

REVIEW

Leptin and hyperleptinemia – from friend to foe for cardiovascular function

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Abstract

The obese gene product, leptin, plays a central role in food intake and energy metabolism. The physiological roles of leptin in human bodily function have been broadened over the past decade since leptin was first discovered in 1994. Evidence has suggested that leptin plays a specific role in the intricate cascade of cardiovascular events, in addition to its well-established metabolic effects. Leptin, a hormone linking adiposity and central nervous circuits to reduce appetite and enhance energy expenditure, has been shown to increase overall sympathetic nerve activity, facilitate glucose utilization and improve insulin sensitivity. In addition, leptin is capable of regulating cardiac and vascular contractility through a local nitric oxide-dependent mechanism. However, elevated plasma leptin levels or hyperleptinemia, have been demonstrated to correlate with hyperphagia, insulin resistance and other

markers of the metabolic syndrome including obesity, hyperlipidemia and hypertension, independent of total adiposity. Elevated plasma leptin levels may be an independent risk factor for the development of cardiovascular disease. Although mechanisms leading to hyperleptinemia have not been well described, factors such as increased food intake and insulin resistance have been shown to rapidly enhance plasma leptin levels and subsequently tissue leptin resistance. These findings have prompted the speculation that leptin in the physiological range may serve as a physiological regulator of cardiovascular function whereas elevated plasma leptin levels may act as a pathophysiological trigger and/or marker for cardiovascular diseases due to tissue leptin resistance.

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Introduction

Obesity is an increasingly prevalent metabolic disorder affecting not only the developed but also the developing countries. The pathogenesis of obesity is multifactorial incorporating both genetics and lifestyle. While heredity explains 30% to 70% of obesity cases with causative genetic mutations being identified in several recognizable pleiotropic obesity syndromes such as the Bardet-Biedl syndrome and melanocortin 4 receptor (MC4R) gene mutation-associated obesity (Farooqi *et al.* 2003), the contribution from lifestyle factors such as diet and satiety may be predominantly responsible for the recent dramatic increase in the prevalence of obesity. In the United States, despite the fact that consumption of fat has been reduced dramatically over the last three decades, a decrease in incidence of obesity has not occurred. This is likely attributable to maintenance of food intake with an increase in total calories and also to reduced physical activity.

Uncorrected obesity, when sustained, often leads to cardiac hypertrophy, ventricular dysfunction, reduced diastolic compliance, increased blood pressure, arteriosclerosis and other vascular complications. In addition, uncorrected obesity dramatically enhances the propensity of a cluster of metabolic diseases such as diabetes mellitus, insulin resistance, hyperinsulinemia, hyperlipidemia, hyperuricemia and low plasma high-density lipoprotein cholesterol, collectively known as the metabolic syndrome (Sowers 1998, Unger & Orci 2002). Although most of the comorbidities relating obesity to cardiovascular dysfunction become more intense as body mass index (BMI) increases in conjunction with body fat distribution, long-term longitudinal studies indicate that obesity not only relates to, but also independently predicts, certain cardiovascular defects such as coronary atherosclerosis (Sowers 1998). This relationship appears to exist for both men and women with minimal increases in BMI (Sowers 1998). Nevertheless, no clear culprits have been identified to link

any product of mutant gene or lifestyle factors to disrupted cardiovascular function under obesity. Although it is generally accepted that activation of the sympathetic autonomic nervous system and impairment of endothelial function are the two pivotal phenotypical traits in obesity (Steinberg *et al.* 1996, Julius *et al.* 2000, Kuo *et al.* 2003), the cellular mechanisms responsible for sympathetic hyperactivity and endothelial dysfunction have not yet been clearly elucidated. To add to the complexity, metabolic syndromes such as diabetes and hypertension often coexist with obesity and may themselves exert independent detrimental effects on cardiovascular function. Therefore, the direct and independent impact as well as the consequence of obesity on cardiovascular function may be largely obscured.

