CHAPTER-2
REVIEW OF LITERATURE

Metabolic syndrome

A group of metabolic disorders like central obesity, insulin resistance, increased insulin level, impaired glucose homeostasis, dyslipidemia, raised blood pressure and low grade chronic inflammation is defined as metabolic syndrome. Other variety of names like Syndrome-X, Metabolic syndrome-X and Insulin resistance syndrome. It is also called as Reaven’s syndrome which was named after Gerald Reaven, who insisted the research in metabolic syndrome. In Australia the syndrome is called as CHAOS which means coronary artery disease, hypertension, atherosclerosis, obesity and stroke. It has been well established that MetS increases the risk of CVD development by two times and increases the risk of T2DM onset by five times in either sex as compared to subjects MetS. Numerous researchers have also evidenced the association of MetS with various diseases like fatty liver, chronic kidney disease, cancer, mental illness, etc.

Historical aspects and Definitions:

The historical aspect of MetS is much longer which was previously called as syndrome X as the pathophysiology of the syndrome was clearly unknown. In late 1980s, G.M. Reaven revealed the involvement of insulin resistance with feature of hyperinsulinemia therefore the syndrome was also called as insulin resistance syndrome. The association of IR with increased level of free fatty acids (FFAs) was also demonstrated by Reaven and then they hypothesized that IR was the fundamental mechanism in progression of MetS. Lastly Reaven projected that a constellation of diverse riskfactors, connected by a common link could constitute the
syndrome which was then called metabolic syndrome in a standard article published on the subject in 1988.\textsuperscript{49}

Thereafter, a group of international organizations and experts have struggled to define MetS by integrating different parameters:

WHO (World Health Organization) in 1998 made first attempt to define the metabolic syndrome as described below in the table.\textsuperscript{50}

**Table 2.1: Definition and diagnosis criteria of MetS by WHO (1998).**\textsuperscript{50}

<table>
<thead>
<tr>
<th>Definition of MetS</th>
<th>12DM, IFG, IGT or IR plus at least two of the criteria below.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Glucose</td>
<td>Impaired Fasting Glucose (IFG), Impaired Glucose Tolerance, Type 2 Diabetes,</td>
</tr>
<tr>
<td>2  Abdominal Obesity</td>
<td>WHR: &gt;0.9 Male and &gt;0.85 in Female or BMI &gt; 30 kg/m(^2)</td>
</tr>
<tr>
<td>3  Blood Pressure (BP)</td>
<td>BP &gt; 140/90 mmHg</td>
</tr>
<tr>
<td>4  Triglycerides (TG)</td>
<td>Triglycerides &gt; 150 mg/dl</td>
</tr>
<tr>
<td>5  HDL-Cholesterol</td>
<td>HDL-C &lt;35 mg/dl in Male and HDL-C &lt; 35 mg/dl in Female.</td>
</tr>
<tr>
<td>6  Microalbuminuria</td>
<td>&gt;20 μg/min.</td>
</tr>
</tbody>
</table>
Shortly thereafter, the European Group for the Study of Insulin Resistance (EGIR) in 1999 excluded microalbuminuria and defined MetS.\textsuperscript{51}

**Table 2.2: Definition and diagnosis criteria of MetS by EGIR (1999).\textsuperscript{51}**

<table>
<thead>
<tr>
<th>Definition</th>
<th>Fasting hyperinsulinemia (highest 25%), plus at least two criteria below</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glucose</td>
<td>Fasting Plasma Glucose (FPG) ≥ 6.1 mmol/L (Excludes diabetes)</td>
</tr>
<tr>
<td>2 Abdominal Obesity</td>
<td>Waist Circumference (WC): ≥ 94 cm in Male and ≥ 80 cm in Female</td>
</tr>
<tr>
<td>3 Blood Pressure (BP)</td>
<td>BP &gt; 140/90 mmHg and/or treated for hypertension</td>
</tr>
<tr>
<td>4 Triglycerides (TG)</td>
<td>TG &gt; 150 mg/dl</td>
</tr>
<tr>
<td>5 HDL-Cholesterol</td>
<td>HDL-C &lt;39 mg/dl and/or treated for dyslipidemia.</td>
</tr>
</tbody>
</table>
The NCEP: ATPIII (National Cholesterol Education Program Adult Treatment Panel III) developed a definition for MetS in 2001.52

Table 2.3: Definition and diagnosis criteria of MetS by NCEP-ATP III (2001).

<table>
<thead>
<tr>
<th>Definition</th>
<th>At least three criteria below.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glucose</td>
<td>Fasting Plasma Glucose (FPG) ≥ 110 mg/dl.</td>
</tr>
<tr>
<td></td>
<td>(Includes Diabetes)</td>
</tr>
<tr>
<td>2 Abdominal Obesity</td>
<td>Waist Circumference (WC): ≥ 102 cm in Male and</td>
</tr>
<tr>
<td></td>
<td>≥ 88 cm in Female</td>
</tr>
<tr>
<td>3 Blood Pressure (BP)</td>
<td>BP &gt; 130/85 mmHg and/or treated for hypertension</td>
</tr>
<tr>
<td>4 Triglycerides (TG)</td>
<td>TG &gt; 150 mg/dl or treated for dyslipidemia</td>
</tr>
<tr>
<td>5 HDL-Cholesterol</td>
<td>HDL-C: &lt;40 mg/dl for men.</td>
</tr>
<tr>
<td></td>
<td>&lt;50 mg/dl for women</td>
</tr>
</tbody>
</table>
Thereafter; again in 2006 the definition was revised by changing the cut-off value of glucose to 100 mg/dl from 110 mg/dl.

Table 2.4: Revised definition and diagnosis criteria of MetS by NCEP-ATP III (2006).

<table>
<thead>
<tr>
<th>Definition</th>
<th>At least three criteria below.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glucose</td>
<td><strong>Fasting Plasma Glucose (FPG) ≥ 100 mg/dl.</strong> (Includes Diabetes)</td>
</tr>
<tr>
<td>2 Abdominal Obesity</td>
<td>Waist Circumference (WC): ≥ 102 cm in Male and ≥ 88 cm in Female</td>
</tr>
<tr>
<td>3 Blood Pressure (BP)</td>
<td>BP &gt; 130/85 mmHg and/or treated for hypertension</td>
</tr>
<tr>
<td>4 Triglycerides (TG)</td>
<td>TG &gt; 150 mg/dl and/or treated for dyslipidemia</td>
</tr>
<tr>
<td>5 HDL-Cholesterol</td>
<td>HDL-C: &lt; 40 mg/dl for Male. &lt;50 mg/dl for Female</td>
</tr>
</tbody>
</table>
International Diabetes Federation (IDF) also published new criteria for MetS in 2005.53

Table 2.5: Definition and diagnosis criteria of MetS by IDF (2005).

<table>
<thead>
<tr>
<th>Definition</th>
<th>Central obesity plus at least two of the criteria below.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glucose</td>
<td>Fasting Plasma Glucose (FPG): ≥100 mg/dl (Includes diabetes)</td>
</tr>
<tr>
<td>2 Abdominal Obesity</td>
<td>Population and country specific.</td>
</tr>
<tr>
<td>3 Blood Pressure (BP)</td>
<td>BP: ≥130/80 mmHg and/or treated for hypertension</td>
</tr>
<tr>
<td>4 Triglycerides (TG)</td>
<td>TG ≥ 150 mg/dl and/or treated for dyslipidemia.</td>
</tr>
<tr>
<td>5 HDL-Cholesterol</td>
<td>HDL-C &lt;40 mg/dl in Male and HDL-C &lt;50 mg/dl in Female. And/or treated for dyslipidemia.</td>
</tr>
</tbody>
</table>
In 2009, representative from IDF and AHA/NHLBI (American Heart Association/National Heart, Lung, and Blood Institute) discussed and tried to resolve the rest of the difference between definitions of MetS. Then they agreed to exclude abdominal obesity as essential criteria for diagnosing Mets as being one of the 5 criteria. They concluded that meeting any three criteria among 5 will be diagnosed as Mets.54

Table 2.6: Definition and diagnosis criteria of MetS by IDF (2009).

<table>
<thead>
<tr>
<th>Definition</th>
<th>At least three of the criteria below.</th>
</tr>
</thead>
</table>
| 1 Glucose           | Fasting Plasma Glucose (TPG): $\geq 100$ mg/dl  
INCLUDE (includes diabetes)                          |
| 2 Abdominal Obesity | Population and country specific.                                                                         |
| 3 Blood Pressure (BP)| BP: $\geq 130/80$ mmHg and/or treated for hypertension                                           |
| 4 Triglycerides (TG)| TG $\geq 150$ mg/dl and/or treated for dyslipidemia.                                                   |
| 5 HDL-Cholesterol   | HDL-C $<40$ mg/dl in Male and  
HDL-C $<50$ mg/dl in Female  
And/or treated for dyslipidemia.                     |
Why NCEP ATP III criteria is widely used for diagnosis of MetS?

