Contribuição Internacional

Mechanisms of obesity-related hypertension: from insulin to leptin

Marcelo L. G. Correia*, William G. Haynes, Allyn L. Mark

Abstract

Hyperinsulinemia has been classically associated with obesity-related hypertension. However, this concept has been challenged given that acute hyperinsulinemia has repeatedly failed to increase arterial pressure in humans.

Most recently, leptin-dependent mechanisms have raised great interest as potential explanations for obesity-related hypertension. Despite potential depressor effects of leptin, most reports have shown that leptin increases arterial pressure probably due to sympathetic activation. Human obesity hypertension is associated with increased sympathetic activity. Thus, it is possible that hyperleptinemia in human obesity could contribute to obesity-related sympathetic activation. However, human obesity is a partial leptin resistant condition. The novel concept of selective leptin resistance may help explain leptin-induced sympathoactivation in obese subjects resistant to the metabolic effects of leptin.

In this review article, we revisit insulin-dependent mechanisms reportedly associated with obesity hypertension. We also discuss leptin actions on the cardiovascular system and show experimental results that support the concept of selective leptin resistance.

Correspondence to:
Marcelo L. G. Correia
From the Hypertension Genetics Specialized Center of Research; and Department of Internal Medicine, University of Iowa College of Medicine, and the Veterans Administration Medical Center, Iowa City, Iowa, USA
*Internal Medicine Department, State University of Rio de Janeiro, Brazil
Av. Lineu de Paula Machado, 850/501 – Jardim Botânico – Rio de Janeiro, RJ – CEP 22470-040

Introduction

The Framingham Heart Study has demonstrated that approximately 70% of the risk of development of hypertension can be related to obesity[1]. Overweight, defined as a body mass index above 25 kg/m^{2}, is increasingly prevalent in the US reaching about 50% of the adult population[2]. In Brazil, the prevalence of overweight is similar to that in the US but obesity is 2.5 times less prevalent[3]. With increasing prevalence of overweight and obesity in Western countries, it is presumed that hypertension prevalence will increase accordingly, magnifying an already critical public health problem.

Despite the severity of the obesity hypertension problem, few practical recommendations have been issued in terms of prevention and treatment of this condition. Unlike other aspects of essential hypertension, treatment of obesity-related hypertension has not been thoroughly studied in clinical trials. Much attention has been paid to weight loss, but modification of lifestyles has been costly, hard to pursue, and ineffective. Only two medications for body weight control are currently approved by the US Food and Drug Administration (FDA), and efficacy is far from ideal and adverse effects are frequent. Pharmacotherapy for obesity-related hypertension has been based on "soft" endpoints like insulin sensitivity and beneficial impact on lipid profile. To our knowledge, no comparative clinical trial has addressed issues like reduction in cardiovascular mortality with optimal anti-hypertensive therapy tailored for patients with obesity and hypertension. As a matter of fact, most clinical trials exclude individuals with higher body mass index.

Many mechanisms for obesity hypertension have been proposed such as activation of the renin-angiotensin-aldosterone system, increased sympathetic drive, augmented sodium sensitivity of arterial pressure, and endothelial dysfunction. Obesity-related hypertension is a syndrome where most of these mechanisms operate in concert, but there might be key systems amenable to optimal intervention.

Classically, obesity-related hypertension has been strongly associated with selective insulin resistance and hyperinsulinemia. In this brief review, we revisit the insulin hypothesis of obesity-related hypertension and address the novel concept of selective leptin resistance that may contribute to the understanding of mechanisms underlying hypertension in obese patients.

The insulin hypothesis of obesity hypertension: pros and cons

Central adiposity distribution appears to be an important marker of obesity-related hypertension[4]. Furthermore, central obesity has been related to hyperinsulinemia and insulin resistance, raising the hypothesis that impairment in glucose metabolism might be linked to obesity hypertension[5]. In 1988, Reaven proposed the so-called Syndrome X that comprises hypertension, central obesity, glucose intolerance, dyslipidemia and atherosclerosis[5]. This hypothesis is supported by several epidemiological studies. First, the National Health Examination Survey III (NHANES III) conducted between 1988 and 1994 demonstrated a strong association of body mass index with systolic and diastolic arterial pressure in American adults at age younger than 60 years[6]. Second, in a cross-sectional study involving 10,303 middle-aged subjects for the *European Prospective Investigation into Cancer and Nutrition* (*EPIC*), central distribution of body fat was independently associated with the prevalence of hypertension[7]. Third, the relationship of hypertension, glucose tolerance, hyperinsulinemia and insulin resistance was established in a cohort of Dutch and Finnish men followed-up for 30 years[8].

Various mechanisms have been implicated in hyperinsulinemic, insulin resistance states to cause hypertension, including activation of the sympathetic nervous system, increased renal reabsorption of sodium, and vascular remodeling and hypertrophy.