Obesity has long been considered as a behavioral and social disorder. It was not until 1994 that the discovery of the 16 kDa obese gene (*ob*) product, leptin, brought a complete revolution to the concept of obesity (Zhang *et al.* 1994, Caro *et al.* 1996, Leibel *et al.* 1997, Friedman & Halaas 1998). Released by adipose tissue, leptin is considered the hormonal signal bridging the peripheral adipose tissue to the central nervous system (CNS) for the control of appetite and energy expenditure (Campfield *et al.* 1995, Caro *et al.* 1996, Friedman 2002). The CNS action of leptin is crucial to maintain energy balance likely through an increased sympathetic tone (Pellemounter *et al.* 1995, Havel 2000, Hall *et al.* 2002). Moreover, localization of leptin receptors in a wide range of tissues has indicated central as well as peripheral actions of leptin (Bjorbaek *et al.* 1997, Hall *et al.* 2002, Jequier 2002). For example, intravenous leptin infusion has been shown to enhance norepinephrine turnover and sympathetic nerve activity (Haynes *et al.* 1997). Interaction with both CNS and peripheral nerves under different conditions reflects the complexity of leptin signaling in the regulation of bodily function. Leptin may act as an endocrine, a paracrine, as well as an autocrine factor. It participates in multiple regulatory mechanisms such as energy expenditure/metabolism, cell proliferation/differentiation, as well as signal interaction with other hormonal regulators of energy and metabolism such as insulin, insulin-like growth factors, growth hormone, glucagon and glucocorticoids (Friedman 2002, Hall *et al.* 2002, Margetic *et al.* 2002, Sweeney 2002). The leptin signaling system is becoming ever more complex than was first envisioned. This review will be focused on the literature dealing with the cardiovascular response of leptin, predominantly on those articles which have appeared in the last five years. It is crucial to enrich our understanding of the complex interactions among leptin, hyperleptinemia and cardiovascular function to understand the pathogenesis of the obesity-related cardiovascular dysfunction so that optimal management and therapy may be achieved for obese individuals who are at high risk of cardiovascular events.

Leptin, leptin receptors and their biological actions

Leptin has structural homology to tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), leukemia inhibitory factor, granulocyte-colony stimulating factor, glycoprotein 130 (gp130) and other cytokine family proteins, and is therefore considered a cytokine-like substance (Ahima & Flier 2000, Sweeney 2002). Nuclear magnetic resonance and crystal structure analysis revealed a four-helix bundled structure for leptin with four antiparallel α -helices linked with two long crossover arms and one short loop. Binding of leptin to its membrane receptor results in homodimerization. Five isoforms of leptin receptors have been identified so far, namely Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd and Ob-Re. The leptin receptor is a transmembrane protein with structural homology to that of the gp130 receptor family. The extracellular domain of the leptin receptor contains two cytokine binding regions with one region being the specific leptin-binding site. The transmembrane domain consists of 23 amino acids and the intracellular domain is variable in length. The intracellular domain is usually associated with one or two box motifs, whereas most gp130 receptors have three box motifs. The cytoplasmic motifs allow leptin receptors to interact with intracellular messengers such as mitogen-activated protein (MAP) kinase, insulin receptor substrate (IRS-1 and IRS-2), nitric oxide (NO), Janus kinase (JAK) and signal transducer and activator transcription factor (STAT) (Ahima & Flier 2000, Sweeney 2002). While the box 1 motif is necessary for the short form of leptin receptor (Ob-Rs) activation of JAK2, the box 2 motif is required for full transduction of the JAK-STAT signal (Bjorbaek *et al.* 1997). Upon binding of leptin, the Ob-Rb receptor has been demonstrated to activate STAT1, STAT3, STAT5, and STAT6 *in vitro* but only STAT3 *in vivo*. The downstream signaling of STAT is not completely understood at this time but has been somewhat revealed in the heart, brain and other cell lines (Bjorbaek *et al.* 1997). Earlier studies identified the MAP kinase pathway as a downstream signaling mechanism of Ob-Rs (Bjorbaek *et al.* 1997). In addition, the extracellular signal regulated kinase (ERK) was activated with leptin stimulation. While this is enlightening for Ob-Rs, the long form Ob-Rb receptor has also been demonstrated to stimulate ERK activity although at a much higher potency (Bjorbaek *et al.* 1997). In vascular endothelial cells, leptin can activate the stress activated protein kinase (SAPK) and Jun NH2-terminal kinase (JNK) transduction pathways of the MAP kinase family (Bouloumie *et al.* 1999). Leptin may also up-regulate the transcription factor activation protein 1 (AP-1) (Bouloumie *et al.* 1999). While the SAPK/JNK pathway appears to be associated with leptin signaling, it may be directly activated by reactive oxygen species (ROS), thus making sustained hyperleptinemia potentially hazardous. Activation of SAPK/JNK may contribute to the pro-oxidant effects of leptin, especially at chronic high

circulating levels (Bouloumie *et al.* 1999, Anderson & Ren 2002).

