1. INTRODUCTION

1.1 OBESITY:

Obesity originates as a consequence of altered normal physiology of the body that regulates energy intake, energy expenditure and energy storage. Changes in lifestyles as well as higher consumption of more energy-dense rich nutrient foods with high levels of sugar, and saturated fats combined with reduced physical activity, have led to obesity rates that have risen three-fold and are contributing considerably to obesity in population around the world. World Health Organization (WHO) figures indicate that this is a "global epidemic". Currently more than 2 billion adults are overweight, at least 600 million of them clinically obese and is a major contributor to the global burden of chronic disease and disability. The obesity epidemic is not restricted to industrialized societies; this increase is often faster in developing countries than in the developed world. In India around 20% of the populations are overweight or obese, 73% adults in urban India are overweight, and 46% are obese. Obesity is a complex condition, with serious social and psychological dimensions, affecting virtually all ages and socioeconomic groups. Obesity and overweight problems have serious life threatening health-related problems such as cardiovascular diseases (CVD), type 2 diabetes and certain types of cancers (WHO, 2009).

Overweight and obesity is frequently measured by computing body mass index (BMI), which is defined as weight in kilograms divided by the square of the height in meters (kg/m²). WHO, as per Asian classification, defines an adult who has a BMI between 23 and 27.49 as overweight; BMI of 27.5 or higher as obese; BMI below 18.5 as underweight and BMI between 18.5 and 22.99 as normal. Overweight
and obesity direct to adverse metabolic effects on blood pressure, cholesterol, triglycerides and insulin resistance. Increased BMI also enhances the risks of cancer of the breast, colon, prostate, endometrium, kidney and gallbladder. Chronic overweight and obesity contribute significantly to osteoarthritis, a major cause of disability in adults. Although obesity should be considered a disease in its own right, it is also one of the key risk factors for other chronic diseases together with smoking, high blood pressure and high blood cholesterol.

1.2 OBESITY AND CARDIOVASCULAR DISEASES:

Obesity is on the rise world over and this will undoubtedly give rise to an increase in the incidence of cardiovascular diseases. There are strong prospective data that even before the diagnosis of Type 2 diabetes, obesity and weight gain are associated with increased risk of coronary heart disease (CHD) which leads to premature illness and death even in younger age (Neil K. Mehta., 2009). Besides a distorted metabolic profile, a variety of alterations in cardiac structure and function take place in the individual as adipose tissue accumulates in excess amounts, even in the absence of co-morbidities of obesity (Paul Poirier et al., 2006). Overweight or obesity is associated with several cardiac complications such as CHD, angina pectoris, myocardial infarction, coronary insufficiency, and sudden or non sudden coronary death through its impact on cardiovascular system.

WHO estimates that 18 million people die every year from cardiovascular disease and might reach 25 million by 2020. CVD is now on the rise in India and WHO estimates that 60 percent of the world's cardiac patients will be Indians by 2010. According to WHO estimates, 16.7 million people around the globe die of cardiovascular diseases each year and by 2020 it might increase to 25 million worldwide with an alarming rise in India.
1.3 ADIPOSE TISSUE AND CVD:

Adipose tissue is not simply a depository system for fat but an endocrine organ that is potential of synthesizing and releasing into circulation a number of peptides and non peptide factors that may play a role in cardiovascular homeostasis (Kazumi, T. et al., 2002, Lee HY et al., 2013). Adipose tissue is an extensive source of leptin, adiponectin, angiotensinogen, tumor necrosis factor-α (TNF-α) (Kern PA et al., 1995), interleukin-6 (IL-6) (Fried SK et al., 1998), plasminogen activator inhibitor-1 (Lundgren CH et al., 1996), resistin (Steppan CM., 2001), insulin-like growth factor-I (IGF-I) (Philip A et al., 1989). Of clinical consideration, circulating concentrations of plasminogen activator inhibitor-1, angiotensin II, C-reactive protein (CRP), fibrinogen, and TNF-α are all related to BMI. It has been estimated that in vivo, 90% of the total circulating concentrations of leptin originate from adipose tissue. This is of importance because leptin modulates IL-6 and CRP production in the liver, and IL-6, CRP may be a marker of chronic inflammatory state that can trigger coronary syndrome.