Augmented sympathetic drive is allegedly the most important mechanism by which insulin increases arterial pressure. Grassi et al. have reported that skeletal muscle sympathetic nerve activity is increased in normotensive obese versus non-obese subjects[9]. Given that hyperinsulinemia is commonly detected in obese subjects, elevated plasma levels of insulin could potentially cause sympathoactivation in human obesity.

Many reports support the concept that insulin activates the sympathetic nervous system. Insulin acts in the hypothalamus to increase sympathetic activity[10]. Increased plasma concentration of insulin dose-dependently augments the turnover of norepinephrine in the skeletal muscles, liver and adipose tissue[11]. Furthermore, systemic administration of insulin in normotensive, non-obese subjects increases sympathetic nerve activity to skeletal muscles and augments plasma norepinephrine concentration, during euglycemic clamp[12].

Despite marked sympathoactivation, insulin treatment causes substantial vasodilation that presumably prevents changes in arterial pressure[12]. Thus, in normal, insulin sensitive subjects, the vasodilatory action of insulin overrides the expected vasoconstrictor response to sympathetic excitation.

Earlier studies have suggested that insulin may cause vascular dilation through a variety of mechanisms including: 1) β-adrenergic mechanisms; 2) hyperpolarization of vascular

smooth muscle by stimulation of sodium/potassium pump; 3) increased calcium-ATPase activity and 4) metabolic vasodilation in response to skeletal muscle oxygen consumption[13]. In addition, insulin attenuates vasoconstrictor responses of phenylephrine and angiotensin II[14]. Most recently, vasodilation associated with insulin has been attributed to endothelium-derived factors. In healthy subjects, increases in forearm blood flow caused by hyperinsulinemic clamp are prevented by NG-monomethyl-L-arginine (L-NMMA), a specific inhibitor of the synthesis of endothelial nitric oxide (NO)[15]. This result supports a physiologic role for insulin to regulate skeletal muscle circulation through endothelial mechanisms. This hypothesis is further supported by results showing that the expression of endothelial nitric oxide synthase, the enzyme responsible for endothelial NO synthesis, dose-dependently increases in response to varying concentrations of insulin, in cultured human aortic endothelial cells[16].

Impaired vascular dilatation in response to insulin has been demonstrated in insulin resistance and could potentially contribute to hypertension. In lean and obese subjects, leg blood flow increases during euglycemic, hyperinsulinemic clamp, but the plasma insulin concentration required to achieve maximal vasodilatory responses is about fourfold higher in obese as compared with lean individuals[17]. These results indicate resistance to the vasodilatory action of insulin in human obesity. Impaired endothelial NO response to insulin may underlie endothelial dysfunction of obesity. To support this concept, Steinberg et al.[18] have demonstrated that increases in leg blood flow in response to endothelial-dependent methacholine but not to endothelial-independent nitroprusside are impaired in obese subjects during hyperinsulinemic euglycemic clamp.

Resistance to the vasodilatory effect of insulin has also been observed in hypertension. Supraphysiological insulin plasma concentrations reaching 450 microunits/ml increase skeletal muscle blood flow by 91% in normotensive subjects but only 33% in the hypertensive group[19]. Nevertheless, in borderline hypertensive subjects increasing plasma insulin to 73 microunits/ml with euglycemic, hyperinsulinemic clamp, increased skeletal muscle sympathetic nerve activity by 36% and arterial pressure decreased slightly[20]. This study suggests that, despite mild hypertension, insulin retains some of its vasodilatory actions and does not increase arterial pressure.

Insulin-resistance impairs metabolic and vascular actions of insulin, but spares its sympathoexitatory actions. Given this, we might expect that insulin-induced sympathoactivation and blunted vasodilation to contribute to increased arterial pressure in response to hyperinsulinemia. However insulin-resistance may only partially impair insulin's vascular responses. Thus, even in insulin resistant states, the vasodilatory

action of insulin may oppose the vasoconstrictor stimulus of sympathetic activation and prevent arterial pressure elevation (Figure 1). As a matter of fact, as we will see later, the responses to acute hyperinsulinemia consistently fails to support the hypothesis that hyperinsulinemia promote hypertension.

A second major mechanism associating hyperinsulinemia with obesity-related hypertension is increased renal absorption of sodium. Salt-sensitive hypertension develops in obese rats and hyperinsulinemia may contribute to this condition[21]. In non-obese, normotensive subjects, modest increases in plasma insulin concentration decrease renal sodium excretion by 40%[22]. In hypertensive individuals, the infusion of insulin during euglycemic clamp also causes a reduction of renal excretion of sodium[23].

