REVIEW ARTICLE

Leptin as a cardiovascular risk factor

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Abstract

The role of leptin in humans is not yet precisely established. Nevertheless there is increasing evidence revealing that this molecule is involved in the pathogenesis of atherosclerosis as an independent risk factor. From another point of view, however, leptin is allready related to known traditional risk factors for accelerated atherogenesis, like obesity.

We herein provide the experimental and clinical data concerning the association between leptin and atherosclerotic disease. Vascular stiffness and calcification, immune responce regulation, fibrinolysis, and oxidative stress, are the main fields to be investigated in relation to leptin in the present study.

Additionally the discription of the main characteristics of leptin and its receptors is included in the introduction of this article, whereas in the end the main clinical data suggesting that this molecule represents an interesting risk factor for atherosclerotic disease are provided. *Hippokratia 2007; 11 (4): 163-170*

Key words: *leptin, cardiovascular events, obesity, coronary heart disease, atherosclerosis*

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Atherosclerotic complications are the leading cause of morbidity and mortality in modern, industrialized societies. Obesity has been characterised as an independent risk factor of atherosclerotic vascular disease. Leptin, an interesting hormone produced by adipocytes and involved in food intake regulation and energy expenditure, increases with obesity. Additionally, many other risk factors for cardiovascular disease have been associated with leptin. The aim of this study is to provide evidence about leptin as a new and independent risk factor for atherosclerotic vascular disease.

Leptin and its receptors

Leptin a 16kDa protein consisting of 167 amino acids is mainly synthesized by adipose tissue in proportion to adipose tissue mass. Although initially considered as a satiety factor leptin is a pleiotropic molecule. Apart from playing a role in energy regulation, this hormone also deserves immune properties while its structure and that of its receptor suggest that leptin might be classified as a cytokine[1] (Figures 1,2).

Leptin was originally identified in 1994 by Friedman and considered to be the gene defect product that was responsible for the obesity syndrome in ob/ob (obese/obese) mice. The gene defect of ob/ob mice which was described in 1950s was considered as the spontaneous mutation that can cause a severe obese phenotype through overeating and decreased energy expenditure.

The gene was defined as ob, it is expressed exclusively in adipocytes[2], and the obese mice carrying the mutation were called ob/ob mice (homozygous for a spontaneous mutation in the ob gene[3]). The ob gene is located on chromosome 7 in humans. Leptin, the protein encoded by the ob gene, comes from the Greek word leptos, which means thin. A defect in leptin rendered to overeating and obesity, suggesting that leptin was a satiety factor[1].

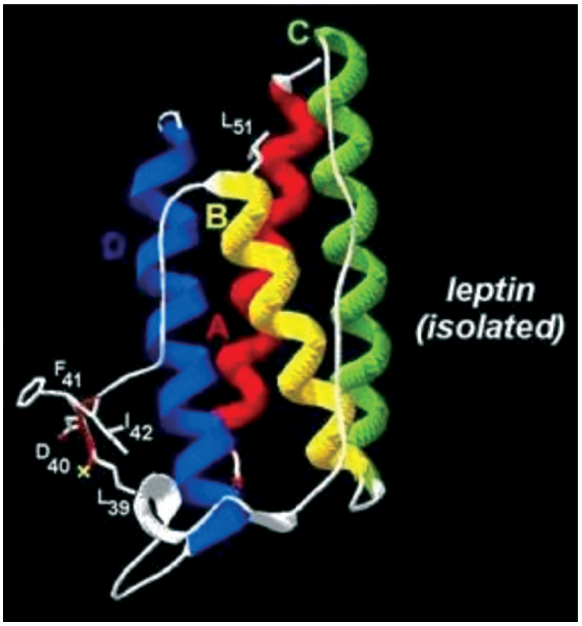

Figure 1. *Tertiary structure of leptin*

Finally it was shown that db/db mice, carrying a mutation in the db gene and whose phenotype is identical to ob/ob mice, synthesize the factor that is missing in ob/ob