Accumulating evidence has suggested a close interaction between leptin signaling and insulin signaling, or between hyperleptinemia and hyperinsulinemia (Ookuma *et al.* 1998). Obese individuals may often display both hyperleptinemia and hyperinsulinemia concurrently, although neither is likely to be permissive to the onset of the other comorbidity (Naderali *et al.* 2001, Hintz *et al.* 2003). Binding of leptin to its receptors directly enhances the activity of IRS-1 and IRS-2 (Bjorbaek *et al.* 1997, Kellerer *et al.* 1997). Phosphorylation of IRS on the serine/threonine residue may lead to decreased phosphatidylinositol-3 (PI-3) kinase and subsequently deficient PI-3 kinase activation (Bjorbaek *et al.* 1997, Kolter *et al.* 1997, Zhao *et al.* 1998, Harvey *et al.* 2000). Therefore, hyperleptinemia may be associated with suppressed PI-3 kinase activity and reduced cardiovascular inotropicity since PI-3 kinase is a known positive inotropic mediator for the heart and vasculature (Northcott *et al.* 2002, von Lewinski *et al.* 2003). This notion is supported by our recent evidence indicating that the leptin-induced cardiac depressant effect was abolished by the JAK2 inhibitor AG-490 (Wold *et al.* 2002). It may be speculated that leptin phosphorylates IRS-1, IRS-2 or both, by first activating JAK2, an upstream signaling molecule of PI-3 kinase activation (Sweeney 2002), which may eventually lead to drained PI-3 activity.

Leptin plays a pivotal role in a wide variety of organ systems including the reproductive, renal and cardiovascular systems (Ahima & Flier 2000, Friedman 2002). Leptin influences the central regulation of food intake and energy expenditure via cerebral leptin receptors. Overeating (hyperphagia) may lead to an elevation whereas fasting or caloric restriction results in a fall in leptin levels (Wang *et al.* 2001). A fall in leptin levels (such as in starvation and malnutrition) may lead to immune deficiency and impaired host defence including lymphoid atrophy and T-lymphocyte dysfunction, which can be restored with leptin supplementation. Reduction in plasma leptin may trigger CNS adaptive responses to starvation by suppressing reproductive and thyroid function (Mancuso *et al.* 2002). Metabolically, leptin promotes the redistribution of intrahepatic glucose fluxes with an increase in gluconeogenesis and a parallel decrease in glycogenolysis (Kamohara *et al.* 1997, Friedman 2002). The signaling mechanism in glucose and energy metabolism is quite different between leptin and insulin although both hormones act on receptors in the same regions of the hypothalamus. Although leptin and insulin may both reduce appetite, the two hormones may combine subadditively in the acute regulation of food intake (Air *et al.* 2002, Kuo *et al.* 2003). While the satiety factor, leptin, facilitates energy expenditure, insulin stimulates glucose uptake for energy utilization or storage. The interaction between leptin and insulin is quite complex.

Release of leptin may be significantly increased when adipocytes are exposed to insulin and glucose (Mueller *et al.* 1998, Levy & Stevens 2001), which underscores the phenomenon of hyperleptinemia in type 2 diabetes. On the other hand, an increase in plasma leptin levels has been shown to reduce insulin release and enhance insulin sensitivity (Barzilai *et al.* 1997, Ookuma *et al.* 1998, Cases *et al.* 2001). Although short-term dietary obesity has been shown to induce endothelial dysfunction without causing insulin resistance (Naderali *et al.* 2001), long-term hyperphagia or hyperleptinemia may eventually down-regulate insulin signaling and induce insulin resistance (Anderson & Ren 2002). It may be speculated that sustained hyperleptinemia-induced over-phosphorylation of IRS-1/IRS-2 and subsequently deficient PI-3 kinase activity may play a role in the onset of insulin resistance (Zhao *et al.* 1998, Harvey *et al.* 2000). The role of leptin in insulin release and action is still debatable with conflicting observations. It is believed that the dose and duration of leptin exposure may be a critical factor in determining the action or sensitivity of insulin.

Regulation of leptin levels by insulin and obesity

Plasma leptin levels are strongly correlated with adiposity. Tissue adiposity, along with gender, is the main determinant of leptin gene expression and release (Trayhurn *et al.* 1998). Change in plasma leptin levels and/or leptin signaling has a profound pathological impact on body weight control. Both leptin deficiency and leptin receptor defect are sufficient to produce obesity of genetic origin (Unger & Orci 2002). However, the most common trigger of obesity is not genetic defects of leptin or its receptor but rather depends on overeating or high fat diet intake, which may rapidly increase plasma leptin levels (Friedman & Halaas 1998, Wang *et al.* 2001). Although it has been speculated that hyperleptinemia leads to tissue resistance of leptin and insulin (Frederich *et al.* 1995), how hyperleptinemia and/or impaired leptin signaling triggers cardiovascular dysfunction is unclear and will be further discussed in the following sections. Several factors have been identified as participating in the regulation of leptin synthesis and release including the sympathetic nervous system, insulin and body adiposity (Havel 2000). Sympathetic nervous system activity is believed to be a key inhibitor of leptin release. Catecholamines including nor-epinephrine, epinephrine and isoprenaline have all been shown directly to inhibit leptin synthesis (Trayhurn *et al.* 1998, Evans *et al.* 1999). Paradoxically, leptin may directly activate sympathetic outflow within the hypothalamus and stimulate adrenal medullary release of epinephrine, thus creating a negative feedback loop between leptin and the sympathetic nervous system (Eikelis *et al.* 2003).