Insulin may induce long-term vascular hypertrophy by acting as a direct mitogen or by promoting the release of insulin-like growth factor 1 (IGF-1)[24]. As early as 1961, Cruz et al.[25] demonstrated intimal and medial vascular hypertrophy in unilateral

Selective insulin resistance

Insulin

Sympathoactivation

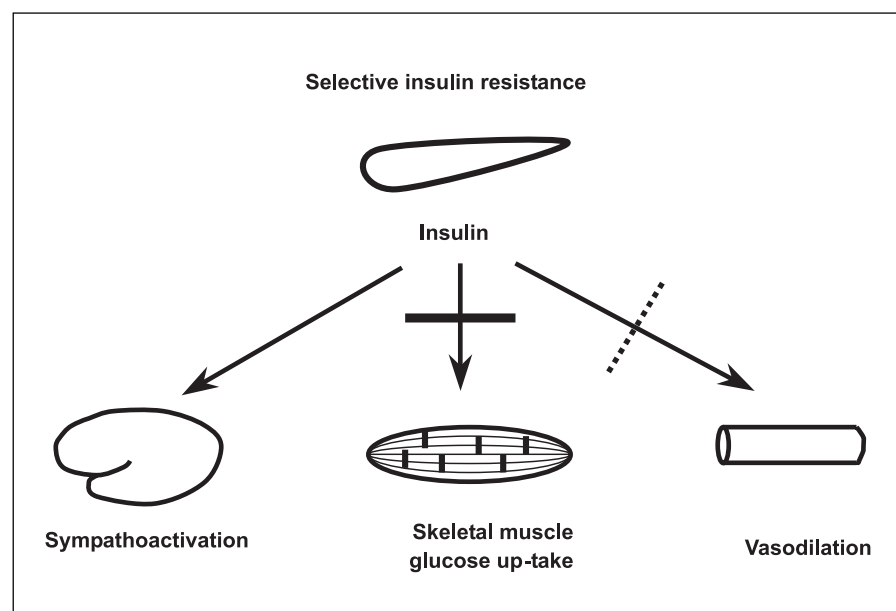Skeletal muscle glucose up-take

Vasodilation

Figure 1 – In selective insulin resistant states, insulin-induced sympathetic activation is preserved, skeletal muscle glucose up-take is substantially inhibited and vasodilation is only partially impaired. Insulin-dependent vasodilation could override sympathetic activation to prevent changes in arterial pressure.

femoral canine arteries chronically perfused with insulin *in vivo*, whereas the non-perfused, contralateral artery remained normal. Vascular remodeling and hypertrophy due to chronic hyperinsulinemia could potentially increase vascular resistance and contribute to obesity-related hypertension.

Despite repeated epidemiological studies linking hypertension, obesity and hyperinsulinemia, this hypothesis has been challenged by some observations in animals and humans. Hall et al. have demonstrated that hyperinsulinemia does not increase arterial pressure in obese dogs that are resistant to the vasodilatory and metabolic actions of insulin[26]. Additionally, neither acute nor chronic hyperinsulinemia has been able to shift the pressure-natriuresis curve in dogs or humans[27]. Moreover, experimental acute hyperinsulinemia failed to increase arterial pressure in obese, insulin-resistant normotensive[28] and hypertensive humans[29]. In obese hypertensives, chronic treatment with NPH insulin significantly raised plasma insulin concentration, but caused a slight decline in 24-hour arterial pressure[30].

Both hyperinsulinemia and obesity increase sympathetic nerve activity to skeletal muscle circulation. However, there might be no direct correlation between insulin-induced sympathoactivation and obesity-related increases in sympathetic activity. Only a weak correlation between fasting insulin and muscle sympathetic nerve activity is observed in obese hypertensive subjects as compared with weight-matched normotensive controls[31]. In addition, baseline muscle sympathetic nerve activity is similar in obese hypertensive and normotensive individuals, suggesting that increased muscle sympathetic nerve activity might not be important for the maintenance of obesity-related increases in arterial pressure[31].

It is well accepted that insulin modulates renal mechanisms of sodium reabsorption and that hyperinsulinemia presumably contributes to sodium retention in obesity. However, other mechanisms have been shown to play a role in obesity-related sodium retention by the kidneys. First, obesity increases plasma renin-angiotensin activity that potentially increases renal sodium reabsorption[32]. Second, accumulation of intrarenal fat and extracellular matrix physically compress the kidneys and cause increases in sodium reabsorption[33]. Thus, the magnitude of the contribution of hyperinsulinemia *per se* to the reduced renal sodium excretion in obesity and obesity-related hypertension is still unclear.

Is there a role for leptin in obesity hypertension?

The discovery of leptin by Jeffrey Friedman's group in 1994[34] revolutionized the understanding of body weight control. Leptin is a peptide hormone mostly expressed in the white adipose tissue that acts on hypothalamic receptors to induce negative energy balance by inhibiting appetite and by promoting sympathetically mediated energy expenditure[35]. In rodents, leptin increases sympathoactivation to interscapular brown adipose tissue and increases the expression of uncoupling protein type 1 (UCP-1), presumably enhancing sympathetically mediated thermogenic metabolism at this organ[36]. Surprisingly, Haynes et al.[37] have shown that intravenous administration of leptin also increases renal, adrenal and hindlimb sympathetic activity that potentially elevates arterial pressure in rats. Conversely, leptin has been associated with effects that could decrease arterial pressure. First, human leptin, but not rodent leptin, increases natriuresis and diuresis in rats[38]. Second, Lembo et al.[39] have demonstrated that leptin causes endothelial-dependent vasodilation, *in vitro*. In addition, leptin

decreased arterial pressure in sympathectomized rats[39]. Fruhbeck[40] has also shown that leptin increases endothelial release of nitric oxide, in rats. Third, leptin decreases rat ventricular myocyte contraction *in vitro*[41]. Fourth, a leptin-dependent angiogenic effect has been demonstrated that potentially decreases peripheral vascular resistance[42]. Fifth, leptin acutely increases insulin sensitivity[43].

