GENERAL INTRODUCTION

I. Atherosclerosis:

Atherosclerosis is major source of morbidity and mortality in the developed world. Atherosclerotic heart disease, involving the coronary arteries (coronary heart disease), is the most common cause of death, accounting for one-third of all deaths. Atherosclerotic interference with blood supply to the brain (stroke) is the third most common cause of death after cancer. Atherosclerosis also causes a great deal of serious illness by reducing the flow of blood in other major arteries, such as to the kidneys, legs, and intestines.

Atherosclerosis is a vascular disease in which the arterial wall thickens, causing narrowing of the lumen and thus, impairing blood flow. The narrowing is caused by the formation of plaques in the inner lining of the arteries. These plaques consist of low-density lipoproteins, decaying muscle cells, fibrous tissue, clumps of blood platelets, cholesterol, and calcium. These plaques can cause several problems. First, plaques can protrude into the artery, eventually causing a partial or complete obstruction to blood flow. Second, plaques can suddenly rupture, causing a blood clot (thrombus) to form, leading to sudden occlusion of the artery. (This condition is called arterial thrombosis.) Third, plaques can weaken the wall of the artery causing a ballooning out of the artery to form what is called an aneurysm. The rupturing of an aneurysm often produces severe internal bleeding. Atherosclerosis commonly affects the coronary arteries, leading to angina and myocardial infarction (heart attack) but it can also affect the cerebrovascular circulation (brain arteries) leading to stroke. The factors involved in atherosclerosis have been intensively studied during the last decades. Based on the experimental and clinical relationship between hypercholesterolemia and atheroma, atherosclerosis was earlier considered mostly as a lipid disease. Currently

inflammation is now considered to be an important factor in the progression of the disease (Libby 2002).

A complex endothelial dysfunction is induced by elevated and modified LDL, free radicals, hypertension, infectious microorganisms, shear stress, toxins from cigarette smoke or other factors causing a proinflammatory stimulus (Ross 1999, Packard & Libby 2008). The dysfunctional endothelium produces adhesion and vasoactive molecules, cytokines and growth factors and is more permeable to lipoproteins and other plasma components as well as having reduced anticoagulant properties (Ross 1999).

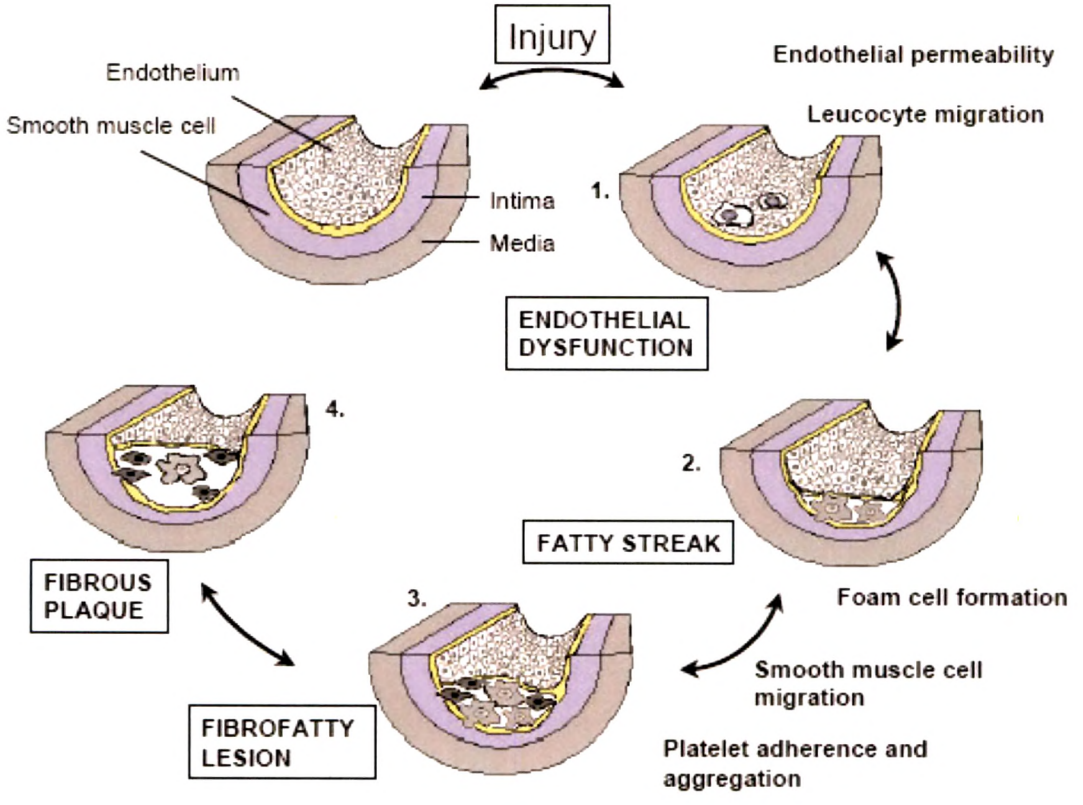


Fig. 1: The progression of atherosclerotic lesion formation according to the response to injury hypothesis of atherosclerosis. 1) The earliest changes occur in endothelium including increased endothelial permeability to lipoproteins, the up-regulation of endothelial adhesion molecules and migration of leukocytes into the artery wall e.g. mediated by Ox-LDL, MCP-1, IL-8 and MCS-F. 2) Fatty streaks consisting initially of cholesterol-ladened monocytes, macrophages (foam cells) and T

lymphocytes are formed. Later, various factors mediate migration of smooth muscle cells, activation of T-cells, formation of foam cells and adherence and aggregation of platelets. 3) As fatty streaks progress to become intermediate and advanced lesions, a fibrous cap is formed covering a mixture of leukocytes, lipid, and debris, which form a necrotic core. These lesions expand their shoulders. 4) Rupture of fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at a site of thinning of the fibrous cap that covers the advanced lesion. (Modified from Ross 1999)

This leads to adhesion of leukocytes to vascular endothelial cells and infiltration of the cells into the intima layer of arterial wall (Ross 1999). The hallmark of early atherosclerotic lesions is the formation of fatty streaks composed of cholesterol laden macrophages called foam cells and T lymphocytes with extracellular lipids

To simplify any discussion of the underlying pathology in these clinical syndromes, coronary artery disease and cerebrovascular disease are commonly referred to by the collective term cardiovascular disease. With respect to the underlying pathology of atherosclerosis, there are a number of environmental and genetic "cardiovascular risk" factors that involved.

II. Factors affecting atherosclerosis

The fundamental underlying cause of atherosclerosis has not been fully established. However, many risk factors that contribute to atherosclerosis have been identified, including:

a) AGE: Age is the most important risk factors among others for predicting incident of cardiovascular disease. This concept is, perhaps, best illustrated if one considers the risk of developing cardiovascular disease over a 10-year period. Based on experience in the United States, the average risk of developing cardiovascular disease for a 30- to 34-year-old male is approximately 3% [Wilson et al., 1998]. This number raises some sevenfold to 21% for a comparable individual aged 60–64 yr. Prediction of coronary heart disease uses risk factor categories [Wilson et al.,

1998]. The exact magnitude of age-related risk compared with other cardiovascular disease risk factors is illustrated by work from the Framingham Heart Study that has resulted in a 14-point scoring system to predict incident 10-yr cardiovascular disease. In this system, increasing risk is characterized by a higher score, and up to 7 points can be attributed to age alone. Thus age is an overriding risk factor for incident cardiovascular disease.

b) GENDER. Numerous observational studies have indicated that males exhibit excess risk for cardiovascular disease compared with age-matched women [Barrett-Connor, 1991]. There has been considerable speculation that estrogens offer a "protective" effect to women, as cardiovascular disease accelerates in women after menopause. However, this speculation has been difficult to substantiate, as the treatment with estrogen has not reduced the incidence of cardiovascular disease of postmenopausal women [Hulley et al., 1998]. Alternatively, some of this apparent protection could be due to the fact that women exhibit relatively higher concentrations of high-density lipoprotein (HDL) cholesterol than do age-matched men. Nevertheless, incident cardiovascular disease is less common in premenopausal women than their age-matched male counterparts.

c) CIGARETTE SMOKING. The notion that cigarette smoking is linked to heart disease dates back to a series of studies that unequivocally linked smoking and the incidence of myocardial infarction [Doll and Hill, 1956; English et al., 1940; Hammond and Horn, 1958]. More recently, the Surgeon General's report estimates that smoking in- creases atherosclerotic disease by approximately 50% and doubles the incidence of coronary artery disease [USA health report, 1989]. There is now considerable confidence that smoking is causally related to coronary artery disease, as smoking cessation is quite effective in lowering the future risk of the disease. In fact, the risk of heart attack in ex-smokers approaches that of nonsmokers in only 2 years [Gaziano, 1996].

d) ROLE OF METABOLIC SYNDROME IN ATHEROSCLEROSIS:

Metabolic syndrome is characterized by obesity, atherosclerosis insulin resistance and hyperinsulinemia, hyperlipidemia, essential hypertension, type 2 diabetes

mellitus, and coronary heart disease. Other features include hyperfibrinogenemia, increased PAI-1, and a proinflammatory state [Fleming, 1999; Rosenson, 2005] This metabolic derangement is also characterized by a low-grade inflammatory state, which is suggested by the high levels of C-reactive protein, tumor necrosis factor-α (TNF-α) and other pro-inflammatory mediators. [Fleming, 1999; Sorrentino, 2005] These key risk factors in the metabolic syndrome have been implicated in endothelial dysfunction such as:

i. SERUM CHOLESTEROL. The association between LDL cholesterol and atherosclerosis has been established based, in part, on an experiment of nature. Familial hypercholesterolemia is an autosomal dominant disorder that affects approximately 1 in 500 persons from the general population. Heterozygotes for this disease manifest a two- to five fold elevation in plasma LDL cholesterol that is due to a functional impairment of the LDL receptor, resulting in a defect in LDL clearance. Homozygotes for this disorder demonstrate a four- to sixfold elevation in plasma cholesterol that produces precocious atherosclerosis. In heterozygotes, 85% of individuals have experienced a myocardial infarction by the age of 60, and this age is reduced to 15 yr in patients homozygous for the disease [Gotto and Farmer, 1988]. In the general population, the cardiovascular disease risk from increased LDL cholesterol is supported by observations that cholesterol-lowering therapy greatly diminishes the clinical manifestations of atherosclerosis, particularly since the advent of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (i.e., statins) that profoundly lower LDL cholesterol [Gotto and Grundy, 1999]. In contrast to the situation with LDL cholesterol, the relation between HDL cholesterol and atherosclerosis is an inverse one [Gordon et al., 1977]. The causal nature of this association is also supported by an experiment of nature, Tangier disease (Oram, 2000) in which autosomal codominant condition is characterized by the essential absence of HDL cholesterol levels due to a defect in the ATP binding cassette transporter-1 [Bodzioch et al., 1999; Marcil et al., 1999; Rust et al., 1999] that impairs cholesterol efflux from cells [Lawn et al., 1999]. Tangier patients demonstrate a tissue cholesterol loading syndrome, characterized by large tonsils,

neuropathy, and premature coronary artery disease in some kindreds [Oram, 2000]. Thus considerable evidence supports the inverse relation between coronary artery disease and serum levels of HDL cholesterol.

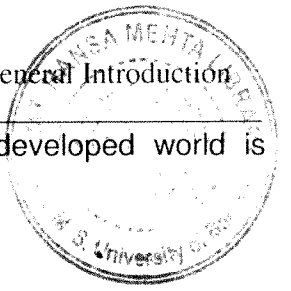
ii. HYPERTENSION. Hypertension is defined as a systolic blood pressure in excess of 140 mmHg or a diastolic blood pressure above 90 mmHg (Pressure Report, USA). The current estimates indicate that the elderly are particularly predisposed to hypertension, with up to 75% of people over 75 years of age qualifying for this diagnosis (Pressure Report). There appears to be an approximately linear relation between blood pressure elevation and the increased incidence of atherosclerotic vascular disease [MacMahon et al., 1990].

Evidence from animal models as well as patients, have indicated that factors regulating hypertension, an established major risk factor for coronary artery disease, has been suggested to exert pro-inflammatory actions through the increased expression of several mediators such as leukocyte adhesion molecules, chemokines, specific growth factors, heat shock proteins, endothelin-1, and angiotensin. Higher C reactive protein levels in patients with hypertension shows low-grade inflammation. In addition angiotensin II (AII) has vasoconstrictor properties and can instigate intimal inflammation. For example, AII elicits the production of superoxide anion, a reactive oxygen species, from arterial endothelial cells and SMCs [Griendling et al., 1997] AII can also increase the expression by arterial SMCs of proinflammatory cytokines such as interleukin (IL)–6 and MCP-1 and of the leukocyte adhesion molecule VCAM-1 on endothelial cells [Kranzhofer et al., 1999; Hernandez-Presa et al., 1997]. In experimental models as well as human studies of atherosclerosis, angiotensin converting enzyme inhibitors or AII receptor blockers has demonstrated the ability to prevent or reverse the progression of atherosclerosis, which was in part associated with decreased expression of inflammatory mediators and improve endothelial functions (Schiffrin, 2002; Virdis and Schiffrin, 2003).

iii. DIABETES MELLITUS. Approximately 17 million people in the United States, or 6.2% of the population, carry the diagnosis of diabetes mellitus [Cowie et al., 2003]. In patients with diabetes, the risk of coronary atherosclerosis is three- to fivefold greater than in nondiabetics despite controlling for other risk factors [Bierman, 1992]. A number of other known risk factors for coronary disease such as hypertension and abnormal lipids are also more common in diabetics than the general population [Bierman, 1992], but despite this association, no more than 25% of the excess coronary atherosclerosis risk from diabetes can be attributed to these known risk factors [Nishigaki et al., 1981].