- The definition of NCEP ATP III is being widely used throughout the world as it more practically applicable compared to other definitions. Some of the definitions like EGIR have made insulin resistance as mandatory criteria in diagnosing metabolic syndrome whereas NCEP ATP III do not support IR as mandatory criteria, hence denies to use the term ‘insulin resistance syndrome’ and suggests ‘metabolic syndrome’.

- NCEP ATP III takes central obesity as a main culprit in the development of metabolic syndrome. Waist Circumference is an ideal anthropometric measurement for the reflection of central obesity which has been used in its definition whereas WHO has used waist hip ratio for the measure of central obesity which is not taken as good as waist circumference.

- NCEP ATP III includes low HDL and raised TGL as different criteria instead of taking dyslipidemia as a single constituent.

- According to definition of MetS given by IDF in 2005, obesity was an obligatory parameter where metabolically obese, normal weight (MONW) individuals were excluded if IDF (2005) definition was used.

- Later, in 2009 a joint interim statement of the IDF; AHA/NHLBI; WHF; IAS; and the IASO decided not to use obesity as mandatory criteria and to use race and gender specific cut-offs for WC recommended by IDF till the proper data of cut-off values for WC from various regions are available. The WC cut-offs evaluated by numerous publications from Japan, Korea, Iran, Iraq and other regions have confused the definition of MetS since there are now various race and gender specific cut-offs. Therefore specific cut-off for WC in NCEP ATP III is more practical and applicable.

- Since the definition of NCEP-ATP III integrates the key features of central obesity, hyperglycaemia/insulin resistance, atherogenic dyslipidemia and raised blood pressure. Anthropometry and lab results used in the definition are readily available to physician. There is no any mandatory criteria in
definition as compared to other definitions. Therefore, the NCEP ATP III criteria is widely used globally.\textsuperscript{63}

**Prevalence of metabolic syndrome**

The prevalence of MetS worldwide varies from less than 10% to 84%, depending upon the region, composition of the population studied with respect to age, sex, race, ethnicity etc. and the definition of the MetS used.\textsuperscript{64, 65} As per NCEP-ATP III principles, 2001 the occurrence of metabolic syndrome globally diverse from 8% to 43% and from 7% to 56% in male and female respectively.\textsuperscript{66}

In India, MetS prevalence is more in urban areas as compared to rural and it has been suggested by numerous surveys that one-third of the people have been suffering from metabolic syndrome.\textsuperscript{67, 68, 69} Data from the survey done in south India has revealed the prevalence of 31.4% central obesity, 45.6% raised triglyceride level, 65.5% diminished HDL, 55.4% increased BP, and 26.7% hyperglycaemia which are all the constituents of metabolic syndrome. A survey done in north India in non-obese subjects revealed that 66% male and 88% female had at least one CVD risk factor.\textsuperscript{70} Whereas survey from east India shows the prevalence of metabolic syndrome higher in women as compared to men which was 48.2% and 31.4%, respectively.\textsuperscript{71} The prevalence of MetS in Indian rural areas found to be lower as compared to urban area in India due to various reasons like sedentary life style, decreased physical activity, increased urbanization etc. Data from central India revealed the prevalence of MetS only 5% in adult population of rural area by using NCEP ATP III criteria. The prevalence of MetS was reported to be higher in females as compared to males which was 10.7% and 8.2% respectively when the cut-off values for WC were modified from NCEP ATP III criteria on the recommendation by Asia Pacific guidelines.\textsuperscript{72} In Indians, the prevalence of metabolic syndrome is predictable to rise further with increasing urbanization, particularly with the adoption of modern lifestyles.\textsuperscript{73, 74, 75}

**Pathophysiology**

The controversy still remains globally regarding the pathophysiological causes that lead to metabolic syndrome and its morbidities. The debate is on central obesity
Figure 2.1: Pathophysiology of Metabolic syndrome
versus IR. Majority of the publications are supporting the obesity as a predominant factor in development of metabolic syndrome. Even though obesity is well established factor to lead insulin resistance, non-obese are also associated with insulin resistance and MetS. There is evidence that there are other causes of insulin resistance like environmental factors and genetics other than obesity (Figure 2.1). Although obese individuals have greater prevalence of risk of MetS and its comorbidities, non-obese or normal weight individuals are also prone to the risk of MetS and its comorbidities, if associated with insulin resistance.

**Obesity**

It is a condition where the weight of the body increases due to deposition or storage of excessive fat content mostly in the form of triglycerides in white adipose tissues. The increased weight of the body in obesity is estimated to be greater than 20% as compared to non-obese healthy control. Obesity is a metabolic disease of pandemic proportion. According to WHO, >300 million adults are obese and >1 billion are overweight globally on the basis of BMI classification.

**Major Causes of obesity**

- Lack of physical activity
- Increased sedentary lifestyle
- Accessible energy dense foods/junk foods
- Hypothyroidism & leptin signalling pathways disorder.
- Genetic factor.

Even though BMI is more popular globally to define obesity, WC has been used in the definition of metabolic syndrome by most of the organizations. WC is established measure of obesity because it is direct measure of abdominal or visceral
obesity. It has also shown a high correlation with presence of metabolic syndrome as compared to BMI and other anthropometry measurement for the measure of central obesity.\textsuperscript{9, 10} On the basis of observation in obese individuals MRI and CT have concluded that, surplus amount of intra-abdominal fat (visceral adipose tissue) has been highly associated with presence of metabolic syndrome rather than subcutaneous abdominal fat. Evidence suggests that individuals with hyperplasia (increase in number of adipose tissues) are less prone to develop features of the metabolic syndrome, as compared to hypertrophic individuals (increase in size of the adipose tissues) in response to positive energy balance with a limited ability to expand, are more prone to insulin resistance and metabolic syndrome.\textsuperscript{77}

**Insulin and insulin resistance**

**Biosynthesis and secretion of insulin**

Insulin is a hypoglycaemic hormone produced by beta cells of pancreatic islets in fed condition, to utilize the glucose by body for production of energy. Initially synthesized, pre-proinsulin is a precursor polypeptide which consists of single chain of 86 amino acids then proinsulin is formed after removal of amino-terminal by consequent proteolysis. Proinsulin further generates the C-peptide and the A and B chains of insulin which have 21 amino acids and 30 amino acids respectively. The proinsulin is degenerated by breakage of an inner 31-residue portion. The A and B chains of insulin are bonded by disulphide bond. The secretory granules of beta cells store the mature insulin molecule and C-peptide and co-secret them when required. During secretion of insulin a 37-amino acid peptide called amyloid polypeptide (IAPP) also called as amylin is secreted together.\textsuperscript{78} Biosynthesis of insulin is depicted below (Figure 2.2).
Figure: 2.2: Biosynthesis of insulin

Regulation of insulin secretion

The glucose homeostasis imparts a crucial part in stimulation of insulin gene transcription and mRNA translation beside multiple factors. Blood glucose level beyond 70 mg/dl increase the protein translation and processing for the synthesis of insulin.
As the transporter of glucose, specifically GLUT2 transports glucose in pancreas beta cells is phosphorylated by glucokinase insulin secretion is stimulated which is a rate limiting step (Figure 2.3).

Figure 2.3: Regulation of insulin secretion.

ATP generated via glycolysis also inhibit the activity of potassium channel consisting two different proteins where oral hypoglycaemic drugs like sulphonylureas and miglinitides have binding site on one of the protein and the other one is kir 6.2 which is rectifying potassium channel. The cell membrane of the beta cell depolarizes, if this potassium channel is inhibited to open the voltage-dependent calcium channels resulting in secretion of insulin along with influx of calcium ions. The hormone insulin is released in pulsatile pattern where in every 10 minutes small secretory bursts occurs, superimposed on greater-amplitude oscillations of about 80-150 minutes. In fed condition GIT release Incretins from their neuroendocrine cells
supress the glucagon and magnify glucose stimulated secretion of insulin. Small intestine synthesizes GLP-1 (Glucagon like peptide-1) which is the most powerful Incretins, from their L-cells when glucose is beyond fasting level to stimulate secretion of insulin. Exena-tide which is one of the analogues of Incretins is used clinically to enhance endogenous insulin secretion.78

**Insulin signalling pathway**

After the secretion of insulin into portal system, liver degenerates approximately 50% of it, rest of it enters the systemic circulation followed by binding with receptors in target sites. When it binds to the receptor the tyrosine kinase is activated recruiting intracellular signalling molecules, such as insulin receptor substrates (IRSs) and auto phosphorylation of receptor. It activates the various cascade or intracellular signalling pathways. The common one is PI-3 kinase pathway. The phosphatidylinositol-3-kinase pathway stimulates translocation of glucose transporter (GLUT 4) especially in skeletal muscle and adipose tissue which uptake the glucose molecules inside the cell from the surface. Some other intracellular signalling pathway activation initiates other metabolic pathways like glycogen synthesis, protein synthesis, lipogenesis, and also gene regulation (Figure 2.4).