Despite potential depressor actions of leptin, most studies directly addressing leptin effects on arterial pressure indicate that a pressor action may predominate. Interestingly, the *ob/ob* obese mouse lacking the *ob* gene responsible for leptin expression has a slightly lower arterial pressure than the lean controls despite extreme obesity[44]. Therefore, the lack of leptin expression appears to alter arterial pressure regulation in mice.

Notably, it has been shown that acute and chronic administration of leptin increases arterial pressure. Acute intracerebroventricular administration of leptin in anesthetized rats has been shown to increase arterial pressure, lumbar and renal sympathetic nerve activity[45], suggesting that the pressor effect of leptin may be mediated by sympathetic mechanisms. Our group was unable to reproduce the pressor effect of leptin after 3 hour-long intravenous infusion of leptin in anesthetized rats[37]. However, we have observed leptin-dependent increases in arterial pressure when our experiments were extended for 6 hours[46].

Shek et al.[47] demonstrated that chronic systemic administration of leptin increases arterial pressure in conscious rats and suggested that this reflects a central neural action of leptin. We have reproduced the pressor effect of leptin by chronically administering the hormone into the third cerebral ventricle of conscious, freely moving rats indicating that leptin-induced increases in arterial pressure are mediated via

central nervous system mechanisms[48]. Importantly, Aizawa-Abe et al.[49] have demonstrated that transgenic mice overexpressing leptin in the liver were hypertensive as compared with wild type controls. Moreover, hypertension in transgenic mice overexpressing leptin was reversed by α and β-adrenergic antagonism and ganglionic blockade. These results strongly suggest that leptin increases arterial pressure in rodents through sympathetic activation.

Being secreted by white adipose tissue, leptin plasma concentration is elevated in human common obesity reflecting augmented adiposity[50]. Thus, it has been postulated that leptin may contribute to obesity-related sympathoactivation and hypertension. In a longitudinal study following a cohort of Japanese men for one year, greater increases of serum leptin due to spontaneous weight gain were observed in the obese subjects who exhibited arterial pressure elevation. These subjects were compared with obese individuals whose arterial pressure remained stable despite similar weight gain[51]. However, at 6 months, arterial pressure elevation following weight gain correlated only with serum norepinephrine whereas at 12 months, the correlation was positive with serum norepinephrine, leptin and insulin. Thus, it appears that sympathetic activation as reflected by increased serum norepinephrine is an initial and lasting determinant of increased arterial pressure that follows increased adiposity, while serum leptin and insulin correlate with a more chronic phase of obesity-related arterial pressure elevation[51].

Leptin has also been associated with hypertension independent of obesity. Several cross-sectional studies have demonstrated that plasma leptin concentration corrected for body mass index is higher in hypertensive than in normotensive males. Such observation has not been shown in women, despite consistent higher body mass index-corrected serum leptin in females[52,53]. These results suggest an association of leptin and hypertension with male gender. In contrast, Suter et al.[54] have found that systolic arterial pressure correlated with leptin serum levels adjusted for body weight in women and non-hypertensive males. In concordance with other reports, hypertensive subjects irrespective of gender presented higher serum leptin concentrations than normotensive controls[54].

A direct role of leptin in the pathogenesis of essential or obesity-related hypertension presumes some sort of leptin action on systems that regulate arterial pressure. However, in addition to insulin resistance, obese subjects are also leptin-resistant[50]. Leptin resistance is defined as an inability of leptin to promote energy expenditure and to inhibit appetite, favoring weight gain. If obese individuals are resistant to the effects of leptin, how could leptin possibly promote sympathetic activation and obesity-related hypertension? Some results in the literature suggest that leptin resistance may not be complete, but instead may spare some effects of leptin. For instance, a reduction in arterial pressure parallels a reduction in leptinemia in hypertensive, leptin-resistant agouti yellow obese mice, suggesting that arterial pressure is under leptin influence despite leptin-resistance[49].

