2. REVIEW OF LITERATURE

2.1 LEPTIN, A LINK BETWEEN OBESITY, INFLAMMATION AND CARDIOVASCULAR DISEASES (CVD):

The relationship between leptin and cardiovascular functions is a complex mechanism. There are data from several different populations suggesting strong positive associations between leptin and CVD (Mohammed I. Hamzah, 2014, Yasuhiro Morita et al., 2013). Epidemiologic evidence (Olga Gruzdeva et al., 2014 Hadi AR Hadi Khafaji et al., 2012, Karthick et al., 2012, 2014) has also implicated elevated leptin levels as a significant predictor of a first ever myocardial infarction and insulin resistance, independent of total adiposity and other classic cardiovascular risk factors. Leptin correlates significantly with markers of the metabolic syndrome, including plasma triglyceride, high density lipoprotein levels (Naglaa F. et al., 2014, Gloria Lena Vega and Scott M. Grundy, 2013). Leptin acts on different types of immune cells, such as monocytes/macrophages, neutrophils, and T cells, to promote the release of inflammatory cytokines (Zarkesh-Esfahani H, et al., 2004, Kiguchi N, et al., 2009). It is generally accepted that leptin acts as a pro-inflammatory adipokine. Numerous studies have indicated that leptin plays an important role in cardiovascular diseases. It has been suggested that leptin could be an important link between obesity and development of cardiovascular disease (Flanagan. DE, et al., 2007). This might be mediated through various effects of leptin including effect on blood pressure (Cooke JP, et al., 2002), platelet aggregation (Chaldakov GN, et al., 2001), formation of arterial thrombosis (Beltowski J, et al., 2002) and inflammatory vascular response (Konstantinides S, et al., 2001). In addition, leptin can promote angiogenesis and induce neovascularization on the vascular endothelial cells (Pucino V et al., 2014). Hence,
hyperleptinemia has also been suggested to be a permissive factor in the pathophysiology of atherosclerosis. Human serum leptin concentrations are independently related with the intima-media thickness of the common carotid artery (Ciccone M et al., 2001). Leptin receptors are found on different cell types in almost every step of atheroma formation such as on vascular smooth muscle cells (Akihiko Oda et al., 2001, Robert J. Gropler, 2012) endothelium (Sierra-Honigmann MR, et al., 1998). In VSMCs, leptin promotes migration, proliferation, and hypertrophy (Martinez-Martinez E, et al., 2014) and probably plays a pro-atherogenic role in the atherogenesis. Chronic Heart Failure are characterized by metabolic abnormalities the process involving several endocrines mainly the adipocytic hormone leptin (Schulze PC, et al., 2003).

Recent studies have demonstrated that leptin-induced local inflammation in vascular endothelium is likely to be involved in the development of advanced atherosclerotic lesions (Hadi AR Hadi Khafaji, et al., 2012, Marco R. Schroeter et al., 2013,). The macrophages densely recruited to the site of injury appeared to be foam cells, expressing plasminogen activator inhibitor-1, and were strongly stained for oxidized LDL (Singhal A, et al., 2002). Intriguingly, the atherogenic diet had no effect on injured vessels from the ob/ob mice and diabetic db/db mice despite their severe obesity and other metabolic dysfunctions. Nevertheless, leptin replacement in the ob/ob mice (but not in the db/db mice) led to dramatically increased recruitment of macrophages and monocytes, neointimal thickness and the severity of luminal stenosis (Schafer et al., 2004). The development of human atherosclerosis plaques is characterized by the conversion of monocyte-derived macrophages into lipid loaded foam cells. This process is mainly controlled by peroxisome proliferators-activated receptor gamma (PPAR-g), which is a key regulator of cholesterol efflux in macrophages (Chinetti et al., 2001, Jin-Feng Zhao, et al., 2013). Leptin treatment
can decrease the mRNA expression of PPAR-g in macrophages and macrophage-derived foam cells, which may accelerate the development of atherosclerotic lesions (Cabrero et al., 2005). Alternatively, leptin-induced reactive oxygen species (ROS) production can enhance the production of MCP-1 in bovine aortic endothelial cells by increasing fatty acid oxidation via activation of protein kinase A (Yamagishi et al., 2001, Kandadi MR, et al. 2014). Because MCP-1 plays an important role in the early phase of atherosclerosis by initiating monocyte/macrophage recruitment to the vessel wall, MCP-1 overproduction provoked by leptin is expected to promote fatty streak formation, the earliest histopathological hallmark of atherosclerosis (Yamagishi et al., 2001). ApoE -/- mice lacking Ob-Rb (apoE -/- db/db) are characterized by a 5-fold higher area of spontaneous atherosclerotic lesions in the aorta than apoE -/- with intact Ob-R. Unlike apoE -/- mice, apoE -/- db/db mice are also obese and insulin resistant, these findings indicate that the hyperleptinemic conditions in obesity may be directly linked to the increased risk of atherosclerosis following a cardiovascular injury (Wei Luo, 2011). Further studies in animal models as well as in clinical subjects are needed to elucidate the contributions from hyperleptinemia and leptin resistance to the inflammatory state of adipose tissues in obesity. Leptin also promotes calcification of cells of the vascular wall and facilitates thrombosis by increasing platelet aggregation. Hyperleptinemia has been associated with coronary atherosclerosis in type 2 diabetes, and this association has been shown to be independent of insulin resistance (Ayaka Tsubo, et al., 2014).