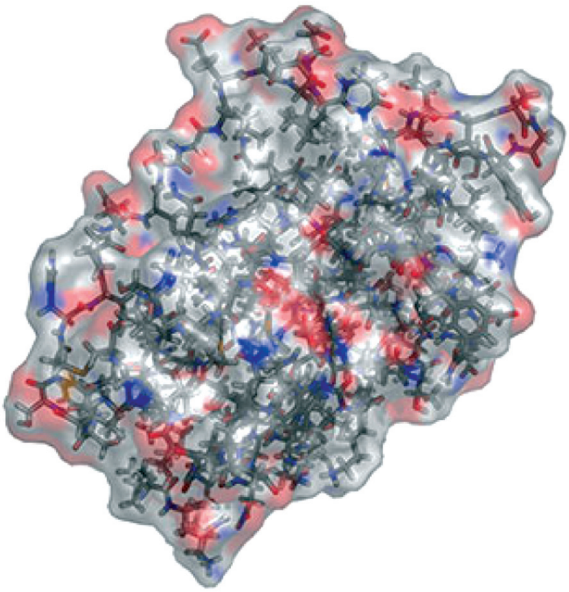

Figure 2. *3D model of leptin*

mice, but they cannot respond to it. This fact suggested that the ob receptor (OB-R) is the product of the db gene. The ob receptor is abundant in the hypothalamus, a region that regulates appetite. The db gene encodes 5 proteins called the long (OB-Rb), the 3 short (OB-Ra, -c, -d) and the soluble (OB-Re) forms that represent the 5 forms of leptin receptor. Among the 5 forms the OB-Rb form is the most important.

The db/db mice have a deletion in the long isoform of the leptin receptor (OB-Rb) and thus are resistant to leptin[1]. Leptin receptors have different patterns of expression in different tissues. OB-Rb seems to be responsible for generating intracellular signals in the hypothalamus in response to leptin through which body weight is regulated (Figure 3).

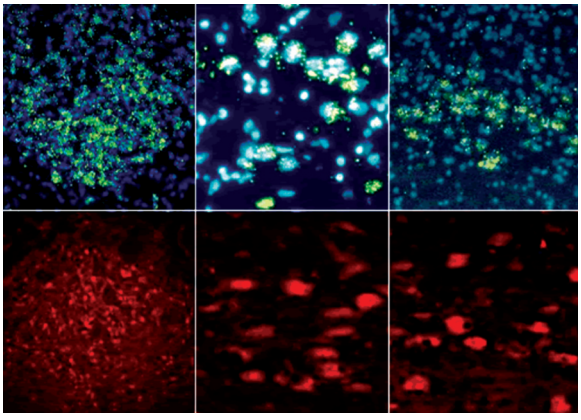

Figure 3. *Leptin receptor (Ob-Rb) in the Area Postrema, Nucleus of the Solitary Tract and Dorsal Motor Nucleus (the top line containing blue colors employs a fluorescent ISH method and the bottom line-red colours-employs immunostaining)*

It is interesting that OB-Rb has been identified also in extrahypothalamic tissues[4], including the lung, spleen, thymus and kidney. Circulating mononuclear cells, T-lymphocytes and macrophages in addition to endothelial cells, express the long isoform of the leptin receptor, as well. However, the role of OB-Rb in regulating extrahypothalamic signal transduction is currently unclear[4].

Circulating leptin concentrations and the role of leptin

Leptin plays a role in the long-term regulation of body weight[5,6]. Circulating leptin levels are directly related to adipose tissue mass. High leptin levels inform the brain, more specifically sites in the central nervous system, about the sufficiency of energy stores. As a result, the appetite is reduced and the energy expenditure is increased. Therefore, leptin signals the nutritional status from the periphery to the hypothalamus (Figure 4).

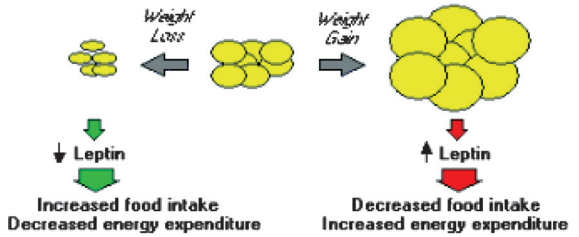

Figure 4. *Regulation of food intake and energy expenditure by leptin, in response to changes in adipose tissue mass*

Leptin works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), as well as by increasing the activity of neurons expressing α-melanocyte-stimulating hormone (α-MSH). The NPY neurons are very important in the regulation of appetite, while, α-MSH is a significant mediator of satiety[7].