Taking into consideration this interesting report, it is conceivable that some other actions of leptin might be preserved, even in the presence of leptin resistance. Therefore, we have proposed the novel concept that leptin resistance can be selective to the metabolic effects of the hormone but spare the sympathoexitatory actions of leptin. We used the agouti yellow obese mouse to test this hypothesis. The agouti yellow obese mouse ubiquitously expresses the agouti peptide that is an endogenous antagonist of melanocortin receptors (MC receptors). Normally, α-melanocyte stimulating hormone (α-MSH) acts on MC type 1 receptors located on hair follicles to produce black hair pigmentation, and on hypothalamic MC type 4 receptors to inhibit appetite along with promotion of energy expenditure. Therefore, the antagonism of MC type 1 and 4 receptors by agouti protein produces yellow coat and late onset obesity in the agouti yellow obese mouse, respectively[55].

Why have we chosen the agouti yellow obese mouse to test the hypothesis that leptin resistance is selective to the metabolic actions of leptin? First, the agouti yellow mouse is hyperleptinemic, reflecting obesity, and does not respond to the leaning and anorectic effects of leptin[56]. Thus, this murine model of obesity can be categorized as a leptin resistant. Second, we have shown that the yellow obese is hypertensive as compared with wild type controls[44].

We reproduced previous results by demonstrating that agouti yellow obese mice have limited food intake inhibition in response to systemic administration of leptin, when compared with wild type, lean controls. However, systemic administration of leptin increased renal sympathetic nerve activity equally in agouti obese and lean mice (Figure 2)[57]. These results indicate that, despite resistance to the anorectic effect of leptin, agouti yellow obese mice retain the leptin-dependent sympathoexitatory response. Therefore, leptin resistance appears to be selective to the metabolic but not to the sympathetic actions of leptin in this murine model of obesity-related hypertension (Figure 3).

It can be argued that systemic administration of leptin activates leptin receptors peripherally located in the white adipose tissue to induce sympathoactivation and overcome leptin resistance[58]. To elucidate this

issue, we conducted experiments to test the hypothesis that selective leptin resistance originates in the central nervous system. Rahmouni et al.[59] have preliminary results showing that direct intracerebroventricular administration of leptin does not inhibit food intake in the agouti yellow obese mice, but the sympathoexcitatory effect of leptin to the kidneys is preserved. These results support the hypothesis that selective leptin resistance develops through mechanisms located in the central nervous system.

To our best knowledge, leptin has been used three times in clinical research. The first study presented a weight reductive effect of leptin in a morbidly obese child with congenital leptin deficiency[60]. In the second study, patients with common obesity were treated with leptin to promote weight loss[61]. In none of these studies, the sympathetic or arterial pressure effects of leptin were thoroughly examined. In the third study, healthy non-obese subjects treated systemically with leptin did not show any evidence of leptin-induced sympathoactivation. Despite reaching high plasma concentrations, leptin did not have any effect on satiety or metabolic rate[62], suggesting that leptin may not have reached effector sites. Thus, the hypothesis that leptin and hyperleptinemia causes obesity-related sympathoactivation is yet to be tested in humans.

Conclusion

Evidence has accumulated that challenge the classical concept linking hyperinsulinemia, hypertension and obesity. Acute hyperinsulinemia in humans has repeatedly failed to produce hypertension. Consequently, whereas insulin resistance may contribute to vascular dysfunction, hyperinsulinemia does not appear to play an important role in obesity hypertension, despite insulin sympathetic and anti-natriuretic effects.
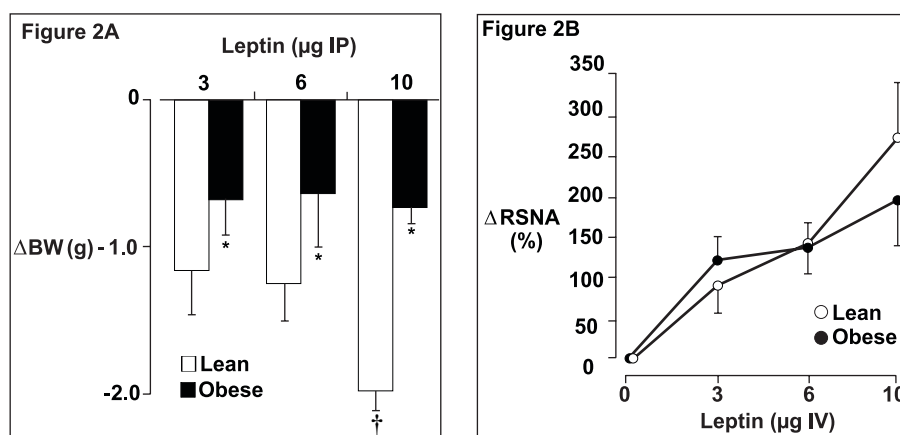