Hypoglycaemic hormone (Insulin) and a number of hyperglycaemic hormones (glucagon, thyroid, PTH, epinephrine, nor epinephrine) maintain the glucose homeostasis by making a balance between hepatic glucose production and peripheral glucose uptake for utilisation. Insulin plays a predominant role for the balanced glucose homeostasis. Under fasting condition, insulin levels are decreased and increases hepatic production of glucose inducing metabolic pathways like gluconeogenesis and glycogenolysis. It results in reduced glucose uptake in tissues and promoting mobilisation of stored substrates like amino acids and free fatty acids as well. Another hyperglycaemic hormone glucagon is synthesized by alpha cells of pancreas under decreased condition of blood glucose and/or insulin levels. It stimulates the metabolic pathways like glycogenolysis and gluconeogenesis (in liver and renal medulla) to surplus the glucose level under fasting condition.
Insulin resistance

Insulin is an anabolic hormone, which helps in the synthesis and storage of carbohydrate, fat and protein synthesis. Skeletal muscle is the major organ which utilizes the major amount of glucose under the influence of glucose whereas brain utilises glucose in an insulin independent fashion. Inability of tissues to response the insulin or derangement in insulin signalling pathway with a characteristic feature of hyperinsulinemia is termed as insulin resistance.

Causes of insulin resistance

- Genetics
- Chronic excess energy intake
- Lack of exercise and physical activity
• Stress
• Chronic sleep deprivation
• Pregnancy
• Obesity
• Chronic inflammation

Insulin resistance is an essential feature of MetS which increases the risk in development of T2DM. It has been well evidenced that obesity leads to IR and increases the risk of T2DM development. Therefore insulin resistance is the link between central obesity and T2DM. Secondly, white adipose tissue is responsive to insulin. It promotes storage of triglycerides in adipose tissue by:  

• Stimulates division of preadipocytes to adipocytes.
• Uprising the glucose uptake and fatty acids
• Inhibiting lipolysis

The Homeostasis Model Assessment of IR (HOMA-IR) is globally used robust tool for alternate assessment of IR. HOMA-IR will be calculated as:  

\[
\text{HOMA-IR} = \frac{\text{Fasting plasma insulin (μIU/ml)} \times \text{Fasting plasma glucose (mg/dl)}}{405}
\]

Explanation of result

Table 2.7: category of Insulin resistance by HOMA-IR.  

<table>
<thead>
<tr>
<th>Category of Insulin resistance</th>
<th>Score of HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Moderate</td>
<td>3-5</td>
</tr>
<tr>
<td>Severe</td>
<td>&gt;5</td>
</tr>
</tbody>
</table>
A huge number of publications have reported significant increased levels of insulin and HOMA-IR with other constituents of MetS in patients with MetS as compared to subjects without MetS. Numerous researchers have evidenced the positive correlation between obesity and insulin or obesity and HOMA-IR.\textsuperscript{82, 83, 84, 85}

**Insulin resistance induced by obesity**

Some of the major consequences of obesity that leads to insulin resistance are described below:

1. Increased production of free fatty acids:
2. Production of pro-inflammatory chemokines and cytokines.
3. Inflammation
4. Hypertension
5. Dyslipidaemia

1) **Increased production of free fatty acids (FFAs):**

Due to increase visceral adipose tissue in obesity free fatty acids (FFAs) are elevated in plasma.\textsuperscript{12, 13} On the surface of visceral adipose tissues, β-adrenergic receptors are densely present and α-1 adrenergic receptors are thinly present which is in contrast to the subcutaneous tissues. This results in increased activity of catecholamine which induces lipolysis followed by release of FFAs into the bloodstream. The major organs whose normal functions are affected by elevated FFAs in circulation are liver, pancreas and muscle.\textsuperscript{86}

**Effect of increased FFAs on pancreas:** Chronic elevation of FFAs may be due to long standing obesity impair the function of beta cells and induces insulin resistance.\textsuperscript{87}
Effect of increased FFAs on liver: Increased FFAs produced by adipocytes in obesity get released into the portal vein. After the FFAs are taken up by hepatocytes, they bind with CoA (Coenzyme A) to form fatty acyl CoA.

The fatty acyl CoA is the precursor for TG synthesis creating VLDL particles which lead to activation of nuclear factor κB (NF-κB) and induce inflammation. The inflammation further causes insulin resistance by phosphorylating the insulin signalling molecules.88

Effect of increased FFAs on skeletal muscle: Raised intra-myocellular fatty acyl CoA (FACoA) in skeletal muscles plays a key role for insulin resistance in it. FACoA helps in activation of serine/threonine kinases and results in phosphorylation of IRS-1 causing defect in insulin signalling in individuals.14

On another hand, obesity helps in releasing of TNFα, IL-1 and IL-6 from infiltrating macrophages in skeletal muscle which ultimately leads to insulin resistance in skeletal muscle (Figure 2.5). Theses proinflammatory cytokine have been evidenced to activate c-Jun N-terminal kinase (JNK) and IκB kinase (IKK) pathway. They result in IR either by increasing IRS-1 phosphorylation or by activating the inflammatory genes like iNOS.

This results in increased production of nitric oxide (NO) and formation of peroxynitrite (ONOO-). NO and ONOO-. These highly reactive derivatives lead to s-nitrosylation or nitration of IRS-1, PI3K and/or Akt. Due to inactivation of these molecules the translocation of GLUT-4 to the cell surface is affected which leads to insulin resistance and hyperglycaemia. Prolonged hyperinsulinemia has also been found to activate mTOR/S6K1 pathway, causing IR by further increase in IRS-1 phosphorylation.

Whereas; physical workout or drugs like TZD and metformin activate AMP-activated protein kinase and inhibits iNOS and mTOR/S6K1 signalling molecules to recover insulin action. AMPK also helps to trigger GLUT4 translocation to improve glucose. The protein tyrosine phosphatases (PTPs) have also been known to dephosphorylate tyrosineresiduesof receptor causing IR.
2) Production of pro-inflammatory chemokines and cytokines.

Adipose tissues play a major role in endocrine functions which secrete multiple adipokines. They include chemokines, cytokines and hormones which cause homeostasis and inflammation. In the obese person, the adipocyte has a role to play in the development of inflammation through increased secretion of chemokines and cytokines. Tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, interleukin-1, interleukin-6 and interleukin-8, are said to promote insulin resistance.
i. Adiponectin:

Adipose tissue is an active metabolic tissue which synthesize ‘adipokines’ which are metabolically important active proteins. They have a crucial role in insulin resistance and cardiovascular complications related with obesity. Various studies have confirmed that decreased adiponectin levels results in decreased insulin sensitivity.

Adiponectin, a 244-amino acid polypeptide with molecular weight of 30 kDa, a gene product of adipose most abundant gene transcript-1 (apM-1) gene: which bears structural homology to collagen VIII, X and complement C1q as well as TNF-α. It is exclusively secreted by adipocytes of white adipose tissue. This plays as a hormone and has anti-inflammatory, anti-atherogenic and insulin sensitizing properties. This also acts as adipocyte complement-related protein (Acrp 30), gelatin-binding protein 28 (Gbp 28) or adipo Q. Adiponectin circulates in different multimeric forms. It is a proteolytic fragment. The globular form of adiponectin has structural similarities with TNF-α. The basic form of adiponectin is a trimer. It can also exist as hexamers. It has multimers-high molecular weight adiponectin (HMW adiponectin). The major configurations in plasma are the hexamers and HMW forms and they have longer half-life. There are different forms of circulating adiponectin, which are found at different target organs like, HMW adiponectin (active in liver) and (endothelial cells). HMW forms are known to be the biologically most active and potent for sensitization of insulin. In normal subjects circulating adiponectin levels are said to be 5-20μg/ml, therefore account for approximately 0.01% of total plasma protein. They are said to be measurable in CSF (cerebrospinal fluid). Adiponectin concentrations in human CSF is said to be typically 0.1% of corresponding plasma concentration. Adiponectin secretion, as compared to secretion of other adipokines, is paradoxically decreased in obesity. Only fat protein is known to be down regulated with weight gain. When there is accumulation of visceral fat that can produce inhibiting factors for synthesis of adiponectin or secretion like TNF-α. Adiponectin concentrations also decrease in various human metabolic and cardiovascular disease conditions like type 2 diabetes mellitus, non-alcoholic hepatic steatosis, lipodystrophy, essential hypertension and coronary artery
disease. During low adiponectin levels there might progress insulin resistance and myocardial infarction. It is known that adiponectin levels progress with age and are raised in type 1 diabetes. It improves activation of insulin-receptor substrate 1 (IRS-1) associated with PI3-kinase and glucose uptake to improve insulin sensitivity and also decreases the mRNA expression gluconeogenesis enzymes (phosphoenolpyruvatecarboxykinase and glucose-6-phosphatase) to decrease the hepatic production of glucose. Adiponectin prevents atherosclerosis and cardiovascular disease by down regulating expression of adhesion molecule on vascular endothelial cells and the conversion of macrophages into foam cells, and by moderating multiplication of smooth muscle cell. Adiponectin decreases inflammatory response which is induced by TNF-α, according to various studies in vitro. Adiponectin decreases macrophage activity and TNF-α production macrophages. Cardio-protective effects of adiponectin have ability to suppress apoptosis, inflammation in cardiomyocytes, oxidative/nitrative stress. Activation of AMPK leads to anti-apoptotic activity of adiponectin. Adiponectin also regulates NO production through eNOS and iNOS. It activates NO production through eNOS activation, leads to the vasodilator and vascular protective effects. Pathologically, adiponectin leads to its anti-nitrative actions in cardiac cells by preventing the induction of iNOS expression and then causing increased NO generation. These effects are understood to avoid plaque formation in apoE-deficient (mice), atherosclerosis (mouse model). The receptors of adiponectin are AdipoR1 and AdipoR2 which are structurally similar to seven transmembrane receptors but dissimilar from G-protein coupled receptor through which adiponectin acts on tissues. The amino terminal of receptor is towards the cytoplasm and carboxyl terminal is extracellular which is short and constituted with around 25 amino acids which have inverted membrane topology. AdipoR1 in humans is highly expressed in heart and skeletal muscle whereas AdipoR2 expression is limited in heart and skeletal muscle in contrast.

