Cardiovascular Diseases

Cardiovascular diseases (CVDs) are globally considered as the leading cause of death with 80% of CVDs related deaths being reported from low- and middle-income countries like India and occur almost equally in men and women. It is expected that by 2030, CVDs would prevail as the leading cause of death and disability over infectious diseases with 23.6 million people projected to die from CVDs. CVDs are a group of disorders of the heart and blood vessels which include:

- Coronary heart disease (CHD) - Disease of the blood vessels supplying the heart muscle.
- Cerebrovascular disease (CBVD) – Disease of the blood vessels supplying the brain.
- Peripheral arterial disease – Disease of the blood vessels supplying the arms and the legs.
- Rheumatic heart disease – Damage to the heart muscle and heart valves from rheumatic fever caused by Streptococci.
- Congenital heart disease – Malformations of heart structure existing at birth.
- Deep vein thrombosis and pulmonary embolism – Blood clots in the leg veins which can dislodge and move to heart and lungs.
CVDs are the number one cause of death globally; more people die annually from CVDs than from any other cause. An estimated 17.3 million people died from CVDs in 2008 representing 30% of all global deaths. Of these deaths, an estimated 7.3 million deaths were due to coronary heart disease and 6.2 million deaths were due to stroke. According to recent statistics, incidences of CVD-related death and disability in low income countries have grown at alarming pace. In India, CVDs are projected to be the largest cause of death and disability. By 2020, 2.6 million Indians are predicted to die due to CHD which constitute 54.1% of all cardiovascular disease related deaths. Nearly half of these are likely to occur among young and middle aged individuals (30-69 years). Published data from a multicentre study of men aged 35-59 years, conducted on behalf of Indian Council of Medical Research during 1990-4, showed rising prevalence rates of coronary heart disease with increasing urbanisation. The prevalence rate in rural Vellore was 3.15, 4.48 in rural Haryana, 5.92 in urban Vellore and 8.72 in urban Delhi per 1000 male population. Tertiary care centres have documented a steep rise in proportion of coronary heart disease related admissions. Studies from rural areas have shown a lower prevalence (3.7%) as compared to urban areas (9.67%) but rising trend is observed there as well. The prevalence of CHD in rural India has risen from 2.03% in 1974 to 3.7% in 1995. In 2008, Gupta et al reported that India alone is burdened with approximately 25% of cardiovascular related deaths and would serve as a home to more than 50% of patients with heart ailments worldwide within next 15 years. Joshi et al
reported that the prevalence of CHD in urban North Indian population is 8.8% as compared to 3.81% in rural areas, indicating the differences in the diet and lifestyle characteristics and other conventional risk factors.\textsuperscript{49}

The Framingham Heart Study and the Seven Countries Study were the two major studies that made substantial contribution in identifying major risk factors for CVD.\textsuperscript{50} Framingham risk score is a widely used tool by clinicians globally to calculate 10-year CVD risk in an individual and for classifying them for risk of coronary death or myocardial infarction.\textsuperscript{51} Substantial evidence have provided the various (conventional) existing risk factors which can be modifiable or non-modifiable and the emerging/new risk factors. Over 300 existing (conventional) risk factors for CVDs have been discovered which includes predominantly high blood pressure, high blood cholesterol, tobacco use (chewing/smoking), diabetes mellitus and obesity in developed nations while in developing countries, in addition to these five risk factors, low vegetable and fruit intake and alcohol abuse ranks first in list of risk factors. More than 100 new/emerging risk factors have been discovered for their ability to improve the global risk assessment like positive family history, lipoprotein (a), serum homocysteine, coronary artery calcium score, C-reactive protein etc.\textsuperscript{52}

Asian – Indian phenotype is marked by combination of clinical (larger waist-to-hip and waist-to-height ratios signalling excess visceral adiposity), biochemical (insulin resistance, lower adiponectin and higher C-reactive
protein levels) and metabolic alterations (raised triglycerides, low high density lipoprotein cholesterol [HDL-C]). In 2006, Gaziano et al predicted that Asian Indians would account for 40-60% of global CVD burden in the coming 10-15 years. Over the past 30 years, there has been an increase in CHD-related incidence from 2-6% in rural population and from 4-12% in urban population. The prevalence of the so called Asian-Indian phenotype in South Asians has led to their increased vulnerability to diabetes and premature CHD. Prevalence of high lipoprotein (a), environmental and lifestyle risk factors explain the growing prevalence of heart disease in India. Higher predisposition to metabolic syndrome characterised by insulin resistance, hyperinsulinemia, type 2 diabetes, impaired glucose tolerance, central obesity, hypertension and dyslipidemia (high triglycerides and low high density lipoprotein levels) is an another important reason for increased CVD incidence in Indian population. The rural-urban differences, public-private health care, low awareness across the region, long term and asymptomatic nature of non-communicable risk factors and disease delays diagnosis of CVD and serve as a roadblock to seek care and self management of risk factors. The genetic and environmental factors act as important etiological clues to diversity in terms of disease presentation, therapeutic needs and responses to treatment. About 30-50% of coronary disease patients have been reported asymptomatic with absence of any conventional risk factor.
In United States, adults with no history of cardiovascular disease have been identified to possess intermediate risk and 10 year CVD risk of 10-20%. In the wake of increasing incidences of cardiovascular events in people who were apparently healthy and asymptomatic, both the American Heart Association and National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) issued directives to identify the individuals who may be sufficiently at high risk of coronary events in the future and justified efforts directed at aggressive risk reduction. It was also reported that even the modest elevation in blood pressure, cholesterol and glucose levels would predispose an individual at a CVD risk. Hence, screening of hyperglycemia for cardiovascular risk purposes should be targeted to high risk individual. Inspite of considerable efforts around the world for the prevention and treatment of cardiovascular diseases, they still remain to be the number one cause of death. This could be attributed to the rapidly increasing incidence of diabetes mellitus (DM), obesity, atherogenic dyslipidemia and elevated blood pressure.

**Diabetes Mellitus**

Diabetes Mellitus (DM) encompasses a range of diseases that are characterised by elevation of the blood glucose level and lead to a reduced quality of life and life expectancy with a greater risk of heart disease, stroke, peripheral neuropathy, renal disease, blindness and amputation. DM is characterised by hyperglycemia, glycosuria, hyperlipidemia, polyuria, polyphagia, polydypsia, negative nitrogen balance and sometimes ketonemia that result from defects in
insulin secretion or defective response of insulin or both.\textsuperscript{59} Four main etiological categories of DM have been identified:

- Type 1 diabetes characterised by deficiency of insulin due to destruction of pancreatic β-cells, progressing to absolute insulin deficiency.\textsuperscript{50}

- Type 2 diabetes characterised by a combination of insulin resistance and β-cell failure in association with obesity (typically with an abdominal distribution) and sedentary lifestyle-major risk factor for type 2 DM.\textsuperscript{61,62}

- Gestational diabetes mellitus develops during pregnancy. After delivery, most returned to euglycemic state, but they are at an increased risk for overt type 2 diabetes mellitus in the future.\textsuperscript{63}

- Other specific types of diabetes include:\textsuperscript{64}
  
  i. Single genetic mutations that lead to rare forms of diabetes mellitus such as maturity onset diabetes mellitus of young.