The hyperglycemia associated with diabetes can lead to modification of macromolecules, for example, advance glycation end products (AGE) [Schmidt et al., 1999]. These AGE-modified proteins by binding surface receptors such as RAGE (receptor for AGE) can augment the production of proinflammatory cytokines and other inflammatory pathways in vascular endothelial cells. Beyond the hyperglycemia, the diabetic state promotes oxidative stress mediated by reactive oxygen species and carbonyl groups [Baynes et al., 1999]. Increased circulating cytokines and growth factors are strongly related to inflammation, which is currently viewed by many researchers as a possible key player in the etiology of atherosclerosis and diabetes [Ross et al., 1999; Yuan et al., 2001]. There are reports of the elevation in adhesion molecules and von Willebrand factor (vWF) in diabetes and their association with the development of atherosclerosis [Gearing et al., 1992; Campbell et al., 1994; Morris et al., 1995].

iv. OBESITY. There is now a growing appreciation that obesity, defined as an excess body weight with an abnormal high preponderance of body fat, is a condition that increases the incident risk of cardiovascular disease. The exact mechanism(s) to explain this phenomenon, however, are controversial. A number of other risk factors for cardiovascular disease, such as hypertension, low HDL cholesterol, and diabetes mellitus, often coexist with obesity [Wilson et al., 1999]. This relation between obesity and cardiovascular disease has become of



considerable concern as the prevalence of obesity in the developed world is increasing at an alarming rate.

III. Role of inflammation in atherosclerosis

Atherosclerosis is generally considered to be a disease associated with lipid disorders but recent advances in basic science have established a fundamental role for inflammation in mediating all stages of this disease from initiation through progression and, ultimately, the thrombotic complications of atherosclerosis.

In a variety of animal models as well as in patients with atherosclerosis, signs of inflammation occur hand-in-hand with incipient lipid accumulation in the artery wall. For example, blood leukocytes, mediators of host defenses and inflammation are localized in the earliest lesions of atherosclerosis [Libby et al., 2002]. The basic science of inflammation biology applied to atherosclerosis has afforded considerable new insight into the mechanisms underlying this recruitment of leukocytes. In general the normal endothelium does not support binding of white blood cells. However, early after initiation of an atherogenic diet, patches of arterial endothelial cells begin to express selective adhesion molecules on their surface that bind to various classes of leukocytes. In particular, vascular cell adhesion molecule-1 (VCAM-1) binds precisely the types of leukocytes found in early human and experimental atheroma, the monocyte and T lymphocyte [Li et al., 1993]. Disturbed blood flow can augment the production of certain leukocyte adhesion molecules (eg, intercellular adhesion molecule-1 [ICAM-1]) [Nagel et al., 1994]. Once adhered to the endothelium, the leukocytes penetrate into the intima. Recent research has identified candidate chemoattractant molecules like monocyte chemoattractant protein-1 (MCP-1) responsible for this transmigration of monocytes into the intima at sites of lesion formation. [Gu et al., 1998; Boring et al., 1998]. A family of T-cell chemoattractants may likewise attract lymphocytes into the intima [Mach et al., 1999]. Once resident in the arterial wall, the blood-derived inflammatory cells participate in and perpetuate a local inflammatory response. The macrophages express scavenger receptors for modified lipoproteins, permitting them to ingest lipid and become foam cells. In addition to MCP-1, macrophage

colony-stimulating factor (M-CSF) contributes to the differentiation of the blood monocyte into the macrophage foam cell [Smith et al., 1995; Qiao et al., 1995]. T cells likewise encounter signals that cause them to elaborate inflammatory cytokines such as gamma-interferon and tumor necrosis factor [TNF]–alpha that in turn can stimulate macrophages as well as vascular endothelial cells and SMCs [Hansson and Libby, 1996]. As this inflammatory process continues, the activated leukocytes and intrinsic arterial cells can release fibrogenic mediators, including a variety of peptide growth factors that can promote replication of SMCs and contribute to elaboration by these cells of a dense extracellular matrix characteristic of the more advanced atherosclerosis lesion [Ross, 1999]. The activated macrophage and lymphocytes abundant in atheroma can produce proteolytic enzymes capable of degrading the collagen that lends strength to the plaque's protective fibrous cap, rendering that cap thin, weak, and susceptible to rupture. Hence inflammatory processes not only promote initiation and progression of atherosclerosis but also cause acute myocardial infarction arises because of a physical disruption of the atherosclerotic plaque.

The role of inflammatory mediators in atherogenesis and the determination of plaque vulnerability, attention have focused on whether plasma levels of inflammatory markers can help predict individuals at increased risk for plaque rupture [Ridker, 1999]. Candidate markers include P-selectin, sICAM-1, IL-6, TNF-alpha, and CRP. IL-6 and TNF-alpha occupy a central role in the amplification of the inflammatory cascade. Of all the plasma markers of vascular inflammation, CRP has been the most extensively investigated in clinical studies. A recent analysis from the WHS sought to compare the risk associated with baseline levels of CRP with other inflammatory and lipid markers of risk (Ridker et al., 2000). Furthermore, even women with low cholesterol levels were found to be at increased risk if CRP or other inflammatory biomarker levels were high. In a subgroup analysis performed on women with LDL,130 mg/dL, women with increased levels of markers of inflammation were found to be at increased risk for subsequent cardiovascular events, an effect that was strongest for CRP (Blake and Ridker, 2001).

IV. The Adipose Tissues: A Key Integrator of Various Metabolic Diseases

The adipose tissue was once thought to be an inert tissue used solely for storage of excess energy. Identification of many bioactive proteins secreted by adipose tissue assigned the adipocyte a more important and central role in the pathophysiology of insulin resistance and the Metabolic Syndrome. This was further underscored by the recognition that many of the so-called adipokines have the ability to influence lipid and glucose metabolism, not only locally in the adipose tissue, but also in the skeletal muscle and liver. In addition, certain adipokines were found to have effects on appetite regulation or to have an important impact on inflammation and vascular biology.

The adipose tissue in mammals consists of 2 types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT and BAT share many metabolic characteristics but there are few differences in their functions. WAT mainly stores excess energy for subsequent needs, BAT functions as an immediate source of energy. In rodents, it is well established that BAT plays an important role in preventing and reducing obesity through increased energy dissipation and heat production. However, the role of BAT is unclear in man. It is well- known that newborns are provided with a considerable amount of BAT which becomes drastically reduced shortly after birth. There is evidence that brown adipocytes are dispersed in the WAT in adult life, with a calculated presence of 1 brown adipocyte for every 100 to 200 white adipocytes [Oberkofler et al., 1997]. Furthermore, the expression of genes characteristic of the brown adipocyte phenotype in human adipose tissue has been shown to correlate negatively with obesity and insulin sensitivity [Semple et al., 2004; Hammarstedt et al., 2003]. Interestingly, treatment with the thiazolidinediones (TZD) has been shown to induce a brown adipocyte phenotype in rodent white adipocytes [Wilson-Fritch et al., 2003].

The WAT in man consists of subcutaneous and visceral depots. The importance of each depot for the dysmetabolic state associated with the Metabolic

Syndrome has been extensively discussed. The visceral depot has been receiving most attention because it is considered to be more metabolically active and because it delivers released factors to the portal venous system and, thus, can directly have an impact on the liver. However, the amount of subcutaneous adipose tissue generally exceeds the visceral adipose tissue by 3 to 4 times [Chowdhury et al., 1994]. It seems that these depots can interact in a coordinate and compensatory manner and both should be considered important for the obesity-related complications [Yang et al., 2007]. The adipose tissue does not only consist of preadipocytes and adipocytes, but also of other cell types such as fibroblasts, vascular cells, inflammatory cells, and mesenchymal stem cells. The mesenchymal stem cells are an important reservoir for recruitment of new preadipocytes within an expanding adipose tissue. Inability to recruit and differentiate new adipocytes is likely to be a key factor regulating degree of adipocyte enlargement in obesity and the associated ectopic lipid accumulation in skeletal muscle and liver when the existing adipocytes are not able to store excess lipids (Gustafson et al., 2007). Adipocyte cell size has been shown to be an independent predictor of insulin resistance and risk for type 2 diabetes and to correlate with different aspects of the Metabolic Syndrome [Weyer et al., 2000; Lundgren et al., 2007]. The adipocyte is the only cell whose size may vary dramatically; around 10-fold in diameter and, thus, 1000-fold in volume! As discussed above, the size of the adipocytes influences the degree of inflammation in the adipose tissue (Figure-2) as well as the rate of lipid mobilization and pattern of adipokine secretion. What initiates the proinflammatory process associated with obesity and adipose cell enlargement is not known. MCP-1 expression and secretion is elevated in large adipocytes [Skurk et al., 2007] in obesity and reduced after weight reduction [Christiansen et al., 2005]. TNF alpha and IL-6 are important inflammatory molecules that are secreted by adipocytes and associated with obesity and insulin resistance.

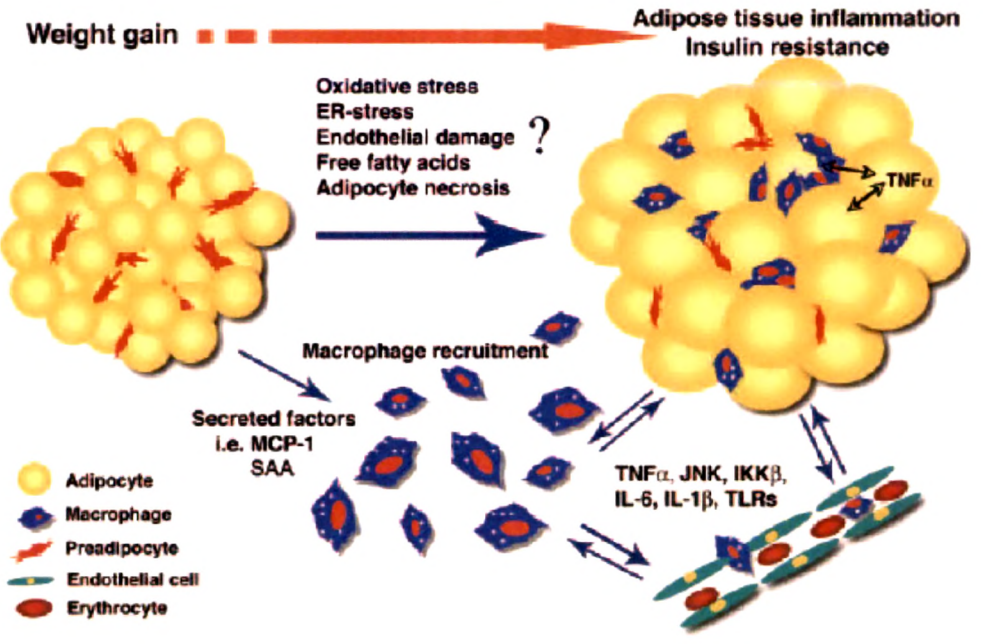


Fig. 2: Schematic representation of the alterations induced in the adipose tissue in obesity. Adipose cell enlargement seems to play a pivotal role, but the precise mechanisms involved are still unclear [Gutafson et al., 2007].

TNF alpha is one of the cytokines whose expression and secretion by the adipose tissue in vitro is elevated in obese subjects [Hotamisligil et al., 1995; Kern et al., 2001]. However, adipocyte TNF alpha does not seem to be cleaved and released by the adipose cells to the systemic circulation in vivo [Mohamed-Ali et al., 1997]. The increased level of TNF alpha in the adipose tissue in obesity is mainly attributable to the infiltrating macrophages. TNF alpha is a potent inducer of cytokine and chemokine expression, and secretion by adipocytes and the expression of TNF alpha, IL-6, and other proinflammatory molecules is positively correlated to adipocyte cell size [Sopasakis et al., 2004].

Adipogenesis is the process by which committed precursor cells (preadipocytes) differentiate into mature adipocytes. Adipocyte differentiation includes morphological changes, cell arrest, lipid accumulation, and acquirement of insulin sensitivity and adipokine expression. More recently, studies of adipogenesis

have proceeded with the hope that manipulation of this process in humans might one day lead to a reduction in the burden of obesity and diabetes. Adipose cell differentiation is under transcriptional control, and induction of differentiation starts a coordinated cascade of events involving the early transcription factors C/EBPβ, C/EBP, and PPAR which then are followed by expression of C/EBP [Rosen and Spiegelman, 2000; Rosen, 2006; Tang et al., 2003; Rosen, 2005; Otto and Lane, 2005] a key transcription factor for full terminal differentiation. However, more than 100 different transcription factors, coactivators, and repressors are expressed in preadipocytes, many of which are necessary for cell differentiation and induction of markers of differentiated adipocytes, eg, lipoprotein lipase (LPL), fatty acid synthase, aP2, adiponectin, GLUT4, and perilipin [Gregoire et al., 1998]. Increased tissue levels of both TNF alpha and IL-6 are detrimental to the normal preadipocyte development and differentiation. Normal adipogenesis is dependent on the inhibition of Wnt signaling. The Wnt-family consists of a number of secreted glycosylated lipoproteins where Wnt10b is expressed in preadipocytes but not in adipocytes. Maintaining Wnt signaling in mesenchymal precursor cells promotes osteoblastogenesis and myogenesis and suppresses adipocyte differentiation [Bennett et al., 2005; Ross et al., 2000]. Overexpression of Wnt10b in adipocytes in transgenic mice reduces the white adipose tissue by around 50% [Longo et al., 2004]. Other Wnts, like Wnt5a, is transiently induced during differentiation and promotes adipogenesis [Kanazawa et al., 2005]. The downstream mediator of the canonical Wnt pathway is beta-catenin. During initiation of preadipocyte differentiation, beta-catenin is sustained in the nucleus for up to 48 hours but then undergoes phosphorylation and degradation before the cells enter terminal differentiation. This coincides with the induction of the adipogenic transcription factors C/EBP alpha and PPAR gamma [Moldes et al 2003]. Wnt-expressing preadipocytes fail to induce PPAR gamma. However, addition of PPAR gamma ligands, the thiazolidinediones, rescues the differentiation and stimulates the degradation of beta-catenin [Moldes et al., 2003]. The cross-talk between inflammation and adipocyte differentiation is further underscored by the recent finding that differentiation of 3T3-L1 preadipocytes in the presence of IL-6 sustained

beta-catenin and the cells maintained a fibroblast-like appearance and accumulated less lipids (Gustafson et al., 2007). In contrast, addition of TNF alpha totally inhibited adipocyte differentiation, including lipid accumulation, and maintained Wnt signaling [Gustafson et al., 2006]. Furthermore, in mature adipocytes, both TNF alpha and IL-6 impair insulin signaling through different mechanisms including decreased tyrosine phosphorylation of key signaling molecules, increased inhibitory serine phosphorylation, and downregulation of the expression of several proteins in the insulin signaling pathway [Rotter et al., 2003; Aguirre et al., 2002] These effects lead to insulin resistance, increased lipolysis and reduced glucose uptake by the adipose tissue.

a. Obesity leads to insulin resistance, diabetes, Dyslipidemia and atherosclerosis

It is well known that obesity is a major health problem that is reaching epidemic proportions, especially in the Western countries, though the prevalence is increasing rapidly also in the developing countries. The results from the latest national FINRISK Study 2007 survey, carried out every five years by the National Institute for Health and Welfare using independent, random and representative population samples from different parts of Finland, have demonstrated that Finns continue to gain weight. Only 33% of Finnish men and 48% of Finnish women can be considered as normal weight and 20% of both genders are obese. Similar trends are observed all over the world. Overweight and obesity can be defined on the basis of body mass index (BMI) which is determined by weight (kg) divided by height squared (m2). An individual with a BMI between 25 and 29.9 is considered as overweight and a person with a BMI ≥30 as obese. However, this might be an oversimplified definition, since it fails to consider fat distribution [Sowers 2003]. The location of adipose tissue may be essential in defining the associated risk, since an excess of abdominal fat is most clearly associated with metabolic risk factors. Hence, the best way to estimate obesity might be the measurement of waist circumference. Measuring percent body fat would provide a more accurate estimate

of the proportion of fat mass in relation to body weight, but, for the time being, it is rarely used since there is no practical and rapid means to measure it reliably in clinical practice [Grundy 2004].