If AdipoR1 is overexpressed, phosphorylation of AMPK is raised and the expression of genes is decreased which causes gluconeogenesis whereas if AdipoR2 is overexpressed it increases PPAR-α(peroxisome proliferator-activated receptor-α) messenger-RNA and the inflammatory cytokines and oxidative stress marker
expression is diminished. It also results in decreased amount of TG in liver.\textsuperscript{110} Adiponectin has also been evidenced that, it binds with a receptor present on vascular endothelium and muscle cell called as T-cadherin. It results in some anti-atherogenic and vascular-protective activity.\textsuperscript{111} This receptor is an extracellular protein anchored by glycosylphosphatidylinositol which has also been reported to act as a co-receptor with AdipoR1 and AdipoR2 in some specialized tissues and cells may be to assist adiponectin signalling. Adiponectin-AdipoRs complex has been evidenced to play a vital role in chronic diseases associated with diabetes.

Yamauchi et al. (2008) proposed an “adiponectin hypothesis” which reveals that genetic factors such as SNP276 in the adiponectin gene itself results in diminished level of adiponectin. Factors like Sedentary life style, increased intake of high fat diet, and obesity have also been reported to cause low adiponectin level. Numerous studies of adiponectin at the biochemical and genetic level has evidenced that the diminished level of adiponectin synthesis results in the development of IR, MetS, T2DM and atherosclerosis.\textsuperscript{112}

Wellen and Hotamisligil have also proposed another hypothesis, “inflammation and insulin resistance in adipose tissue” which reveals that MCP-1 secreted by hypertrophic adipocytes and preadipocytes recruit macrophages. It synthesizes adipokines like TNF-\( \alpha \), CRP, IL-1, IL-6 and FFAs which leads to inflammation and ultimately insulin resistance. IRS-1 phosphorylation by TNF-\( \alpha \) inhibits the receptor kinase activity & downstream signalling via PI3K activation.\textsuperscript{113} TNF-\( \alpha \) along with interleukin-6 in 3T3-L1 adipocytes are responsible to decrease IRS-1, GLUT-4 and PPAR-\( \gamma \) expression.\textsuperscript{18} IL-6 is also reported to bind with insulin receptor and IRS-1 which results in activation of suppressor of cytokine signalling.\textsuperscript{114} In case of obesity related hypertrophic adipocytes the diminished activity of adiponectin and increased activity of MCP-1 primarily cause insulin resistance followed by MetS.\textsuperscript{115,116}

Various researchers have established the association of adiponectin with the obesity which has a negative correlation. Adiponectin synthesis is comparatively lower in case of omental adipose tissue than in subcutaneous adipose tissue.\textsuperscript{117} When adiponectin levels were measured in insulin-sensitive subjects taking high fat diet the elevated levels of adiponectin suggests that its elevation was to increase the fat
oxidation capacity and to protect T2DM development.\textsuperscript{118} The negative correlation between adiponectin and insulin resistance was also reported in non-diabetic subjects independent to age, BP, obesity and lipid profiles.\textsuperscript{119} In another study, adiponectin levels were also found to be negatively correlated with other parameters of MetS like BMI, SBP and DBP, glucose, insulin, insulin resistance, LDL, TG, uric acid and positively correlated with HDL.\textsuperscript{120} Adiponectin levels are higher in Caucasians than Indo-Asians.\textsuperscript{121} Interestingly, adiponectin levels have been found to increase in type 1 diabetic patients compared with healthy subjects.\textsuperscript{122} Adiponectin has been claimed to be with anti-atherosclerotic property from various experiments.\textsuperscript{123} Raised insulin level causes significant adiponectin decrease under euglycemia. In MetS decreased adiponectin level may have link between vascular disease and insulin resistance.\textsuperscript{124} Adiponectin was also negatively correlated with SBP and DBP which suggests the role of adiponectin to increase the blood pressure.\textsuperscript{125} In severe insulin resistant subjects adiponectin were found to be decreased 5 times in blood circulation and the probable cause is dominant-negative PPAR-\(\gamma\) mutations which suggests \textit{in vivo} PPAR-\(\gamma\) activation due to adiponectin.\textsuperscript{126}

Obesity is the main culprit to decrease adiponectin gene expression.\textsuperscript{127, 128} Hypoadeponectinemia has also been reported by numerous researchers in cardiovascular disease and hypertension, which are two of the major components of metabolic syndrome.\textsuperscript{91} The major cytokines responsible for decreasing adiponectin are TNF-\(\alpha\) and IL-6.\textsuperscript{129}

\textbf{ii. Leptin:}

It is a peptide hormone, an \textit{ob} gene product released by white adipocytes. Out of total leptin in circulation, 80\% of it is synthesized by subcutaneous fat. Leptin, discovered by Friedman et al in 1994 through positional cloning, is 16-kDa hormone found as free and bound form in circulation.\textsuperscript{130} Leptin, a 167 amino acids peptide, has structural homology to granulocyte-colony stimulating factor, leukaemia inhibitory factor, glycoprotein 130 (gp130), TNF-\(\alpha\) and IL-6. Leptin is also produced in placenta, mammary epithelium, lymphoid tissues, ovaries and bone
marrow in less concentration whereas it is principally synthesized by adipose tissues in high concentration. Leptin acts by binding to its specific receptors in the hypothalamus altering the expression of numerous neuropeptides including melanocortin system, neuropeptide Y and corticotrophin releasing factor which helps in energy expenditure and regulation of neuroendocrine function. Leptin acts as a hormonal bridge between adipocytes and CNS to balance the appetite and energy expenditure.\textsuperscript{131}

If serum leptin level is reduced, appetite centre is activated initiating response for energy consumption which results in increase food intake and decrease energy expenditure.\textsuperscript{132}

Various studies have shown the mechanism of leptin resistance. Causes of leptin resistance are:

- Impaired leptin transport across the BBB.
- Impaired leptin signal transduction in neurons,
- Suppression of cytokine signalling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B).

Some other mechanisms are also involved like role of endoplasmic reticulum stress, the hypothalamic inflammation, and defective autophagy.\textsuperscript{133} Obesity has a role to play in increasing circulating levels of leptin and IL-6. They activate intracellular activators of transcription 3 (JAK-STAT3) and Janus kinase - signal transducerssignalling. Leptin (Lep) acts mainly in the CNS and IL-6 functions in peripheral organs, though both factors can also act vice versa.

Leptin and IL-6 induce Chronic JAK-STAT3 signalling which results in enhanced expression of the negative regulator SOCS3. This SOCS3 regulates leptin and IL-6 signalling negatively. SOCS3 has also been evidenced to impair the action of insulin resulting in obesity and IR.\textsuperscript{134}
iii. **Tumor necrosis factor-alpha (TNF-α):**

Proinflammatory cytokine, TNF-α, produced by numerous cells, but mainly lymphocytes and macrophages. They stimulate leukocytes and endothelial cells, and they secrete cytokines and growth factor. TNF-α is also produced by rodent, in low quantity in humans which is cause of obesity and insulin resistance.\(^{15, 135, 136}\) Increased expression of TNF-α leads to increased cytokine release in obesity, in the adipose tissue in obese mice as studies in early 1990s. Levels of TNF-α correlate with the degree of adiposity and have association with resistance of insulin.\(^{137}\) Kern et al., found in a study, there is 3.0-fold increase in levels of TNF-α in patients having low insulin sensitivity.\(^{138}\) There are various possible mechanisms which describe that TNF-α cause insulin resistance, like:

- TNF-α interferes with insulin signal transduction through abnormal serine phosphorylation of insulin receptor substrate-1 (IRS-1) that prevents the normal tyrosine phosphorylation if the IRS-1 and leads to insulin resistance.\(^{139}\)

- TNF-α leads to induction of suppressor of cytokine signaling-3 (SOCS-3)\(^{140}\) in turn interfering with signal transduction of cytokine, tyrosine phosphorylation of the IRS-1 and insulin receptor. It causes ubiquitination and degradation of proteosomes in IRS-1.\(^{141}\) There is decreased activation of Akt (Protein Kinase B) leading to the translocation of the insulin-responsive glucose transporter (GLUT-4 to the plasma membrane).\(^{142}\)

- Adiponectin expression is decreased by TNF-α in adipose tissue and cause insulin resistance.\(^{143}\)

- TNF-α leads to lipolysis and increases hepatic lipogenesis causing increase in free fatty acid level and there is insulin resistance.\(^{144}\)

iv. **Interleukin-6 (IL-6):**

IL-6 is secreted by adipocytes and macrophages are pro and anti-inflammatory cytokines. There is stimulation of its expression that is stimulated by increased levels of catecholamine through (adrenergic β2- and β3-receptors) (WAT) white
adipose tissue. Various studies show major site of IL-6 secretion is adipose tissue that accounts for (15–35%) of the circulating levels. IL-6 and waist circumference are positively associated, showing that there is increased body fat, mainly central obesity, leading to insulin resistance and metabolic syndrome.