  ii. Diabetes mellitus secondary to other pathological conditions or diseases (pancreatitis, trauma or surgery of pancreas) and

  iii. Drug or chemically induced diabetes mellitus (such as in the treatment of HIV/AIDS or after organ transplantation)

Diabetes mellitus is a chronic complex metabolic disorder associated with altered metabolism of carbohydrate, protein and lipid, and increased risk of vascular complications like cardiovascular, renal, neural and visual disorders which are related to duration of the disease -
- Diabetic Retinopathy leading to blindness.
- Diabetic Nephropathy resulting in kidney failure.
- Damage to the nerves from diabetes known as diabetic neuropathy, is a leading cause of foot wounds and ulcers which frequently lead to foot and leg amputations. It can also lead to paralysis of stomach (gastroparesis), chronic diarrhoea and an inability to control heart rate and blood pressure during postural changes.
- Diabetes accelerates atherosclerosis (the formation of fatty plaques inside the arteries) which can lead to blockages or clot (thrombus). Such changes can then result in heart attack, stroke and decreased circulation in the arms and legs (peripheral vascular disease).
- Diabetes predisposes people to elevated blood pressure, high levels of cholesterol and triglycerides. These conditions, both independently and together with hyperglycemia, increase the risk of heart disease, kidney disease and other blood vessel complications.

Diabetes can contribute to a number of acute (short lived) medical problems like many infections are associated with diabetes due to impairment of body’s natural ability to fight infection. Hypoglycemia (low blood sugar), diabetic ketoacidosis (DKA) and hyperosmolar nonketotic syndrome are other associated acute medical conditions.59

DM is one of the most common non-communicable diseases globally. It is one of the leading causes of death in most high income countries and there is
substantial evidence that it is epidemic in many economically developing and newly industrialised countries. DM is undoubtedly one of the most challenging health problems in the 21st century. Around the globe, 382 million people (8.3%) suffered from DM in 2013 which is projected to rise to 592 million by 2035 with 80% of cases in low and middle income countries. The greatest number of people with DM is between 40-59 years of age. DM caused 5.1 million deaths in 2013 while 175 million people remained undiagnosed. In India, prevalence of diabetes in 2013 was 9.09% with 65.1 million being adults and 1.06 million deaths were reported. An interesting data pertaining to DM in India is depicted in Figure 1. Overall in South East Asia, half of people with diabetes are undiagnosed with 72.1 million people having diabetes in 2013 and predicted to increase to 123 million by 2035.

Type 2 DM develops following a prolonged period of euglycemic insulin resistance which progresses with the development of beta-cell failure to frank diabetes with increased risk of vascular complications. The present definition of diabetes is based on the level of glucose at which retinopathy occurs, but macrovascular complications such as coronary, cerebrovascular and peripheral artery disease appear earlier and using current criteria, are often present at the time when T2DM is diagnosed. Over 60% of people with type 2 diabetes develop CVD, a more severe and costly complication than retinopathy. Thus, CVD risk should be given a higher priority when cut points for hyperglycemia are defined and should be re-evaluated based on CVD risk. T2DM does not
cause specific symptoms for many years which explain why approximately half of the cases of T2DM remain undiagnosed at any time. Both undiagnosed type 2 diabetes and other disorders of glucose metabolism such as impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) are risk factors for CVD. The most convincing evidence for such relationships was provided by the collaborative DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) study, analysing several European cohort studies with baseline oral glucose tolerance test (OGTT) data. Women with newly diagnosed T2DM have a relatively higher risk for CVD mortality than their male counterparts. A recent British study revealed a greater adverse influence of DM per se on adiposity, HOMA-IR and downstream blood pressure, lipids, endothelial dysfunction and systemic inflammation in women compared with men, which may contribute to their greater relative risk of CAD.

Type 2 diabetes mellitus is characterised by a state of long standing insulin resistance, compensatory hyperinsulinemia and varying degrees of elevated plasma glucose, associated with clustering of cardiovascular risk and development of macrovascular disease prior to diagnosis (Figure 2). The development of cardiovascular disease in people with insulin resistance is a progressive process, characterised by early endothelial dysfunction and vascular inflammation leading to monocyte recruitment, foam cell formation and subsequent development of fatty streaks. Over many years, this leads to
atherosclerotic plaques, which in presence of enhanced inflammatory content, become unstable and rupture to promote occlusive thrombus formation. Atheroma from people with DM has more lipid, inflammatory changes and thrombus than those free from diabetes. These changes occur over a 20-30 years period and are mirrored by the molecular abnormalities seen in untreated insulin resistance and T2DM. Insulin resistance has an important role in the pathophysiology of T2DM and cardiovascular disease and both genetic and environmental factors facilitate its development. More than 90% of people with T2DM are obese and the release of free fatty acids (FFAs) and cytokines from adipose tissues directly impairs insulin sensitivity. In skeletal muscle and adipose tissue, FFA-induced reactive oxygen species (ROS) production blunts activation of insulin receptors substrate 1 (IRS-1) and PI3K-Akt signalling leading to down regulation of insulin responsive glucose transporter-4 (GLUT-4)\textsuperscript{75,76} (Figure 3). Thus, oxidative stress plays a major role in the development of micro-and macrovascular complications. Accumulation of free radicals in the vasculature of patients with DM is responsible for the activation of detrimental biochemical pathways leading to vascular inflammation and ROS generation. Since cardiovascular risk burden is not eradicated by intensive glycemic control associated with multifactorial treatment mechanisms based therapeutic strategies are needed. Specifically, inhibition of key enzymes involved in hyperglycemia-induced vascular damage, or activation of pathways which can improve insulin sensitivity, may represent promising approaches.
Obesity

The progressive impairment of carbohydrate intolerance with normal aging is generally accompanied by obesity and an increase in physical inactivity. These conditions promote hyperinsulinemia, insulin resistance and the development of type 2 diabetes and cardiovascular disease. Obesity is a growing health concern because of these associated co-morbidities. Obesity, defined as excess fat accumulation, is a most common cause of cardiovascular morbidity and mortality in industrialised country. An ongoing epidemic of obesity has been described worldwide and is replacing under nutrition and infectious diseases as the most significant global contributor to health problems. In 2005, 23.2% of the adult global population was estimated to be overweight (body mass index, BMI 25.0-29.9kg/m²) and 9.8% to be obese (BMI ≥ 30.0kg/m²), corresponding to 937 million and 396 million people, respectively. In 2030, the projected number for overweight and obese persons is 2.16 billion and 1.12 billion, respectively. Estimates from 2008 mention even high numbers with 1.46 billion adults being overweight and 502 million being obese worldwide.

In low and middle income countries, the increase in obesity is seen mostly in towns among the more wealthy, and in particular among women. In high income countries, obesity is more equally distributed among men and women but more prevalent in rural areas, socially and economically disadvantage group and neighbourhoods. An increasing prevalence of obesity has also
been reported from Asia. Using BMI 30kg/m² as a cut-off, the prevalence in urban areas in Western India was 4.8% in men and 7.8% in women in 1992. In 2004, urban areas in North India showed prevalence of 20.8% and 32.3% among males and females respectively. Using BMI of 25kg/m² as a cut-off, obesity prevalence in urban Northern India (2007) was 37.8% in men and 50.3% in women. Abdominal obesity carries particularly increased risk of cardiovascular disease (CVD) and type 2 diabetes and the risk seems to be associated with intra-abdominal (visceral) and not subcutaneous fat accumulation. Projected prevalence of overweight in India is depicted in Figure 4.

