Role of Leptin in Cardiovascular Disease

Introduction:

Cardiovascular disease (CVD) has an enormous impact on global health, particularly in developed nations like the United States. CVDs currently afflict more than 70 million Americans.[1] In addition, nearly 910,000 Americans die of such diseases every year.[1] These diseases cumulatively result in over 6 million hospitalizations yearly and over $403 billion in health care expenditures and lost productivity.[1] Thus, the need to develop new treatments to quash CVDs is great. One way to prevent cardiovascular disease is to reduce risk factors leading to onset of the disease. The development of cholesterol-lowering statins and anti-hypertensive angiotensin-converting-enzyme inhibitors have been important steps toward eradicating CVDs. Though numerous pharmacologic agents exist to reduce risk factors, the identification of novel risk factors and their stimulatory conditions could lead to more effective treatments. Current examinations of critical factors in CVD development often converge on leptin. Worldwide research has recently elucidated the significance of this small molecule in CVD progression through widespread and dynamic mechanisms. A review of current findings on leptin's role in CVD will be at the center of this report.

The Biology of Leptin:

The Hormone:

Leptin is a 16-kDa peptide hormone product of the obese (Ob) gene secreted primarily by white adipocytes (Figure 1).[2] This pleiotropic hormone has been shown to impact regulation of food intake, energy expenditure, and reproduction in mammals.[3] Leptin functions as a hormonal mechanism for sensing fat deposition, whereby greater adipocyte density and size yields more leptin which subsequently curbs apetite and increases energy expenditure.[3] Leptin synthesis is under further regulatory control of factors such as glucocorticoids, insulin, and melanin-concentrating hormone.[4,5] Research into leptin's role in obesity and energy metabolism ultimately led to the discovery of its importance in cardiovascular disease.

The Receptor:

As a peptide hormone, leptin exerts its physiologic effects through a cell-surface receptor. At least six isoforms of the Ob receptor exist, with only one (Ob-Rb) capable of undergoing a fully functional response to stimulation.[6] The Ob receptor is a transmembrane protein sharing structural similarity to gp130 receptors utilized by various cytokines.[7] Chemical cross linking studies demonstrated that Ob-Rb homodimerizes following leptin stimulation.[8] Once triggered, this complex is capable of activating several intracellular signaling pathways via cytoplasmic box motifs present in the receptor's amino acid structure. Leptin utilizes JAK2 kinase activity to modulate its effects in different pathways including JAK/STAT, MAPK, and PI3K (Figure 2).[7,8] These intracellular signal cascades often induce transcription that leads to regulation of the anorexigenic and other effects of leptin.[7] Actions mediated by the leptin receptor occur throughout organisms spanning from the CNS to peripheral tissues with Ob-Rb identified in lung, kidney, testis, and skeletal muscle tissue.[9] With such widespread receptor distribution, new functions of leptin are being discovered frequently.

Role in Cardiovascular Disease:

Clinical research has demonstrated an association between elevated plasma leptin levels and cardiovascular complications in humans. One such study examined moderately hypercholesterolemic men with no history of myocardial infarction and/or coronary artery disease in an effort to identify new risk factors for CVD.[10] This study discovered a statistically significant relationship between the likelihood of a coronary event (defined as myocardial infarction and need for coronary revascularization) and circulating leptin levels. When controlling for prominent risk factors such as age, lipids, and systolic blood pressure, for each 1 SD increase in circulating leptin concentration the relative risk of a cardiovascular event increased by 1.20. This preliminary study suggested a correlation between high leptin levels and cardiovascular disease that warranted further studies into the potential role of leptin in cardiovascular disease.

Experiments conducted at the University of Michigan directly implicated leptin in atherosclerosis and thrombosis.[11] In these studies, APOE -/- mice (prone to developing

atherosclerotic plaques) were subjected to chronic elevated leptin levels through daily injections of recombinant murine leptin. Following a 4 week injection period, mice were examined to determine total atherosclerotic lesion as well as the time to vessel occlusion as a measure of atherosclerosis and thrombosis. Atherogenesis measurements revealed significantly greater atherosclerotic lesion and aortic intima thickness in mice that received leptin relative to vehicle, corresponding to increased severity of disease. Mice that received leptin treatment also achieved occlusive thrombus due to photochemical injury significantly faster than vehicle. Minimal changes in total cholesterol and triglycerides revealed a potentially direct effect of leptin on disease progression. These experiments clearly implicate leptin in cardiovascular disease development.

