

Role of leptin and obesity-related leptin resistance in the pathophysiology of metabolic syndrome

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ABSTRACT

The prevalence of metabolic syndrome is high and will likely increase further as the obesity epidemic accelerates. Leptin is a peptide hormone mostly derived from adipose tissue that promotes negative energy balance. Hyperleptinemia is common in obesity and reflects increased fat mass and leptin resistance. Nevertheless, leptin resistance might not be complete as several actions of leptin, like cardiovascular sympathetic activation, might be preserved in obese subjects that are resistant to the metabolic actions of leptin (i.e. selective leptin resistance).

Leptin may contribute to elevated blood pressure, hyperglycemia, dyslipidemia, pro-thrombotic and pro-inflammatory states found in metabolic syndrome. Notably, the renal and sympathetic actions of leptin appear to play a major role in the pathogenesis of hypertension related to obesity and the metabolic syndrome. Furthermore, the lipotoxic effect of leptin resistance is thought to be a major determinant of insulin resistance and may increase the risk of type 2 diabetes.

KEY WORDS

Metabolic syndrome, obesity, leptin, selective leptin resistance, diabetes, hypertension, lipotoxicity.

INTRODUCTION

According to the Third National Health and Nutrition Examination Survey (NHANES III), between 1988 and 1994 the average prevalence of metabolic syndrome was 22% in the United States adult population. Also, 22% and 59% of overweight and obese adults, respectively, exhibited metabolic syndrome. However, this condition is not confined exclusively to overweight and obese subjects and was found in about 5% of lean NHANES III participants¹.

The National Cholesterol Education Program's Adult Treatment Panel III indicates 6 pathologic components of the metabolic syndrome that are relevant to cardiovascular disease: 1) abdominal obesity, 2) atherogenic dyslipidemia, 3) increased blood pressure, 4) insulin resistance with or

without glucose intolerance, 5) pro-inflammatory state, and 6) pro-thrombotic state².

Extensive investigative efforts have been aimed at the interactions between the endocrine abnormalities of obesity and the pathologic components of metabolic syndrome. Leptin is a peptide hormone mainly produced by white adipose tissue. It promotes satiety and increases metabolic expenditure through activation of central neural leptin receptors and favors negative energy balance. Plasma leptin reflects adipose tissue mass and is greatly increased in obesity. Hyperleptinemia also parallels leptin resistance that is commonly present in obesity.

Experimental results from animal models of obesity indicate that leptin resistance might not be invariably complete. In these models,

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leptin resistance appears to be selectively restricted to the metabolic actions of leptin whereas the sympathoexcitatory effects of leptin are preserved, the so called selective leptin resistance.

In this article we briefly review the associations between leptin and various pathologic components of metabolic syndrome, with emphasis on the modulation of blood pressure, insulin sensitivity and pancreatic endocrine function.

LEPTIN EFFECTS ON BLOOD PRESSURE

There is large evidence from animal studies indicating that leptin might play an important role in modulating blood pressure mainly through its vascular, renal and sympathetic actions. Although depressor and pressor actions of leptin have been reported, the pressor effects of leptin appear to predominate.

As mentioned above, leptin has been shown to promote nitric oxide release by the vascular endothelium that could potentially decrease blood pressure^{3,4}. However, this effect may be blunted by leptin resistance and endothelial dysfunction that often accompanies human obesity. In addition, leptin has been shown to promote endothelin 1 secretion from endothelial cells isolated from human umbilical veins⁵. It is conceivable that under conditions of endothelial dysfunction, leptin-dependent secretion of endothelin 1 may predominate and increase vascular tone.

Even though acute pharmacologic administration of leptin to rats causes diuresis and natriuresis^{6,7}, chronic administration of leptin appears to shift the pressure-natriuresis curve to a higher blood pressure setting, presumably through activation of the renal sympathetic nerves that promote renal tubular sodium reabsorption⁸. Shifts in the pressure-natriuresis curve could favor long-term sodium retention and may contribute to elevation of blood pressure.

Leptin exhibits unequivocal sympathoexcitatory actions in rodents. It has been shown that acute systemic administration of leptin to rats increases sympathetic nerve activity (SNA) to the brown adipose tissue, kidneys, adrenal glands and hindlimbs^{9,10}.