1.4 LEPTIN:

Leptin is an adiposity peptide hormone that controls the central regulation of food intake and energy expenditure through cerebral leptin receptors. In humans, leptin is encoded by a gene located in human chromosome 7q31.3 (Yiying Zhang et al., 1994). Obesity is associated with increased circulating leptin concentrations. High levels of leptin have been documented in individuals with obesity and found to be correlated with endothelial cell dysfunction (Mykhaylo Korda et al., 2008), smooth muscle cells proliferation and migration (Akihiko Oda et al., 2001), Macrophages formation (Lisa O’Rourke et al., 2001), monocytes activation (Hamid Zarkesh-Esfahani, et al., 2001), neovascularisation (Eri Suganami, et al., 2004).
calcification etc., (Melec Zeadin et al., 2009). In addition, leptin induces oxidative stress in endothelial cells, and this action triggers transcription of oxidant-sensitive genes that contribute in atherogenesis (Atsushi Suzuki, et al., 2009). Leptin also augments sympathetic nervous activity (John E. Hall, et al., 2010) and increased BP observed in elevated leptin levels (Karthick. R et al., 2012) in cardiovascular diseases. It is possible that the high levels of leptin observed with obesity could contribute to its adverse effects on CV health. It also has been proposed that an increase in circulating leptin may contribute to the development of CVD in obese individuals.

1.4.1 STRUCTURE OF LEPTIN:

Leptin is an adipocytic protein hormone consisting of 167 amino acids with an amino-terminal secretory signal sequence of 21 amino acids (Figure 1.1). The signal peptide of leptin is removed during maturation process into microsomes followed by subsequent secretion into the bloodstream (Y. Zhang et al., 1994). Circulating leptin is 146 amino acids long with a molecular mass of 14±16 kDa. Human leptin is 84% homologous to mouse and 83% homologous to rat. The crystal structure of leptin reveals a four-helix bundle similar to the long-chain helical cytokine family that includes IL-6, IL-11, IL-12, LIF, G-CSF, CNTF, and oncostatin M.

1.4.2 LEPTIN RECEPTOR:

Leptin Receptor (LR or OB-R) is encoded by the db gene. OB-R is identified from mouse choroid plexus through an expression cloning strategy (Tartaglia et al., 1995). Leptin receptors are expressed in a variety of peripheral tissues and are widely distributed throughout the body including the brain (Funahashi. H, et al., 2003), liver (Cohen. P, et al., 2005), pancreas (Emilsson. V, et al., 1997), placenta
Leptin acts via its cell surface receptor (OB-R), multiple OB-R isoforms exist in the body; all are produced from a single lepr gene and result from alternative mRNA splicing and/or proteolytic processing (Tartaglia, 1997). All isoforms fall into three classes: secreted form (OB-Re), short form (e.g., OB-Ra, OB-Rc, OB-Rd), and long form (OB-Rb). The secreted forms are alternative splice products (e.g., human OB-Re) or proteolytic cleavage products of membrane-bound OB-R forms. OB-Rb form contains 1165 amino acids, OB-Ra contains 896 amino acids, OB-Rc contains 958 amino acids, OB-Rd contains 906 amino acids, OB-Re contains 839 amino acids. Soluble form of OB-R contains only extracellular leptin-binding domains and complex with circulating leptin, perhaps regulating free leptin concentrations (Ge et al., 2002).

1.4.3 LEPTIN RECEPTOR STRUCTURE:

Leptin is known to exert its action in the hypothalamus by binding to the long form of its receptor OB-Rb. OB-Rb has homology to members of the gp130 cytokine receptor superfamily (Tartaglia et al., 1995), which comprises the receptors of IL-2, -3, -4, -6, -7, LIF, G-CSF, GRH, prolactin, and eritropoietin in addition to hypothalamic receptors. All receptors in this family are known to poses characteristic extracellular motifs of four cysteine residues and WSXWS motif (Bazan, 1990) and they contain different number of fibronectin type III domains.

OB-Rb consists of an extracellular domain, transmembrane domain and intracellular domain (Figure 1.2). Extracellular domain is composed of two so-called cytokine receptor homology (CRH) domains, a membrane distal CRH1 and a membrane proximal CRH2, both domains are separated by a fibronectin type III
domain, an immunoglobulin-like (Ig) domain and are followed by three fibronectin type III (FNIII) domains proximal to the membrane (Mitsuru Haniu, et al., 1998). OB-Rb becomes activated upon simple ligand-induced dimerization, the JAK-STAT pathway seems to be the main route by which OB-Rb transmits the extra cellular signal it receives. Similar to other type I cytokine receptor members, OB-Rb contains a highly conserved, proline-rich box1 (aminoacid 6 to 17) (Bjorbaek et al., 1997; White et al., 1997) and two putative less conserved box2 motifs (intracellular amino acids 49 to 60 and 202 to 213) (Chua S et al., 1996; Kloek et al., 2002). Box1 and box2 motifs are considered to engage and bind Jaks (Murakami et al., 1997).