A closer examination of the central and peripheral effects of leptin will clarify its observed role in CVD progression.

Hypertension and Tachycardia

Elevated blood pressure, or hypertension, is diagnosed in humans with a systolic blood pressure of over 140 mm Hg and a diastolic blood pressure of over 90 mm Hg. Though no perceptible symptoms are usually present, secondary complications of hypertension can be severe. Secondary conditions such as myocardial infarction, hypertensive cardiomyopathy, and hypertensive retinopathy often occur. A Japanese investigation into the potential role of leptin in development of hypertension revealed a statistically significant correlation between circulating leptin levels and incidence of hypertension.[12] The study examined adolescents and excluded individuals with prior metabolic or cardiovascular disorders. After adjustment for age and BMI, researchers found that elevated leptin levels correlated significantly with increased blood pressure in obese individuals. These results suggested a possible role of leptin in hypertension.

The development of transgenic “skinny” mice that overexpress leptin under the control of a liver-specific promoter provided a new experimental model to examine leptin’s role in hypertension without the confounding variable of obesity.[13] The power of this model stems from its ability to achieve chronically high leptin levels without high amounts of adipose tissue. Plasma leptin concentrations in the transgenic mice used in

the experiment were 60.6 ± 14.4 ng/mL, approximately 10-fold higher than those in nontransgenic littermates (4.8 ± 0.6ng/mL). Tail-cuff systolic BP measurements revealed significantly elevated BP in transgenic skinny mice compared with nontransgenic littermates (120.0 ± 1.6 vs.102.7 ± 2.7 mmHg; $n = 12$; $P < 0.001$). Further analysis revealed roughly 3 fold higher epinephrine and norepinephrine levels in urine of transgenic vs. nontransgenic mice with comparable total urine output. This data suggested a leptin-mediated chronic sympathetic activation, which may have led to the observed hypertension in these mice. The investigators tested this theory though a sympathetic blockade of α_1-adrenergic, β-adrenergic, or ganglionic blockers. This blockade effectively abrogated the observed hypertension of the skinny mice and had no effect on the blood pressure of nontransgenic mice. This result suggests that the observed leptin-mediated hypertension does rely on sympathetic activation (Figure 3). Additional studies examining the importance of sympathetic activation on leptin-mediated cardiovascular effects through direct IV leptin injections on rats revealed tachycardia as an additional effect of leptin.[14] This research group was able to reduce this observed elevated heart rate with an adrenergic blockade that suggested that leptin mediates tachycardia through sympathetic activation as well.

Leptin may also contribute to hypertension through its effects on circulating white blood cells. Flow cytometric analysis of blood samples from healthy human subjects with an anti-leptin receptor mAb showed that 25 +/- 5 % of monocytes, 12 +/- 4% of polymorphonuclear cells, and 5 +/- 1% of lymphocytes express the Ob-R.[15] This expression pattern would allow leptin to mediate a robust effect on white blood cells. Researchers observed leptin's ability to stimulate cytokine production through experiments on peripheral blood mononuclear cells (PBMC).[15] Leptin treatments of 50-1000 ng/ml on these cells significantly induced the production of IL-6 and TNF-alpha as observed through flow cytometric measurements. Leptin's ability to stimulate cytokine production may be important to hypertension because several studies have demonstrated that increased levels of these cytokines stimulates hypertension.[16] This data demonstrates the dynamic effects of leptin and its ability to affect CVD in multiple ways through indirect effects on different tissues.