Two generally used clinical definitions of abdominal obesity exist, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III definition (2001) and International Diabetes Federation (IDF) definition (2005). Both use waist circumference (WC), and NCEP cut-offs are greater than 102 cm in men and more than 88 cm in women, although for men at an increased metabolic risk with multiple risk factors, WC more than 94 cm can be used. In South Asians, WC ≥ 90 cm for men while WC ≥ 80 cm are recommended cut-offs as per Indian recommendations. Waist to hip ratio (WHR) is also used to define abdominal obesity mostly with cut-off >0.9 in males and >0.85 in females. Hip circumference in some studies, has been inversely related to CVD, type 2 diabetes, hypertension, dyslipidemia and death due to association with increased subcutaneous fat mass. Because WHR
also includes measurements of hip circumference, the ratio has been suggested to be more useful than waist circumference. WHR, however, is less reproducible.\textsuperscript{87}

DM and obesity are closely related in terms of pathogenesis and pathophysiology. Both diabetes mellitus and obesity result in vascular complications through unknown mechanisms. This increased risk of CVD is due to complex cluster of risk factors which include insulin resistance, hyperglycemia, dyslipidemia, hypertension, hyperinsulinemia, systemic inflammation and adipose tissue-derived factors.\textsuperscript{88} Changes in the mass and metabolism of adipose tissue may be related to insulin resistance – a common pathogenic mechanism in T2DM and obesity.\textsuperscript{89}

**Obesity, DM and CVDs: The Biochemical Orchestration**

The classical perception of adipose tissue as a storage site for fatty acids has been replaced over the last years by the notion that adipose tissue has a central role in lipid and glucose metabolism and produces a large number of hormones and cytokines e.g., angiotensinogen, tumour necrosis factor–α (TNF-α), interleukin-6 (IL-6), adiponectin, leptin, plasminogen activator inhibitor-1 (PAI-1).\textsuperscript{90-92} The close relationship between an increased quantity of visceral fat, metabolic disturbances and cardiovascular diseases, and the unique anatomical relation to hepatic portal circulation has led to an intense endeavour to unravel the specific endocrine functions of this visceral fat depot. From a pathophysiological point of view, the “quality” of adipose tissue is
more important than the “quantity”. Nevertheless, a major driver of adipose tissue function is the quantity of visceral fat. Adipose tissue exists in adipocytes and a vascular-stromal fraction in which macrophages, fibroblast, endothelial cells and pre-adipocytes are present. The primary and classical roles of adipose tissue are to insulate and cushion the body, to store free fatty acids (FFAs) after food intake and to release FFAs during fasting state to ensure sufficient energy status. During the post-prandial phase, FFAs are taken up from the blood in adipose tissue after hydrolysis of triglycerides (TGs) from VLDL-cholesterol, chylomicrons and their remnants by lipoprotein lipase (LPL). Mobilisation of this reserve occurs by hydrolysis of adipocyte TG by hormone sensitive lipase (HSL). Insulin is the main regulator of adipocyte fat content, since it is both a potent inhibitor of HSL and an important activator of LPL, thereby enhancing FFA uptake and TG synthesis in adipocytes.

Adipocytes and adipose tissue produce a wide range of hormones and cytokines involved in glucose metabolism (e.g. adiponectin, resistin), lipid metabolism (e.g. cholesteryl ester transfer protein, CETP), inflammation (e.g. TNF-α, IL-6), coagulation (PAI-1), blood pressure (e.g. angiotensinogen, angiotensin II) and feeding behaviour (leptin) thus affecting metabolism and function of many organs and tissues including muscle, liver, vasculature and brain (Figure 5). Plasma adipokine levels rise with an increase in adipose
tissue and adipocyte volume except for plasma adiponectin which is lower in obesity.\textsuperscript{94}

Obesity is associated with the appearance of chronic, low grade inflammatory state due to changes in function of adipocytes and macrophages.\textsuperscript{95} This indicates that there is not merely an increase in secretion of proteins but also that inflammation ensues from changes in secretory function. The term “adipose tissue dysfunction” has been used for this state of hypersecretion of pro-atherogenic, pro-inflammatory and pro-diabetic adipocytokines which is accompanied by decreased production of adiponectin. Obesity has a strong genetic predisposition and results from an excess energy intake and/or too little energy expenditure. It is associated with marked changes in secretory function of adipocytes and macrophages, together with chronic low grade inflammation and an increased risk to develop insulin resistance, diabetes and/or vascular disease.\textsuperscript{96} Tumour necrosis factor-alpha (TNF-\(\alpha\)), IL-6, FFAs induced serine phosphorylation of insulin receptor substrate-1 (IRS-1) and IRS-2 which reduces the capacity of insulin receptors substrate proteins to be phosphorylated by insulin receptor \textit{in vitro} and may even inhibit insulin receptor auto-phosphorylation (tyrosine kinase) activity thereby further attenuating the insulin signalling cascade. FFAs presumably acts through activation of protein kinase C (PKC) isoforms after formation of diacylglycerol while TNF-\(\alpha\) acts via activation of c-jun N-terminal kinase 1. In muscle, FFA related generation of acyl CoA derivatives (e.g. ceramide) can
diminish Akt 1 activity and hence insulin action. In liver, IRS-2 is involved in inhibition of gluconeogenesis which is often augmented in an insulin-resistant state, possibly via activation of both PKC and c-jun N-terminal kinase 1 by FFA and TNF-α\textsuperscript{97,98} (Figure 6). Thus, both FFAs and TNF-α, secreted in large quantities by enlarged adipocytes play a prominent role in the development of insulin resistance.\textsuperscript{99} Since insulin is the main regulator of hormone sensitive lipase, the rate limiting enzyme for hydrolysis of triglyceride, the inhibitory effect of free fatty acids on insulin sensitivity leads to enhanced lipolysis in adipocytes. This effect is further augmented by upregulation of triglyceride hydrolysis by TNF-α in adipose tissue. Besides, TNF-α also contributes to insulin resistance by inhibiting the expression of genes, essential for insulin signalling and adipocyte differentiation (CCAAT-enhancer binding protein-alpha, peroxisome proliferator activated receptor-gamma [PPAR-γ], glucose transporter type 4, IRS-1 protein, adiponectin and long chain fatty acid acyl CoA synthase) providing another molecular basis for insulin resistance.\textsuperscript{100} Adiponectin increases insulin sensitivity by inhibiting hepatic gluconeogenesis and increasing fatty acid oxidation in both liver and muscle as a result of improved 5’-AMP activated protein kinase activity.\textsuperscript{101} Single-nucleotide polymorphisms (SNPs) of the promoter region of adiponectin gene may relate to the development of insulin resistance, obesity and type 2 diabetes.\textsuperscript{102} In most studies, low adiponectin and elevated levels of other adipocytokines (e.g. leptin, TNF-α, IL-6) are associated with an increased risk of diabetes
presumably not related to their effects on insulin sensitivity but also to their effects on pancreas leading to β cell failure.\textsuperscript{103,104}