Several studies indicate that leptin may modulate arterial pressure through sympathetic mechanisms. Chronic intracarotid and intracerebroventricular administration of leptin increase blood pressure in rats^{11,12}. Also, skinny transgenic mice overexpressing leptin in the liver develop hypertension that is reversed by α -adrenergic antagonists and ganglion blockers¹³. Furthermore, despite severe obesity, leptin-deficient *ob/ob* mice have lower blood pressure than lean controls¹⁴. Administration of exogenous leptin to *ob/ob* obese mice increases blood pressure to that observed in lean controls, despite food intake and body weight reduction¹³.

In humans, pathological conditions associated with high sympathetic activation such as congestive heart failure, hypertension and obesity, the renal norepinephrine spillover correlates with

plasma leptin after adjustments for fat mass¹⁵. Despite these observational findings, leptin administration to lean subjects for 6 days did not alter norepinephrine, dopamine, and epinephrine levels in 24-hour urine collections¹⁶. However, plasma and urine catecholamine measurements may not detect increased sympathetic outflow to specific organs. Conclusive evidence of the sympathetic and blood pressure actions of leptin in humans is not currently available.

POTENTIAL ROLE OF SELECTIVE LEPTIN RESISTANCE IN THE PATHOPHYSIOLOGY OF OBESITY-RELATED HYPERTENSION

Obesity is known to be associated with hyperleptinemia, reflecting resistance to leptin because obese subjects remain overweight despite the high circulating levels of leptin¹⁷. This was confirmed by clinical trials where leptin was administered to obese patients causing only modest effects on body weight¹⁸. One important question was raised from these observations. Indeed, if obesity is a condition associated with leptin resistance, how could leptin modulate sympathetic function in obese subjects? We have introduced and tested the novel concept of selective leptin resistance that may partly explain this important question.

The concept of selective leptin resistance originated from studies with the obese agouti mouse. Obesity in the agouti mouse is caused by ubiquitous overexpression of agouti protein, a potent endogenous melanocortin-4 receptor antagonist. In addition to obesity, these animals exhibit profound resistance to the anorexigenic and weight lowering effects of leptin. We have demonstrated that, despite severe leptin resistance, the sympathoexcitatory effect of leptin, as measured by neurography of renal sympathetic nerves, is preserved after either systemic or central neural administration of leptin^{19,20}. Similar results have been found in C57BL/6J mice with diet-induced obesity²¹.

Importantly, we have demonstrated that the preservation of the renal sympathoexcitatory response of leptin translates into increased arterial pressure in mice with dietary obesity. Indeed, we found that arterial pressure in mice on high fat diet was responsive to leptin despite resistance to the anorexigenic effect of leptin. Twelve days of systemic leptin treatment caused substantial increases in arterial pressure in the high fat-fed mice. Furthermore, the leptin-dependent increases in arterial pressure were of similar magnitude in mice fed either high fat diet (about 10 mmHg) or normal chow (about 11 mmHg)²². These findings highlight the potential role of selective leptin resistance in hypertension in common, polygenic obesity.

We speculate, if selective leptin resistance occurs in human obesity, that hyperleptinemia may contribute to sympathetic modulation of blood pressure in obese subjects despite leptin resistance, particularly in those presenting metabolic syndrome

along with hypertension. Eikelis *et al.*¹⁵ have shown the existence of a strong correlation between leptin plasma concentration and renal SNA across a broad range of leptin values in men of widely differing adiposity. This indicates that leptin may cause sympathetic activation in human obesity.

ANATOMICAL AND MOLECULAR BASES OF SELECTIVE LEPTIN RESISTANCE

The mechanisms underlying selective leptin resistance are currently under intense investigation, and may involve differential levels of leptin resistance found in distinct central neural networks or post-receptor intracellular pathways targeted by leptin²³. In obese mice, the inability of leptin to activate intracellular signaling pathways such as the signal transducer and activator of transcription 3 protein (STAT-3) appears to be restricted to the arcuate nucleus of the hypothalamus. Indeed, after 16 weeks of high fat diet feeding, leptin-activation of STAT-3 within the arcuate nucleus was dramatically decreased. In contrast, other hypothalamic and extra-hypothalamic nuclei remained leptin sensitive²⁴. Interestingly, the level of suppressor of cytokine signaling 3 protein (SOCS-3) is specifically increased in the arcuate nucleus of diet-induced obese mice²⁴. Increased expression of SOCS-3 inhibits leptin signaling and is probably a molecular component of leptin resistance in the arcuate nucleus.

The arcuate nucleus is known to be a major site of leptin action to control food intake and body weight. By suppressing the activity neuro-peptide- γ neurons in the arcuate nucleus, leptin presumably regulates sympathetically driven thermogenesis in the brown adipose tissue of rodents²⁵. In contrast, the cardiovascular sympathetic effects of leptin appear to be mediated by other hypothalamic nuclei. Indeed, using brain site-specific injections, Marsh *et al.*²⁶ found that the ventromedial and dorsomedial hypothalamus are important sites where leptin activation leads to increased sympathetic vasomotor activity and heart rate.