**1.4.4 LEPTIN SIGNALING:**

OB-Rb is a type I cytokine receptor and hence the possibility that leptin signaling might mediate cytokine receptor-like signals, including the activation of Jaks and STATs that communicate cell signaling via a member of the Jak family of tyrosine kinases (Gregor Bahrenberg et al., 2002). Leptin binding activates Tyr1138 of OB-Rb and mediates cytokine receptor-like signals, including those mediated by the latent transcription factor, signal transducer and activator of transcription 3 (STAT3) (Tartaglia, 1997; White et al., 1997, Baquero AF, 2014), Tyr985 recruits the tyrosine phosphatase SHP-2 (Carpenter L R, et al., 1998, Jia You, et al., 2010). The blockade of OB-R function by antibodies is proposed to impair IL-1 functions in the endometrium and trophoblast (Tena-Sempere. M, et al., 2003).

So far, relatively little is known on the precise mechanisms of activation of the OB-R due to lack of structural data for the obesity receptor (OB-R) and its complex with leptin. More recently, the crystal structure of a leptin binding domain of OB-R (Byron Carpenter, et al., 2012) has been determined and this will facilitate the understanding of leptin with its receptor and the signaling induced by leptin.
1.5 PHYSIOLOGICAL ROLE OF LEPTIN:

The ability of leptin to reduce body weight and fat content led to the general view that leptin is an anti-obesity hormone. Administration of leptin to rodents reduces food intake and enhance energy expenditure (Velkoska. E et al., 2003, Jae Geun Kim et al., 2014). Leptin concentrations increase with adiposity in the body, most probably to inform the brain regarding the quantity of stored fat but the wide expression of leptin receptors on diverse types of cells explained the pleiotropic biological effects of leptin (Figure 1.3).

Leptin plays an important role during development, as evidenced by enhanced expression of leptin in placenta, widespread expression of leptin and its receptors in the fetal tissues (Akerman F et al., 2002, Ubags ND et al., 2014). Leptin receptors are expressed by various types of endothelial cell (Sierra-Honigmann. M. R, et al., 1998, Knudson. J. D et al., 2005) and leptin has been stimulating endothelial cell proliferation and formation of capillary-like tubes in vitro (Rita Ferla et al., 2011). In addition, leptin is known to induce formation of new blood vessels in chick chorioallantoic membrane (Reji B.R et al., 2012).

Leptin is known to be involved in signaling to the brain about the sufficiency of fat requirement during the onset of puberty and a threshold level of leptin is required for pubertal development (Carol F. Elias, 1998, Rexford S. Ahima. 2011). Expression of leptin and its receptors (Ryan N. K, et al. 2003) have been reported in human ovary. Serum leptin concentrations are reported to be higher in women with PCOD than in regularly menstruating women (Ravishankar Ram et al., 2004).
1.6 LEPTIN RESISTANCE:

Reports have shown that fundamental of leptin resistance causes obesity and that obesity-induced leptin resistance affects numerous peripheral tissues, including myocardium, liver, pancreas, vasculature, and platelets (Lukasik. M et al., 2012). This metabolic and inflammatory-mediated injury may be a consequence of either resistance to leptin’s action in selective tissues, or excess leptin action from adiposity associated hyperleptinemia. Leptin resistance may reflect the noticeable metabolic dysregulation that occurs in as the result of leptin’s key homeostatic physiological functions.