Chronically elevated free fatty acids inhibit insulin secretion. \textit{In vitro}, long chain fatty acyl CoA and free fatty acid can open β cell potassium channels which diminish insulin secretion. Free fatty acids enhance expression of uncoupling protein-2 (UCP-2) which decreases ATP production necessary for secretion of insulin. FFAs can also induce β cell apoptosis via endoplasmic reticulum stress response and by inhibition of expression of anti-apoptotic factor Bcl-2. Leptin has also been shown to be anti-apoptotic which may be diminished in obese/leptin – resistant state. Anti-apoptotic effects of leptin include inhibition of nitric oxide (NO) production via reduction triglyceride content. NO has been proposed to induce apoptosis via depletion of calcium stores in endoplasmic reticulum (ER) leading to ER stress response. By inhibiting insulin signaling in β cell and by induction of NO synthesis, TNF-α may reduce insulin secretion \textit{in vitro}. At the same time, NO may cause damage to DNA enhancing β cell apoptosis.\textsuperscript{105,106}

Atherosclerotic vascular disease may also be an important clinical result of adipose tissue dysfunction. Dysfunctional adipocytes contribute directly and indirectly (through insulin resistance) to the development of vascular risk factors and vascular disease. Elevated levels of IL-6, TNF-α and presence of insulin resistance lead to decrease in production and availability of eNOS resulting in endothelial dysfunction. Increased concentration of adipocyte
derived cholesteryl ester transfer protein (CETP) results in lower levels of HDL-C and increase in small dense LDL-C particles. Adiponectin has inhibitory effects on the development of atherosclerosis by inhibiting the expression of adhesion molecules [intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)], induced by IL-6 and TNF-α on endothelial cells by activating 5’AMP-activated protein kinase (AMPK) in vitro by inhibiting NF-κβ and by inhibition of scavenger receptor class A-1 which leads to reduction of cholesterol uptake in macrophages and 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 plasminogen activator inhibitor-1 (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.

Waist-to-hip ratio (WHR) and waist circumference (WC), good indicators of abdominal obesity, are more closely associated with atherosclerosis and the risk of myocardial infarction than BMI. After controlling for cardiac risk factors, including BMI, women with WHR of atleast 0.76 were more than twice as likely to develop coronary heart disease compared with women
having WHR <0.72. Women with WHR >0.88 were even more than three times as likely to develop coronary heart disease.\textsuperscript{109} Thus, increasing physical activity and weight reduction are two important lifestyle changes to reduce insulin resistance and visceral obesity (mainly by decreasing the size of existing adipocytes) and are therefore central in modifying adipose tissue dysfunction. However, changes in lifestyle are difficult to achieve and to maintain in clinical practice, but are required for a constant improvement of a patient’s metabolic profile.\textsuperscript{110}

**Adiponectin**

Abdominal obesity is associated with decreased levels of the vascular protective adipokine, adiponectin. It is the gene product of adipose most abundant gene transcript-1 (apM-1) gene: bearing structural homology to collagen VIII, X and complement C1q as well as TNF-\(\alpha\). It is a 244-amino acid polypeptide with molecular weight of 30 kDa i.e. exclusively secreted by adipocytes of white adipose tissue and acts as a hormone with anti-inflammatory, anti-atherogenic and insulin sensitizing properties. It is also known as adipocyte complement-related protein (Acrp 30), gelatin-binding protein 28 (Gbp 28) or adipo Q.\textsuperscript{111} Adiponectin circulates not only indifferent multimeric forms but also as a proteolytic fragment: the globular form having structural similarities with TNF-\(\alpha\). The basic form is a trimer but can exist as hexamers and multimers-high molecular weight adiponectin (HMW adiponectin).\textsuperscript{112-114} The hexamers and HMW forms are the major
configurations in plasma having longer half-life. The different forms of circulating adiponectin are found at different target organs e.g. HMW adiponectin is particularly active in liver and in endothelial cells.\textsuperscript{112} HMW form has been shown to be the biologically most active and potent at insulin sensitization.\textsuperscript{115,116} Circulating adiponectin levels in normal subjects has been reported as 5-20μg/ml, thus accounting for approximately 0.01% of total plasma protein.\textsuperscript{117} It is detectable in cerebrospinal fluid (CSF) with CSF concentrations in humans typically 0.1% of corresponding plasma concentration. Adiponectin secretion, in contrast to secretion of other adipokines, is paradoxically decreased in obesity. It is the only fat protein that is down regulated with weight gain and it is possible that accumulation of visceral fat might produce inhibiting factors for adiponectin synthesis or secretion such as TNF-α.\textsuperscript{94} Besides obesity, adiponectin concentrations are decreased in a variety of human metabolic and cardiovascular disease states including type 2 diabetes mellitus, lipodystrophy, non-alcoholic hepatic steatosis, essential hypertension and coronary artery disease. Low adiponectin levels precede the development of insulin resistance and myocardial infarction in humans. Interestingly, adiponectin levels increase with age and are elevated in type 1 diabetes.\textsuperscript{118} (Table 1)

Adiponectin has insulin-sensitizing action in the liver, and lowers blood glucose levels by improving insulin-mediated suppression of gluconeogenesis.\textsuperscript{119} (Figure 7a) In liver and skeletal muscle, adiponectin also
improves glucose utilisation and stimulates fatty acid oxidation via a pathway that involves AMP kinase (AMPK) and acetyl CoA carboxylase (ACC). It also stimulates food intake by activating hypothalamus.\textsuperscript{120} (Figure 7b) It also prevents TNF-α stimulated expression of adhesion molecules in cultured human endothelial cells by inhibiting IKKβ phosphorylation and NF-κβ activation and inhibits transformation of macrophages into foam cells.\textsuperscript{121-123} In addition, the cardio-protective effects of adiponectin are attributed to its ability in suppressing apoptosis, oxidative/nitrative stress and inflammation in cardiomyocytes. The anti-apoptotic activity of adiponectin is dependent on the activation of AMPK.\textsuperscript{124} Adiponectin differentially regulates NO production by eNOS and iNOS. It stimulates NO production via eNOS activation, thereby contributing to its vasodilator and vascular protective effects. However, under pathological conditions adiponectin exerts its anti-nitrative actions in cardiomyocytes by preventing the induction of iNOS expression and the resulting excess NO generation.\textsuperscript{125} Together, these effects have been shown to prevent plaque formation in apoE-deficient mice, a mouse model of atherosclerosis.\textsuperscript{126}

Adiponectin’s diverse actions in these tissues are mediated by its receptors, AdipoR1 and AdipoR2. Both are structurally related seven transmembrane receptors which are distinct from classical G-protein coupled receptors. They have an inverted membrane topology with a cytoplasmic NH\textsubscript{2} terminus and a short extracellular COOH terminus of approximately 25 amino acids. In
humans, AdipoR1 is ubiquitously expressed, with highest levels of expression in heart and skeletal muscle; while AdipoR2 expression is more restricted to skeletal muscle and liver. Overexpression of AdipoR1 increased AMPK phosphorylation and reduced the expression genes involved in hepatic gluconeogenesis; while overexpression of AdipoR2 increased peroxisome proliferator-activated receptor-α (PPAR-α) mRNA and reduced the expression of inflammatory cytokines and markers of oxidative stress besides reducing hepatic triglyceride content.