Figure 2 – (A) Absolute changes in body weight (BW) induced by leptin administered intraperitoneally (IP). Leptin causes dose-dependent weight loss in wild type, agouti lean mice. Agouti obese mice are resistant to weight loss produced by leptin. * $P < 0.05$ obese *versus* lean, † $P < 0.05$ lean 3 & 6 μg *versus* lean 10 μg. **(B)** Percent changes in renal sympathetic nerve activity (RSNA) induced by leptin administered intravenously (IV). Leptin causes dose-dependent increases in RSNA that are equal in agouti lean and obese mice.
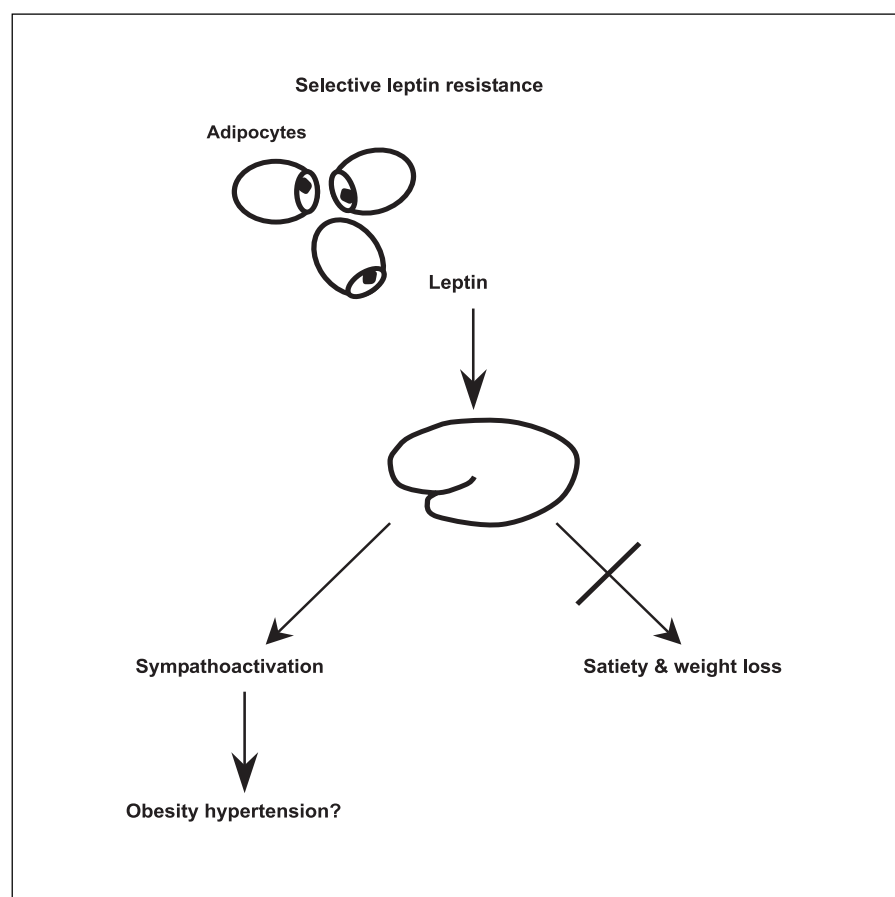

Figure 3 – In selective leptin resistant states, sympathoactivation caused by leptin is preserved but leptin effects on metabolism (satiety and weight loss) are substantially inhibited. Leptin-induced sympathoactivation could contribute to obesity-related hypertension.

The discovery of the adipose tissue-derived leptin has led to alternative explanations of obesity-related hypertension. It is true that leptin has potential depressor effects but most reports have shown that leptin increases arterial pressure in rodents probably due to sympathetic mechanisms. Given that human obesity-related hypertension is associated with increased sympathetic drive, it is conceivable that hyperleptinemia in human obesity contributes to the activation of the sympathetic nervous system. Nevertheless, human obesity is a partial leptin resistant condition. The novel concept of selective leptin resistance may help explain leptin-induced sympathoactivation in obese subjects resistant to the metabolic effects of leptin. At present, this novel concept may be useful for the understanding of hypertension in murine models of obesity. Unequivocal evidence of leptin-induced sympathoactivation in human obesity has not been demonstrated yet. However, research with these exotic rodent models of obesity and hypertension may indicate promising directions to be pursued by clinical physiologists.

References

1. Garrison RJ, Kannel WB, Stokes J, Castelli WP. Incidence and precursors of hypertension in young adults: the Framinghan offspring study. *Prev Med* 16: 234-51, 1987.

2. Najjar M, Rowland M. Anthropometric reference data and prevalence of overweight: United States, 1976-80. Washington DC, US Government Printing Office, Department of Health and Human Services Publication (PHS) 87-1688, 1987.

3. Guimarães AC. Sobrepeso e obesidade: fatores de risco cardiovascular (aspectos clínicos e epidemiológicos). *Rev Soc Bras Hipertens* 4(3): 94-7, 2001.

4. Kissebah AH, Krakower GR. Regional adiposity and morbidity. *Physiol Rev* 11: 255-61, 1994.

5. Reaven GM. Banting lecture 1988: role of insulin resistance in human disease. *Diabetes* 37: 1595-607, 1988.

6. Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. Body mass index and the prevalence of hypertension and dyslipidemia. *Obes Res* 8: 605-19, 2000.

7. Kroke A, Bergman M, Klipstein-Grobusch K, Boeing H. Obesity, body fat distribution and body built: their relation to blood pressure and prevalence of hypertension. *Int J Obes Relat Metab Disord* 22: 1062-70, 1998.