It is well established that obesity is associated with an increased incidence of atherosclerotic CVD. The reason for this association is that obesity is usually accompanied by risk factors for atherosclerotic CVD, like atherogenic dyslipidemia, insulin resistance, a proinflammatory state, a prothrombotic state and hypertension (Figure-2). A significant part of increased prevalence of CVD in obesity is mediated by T2D [Grundy 2004].

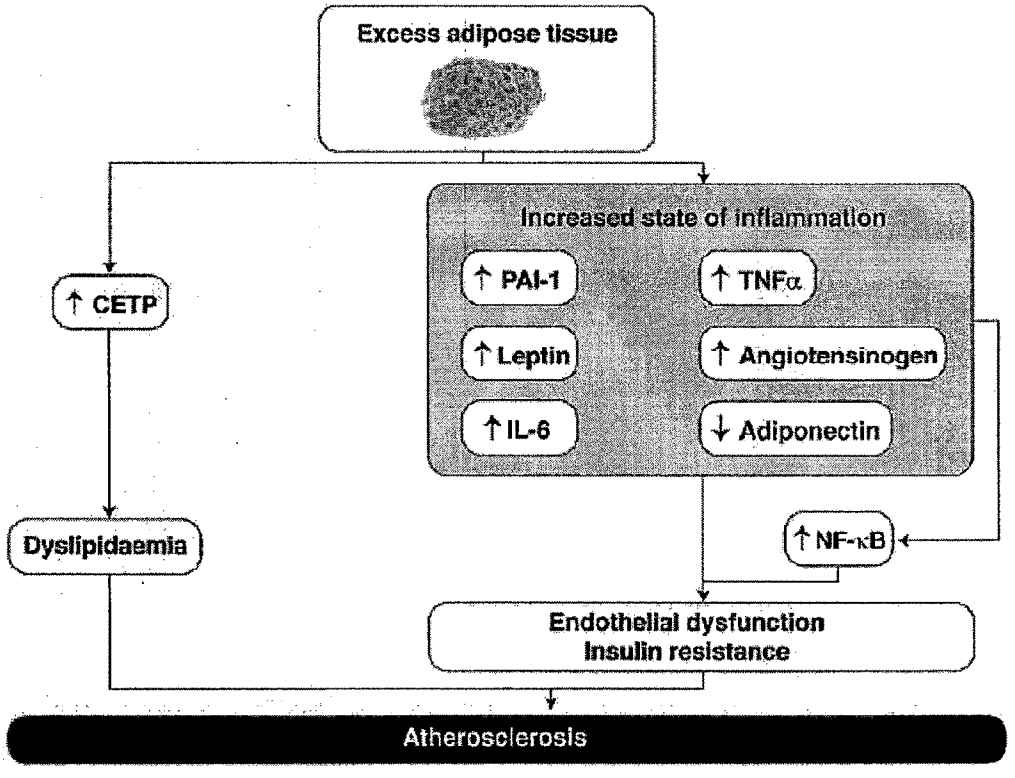


Fig.3: Obesity associated with a state of low level systemic inflammation and dyslipidaemia leading eventually to atherosclerosis

i. The link between obesity and type 2 diabetes

Insulin resistance is associated with both obesity and T2D. In this state, the pancreatic β-cells are not able to compensate for the decreased insulin action by increasing their secretion of insulin. The abnormally high plasma NEFA concentrations are probably the single most critical factor modulating insulin sensitivity [Kahn et al. 2006]. The excess NEFAs lead to insulin resistance in muscle and liver which further promotes the release of NEFA, as the insulin levels are high but not sufficient to suppress adipose tissue lipolysis [Grundy 2004]. In this way, a vicious cycle is created. There are various reasons why the hyperglycemia associated with T2D may promote atherosclerosis. These include harmful effects on the vascular wall that are caused by the activation and adhesion of monocytes, inhibition of NO production by endothelial cells, and the stimulation of VSMC proliferation.

Adipose tissue is a prominent source of CETP, and the activity and mass of CETP is increased in obesity [Arai et al., 1994]. CETP transfers cholesteryl esters from HDL to VLDL and LDL, and triglycerides from VLDL to LDL and HDL [Barter et al., 2003]. When the activity of CETP is high and the level of VLDL particles is increased (resulting from increased NEFA influx into the liver) HDL cholesteryl esters are preferentially transferred to VLDL, increasing the cholesterol content and making them more atherogenic. In addition, the HDL particles become smaller and denser, and are cleared from the circulation more rapidly. CETP also interacts with hepatic lipases that promote the formation of small, dense LDL particles. The net effect of increased CETP activity is the pattern of dyslipidaemia often referred to as the 'atherogenic lipid triad' seen in patients with metabolic syndrome or type 2 diabetes—increased triglyceride levels, decreased HDL levels and a greater proportion of small, dense LDL. This pattern of dyslipidaemia is particularly atherogenic in particular small, dense LDL particles have a greater propensity to form oxidized LDL and are less rapidly cleared from the circulation [Nesto, 2005].

b. Adipose Tissues and its role of inflammation

Macrophages are known to be crucial contributors to inflammation, but more recently, it has been recognized that adipocytes demonstrate significant intrinsic

inflammatory properties as well. Like macrophages, the adipocyte is exquisitely sensitive to infectious disease agents and cytokine-mediated inflammatory signals; it expresses a host of receptors, enabling it to sense the presence of pathogens and inflammation and on stimulation of these receptors, it activates multiple inflammatory signal transduction cascades, and induces and secretes a number of potent inflammatory cytokines and acute phase reactants. Adipocytes are sensitive to the effects of tumor necrosis factor-alpha (TNF-alpha), which, through its p55 and p75 TNF receptors, stimulates NF-kB, extracellular signal-regulated kinase, and p38 mitogen-activated protein kinases PI-3 kinase and jun- N-terminal kinase cascades [Ryden et al., 2002]. The mammalian toll-like lipopolysaccharide (LPS) receptor TLR4 is expressed in tissue and in vitro cultured adipocytes. When stimulated with endotoxin, these receptors activate p65/p50 and p68/p52 NF-kB signal transduction pathways [Berg et al., 2004]. In turn, these pathways induce the expression of inflammatory mediators such as interleukin-6 (IL-6), TNF-alpha, and serum amyloid A3 (SAA3). At the same time, endotoxin further sensitizes the adipocytes to infectious pathogens, inducing expression of the toll-like receptor for fungal wall components (TLR2). It has been demonstrated that adipocytes are responsive to IL-1beta, IL-4, IL-6, IL-11, interferon-gamma (IFN-gamma), and fungal cell wall components, with a downstream activation of inflammatory signaling cascades [Rajala and Sherer, 2003; Lin et al., 2000]. These inflammatory signaling pathways are differentially regulated during adipogenesis; adipocytes differentiation entails a dramatic induction of expression of NF-kB subunits p65, p68, p52, and the inhibitor of NF-kB (IkB), as well as changes in NF-kB constitutive nuclear localization/promoter binding, constitutive IL-6 secretion, and modulation of LPS responsiveness [Berg et al., 2004]. The induction of p52 and p68, their constitutive nuclear localization and increased activity, and a decrease in LPS-inducible p65/p50 activity after differentiation is reminiscent of activated dendritic cells, suggesting a similar immunomodulatory switch and inflammatory function for these 2 cell types [Charriere et al., 2003]. In response to infectious and inflammatory signals, adipocytes have been shown to induce expression and secretion of several acute phase reactants and mediators of inflammation, including TNF-alpha,

plasminogen activator inhibitor-1 (PAI-1), IL-1beta, IL-6, IL-8, IL-10, and IL-15, leukemia inhibitory factor, hepatocyte growth factor, SAA3, macrophage migration inhibitory factor, haptoglobin, complement factors B, D, C3, prostaglandin E2, and potential inflammatory modulators such as leptin, adiponectin, and resistin (Fain et al., 2004). Although many of these activities are restricted to autocrine and paracrine effects, some of these cytokines secreted from adipocytes and adipose-resident macrophages make significant contributions to systemic inflammation.

i. Macrophage accumulation in Adipose tissue & its role in disease pathophysiology

It has been shown that adipocytes possess many features of immune cells and adipocyte precursors have phagocytic capacity and can be converted into macrophage-like cells in response to certain stimuli [Cousin et al 1999; Charriere et al 2003]. Two studies in 2003, which used transcription profiling approach to compare adipose tissue from lean and several rodent obesity models, or adipose tissue from obese animals before and after treatment with compounds of the thiazolidinedione class of anti-diabetic agents, have showed that the majority of genes significantly upregulated in obese adipose tissue are macrophage and inflammatory genes, which can be repressed by rosiglitazone [Weisberg et al 2003; Xu et al 2003]. By separating adipocytes from stromal-vascular cells and histological examination, these studies revealed massive macrophage accumulation in adipose tissue in the obese state. Bone marrow transplant study further demonstrated that these macrophages are predominantly infiltrated, rather than converted from local preadipocytes. Activated macrophages are known to secrete a variety of pro-inflammatory cytokines and chemokines. In these two studies [Weisberg et al 2003; Xu et al 2003], isolated stromal-vascular cells, the fraction containing infiltrated macrophages, are demonstrated to be the predominant source for producing proinflammatory molecules in comparison to adipocytes, including TNFα, MCP-1, IKKβ, IL-1β, and inducible nitric oxide synthase (iNOS). These results suggest that infiltrated macrophages in adipose tissue in state of obesity are likely an important source contributing to elevation of

circulating inflammatory marker proteins. Blood mononuclear cells (MNCs) in the obese state are also reported to be present in a proinflammatory state, as reflected by increased nuclear factor kappa-B (NF-κB) binding activity, decreased IκB content and increased transcription of NF-κB controlled proinflammatory genes, indicating that inflamed circulating MNCs may also contribute to elevation of circulating inflammatory marker proteins as well [Ghanim et al 2004].

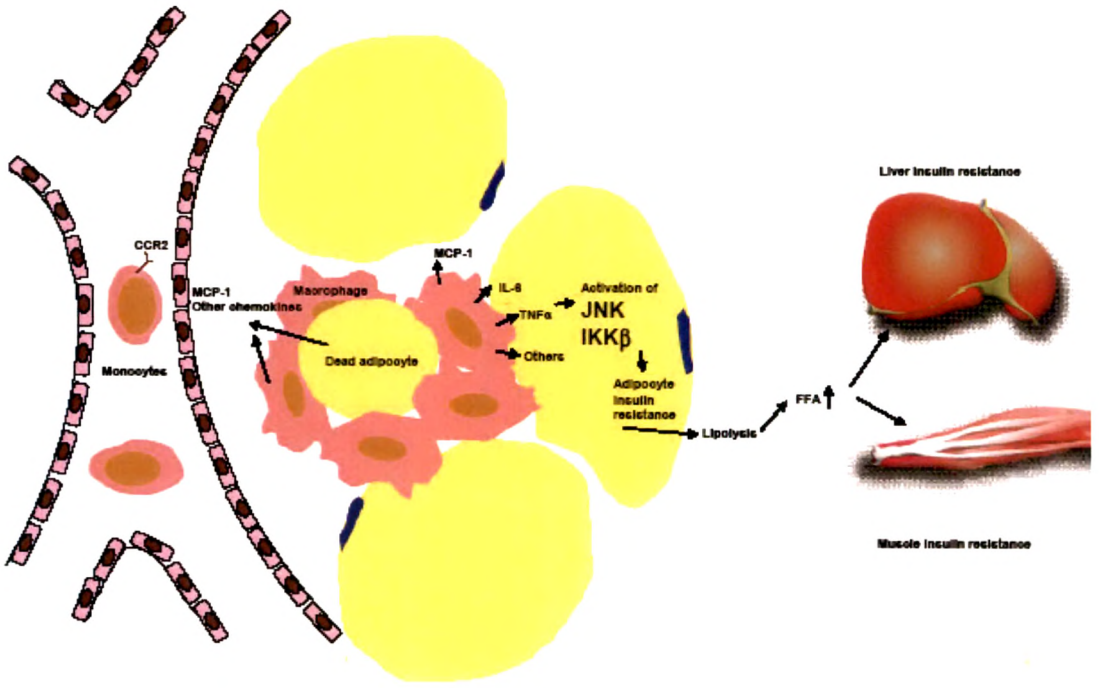


Fig. 4: Mechanism of adipose macrophage infiltration and the role of adipose inflammation in systemic insulin resistance. Accumulation of triglycerides in adipocytes causes increased production of MCP-1 and the other monocyte chemotactic factors, which attract circulating monocytes to migrate into adipose tissue. These inflamed monocytes convert into activated macrophages and secrete a variety of proinflammatory factors that can impair insulin signaling in adipocytes. Dysregulated adipocytes lipolysis contributes to elevation of circulating FFA level which subsequently induces insulin resistance in other tissues such as liver and muscle (Jiao and Xu, 2008).