Circulating leptin levels are related to body fat mass, sex hormone levels, exposure to bacterial lipopolysaccharide, dietary fat and age[4]. Increased BMI, female gender, inflammation, fatty food and advanced age are related to high leptin levels. In a large epidemiological study (third National Health and Nutrition Examination Survey-NHANES III)[8] in a representative sample of the US population (6303 women and men aged ≥ 20 years), leptin concentrations were found to vary in a large range both between men and women, but in women were much higher than in men. More specifically, mean concentrations in women were 12.7 μg/L (12.7 ± 0.37), while they were 4.6 μg/L (4.6 ± 0.12) in men. Leptin concentrations also vary between different ethnicities. For example, in the above study non-Hispanic white men had higher leptin concentrations when compared to non-Hispanic blacks (4.7 ± 0.14 vs. 4.1 ± 0.17 μg/L respectively). Conversely, non-Hispanic black women were found to have higher leptin concentrations than non-Hispanic whites

(16.4 ± 0.55 vs. 12.2 ± 0.41 μg/L respectively). Mexican Americans, men and women, had intermediate leptin concentrations when compared to non-Hispanic white and blacks (4.4 ± 0.14 and 14.3 ± 0.57 μg/L respectively). Nevertheless, these differences are small and not significant, with uncertain physiologic significance. We must note that serum leptin concentrations should be measured in the morning, after an overnight fast. This is standard for all the levels of leptin mentioned in the studies used to realise the aim of our study.

It is significant that leptin levels are also modulated acutely. For example, leptin levels change rapidly in the presence or absence of food intake, disproportionately to the changes in fat depot[9,10]. Therefore, leptin does not represent only the stored body fat.

On the other hand, this molecule plays a pleiotropic role in mammalian physiology. For example, leptin-deficient ob/ob mice and leptin receptor-deficient db/db mice are not only obese, but they also manifest insulin resistance with hyperinsulinaemia accompanied by noninsulin dependent diabetes mellitus and abnormalities in reproductive function, hormone levels, woundhealing, bone structure and immune function, along with cold intolerance[11].

Blood concentrations of leptin are usually increased in obese humans, which means they are leptin resistant, rather than suffering from leptin deficiency. Mutations in ob or db genes appear to be a very rare cause of extreme obesity in humans, but both have been described.

The precise physiological role of leptin in humans has not been established yet. It has been suggested that leptin is important for adequate neuroendocrine responses to undernutrition. In contrast, recent findings suggest that hyperleptinaemia may contribute to the development of the metabolic syndrome. Leptin also exhibits significant effects on lipid metabolism, platelet aggregation, hematopoiesis, smooth muscle cell proliferation, pancreatic cell function, thermogenesis as well as on the response to bacterial lipopolysaccharide[4]. Other emerging data suggest that leptin may accelerate vascular disease in an "over-flow" situation, i.e. in association with increased bodyweight when leptin levels are elevated[12]. Moreover, in experimental models, leptin has been shown to have angiogenic activity, to increase oxidative stress in endothelial cells as well as to promote vascular cell calcification and smooth muscle cell proliferation and migration[13]. Increased circulating leptin levels have also been correlated with aging and higher inflammatory markers independent of body fat mass[14].

In the present study we provide evidence about the effects of leptin in atherosclerosis through its effects on the vascular wall, the immune system, the fibrinolytic activity and the oxidative stress whereas we speculate the relation of this molecule to major cardiovascular events.

Leptin, Vascular calcification and Arterial Stiffness

As it was already mentioned, increased circulating leptin levels have been correlated with obesity, infection, dietary fats, aging and sex hormons. All these factors have also been correlated with increased vascular calcification, which is an emerging factor in the process of atherosclerotic vascular disease. Additionally, the differentiation of marrow osteoprogenitor cells is regulated by leptin and thus it was hypothesized that leptin could regulate the calcification of vascular cells. Parhami et al, demonstrated the expression of leptin and its receptor in the artery wall cells and a direct effect of leptin on osteogenic differentiation of a subpopulation of vascular cells, called calcifying vascular cells (CVC)[4].

When treated with leptin for 4 days, these CVC had a significant 5- to 10-fold increase in alkaline phosphatase activity. Alkaline phosphatase, a marker of osteoblastic differentiation, mediates the differentiation process and provides phosphate ions for calcium phospate mineral formation[15,16]. After 10 days of leptin treatment, calcification in the CVC cultures was also dramatically increased. This finding supports even more the pro-osteogenic differentiation effects of leptin.