Hypertension is one of the major risk factors for atherosclerosis. Although the association of obesity and hypertension is well established, information about the underlying mechanism is limited. Leptin acting on the hypothalamus reduces food intake and stimulates thermogenesis (Woods AJ, Stock MJ. (1996). The primary mechanism of this thermogenesis is the activation of the
sympathetic nervous system by leptin (John E, et al., 2010, Jintao Wang, et al., 2013). Chronic intravenous or intracerebroventricular infusion of leptin increases blood pressure in normotensive rats by increasing sympathetic activity in the kidneys, and in adrenal and brown adipose tissue (Farhana K, et al., 2014). These observations show that hyperleptinemia may increase sympathetic activity and contribute to obesity hypertension. Leptin has been shown to stimulate the secretion of endothelin by endothelial cells in vitro, and several studies have shown that the serum concentrations of endothelin-1 are increased in patients with hypertension (Francesca Schinzari, et al., 2013). It has also been shown that the function of the renin-angiotensin system may be associated with metS and leptin secretion (Takuya Kishi and Yoshitaka Hirooka, 2013) and there is a significant positive correlation between hyperleptinemia and plasma renin activity in essential hypertension (Shannon M. Harlan, et al., 2011). Therefore, leptin seems to play an important role in the regulation of blood pressure by influencing the activity of the sympathetic nervous system, endothelial function and the renin-angiotensin system.

2.2 LEPTIN AND INFLAMMATORY MARKERS:

Leptin is associated with vascular remodeling, decreased arterial distensibility, oxidative stress and calcification. Thus, plays a critical role from initiation process of atherosclerosis to severe form of CVD. Increased level of leptin are always associated with many acute phase cytokines, such as tumor necrosis factor alpha (TNF-a), interleukin-1 (IL-1), interleukin-6 (IL-6), and so forth (Gilberto Paz-Filho, et al., 2012).
2.2.1 INTERLEUKIN-6:

Interleukin-6 (IL-6) is a 27-kDa member of the cytokine family. IL-6 is described as an endocrine cytokine and is secreted from many different cell types including monocytes, fibroblasts, lymphocytes, and glial cells (Tadamitsu Kishimoto, 2010, Jurgen Scheller, et al., 2011). This pro-inflammatory cytokine is probably the most consistent biomarker of peripheral arterial disease (Tzoulaki I, et al., 2005) identified thus far IL-6 has numerous biological activities and is considered as the major player that regulates the innate immune response, haemopoiesis, and inflammation (Graeve L, et al., 1993, Grimble RF et al., 1998). Leptin induces IL-6 transcription and secretion in different cell types such as dendritic cells (Mattioli.B, et al., 2005), human leukocytes (Zarkesh-Esfahani.H, et al., 2001), colon epithelial cell line (Jenifer I. et al., 2006). Human IL-6, even at concentrations more than tenfold above the ranges observed in obese individuals, suggesting that leptin potentially has a more prominent effect on IL-6 concentrations under the conditions of chronic low-grade inflammation. OB-R has been shown to have signaling capabilities similar to IL-6-type cytokine receptors. Therefore, it is likely that leptin acts via IL-6, or perhaps even via OB-R (Muraoka. S, et al., 2013).