The triggering factors for macrophage infiltration into adipose tissue and the roles of infiltrating macrophages are still not well understood. One speculation is

that enlargement of fat cells, which commonly occurs in obese state at relatively early stage, may trigger the initial secretion of critical monocyte chemotactic factors.

Once macrophages infiltrate into adipose tissue and get activated, they become the major source of inflammatory molecules and continue to attract more macrophages to further amplify the harmful cycle. The prototype monocyte chemotactic factor, MCP-1, has been under intensive investigation as such a candidate for recruiting macrophages into adipose tissue in obesity since its expression is upregulated in adipose tissue in early stage of diet-induced obesity. However, controversial results have been reported for the role of MCP-1. One publication showed that mice deficient in MCP-1 have partially reduced adipose tissue macrophage accumulation upon high fat diet and are accompanied with improved insulin sensitivity and hepatic steatosis [Kanda et al 2006]. Acute expression of a dominant-negative MCP-1 mutant also ameliorated insulin resistance in both diet-induced obese mice and db/db mice [Kanda et al 2006]. Another publication demonstrated no change of adipose macrophage content in MCP-1 knock out mice [Inouye et al 2007]. These results suggest the roles of other monocyte chemotactic factors. Nevertheless, reports from two independent laboratories reported that mice overexpressing MCP-1 transgene in adipose tissue under the promoter of adipocyte fatty acid binding protein have increased macrophage accumulation in adipose tissue and are insulin resistant [Kamei et al 2006; Kanda et al 2006]. The major receptor of MCP-1 is CCR2, which also mediates signaling of other monocyte chemotactic factors such as MCP-2 and MCP-3. Macrophages recruited into adipose tissue in obese state have increased expression of CCR2. Mice deficient in CCR2 have a lean phenotype when fed on a high fat diet due to reduced food intake. When high fat-diet fed CCR2 deficient mice and control mice are matched for adiposity, partially decreased adipose macrophage content was observed. These mice have improved inflammatory profile in adipose tissue, along with improved systemic insulin sensitivity and ameliorated hepatic steatosis [Weisberg et al 2006]. DIO mice treated with CCR2 antagonist have a 28% reduction of adipose macrophage content and have

improved hyperglycemia [Weisberg et al 2006]. These data suggest that CCR2 plays a role in adipose tissue macrophage recruitment in obese state and also point out the potential role(s) of other chemokine receptor(s) since absence of CCR2 only partially blocked macrophage infiltration upon high fat diet. Macrophages are phagocytic cells that clear pathogens, dead cells and cell debris in innate immunity and wound healing. It has been shown that adipocyte death increases significantly in obese humans and mice via the mechanism of necrosis. The majority of infiltrated macrophages in adipose tissue are localized around dead adipocytes to form crown like structure (CLS) [Cinti et al., 2005]. This scenario highly resembles atherosclerosis, which is caused by macrophage infiltration into aorta to clean excessive cholesterol and subsequent formation of foam cells. Interestingly, frequency of adipocyte death in epididymal fat pad correlates with the extent of macrophage infiltration [Strissel et al., 2007]. These data indicate that infiltrated macrophages are involved in clearance of cell debris, leaked lipids and perhaps are important for adipose tissue remodeling. Macrophages are heterogeneous in function and their properties and activation state are dependent upon local environment factors. Two polarization states, M1 or "classically activated" and M2 or "alternatively activated", have been defined for macrophage activation. M1 macrophages have high proinflammatory cytokine expression and produce reactive oxygen species; M2 macrophages express high levels of anti-inflammatory cytokines, such as IL-10. IL-10 can selectively inhibit transcription rates of inflammatory gene and activate STAT3 to attenuate inflammatory signals induced by TNFα. Macrophages infiltrated into adipose tissue in obese state possess features of M1 macrophages and more importantly, macrophages in CLS express protein of TNFα and IL-6 [Lumeng et al., 2007; Strissel et al., 2007]. In contrast, resident macrophages in adipose tissue of lean mice have many features of M2 macrophages, including expression of M2 macrophage characteristic genes such as Ym1, arginase 1, and IL-10. High fat diet is sufficient to promote macrophage phenotypic switch from anti-inflammatory M2 polarization to proinflammatory M1 polarization. Peroxisome proliferator activated receptor gamma (PPAR gamma), the molecular target for the anti-diabetic thiazolidinedione class of compounds, has

been reported to play a key role in maturation of alternatively activated macrophages with anti-inflammatory properties [Bouhlel et al., 2007]. Mice deficient of PPAR gamma in myeloid cells have impaired activation of M2 macrophages, as demonstrated by decreased expression and activity of arginase, hallmarks of alternatively activated macrophages [Hevener et al., 2007; Odegaard et al., 2007]. These mice have lower content of alternatively activated macrophages, increased local inflammation in adipose tissue, impaired insulin signaling in adipose tissue, muscle, and liver on chow diet. These mice are also prone to the development of obesity-related insulin resistance. These results indicate that phenotypic switch from M2 to M1 macrophages are critical for impairing insulin sensitivity.

Transient neutrophil infiltration into adipose tissue in obesity is also reported, which occurs 3 and 7 days upon high fat diet in epididymal but not subcutaneous fat and disappears on all subsequent times points [Elgazar-Carmon et al., 2008]. A potential chemotactic factor for neutrophils infiltration has been proposed to be IL-8, which can be secreted by adipose tissue in obesity. Neutrophils have been demonstrated to directly adhere to adipocytes and this process can be influenced by cations and activation state of neutrophils. The time course of adipose tissue neutrophils infiltration precedes macrophage infiltration, which does not start increase till 8 weeks upon high fat diet [Strissel et al., 2007]. Neutrophil infiltration reflects acute inflammation while macrophage infiltration symbols chronic inflammation. Activated neutrophils can release ROS and TNFα, whether infiltrated neutrophils contribute to adipose inflammation remains a question to be studied.

ii. Key inflammatory molecules in white adipose tissue

Intensive investigations with various animal models deficient in key molecules in the inflammatory pathway further provided solid evidence for the causative role of inflammation in the development of obesity-related insulin resistance. The first proof of principle animal model is TNF alpha-deficient mice. In the absence of TNFα, mice are partially protected from development of hyperlipidymia, hyperglycemia and hyperinsulinemia without an effect on body weight in obesity models induced by high fat diet, deficiency of leptin, or gold-thioglucose injection [Uysal et al., 1997;

Ventre et al., 1997]. Increased tyrosine phosphorylation of insulin receptor was observed in both adipose tissue and muscle in obese mice deficient in TNF alpha. In addition, KB-R7785 a novel MMP inhibitor that inhibits TNFα production, can improve insulin sensitivity in obese/diabetic KKAy mice [Morimoto et al., 1997]. These in vivo data support a role of TNF alpha as a candidate mediator of obesity-induced insulin resistance but also indicate the involvement of other factors since the protection is partial. TNFα signals through two receptors, p55 and p75. The role of TNF alpha receptors in mediating the effect of TNF alpha on obesity-related insulin resistance is controversial in two independent studies, which might be attributable to differences in genetic backgrounds [Schreyer et al., 1998; Uysal et al., 1998].

Recently, the **c-Jun NH2-terminal kinase (JNK)** has appeared as a vital regulator in obesity and insulin resistance. Both TNF alpha and FFA are potent stimulators for JNK activation. Indeed, JNK activity is elevated in liver, adipose tissue and muscle in both diet-induced obese mice and ob/ob mice [Hirosumi et al., 2002]. Interestingly, different JNK isoforms seem to play distinct roles. JNK1 defi cient mice have decreased adiposity and adipocyte size, increased serum adiponection and lowered serum resistin concentrations, lowered glucose and insulin concentrations, enhanced insulin signaling in the liver and improved whole body insulin sensitivity upon high fat diet [Hirosumi et al., 2002]. Absence of JNK1 also partially protected ob/ob mice from developing hyperglycemia and hyperinsulinemia [Hirosumi et al., 2002]. In contrast, deficiency of JNK2 does not have any influence on diet-induced insulin resistance. These results demonstrate that JNK1 accounts for the majority of increased JNK activity in insulin target tissues and is an important regulator in obesity-related insulin resistance.

IKK beta, the master regulator of inflammation which can also be activated by TNFalpha and FFA, is another important serine kinase involved in the development of obesity-related insulin resistance. Homozygous IKK beta deficiency is lethal due to increased liver apoptosis but heterozygous IKK beta+/– mice appear normal. Lowered fasting glucose and insulin concentrations were observed in IKK beta+/– mice on both standard diet and high-fat diet compared to wild-type

littermates [Yuan et al., 2001]. Furthermore, absence of one IKK beta allele is sufficient to lower blood glucose and plasma FFA levels as well as improve glucose tolerance in ob/ob mice [Yuan et al., 2001]. Arkan and colleagues (2005) further selectively deleted IKK beta in hepatocytes or myeloid cells and showed that IKK beta deficiency in hepatocytes locally protected liver from development of obesity-related insulin resistance, but fat and muscle still develop insulin resistance upon high fat diet. Consistent with these results, transgenic mice selectively expressing the constitutively active form of IKK beta in the liver have increased hepatic production of proinflammatory cytokines, which caused profound hepatic insulin resistance [Cai et al., 2005]. In addition, these proinflammatory cytokines also cross talk with other tissues and lead to hyperglycemia, and modest systemic insulin resistance [Cai et al., 2005]. Interestingly, specific deletion of IKK beta in myeloid cells of diet-induced obese mice or ob/ob mice is sufficient to improve systemic insulin sensitivity, which is attributable to decreased production of proinflammatory cytokines or chemokines that are known to inhibit insulin signaling in cultured cells [Arkan et al., 2005]. These data demonstrate that IKK beta plays an important role in the development of obesity-related insulin resistance by acting in multiple cell types, particularly myeloid cells.

TLR4, a family member of toll-like receptors (TLRs), plays a critical role in pattern recognition and activation of innate immunity [Medzhitov et al., 1997]. Lipopolysaccharide (LPS) is the well characterized ligand for TLR4 [Poltorak et al., 1998]. Several recent studies demonstrate that FFAs, whose levels are elevated in obesity, can also activate TLR4 [Lee et al., 2001]. Deficiency of TLR4 can prevent FFA-induced production of inflammatory cytokines in both macrophages and adipocytes. In lipid-infused animals, absence of TLR4 significantly improved insulin signaling in muscle and decreased production of inflammatory cytokines in adipose tissue [Shi et al., 2006]. Absence of TLR4 also modestly improved systemic insulin sensitivity in female mice upon high fat challenge, presumably through reduced production of proinflmatory cytokines in liver and adipose tissue. Similar phenotypes are also observed in diet-induced obese mice with a loss-of-function

mutation in TLR4, confirming a role of TLR4 in linking innate immunity and lipid-induced insulin resistance [Tsukumo et al., 2007].

iii. Adipocyte derived cytokine (adipokine) as mediators of inflammation and immune responses

The term adipocytokine is used to describe certain cytokines that are mainly produced by adipose tissue, although it is important to note that they are not all exclusively derived from this organ. Adiponectin, leptin, resistin and visfatin are adipocytokines and are thought to provide an important link between obesity, insulin resistance and related inflammatory disorders [Wellen et al., 2005; Mannino et al., 2006; La Cava and Matarese, 2004; Kusminski et al., 2005; Weisberg 2006]. Various other products of adipose tissue that have been characterized include: certain cytokines, such as tumour-necrosis factor (TNF), interleukin-6 (IL-6), IL-1 and MCP1; mediators of the clotting process, such as plasminogen-activator inhibitor type 1; and certain complement factors [Wellen et al., 2005]. These products have well-known roles in the immune system, and although some of them are also produced by adipocytes. They are not normally considered to be adipocytokines; nonetheless, they have important roles at the interface between the immune and metabolic systems. Obesity is associated with a chronic inflammatory response, which is characterized by abnormal cytokine production, increased synthesis of acute-phase reactants, such as C-reactive protein (CRP), and the activation of pro-inflammatory signalling pathways [Wellen et al., 2005]. Visfatin and

resistin are marker of inflammation [Moschen et al., 2007; Lu et al., 2002] and their expression is increased in different inflammatory condition like atherosclerosis [Dahl et al., 2007; Jung et al., 2005] and inflammatory bowel disease [Moschen et al., 2007]. Although there is no doubt that pro-inflammatory pathways are activated in the adipose tissue itself in cases of obesity, the relative contribution of adipocytes as a source of the circulating and systemically active cytokines, adipocytokines and chemokines remains unclear. The adipose tissue of obese individuals also contains

a large number of macrophages, which are an additional source of soluble mediators in the adipose tissue [Weisberg et al., 2003; Xu et al., 2003].

Table 1. Adipose tissue derived proteins known to affect inflammation

TNF alpha
IL-6
IL-1 beta
Leptin
Adiponectin
Resistin
Acylation stimulating protein
SAA3
Pentraxin-3
IL-1 receptor antagonist
Macrophage migration inhibitor factor

In humans, adipocytokines function as hormones to influence energy homeostasis and to regulate neuroendocrine function. As cytokines, they affect immune functions and inflammatory processes throughout the body. The field of adipocytokines has attracted tremendous interest recently and the knowledge that has accumulated might lead to the development of new therapeutics. Here, we provide an overview of recent advances in our view of the role of adipocytokines in inflammation and immunity.