IL-6 reduces the synthesis of glycogen and uptake of glucose in adipocytes by increasing expression of SOCS-3. It is a protein which binds with and inhibits receptor of insulin. There is negative regulation of IRS-1 phosphorylation and its transcription. There is reduced adiponectin secretion by IL-6 from adipocytes and cause insulin resistance.

v. Resistin:

It is a putative adipocyte-derived signalling polypeptide which was identified by three independent groups using various techniques. The expression of resistin was first described in adipose tissue, with circulating levels detected in rodents and humans for the first time.

Resistin is a 114-amino acid polypeptide gene, encoded by resistin gene stated as Retn, and secreted as disulphide-linked homodimer. There are studies which show increased resistin expression in adipose tissue, (particularly abdominal depots), this is shown that positive correlations between serum resistin and body fat content exists. Central obesity causes IR (insulin resistance) and ultimately to type-2 DM. Lazar (et al) studied that resistin inhibits insulin signalling by increasing the expression of suppressor of cytokine signalling(SOCS)-III that in turn impairs resistin from opposing insulin action in adipocytes. This shows that the insulin-independent action of resistin on adipocytes is mediated by SOCS-3 which has an impact on normal homoeostasis of glucose.
3) Inflammation:

A physiological response of the organism against harmful chemical, physical, or biological stimuli for the re-establishment of homeostasis is inflammation. There are pro-inflammatory mediators such as:

- Bradykinin
- Serotonin
- Histamines
- Prostaglandins
- Nitric oxide.

The symptoms of inflammation are heat, pain, redness, swelling and loss of function. There is dilation of the blood vessels and increased blood supply and increased intercellular spaces. There is movement of fluids, leukocytes, protein into the inflamed areas. There is an initial response of the immune system against pathogens and tissue injury called as acute inflammation. Eicosanoids and vasoactive amines increase the movement of leucocytes and plasma towards infected site, characterized by heat, pain reddening, oedema and loss of function. When there is increase of higher order immune cells such as leukocytes, lymphocytes and fibroblasts, it is chronic inflammation. The inflammation may cause persistent damage of this cells. Chronic inflammation is related with hay fever, rheumatoid arthritis, arteriosclerosis, periodontitis, cardiovascular diseases, diabetes, neurologic diseases, obesity, pulmonary diseases and cancer. There is increased expression of proinflammatory cytokines in obesity causing inflammation and insulin resistance (Figure 2.6). This relation between obesity and adipocyte enlargement, and reduced blood supply to adipocytes causes hypoxia. Hence there is necrosis and infiltration of macrophage into adipose tissue. There is overproduction of inflammatory chemokines. There is localized inflammation and ultimately systemic inflammation because of obesity-related complications. Lipid accumulation occurs because of obesity in adipocytes. There is activation of nuclear factor kappa B (NF-κB) and c-Jun N-terminal kinase (JNK) signalling pathways. Subsequently, there is increase in the production of proinflammatory cytokines like interleukin-6 and tumour necrosis factor-alpha (TNF-α). Major cytokines like
Leptin, adiponectin, TNF-α, Interleukin 1β, interleukin-6, Resistin, Monocyte chemo attractant protein-1 (MCP-1) are the inflammation markers for insulin resistance through various pathways like \( \text{IKK}\beta/\text{NF-κB} \) pathway, Inflammasome pathway and JNK pathway.

i. \( \text{IKK}\beta/\text{NF-κB} \) pathway:

NF-κB binds with IκB proteins. The complex is responsible results in inflammation and innate immunity.\(^{167, 168}\) IKK complexes has two subunits IKKα and IKKβ. They phosphorylate IκB\(\alpha\). It results in breakdown of IκB\(\alpha\) and release of active NF-κB.
This active NF-κB complex transloactes to the nucleus whichupregulates target genes for the production of cytokines like IL-1β, TNF-α, and IL-6 (Figure 2.7). Deficient IKKβ in adipocytes it prevents the expression of IL-6 and TNF-α. Whereas; if IKKβ is activated it suppresses the expression adiponectin and leptin.

It confers that glucose tolerance and insulin sensitivity is improved if IKKβ is deleted or therapeutic treatment to inhibit NF-κB is done. Therefore it has been well supported by numerous studies that NF-κB plays a crucial role in IR induced by inflammation.

Figure 2.7: Production of inflammatory cytokines by IKKβ/NF-κB pathway.
ii. JNK

3 isoforms of JNK (JNK-1, JNK-2, and JNK-3) regulate the production of karyomitosis, production of cytokines, and apoptosis of cells. 172, 173, 174 JNK has been evidenced with the obesity, inflammation, IR and MetS by various studies. Endoplasmic reticulum (ER) stimulates JNK by stress and causes serine phosphorylation of IRS1. It further phosphorylates the ‘c-Jun’ constituent of transcription factor AP-1. The interrelationship between JNK-reduced IR and transcriptional pathway has not been evidenced. Under diabetic conditions JNK pathway is activated, which increases IR (Figure 2.8). But if JNK pathway is suppressed that improves glucose tolerance and IR. 175 JNK inhibits insulin secretion from pancreatic β-cells by stimulating proinflammation and IL-1 from and causes IR. It was found that if the JNK is inhibited it will reduce the release of TNF-α and MCP-1. 176, 177, 178 It is known that if JNK-1 is deficient in adipose tissue, this prevents hepatic steatosis and glucose intolerance, insulin clearance and IR, is promoted. 174, 179

Figure 2.8: Role of JNK in insulin resistance
iii. **Inflammasome:**

It regulates the production of IL-18 and IL-1β. Hence, it plays role in and metabolic syndromes and innate immunity like IR and obesity.\(^\text{180, 181}\) NOD includes NLRPs, NALPs, apoptosis associated speck such as caspase-1, ASC. These are essential constituents of inflammasome complexes.\(^\text{169}\) Inflammasome NLRP3 (leucine-rich containing family, nucleotide binding domain and pyrin domain containing-3), they link chronic inflammation and saturated FFAs (Figure 2.9).

![Figure 2.9: Role of Inflammasome pathway in inflammation and IR.](image)

It is found that as it is very sensitive to non-microbial stress, mitochondrial dysfunction can activate it. Moreover, in obesity the reduced expression of NLRP3 can cause improved insulin sensitivity, enhanced insulin signalling and decreased inflammation.\(^\text{181, 182}\) Caspase-1 interferes in the metabolic activity of adipocytes and lead to IR. The key reason is may be due to infiltration of macrophages in the adipocytes.\(^\text{183, 184}\)
Supression of ASC and caspase-1 have been evidenced to drop the levels of leptin, resistin and insulin in blood. The deficiency of ASC shields against hepatic steatosis, IR and obesity. Above studies when put together, suggest that the inflammasome is important in obesity-induced IR and therapeutically important target in treatment of IR.\textsuperscript{184}

**CRP (C - Reactive Protein)**

William Tillet and Thomas Francis were the first who made CRP discovery in 1930, in New York. Researchers noted that acute streptococcus pneumonia infected sera chemically reacted with streptococcal bacterium extract (which formally was tagged as Fraction C but was polysaccharide as understood) so as to produce visible precipitate of precipitin. Therefore, CRP was coined due to its reactive nature with C polysaccharide extract from cell wall of Streptococcus.\textsuperscript{185}

After 10 years, “acute-phase reactant” was designed for CRP by the team lead by Oswald Avery and Maclyn Macarty who also forwarded “transforming principle” since myocarditis like inflammatory stimuli and rheumatic fever associated inflammation showed increased CRP level.\textsuperscript{186-188}

In 1943, at the State Bacteriologic Laboratory situated in Stockholm, Gunnar Lofstrom identified 2 unique patients and their reports showed ideas of linkage between biomolecular indicator of inflammation and atherothrombosis.\textsuperscript{189}

In the middle years of 1950-60, many scientists including Irving Kroop pointed out the serum value of CRP was comparatively and consistently high after onset of coronary ischemia and necrosis.\textsuperscript{190} Not as early as 1990s, scientists showed dedicated interest and went through new researches linking cardiovascular disease and CRP. In the middle of 1990s, new technique of immunoassay for CRP that had more sensitivity compared to the older one was discovered. The new technique estimated hs-CRP value in the blood. This hs-CRP value showed increased prediction of coronary events to be happened in future, even though the readings were within normal range according to the older technique.
Structure

As per classification, CRP falls under sub-family pentraxin which again is under calcium dependent ligand-binding plasma proteins family. The word ‘pentraxin’ comes from Greek words; *penta* meaning five and *ragos* meaning berries and the nomenclature is due to its physical appearances seen under electron micrographic study which also suggests preservation of properties during evolution. The Homo sapiens biomolecule ‘CRP’ is chemically made of five structurally similar non-glycosylated polypeptide fractions; each of which again comprise of 206 amino acid remnants (Figure 2.10).