2.2.2 MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1):

Monocyte chemoattractant protein-1 (MCP-1) is one of the critical factors attracting macrophages to adipocytes. MCP-1 selectively promotes the chemotaxis of monocytes. Gene deletion of MCP-1 leads to diminished macrophage infiltration in the white adipose tissue (Kanda Hajime. et al., 2006). Genetically modified mice lacking MCP-1 or its receptors (chemokine [C-C motif] receptor 2) have delayed and attenuated atheroma formation when crossed with an atherosclerosis-prone hyperlipidemic genetic background (Long Gu, et al., 1998). Expression of MCP-1 is
altered by leptin through various mechanisms. In Human umbilical vein endothelial cells (HUVECs), leptin increases the generation and accumulation of ROS by activating AP-1, C-jun-amino-terminal kinase and nuclear factor-kappa B pathways (Bouloumie A et al., 1999). In turn, ROS enhances the induction of adhesion molecules, such as vascular cell adhesion molecule-1 and monocyte chemoattractant molecule-1 (MCP-1) (Chang Yeop Han, et al., 2012). MCP-1 selectively encourages the chemotaxis of monocytes. Genetically mutant mice lacking MCP-1 or its receptors (chemokine receptor 2) have delayed and attenuated atheroma formation when crossed with an atherosclerosis-prone hyperlipidemic genetic background (Boring L et al., 1998). Oxidative stress may also operate as an indirect factor to increase serum atherogenic factors. By increasing oxidative stress and activating protein kinase C, leptin increases the secretion of atherogenic lipoprotein lipase (LPL) from macrophages in vitro (Maingrette F et al., 2003). The stimulation of MCP-1 in the endothelium of the blood vessels is expected to facilitate the recruitment of macrophages into the adipose tissue.

2.2.3 C-REACTIVE PROTEIN (CRP):

Human C-reactive protein (CRP), one of the hepatic acute phase reactants, is a negative regulator of functions of human leptin (Chen et al., 2006). CRP can bind directly to leptin and inhibit the ability of leptin to interact with its receptors and to activate signal-transducer and activator of transcription-3 (STAT3) and phosphatidylinositol-3 kinase (PI3K). Human CRP, although traditionally considered an acute inflammatory marker, has now been recognized as an independent risk factor that positively associates with obesity, insulin resistance, central fat disposition, hepatic steatosis and cardiovascular diseases (Kazumi et al., 2003; Anty et al., 2006; Park et al., 2004; Schulze et al., 2004). Both CRP and leptin
are increased in AMI (Karthick R. et al., 2012, Yasuyuki Nakamura., et al., 2013). There is also evidence that leptin may have proinflammatory effects by increasing C-reactive protein (Singh P, et al., 2007, De Rosa S, et al., 2009), an acute phase reactant that has been consistently associated with an increased risk of cardiovascular events. Hepatic synthesis of CRP is influenced by proinflammatory cytokines, such as IL-1, IL-6 and TNF-α. The findings of human CRP blocking physiological functions of leptin offer an additional mechanism underlying leptin resistance in human obesity. They also help explain in part about why recombinant human leptin fails to effectively reduce the body weight of participants from the previous clinical trials. Overall, the direct actions of leptin in the liver promote the regional inflammatory response and stimulate the production of a classical inflammatory marker, CRP that in turn influences energy balance and metabolism. Reports suggested that CRP is not only an inflammatory marker, but also a direct cause of CVD (Jian-Jun Lie, Chun-Hong Fang, 2004). In humans, an increase of body fat content leads to elevated concentrations of leptin and other proinflammatory cytokines (such as IL-6), which in turn can stimulate the production of human CRP in the hepatocytes. Blood CRP in turn binds to leptin and attenuates the physiological functions of leptin, rendering the body resistant to leptin.

**2.2.4 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF):**

VEGF is a master regulator of the growth of blood vessels required for tissue differentiation and function. Leptin signaling upregulates the expression of several molecules involved in proliferation, survival, inflammation and angiogenesis. Leptin initially identified as a pro-angiogenic factor (Sierra-Honigmann MR et al., 1998) is also a positive regulator of VEGF in endometrial (Gonzalez RR et al., 2004). High
concentration of leptin within vasa vasorum and plaque itself, may influence inflammatory and vascular neovascularization coupling with functional upregulation of the vascular endothelial growth factor (VEGF). Hence VEGF has identified as a potential factor of pathological neovascularization induced by leptin (Hyun-Young Park, et al., 2001). In atherosclerosis, elevating plasma VEGF levels in relative to healthy controls observed in subjects with CVD risk factors and in patients with established atherosclerotic diseases (Blann AD, et al., 2002) is suggested as an attempt to compensate for tissue damage or hypoxia, or simply a reflection of endothelial cell damage (Felmeden DC, et., 2003). The immunoreactivity for OB-R, VEGF increased in atherosclerotic plaque, predominantly in the endothelial lining of the intimal neovessel and macrophages/foam cells. VEGF may promote atherosclerosis progression through its ability in enhancing plaque inflammatory infiltration and plaque neovascularization (Holm PW, et al., 2009). A prospective study on association of VEGF with coronary heart disease mortality reveals that subjects with large CVD risk burden and elevated VEGF levels at baseline are at greater risks of death than those with lower VEGF levels and also less CVD burden (Eaton CB, et al., 2008).