Adiponectin: Although adiponectin is synthesized mainly by adipocytes, it is also expressed by skeletal muscle cells, cardiac myocytes and endothelial cells [Pineiro et al., 2005; Delaigle et al., 2004; Wolf et al., 2006]. It has sequence homology with a family of proteins that are characterized by an amino-terminal collagen-like region and a carboxy-terminal, complement factor C1q-like globular domain [Scherer et al., 1995; Maeda et al., 1996; Hu et al., 1996]. Expression of adiponectin is regulated by pro-inflammatory mediators such as IL-6, which suppresses adiponectin transcription and translation in an adipocyte cell line

[Fasshauer et al., 2003]. Weight loss is a potent inducer of adiponectin synthesis [Bruun et al., 2003], as is activation of peroxisomeproliferator- activated receptor-γ (PPARγ) by its ligands thiazolidinediones, which are important for the treatment of type 2 diabetes mellitus [Maeda et al., et al., 2001; Iwaki et al., 2003]. Circulating levels of adiponectin, however, are affected by many other factors including gender, age and lifestyle. Early studies indicated that adiponectin had an anti-inflammatory effect on endothelial cells through the inhibition of TNF-induced adhesion-molecule expression [Ouchi et al., 1999]. In addition, adiponectin-deficient mice have higher levels of expression of mRNA encoding TNF in adipose tissue and higher TNF concentrations in plasma compared with adiponectin-sufficient mice [Maeda et al., 2002]. Adiponectin inhibits NF-κB activation in endothelial cells and interferes with the function of macrophages [Ouchi et al., 1999; Yokota et al., 2000] treatment of cultured macrophages with adiponectin markedly inhibited their phagocytic activity and production of TNF in response to stimulation with lipopolysaccharide (LPS)[Yokota et al., 2000]. Adiponectin also induces the production of important anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonist (IL-1RA), by human monocytes, macrophages and dendritic cells (DCs), and suppresses the production of interferon-γ (IFNγ) by LPS-stimulated human macrophages (Wolf et al., 2004). Through ADIPOR1, globular adiponectin suppresses TLR-induced NF-κB activation [Yamaguchi et al., 2005], indicating that adiponectin negatively regulates macrophage responses to TLR ligands, which is probably of relevance in innate immune responses.

The presence of adiponectin in T-cell proliferation assays resulted in a decreased ability to evoke an allogeneic T-cell response [Wolf et al., 2004], and adiponectin also markedly reduced the phagocytic capacity of macrophages. However, stimulation of DCs with adiponectin did not result in any changes in cell-surface marker expression, phagocytic capacity or ability to stimulate allogeneic T-cell proliferation, which might indicate that adiponectin mainly affects the function of macrophages and not DCs [Wolf et al., 2004]. There might, however, be certain situations in which adiponectin has a pro-inflammatory effect. In the presence of

LPS, high-molecular-weight adiponectin was shown to augment the translation of CXC-chemokine ligand 8 (CXCL8; also known as IL-8) by human macrophages [Saijo et al., 2005]. Low- and high-molecular-weight adiponectin share some biological effects on monocytes, such as the induction of apoptosis, the activation of AMP-activated protein kinase (AMPK) and the suppression of scavenger receptor expression by macrophages [Neumeier et al., 2006]. However, in this study [Neumeier et al., 2006], high-molecular-weight adiponectin also induced the secretion of IL-6 by human monocytes, whereas only the low-molecular-weight form had anti-inflammatory effects by decreasing IL-6 production in response to LPS and inducing IL-10 synthesis. The exact roles of the different full-length and globular forms of adiponectin in inflammation and immunity remain to be defined.

Leptin: The adipocyte-derived hormone leptin is a critical mediator of energy balance that relays information regarding the depletion or accumulation of fat stores to the brain [Schwartz et al., 2000; Flier, 2004]. Although identified as a classic peptide hormone, the four alpha helix domains in the folded structure make leptin most similar to cytokines such as IL-2. Moreover, the leptin receptor (lep^r) bears significant homology to type 1 cytokine receptors; therefore, the hormone leptin is in many ways more appropriately identified as a cytokine. Although the role of leptin in controlling energy homeostasis is increasingly well defined [Schwartz et al., 2000; Flier, 2004], it remains unclear whether leptin plays a role in the inflammatory syndrome caused by abdominal obesity. Clearly, serum leptin concentrations rise in proportion to body adiposity [Considine et al., 1996] therefore, obese individuals with the metabolic syndrome generally have higher circulating leptin concentrations.

A salient aspect of the effects of leptin in the immune system is its action as a pro-inflammatory cytokine: it is produced by inflammatory cells [Sanna et al., 2003], and leptin mRNA expression and circulating leptin levels are increased by a number of inflammatory stimuli, including IL-1, IL-6 and lipopolysaccharide (LPS) [Faggioni et al., 2001]. Leptin-deficient mice are less prone than non-leptin-deficient mice to develop inflammatory diseases, regardless of whether these involve innate or adaptive immunity; reported conditions include experimentally induced colitis, experimental autoimmune encephalomyelitis, type I diabetes and experimentally

induced hepatitis [Otero et al., 2005]. In the innate case, a reported imbalance between pro- and anti-inflammatory cytokines [Faggioni et al., 1999] suggests that leptin is able to modify the cytokine secretion pattern of monocytes and macrophages through a STAT3-mediated mechanism [Williams et al., 2004]. In the adaptive case, resistance may be due to the above-noted influence of leptin deficiency on TH1/TH2 balance [Busso et al., 2002]. db/db mice, which lack leptin receptors, suffer from thymus atrophy [Kimura et al., 1998], and ob/ob mice, which lack leptin, are immunodeficient. Leptin must therefore play a role in immunity. This presumably explains why the murine immune system is depressed by acute starvation and reduced caloric intake, both of which result in low leptin levels [Howard et al., 1999], and why this depression is reverted by administration of exogenous leptin.

Known direct actions of leptin on immune responses include the following [Otero et al., 2006; Tilg et al., 2006; Matarese et al., 2005].

(I) It promotes phagocyte function [Zarkesh-Esfahani et al., 2001] and induces the synthesis of eicosanoids [Mancuso et al., 2004], nitric oxide [Raso et al., 2002] and several pro-inflammatory cytokines [Raso et al., 2002] in macrophages and monocytes.

(II) It increases IFN gamma-induced production of nitric oxide synthase in murine macrophages [Raso et al., 2002].

(III) It induces chemotaxis and the release of reactive oxygen species by neutrophils [Caldefie-Chezet et al 2001, Caldefie-Chezet et al 2003].

(IV) It influences the proliferation, differentiation, activation and cytotoxicity of natural killer (NK) cells [Tian et al., 2002].

(V) It may protect dendritic cells from apoptosis and promote their lipopolysaccharide-induced maturation and a cytokine production profile featuring low levels of IL10 and high levels of IL12, TNFa and costimulatory molecules, which favours the proliferation of allogeneic CD4+ T cells (whereas leptin receptor deficiency and sequestration of leptin have the opposite effects and result in depressed proliferation of allogeneic CD4+ T cells) [Lam et al., 2006].

(VI) It modifies T-cell balance, induces T-cell activation, and alters the pattern of T-cell cytokine production by directing T-cell differentiation towards a TH1 response [Farooqi et al., 2002; Lord et al., 1998].

Resistin is a dimeric protein that received its name from its apparent induction of insulin resistance in mice. It belongs to the FIZZ (found in inflammatory zones) family (now also known as RELMs, i.e. resistin-like molecules). The first member this family to be discovered, FIZZ1 (also known as RELM alpha), is a protein that is found in above normal levels in the bronchoalveolar fluid of mice with experimentally induced asthma [Holcomb et al., 2000]. FIZZ2 (RELM beta) was discovered in the proliferating epithelium of intestinal crypt [Rajala et al., 2003]. Resistin (FIZZ3) has been found in adipocytes, macrophages and other cell types. In rodents, a fourth FIZZ protein, RELM gamma, has been identified in WAT and haematopoietic tissues [Gerstmayer et al., 2003]. As noted above, it has been postulated that resistin mediates insulin resistance, but this role may be limited to rodents. Initial enthusiasm for this theory, which provides a direct link between adiposity and insulin resistance [Steppan et al., 2001], was quickly quenched by contradictory findings in both mice and humans [Way et al., 2001; Savage et al., 2001; Patel et al., 2003]. Resistin levels depend upon both nutritional state and hormonal environment; that they are low during fasting and restored by refeeding; and that growth hormone, catecholamines and endothelin 1 are all able to increase resistin secretion [Koerner et al., 2005]. This hypothesis that resistin is involved in inflammatory conditions in humans is suggested by its secretion in appreciable quantities by mononuclear cells. Resistin levels are correlated in atherosclerotic patients with other markers of inflammation, such as soluble TNF-R type II and lipoprotein-associated phospholipase A2 [Reilly et al., 2005]. Furthermore, LPS has been reported to induce resistin gene expression in primary human and murine macrophages via a cascade involving the secretion of pro-inflammatory cytokines [Lehrke et al., 2004]; and in human peripheral blood mononuclear cells resistin appears both to induce [Bokarewa et al., 2005] and be induced by [Pang et al., 2006] IL-6 and TNFalpha (induction of these cytokines by resistin occurring via the

NFkB pathway [Bokarewa et al., 2005]. However, there are few reports that both TNF alpha and IL6 downregulate resistin or have no effect in adipocytes [Pang et al., 2006]. A pro-inflammatory role of resistin in atherosclerosis is suggested by reports that in vascular endothelial cells it induces the inflammation marker long pentraxin 3 [Kawanami et al., 2004] and promotes the release of endothelin 1 and production of VCAM1, ICAM1 and monocyte chemotactic protein 1 (MCP1) [Verma et al., 2003]. In murine models of atherosclerosis, resistin is present in sclerotic lesions at levels that are proportional to the severity of the lesion [Senolt et al., 2007]. In humans resistin is associated with coronary artery calcification, a quantitativemarker of atherosclerosis [Burnett et al., 2005]. There are indications that resistin may also be involved in the pathogenesis of rheumatoid arthritis: resistin has been found in the plasma and the synovial fluid of rheumatoid arthritis patients [Senolt et al., 2007], and injection of resistin into mice joints induces an arthritis-like condition, with leukocyte infiltration of synovial tissues, hypertrophy of the synovial layer and pannus formation [Bokarewa et al., 2005].

Visfatin is an insulin-mimetic adipokine that was originally discovered in liver, skeletal muscle and bone marrow as a growth factor for B lymphocyte precursors (whence its alternative name, pre-B-colony enhancing factor, or PBEF). It is up-modulated in acute lung injury and sepsis [Ye et al., 2005; Jia et al., 2004]. It was re-discovered by Fukuhara et al. [Fukuhara et al., 2005] using a differential display technique to identify genes that are relatively specifically expressed in abdominal fat. Circulating visfatin levels are closely correlated with WAT accumulation, visfatin mRNA levels increase in the course of adipocyte differentiation, and visfatin synthesis is regulated by several factors, including glucocorticoids, TNF alpha, IL-6 and growth hormone [Kralisch et al., 2005]. In an experimental model of obesity-associated insulin resistance, circulating visfatin levels increased during the development of obesity, apparently due solely to secretion by abdominal WAT (since visfatin mRNA increased only in this tissue, not in subcutaneous WAT or liver) [Fukuhara et al., 2005]. However, visfatin is not only produced by WAT, but also by endotoxin-challenged neutrophils, in which it

prevents apoptosis through a mechanism mediated by caspases 3 and 8 [Jia et al., 2004]. Also, patients with inflammatory bowel diseases have increased circulating visfatin levels and increased levels of visfatin mRNA in their intestinal epithelium. Visfatin has been shown to induce chemotaxis and the production of IL1beta, TNF alpha, IL6 and costimulatory molecules by CD14+monocytes, and to increase their ability to induce alloproliferative responses in lymphocytes, effects which are mediated intracellularly by p38 and MEK1 [Moschen et al., 2007]. Visfatin is therefore certainly pro-inflammatory in some circumstances. In addition, circulating visfatin is higher in patients with rheumatoid arthritis than in healthy controls [Otero et al., 2006]. Even though it is currently unclear what is visfatin physiological role or relevance in the context of rheumatoid arthritis, it may reflect modulation of the inflammatory or immune response by visfatin; or it may forms part of a compensatory mechanism that facilitates the accumulation of intraabdominal fat so as to prevent rheumatoid cachexia; or it may simply be an epiphenomenon.

Serum retinol-binding protein 4 (RBP4) is another recently characterized adipocytokine [Heinonen et al., 2005]. Until recently, the sole function of RBP4 was thought to be the delivery of retinol to tissues. However, in patients with type 2 diabetes mellitus, serum levels of RBP4 are increased. Expression of RBP4 is also increased in adipose tissue of mice lacking the glucose transporter GLUT4, and consistent with this, expression of GLUT4 is selectively decreased in adipocytes from obese individuals or individuals with type 2 diabetes [Yang et al., 2005]. Treatment with fenretinide, a synthetic retinoid that increases urinary excretion of RBP4, normalizes serum levels of RBP4 and decreases insulin resistance in mice with obesity induced by a high-fat diet [Yang et al., 2005]. Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance [Yang et al., 2005]. Therefore, decreasing the concentration of RBP4 could be an interesting strategy for the treatment of individuals with type 2 diabetes mellitus.

V. The Role of adipokines in metabolic diseases

Increased circulating cytokines and growth factors are strongly related to inflammation, which is currently viewed by many researchers as a possible key player in the etiology of atherosclerosis and diabetes [Ross, 1999; Yuan et al., 2001]. Apart from inflammation, adipokines involved in glucose metabolism (e.g. adiponectin, resistin), lipid metabolism (e.g. Cholesteryl Ester Transfer Protein, CETP), inflammation (e.g. TNF alpha, IL-6), coagulation (PAI-1) and feeding behaviour (leptin) thus affecting metabolism and function of many organs and tissues including muscle, liver, the vasculature and brain (Table-1). Although little is known about the extent of involvement and interaction between inflammation and adipose tissue in relation to the etiology and the progression of insulin resistance, adipokines may emerge as possible links between inflammation and insulin resistance and diabetes.