Furthermore, the presence of leptin receptor was demonstrated on CVC as well as in the mouse artery wall, localized in subpopulations of medial and adventitial cells (Figure 5). With immunostaining, leptin receptors were found along the outer circumference of the vessel wall, mainly specific to subpopulations of medial and adventitial cells near the external elastic lamina. Leptin receptors were found in the endothelium of the adventitial vessels but not in the aortic endothelium. Leptin receptors were identified in atherosclerotic coronary arteries, predominantly in the endothelial cells of intimal neovessels, macrophages/foam cells and vascular smooth muscle cells[17]. It is very interesting that Parhami et al reported the absence of leptin receptors in the aortic endothelium. This suggests specificity to microvascular endothelium and constitutes an important difference between the endothe-

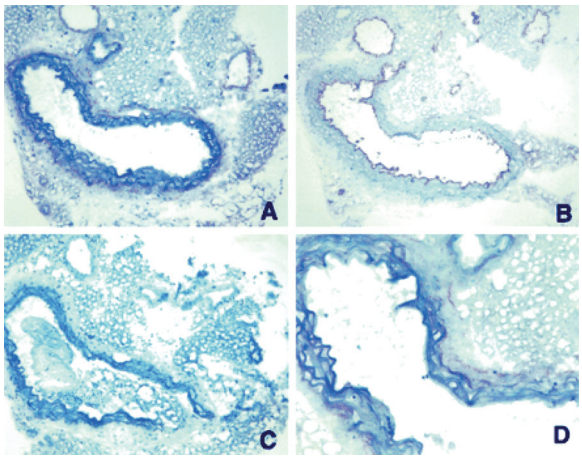

Figure 5. *Leptin receptor expression in mouse aortic wall. Frozen serial sections of mouse aortas were stained (A, D) for leptin receptor and (B) von Willebrand factor (for identification of the endothelium). C. Negative control using nonimmune rabbit IgG. Counterstaining was done with hematoxylin.*

lium of large and small vessels.

The expression of leptin by artery wall cells and atherosclerotic lesions in mice has also been shown[13]. As it was demonstrated, that leptin was present in atherosclerotic lesions in mice, but there was significantly less or no expression in normal aortas. Although leptin was not evident on immunohistochemistry of the normal mouse aorta, the authors hypothesized that possibly the endothelial cells secreted the protein into the circulation, while leptin is trapped in the abnormal matrix of atherosclerotic lesions and it may also be produced by monocytes and macrophages within the lesion. As far as the distensibility of the vascular wall is concerned, higher leptin concentrations are associated with impaired arterial distensibility, as it was shown by Singhal et al on healthy adolescents with a broad range of body mass index[13].

Arterial distensibility is a capable index of the elastic properties of the vessel wall and its functional capacity against the atherosclerotic process[18,19], given that arterial stiffness is known to correlate well with the extent of atherosclerosis[18,19] and other atherosclerotic risk factors[20-23], such as obesity, plasma lipid concentrations and insulin resistance. In the last of the aforementioned studies, Singhal et al showed that arterial stiffness was significantly associated with body fat mass, independently of potential confounding factors, such as pulse pressure, age, sex, z score for height and weight, and diastolic arterial diameter. The association remained significant after adjustment for the above potential confounding factors and for insulin or LDL cholesterol concentration.

A strong inverse association was also revealed between arterial distension and plasma leptin concentration, which was independent of potential confounding factors, as well. The association remained significant after adjustment for potential confounding factors like fasting insulin, LDL/HDL cholesterol, CRP concentration, mean arterial blood pressure and for three different measures of fat mass.

The aforementioned findings about the role of leptin on the arterial wall in healthy adolescents are significant, as they prove that elevation in leptin levels is independently assosiated with impaired vascular function, even during an early stage of the atherosclerotic process, and that the effect of leptin on the arterial condition is clinically relevant and not just an epiphenomenon[13].

Taken together, these results suggest a role for leptin as an important factor in the calcification of vascular cells and as a possible regulator of the vascular wall.

Leptin and the Immune response regulation

Atherosclerosis is a combination of a variety of changes to the inner arterial layer, accompanied by changes to the medium arterial layer. The cells that take part in this process are endothelial cells, smooth muscle cells, platelets, macrophages and T-lymphocytes. Macrophages are multiplied in the atherosclerotic lesions and along with smooth muscle cells constitute the main source of foam cells. T-lymphocytes take part in all the stages of atherogenesis. It appears that oxidised LDL is a possible antigen that causes the interaction between macrophages and lymphocytes and the release of cytokines and growth factors.

Leptin was recently found to play a role in the immune response and the development of a pro-inflammatory state in relation to increased body weight[24]. We will hereby present some evidence of leptin effects on the immune system and its components, to reveal another mechanism through which leptin promotes atherosclerosis.