In addition to AdipoR1 and AdipoR2, adiponectin also binds to T-cadherin, a receptor localised on vascular endothelium and muscle cell which may underline some anti-atherogenic and vascular-protective actions of adiponectin. T-cadherin is a glycosylphosphatidylinositol-anchored extracellular protein which may function as co-receptor with AdipoR1 and AdipoR2 to facilitate adiponectin signalling in specific tissues or cell types. Adiponectin and its receptor (AdipoRs) have been found to play significant roles in the etiology of obesity-related chronic diseases. Yamauchi et al (2008) proposed an “adiponectin hypothesis” according to which reduced adiponectin levels can be caused by genetic factors such as SNP276 in the adiponectin gene itself. Reduced adiponectin levels can also be due to changes in lifestyle causing obesity, such as high fat diet. Both functional and genetic studies on adiponectin strongly suggest that reduced adiponectin levels play a causal role in the development of insulin resistance, metabolic syndrome, type 2 diabetes
and atherosclerosis. Another hypothesis entitled “inflammation and insulin resistance in adipose tissue” has been proposed by Wellen and Hotamisligil. According to their hypothesis, hypertrophic adipocytes, preadipocytes and endothelial cells may secrete monocyte chemoattractant protein-1 (MCP-1) which recruits macrophages, thereby inducing inflammation and insulin resistance through secretion of adipokines such as TNF-α, CRP, IL-6, FFA etc. TNF-α induces serine phosphorylation of insulin receptor substrate-1 (IRS-1) which inhibits insulin receptor kinase activity and downstream signalling via PI3K activation. Both IL-6 and TNF-α reduce the expression of IRS-1, GLUT-4 and PPAR-γ in 3T3-L1 adipocytes. Furthermore, IL-6 induces expression of suppressor of cytokine signalling by binding to the insulin receptor and IRS-1. In hypertrophic adipocytes observed in obesity, decrease adiponectin action and increased MCP-1 form a vicious adipokine network to cause obesity-linked insulin resistance and metabolic syndrome. (Figure 8)

Clear relationship exists between adiponectin and fat mass in humans. Adiponectin release is positively correlated with fat cell size and negatively with BMI. Adiponectin release is significantly lower in omental than in subcutaneous adipose tissue. The levels of adiponectin were found to be increased in more insulin-sensitive subjects on a high fat diet suggesting increased capacity for fat oxidation and may seem to be protective against development of type 2 diabetes. Adiponectin was inversely associated with
insulin resistance in non-diabetic subjects, independently of age, blood pressure, adiposity and serum lipids. Another study performed in subjects with normal weight, has shown that plasma adiponectin is negatively correlated with BMI, systolic and diastolic blood pressure, fasting plasma glucose, insulin, insulin resistance, total and LDL-cholesterol, triglycerides, uric acid and positively correlated with HDL-cholesterol. Caucasians have higher serum adiponectin levels compared with Indo-Asians. On the contrary, plasma adiponectin concentrations are significantly increased in type 1 diabetic patients compared with healthy controls. Experimental evidence suggest that adiponectin may play a protective role against atherosclerosis. Hyperinsulinemia caused significant decrease of adiponectin plasma levels under euglycemic conditions. Hypoadiponectinemia might atleast partly be a link between hyperinsulinemia and vascular diseases in metabolic syndrome. A significant negative correlation found between plasma adiponectin concentration and mean systolic and diastolic blood pressure, suggested that adiponectin contributes to the clinical course of hypertension. Circulating adiponectin levels were found to be suppressed five fold in patients with severe insulin resistance due to dominant-negative PPAR-γ mutations, thus suggesting that adiponectin may be a biomarker of in vivo PPAR-γ activation.

Adiponectin is also one of the strongest biochemical predictors of T2DM as risk of type 2 diabetes appeared to decrease with increasing adiponectin levels
in number of studies.\textsuperscript{146-148} Even a low plasma adiponectin concentration was reported as a sensitive predictor of impaired fasting glycemia (IFG) in the development of diabetes.\textsuperscript{149} Besides, certain previous studies have found negative correlation of serum adiponectin with HbA\textsubscript{1c} levels while no significant correlation was found between adiponectin and age, DM duration, glucose and insulin levels.\textsuperscript{150} Thus, higher levels of adiponectin are reported to improve insulin sensitivity.\textsuperscript{151}

**Leptin**

Various other adipokines like TNF-\(\alpha\), IL-6 and leptin are able to induce insulin resistance.\textsuperscript{38} Leptin, the protein product of the \textit{ob} gene, is a multifunctional peptide hormone, the circulating concentration of which is proportional to fat mass since adipocytes are the primary source of leptin. Leptin, discovered by Friedman et al in 1994 through positional cloning, is a 16-kDa adipocyte-secreted hormone that circulates in the serum in the free and bound form.\textsuperscript{152} Leptin, a 167 amino acids peptide, has structural homology to TNF-\(\alpha\), IL-6, leukemia inhibitory factor, granulocyte-colony stimulating factor, glycoprotein 130 (gp130) and other cytokine family proteins. It is produced predominantly in the adipose tissue but is also expressed in a variety of other tissues including placenta, ovaries, mammary epithelium, bone marrow and lymphoid tissues. The normal plasma leptin concentration in females varies from 3.7-11.1 ng/ml while in males, it ranges between 2.0-5.6 ng/ml; the concentration is directly proportional to the amount of adipose tissue. Leptin acts by binding
to specific receptors in the hypothalamus to alter the expression of several neuropeptides including melanocortin system, neuropeptide Y and corticotrophin releasing factor that regulate neuroendocrine function and energy expenditure. In fact, leptin is considered to be the hormonal signal bridging the peripheral adipose tissue to the central nervous system for the control of appetite and energy expenditure. The CNS action of leptin is important for the maintenance of energy balance, most likely through an increased sympathetic tone. In addition, localization of leptin receptors in a wide range of tissues has indicated central as well as peripheral actions of leptin.153

In humans, the release of leptin into the circulation is pulsatile. Its concentration follows a circadian rhythm and is affected by sleep patterns. Women have greater leptin concentration than men for the same age and body mass index (BMI). The sex difference in leptin concentration may be attributed to difference in sex hormones, e.g. estrogen and testosterone beside BMI. Leptin influences the central regulation of food intake and energy expenditure via cerebral leptin receptors (Figure 9). Overeating (hyperphagia) may lead to an elevation whereas fasting or caloric restriction results in a fall in leptin levels. Studies have demonstrated that leptin stimulates the expression of pro-opiomelanocortin (POMC) which is extensively modified post-transitionally in primary neurons of hypothalamus to produce few smaller biologically active peptides like melanocortins, which act at respective
receptors and mediate anorectic response. In contrast, orexigenic pathways mediated by neurons expressing melanocortin antagonist Agouti-related peptide and neuropeptide Y are inhibited by leptin. Both these pathways interact with other brain centres to coordinate appetite. Reduced serum leptin acts as a signal of nutritional deprivation initiating an adaptive response to conserve energy, which is manifested by increased food intake, decreased energy expenditure and suppression of reproductive and certain other endocrine axes thereby establishing that starvation/fasting could be considered a state of relative leptin deficiency.  