Table 2: Adipokines and their functions

Adipocytokine	Full name	Effects on
Leptin	Leptin	Food intake, fat mass
Adiponectin	Adiponectin	Insulin resistance, inflammation
Resistin	Resistin	Insulin resistance, inflammation
Visfatin	Visfatin	Insulin resistance
Omentin	Omentin	Insulin resistance
Vaspin	Visceral adipose tissue-derived serpin	Insulin resistance
Apelin	Apelin	Vasodilatation
CETP	Cholesteryl ester transfer protein	Lipid metabolism
LPL	Lipoprotein lipase	Lipid metabolism
HSL	Hormone sensitive lipase	Lipid metabolism
A-FABP (aP2)	adipocyte fatty acid binding protein	Lipid metabolism
Perilipin	Perilipin	Lipid metabolism
RBP (4)	Renitol-binding protein (4)	Lipid metabolism

AT II	Angiotensin II	Blood pressure
ACE	angiotensin converting enzyme	Blood pressure
AGT	Angiotensinogen	Blood pressure
TNF-α	Tumor Necrosis Factor-α	Inflammation
IL-6	Interleukin-6	Inflammation
CRP	C-reactive protein	Inflammation
Adipsin	Adipocyte trypsin / Complement Factor D	Inflammation
MCP-1	Macrophage Chemo attractant Protein-1	Macrophage attraction
ICAM-1	InterCellular Adhesion Molecule-1	Macrophage activation
PAI-1	Plasminogen Activator Inhibitor-1	Fibrinolysis

Levels of the proinflammatory cytokines, IL-6 and TNF alpha, the most widely studied cytokines in adipose tissue, were shown to be correlated with all measures of obesity and were strongly related to insulin resistance [Ventre et al., 1997; Katsuki et al., 1998; Katsuki et al., 2000; Stephens et al., 1991; Hotamisligil et al., 1996]. The direct correlation between the increase in circulating levels of adipokines and the increase in visceral adiposity further supports the assumption that adipokines potentially play in resistance [Bergman and Mittelman 1998]. Increased gene expression and protein production of TNF alpha and its receptors 1 and 2 in adipose tissue were observed in obese insulin-resistant rodent models and obese subjects [Hotamisligil et al., 1995]. Several studies in obese mice with homozygous null mutation at the TNF-α, or its receptor loci, demonstrated that genetic absence of TNF-α signaling leads to significant improvement in insulin receptor signaling capacity and, consequently, insulin sensitivity [Ventre et al., 1997]. In humans, TNF alpha levels inversely correlate with insulin sensitivity assessed by euglycemic-hyperinsulinemic clamp [Katsuki et al., 1998]. In addition, treatment with troglitazone reduces plasma levels of TNF alpha and simultaneously improves insulin sensitivity in patients with type 2 DM [Katsuki et al., 2000]. The mechanism by which TNF alpha induces insulin resistance is still not fully understood. Evidence suggests that TNF alpha is associated with the

downregulation of GLUT4 mRNA in adipose tissue and skeletal muscles and reduction in insulin receptor substrate-1 [Stephens et al., 1991; Hotamisligil et al., 1996]. Similarly, elevated levels of IL-6 reduce insulin sensitivity by inhibiting GLUT4 [Strassmann et al., 1993]. Pradhan et al. [Pradhan et al., 2001] recently showed that elevated IL-6 is associated with doubling the risk for developing diabetes. Although IL-6 plasma levels decrease following lifestyle modification [Bastard et al., 2000], it is still not known whether modifications of the IL-6 system are involved in the improvement of insulin sensitivity. A growing body of evidence implicates adiponectin as an important factor in the pathophysiology of insulin resistance [Maeda et al., 2002; Yamauchi et al., 2001]. Adiponectin knockout mice exhibit diet-induced insulin resistance [Maeda et al., 2002]. Furthermore, adiponectin decreases insulin resistance in mouse models of obesity and lipoatrophy [Yamauchi et al., 2001]. Its plasma levels correlate significantly with insulin sensitivity assessed by hyperinsulinemic and euglycemic clamps in monkeys and humans [Maeda et al., 2002]. Although it is unclear how adiponectin affects insulin resistance, some evidence indicates that adiponectin improves the peripheral action of insulin by accelerating β oxidation of free fatty acids in skeletal muscle [Yamauchi et al., 2001]. Plasma levels of PAI-1 were shown to be elevated in the insulin-resistance state and were thought to be directly related to higher levels of proinsulin and insulin [Calles-Escandon et al., 1998]. In humans, it has been shown that improvement in insulin sensitivity by either weight reduction or medications lowers circulating levels of PAI-1 [McGill et al., 1994]. The leptin role in the etiology of insulin resistance is also not fully understood. Leptin may affect insulin sensitivity in adipose tissue, skeletal muscle, liver, and pancreatic islets [Friedman, 2002]. Both insulin and TNF alpha regulate the secretion and mRNA expression of leptin in adipocytes [Havel et al., 1996, Hube and Hauner, 1999]. Its role in the treatment of diabetes and insulin resistance is not exactly known, owing to limited human interventional studies. Resistin has recently been implicated in human obesity and insulin resistance [Steppan et al., 2001]. Several observations showed increased serum level in genetic and diet-induced obese mice [Ehtisham, 2002]. The observations that insulin action was reduced in mice treated with

exogenous resistin, and that reduction in resistin levels in ob/ob and Zucker diabetic obese mice treated with peroxisome proliferator-activated receptor gamma (PPAR gamma) agonists was associated with improved insulin sensitivity, raised the assumption of a possible link between resistin and insulin resistance [Stepan et al., 2001; Nagaev and Smith, 2001]. Subsequent data failed to confirm this association [Ehtisham, 2002; Nagaev and Smith, 2001].

VI. Adipokines as Biomarkers of Metabolic Diseases: A clinical overview

In adults, most organ systems have reached their final size and are programmed to be maintained at steady state. However, WAT is unique because of its almost unlimited expansion potential. Thus, WAT can become one of the largest organs in the body, and the total amount of an adipokine secreted from WAT may affect whole-body homeostasis. WAT contains various types of cells that include preadipocytes, adipocytes and stromal vascular cells. Moreover, bone marrow derived macrophages home to WAT in obesity [Weisberg et al., 2003; Xu et al., 2003]. The massive increase in fat mass leads to a dysregulation of circulating adipokine levels that may have pathogenic effects associated with obesity. Thus, dysregulated secretion of adipokines, not only from adipocytes but also from macrophages in WAT, will contribute to the pathogenesis of obesity by triggering IR and systemic inflammation. It is expected, therefore, that circulating levels of adipokines can be used as a high-throughput biomarker to assess obesity-related health problems.

Adiponectin is the only adipokine that is known to be down-regulated in obesity. Plasma concentrations are negatively correlated with body mass index (BMI) [Arita et al., 1999]. A longitudinal study in primates suggests that adiponectin decreases with weight gain as animals become obese [Hotta et al., 2001]. In contrast, weight loss results in significant increases in circulating adiponectin levels [Yang et al., 2001; Bruun et al., 2003]. In addition to the association with whole-body fat mass, adiponectin levels differ with the distribution of body fat. Plasma

levels of adiponectin exhibit strong negative correlations with intra-abdominal fat mass [Cnop et al., 2003]. Visceral, but not subcutaneous abdominal fat, was reported to be inversely associated with plasma adiponectin levels in healthy women [Kwon et al., 2005]. A low waist to hip ratio has been reported to be associated with high levels of plasma adiponectin independent of the body fat percentage [Staiger et al., 2003].

Plasma adiponectin concentrations are lower in people with T2DM than in BMI-matched controls [Hotta et al., 2000]. The plasma concentrations have been shown to correlate strongly with insulin sensitivity, which suggests that low plasma concentrations are associated with IR [Stefan et al., 2002]. In a study of Pima Indians, a population that has one of the highest prevalence of obesity, IR and T2DM, individuals with high adiponectin levels were less likely to develop T2DM than those with low concentrations [Lindsay et al., 2002]. The high adiponectin concentration was, therefore, a predictive marker for the development of T2DM. Plasma concentrations of adiponectin are also reported to be associated with components of MS. High plasma concentrations of adiponectin were found to be related to an advantageous blood lipid profile [Tschritter et al., 2003; Baratta et al., 2004]. Plasma adiponectin levels are decreased in hypertensive humans, irrespective of the presence of IR [Iwashima et al., 2004]. Endothelium-dependent vasoreactivity is impaired in people with hypoadiponectinemia [Ouchi et al., 2003], which might be one of the mechanisms involved in hypertension in visceral obesity. A reciprocal association between CRP and adiponectin mRNA levels was reported in human WAT, suggesting that hypoadiponectinemia appears to contribute to low-grade systemic chronic inflammation [Ouchi et al., 2003]. All these mechanisms may underlie the protective effects against the progression of atherosclerosis of adiponectin. A recent study revealed that adiponectin may function as a biomarker for MS, even in childhood obesity [Winer et al., 2006]. Collectively, adiponectin has been recognized as a key molecule in MS and has the potential to become a clinically relevant parameter to be measured routinely at general medical check ups. Plasma concentrations of adiponectin are also known to be lower in people with CVD than in controls, even after matching for BMI and age [Ouchi et al., 1999].

A case-control study performed in Japan revealed that the people with hypoadiponectinemia with the plasma levels less than 4μg/ml had increased risk of CVD and multiple metabolic risk factors, indicating that hypoadiponectinemia is a key factor in MS [Kumada et al., 2003]. Retrospective case-control studies have demonstrated that patients with the highest levels of adiponectin have a dramatically reduced 6-year risk of myocardial infarction compared with case controls with the lowest adiponectin levels, and this relationship persists even after controlling for family history, BMI, alcohol, history of diabetes and hypertension, hemoglobin A1c, CRP, and lipoprotein levels [Pischon et al., 2004]. An inverse relationship between serum adiponectin levels and the intima media thickness of common carotid arteries was also reported [Pilz et al 2005]. These clinical studies clearly indicate that hypoadiponectinemia is a strong risk factor for CVD. Although the above studies support the notion that adiponectin would protect against vascular diseases, recent epidemiological studies have failed to support this notion [von Eynatten et al., 2008; Kistorp et al., 2005; Lawlor et al., 2005; Lindsay et al., 2005; Kanaya et al., 2006]. A recent prospective study reported adiponectin levels were not significantly associated with future secondary CVD events [von Eynatten et al., 2008]. Thus, measurement of adiponectin may add no significant value to risk stratifications in patients with incident CVD, and effects of adiponectin may be more of importance in the early phases of atherosclerosis. Kistorp et al. reported that adiponectin was positively related to increased mortality in patients with chronic heart failure [Kistorp et al., 2005]. These authors suspect that the high adiponectin concentrations may reflect a wasting process in subjects with increased risk of death. Pilz et al. reported that high adiponectin levels predict all-cause, cardiovascular and noncardiovascular mortality [Pilz et al., 2006]. A recent study also reported that a high adiponectin level was a significant predictor of all-cause and CVD mortality [Dekker et al., 2008]. These authors hypothesized that a counter-regulatory increase in adiponectin occurs, which represents a defense mechanism of the body against cardiovascular alterations and a pro-inflammatory state associated with CVD. Thus, yet-unknown mechanisms may underlie the association between adiponectin and the risk of death, the prognostic value of

adiponectin remains unresolved. Further prospective studies will be required to provide conclusive results about the association of adiponectin and mortality. It is also necessary to understand the underlying molecular mechanisms of elevated adiponectin concentrations in these disease states. It must be highlighted that several physiological factors affect the circulating levels of adiponectin. First, aging, gender and puberty have effects on circulating adiponectin levels [Butte et al., 2005; Ong et al., 2006]. An age-associated elevation of plasma adiponectin levels has been reported [51, Isobe et al., 2005]. Plasma adiponectin levels were significantly higher in female subjects, indicative of a sex hormone affect on circulating adiponectin levels [Dekker et al., 2008; Aso et al., 2006]. Adiponectin levels tend to decrease throughout puberty, which parallels the development of IR [Winer et al., 2006; Bottner et al., 2004]. All of these factors must be considered when evaluating the clinical significance of circulating adiponectin levels in MS or vascular diseases related to obesity. Circulating adiponectin forms several different complexes in the adipocyte before being secreted into the blood [Pajvani et al., 2003].

RBP4, although largely produced in liver, is also made by adipocytes, with increased levels in obesity contributing to impaired insulin action [Yang et al., 2005]. Studies in transgenic rodent models showed overexpression of human RBP4 or injection of recombinant RBP4 induced IR in mice, whereas RBP4 knockout mice showed enhanced insulin sensitivity [Yang et al., 2005]. The same authors reported that high plasma RBP4 levels are associated with IR states in humans and suggested that RBP4 is an adipokine responsible for obesity-induced IR and, thus, a potential therapeutic target in T2DM [Yang et al., 2005; Graham et al., 2006]. Cho et al. reported that plasma concentrations of RBP4 were higher in people with impaired glucose tolerance (IGT) or T2DM than in people with normal glucose tolerance [Cho et al., 2006]. A recent cross-sectional study of 3289 middle-aged population showed that plasma RBP4 levels increased gradually with increasing numbers of MS components [Qi et al., 2007]. RBP4 was reported to be more highly correlated with waist-to-hip ratio or visceral fat areas than with BMI [Grahm et al., 2006; Lee et al., 2007; Jia et al., 2007]. Two recent studies have indicated that high

circulating RBP4 is associated with elevated liver fat and, presumably, hepatic insulin resistance [Stefan et al., 2007; Perseghin et al., 200]. In rodents, only 20% of systemic RBP4 is produced by adipocytes, and RBP4 gene expression in adipocytes was 20% compared with expression in the liver [Tsutsumi et al., 1992]. Thus, it is possible that the increase in systemic RBP4 concentrations is not explained by increased RBP4 production in WAT. RBP4 is a transporter for retinol, which serves as a precursor for the synthesis of ligands for nuclear hormone receptors such as retinoid X receptor and retinoic acid receptor. Thus, circulating RBP4 can modulate metabolic pathways via these nuclear hormone receptors. Certainly, future prospective studies are needed to clarify whether a high RBP4 level plays a causal role in the development of MS, T2DM, and eventually for the development of CVD.

The role of resistin in the pathophysiology of obesity and IR in humans is controversial. Several studies have shown positive correlations of circulating resitin levels with body fat mass [Savage et al., 2001, Yannakoulia et al., 2003] or IR [Silha et al., 2003; Zhang et al., 2003]. However, the other studies found no relationship between resistin gene expression and body weight or insulin sensitivity [Janke et al., 2002; Fehmann et al., 2002; Shadid et al., 2006]. These conflicting data may reflect variations in the study design and the lack of adjustment for potential confounding factors. It also seems possible that resistin is a marker for, or contributes to, IR in a specific population. The predominantly paracrine role of resistin might explain the weakness of the correlations between circulating resistin levels and some of the metabolic variables. Two studies have shown that among the blood markers, the most significant association of the circulating resistin level was with plasma CRP [Shetty et al., 2004; Pischon et al., 2005]. The circulating resistin level is also reported to be an inflammatory marker of atherosclerosis [Reilly et al., 2005]. Considering that the resistin concentration is elevated in the patients with severe inflammatory disease [Stejskal et al., 2003], hyperresistinemia may be a biomarker and/or a mediator of inflammatory states in humans. Overall, the resistin levels in humans are thought to correlate more closely with inflammation than with IR.