Leptin can be considered as a cytokine, taking into consideration its structure and the structure of its receptor. Secondary structure of leptin is like the long-chain helical cytokines family, which includes interleukin 6 (IL-6), IL-11, Ciliary Neurotrophic Factor (CNTF), and Leukemia Inhibitory Factor (LIF), whereas the leptin receptor is similar to the gp-130 signal-transducing subunit of the IL-6-type cytokine receptors[1]. As for its tertiary structure, leptin has a four-helix bundle which resembles that of the long-chain helical cytokine family, in which IL-6, IL-11, IL-12, LIF, Granulocyte-Colony Stimulating Factor (G-CSF), CNTF, and oncostatin M25,26,27 are included (Figure 6).

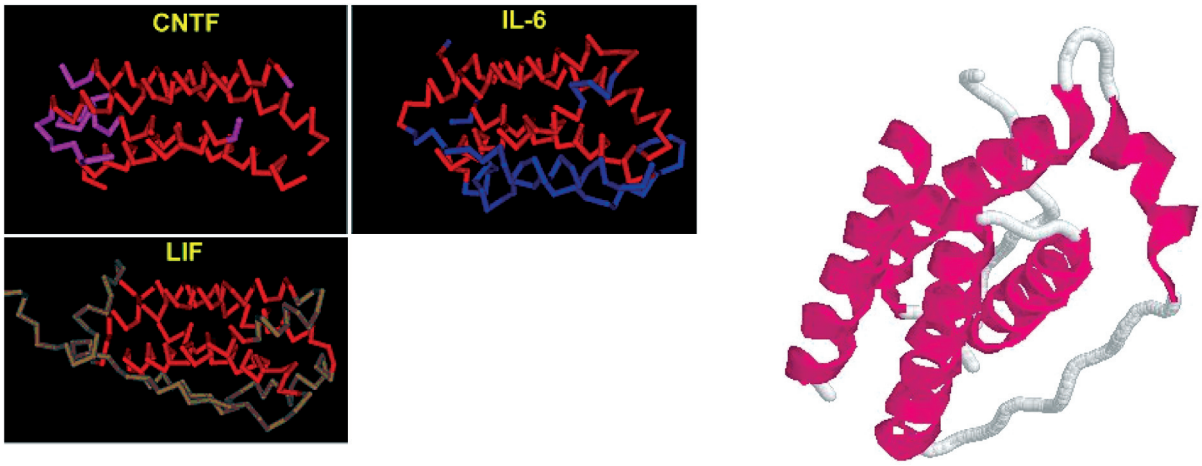

Figure 6. *Similarities in the tertiary structure between leptin (on the right) and CNTF, IL-6 and LIF (on the left)*

Leptin contributes to innate and acquired immunity. There are several studies to support this theory. Leptin exerts proliferative and anti-apoptotic activities in a variety of cell types, including T lymphocytes, leukemia cells and hematopoietic progenitors. It can affect cytokine production as well as monocytes-macrophages activation and was found to induce the Macrophage-Colony Stimulating Factor (M-CSF)[28,29] production. Additionally, leptin receptors have been identified on macrophages[29]. The fact that leptin regulates inflammatory responses is clearly indicated by the increased sensitivity to proinflammatory monocyte/macrophage-activating stimuli and the alterations in immune and inflammatory responses observed in ob/ob or db/db mice. Leptin deficiency aggravates susceptibility to LPS and TNF -induced lethality and liver injury[30,31]. Consequently, a functional leptin system appears to be protective against inflammation from external or autoaggressive effects.

Furthermore, leptin acts as a regulator of T-cell mediated inflammation in vivo, and regulates the number and activation of lymphocytes through: 1) enhancing the alloproliferative response of human peripheral blood lymphocytes by acting on naive T lymphocytes, 2) enhancing phytohemagglutinin and concanavalin A-induced proliferation of human T lymphocytes, 3) increasing the expression of activation markers CD69, CD25, and CD71 in CD4+ and CD8+ cells, 4) regulating cytokines production by T lymphocytes, 5) polarizing T helper cells toward a Th1 phenotype by enhancing proliferation and IL-2 production of naive T cells, 6) increasing TNF-α and inhibiting IL-4 production in memory CD4+ T cells and 7) up-regulating the expression of adhesion molecules, such as ICAM-1, on CD4+ T cells[1].

Studies in rodents with genetic abnormalities in leptin or leptin receptors revealed impaired phagocytic function by macrophages and expression of proinflammatory cytokines both in vivo and in vitro[3]. More specifically, macrophages from ob/ob and db/db mice present a deficiency to phagocytose and kill Candida (Figure 7). Recombinant leptin restores this deficit in cells from ob/ob mice, but not from db/db mice, indicating that leptin regulates macrophage phagocytosis through direct interaction with its receptors. The results from this study in lean, nondiabetic mice, in combination with those already mentioned[29], suggest that leptin deficiency is directly responsible for the impaired phagocytic function in ob/ob and db/db mice and not as a parameter of obesity-related diabetes.