Therefore, it is very likely that selectivity in leptin resistance is due to the inability of leptin to activate downstream signaling pathways in the arcuate nucleus, but preservation of leptin actions in other cardiovascular-related hypothalamic areas (Figure 1).

LEPTIN RESISTANCE AND LIPOTOXICITY MAY CONTRIBUTE TO THE DEVELOPMENT OF INSULIN RESISTANCE AND TYPE 2 DIABETES

Roger Unger and collaborators have advanced the concept that the lack of leptin actions, either due to leptin deficiency or leptin resistance, causes dysfunction of critical non-adipose tissues through cellular lipotoxicity.

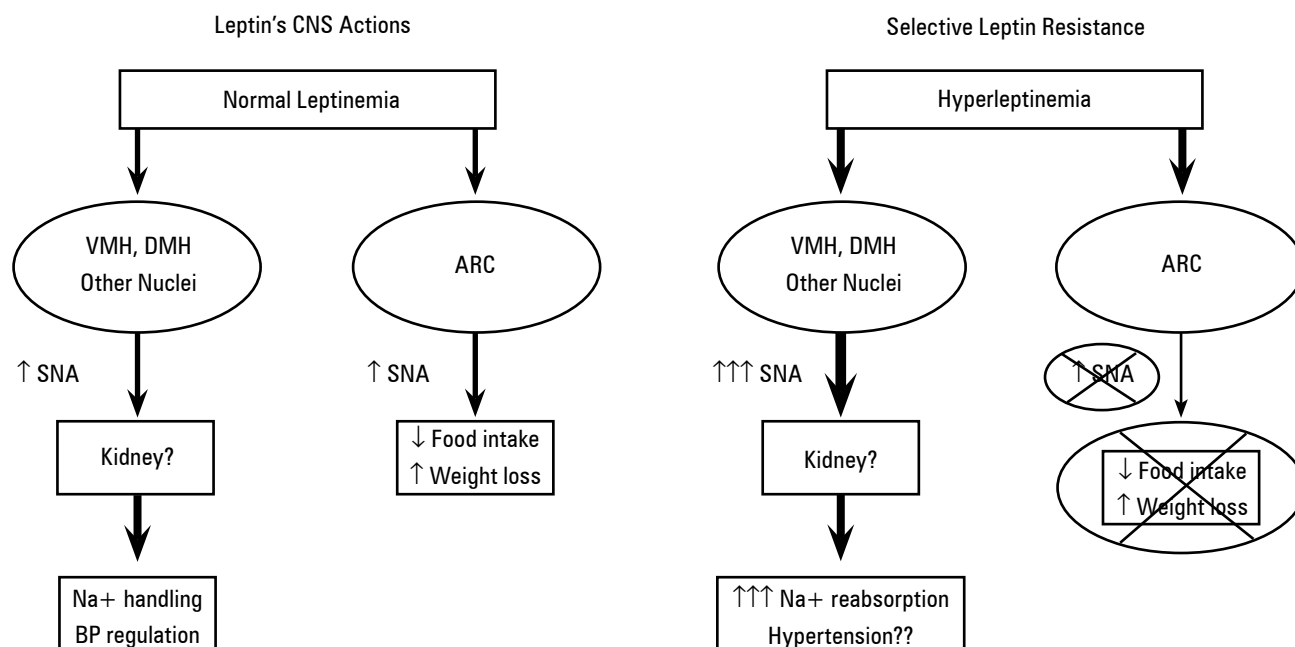


Figure 1. Mechanisms of selective leptin resistance. Leptin acts in the hypothalamus to decrease food intake and increase thermogenic metabolism, as well as increase sympathetic nerve activity to non-thermogenic organs. These actions can be dissociated in obesity, with resistance to the anorexic and thermogenic effects of leptin but preservation of cardiorenal sympathoactivation. These effects might explain how hyperleptinemia could be associated with partial loss of appetite and metabolic actions of leptin in obesity but still contribute to sympathoexcitation and presumably obesity-related hypertension (CNS, central nervous system; SNA, sympathetic nervous system; BP, blood pressure; ARC, arcuate nucleus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus).