Plasma leptin levels appear to be associated with the propensity of certain cardiac damage in patients with acute

myocardial infarction (Fujimaki *et al.* 2001). Elevated plasma leptin levels and its soluble receptor were found in patients with congestive heart failure (Christian Schulze *et al.* 2003, Schulze *et al.* 2003), suggesting that leptin may participate in the catabolic cardiac cachexia en route to congestive heart failure. On the other hand, plasma leptin levels were reduced in patients with idiopathic sympathetic nerve degeneration and low sympathetic tone. However, intravenous infusion of the central sympathetic nervous system inhibitor, clonidine, reduced sympathetic nervous activity without affecting plasma leptin levels. This apparently discrepant response may be related to decreased renal clearance of leptin as a result of clonidine-induced reduction in renal blood flow.

There seems to be a positive correlation among pro-insulin, insulin and leptin levels, all of which were elevated in hypertensive patients when indexed for body size (Malmqvist *et al.* 2002). A significant negative correlation was found between the levels of leptin and IL-6. In a multivariate analysis, plasma leptin levels were found in correlation with diet, independent of age, BMI, body fat, alcohol consumption or insulin. Fish eaters exhibited lower plasma leptin levels than vegetable eaters with similar BMI (Winnicki *et al.* 2002). This is consistent with the epidemiological indication of reduced cardiovascular risk with a high-fish diet.

Leptin, vascular function and blood pressure

Like other metabolic hormones such as insulin, leptin possesses potent vascular effects and participates in the regulation of sympathetic tone and arterial blood pressure (Haynes *et al.* 1997). The regulatory effects on vascular tone and blood pressure have been demonstrated by both intracerebroventricular and intravenous administration of leptin, indicating central as well as peripheral actions of the hormone. Intracerebroventricular administration of leptin was found to elicit an increase in arterial blood pressure (Matsumura *et al.* 2000), consistent with its sympatho-excitatory property. The pressor effect of leptin was proportional to its levels in the cerebrospinal fluid (Shirasaka *et al.* 2003), suggesting that the pressor and sympathoexcitatory properties of leptin are attributed to a central mechanism. This is supported by the fact that leptin administered intravenously or intracerebroventricularly significantly increases the sympathetic outflow to kidneys, adipose tissue, skeletal vasculature and adrenal medulla in rodents. Interestingly, the dose of leptin required to enhance catecholamine release is at least 100 times higher for intravenous than for intracerebroventricular injection (Satoh *et al.* 1999), suggesting that leptin activates sympathetic outflow mainly via a central mechanism. On the other hand, chronic intravenous infusion of leptin also significantly enhanced arterial blood pressure associated with an elevated circulating plasma leptin level

(Shirasaka *et al.* 2003). The intravenous infusion of leptin induced an increase in blood pressure in conjunction with overt weight loss, which itself is expected to lower arterial blood pressure (Shek *et al.* 1998). The hypertensive effect of chronic intravenous leptin infusion disappeared when leptin infusion was stopped. It should be mentioned that acute leptin administration does not consistently elicit a hypertensive effect (Haynes *et al.* 1997, Shirasaka *et al.* 2003). Such discrepancy may be attributed to the small and inconsistent changes in plasma leptin following bolus injection or short-term infusion of leptin. Although accumulating evidence suggests a naturalistic linkage between sympathetic activity and plasma leptin levels, certain mechanisms other than leptin, particularly hyperinsulinemia, have been suggested to be responsible for the enhanced sympathetic activation in obesity. Further study is warranted to examine the interaction between leptin and other signaling pathways in the elevated sympathetic nervous activity in obesity.

In addition to its central neuronal sympathoexcitatory action, leptin may exert direct peripheral vascular actions on certain vasoactive mediators such as NO and endothelin-1 (ET-1). Leptin has been shown directly to induce vasorelaxation through NO-dependent as well as NO-independent mechanisms. Leptin elicits an NO-dependent arterial vascular relaxation with the chloride ion being an important regulator in leptin-induced endothelial NO release (Kimura *et al.* 2000). Leptin has been shown to stimulate endothelial NO synthesis, upregulate ET-1 production and promote accumulation of ROS in human umbilical vein endothelial cells (Bouloumie *et al.* 1999, Lembo *et al.* 2000, Yamagishi *et al.* 2001, Quehenberger *et al.* 2002, Vecchione *et al.* 2002). The leptin-induced vascular NO production was further proven to be mediated through PI-3 kinase-independent endothelial nitric oxide synthase (eNOS) phosphorylation by the Akt pathway (Vecchione *et al.* 2002). Recent evidence also indicated the possible involvement of AMP-activated protein kinase (AMPK) in enhanced eNOS phosphorylation and NO production in response to leptin. AMPK, activated by an increase in the AMP-to-ATP ratio, and/or a decrease in phosphocreatine, is known to mediate the metabolic effects of leptin including stimulation of glucose uptake and fatty acid oxidation (Minokoshi *et al.* 2002). AMPK has been shown to promote phosphorylation of eNOS either independently or in concert with Akt in a number of cell types including endothelial cells and myocytes (Chen *et al.* 1999), thus providing an important regulatory link between metabolic stress and cardiovascular function. What makes AMPK more attractive is the recent observation of elevated expression and phosphorylation of AMPK following chronic leptin treatment (Steinberg *et al.* 2003). It is therefore reasonable to postulate that leptin-induced regulation of NO in the cardiovascular system may be related to the enhanced expression and activation of AMPK. Nevertheless, the