Moreover, according to this finding, leptin up-regulates phagocytic function and cytokine release only after stimulation by an inflammatory agent, such as LPS. In the atherosclerotic process, this inflammatory agent could be the oxidized LDL, as mentioned above.

Data regarding the regulation of leptin expression in response to infection and inflammation, especially in humans, and the exact role of leptin in the immune response are ambiguous and non conclusive. Nevertheless, all the former findings identify an important and novel function for leptin, that of up-regulation of inflammatory immune responses, which may constitute a pathogenetic mechanism responsible for some of the major complications of obesity and especially of the atherosclerotic process, an issue that remains to be investigated.

Leptin and Fibrinolysis

Another possible mechanism through which leptin promotes atherosclerosis is its association with abnormal fibrinolytic activity, as recent studies have shown. It has been proved that impaired fibrinolysis plays a role in the development of atherothrombotic disease[32] and that it increases the risk of myocardial infarction[33], as well as the possibility of reinfarction and death after myocardial infarction[34,35]. The main regulators of fibrinolytic activity are tissue plasminogen activator (tPA) which up-regulates fibrinolysis, and plasminogen activator inhibitor-1 (PAI-1), which has the opposite effect, that is inhibition of the fibrinolytic process.

Leptin was shown to be asssociated with defective fibrinolytic activity and more specifically, with low t-PA and high PAI-1 activity. Söderberg et al[2] showed that leptin levels in males were significantly associated with increased waist circumference, low HDL cholesterol, low tPA activity and high PAI-1 activity, after adjustments for age, BMI and baseline insulin levels in healthy subjects from the Northern Sweden MONICA population. Accordingly, an important association between leptin and low tPA activity/high PAI-1 activity was shown amongst postmenopausal females, after adjustment for age and BMI. More specifically, leptin correlated significantly with serum fibrinogen, t-PA activity and PAI-1 activity in both sexes.

After adjustments, in males and postmenopausal women the strong associations between high leptin levels and high PAI-1 activity/low tPA activity did not change, while tPA, PAI-1 activity and postload insulin levels did not correlate to leptin levels amongst premenopausal women.

Leptin and Oxidative Stress

As it was previously described, atherosclerosis is the result of an excessive proliferative and inflammatory response involning several cellular events. There are emerging data that oxidative stress may play a regulatory role in such events, through the formation of reactive oxygen species (ROS). ROS can activate cell migration and proliferation[36] and appear to control the expression of adhesion molecules and chemotactic factors, which are proinflammatory molecules in endothelial cells. Furthermore, ROS are able to activate transcriptional factors, such as Nuclear Factor kappa B (NF-κB) and can affect intracellular transduction pathways functioning as signaling messengers[37,38]. It seems probable that leptin plays a further role in the atherosclerotic process through its effects on ROS.

Bouloumi et al[7] showed that leptin plays a role in the atherosclerotic process through the generation of oxidative stress in endothelial cells and that ROS are second

messengers involved in leptin-induced transduction in endothelial cells. In human umbilical vein endothelial cells (HUVECs) leptin increased the accumulation of ROS, as assessed by the oxidation of 2',7'- dichlorodihydrofluorescein (DCHF), which is indicative of increased intracellular H2O2 or HO generation. When stimulating DCHF-loaded HUVECs with increasing concentrations of human recombinant leptin (1 to 100 ng/ml) they observed a concentration- and time-dependent increase in DCHF fluorescence.

Additionally, leptin activated the NH2-terminal c-Jun kinase (JNK)/stress-activated protein kinase pathway, which is a stress-dependent intracellular pathway, and its downstream target, the transcription factor AP-1, which is an inducible transcription factor. A time-dependent increase in JNK activity was shown through immunoreactivity assays on protein extracts from cells stimulated with leptin. Leptin (10 ng/ml) enhanced the JNK-mediated phosphorylation of the GST (Glutathione-S-transferase)-Jun fusion protein, with a maximal effect after 30 min. GST-Jun fusion protein was used as substrate for JNK.