Leptin is a key regulator of glucose metabolism in mammals. It acts through peripheral and/or central pathways probably by increasing plasma glucagon and growth hormone which may improve insulin sensitivity and mediate the restoration of euglycemia and by inhibiting hepatic glucose production due to down regulation of expression of gluconeogenic genes in liver such as glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. It has also been shown to increase skeletal muscle glucose and fatty acid oxidation both in vitro and in vivo. These effects may be especially important because excessive deposition of lipids in skeletal muscle has been implicated in the development of insulin resistance. The proposed mechanism for this effect involves cross-talk between the leptin and insulin signaling pathways especially phosphatidyl inositol–3 kinase (PI3K) and activation of 5’-AMP activated protein kinase (AMPK). It also directly affects glucose metabolism
in liver, one of the primary tissues where leptin acts. It may alter the action of insulin in adipocytes and may promote glucose and fatty acid oxidation and lipolysis. Overall, leptin promotes energy dissipation and decreases lipid deposition in adipose tissue. Nevertheless, in human obesity, excessive lipid deposition in adipose tissue cannot be corrected despite the elevated circulating leptin levels due to suppressive mechanisms that impair leptin action in adipose tissue. It is also generally accepted that leptin significantly reduces insulin release from pancreatic beta cells under physiological conditions. There are several mechanisms through which it may suppress insulin secretion by acting directly on pancreas. It affects ATP sensitive potassium channels through PI3K dependant activation of phosphodiesterase 3B (PDE3B) and reduces glucose transport into beta cells. It has also been shown to suppress preproinsulin mRNA and insulin promoter activity in vitro and in vivo. It effectively inhibits glucagon secretion from pancreatic alpha cells. Thus, leptin represents a signaling molecule from adipose tissue to endocrine pancreas that suppresses insulin secretion according to the needs dictated by body fat stores; this connection establishes a classic feedback loop, the “adipo-insular axis” (Figure 10).\textsuperscript{153}

Like other metabolic hormones such as insulin, leptin possesses potent vascular effects and participates in the regulation of sympathetic tone and arterial blood pressure. It has been shown directly to induce vasorelaxation through nitric oxide (NO)-dependent as well as NO-independent mechanisms.
Leptin elicits an NO-dependent arterial vascular relaxation with the chloride ion being an important regulator in leptin-induced endothelial NO release. The leptin-induced vascular NO production was further proven to be mediated through PI-3 kinase-independent endothelial nitric oxide synthase (eNOS) phosphorylation by the Akt pathway. Recent evidence indicated the possible involvement of AMP-activated protein kinase (AMPK) in enhanced eNOS phosphorylation and NO production in response to leptin. AMPK, activated by an increase in the AMP-to-ATP ratio, and/or decrease in phosphocreatine, is known to mediate the metabolic effects of leptin. Leptin directly induces vasodilation through an NO-independent pathway in healthy men. Further, leptin has been shown to stimulate endothelial NO synthesis and upregulate endothelin-1 (ET-1) production. Leptin promotes angiogenesis, enhances the calcification of vascular cells and potentiates the pro–thrombotic platelet aggregation through a novel leptin receptor-dependant mechanism. Plasma leptin levels are strongly correlated with adiposity. Tissue adiposity, along with gender, is the main determinant of leptin gene expression. Change in plasma leptin levels and/or leptin signaling has a profound pathological impact on body weight control. Both leptin deficiency and leptin receptor defect are sufficient to produce obesity of genetic origin. However, the most common trigger of obesity is not genetic defect of leptin or its receptors but rather depends upon overeating especially high fat intake, which may rapidly increase plasma leptin levels. Although it has been speculated that hyperleptinemia leads to tissue resistance of leptin and insulin, how
hyperleptinemia and/or impaired leptin signaling triggers cardiovascular dysfunction is unclear. Several factors have been identified as participating in the regulation of leptin synthesis and release including the sympathetic nervous system, insulin and body adiposity. Sympathetic nervous system activity is believed to be a key inhibitor of leptin release; catecholamines directly inhibit leptin synthesis. Paradoxically, leptin may directly activate sympathetic outflow within the hypothalamus and stimulate adrenal medullary release of epinephrine, thus creating a negative feedback loop between leptin and the sympathetic nervous system. There is a positive correlation among plasma proinsulin, insulin and leptin levels, all of which were elevated in hypertensive patients when indexed for body size. A significant negative correlation was found between the levels of leptin and IL-6. In a multivariate analysis, plasma leptin levels were found in correlation with diet, independent of age, BMI, body fat, alcohol consumption or insulin.\textsuperscript{153}

In contrast to obese subjects with congenital leptin deficiency, garden-variety obese individuals have greater leptin concentrations than lean individuals and are resistant or tolerant to the effects of leptin. Leptin resistance or tolerance was first thought to be due to mutations of the leptin receptor and other rare monogenic obesity syndromes. However, most instances appear to be multifactorial; only a few cases of human obesity are due to monogenic syndromes. Although it was determined that the exact defect in the leptin receptor present in db/db mice is not present in obese humans, several genetic
variants are associated with hyperleptinemia, including the Lys109Arg or Gln223Arg mutation in the leptin receptor (LEPR) gene. Mutations of other genes downstream of leptin, including POMC and the melanocortin 4 receptor (MC4R), also result in an obese phenotype with associated neuroendocrine dysfunction. Leptin transport across the blood-brain barrier is impaired in obesity. This is partially due to saturation of the transporter as a result of hyperleptinemia and a subsequent decrease in transport activity. Moreover, different brain regions may saturate at different concentrations. In addition, the soluble leptin receptor isoforms ObRe antagonizes leptin transport by inhibiting surface binding and endocytosis of leptin. Some investigators have found that in more obese patients with type 2 DM, the leptin levels were comparatively low in patients with poorly controlled diabetes than in those with well controlled diabetes. Yet, few other studies found no significant difference in the plasma levels of leptin between diabetic and non diabetic patients, thus indicating no association with the risk of type 2 diabetes. Leptin relationship with total body fat and insulin resistance has been observed to be independent of age and gender. Leptin-induced increases in sympathetic nerve activity have been suggested to contribute to hypertension, and leptin has been observed to increase oxidative stress in cultured endothelial cells. Many of these pathophysiologic effects of leptin on the vasculature are most likely of importance when leptin levels are elevated in obese subjects due to resistance to the weight-reducing effects of the hormone.156
Insulin, C-peptide and Insulin resistance