Visfatin is an adipokine identified as being predominantly produced by abdominal adipose tissue [Fruzzetti et al., 2002]. Plasma levels of visfatin are closely related to white adipose tissue accumulation and it was originally shown to have insulin-mimetic properties, although this is now under debate. Levels of the adipokine visfatin are higher in overweight or obese patients with additional cardiovascular risk factors, such as increased waist circumference, blood pressure and triglyceride levels, than in patients without these factors [Filippatos et al., 2007]. Visfatin is considered to be a proinflammatory adipokine and has been shown to upregulate expression of TNF-alpha and IL-6 [Moschen et al., 2007].

A number of studies have reported that several humoral markers of inflammation are also elevated in people with obesity and T2DM [Pickup and Crook, 1998; Yudkin et al., 2000]. Pfeiffer et al. showed that men with T2DM had higher TNFα concentrations compared with nondiabetic subjects [Pfeiffer et al., 1997]. However, several studies reported no association between circulating levels of TNFα and insulin sensitivity [Kellerer et al., 1996; Kern et al., 2001]. Since there was no arteriovenous difference with TNF alpha [Mohamed-Ali et al., 1997], TNF alpha is considered to work mainly in an autocrine or paracrine manner, where the local concentrations would be more likely to exert its metabolic effects [Mohamed-Ali et al., 1997, Yudkin et al., 1999]. Moreover, circulating TNF alpha has been reported to be associated with a soluble receptor that inhibits its biological activity [Engelberts et al., 1991], suggesting that the action of TNF alpha is primarily a local one. Therefore, it seems unlikely that the circulating levels of TNFalpha would be a good biomarker to reflect the IR state of the whole body.

A considerable proportion of circulating IL-6 is derived from WAT, and WAT is estimated to produce about 25% of the systemic IL-6 in vivo [Mohamed-Ali et al., 1997]. Fasting plasma IL-6 concentrations were negatively correlated with the rate of insulin-stimulated glucose disposal in Pima Indians [Vozarova et al., 2001]. Bastard et al. reported that the IL-6 values were more strongly correlated with obesity and IR parameters than TNFalpha, and a very low-calorie diet induced significant decreases in circulating IL-6 levels in obese women [Bastard et al., 2000]. Other studies have also showed that weight loss results in decreased

circulating levels of IL-6 [Esposito et al., 2003; Kopp et al., 2003; Giugliano et al., 2004]. Overall, the association of IL-6 and IR seems complex and IL-6 alone might not be an appropriate marker of IR or MS [Salmenniemi et al., 2004, Matsushita et al., 2006].

IL-6 derived from visceral adipose tissue draining directly into the portal system and causes the obesity-associated rise of liver CRP production [Mortensen, 2001]. Although CRP was traditionally thought to be produced exclusively by the liver in response to inflammatory cytokines, emerging data indicate that CRP can also be produced by nonhepatic tissues. Adipocytes isolated from human WAT produced CRP in response to inflammatory cytokines [Calabro et al., 2005]. CRP has been reported to be associated with body fat and other inflammatory markers [Shadid et al., 2006; Lemieux et al., 2001]. Abundant evidence has accumulated to show that CRP is associated with MS and predicts T2DM and CVD events independently of traditional risk factors [Ridker et al., 2003, Freeman et al., 2002]. Thus, elevated CRP levels in obesity, and the decreases associated with weight loss indicate a link between CRP and obesity-associated risks for CVD [Esposito et al., 2003, Heilbronn et al., 2001, Tchernof et al., 2002]. Further, monocyte chemoattractant protein-1 (MCP-1) is a chemokine, which plays a pivotal role in the recruitment of monocytes and T lymphocytes to the sites of inflammation. MCP-1 is expressed in adipocytes and considered to be an adipokine [Gerhardt et al., 2001; Dietze-Schroeder et al., 2005]. MCP-1 mediates the infiltration of macrophages into WAT in obesity and may play an important role in establishing and maintaining a proinflammatory state that predisposes to the development of IR and MS [Van Gaal et al., 2006]. Macrophage infiltration into WAT is increased by the secretion of MCP-1, which is expressed by adipocytes, as well as by macrophages and other cell types, especially in obese, insulin-resistant subjects [Christiansen et al., 2005]. A number of studies have reported significantly higher circulating MCP-1 levels in obese [Christiansen et al., 2005; Kim et al., 2006] or T2DM patients [Nomura et al., 2000; Piemonti et al., 2003]. Previous studies showed that plasma MCP-1 levels were influenced by numerous factors, including aging [Inadera et al., 1999],

hypertension [Parissis et al., 2000], hypercholesterolemia [Garlichs et al., 2001], vascular disease [de Lemos et al., 2003], and renal failure [Papayianni et al., 2002].

IL-8 is another chemokine responsible for the recruitment of neutrophils and T lymphocytes into the subendothelial space and considered to be an atherogenic factor that leads to intimal thickening. IL-8 is produced and secreted by human adipocytes [Bruun et al., 2001]. Plasma IL-8 levels are increased in obese subjects, linking obesity with increased cardiovascular risk [Straczkowski et al., 2002]. The circulating IL-8 level is associated with obesity-related parameters such as BMI, waist circumference and CRP [Kim et al., 2006]. However, Herder et al. reported that, among the seven immunological mediators (IL-6, IL-18, TNFalpha, IL-8, MCP-1, IP-10, and adiponectin) expressed and secreted by WAT, high BMI was significantly associated with elevated circulating levels of IL-6, IL-18, and IP-10 as well as lower levels of adiponectin [Herder et al., 2007]. Thus, the clinical relevance of circulating levels of MCP-1 and IL-8 to predict obesity-related disease conditions is still unresolved.

Other molecules like Plasminogen activator inhibitor-1 (PAI-1) is an important endogenous inhibitor of tissue plasminogen activator and is a main determinant of fibrinolytic activity. PAI-1 contributes to the pathogenesis of atherothrombosis and CVD. Experimental data indicate that WAT has a capacity to produce PAI-1 [Alessi et al., 1997]. Much of the elevation of circulating levels of PAI-1 in obesity is attributable to upregulated production from WAT [Alessi et al., 1997; Loskutoff et al., 1998; Skurk et al., 2004]. The increased plasma PAI-1 levels in obesity and positive correlations with visceral fat depots are reported in several studies [McGill et al., 1994; Eriksson et al., 1998; Giltay et al., 1998; Mertens et al., 2001]. Conversely, weight loss is associated with reduced PAI-1 activity in obese subjects [Primrose et al., 1992]. Hyperinsulinemia caused by IR may increase both adipocyte and hepatic synthesis of PAI, which could play a role in the development of the vascular complications [Hamsten et al., 1985; Juhan-Vague et al., 1989].

VII. Adipokines as therapeutic targets for new drug development for atherosclerosis

The evolving role of augmented adipokine production in obese and insulin resistant state in cardiovascular disease risk opens new dimensions for therapeutic interventions. Adipose tissues have been targeted in several ways, hoping to improve the metabolic and cardiovascular parameters. There is a strong association between changes in adipokines, endothelial function, and prevention of diabetes through lifestyle modifications. Exercise and weight loss improve endothelial function and prevent diabetes [Clarkson et al., 1999; Brendle et al., 2001]. Reducing adipocyte mass is associated with a reduction in proinflammatory TNF alpha, IL-6, haemostatic factors associated with CVD, including PAI-1 antigen and a rise in circulating adiponectin.

For example, plasma IL-6 levels, which are 30% higher in obese people, decline toward basal levels with weight loss [Bruun et al., 2003; Mohamed-Ali et al., 1997; Roytblat et al., 2000]. Many of the proinflammatory adipokines exert multiple actions in a variety of cellular processes leading to a complex array of abnormalities characteristic of metabolic syndrome. Evidence is mounting to suggest that adipokines may directly influence endothelial function through their proinflammatory properties. While the majority of the data are based on in vitro studies, they link between obesity, insulin resistance, and endothelial dysfunction. Various adipokines modulate the several key mechanisms signaling which can affect vascular homeostasis and atherosclerosis. Emerging data also link leptin to cardiovascular disease (CVD).

Leptin, like CRP, upregulates ET-1 and endothelial NO synthase production in endothelial cells and promotes accumulation of reactive oxygen species [Cooke and Oka., 2002; Konstantinides et al., 2001]. Furthermore, leptin increases platelet aggregation and arterial thrombosis via a leptin receptor-dependent pathway [Cooke and Oka., 2002; Konstantinides et al., 2001], has a direct action on macrophages by increasing the release of monocyte colony-stimulating factor

[Loffreda et al., 1998], promotes cholesterol accumulation in macrophages under high glucose conditions [O'Rourke et al., 2002], and stimulates angiogenesis [Sierra-Honigmann et al., 1998]. Leptin has been shown to play an important role in the development of atherosclerosis. Indeed, leptin deficient mice (ob/ob) who exhibit an early onset morbid obesity, are markedly resistant against diet-induced atherosclerosis, whereas exogenous administration of leptin promotes arterial neointimal proliferation [Schafer et al., 2004].

TNF alpha, an inflammatory cytokine released in greater quantities by obese humans and patients with insulin resistance, not only initiates but also propagates atherosclerotic lesion formation. TNF alpha activates the transcription factor nuclear factor-kB (NF-kB), which accelerates experimental atherogenesis, in part by inducing the expression of VCAM-1, ICAM-1, MCP-1, and E-selectin in aortic endothelial and vascular smooth muscle cells [Ouchi et al., 1999]. TNF alpha reduces NO bioavailability in endothelial cells and impairs endothelium-dependent vasodilatation, promoting endothelial dysfunction [Bhagar and Vallance, 1997; Wang et al., 1994].

Resistin is a fat-specific hormone that directly induces insulin resistance in muscle and the liver. Circulating resistin levels are increased in diet-induced and genetic forms of obesity in rodents [Steppan et al., 2001] Resistin exerts direct vasoactive effects in cultured endothelial cells [Verma et al., 2003]. Resistin treatment activated endothelial cells by promoting ET-1 release, in part by inducing ET-1 mRNA expression, suggesting it participates in the endothelial dysfunction observed in patients with insulin resistance. Resistin also significantly augmented the expression of the cell adhesion molecule VCAM-1 and the chemoattractant chemokine MCP-1, key processes in early atherosclerotic lesion formation.

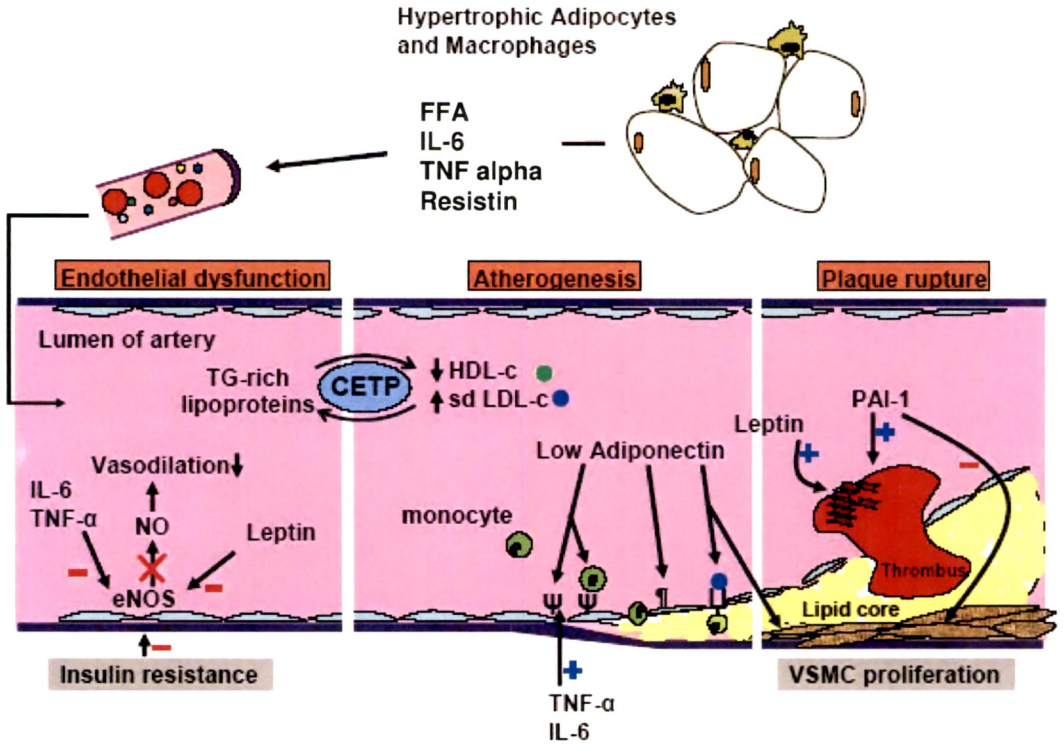


Fig.5 Adipocyte dysfunction and the involvement of adipokines in atherosclerosis

Elevated levels of IL-6, TNF alpha and presence of insulin resistance lead to a decrease in production and availability of eNOS resulting in endothelial dysfunction. Increased adipocyte derived CETP plasma concentrations lead to lower levels of HDL-c and an increased number of small dense LDL-c particles. Adiponectin has inhibitory effects on the development of atherosclerosis by inhibiting the expression of adhesion molecules (ICAM-1, VCAM-1)(induced by IL-6 and TNF alpha) on endothelial cells by activating AMPK and by inhibiting NF-κB and by the inhibition of scavenger receptor class A-1. The latter leads to reduction of cholesterol uptake in macrophages and to transformation of macrophages into foam cells. Furthermore, adiponectin reduces vascular smooth muscle cell proliferation (VSMCs), migration and apoptosis by attenuating DNA synthesis inducing effects of growth factors including platelet-derived growth factor and fibroblast growth factor. Increased levels of PAI-1 can inhibit plasminogen-induced migration of VSMCs leading to plaques prone to rupture with thin fibrous caps, necrotic cores and rich in macrophages. Leptin is capable to induce ADP-dependent platelet activity and aggregation in healthy subjects.