Normally, leptin promotes fatty acid oxidation through increased expression of peroxisomal proliferation-activated receptor α (PPAR α)²⁷. Impaired leptin action causes ectopic accumulation of triglycerides in non-adipose organs and tissues such as skeletal muscles and pancreas. Accumulation of triglycerides and long-chain free fatty acids in these organs triggers ceramide synthesis that causes apoptosis through stimulation of inducible nitric oxide synthase. Lipotoxicity developing in skeletal muscle and pancreas cause insulin resistance and beta cell dysfunction, respectively, and could be responsible for the development of type 2 diabetes.

Studies in leptin-resistant, obese Zucker rats illustrate the physiologic relevance of leptin action to normal pancreatic β cell function. Obese Zucker rats exhibit non-functional, mutated leptin receptors. In addition to the obesity caused by leptin resistance, obese Zucker rats develop diabetes partly due to pancreatic endocrine failure presumably caused by a 50-fold increase in triglyceride content in islet β cells. Wang *et al.*^{28,29} induced the expression of functional leptin receptors by adenoviral transfection in isolated pancreatic β cells from obese Zucker rats. Leptin administration after receptor expression decreased intracellular triglyceride by 87%²⁸ and improved glucose-stimulated insulin secretion²⁹. Overproduction of inducible nitric oxide was also reduced by leptin treatment in this *in vitro* experimental model²⁸. Reduced inducible nitric oxide production may decrease lipotoxicity by preventing ceramide synthesis and, consequently, apoptosis.

Increased expression of PPAR α appears to play a critical role in the maintenance of the normal, non-toxic content of intracellular lipids. Troglitazone is an anti-diabetic drug that, like leptin, increases PPAR α expression. Not surprisingly, the administration of troglitazone to pre-diabetic obese Zucker rats *in vivo* prevented pancreatic accumulation of triglycerides and diabetes³⁰.

Strongly corroborating the validity of lipotoxicity concept in human disease, it has been demonstrated that decreased leptin actions partly underlie the development of type 2 diabetes in human lipodystrophy. This syndrome is characterized by severe hypothrophy of adipose tissue and very low serum levels of adipocyte-derived leptin. Human lipodystrophy is also accompanied by ectopic accumulation of lipids in the skeletal muscles and liver, insulin resistance and diabetes. Leptin treatment in subjects with lipodystrophy decreased muscle and liver steatosis and dramatically improved diabetes by increasing insulin sensitivity^{31,32}.

Leptin promotes fatty acid oxidation in isolated human skeletal muscles *in vitro*. It has been demonstrated that leptin-dependent fatty acid oxidation in isolated muscle is blunted in obese as compared to lean subjects. Furthermore, under basal conditions, fatty acid uptake and esterification was increased

in muscles obtained from obese subjects³³. Therefore, skeletal muscle leptin resistance could potentially contribute to muscular fatty acid accumulation in common human obesity, simulating the muscle steatosis found in lipodystrophy patients.

In summary, lipotoxicity may set the pathologic ground to the development of type 2 diabetes in metabolic syndrome subjects with impaired leptin actions due to obesity-related leptin resistance³⁴. Interventions leading to increase skeletal muscle and pancreatic leptin sensitivity could potentially improve insulin metabolic actions and prevent pancreatic endocrine dysfunction in subjects with metabolic syndrome.

LEPTIN ROLE IN DYSLIPIDEMIAS, INFLAMMATION, THROMBOGENESIS, AND OXIDATIVE STRESS

In a major prospective cohort, the West of Scotland Coronary Prevention Study (WOSCOPS), serum leptin levels have been independently associated with coronary heart disease³⁵. In obese women, leptin levels are associated with atherogenesis markers such as vascular adhesion molecule-1, and thrombomodulin³⁶. In addition, leptin levels independently predict future cardiovascular events in subjects with established angiographic coronary lesions³⁷.

Although dyslipidemias do not appear to be strongly associated with leptin levels in humans³⁸, hypercholesterolemia and hypertriglyceridemia in mice lacking the low density lipoprotein receptor are considerably aggravated when the leptin receptors are also absent³⁹.

There are other leptin-dependent actions that have been demonstrated and could contribute to the epidemiologic associations between leptin and the atherogenic component of metabolic syndrome. These include: 1) proliferative, 2) pro-inflammatory, 3) pro-thrombotic, and 4) oxidative actions.

These actions of leptin have been mostly studied in experimental models that retain leptin sensitivity. Whether leptin resistance also impairs these actions of leptin in obesity is an issue that needs clarification.

