emerging in which the risk of obesity is not merely attributable to the physical burden of extra weight but is, rather, a complex condition of hormonal dysregulation. Improved understanding of the actions of leptin within the cardiovascular system will greatly improve our understanding of obesity-associated heart disease.

Sources of Funding

This work was supported in part by the Donald W. Reynolds Foundation, NIH grant K08-HL076220, the W.W. Smith Charitable Trust, and the Irvin Talles Endowed Fund for Cardiomyopathy Research.

Disclosures

None.

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Leptin Signaling and Obesity: Cardiovascular Consequences
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Circ Res. 2007;101:545-559
doi: 10.1161/CIRCRESAHA.107.156596

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