![Figure 2.10: Pentamer structure of CRP](image)

There is non-covalent association between the protomers in a ring-shaped arrangement with pentameric conformity. The chemical arrangement of protomer has been documented as 2 antiparallel Beta-sheets and flattened jelly-roll topography under X-ray crystallography study same as of lectins, principally concanavalin.
Each fraction consists of a recognizing face having a binding site for phosphocholine. It is comprised of 2 coordinated Ca\textsuperscript{2+} adjoining to a hydrophobic pouch (Figure 2.11).

![Binding sites of CRP](image)

**Figure 2.11: Binding sites of CRP**

The binding between phosphocholine and CRP is mediated by two chief fractions; Phe-66 and Glu-81. The hydrophobic interaction with the three methyl groups of choline (part of phosphocholine) is provided by Phe-66 whereas the phosphate group (the other part of phosphocholine) directly forms coordinate bond with the two Ca\textsuperscript{2+} ions. Glu-81 is present on another side of the hydrophobic pouch of CRP. Glu-81 makes interaction with nitrogen ion phosphocholine\textsuperscript{187}. The chemical attachment of the complement C1q with the effector face of CRP leads to the
formation of Fcγ receptor. After attachment it results in activation of classical complement pathway up to the level of C3 convertase.

**Synthesis of CRP**

CRP is mainly synthesized by the liver along with other organs in our body. Liver synthesizes CRP on activation by cytokines (like interleukin-6 and many more). It has been reported that monocyte activated products also induces the Hep 3B cells and results in synthesis of CRP and human serum amyloid A (SAA) protein (Figure 2.12).

![Figure 2.12: Synthesis of CRP in inflammation](image)

Other than liver cell the other tissues or cells like monocytes, Kupffer cells and lymphocytes, atherosclerotic plaques, neurons are also reported to produce CRP but in very less concentration. Interestingly some studies also have reported the production of CRP by epithelial cells of both respiratory tract and renal but only under certain condition. Recently production of CRP by the smooth muscle cells of coronary artery under stimulation of the cytokines has been evidenced.
The compelling data shows the synthesis of CRP by alveolar macrophages, neurons, kidneys and atherosclerotic lesions also takes place. Some of the studies have revealed that lipid peroxidation also helps in activation of a pro-inflammatory cytokine cascade which leads to synthesis of CRP. In case of viral infection specifically by cytomegalovirus has been evidenced to produce CRP by triggering pro-inflammatory cytokine cascade. Active human peripheral blood monocytes may synthesize CRP whereas peripheral blood mononuclear cells (PBMC) were not reported in significant synthesis of protein.

It has been advised that if the synthesis of CRP by alveolar macrophages and respiratory epithelial cells takes place it is associated with immune response and pulmonary host mechanism.

The major volume of CRP concentration in blood circulation is from liver even though numerous tissues and organs have been reported for CRP production. It is also produced locally by SMCs lymphocytes and monocytic cells in atherosclerotic lesions. The key regulator for CRP synthesis is IL-6 and later it is up regulated by other cytokines like IL-1 and tumour necrosis factor (TNF-α).

**Biological functions of CRP**

Liver synthesize a protein called CRP as a response against inflammation causing stimulus. It is the first line of defence of pathogens. CRP is produced even before specific antibodies like IgM or IgG synthesis by immune response. It binds to pathogens to induce the complement cascade and increases the opsonisation and clearance process.

It binds only to dead or damaged cells because lysophosphatidyl choline is present in high concentration in dead or damaged cells. The binding of CRP on surface of living healthy cells has not been evidenced. Even though CRP is different in structure from immunoglobulin; it shares the similar functions like promotion of agglutination, classical complement pathway activation and swelling of bacterial capsule etc. against the antigen.
The various physiological functions of CRP are:\textsuperscript{203}

- After the attachment with the cells and antigen, CRP coordinates with neutrophil and monocytes and results in cells’ tumoricidal activities.
- Adhesion molecules chemokines and cytokines are expressed by endothelial cells as the result of activation by CRP.
- An enzyme known as nitric oxide synthase of endothelium is down regulated by CRP which leads to inhibition of NO production.
- Helps in expression of Angiotensin receptor-1 (AT1-R) protein by up regulation. Amplifies AT1-R number on vascular smooth muscle cell. It results in migration and proliferation of vascular smooth muscle enhancement \textit{in vitro}.
- CRP acts as chemo attractant for monocytes.
- It also initiates tissue factor expression in macrophages.
- Phagocytosis of native LDL into macrophages is also mediated by CRP.
- Complement pathway up to C5 convertase can be activated by CRP for enhancement of phagocytosis.
- CRP has been evidenced for inflammatory responses.

\textbf{The role of CRP end products:}

The degraded products of CRP are small and soluble peptides which have been reported to be biologically active. They inhibit the proinflammatory functions of neutrophil. They also reduce the tissue destruction capacity of neutrophil.\textsuperscript{203}

The major degradation products of CRP along with their residues are:

- CRP III (201-206):
- CRP IV (83-90)
- CRP V (77-82)
- CRP (174-185)

The role of these CRP degradation products that have been reported from various \textit{in vitro} studies are:\textsuperscript{204, 205, 206}

- Synthesis of super oxide from activated neutrophil is reduced
- Neutrophil chemotaxis is affected.
- Promotes in shedding of IL-6 receptor from neutrophil.
Reduces neutrophil adhesion to the endothelium and expression of L-selectin.

**Regulation of CRP gene expression**

Various human hepatoma cell lines have been used to study transcriptional regulation of the CRP gene. Studies from multiple labs have shown that cytokines like IL-1β, IL-6, IL-17, TGFβ, and TNFα adjust the transcription of CRP gene in these cell lines. The most commonly used model to study CRP gene expression has been the human hepatoma Hep-3B cell line, and in this cell line CRP gene expression is primarily regulated by the IL-6 and IL-1β cytokines. In human hepatoma Hep-3B cells, IL-6 activates the transcription factors C/EBPβ and STAT3 and induces CRP gene transcription. IL-1β alone can not induce CRP gene transcription, but synergistically enhances IL-6 induced CRP gene transcription by activating NF-κB. In addition to these cytokine-induced transcription factors, 5 constitutively expressed transcription-factors (C/EBPδ, RBP-Jκ, HNF-1, HNF-3, and OCT-1) are known to have a role in CRP gene regulation. The initial 157 bp of the CRP promoter has been shown to be sufficient for this synergistic interaction between IL-6 and IL-1β cytokines. The IL-6 induced transcription factor C/EBPβ binds the CRP promoter at 2 sites centred at -52 and -219, while STAT3 binds the promoter at -108. The NF-κB p50-p50 homodimer binds to an onconsensus site at -47, which overlaps the proximal C/EBPβ binding site, and the NF-κBp50-p65 heterodimer binds to a site located at -69. The constitutively expressed transcription factors C/EBPδ and RBP-Jκ bind to the proximal C/EBPβ and NF-κB p50-p50 sites respectively, while the HNF-1 and HNF-3 binding sites are at -67 and -62. The Oct-1 binding site is centred at -63, and overlaps the HNF-1, HNF-3, and NF-κB p50-21p65 binding sites.
**CRP and Insulin resistance**

Various studies have demonstrated association between elevated CRP level and Insulin resistance but the causal role played by CRP is unclear.\(^{222, 223, 224, 225}\) Tanigaki et al. (2013) have demonstrated in mice that CRP Causes Insulin resistance through Fc\(\gamma\) Receptor IIB, which defects the glucose uptake by skeletal muscle. In both CRP transgenic mice and wild-type mice, administered with recombinant CRP were found insulin resistant. Tanigaki et al. made a conclusion that mice with deficient inhibitory Fc\(\gamma\) receptor IIB (Fc\(\gamma\)RIIB) were protected from insulin resistance induced by CRP. The presence of Fc\(\gamma\)RIIB expression in skeletal muscle microvascular endothelium and absence in skeletal muscle myocytes, adipocytes, and hepatocytes was confirmed by immunohistochemistry. Primarily CRP in skeletal muscle disrupts the glucose delivery, and attenuation of insulin induced blood flow in skeletal muscle. In Fc\(\gamma\)RIIB/2 mice or in endothelial nitric oxide synthase knock-in mice with phosphomimetic modification of Ser1176, CRP was unable to impair skeletal muscle glucose delivery. To induce skeletal muscle blood flow and glucose delivery mediated by NO, it is normally phosphorylated by insulin signalling. It is dephosphorylated by CRP/Fc\(\gamma\)RIIB. Therefore, CRP has been reported to cause insulin resistance in mice by inhibition of skeletal muscle glucose delivery which is mediated through Fc\(\gamma\)RIIB.\(^{226}\)