precise role of NO in cardiovascular regulation elicited by leptin still remains ambiguous. Leptin was shown to induce an acute hypotensive effect in 6-hydroxydopamine sympathectomized rats, which was unaffected by NOS inhibition, suggesting a predominant role of the endothelium-derived hyperpolarizing factor mechanism in the hypotensive effect of leptin (Lembo *et al.* 2000). The direct vascular response of leptin in rodents was further confirmed in humans. Leptin directly induces vasodilation through an NO-independent pathway in healthy men (Nakagawa *et al.* 2002). The discrepancy in the NO dependence on the vascular effect of leptin is still largely unclear but may be related to the source of leptin (human versus murine), species, and the nature of the vascular beds tested. Considering pressor (sympathetic activation) and depressor (increased NO) actions, the integrated mechanism of actions of leptin on vascular tone and arterial blood pressure regulation is rather complex. State-of-the-art transgenic models of leptin and its receptor have provided some useful information regarding the vascular regulatory role of leptin. Transgenic mice overexpressing leptin displayed high arterial blood pressure compared with wild-type control mice, despite decreases in body fat (Aizawa-Abe *et al.* 2000). The increase in blood pressure was accompanied by an increase in urinary norepinephrine excretion and was normalized after α -adrenergic or ganglionic blockade (Aizawa-Abe *et al.* 2000). These observations support the sympathetic pressor property of leptin (Shek *et al.* 1998, Haynes 2000). However, it was recently reported that moderate hyperleptinemia produced by adenoviral gene transfer to obese mice reduced body weight without affecting blood pressure (Zhang *et al.* 2002). On the other hand, the leptin-deficient ob/ob obese mice showed slightly lower arterial blood pressure than their wild-type controls, despite profound obesity that would be expected to increase blood pressure. Administration of leptin to the ob/ob mice (the 'leptin reconstitution') resulted in elevated blood pressure despite reduced food intake and body weight (Aizawa-Abe *et al.* 2000, Mark & Sivitz 2002). In contrast to the leptin-deficient ob/ob mice, agouti yellow obese mice display partial leptin resistance, compensatory hyperleptinemia and elevated blood pressure (Mark *et al.* 1999, Correia *et al.* 2002). One other interesting note is that the vascular response of leptin seems to be independent of that of insulin or insulin resistance. Dietary obesity, which itself is associated with hyperleptinemia and leptin resistance (Wang *et al.* 2001), induces endothelial dysfunction without causing insulin resistance (Naderali *et al.* 2001). Studies from our laboratory, on the other hand, indicated that insulin resistance itself is not permissive to, or a prerequisite for the onset of cardiac leptin resistance (Hintz *et al.* 2003). It may thus be speculated that leptin and insulin possess distinct signaling mechanisms in the regulation of vascular tone.

Leptin receptors are localized in the hypothalamus and peripheral vascular tissues such as endothelial cells and

platelets (Lembo *et al.* 2000). The hypothalamic receptors for leptin are expected to assist the transfer of the hormone across the blood-brain barrier. The transfer is believed to occur at a low rate. Therefore, the levels of leptin in the cerebrospinal fluid are much lower than those in the plasma. The central leptin action is usually associated with elevated cerebrospinal leptin and an increased JAK/STAT3 signaling, leading to increased sympathetic tone and reduced food intake. JAK/STAT and IRS-2 may mediate leptin-induced activation of PI-3 kinase (Kellerer *et al.* 1997), which is a critical signal regulating vascular tone (Northcott *et al.* 2002). The specific pathway may be activation of JAK-2 by leptin followed by tyrosine phosphorylation of IRS-2 which would result in an increase in PI-3 kinase activity. The enhanced sympathetic nervous tone is thought to play a role in the cardiovascular response to leptin. Although this may be the case, the contribution from the peripheral response to leptin vascular activity and arterial blood pressure cannot be ignored. While the enhanced sympathetic nervous activation is expected to increase the vascular tone and blood pressure, leptin has been shown to elicit peripheral vascular relaxation mediated by NO-dependent or -independent mechanisms when the sympathetic control is removed (Bjorbaek *et al.* 1997, Fruhbeck 1999, Kimura *et al.* 2000, Lembo *et al.* 2000). The leptin-induced peripheral NO release and vasorelaxation may serve to counter-balance the enhanced sympathetic activity in response to leptin. While there was an increase in lumbar sympathetic nervous activity in response to low physiological levels of leptin, no acute response in blood pressure or heart rate was observed (Mitchell *et al.* 2001).