They proceeded further on to study the DNA binding activity of the transcription factor AP-1, which is as mentioned the downstream target of the JNK-dependent pathway. Electrophoretic mobility shift assays using [32P]AP-1 consensus sequence resulted in a time-dependent appearance of two bands, in nuclear extracts from cells stimulated with leptin. This shows a strong association of nuclear proteins with the AP-1 consensus sequence. The stimulatory effect reached a peak within 30 min of stimulation (4.2-fold increase) and gradually decreased after 1 h. NF-κB, a protein complex which works as a transcription factor and is sensitive to redox changes, was also activated by leptin stimulation in an oxidant-dependent manner. Thus, they invastigated the DNA binding activity of NF-κB as an index of oxidative stress after stimulation of endothelial cells by leptin. Nuclear extracts from cells stimulated by leptin resulted in a change in the mobility of the NF-κB consensus sequence in a time-dependent manner. The activation peaked after 30 min stimulation and remained elevated for over 1 h, nevertheless it was less intense than that of AP-1.

In all the above experiments, when the cells were pretreated with antioxidants, leptin failed to exert its stimulatory effects.

Leptin and Major Cardiac Events

One of the most significant prospective studies on the role of leptin as a major and independent risk factor for coronary heart disease is a part of the West of Scotland Coronary Prevention Study (WOSCOPS)[39], whose original aim was to investigate the effectiveness of Pravastatin in preventing coronary morbidity and mortality. Plasma leptin levels were measured at baseline in 377 men who then experienced a coronary event, and in 783 men (controls) who did not experience any event during the 5-year follow-up period of the study. Controls shared the same characteristics with cases concerning age and smoking history and were representative of the entire WOSCOPS cohort.

According to the study, men who experienced a coronary event during the 5 years follow-up were older, more likely to be smokers, had higher blood pressure and cholesterol levels and lower HDL cholesterol levels. Baseline leptin levels were significantly, higher (16%) in the cases compared to controls (5.87±2.04 ng/mL versus 5.04±2.09 ng/mL, P<0.001). In a univariate analysis, for each 1 SD increase in leptin levels, the relative risk (RR) of a coronary event increased by 1.25. When the group

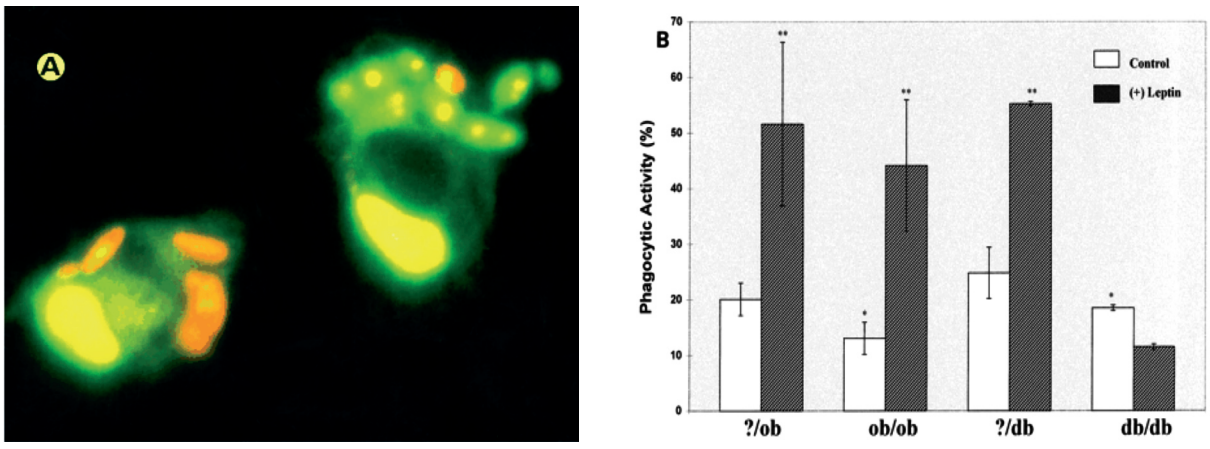

Figure 7. *Phagocytic function of macrophages from lean (?/ob, ?/db) and obese (ob/ob, db/db) mice. Thioglycollate-elicited peritoneal macrophages were plated in medium with or without leptin (500 ng/ml), and then incubated for 2 h with Candida parasilopsis. Staining with acridine orange/crystal violet was performed to reveal live (green) and dead (orange) intracellular microorganisms so that differences in the proportion of phagocytically active cells, the number of intracellular Candida/phagocytically active cell and the ability of individual phagocytically active cells to kill ingested Candida could be evaluated (A). Phagocytic activity of duplicate cultures differed in the absence (open bars) and presence (hatched bars) of leptin (500 ng/ml) (B).*

was divided into quintiles of baseline leptin level, it was found that the risk of a coronary event almost doubled when they compaired the highest 2 quintiles with the lowest quintile.