1) PROLIFERATIVE EFFECTS OF LEPTIN

The vascular proliferative actions of leptin depend mostly on the activation of mitogenic factors. For instance, leptin administration to culture media produced a dose-dependent increase in migration and proliferation of rat vascular smooth muscle cells through activation of phosphatidylinositol-3-kinase, and mitogen-activated protein kinases⁴⁰. These proliferative actions of leptin may contribute to formation of obstructive plaque either by promoting neointimal formation after vascular injury⁴¹ or calcification⁴².

In vitro activation of fibroblast growth factor-2 partly explains the angiogenic effect of leptin⁴³. Also, leptin stimulates angioge-

nesis by inducing endothelial cell and matrix proliferation through stimulation of endothelial expression of vascular endothelial growth factor receptors, matrix metalloproteinases, and tissue inhibitors of metalloproteinases in atherosclerosis⁴⁴. Therefore, leptin-dependent angiogenesis may be important in diverse physiologic and pathologic processes ranging from regulation of adipose tissue oxygen supply to vascular remodeling under atherosclerotic conditions.

2) PRO-INFLAMMATORY EFFECTS OF LEPTIN

Leptin modulates several inflammatory mediators and cells and may be associated with the low-grade inflammation encountered in obese subjects. In vitro leptin administration to macrophages potentiates the lipopolysaccharide-induced synthesis of tumor necrosis factor α , interleukin 6, and interleukin 12⁴⁵. In addition, leptin treatment promotes macrophage phagocytic function only in cells collected from ob/ob (leptin deficient) but not from db/db (leptin resistant) mice⁴⁵. These findings strongly suggest a physiological role of leptin in the modulation of inflammatory process.

Leptin increases the expression of monocyte chemo-attractant protein-1⁴⁶, and macrophage lipoprotein lipase, a potent atherogenic cytokine in type 2 diabetic subjects⁴⁷. Furthermore, leptin decreases hydrolysis of cholesterol esters in macrophages which could directly contribute to foam cell formation⁴⁸.

Currently, the knowledge about potential associations between leptin and human inflammatory response is scarce. In a cross-sectional study involving young healthy men, leptin was independently associated with C reactive protein⁴⁹, a strong predictor of atherosclerosis and its complications.

3) PRO-THROMBOTIC EFFECTS OF LEPTIN

Obese subjects are at increased risk for deep venous thrombosis and pulmonary embolism. Leptin may contribute to the pro-thrombotic state in obesity. The physiologic relevance of leptin as a pro-coagulant factor is suggested by experimental results showing that platelet aggregation is attenuated in ob/ob and db/db mice but only normalized by leptin in ob/ob mice⁵⁰.

Leptin accelerates thrombogenesis by acting on platelets of ob/ob mice after experimental arterial injury⁵¹. This result suggests that leptin may contribute to thrombotic events after plaque rupture.

Leptin also modestly decreases the expression of thrombomodulin, an anti-thrombotic protein, in endothelial cells from the human umbilical vein⁵².

4) PRO-OXIDANT EFFECTS OF LEPTIN

Oxidative stress is not considered a primary pathogenic component of metabolic syndrome as defined by the National Cholesterol Education Program's Adult Treatment Panel III. However reactive free oxygen radicals may play a decisive

role on the pathogenesis of atherosclerosis and hypertension. Increased oxidative stress has been described in experimental and human obesity and may contribute to the pathogenesis of metabolic syndrome⁵³.

Leptin increases oxygen reactive species by promoting increased fatty acid oxidation⁵⁴ and by reducing lipoprotein-associated antioxidant enzymes such as paraoxonase 1⁵⁵.

Oxidative stress can cause direct endothelial or vascular smooth muscle damage but may also operate as an indirect factor to increase serum atherogenic factors. By increasing oxidative stress and activating protein kinase C, leptin appears to increase secretion of atherogenic lipoprotein lipase from macrophages in vitro⁴⁷.

Increased systemic and renal oxidative stress is associated with reductions in nitric oxide availability and increased renal tubular absorption of sodium in Wistar rats with leptin-induced hypertension⁵⁶ and could indirectly contribute to the progression of atherosclerosis.

CONCLUSIONS

We have reviewed evidence that leptin and selective leptin resistance may contribute to several components of the metabolic syndrome. Most evidence shown in this manuscript originated from animal and "in vitro" studies but provides important insight into potential mechanisms operating in human disease. Clinical studies are eagerly awaited so that the effects of leptin on renal function, the sympathetic nervous system, blood pressure, and atherogenesis can be clarified. A better understanding of the anti-lipotoxic effects of leptin may prove useful to the development of effective strategies to prevent and treat insulin resistance and type 2 diabetes that often complicate the metabolic syndrome.

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