Atherosclerosis

Atherosclerosis is the process of accumulating fatty substance, cholesterol, cellular waste, calcium and fibrin on the inner lining of arteries. These vessel-narrowing deposits, called plaques, can be compensated for by artery enlargement, but inevitably lead to plaque ruptures and arterial stenosis. Many factors contribute to atherogenesis including smooth muscle cell proliferation and migration, neovascularization, and calcification of vascular cells. Leptin has been shown to play a key role in each of the aforementioned processes involved in atherogenesis.

Arterial intima thickening observed in atherosclerosis has been attributed in part to proliferation of vascular smooth muscle cells (VSMC) that migrate into the intima of the artery. Leptin has been shown to increase both VSMC proliferation and migration. A study to determine leptin's effects on these processes treated VSMC isolated from rat aorta with several doses of leptin in different assays.[17] Researchers observed an approximately 20% increase of maximal cell number in VSMC cultures treated with leptin (100ng/ml) vs. vehicle over a three day time course. A chemotaxis assay that examined the propensity of VSMC to migrate towards a chemoattractant solution of leptin through a microchemotaxis chamber revealed that 3 fold more cells were able to migrate through a polycarbonate membrane following a leptin trail than with no leptin trail. The use of specific inhibitors of PI3K pathway (wortmannin and LY 294002) showed that this pathway was critical for the migratory effect of leptin. Several other reports suggested that leptin mediates proliferation through the MAPK pathway, though it was not explicitly examined in this publication. These results clearly describe leptin's ability to stimulate VSMC proliferation and migration.

Studies have shown neovascularization within atherosclerotic plaque to be critical to plaque formation.[21] Neovascularization may act as a gateway for leukocyte entry into sites of chronic inflammation in atherosclerotic plaques. Angiogenisis inhibitors were found to inhibit both atherosclerosis and macrophage infiltration into plaques.[21] Other complications of angiogenesis occur primarily because arterial walls are normally free of microvessels except in the atherosclerotic plaques where there are dense networks of capillaries known as the vasa vasorum. These fragile vessels can cause hemorrhages that

can significantly impair cardiac function. Leptin's ability to stimulate neovascularization was demonstrated both in vivo and in vitro by a group of researchers.[18] Leptin-infused matrigel was used to plate human umbilical vein endothelial cells (HUVECS), which were then cultured in serum free media and examined qualitatively four hours after plating. The HUVECs cultured on matrigel containing 100 ng/ml of leptin formed capillary like structures that were not present in control groups plated without leptin. This induced structure corresponds to leptin's ability to promote neovascularization within atherosclerotic plaques. In vivo studies of leptin's effects were achieved through implantation of a pellet composed of hydratable hydrogel and PBS or 125 ng of leptin into a corneal pocket of rat eyes. Examination of these eyes seven days following implantation showed that the leptin pellet promoted substantial new vessel growth within the eye (Figure 4). Exploration into the mechanism of leptin-mediated vascularization revealed an upregulation in VEGF in leptin treated HUVECS. Experiments also revealed increased production of the matrix metalloproteinases MMP-2, MMP-9, and TIMP-1 in response to leptin. VEGF and the MMPs have previously been described as important factors in initiating angiogenesis through tissue remodeling and endothelial cell proliferation. Histological examination of atherosclerotic plaque revealed the presence of OB-Rb, VEGFR-1, and MMP activity. Thus, leptin's ability to upregulate VEGF and MMP is likely responsible for its effects on angiogenesis within atherosclerotic plaques.

Vascular calcification is an actively regulated process involving calcifying vascular cells (CVC) that reside in the arterial walls and undergo osteoblastic differentiation and form hydroxyapatite mineral. This calcification corresponds to severity of atherosclerosis and may play a role in plaque instability. One group of researchers showed leptin's ability to facilitate this calcification in CVC cells.[19] Alkaline phosphatase is a marker of osteoblastic differentiation in CVC that mediates the differentiation process and provides phosphate ions for calcium phosphate mineral formation. Treatment of CVC with leptin for 4 days caused a dose dependent 5-10 fold induction of alkaline phosphatase activity as measured by a cell-associated alkaline phosphatase activity assay. This suggests an ability of leptin to prepare these cells for osteoblastic differentiation. In another study, a ^{45}Ca incorporation assay showed leptin's

stimulatory effect on calcium uptake into CVC. Taken together, these data suggest a predominant role of leptin in vascular calcification.