2.2.5 OTHER INFLAMMATORY MARKERS:

Tumor Necrosis Factor α (TNF – α) is a 26-kDa protein that is a member of the cytokine family. TNF– α is most often associated with its role in inflammation (Tzanavari. T, et al., 2010). During an inflammatory response, inflammatory stimuli such as lipopolysaccharide stimulate TNF-α production by macrophages in the blood (Olszewski. M. B, et al., 2007.). Although the normal physiological effect of leptin on the regulation of TNF– α expression seems to be suppressive, the
hyperleptinemic condition and leptin resistance may have both contributed to the rise of TNF–α in the adipose tissue in obesity (Se-Min Lee, et al., 2014).

Matrix metalloproteinases are endoproteases that are regulators of the extracellular matrix (Sternlicht MD and Werb Z. 2001) and play an important role in the progression of atherosclerosis and plaque rupture by degradation of the extracellular matrix (Yoon YW, et al., 2005). In experimental animal models, the chymase enzyme, released from degranulating mast cells after interaction with leptin, stimulates monocyte to produce matrix-degrading MMPs and activates MMP-9 (Zhaogang Dong, et al., 2013, Marco R. Schroeter, et al., 2012).

Acute leptin administration has little or no effect on arterial pressure despite an increase in sympathetic activity (Jay J. et al., 2001). This is because acute leptin stimulates endothelial NO production (Francesca Schinzari, et al., 2013) by activating protein kinase B/Akt, which phosphorylates endothelial NOS and increases its activity even at low calcium concentrations (Vecchione C et al., 2002), and the presser effect of sympathetic activation is counterbalanced by the depressor effect of endothelial-derived NO (Lembo G et al., 2001).

Resistin was suggested to be a link between obesity and insulin resistance but until now its role is unclear. Serum resistin was positively correlated with changes of BMI and body adipose mass. Circulating resistin levels increase with age, probably reflecting the increase in the body fat content (Oliver P et al., 2003). Resistin is poorly expressed in human fat cells. Since it is produced by blood monocytes (Laudes.M., 2010) its inflammatory activity and contribution to development of endothelial dysfunction has been suggested.
2.3 THE MECHANISM OF ACTION OF LEPTIN:

2.3.1 THE BINDING OF LEPTIN WITH LEPTIN RECEPTOR (OB-R):

Fong and co-workers constructed a panel of OB-R deletion and substitution variants, and tested these receptors for the ability to bind leptin and to signal in response to leptin. They showed that the membrane proximal CRH2 domain is sufficient and absolutely necessary for leptin binding (Fong TM, 1998). Despite the lack of any affinity for the ligand, the FNIII domains are also needed for activation of the receptor. More recently, researcher could show that a OB-R deletion-variant, which lacks both CRH1 and Ig-like domains, is unable to activate the JAK kinases and therefore cannot generate a STAT3-dependent signal in response to leptin and also could demonstrate that the membrane distal CRH1 is not strictly required for signalling, but allows optimal signalling (Zabeau L, et al., 2004). Several lines of evidence suggest that the OB-R exists as a preformed (i.e. in the absence of the leptin ligand) complex. Chemical cross-linking and Western blot analysis indicate that the receptor forms dimers in solution and on the cell-surface (White DW et al., 1997). They could demonstrate that 60% of the receptors are expressed as dimers, and this dimerisation is not increased after addition of leptin (Couturier et al., 2003). It is therefore reasonable to assume that the OB-R becomes activated upon conformational changes, more than by a simple leptin-induced receptor oligomerisation. Recently it has been reported that both the membrane proximal CRH2 module as well as the FNIII domains could be involved in this ligand-independent dimerisation (Zabeau et al., 2005). The second fibronectin type-III domain consists a WSXWS motif, which plays a role in leptin receptor dimerization and activation (Dagil R, et al., 2012) and is conserved among birds, humans, and fish (Prokop JW, et al., 2012). The WSXWS motif is also necessary for proper receptor folding, but is...
not directly involved in ligand binding (Yawata et al. 1993, Taga & Kishimoto 1997). Intracellularly, the membrane-spanning leptin receptor isoforms contain a highly conserved proline-rich box 1 (intracellular amino acid 6-17 for the human receptors), a highly conserved proline-rich box region that recruits and binds Janus kinases (JAKs) (Bjorbaek, C, et al., 1997, BahrenbergG, et al., 2002), which may have some signaling function in vitro (Murakami, T, et al., 1997). A model wherein leptin-induced clustering activated LR complex has shown that disulfide bridge formation involving Cys-672 and Cys-751 may be necessary for JAK activation and hence signaling (Lennart Zabeau, et al., 2005).