**High sensitivity CRP (hs-CRP)**

Previous traditional methods were not enough sensitive to detect blood CRP level within the normal range or below 10 mg/L. These days by using advance techniques like high-sensitivityELISA(enzyme-linked immunosorbent assay), RAP(resonant acoustic profiling), chemiluminescence and immune assay C-reactive protein with a sensitivity range of 0.01 to 10 mg/L can be detected which is now termed as hs-CRP.\(^{37}\) The Centres for Disease Control and Prevention (CDC) along with American Heart Association (AHA) has put forth the recommendations for the measurement of hs-CRP as given in table below:\(^{227}\)
Table 2.8: Recommendations by CDC and AHA for clinical measurement of hs-CRP

<table>
<thead>
<tr>
<th>S. No</th>
<th>Recommendations by CDC and AHA for clinical measurement of hs-CRP.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hs-CRP can be measured both in fasting and non-fasting state.</td>
</tr>
<tr>
<td>2</td>
<td>When the test values are more than 10 mg/L then avoid the results and repeat the test after 2 weeks.</td>
</tr>
<tr>
<td>3</td>
<td>For a reliable estimate, mean of two results performed 2 weeks apart should be considered.</td>
</tr>
<tr>
<td>4</td>
<td>When hs-CRP levels are &gt; 10 mg/L on two consecutive tests, search for other infections and inflammation has to be performed.</td>
</tr>
</tbody>
</table>

Universally the hs-CRP is estimated as an ideal biomarker for the risk prediction of cardiovascular disease (CVD). American Heart Association/Centres for Disease Control is a working assembly for indicators of inflammation in CVD. Data from population based study it has classified serum hs-CRP levels for global CVD risk prediction into three different groups as given below:

Table 2.9: Classification of hs-CRP levels for global CVD risk.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration of hs-CRP (mg/L)</th>
<th>Risk stratification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 1.0 mg/L (µg/ml)</td>
<td>Low risk</td>
</tr>
<tr>
<td>2</td>
<td>1.0 - 3.0 mg/L (µg/ml)</td>
<td>Average/Intermediate risk</td>
</tr>
<tr>
<td>3</td>
<td>&gt;3.0 mg/L (µg/ml)</td>
<td>High risk</td>
</tr>
</tbody>
</table>

Increased CRP level is associated with following conditions.228
i. Acute infections: etc.
   - Bacterial infections like pneumococcal staphylococcal osteomyelitis, bacterial endocarditis

ii. Avery rare condition like acute rheumatic fever

iii. Autoimmune disease like:
   - Systemic lupus erythematosis
   - Rheumatoid arthritis
   - Reiter’s syndrome

iv. Bone diseases like:
   - Osteoarthritis
   - Psoriatic arthropathy
   - Arthritis following jejunileal bypass

v. Cardiovascular diseases:
   - Disseminated polyarteritis nodosa
   - Systemic vasculitis
   - Cutaneous vasculitis
   - Acute myocardial ischemia

vi. GIT diseases:
   - Crohn’s disease
   - Ulcerative colitis

vii. Muscular disease like polymyalgia rheumatic.

viii. Skin disease like dermomyositis

ix. Neoplastic diseases

x. Obesity & Diabetes

xi. Smoking
hs-CRP in metabolic syndrome, diabetes and cardiovascular disease

hs-CRP in Metabolic Syndrome
Numerous studies have established the association of Mets with inflammation (markers such as CRP, IL-1, IL-6, IL-8, serum amyloid A etc.) therefore, MetS is also known with the term low grade chronic inflammation.\(^{229}\) Regarding hs-CRP, it has been correlated significantly with WC, raised TG, low HDL, IR, hyperglycaemia by numerous studies. Therefore, being the constituents of MetS it is evidenced that hs-CRP or inflammation is highly associated with MetS.\(^{38, 39, 40}\) Some of the researchers have demonstrated CRP as a factor in MetS development.\(^{41, 42}\) It has also been reported that high hs-CRP levels in MetS subjects is associated with increased risk for CVD.\(^{230}\) Given the evidence, CRP has also been proposed as constituent of MetS. Whereas, Chopra et al. have suggested that hs-CRP can be used for prediction of metabolic syndrome when cut off value 2.83 mg/L is adopted.\(^{43}\)

hs-CRP in Diabetes
Several studies have suggested the prediction of diabetes from their finding of increased hs-CRP and IL-6 levels.\(^{231, 232, 233, 234}\) In fact, IL-6 induces proteins to bind the receptor of insulin and may interfere with insulin signalling.\(^{235}\) The hypothesis that ‘insulin sensitivity at peripheral tissues is decreased by chronic systemic inflammation’ has been well evidenced as it is supported by number of studies.\(^{236, 237, 238}\) Several cross-sectional studies have reported an increase level of hs-CRP, IL-6, and TNF-α in IGT subjects\(^{236, 239}\) and increase of hs-CRP levels in diabetics.\(^{231, 232, 240}\) In addition, it has been shown that the lowgrade chronic inflammation is guilty for the derangement of glucose homeostasis in IGT subjects.\(^{241, 242}\) Diabetic subjects with hs-CRP>3mg/L have risk of all types of mortality by 51% high and risk of CVD mortality by 44% as compared to diabetics with hs-CRP <3mg/L.\(^{243}\)
hs-CRP in Cardio Vascular Diseases (CVD)

Various studies have been done on CRP, IL-1, IL-6, IL-8, TNF-α, adiponectin etc. since long for the prediction of CVD and for association with inflammation with CVD. CRP has been established as the best marker for both conditions. Increased level of hs-CRP in circulation reveals the increased risk of CVD development. A study group called European Concerted Action on Thrombosis and Disabilities Angina Pectoris also reported raised CRP levels in subjects with coronary events as compared to subjects without it. Later on the report of study ‘Cholesterol and Recurrent Events Trial’ also supported the association of increased hs-CRP with coronary events risk after MI. In 1982 the binding of CRP with VLDL and LDL was discovered and detected in atherosclerotic plaque as well. These findings have suggested the proatherogenic actions of CRP also.

In progression of different stages of atherosclerosis starting from recruitment of circulating leukocytes to the arterial wall to the rupture of unstable plaques inflammatory mechanisms play a crucial role. CRP is found to be present in the vascular intima of atherosclerotic lesion along with monocytes, monocyte-derived macrophages and lipoproteins.

CRP plays a pivotal role in many aspects of atherogenesis as described briefly below:

- The classical pathway of the complement system is activated by CRP followed by direct amplification of innate immunity. This results in initiation and progression of CHD.
- Macrophages scavenge the LDL particle and convert into foam cells in assistance of CRP.
- CRP inhibits endothelial NO synthase expression in ECs. NO has important anti-atherogenic effects.
- Macrophages secrete tissue factor (strong procoagulant) on activation by CRP. It results in dispersed intravascular coagulation followed by thrombosis.
- Expression of adhesion molecules are up regulated by CRP in ECs which attracts monocytes to the injury sites.
Helps in PAI-1 expression which is a PAI-1 is a protease inhibitor. It regulates fibrinolysis by tissue plasminogen activator inhibition. The consequences leads to atherogenesis.36

CRP also indirectly affects specific immune response in atherogenesis. Macrophages increase the production of IL-12 succeeding induction and proliferation of CD4 + T lymphocytes as well as Interferon gamma production.247

Numerous studies have evidenced CRP as a mediator in the development of cardiovascular disease and hence it has been established as emerging CVD risk factors against other conventional one.248, 249, 250, 251

4) Hypertension

Raised in SBP > 140 mmHg and/or DBP > 90 mmHg is defined as hypertension. It has been recognized globally as a foremost growing health problem. Every year the toll death of 7.5 million is estimated worldwide due to hypertension. It is classified into two groups: primary (or essential) HTN and secondary HTN. Primary hypertension accounts for 95% of cases and only 5% by secondary. Essential HTN typically begins after fifty or sixty years of life. The main causes of essential HTN are increased intake of table salt, obesity, historical background with HTN in family and genetic predisposition. Whereas causes of secondary HTN are diseases related to adrenal gland, chronic renal failure, stenosis in the artery supplying kidney, and sleeps apnoea. The common mechanisms for maintaining the blood pressure within normal are:

- Sympathetic nervous system
- Renin-angiotensin-aldosterone system
- Endothelial function
- Water retention
Any disorder in above mentioned mechanism leads to both types of hypertension development (Figure 2.13).  