The leptin axis has functional interactions with elements of metabolism such as insulin and inflammation, including mediators of innate immunity such as interleukin-6. Leptin is even purported to physically interact with C-reactive protein, resulting in leptin resistance, which is particularly intriguing, given C-reactive protein’s well-studied relationship to cardiovascular disease (Ke Chen et al., 2006). Given that plasma levels of leptin and inflammatory markers are correlated and also predict cardiovascular risk, it is conceivable that part of this risk may be mediated through leptin resistance-related insulin resistance, chronic inflammation, type II diabetes, hypertension, atherothrombosis, and myocardial injury. Leptin resistance and its interactions with metabolic and inflammatory factors, therefore, represent potential novel diagnostic and therapeutic targets in obesity-related cardiovascular disease.
1.7 LEPTIN, INFLAMMATION AND CARDIOVASCULAR DISEASES:

A growing body of evidence suggests that alterations in adipose tissue-derived factor may mediate the pathophysiological changes in obesity associated CVD (Kazuto Nakamura, et al., 2014) (Figure 1.4). Owing to the general elevation of cytokines and inflammatory markers obesity is considered a chronic inflammatory disease. As a member of the adipokine family, leptin is an indispensable hormone in the physiological control of energy balance. However, recent evidences suggest that the proportional increase of plasma leptin with adiposity might also plays important role in regulation of obesity associated CVD (Raskin Erusan. R, et al., 2012). The reported increase in serum leptin concentrations following acute infection and in chronic inflammation suggests that leptin may actively participate in immune network and host defense.

Leptin plays regulatory roles of immunity and inflammatory states in liver (Shen J, et al., 2005), blood vessels and (Bodary PF et al., 2002). In innate and adaptive immune systems, leptin can induce chemotaxis of neutrophils, increase the number of CD4+/CD8+ T lymphocytes, facilitate the development of NK cell, and promote T helper 1 cell differentiation. In adipose tissue, the paracrine/autocrine actions of leptin can be either pro- or anti-inflammatory. In addition to that, leptin is known to promote platelet aggregation, to enhance neointimal thickening, and to induce the conversion of macrophages into foam cells, all of which contribute to the development of atherosclerosis. Leptin can contribute to macrophage accumulation in fat tissue via several mechanisms. Accordingly, leptin has been shown to stimulate the transport of macrophages to adipose tissue by increasing diapedesis of blood monocytes across the capillary endothelial cells of the adipose tissue (Cyrile A. Curat, et al., 2004). Leptin has been shown to induce the release of cytokines.
such as TNF-α, IL-6, in human adipose tissue (Neda Rasouli and Philip A. Kern, 2008), human B cells (Sudhanshu Agrawal, et al., 2011). IL-6 is a pleiotropic cytokine with proinflammatory and procoagulant properties that might be involved in the pathogenesis of acute cardiovascular events (Baumann H, et al., 1994, De Maat MP, et al., 1996). Clinical studies have shown that circulating IL-6 levels are elevated in patients with acute coronary syndrome (Wang. X.H et al., 2014). IL-6 is modulated by leptin, studies have shown that PCOD women had higher serum levels of IL-6, as well as many other markers of vascular inflammation, when compared with normal women (Ravishankar Ram et al., 2005). The acute-phase response protein CRPs is synthesized by liver and regulated by cytokines, in particularly by IL-6 (Livia Helena M et al., 1990) is found to be elevated in CVD. CRP is an independent risk factor for different types of CVD and therefore, it is likely that adipocytes secreting leptin is indirect causative of CRP synthesis.

Leptin is found to have multiple roles in the cardiovascular system (Figure 1.5). The proatherogenic action of leptin is attributable to a combination of its effects on various cell types. In blood vessels, leptin can promote endothelial cell proliferation and neovascularization, increase MCP-1 production (Sho-ichi Yamagishi et al., 2001, Jian Zhao, et al., 2014) and enhance macrophage recruitment. In hepatic stellate cells, leptin induces the expression of multiple factors including MCP-1, VEGF, and angiopoietin-1. In United States population, increased level of leptin was strongly associated with increased risk of myocardial infarction in men and women, independent of traditional cardiovascular risk factors and obesity status (Sierra-Johnson J, et al., 2007). In another study, serum leptin is reported to act as an acute phase reactant in AMI patients. Significant correlation was found to exist between mean serum leptin level with BMI, CK, and VEGF (Hadi AR Hadi Khafaji, et al., 2012) in CVD.
NEED FOR THE STUDY

Obesity is the leading risk factor for increased incidence of cardiovascular diseases. Adipocytes secreting a number of adipokines, including leptin, are known to modulate atherosclerosis and are candidate risk factors for CVD. Leptin, a major hormone, stimulates atherosclerosis through various effects such as endothelial dysfunction, cellular proliferation and inflammation. However, the causal mechanisms explaining the relationship between leptin and other risk factors such as IL-6, hs-CRP is not clear. This is in part due to lack of large case-control study in Chennai based population. Elucidation of molecular link of between these factors and obesity should lead to development of effective strategies against life threatening - diseases. Hence in the present study, we studied the role of leptin in the pathogenesis of CVD by measuring its levels in AMI and atherosclerosis subjects and analyzing its correlation to anthropometric variables such as WHR, BMI; biochemical parameters such as lipids, and inflammatory marker such as IL-6, hs-CRP.