Thrombosis

Thrombosis is the formation of a clot within a blood vessel that obstructs the flow of blood through the circulatory system. Thrombi normally form following injury to the vessel's wall and stagnation of blood flow past the point of injury. Abnormalities in coagulation are also responsible for thrombus formation. Thrombosis is a particularly dangerous CVD because it can lead to ischemia that can severely damage tissue. Thrombi also can detach and enter systemic circulation as an embolus that can obstruct critical vessels near the heart or brain. Leptin's pro-thrombotic effects described previously in this review are likely due to its role in promoting aggregation of platelets. The presence of the Ob-Rb on platelets suggested that it may play a direct role in aggregation. To test this theory investigators studied the effects of leptin on platelet aggregation in vitro.[20] Investigators used Platelet Rich Plasma (PRP) isolated from murine blood. These isolated platelets were dosed with leptin (100ng/ml) and analyzed for platelet aggregation with a microplate reader at 560-nm wavelength. Higher transmittance corresponded to increased platelet aggregation. Though leptin alone did not have any effect on platelet aggregation, leptin significantly enhanced Adenosine diphosphate- (a known platelet agonist) induced aggregation (transmittance of leptin-treated 34.5% ± 0.6% vs. vehicle-treated 17.9% ± 0.2% ; $P < 0.001$). To ensure that this was a specific effect of leptin, the investigators examined platelets with a non-functional leptin receptor (db/db). Results of their experiments revealed no leptin enhanced platelet aggregation with db/db cells, and confirmed that it was in fact a leptin receptor mediated action. Overall, these results demonstrate the specific effect of leptin in enhancing platelet aggregation.

Conclusion:

Leptin plays a significant role in many processes related to the development of CVD. Though it appears to be an important factor in disease progression, treatment options related to significantly decreasing leptin levels could be problematic. Since leptin plays a critical role in normal physiologic function, antibody treatments against leptin could be more harmful than beneficial. Significant research must still be performed into testing the viability of leptin as a therapeutic target. One potent way to reduce leptin levels and consequently risk for CVD, however, is to reduce adipose tissue. Thus, research on leptin's role in CVD should act as an impetus towards increasing education regarding proper diet and exercise.

References

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Figures

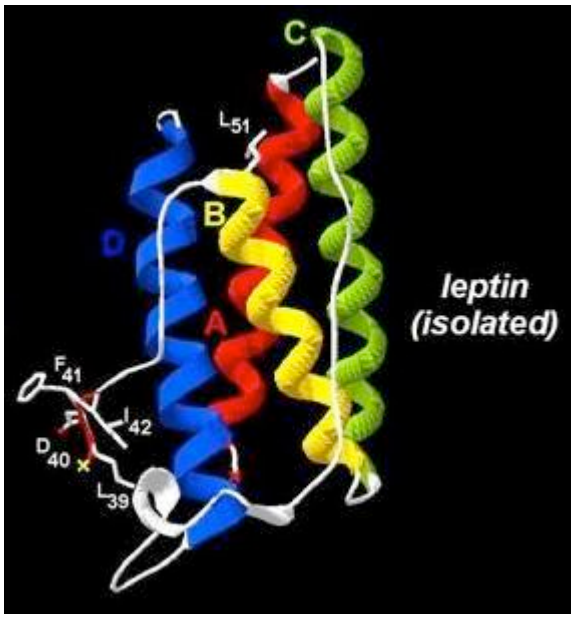

Figure 1.
Representation of three dimensional structure of the four-helix bundle leptin molecule. Leptin has 146 amino acids with a molecular weight of approximately 16 kDa.