Insulin resistance, characterized by hyperinsulinemia, hyperglycemia and alterations in levels of adipokines, could lead to vascular endothelial dysfunction, an abnormal lipid profile, hypertension and vascular inflammation, all of which promote the development of cardiovascular disease. Insulin is produced in the beta cells of the pancreatic islets. It is initially synthesized as a single-chain 86-amino acid precursor polypeptide, preproinsulin. Subsequent proteolytic processing removes the amino-terminal signal peptide, giving rise to proinsulin. Proinsulin is structurally related to insulin-like growth factor I and II which bind weakly to the insulin receptor. Cleavage of an internal 31-residue fragment from proinsulin generates the C-peptide and the A (21 amino acids) and B (30 amino acids) chains of insulin, which are connected by disulphide bonds (Figure 11). The mature insulin molecule and C-peptide are stored together and co-secreted from secretory granules in the beta cells. Pancreatic beta cells co-secrete islet amyloid polypeptide (IAPP) or amylin, a 37-amino acid peptide, along with insulin. The role of IAPP in normal physiology is unclear but it is the major component of the amyloid fibrils found in the islets of patients with type 2 diabetes, and an analogue is sometimes used in treating both type 1 and type 2 DM.157

Glucose is the key regulator of insulin secretion by the pancreatic beta cell, although amino acids, ketones, various nutrients, gastrointestinal peptides, and
neurotransmitters also influence insulin secretion. Glucose level >3.9mmol/l (70mg/dl) stimulate insulin synthesis, primarily by enhancing protein translation and processing. Glucose stimulation of insulin secretion begins with its transport into the beta cell by the GLUT 2 glucose transporter. Glucose phosphorylation by glucokinase is the rate limiting step that controls glucose-regulated insulin secretion. Further metabolism of glucose-6-phosphate via glycolysis generates ATP, which inhibit the activity of an ATP-sensitive potassium channel. This channel consists of two separate proteins: one is the binding site for certain oral hypoglycemics (e.g. sulphonylureas, miglinitides); the other is an inwardly rectifying potassium channel protein (Kir 6.2). Inhibition of this potassium channel induces beta cell membrane depolarisation, which opens voltage-dependent calcium channels (leading to an influx of calcium) and stimulates insulin secretion. Insulin secretory profiles reveal a pulsatile pattern of hormone release, with small secretory bursts occurring about every 10 minute, superimposed on greater-amplitude oscillations of about 80-150 minutes. Incretins are released from neuroendocrine cells of the gastrointestinal tract following food ingestion and amplify glucose-stimulated insulin secretion and suppresses glucagon secretion. Glucagon-like peptide 1 (GLP-1), the most potent incretin, is released from L-cells in the small intestine and stimulates insulin secretion only when blood glucose is above the fasting level. Incretin analogues such as exena-tide, are being used to enhance endogenous insulin secretion.
Once insulin is secreted into the portal venous system, about 50% is degraded by the liver. Unextracted insulin enters the systemic circulation where it binds to receptors in target sites. Insulin binding to its receptor stimulates intrinsic tyrosine kinase activity, leading to receptor autophosphorylation and the recruitment of intracellular signalling molecules, such as insulin receptor substrates (IRSs). IRSs and other adaptor proteins initiate a complex cascade of phosphorylation and dephosphorylation reactions, resulting in the widespread metabolic and mitogenic effects of insulin. As an example, activation of the phosphatidylinositol-3-kinase (PI-3 kinase) pathway stimulates translocation of glucose transporter e.g. GLUT 4 to the cell surface, an event that is crucial for glucose uptake by skeletal muscle and fat. Activation of other insulin receptor signalling pathways induces glycogen synthesis, protein synthesis, lipogenesis, and regulation of various genes in insulin responsive cells. Glucose homeostasis reflects a balance between hepatic glucose production and peripheral glucose uptake and utilisation (Figure 12). Insulin is the most important regulator of this metabolic equilibrium, but neural input, metabolic signals, and other hormones (e.g. glucagon) result in integrated control of glucose supply and utilisation. In the fasting state, low insulin levels increase glucose production by promoting hepatic gluconeogenesis and glycogenolysis and reduce glucose uptake in insulin sensitive tissues, thereby promoting mobilisation of stored precursors such as amino acids and free fatty acids (lipolysis). Glucagon, secreted by pancreatic alpha cells when blood glucose or insulin levels are low, stimulates
glycogenolysis and gluconeogenesis by the liver and renal medulla. Postprandially, the glucose load elicits a rise in insulin and fall in glucagon, leading to a reversal of these processes. Insulin, an anabolic hormone, promotes the storage of carbohydrate and fat and protein synthesis. The major portion of postprandial glucose is used by skeletal muscle, an effect of insulin stimulated glucose uptake. Other tissues, most notably the brain, utilises glucose in an insulin independent fashion.\(^{157}\)

Fasting insulin levels have been reported to be higher in overweight/obese subjects as compared to normal weight subjects.\(^{12}\) It is also known that significantly high insulin levels (hyperinsulinemia) with normal fasting blood glucose are features of insulin resistance which may further be implicated in the development of CVD.\(^{158}\) It is well documented that high levels of insulin are associated with elevated “connecting peptide” (C-peptide) levels as both are produced in equimolar amounts. C-peptide was initially considered an inert substance, but recent research findings have proven that it is a biologically active peptide that affects various cell membranes, including endothelial, renal and nerve cells.\(^{12}\)

In general, C-peptide is produced by a series of enzymatic cleavages of the precursor molecules-preproinsulin and proinsulin (Figure 11). Preproinsulin, a precursor of proinsulin, is produced in the endoplasmic reticulum of pancreatic beta cells in response to elevated blood glucose levels in healthy individuals; it is then cleaved by microsomal enzymes into proinsulin.\(^{159}\)
Proinsulin is the precursor of insulin and C-peptide. It consists of the alpha chain and beta chain of insulin linked together by C-peptide. C-peptide is composed of 31 amino acids and facilitates the correct folding of proinsulin to allow the cysteine disulphide bridges between the alpha chain and beta chain of insulin to form. Enzymatic cleavage of proinsulin by proconvertases and carboxypeptidases produces insulin and C-peptide which are released from beta cells into portal circulation. C-peptide has several conserved sequences despite some variability in different species, e.g. N-terminal acidic region, glycine rich central segment, and C-terminal pentapeptide.\(^{160}\) C-terminal pentapeptide gives full replacement of the entire molecule which is similar to other peptides with hormone function like gastrin and cholecystokinin. C-peptide is cleared by the kidney and has a half life of about 20-30 minutes compared to insulin which is cleared through liver and has a half life of about 3-5 minutes.\(^{159}\) The longer half life, renal clearance, and equimolar release of C-peptide make it an attractive proxy for estimating insulin secretion and beta cell function.