Leptin, heart rate and cardiac function

While acute leptin infusion failed significantly to alter the heart rate over a span of 90 min (Fruhbeck 1999), chronic leptin treatment over one week and cerebral injection of leptin elicited a significant increase in heart rate along with an increase in sympathetic nervous activity (Shek *et al.* 1998). An astonishing positive correlation between hyperleptinemia and tachycardia has been confirmed in mildly obese or mildly hypertensive human subjects (Narkiewicz *et al.* 1999). While an increase in heart rate may enhance cardiac output and provide short-term beneficial effects, sustained tachycardia may cause cardiac hypertrophy and ultimately heart failure. The higher heart rate in the hyperleptinemic individuals will impose a greater myocardial workload and therefore predispose the heart to pathophysiological changes, leading to congestive heart failure and myocardial infarction. This is supported by the clinical and experimental observations of obesity-induced cardiac hypertrophy, and the direct correlation between BMI and the left ventricular size in human and animal models (Sowers 1998, Ren *et al.* 2000). Such a relationship

may be transposed into a direct correlation between left ventricular size and plasma leptin levels. While it seems reasonable that the higher heart rate under hyperleptinemia may be due to leptin-induced sympathetic activation, an independent association between leptin levels and heart rate was observed in heart transplant recipients with sympathetic denervation (Winnicki *et al.* 2001). This finding suggests a direct effect of leptin on heart rate conceivably through cardiac leptin receptors, although direct effects of leptin on the cardiac conducting system and cardiac growth still warrant further study.

Leptin has been demonstrated to elicit concentration-dependent negative inotropic effects on myocyte contraction and intracellular Ca^{2+} release (Nickola *et al.* 2000). These effects were proven to be NO-dependent. However, the effects were blunted in ventricular myocytes from SHR hypertensive rats. A similar protein expression of the leptin receptor (Ob-R) was demonstrated between the WKY normotensive and the SHR hypertensive animals. Interestingly, leptin elicited overt post-receptor signaling pathway STAT3 phosphorylation in WKY but not SHR myocytes which was probably related to an up-regulated basal phosphorylation of STAT3 in SHR myocytes (Wold *et al.* 2002). The leptin-induced negative cardiac contractile response was blunted by the JAK2 inhibitor, AG-490, or the MAP kinase inhibitor, SB203580 (Wold *et al.* 2002), suggesting involvement of JAK2/STAT and p38 MAP kinase in the leptin-induced cardiac response. It is possible that NO may serve as the downstream mediator or the final instigator for JAK2/STAT and p38 MAP kinase in cardiac myocytes. Our earlier evidence suggested that leptin increases the NOS activity in cardiac myocytes (Nickola *et al.* 2000). However, the leptin-induced increase in NOS activity was blunted under hypertension (Wold *et al.* 2002), which may be directly responsible for the loss of the leptin response in SHR myocytes.

Other signaling pathways have also been implicated in the leptin-induced cardiac effect. The adenylate cyclase complex has been shown to be affected by leptin in the H9c2 cardiac cell line. Differences in adenylate cyclase activity after short- and long-term exposure to leptin and the interaction between leptin and the sympathetic nervous system catecholamine neurotransmitters is believed to play a key role in the development of hypertension and congestive heart failure in obese patients (Illiano *et al.* 2002). Leptin has also been demonstrated to activate fatty acid oxidation and decrease triglyceride content without affecting glucose oxidation over a 60-min perfusion period. Although leptin did not affect cardiac work, it increased oxygen consumption and decreased cardiac efficiency (Atkinson *et al.* 2002). The role of leptin in lipid metabolism is of interest and it may participate in the cardiac regulation of the hormone. Lipoapoptosis is a metabolic cause of tissue injury and death under obesity. As mentioned earlier, leptin is capable of oxidizing

excessive long-chain fatty acids to improve cardiac function. However, the leptin-induced fatty acid oxidation may become inefficient under hyperleptinemia or leptin resistance, allowing unoxidized fatty acids to enter non-oxidative pathways, eventually leading to cellular injury (Unger & Orci 2002). The ceramide/sphingosine pathway has been implicated in the lipoapoptosis of β -cells and cardiac myocytes of genetically obese rats under which condition the leptin malfunctions. The leptin-induced liporegulation serves to prevent lipid accumulation and lipoapoptosis. Disruption of liporegulation in response to leptin is speculated to be essential under hyperleptinemia and may attribute to the metabolic syndrome in obesity (Unger & Orci 2002).