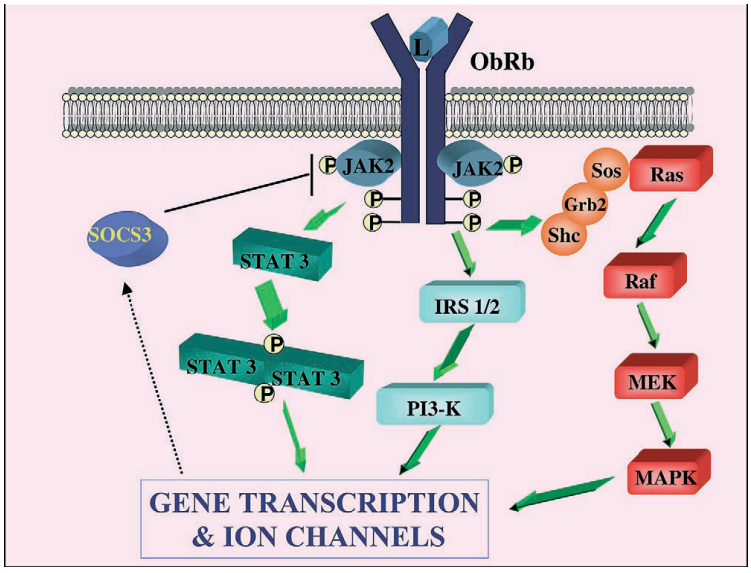

Figure 2.
Representation of the leptin receptor (Ob-Rb) and the signaling pathways activated following leptin stimulation.

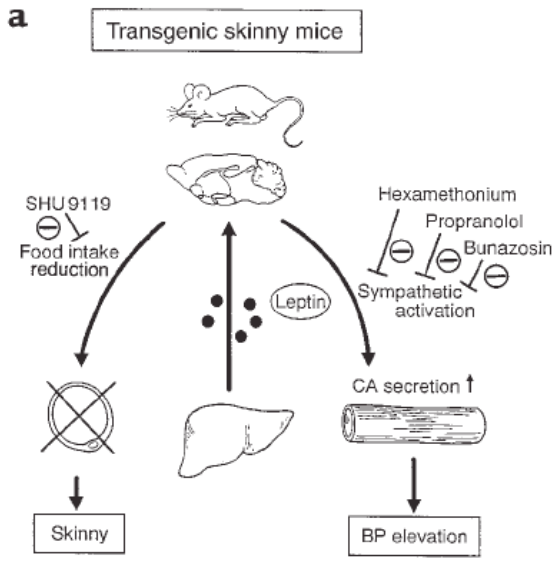

Figure 3.
Depiction of leptin-mediated increase in blood pressure.

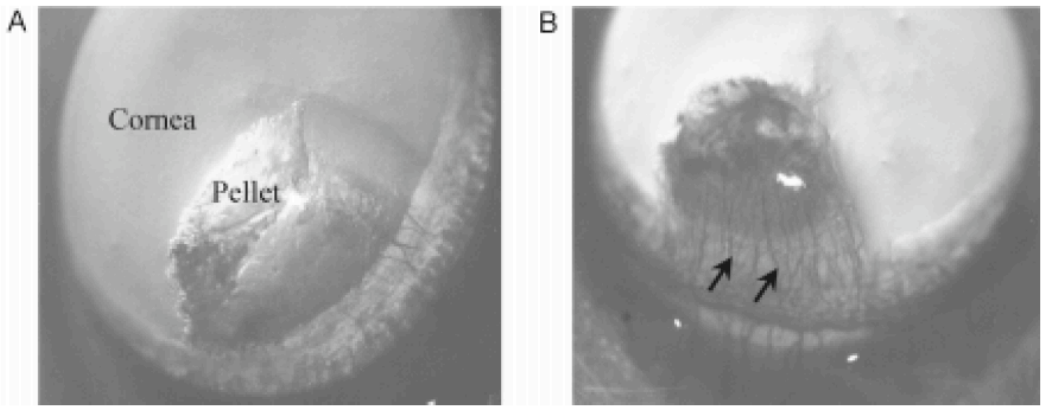

Figure 4.
In vivo angiogenic activity of leptin. Corneal response 7 days after implantation of a pellet containing PBS (A) or 125 ng of leptin (B). Arrow
indicates neovessel development.