Using leptin as a continuous variable in several tests proved that it is a univariate predictor of risk of a coronary event. The independence of leptin as a predictor of such events was displayed after adjustment in the analysis for BMI, plasma lipids, systolic blood pressure, glucose and CRP. More specifically, adjustment for BMI hardly changed leptin's association with risk (RR, 1.24), while further addition to this model of baseline lipids and systolic blood pressure also had only a minor effect (RR, 1.21). Moreover, adding glucose to the model had a similar effect (RR, 1.20). Finally, further addition of CRP resulted in the association of leptin with coronary risk shifting to borderline significance (RR, 1.18). It should be mentioned that, although CRP achieved borderline significance (P=0.05) as a predictor of a coronary event in a model including major risk factors, such the above, but not leptin, when leptin was added CRP lost its property as an independent predictor (P=0.12).

The WOSCOPS study is the first prospective study to show that high plasma leptin concentrations in hypercholesterolemic men can predict a future coronary event and that these men are of high risk. A very important result is that the significance of leptin as a predictor of a coronary event was as strong as classic risk factors in univariate analysis, and the RR for a 1 SD change was as high as the RR for a 1 SD change in systolic blood pressure or HDL cholesterol. A finding of considerable interest was that after further adjustment for CRP, as mentioned above, leptin retained a borderline significant association with risk, at the expense of CRP. Plasma leptin concentration can, therefore, be considered as a new, major, independent risk factor for coronary events.

In another study[12], supporting the above findings, sixty-two men with first-ever acute myocardial infarction (AMI) took part and it was found that they had BMI, plasma insulin and leptin levels, as well as diastolic blood pressure higher than their matched controls. Men who did not smoke and experienced an AMI had significantly higher leptin levels (9.0 vs. 5.8 ng/ml, respectively, P = 0.03) and BMI (28.6 vs. 25.9 kg/m2, respectively, P = 0.01) than men with AMI who smoked. This difference did not exist between smoking and non-smoking controls.

To show the role of leptin as a significant and independent risk factor two separate multivariate regression models were conducted. In the first one, traditional risk factors, such as BMI, hypertension, history of diabetes, daily smoking habits and cholesterol levels were tested together. Smoking and total cholesterol levels emerged as significant risk factors for a first-ever AMI. In the second model, leptin, apo A-1, apo B, insulin and PAI-1 were added firstly one at a time in multiple steps and then were tested together. When added to multivariate model 1 insulin and PAI-1 lost their predictive value while, high levels of leptin and low levels of apo A-1 were strongly associated with increased risk for AMI (when added both separately and combined), irrespective of other co-tested parameters/factors. In the final analysis, high leptin levels and total cholesterol levels together with low levels of apo A-1 emerged as significant predictors for development of a future AMI event. To futher support the above results, the proportion of AMI cases out of all cases and controls in the right lower quadrant (low apo A-1 and high leptin) was 55.2% compared to 20.6% in the upper left quadrant (high apo A-1 and low leptin). As a result, leptin (together with high levels of total cholesterol and low levels of apo A-1) can be considered as the strongest independent risk factor.

Furthermore, there are recent studies that relate leptin to other cardiac events, such as chronic stable angina pectoris (CSAP). Interesting findings emerged in a late study by Taneli et al[40], whose aim was firstly to investigate serum leptin concentrations in patients with ST-elevated myocardial infarction (STEMI) and CSAP, and secondly, to find if leptin correlates to other atherosclerotic risk factors, such as BMI, serum high sensitive C-reactive protein, serum homocysteine and fibrinogen. This prospective study included thirty-five patients with CSAP, forty with acute STEMI and thirty controls with normal angiographies. Patients who experienced a STEMI had higher serum concentrations of leptin when compared to controls (8.22±3.13 vs 6.37±1.85 ng/ml, P = 0.023) and higher concentrations of homocysteine. Patients suffering from CSAP also had higher concentrations of leptin when compared to controls (7.74±1.34 vs 6.37±1.85 ng/ml, P = 0.025) and higher concentrations of fibrinogen and homocysteine. Leptin, interestingly, showed no correlation to other risk factors of coronary heart disease, such as homocysteine, which was also significantly increased in patients with STEMI and CSAP. This proves that leptin can be evaluated as one of the independent atherosclerotic risk factors.

In conclusion, from the aforementioned clinical and experimental evidence leptin appears to be an established risk factor for atherosclerotic disease, even though more clinical data may be needed to confirm this interesting view.

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