While the physiological functions of insulin are well established and understood, the functions of C-peptide are still being established and investigated. C-peptide has been shown to bind to specific cell surface receptors in cultured cells derived from human renal tubules, mesangium, skin fibroblast and saphenous vein endothelium.\(^{159}\) C-peptide interaction with cell membranes is accompanied by activation of a pertussis toxin-sensitive G-
protein. Subsequently, there is influx of calcium ions and activation of eNOS resulting in NO formation. Phospholipase C and specific isomers of protein kinase C (PKC) are also activated as well as MAPK complex. This leads to activation and induction of Na\(^+\)-K\(^+\) ATPase as well as DNA binding of several transcription factors, resulting in augmented eNOS mRNA formation and increased eNOS protein synthesis. Phosphoinositide 3-kinase (PI3-K) gamma is also activated, giving rise to PPAR-γ mediated transcriptional activity. In addition, there is evidence to indicate that C-peptide may interact synergistically with insulin signalling pathway.\(^{160-165}\)

Inflammation is increasingly being recognised as an important factor contributing to vascular damage in diabetes. There is evidence indicating that hyperglycemia may precipitate an inflammatory vascular response and play a significant role in the development of endothelial dysfunction. The multiple effects of C-peptide on inflammatory processes are shown in figure 13.\(^{166-168}\)

Endothelial dysfunction and compromised microvascular circulation is common denominators in the development of microvascular complications of diabetes. C-peptide exerts its circulatory effects by stimulating calcium uptake by endothelial cells leading to increased expression and activity of eNOS and increased production of NO. NO elicits relaxation of smooth muscle cell and vasodilation. In RBCs, it increases uptake of glucose and subsequent release of ATP which in turn stimulates NO production in endothelial cells. In
addition, C-peptide improves the erythrocyte deformability by enhancing Na\(^+\)-K\(^+\)-ATPase activity.\(^{163, 169, 170}\)

There is a certain controversy regarding reported effects of the C-peptide. Its beneficial effects have been demonstrated in long term complications in type 1 diabetes mellitus. In contrast, C-peptide in type 2 diabetes mainly shows pro-inflammatory and pro-atherogenic effects.\(^{171}\) A study done by Kim et al (2011) suggested that basal C-peptide levels in type 2 DM related to metabolic syndrome correlates to intima-media thickness and C-peptide could be used as surrogate marker of sub-clinical atherosclerosis.\(^{172}\) Since C-peptide and insulin are released in equimolar amounts from the beta cells of pancreas, the measurement of C-peptide has been used as a marker of beta cell function and an index of insulin secretion. The great interest in C-peptide over insulin is due to limitations of the use of serum insulin as a measure of insulin secretion. After its secretion into portal vein, insulin passes through liver where approximately 50% of the insulin delivered is extracted. Peripheral insulin concentration therefore reflects post hepatic insulin delivery rather than actual secretory rates of insulin. Until the development of C-peptide assays, evaluation of beta cell function in insulin treated patients was impossible as insulin assay is unable to discriminate between secreted and injected insulin. Further, C-peptide determination is disturbed, to lesser extent, than insulin measurement by the presence of insulin binding antibodies and insulin analogues that the patient undergoing insulin therapy uses.\(^{173}\) Thus, plasma C-
peptide concentrations provide an indirect measure of the endogenous insulin secretory reserve.

Abdullah et al considered fasting C-peptide level of <0.6 ng/ml as an indicator of poor insulin reserve; thus suggesting the role of C-peptide as a useful guide in initiating therapy to prevent diabetic complications. The study also reported higher fasting C-peptide levels in the obese type 2 diabetics than in non obese diabetics. This increase in C-peptide levels was associated with increased plasma glucose due to insulin resistance inferring that majority of patients with elevated fasting blood sugar and fasting C-peptide levels were obese and also that obese patients were more insulin resistant than non obese.\textsuperscript{174} Number of other studies also observed that basal plasma glucose, insulin and C-peptide concentrations are higher in obese than non obese patients.\textsuperscript{12,175} Beta cell function deteriorates with time. So, duration of diabetes is one of the important confounding factors in worsening glycemic control. It has been observed that as duration of diabetes increases, the insulin secretion decreases. But duration of diabetes had no significant association with obesity.\textsuperscript{12,176} In contrast, Sari et al found no correlation between the duration of diabetes and C-peptide levels.\textsuperscript{177} So, the significance of routine C-peptide testing in obese and individuals with poor glycemic control resides in assessment of endogenous insulin reserve and also in altering the treatment modality based on it. It may help in early screening of subjects with positive family history and in creating awareness about lifestyle modifications and education to prevent obesity.
Furthermore, the possible role of C-peptide as an additional biomarker in the prediction of the early development of CVD in obese subjects besides insulin has also been highlighted. Additionally, the use of C-peptide and insulin measurements together provides valuable information for the evaluation of hypoglycemia and the diagnosis of insulinoma.

**Dyslipidemia**

Obesity, especially abdominal obesity, is associated with atherogenic lipid profile; with increased low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides (TG) and decreased high density lipoprotein (HDL). Dyslipidemia, an important component of insulin resistance syndrome and type 2 DM is strongly associated with CVD risk. The lipid abnormalities are prevalent in diabetes mellitus because insulin resistance or deficiency affects key enzymes and pathways in lipid metabolism. In particular, the processes affected include apoprotein production, regulation of lipoprotein lipase, action of cholesteryl ester transfer protein (CETP) and hepatic and peripheral actions of insulin. Even more, it has been proposed that the composition of lipid particles in diabetic dyslipidemia is more atherogenic than other types of dyslipidemias. This means that even normal lipid concentrations might be more atherogenic in diabetic than in non-diabetic people. In diabetes, the associated hyperglycemia, obesity and insulin changes highly accelerate the progression to atherosclerosis. In type 2 DM patients who are centrally obese,
increased lipolysis causes the liver to increase the glucose and VLDL output, while muscle uses less. This leads to increase in blood glucose and triglycerides, a drop in HDL-C and an increase in small, dense LDL particles.\textsuperscript{186}

The relation between diabetes mellitus and serum lipid profile had been much discussed during the past decades. The prevalence of dyslipidemia is variable with over 70\% of patients with type 2 DM having one or more types of dyslipidemia. Even race and sex differences in patterns of serum lipids have been noted in diabetes.\textsuperscript{187} Furthermore, the type of lipoprotein abnormality in diabetes depends upon many factors such as type of diabetes, endogenous insulin reserve, degree and distribution of obesity, degree of glycemic control, type of therapy and the presence or absence of nephropathy.\textsuperscript{188} Chandalia et al found significantly elevated levels of total cholesterol and TG in diabetics as compared to non-diabetics with most characteristic lipid abnormality being hypertriglyceridemia with or without associated increase in plasma cholesterol. Reduction of HDL cholesterol was also seen.\textsuperscript{189} All lipid fractions (except HDL) namely serum total cholesterol, TG, LDL-cholesterol and phospholipids are abnormally elevated in obese diabetics when compared with obese non-diabetics as reported by Zargar et al.\textsuperscript{190} Mukhyaprana et al revealed the conspicuous absence of hyperlipidemia in non obese diabetic individuals.\textsuperscript{191} A study by Sinharoy et al showed elevated TG in lean type 2 diabetics compared to normal weight and obese type 2 diabetics.\textsuperscript{192}
In addition, the study conducted by Brehm et al demonstrated that TG/HDL ratio positively correlates with insulin resistance in severely obese non-diabetic individual than in patients with overt diabetes and may serve as a marker that is easy to determine an links insulin resistance and CVD risks in non-diabetic individuals.\textsuperscript{193} Because detection and treatment of dyslipidemia is one means of reducing cardiovascular disease risk, determination of serum lipid levels in patients with diabetes mellitus is now considered a standard of care. Aggressive lifestyle changes, such as weight reduction and physical exercise should be initiated first followed by medication with lipid lowering drugs.

Keeping in view the above facts, the present study was planned to evaluate the serum levels of insulin, C-peptide, leptin, adiponectin along with lipid profile in patients with type 2 diabetes mellitus with and without obesity.