Circulating leptin levels have been postulated to be a marker of the cardiovascular and neuroendocrinological stress response. Patients with advanced congestive heart failure exhibit elevated plasma levels of leptin and its soluble receptor, indicating that leptin may participate in the catabolic state leading to the development of cardiac cachexia (Schulze *et al.* 2003). Critically ill patients display elevated leptin levels in response to stress-related cytokines (TNF- α , interleukins) and loss of the circadian rhythm in leptin secretion, suggesting that the hormone plays a role in cardiac stress response (Modan-Moses *et al.* 2001). Cardiopulmonary bypass surgery was found to be associated with acute changes in circulating leptin levels inversely related to those of cortisol (Modan-Moses *et al.* 2001). Patients undergoing major surgery show a similar increase in plasma leptin levels compared with those following cardiopulmonary bypass, indicating that a sepsis-like inflammatory syndrome does not further increase elevated leptin concentrations following major surgery (Dotsch *et al.* 2001).

Leptin and circulatory function

High levels of leptin are believed to be associated with lower arterial distensibility, an index of circulatory function relevant to the atherosclerotic process through mechanisms other than vascular relaxation (Singhal *et al.* 2002). Leptin promotes angiogenesis, regulates osteoblastic differentiation, enhances the calcification of vascular cells and potentiates the pro-thrombotic platelet aggregation through a novel leptin receptor-dependent mechanism (Sierra-Honigsmann *et al.* 1998, Konstantinides *et al.* 2001, Parhami *et al.* 2001). This is consistent with the observation that leptin-deficient ob/ob mice display delayed thrombus formation after arterial injury, which may be canceled by leptin replacement (Konstantinides *et al.* 2001). Obese individuals possess higher plasma levels of pro-thrombotic factors such as fibrinogen, von Willebrand factor, factor VII and plasminogen activator inhibitor-1 (PAI-1), which impose a higher risk of thrombosis and arteriosclerosis. The levels of pro-thrombotic factors and

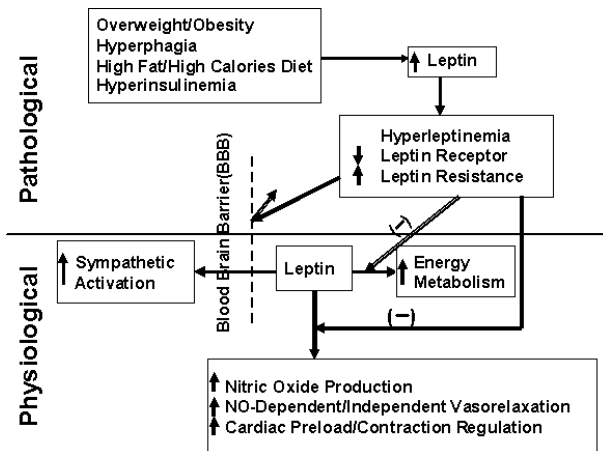


Figure 1 Regulation of the sympathetic, metabolic and cardiovascular functions of leptin under physiological 'euleptinemic' (lower half) or pathological 'hyperleptinemic' (upper half) conditions. Note that leptin cannot go through the blood-brain barrier (BBB) and therefore overweight, hyperphagia and hyperinsulinemia are unlikely to down-regulate leptin receptors and trigger leptin resistance in the CNS. However, the peripheral metabolic and cardiovascular actions of leptin will be interrupted by leptin resistance. (–), inhibition or interruption.

PAI-1 are directly correlated with fat composition and therefore leptin levels. Paradoxically, higher plasma levels of anti-thrombotic factors such as tissue-type plasminogen activator and protein C have also been demonstrated in obesity to counteract the increased pro-thrombotic factors, probably through a compensatory mechanism. It is speculated that leptin-associated secretion of TNF- α and IL-6 from the adipose tissue may be responsible for the enhanced thrombosis formation (De Pergola & Pannaciuoli 2002), which predisposes to the pathogenesis of endothelial dysfunction and coronary heart disease. Similar to the common anti-obese measures, dietary (i.e. low-fat, high-fiber diet) and lifestyle (i.e. physical activity) factors seem to effectively lower these obesity-associated pro-thrombotic risks (De Pergola & Pannaciuoli 2002).

Leptin/Leptin Resistance: an Anti-/Pro-Obesity Pair?