Retinol binding protein-4 (RBP-4) is preferentially expressed in visceral adipose tissues versus subcutaneous [Kloting et al., 2007] and appears to be up regulated in obese rodents [Yang et al., 2005]. Von Eynatten et al. stated RBP4 is a

valuable marker for the metabolic syndrome and associated with pro-atherogenic lipoproteins and enzymes of lipoprotein metabolism, suggesting a possible role of RBP4 in lipid metabolism [von Eynatten et al., 2007]. RBP4 provides an important link between obesity, insulin resistance and inflammation, and there are emerging evidences that they also are closely involved in the development of cardio vascular disease [Matsuzawa et al., 2006; Ingelsion et al., 2009].

Adiponectin is abundantly expressed in adipocytes that increase fat oxidation and insulin sensitivity (Yamuchi et al., 2001). Subjects with coronary heart disease have lower adiponectin levels compared with age and body mass index-adjusted controls (Ouchi et al., 1999), suggesting that adiponectin, in contrast to other adipokines, confers a protective effect against atherosclerosis. Furthermore, adiponectin protected apolipoprotein E (APOE)-deficient mice (mice lacking a key component in cholesterol metabolism) from atherosclerosis (Okamoto et al., 2002; Ouchi et al., 2000). Adiponectin exerts antiatherogenic properties by suppressing the endothelial inflammatory response, inhibiting vascular smooth muscle proliferation, and decreasing VCAM-1 mRNA expression, all of which are associated with endothelial injury and the subsequent development of atherosclerotic lesions [Okamoto et al., 2002; Ouchi et al., 2000]. Adiponectin also suppresses the transformation of macrophages to foam cells [Arita et al., 2001]. Further, macrophage adiponectin expression improves insulin sensitivity and protects against inflammation and atherosclerosis [Luo et al., 2010].

Replenishment of recombinant forms of different adipokines that are down regulated in obesity or the depletion of selective adipokines that are produced in excess in the obese state might have a powerful therapeutic impact. Alternatively the stimulation or the reduction of the release of these proteins from adipocytes could represent an additional approach to alter circulating levels of these proteins.

The most prominent examples of drugs favoring a 'healthy' adipokine profile are the TZDs. TZDs are the first class of agents that directly target the adipocyte. These drugs (rosiglitazone and pioglitazone) are widely used to ameliorate insulin sensitivity in patients with type 2 diabetes. They are potent inducers of

adipogenesis, and many metabolically relevant adipocyte-derived proteins are regulated in response to TZDs (adiponectin, resistin, tumor necrosis factor alpha (TNF alpha), 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the fatty acid-binding protein aP2, and others). TZDs are selective ligands of the nuclear transcription factor PPAR gamma. PPAR gamma is expressed at highest concentrations in adipocytes and it is considered the 'master switch' of adipocyte differentiation [Forman et al., 1995; Berger and Moller, 2002]. Several endogenous, mostly hydrophobic ligands for PPARγ have been identified with various affinities for the receptor. Although the precise mechanisms of the insulin sensitizing action of TZDs are not well understood, it is clear that adipose tissue is the critical tissue for the glucose-lowering effects of TZDs. Most certainly, a reduction of lipotoxicity in other tissues by partitioning lipids into adipose tissue is one aspect of TZD action [Yki-Jarvinen, 2004]. However, an indirect mechanism, i.e. through the induction or repression of adipokines such as adiponectin and resistin, is another likely mechanism of action. Treatment with TZDs are reported to decrease serum hsCRP [Haffner et al., 2002], leptin [Zhang et al., 1996], PAI-1 [Kato et al., 2000], TNFalpha [Katsuki et al., 2000] levels and RBP-4 [Yang et al., 2005], resistin [Moore et al., 2001] and to increase circulating adiponectin [Maeda et al., 2001; Yu et al., 2002] and increase visfatin mRNA in WAT [Choi et al., 2005]. The PPAR gamma ligands currently in clinical use, rosiglitazone and pioglitazone, are being tested in clinical trials for their ability to prevent diabetes and decrease CAD events. In mice that are genetically prone to develop atherosclerosis, PPAR gamma ligands consistently attenuate vascular lesions [Collins et al., 2001; Chen et al., 2001]. Clinical studies have shown that improvements in insulin sensitivity after treatment with rosiglitazone are strongly associated with an increased release of the HMW form of adiponectin [Pajvani et al., 2004; Tonelli et al., 2004]. The ratio between the HMW over the total amount is directly related to the biological activity of adiponectin [Pajvani et al., 2004]. The hypothesis that adiponectin might be a mediator of TZD action is underlined by the observation that a chronic elevation of adiponectin in a mouse model has many features similar to prolonged TZD treatment in diabetic patients [Combs et al., 2004]. These might include wanted and unwanted effects

such as increased weight gain or, on the beneficial side, reduced liver fat content, increased lipid clearance, reduction of inflammatory markers and protection of the vascular endothelium from the development of atherosclerotic plaques. TZDs as well as adiponectin might exert direct effects on lipid metabolism and the vascular wall. Low levels of PPAR gamma are found in macrophages, endothelial cells, vascular smooth muscle cells and other cell types. These low levels can change under pathophysiological conditions. For instance, PPAR gamma is elevated in macrophage foam cells of atherosclerotic lesions and appears to be directly involved in the regulation of both the anti- and pro-atherogenic genes [Hsueh et al., 2001;Li et al., 2000]. The only FDA-approved clinical indication for the prescription of TZDs is type 2 diabetes, however the range of TZD effects might be considerably wider than just regulating insulin sensitivity [Yki-Jarvinen et al., 2004].

The endocannabinoid system (ECS) plays a role in the regulation of energy intake [Di Marzo et al., 2005]. Stimulation of the ECS induces food intake and the ECS may become hyperactive in response to a high-fat diet as endogenous endocannabinoids are synthesized from arachidonic acid, which is in turn derived from essential fatty acids obtained in the diet. Endocannabinoid tone has been increased in adipose tissues of obese patients [DiMarzo et al., 2005]. Sustained hyperactivity of the ECS may, therefore, contribute to the development of obesity and related cardiometabolic risk factors [Di Marzo et al., 2005]. CB1 receptors are expressed in adipocytes [Steinberg et al., 2007, Gary-Boboo et al., 2006] and appear to be upregulated in adipose tissue of genetically modified obese animals [Bensaid et al., 2003]. CB1 receptor activation induces adipocyte differentiation, increases the activity of lipoprotein lipase and stimulates lipogenesis in vitro, while CB1 antagonism by rimonabant respectively blocks these effects [Engeli et al., 2005; Bensaid et al., 2003] indicating the role of CB1 receptors in adipocytes. The selective cannabinoid receptor 1 antagonist, rimonabant, blocks activation of the ECS thus reducing food intake and facilitating weight loss. The efficacy of rimonabant has been shown in four large, placebo-controlled randomized studies, the Rimonabant in Obesity trials, in which patients receiving rimonabant achieved

weight loss of 3.9–5.4 kg greater than placebo while the drug was being administered [Pi-Sunyer et al., 2006; Van Gaal et al., 2005; Despres et al., 2005; Scheen et al., 2006]. While patient tolerance was high for rimonabant, psychiatric disorders (depression, anxiety, irritability, aggression) were 3% more likely to occur in patients treated with rimonabant compared with placebo [Rucker et al., 2007]. In addition to weight loss, rimonabant displayed beneficial effects on several other cardiometabolic risk factors. In overweight and obese patients with type 2 diabetes on monotherapy, rimonabant reduced HbA1c by 0.7% more than placebo ($P<0.0001$), an effect only partly explained by the observed weight loss alone.83 Rimonabant increased HDL-C levels and reduced triglyceride levels by 8.1 and 12.4%, respectively, more than placebo ($P<0.001$ for both) [Despres et al., 2005]. While overall levels of LDL-C did not change significantly, the distribution of LDL particle size moved towards a lower proportion of small-dense LDL [Despres et al., 2005]. In two of the Rimonabant in Obesity trials, the effects of rimonabant on HDL-C and triglyceride levels were greater than could be attributed to weight-loss alone [Pi-Sunyer et al., 2006; Scheen et al., 2006]. Rimonabant also significantly increased adiponectin levels, an effect that was partly independent of weight loss. This weight loss-independent effect has also been shown in vitro, where addition of rimonabant to adipocyte cultures produced a rapid increase in adiponectin levels [Bensaid et al., 2003]. Rimonabant is the third agent licensed as an adjunct to diet and exercise for the treatment of obese adults (body mass index >30 kg/m2) or overweight adults (body mass index >27 kg/m2) with associated risk factor(s) such as type 2 diabetes or dyslipidaemia. Recently, the National Institute for Health and Clinical Excellence (NICE) published its Final Appraisal Determination (FAD), which recommended that rimonabant be made available as an adjunct to diet and exercise to overweight or obese patients who have not responded to, are intolerant of, or are contraindicated to sibutramine and orlistat [NIH and Clinical Excellence, 2008]. While several agents have been licensed directly for weight loss, data on long-term safety, tolerability and more importantly cost effectiveness is still being compiled [Caro et al., 2007]. Moreover, these current agents are effective only while being taken, with weight regain on discontinuation. Newer more potent agents with

the ability to help sustain lifestyle modifications even after discontinuation would be more ideal targets of the future.

Treatment with rimonabant is reported to decrease adiposity associated with increase in adiponectin [Bensaid et al., 2003] mRNA levels in WAT of obese fa/fa rats. Further it is also reported to reduce TNF alpha [Croci et al., 2003] mRNA expression in different systems. In a clinical study (STRADIVARIUS) 20 mg/day rimonabant ameliorated the normalized total atheroma volume (TAV, secondary endpoint), however, failed to show a decrease in percent atheroma volume (PAV, primary endpoint). These controversial results indicated that the use of rimonabant in the management of coronary disease in patients with central obesity or metabolic syndrome requires further investigation.

Despite of extensive *in vitro* work and lot many evidences available, how adipokines link to insulin resistance, inflammation, dyslipedemia and atherosclerosis. However, the cellular mechanisms linking obesity and metabolic syndrome and atherosclerosis are complex and remains to be clarified. Clearly, much more investigation is needed, but insight into mechanisms by which the adipocyte communicates with both insulin target tissues and the vasculature allows us to better understand the relationships between obesity and cardiovascular disease. Furthermore, study of the regulation of these mechanisms will help us to develop treatment strategies to prevent diabetes and heart disease in the growing epidemic of obesity.

THESIS RATIONALE

Obesity with its associated increased risks of diabetes mellitus and atherosclerosis is rapidly increasing in Western societies. However, the key mediators involved in the crosstalk between metabolically active organs and atherosclerosis are still poorly defined. This project will clarify the role of adipose tissue as a source of inflammatory signals in the context of atherosclerosis. Beyond its diagnostic level, the project may identify novel treatment targets aiming at inhibition of atherogenesis and/or obesity. So the general objective of the research work was to investigate the role of various adipocyte derived cytokines (adipokines) in atherosclerosis.

The following specific objectives were perused:

- WAT is now regarded as a pro-inflammatory state, several markers of inflammation have been found to be elevated in obese subjects. It is thought that excess WAT can contribute to the maintenance of this state in three ways: through inflammation-inducing lipotoxicity; by secreting factors that stimulate the synthesis of inflammatory agents in other organs; and by secreting inflammatory agents itself. Danforth et al proposed that impaired adipocyte proliferation and differentiation may cause the progressive filling of existing adipocytes, leading to overflow of excess calories as fat into other tissues and insulin resistance. Obesity with an excess body fat is a condition that increases the incident risk of cardiovascular disease. Thus, it is hypothesized that insulin resistance and eventually full-blown type 2 diabetes can be triggered due to failure of adipocytes to differentiate because of increase in pro-inflammatory condition. *It is aimed to characterize the effects of adiposity on inflammatory cytokines and to determine how pro-inflammatory condition modulates adipogenic potential in vivo, to examine the expression of transcription factors that regulate*

pathways of adipogenesis and inflammatory cytokines genes responsible for maintaining normal state while repressing individual cell hypertrophy.

- Increasing evidences support the role of adipose tissue in the development of a systemic inflammatory state, which contributes to obesity-associated vasculopathy and cardiovascular risk. This production of pro inflammatory cytokine by adipose tissue is of particular interest, because their local secretion by perivascular adipose depots may provide a new mechanistic link between obesity and its associated vascular complications. *So the second objective of the present study is to test the hypothesis that adipose tissue, rather than circulating mononuclear cells, is a key player in causing the systemic inflammation associated with obesity. We also speculate that the mononuclear cells from the obese animals display a pre-activated phenotype which is more vulnerable to aggravate vascular complications than that of lean populations.*

- Obese individuals have a high risk of cardiovascular disease at least two to three times that of the general population; obese type 2 DM patients have a risk of cardiovascular disease three to four times that of nondiabetic individuals. Obesity is an important determinant of cardiovascular risk, because it is associated with insulin resistance, hyperglycemia, dyslipidemia, hypertension, prothrombotic states and proinflammatory states. It has been reported that chronic inflammation in white adipose tissue (WAT) may cause whole-body insulin resistance in obese diabetic animals. Moreover, inflammatory markers such as C-reactive protein are associated with insulin resistance, adiposity, and type 2 diabetes in human subjects. Therefore, it has become important to investigate the mechanisms of anti-obesity and insulin-sensitizing drugs by focusing on the regulation of risk factor for inflammation and

atherosclerosis. *The third objective is to test our hypotheses that obesity or obese diabetic associated pro-inflammatory state can be reversed by different classes of drugs through their actions on the adipokines which will ameliorate the insulin resistance and thus the metabolic syndrome.* In this study, we examined the effects of CB1 antagonist a weight reducing agent and PPAR gamma agonist, pioglitazone an antidiabetic drug in obese (ob/ob) and obese diabetic (db/db) mice respectively, and we investigated the mechanisms by which they improve insulin resistance, especially in WAT.

- RBP-4 is preferentially expressed in visceral adipose tissues versus subcutaneous. Elevated serum RBP-4 is associated with insulin resistance, type 2 diabetes, and metabolic abnormalities such as obesity, glucose intolerance, dyslipidemia, and hypertension. So we hypothesize that RBP-4 provides an important link between obesity, inflammation, and there are emerging evidences that they also are closely involved in the development of cardio vascular disease. *So the fourth objective was to examine the relationship between the expression of retinol binding protein-4 (RBP-4), a novel adipokine with obesity, adipose inflammation and atherosclerosis.*