8. Feskens EJ, Tuomilehto J, Stengard JH, Pekkanen J, Nissinen A, Kromhout D. Hypertension and overweight associated with hyperinsulinaemia and glucose tolerance: a longitudinal study with Finnish and Dutch cohorts of the Seven Countries Study. *Diabetologia* 38: 839-47, 1995.

9. Grassi G, Seravalle G, Cattaneo BM, Bolla GB, Lanfranchi A, Colombo M, Giannattasio C, Brunani A, Cavagnini F, Mancia G. Sympathetic activation in obese normotensive subjects. *Hypertension* 25: 560-3, 1995.

10. O'Hare JA. The enigma of insulin resistance and hypertension: insulin resistance, blood pressure and the circulation. *Am J Med* 84: 505-1, 1988.

11. DeFronzo RA. Insulin resistance, hyperinsulinemia, and coronary artery disease: a complex metabolic web. *J Cardiovasc Pharmacol* 20 (suppl 11): S1-S16, 1992.

12. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87: 2246-52, 1991.

13. Anderson EA, Mark AL. The vasodilator action of insulin. Implications for the insulin hypothesis of hypertension. *Hypertension* 21: 136-41, 1993.

14. Sakai K, Imaizumi T, Masaki H, Takeshita A. Intra-arterial infusion of insulin attenuates vasoreactivity in human forearm. *Hypertension* 22: 67-73, 1993.

15. Scherrer U, Randin D, Vollenweider P, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94: 2511-5, 1994.

16. Aljada A, Dandona P. Effect of insulin on human aortic endothelial nitric oxide synthase. *Metabolism* 49: 147-50, 2000.

17. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest* 85: 1844-52, 1990.

18. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J Clin Invest* 97: 2601-10, 1996.

19. Laine L, Knuuti MJ, Ruotsalainen U, Raitakari M, Iida H, Kapanen J, Kirvela O, Haaparanta M, Yki-Jarvinen H, Nuutila P. Insulin resistance in essential hypertension is characterized by impaired insulin stimulation of blood flow in skeletal muscle. *J Hypertens* 16: 211-9, 1998.

20. Anderson EA, Balon TW, Hoffman RP, Sinkey CA, Mark AL. Insulin increases sympathetic activity but not blood pressure in borderline hypertensive humans. *Hypertension* 19: 621-7, 1992.

21. Suzuki H, Ikenaga H, Hayashida T, Otsuka K, Kanno Y, Ohno Y, Ikeda H, Saruta T. Sodium balance and hypertension in obese and fatty rats. *Kidney Int* 55: S150-S153, 1996.

22. DeFronzo RA, Cooke CR, Andres R, Faloona GR, Davis PJ. The effect of insulin on renal handling of sodium, potassium, calcium and phosphate in man. *J Clin Invest* 55: 845-55, 1975.

23. Muscelli E, Natali A, Bianchi S, Bigazzi R, Galvan AQ, Sironi AM, Frascerra S, Ciociaro D, Ferrannini E. Effects of insulin on renal sodium and uric acid handling in essential hypertension. *Am J Hypertens* 9: 746-52, 1996.

24. Froesh ER, Schmid C, Schwander J, Zapf J. Actions of insulin-like growth factors. *Ann Rev Physiol* 47: 443-67, 1985.

25. Cruz AB, Amatuzio DS, Grande F, Hay LJ. Effect of intraarterial insulin on tissue cholesterol and fatty acids in alloxan-diabetic dogs. *Circ Res* 9: 39-43, 1961.

26. Hall JE, Brands MW, Zappe DH, Dixon WN, Mizelle HL, Reinhart GA, Hildebrandt DA. Hemodynamic and renal responses to chronic hyperinsulinemia in obese, insulin resistant dogs. *Hypertension* 25: 994-1002, 1995.

27. Hall JE. Hyperinsulinemia: a link between obesity and hypertension? *Kidney Int* 43: 1402-17, 1993.

28. Baron AD, Brechtel G. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol* 265: E61-67, 1993.

29. Rocchini AP. Insulin resistance and blood pressure regulation in obese and nonobese subjects. *Hypertension* 17: 837-42, 1991.

30. Heise T, Magnusson K, Heinemann L, Sawicki PT. Insulin resistance and the effect of insulin on blood pressure in essential hypertension. *Hypertension* 32: 243-8, 1998.

31. Gudbjornsdottir S, Lonnroth P, Sverrisdottir YB, Wallin BG, Elam M. Sympathetic nerve activity and insulin in obese normotensive and hypertensive men. *Hypertension* 27: 276-80, 1996.

32. Tuck ML, Sowers J, Dornfield L, Kledzik G, Maxwell M. The effect of weight reduction on blood pressure, plasma renin activity, and plasma aldosterone levels in obese patients. *N Engl J Med* 304: 930-3, 1981.