A schematic diagram depicting the neuronal regulatory, cardiovascular and metabolic effects of leptin under both physiological and hyperleptinemic pathological conditions is summarized in Fig. 1. Using plasma norepinephrine spillover as a criterion, plasma leptin levels are a stronger predictive factor for obesity than percentage body fat, suggesting that hyperleptinemia is the prime drive underlying the sympathetic stimulation and pathophysiology of obesity-related cardiovascular dysfunctions (Grassi *et al.* 1998, Anderson & Ren 2002). While leptin may be considered as an anti-obesity hormone due to its metabolic

properties, hyperleptinemia and subsequently leptin resistance have surely made the ugly switch or transition. It is unlikely that leptin has evolved to counteract obesity since overweight individuals often become resistant to leptin action. On the other hand, due to the poor transport of leptin across the blood-brain barrier, hyperleptinemia cannot effectively signal the CNS to initiate sufficient negative feedback inhibition of the leptin signaling (Bjorbaek *et al.* 1999). While secretion of leptin may not be acutely determined by a single meal, a more chronic stimulus of food intake does modulate and affect leptin secretion. Elevated plasma leptin levels and severe resistance to leptin and insulin have been shown to develop after only 3 days of overfeeding. With the presence of insulin clamps, glucose production was decreased by 70% in control rats and by 28–53% in overfed rats. Similarly, leptin infusion doubled the contribution of gluconeogenesis to glucose output in control rats but failed to modify gluconeogenesis in overfed animals (Wang *et al.* 2001). On the contrary, fasting over several days induced a decrease of 10% in body weight associated with a 53% reduction in plasma leptin (Boden 1997). The reduced leptin levels may be a survival response to minimize the energy expenditure during weight loss. The lowered plasma leptin levels also facilitate mobilization of energy stores by promoting secretion of glucocorticoids (Legradi *et al.* 1997). It is therefore postulated that a drop in plasma leptin levels may be a key short-term adaptation mechanism to starvation or famine situations (Jequier 2002).

Hyperphagia and elevated levels of leptin (along with those of insulin) are common features of obesity (Igel *et al.* 1997, Lu *et al.* 1998, Marsh *et al.* 1999). This seems to be paradoxical since leptin itself is a potent inhibitor of food intake and is expected to decrease insulin levels via improved insulin action and inhibition of insulin secretion (Flier 1997, Rossetti *et al.* 1997, Sivitz *et al.* 1997, Shimomura *et al.* 1999, Wang *et al.* 1999). It is plausible that leptin may have been somewhat mislabeled as a metabolic hormone that can limit the excessive weight gain of individuals exposed to an environment of plenty. In fact, the hormone may never be able to achieve this goal with the epoch of hyperleptinemia and leptin resistance associated with obesity. Recently, the concept of leptin resistance has been articulated to be 'selective leptin resistance' largely based on the preserved sympatho-excitatory actions of leptin, despite resistance to the satiety and weight-reducing properties of the hormone (Mark *et al.* 2002). The 'selective leptin resistance' theory is feasible to explain why hyperleptinemia contributes to increased sympathetic activity and arterial pressure in obesity, whereas at the same time there is resistance to the metabolic (satiety and weight-reducing) actions of leptin. If selective leptin resistance occurs in obese humans, then leptin could contribute to the sympathetic overactivity and hypertension despite resistance to its metabolic actions

(Mark *et al.* 2002). Although leptin or selective leptin resistance may be accepted as the causative factor for metabolic as well as cardiovascular dysfunctions, the mechanism(s) leading to this resistance has never been elucidated. Using transgenic techniques to overexpress uncoupling protein-1 (UCP-1) in skeletal muscle of agouti yellow obese mice, it was found that the UCP-1 mice displayed increased metabolism, promoted weight loss and increased insulin sensitivity. More importantly, the UCP-1 mice showed decreased arterial pressure, serum leptin and urinary catecholamines. The hypotensive effect of UCP-1 may be blunted by leptin administration (Bernal-Mizrachi *et al.* 2002). Uncoupling proteins are known to dissipate food metabolism-derived energy as heat rather than being stored as high-energy phosphates. UCP-1 is expressed only in brown adipose tissue where leptin is released. Therefore, it is possible that UCP-1-induced regulation of thermogenic metabolism may play a role in the leptin signaling cascade although further study is warranted (Mark & Sivitiz 2002). Last, but not least, leptin resistance can be beneficial in certain situations since it may facilitate energy storage to avoid the consequences of starvation when food resources are limited.

Leptin plays a physiological role but leptin resistance may be pathophysiological for metabolic and cardiovascular dysfunction under obesity. While the role of leptin has been determined in elevation of sympathetic nervous system activity, many of its action on the cardiovascular system such as cardiac growth and ventricular function have not been carefully studied. Understanding the signaling mechanisms behind selective leptin resistance and searching for strategies to alleviate this hormone resistance should have significant clinical value in managing obesity and obesity-associated cardiovascular dysfunction.

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