Elevated level of leptin is able to activate of human monocytes and to enhance activation and proliferation of preactivated T lymphocytes (Santos-Alvarez J, et al., 1999). Moreover, leptin can induce the synthesis of proinflammatory cytokines by human monocytes and the production of Th1-type cytokines by human T lymphocytes cultured in vitro (Martin-Romero C, et al., 2000). Effects of leptin on human peripheral blood mononuclear cells (PBMC) are activated through leptin receptor which is present in peripheral blood monocytes and T lymphocytes (Sanchez-Margalet V, et al., 2001). To analyse the role of leptin in the expression of IL-6, we have studied the expression of the long isoform of the leptin receptor and IL-6 in PBMC of CVD and non-CVD subjects.
Leptin exclusively binds to its receptor, OB-R. Several isoforms of OB-R are found in diverse tissues and long isoform (OB-Rb) is proposed to be involved in the signaling process during leptin binding to the receptor. We studied the function of OB-Rb function by using specific OB-Rb inhibitor and analysed the IL-6 expression in peripheral blood mononuclear cells (PBMCs).

Leptin is also, defined as a potent proatherogenic factor (Maria Balasoiu, et al., 2014) and understanding of the role of leptin and its receptor in CVD could be important in medical management and devising leptin based therapeutic strategy. Hence we extended our work to evaluate factors such as VEGF and MCP-1 in CVD and non-CVD.

Understanding the molecular mechanism of leptin:leptin receptor (OB-R) interaction and the OB-R activation is crucial for the signaling of leptin. This requires a detailed understanding of the structure of leptin and its receptor at molecular level. Recent crystal structure determination of Leptin Binding Domain (LBD) of OB-R (Byron Carpenter, et al., 2012) could throw new insights into the leptin activation mechanism. Conserved residues and their interaction may contribute to leptin/OB-R interaction and these have been analysed using docking and Molecular Dynamics (MD) simulation.
OBJECTIVES

CHAPTER I:

To analyse the relationship between serum leptin concentration in non-CVD subjects including normal weight, overweight, obese and study its relationship to BMI, BP and biochemical parameters namely serum levels of total cholesterol, triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very low Density Lipoprotein (VLDL) and inflammatory proteins such as IL-6, high sensitivity C - reactive protein (hs - CRP).

CHAPTER II:

To estimate serum leptin concentration in CVD diagnosed for (1) Acute Myocardial Infarction (AMI) subjects, those were admitted immediately to ICCU, (2) Atherosclerosis subjects, those were visited to OPD, including normal weight, overweight, obese subjects and to study its relationship to BMI, BP and biochemical parameters namely serum levels of glucose, total cholesterol, triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very low Density Lipoprotein (VLDL) and inflammatory cytokines such as IL-6, high sensitivity C - reactive protein (hs-CRP).

CHAPTER III:

Obesity is regarded as a pro-inflammatory state, in order to understand this we have analysed the expression of long form of leptin receptor (OB-Rb), IL-6 in peripheral blood mononuclear cells (PBMCs). We have also evaluated the amount of circulating VEGF, MCP-1 in CVD and Non-CVD subjects to understand the atherogenic role of leptin.
CHAPTER IV:

Leptin is known to bind to its receptor in their extracellular domains during signal transduction. It has been of our interest to study the activation of OB-Rb and IL-6 expression in CVD subjects and hence, PBMCs were isolated from CVD subjects. To study the precise role of leptin receptor, we have used a specific inhibitor of OB-Rb (human super-active leptin antagonist).

CHAPTER V:

In order to understand the mechanism of interaction leptin with its receptor, a detailed understanding of their structure is required. Therefore, amino acid sequences of leptin receptor from various species were aligned and the homology between sequences was evaluated using multiple sequence alignment tool clustal w. Conservation of WSXWS motif, box 1 motif, prolines and cysteines both free and those involved in disulphide bonds were analysed using the same tool. Leptin regulates its action through binding to its receptor and during this cysteines have been known to play a major role. Molecular Dynamic (MD) simulation studies on leptin binding to its receptor were performed to evaluate the role of conserved features such as cysteines during leptin recognition interaction.