33. Hall JE, Brands MW, Henegar JR. Mechanisms of hypertension and kidney disease in obesity. *Ann N Y Acad Sci* 892: 91-107, 1999.

34. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 372: 425-32, 1994.

35. Campfield LA, Smith FJ, Brun P. The OB protein (leptin): a link between adipose tissue mass and central neural networks. *Horm Metab Res* 28: 619-32, 1996.

36. Haynes WG, Sivitz WI, Morgan DA, Walsh SA, Mark AL. Sympathetic and cardiorenal actions of leptin. *Hypertension* 30: 619-23, 1997.

37. Haynes WG, Morgan DA, Walsh SA, Mark AL, Sivitz WI. Receptor-mediated regional sympathetic nerve activation by leptin. *J Clin Invest* 100: 270-8, 1997.

38. Jackson EK, Herzer WA. A comparison of natriuretic/diuretic effects of rat vs. human leptin in the rat. *Am J Physiol* 277: F761-F765, 1999.

39. Lembo G, Vecchione C, Fratta L, Marino G, Trimarco V, d'Amati G, Trimarco B. Leptin induces direct vasodilation through distinct endothelial mechanisms. *Diabetes* 49: 293-7, 2000.

40. Fruhbeck G. Pivotal role of nitric oxide in control of blood pressure after leptin administration. *Diabetes* 48: 903-8, 1999.

41. Nickola MW, Wold LE, Colligan PB, Wang GJ, Samson WK, Ren J. Leptin attenuates cardiac contraction in rat ventricular myocytes. Role of NO. *Hypertension* 36: 501-5, 2000.

42. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science* 281: 1683-6, 1998.

43. Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG. Effects of leptin on insulin sensitivity in normal rats *Endocrinology* 138: 3395-401, 1997.

44. Mark AL, Shaffer RA, Correia MLG, Morgan DA, Sigmund CD, Haynes WG. Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *J Hypertens* 17: 1949-53, 1999.

45. Dunbar JC, Hu Y, Lu H. Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. *Diabetes* 46: 2040-3, 1997.

46. Correia MLG, Morgan DA, Mitchell JL, Sivitz WI, Mark AL, Haynes WG. Role of corticotrophin-releasing factor in effects of leptin on sympathetic nerve activity and arterial pressure. *Hypertension* 38: 384-8, 2001.

47. Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. *Hypertension* 31: 409-14, 1998.

48. Correia MLG, Morgan DA, Sivitz WI, Mark AL, Haynes WG. Leptin acts in the central nervous system to produce dose-dependent changes in arterial pressure. *Hypertension* 37: 936-42, 2001.

49. Aizawa-Abe M, Ogawa Y, Masuzaki H, Ebihara K, Satoh N, Iwai H, Matsuoka N, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Nakao K. Pathophysiological role of leptin in obesity-related hypertension. *J Clin Invest* 105: 1243-52, 2000.

50. Chu NF, Spiegelman D, Yu J, Rifai N, Hotamisligil GS, Rimm EB. Plasma leptin concentrations and four-year weight gain among US men. *Int J Obes Relat Metab Disord* 25: 346-53, 2001.

51. Masuo K, Mikami H, Ogihara T, Tuck ML. Weight gain-induced blood pressure elevations. *Hypertension* 35: 1135-40, 2000.

52. Sheu WH, Lee WJ, Chen YT. High plasma leptin concentrations in hypertensive men but not in hypertensive women. *J Hypertens* 17: 1289-95, 1999.

53. Mallamaci F, Cuzzola F, Tripepe G, Cutrupi S, Parlongo S, Tripepi R, Zoccali C. Gender-dependent differences in plasma leptin in essential hypertension. *Am J Hypertens* 13: 914-20, 2000.

54. Suter PM, Locher R, Hasler E, Vetter W. Is there a role for the ob gene product leptin in essential hypertension? *Am J Hypertens* 11: 1305-11, 1998.

55. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and agouti obesity syndrome. *Nature* 385: 165-8, 1997.

56. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton D, Friedman J. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci* 94: 8878-83, 1997.

57. Correia MLG, Haynes WG, Rahmouni K, Morgan D, Sivitz WI, Mark AL. The concept of selective leptin resistance: evidence from agouti yellow obese mice. *Diabetes*. In Press, 2002.

58. Niijima A. Reflex effects from leptin sensors in the white adipose tissue of the epididymis to the efferent activity of the sympathetic and vagus nerve in the rat. *Neurosci Lett* 262: 125-8, 1999.

59. Rahmouni K, Haynes WG, Morgan DA, Mark AL. Selective resistance to central neural administration of leptin in agouti obese mice. *Hypertension* 38: 506 (abstract), 2001.

60. Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. *N Engl J Med* 341: 879-84, 1999.

61. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalating trial. *JAMA* 282: 1568-75, 1999.

62. Mackintosh RM, Hirsch J. The effects of leptin administration in non-obese human subjects. *Obes Res* 9: 462-9, 2001.