Figure 2.13: Mechanisms and hormonal systems involved in obesity associated Hypertension.  

It is estimated that at least 75% of the incidence of hypertension is related directly to obesity. The association of obesity and hypertension was first demonstrated by measuring body weight and BP in the Framingham Heart Study in the 1960s. It has already been discussed that obesity leads to increase in FFA, insulin, leptin and aldosterone and decrease in NO (Nitric Oxide) levels. Obesity has also been demonstrated with activation of RAS (Renin Angiotensin System). On one hand increased level of FFA, insulin, leptin, aldosterone and activated RAS further
activates the Sympathetic Nervous System. Activated Sympathetic Nervous System along with increased level of leptin, aldosterone and activated RAS acts on kidney which leads to sodium and water retention followed by hypertension. On another hand decreased NO, increased aldosterone and activated RAS helps in vasoconstriction and lead to hypertension.254

5) Dyslipidaemia

Obesity is a major constituent of MetS which is also associated with an increased prevalence of dyslipidemia. Dyslipidaemia is characterized with hypercholesterolemia and increased LDL, low HDL and raised triglycerides which may be present alone or in combination.23 Decreased HDL levels and raised triglycerides levels are metabolically interweaved and their combination is defined as “atherogenic dyslipidaemia”. It has also a feature of increased of small-dense LDL particles with fairly normal LDL-Cholesterol, and IR. In South Asians atherogenic dyslipidaemia seems to be very common. Numerous studies have evidenced a strong association of atherogenic dyslipidaemia with T2DM, MetS and CVD.22

In obesity, adipocytes increase the synthesis of FFAs. The FFAs fluxes towards the liver through portal vein and up taken by hepatocytes. Increased concentration of FFAs results in increased synthesis of triglycerides by hepatocytes. Increased availability of triglycerides in hepatocytes further increases the synthesis of VLDL. Increased TG and VLDL in liver increases the demand of an enzyme lipoprotein lipase (LPL), required in lipolysis that ultimately results in diminished lipolysis of chylomicrons.21 Obesity has also been evidenced in reduction of mRNA expression of Lipoprotein Lipase (LPL) in adipocytes255 and skeletal muscle256 which leads to decrease lipolysis in mentioned tissues. Under the condition of raised triglycerides, Cholesterylester-transfer-protein (CETP) induces the exchange of TG from VLDL and LDL towards HDL with cholesterolesters (CE) from HDL to VLDL and LDL (Figure 2.14). This results in the consequences like:
• HDL with highly content of TG is transported from circulation to liver for regeneration which leads to decreased level of HDL in circulation.
• Diminished content of TG in LDL.
• Enzyme called hepatic lipase (HL) clears LDL and TG from LDL and results in conversion to small dense LDL.

![Figure 2.14: Obesity induced dyslipidemia](image)

With the background of hypertriglyceridemia, hepatic lipase transforms LDL to small dense lipoproteins by clearing off the TG and phospholipid content of LDL. It takes 5 days residence time duration to get metabolized which plays a major role in the atherogenicity. LDL and Chylomicron remnants get trapped in the sub-
endothelial space if they are migrated towards sub-endothelium space. Monocyte/macrophages take up these particles. There is high affinity between small dense LDL and proteoglycans present in arteries. This results in increased retention of sub-endothelial lipoproteins. In contrast to native LDL, subendothelial remnants of chylomicrons and VLDL are directly taken up by macrophages and monocytes without any modification of receptors. The content of free cholesterol and antioxidant in small dense LDL is very low. This is the reason which makes these particles more prone for oxidation.

The size of the lipoprotein is limiting factor to endothelium migration. Comparatively LDL migrate more conveniently than chylomicrons remnants. However, the migrated particles do not convert into more cholesterol deposition because the content of chylomicrons remnants is around 40 times more cholesterol per particle as compared to LDL. But in contrast the chylomicrons remnants and VLDL which are rich in LPL move towards the liver to interact with lipoproteins and proteoglycans receptors which functions as antiatherogenic activity. Whereas in other tissues atherosclerotic plaque are formed due to decreased removal and accumulation of cholesterol.21

**Morbidities of Metabolic syndrome**

A patient with MS has, increased three times risk of a heart attack or stroke, two times risk of CVD or dying from such events, and five times greater risk of T2DM development in either sexes as compared to subjects without it.

**Diabetes Mellitus**

DM is a condition which is characterized by hyperglycemia, glycosuria, hyperlipidemia, polyuria, polyphagia, polydypsia, negative nitrogen balance and sometimes ketonemia. The cause of DM is due to partial or absolute defect in insulin secretion or decreased response of insulin by the body cells or both. Major categories of DM are as follow:
• **Type 1 Diabetes**
It is characterized by absolute and/or diminished insulin level due to destruction of pancreatic β-cells.²⁵⁷

• **Type 2 Diabetes**
It is characterized by hyperglycaemia due to IR. The major risk factors are sedentary life styles, obesity and may be family history.²⁵⁸, ²⁵⁹

• **Gestational Diabetes Mellitus**
It may develop during pregnancy. In most of the cases, glucose level returns to normal after delivery. They may have risk in development of T2DM in the future.²⁶⁰

• Other miscellaneous types of diabetes are:²⁶¹
  i. Maturity onset diabetes mellitus of young: It is caused due to single genetic mutations. A rare form of diabetes mellitus.
  ii. Secondary diabetes mellitus: It is caused due to other pathological cause like pancreatitis, trauma or surgery of pancreas.
  iii. Drug/chemically induced diabetes mellitus: During treatment of HIV/AIDS or after organ transplantation.

Diabetes mellitus is a chronic complex metabolic disorder associated with derangement of carbohydrate, protein and lipid metabolism, and increased risk of CVD, renal, neural and visual disorders.

The major complications of diabetes are:

• Diabetic Retinopathy leading to blindness.
• Diabetic Nephropathy resulting in renal failure.
• Diabetic neuropathy: Damage to the nerves causing foot wounds and ulcers which frequently lead to foot and leg amputations. It can also lead to chronic
diarrhoea, paralysis of stomach (gastroparesis), and may lose control on heart rate and blood pressure during postural changes.

- Diabetes also helps in promotion of atherosclerosis which can lead to blockages or clot (thrombus) in blood vessels. This results in increased risk of heart attack, stroke and decreased blood circulation in limbs.
- Diabetic subjects may predispose with raised blood pressure, atherogenic dyslipidemia. These conditions, along with hyperglycemia, increase the risk of heart disease, kidney disease and other blood vessel complications.

Diabetes may result in acute complications also. Acute infections have also been found to be associated with diabetes due to impairment of body’s natural ability to fight infection. Hypoglycemia, diabetic ketoacidosis (DKA) and hyperosmolar nonketotic syndrome are other associated acute medical conditions.52

DM is one of the most common diseases with high prevalence globally. This non-communicable disease is the leading causes of death in most developed countries. It is epidemic in many developing and industrializing countries like India. DM is being one of the most challenging problems in health and disease in the 21st century.

Around the globe, 415 million people (8.8%) suffered from DM in 2015 which is estimated to rise to 642 million by 2040. DM caused 5.0 million deaths in 2015 while 193 million or close to half 46.5% people remained undiagnosed. In India, prevalence of diabetes in 2015 was 8.7 % and 1.02 million deaths were reported. Overall in South East Asia, 8.5 % of the adult population i.e. equivalent to 78.3 million people have diabetes and over half 52.1 % are undiagnosed in 2015 and it is predicted the prevalence of diabetes to increase to 140 million by 2040.30

Development of T2DM initially starts with the insulin resistance and normal glucose level for a long time and progress to beta-cell failure and hyperglycaemia and results in vascular complications. The level of blood glucose and occurrence of retinopathy have been given priority in present definition of diabetes mellitus but other important complications like peripheral artery disease, coronary artery disease and cerebrovascular disease also often present when T2DM is diagnosed. Evidence says >60% subjects with T2DM have risk of CVD development which is more severe than retinopathy. Therefore CVD risk must be given a higher priority when cut-off
for blood glucose are defined and should be re-evaluated based on CVD risk. Most of the time T2DM is asymptomatic for many years therefore approximately 50% of the cases of T2DM remain undiagnosed at any time.\textsuperscript{262, 263} Undiagnosed T2DM including IFG and IGT is a very high risk factor for CVD. DECODE (Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) study analysed several European cohort studies with baseline oral glucose tolerance test (OGTT) data demonstrates the substantial evidence for such relationships.\textsuperscript{264, 265, 266} Numerous researchers have claimed the risk of CVD mortality in women with T2DM is relatively higher as compared to male subjects.\textsuperscript{262, 266} Treatment with only glycaemic control is not lowering the risk burden of CVD. Treatment with multifactorial or wide spectrum associated with complications based therapeutic strategies should be developed. Treatment related with inhibition of key enzymes associated with hyperglycemia-induced vascular damage, or improving insulin signalling pathway in IR, may be the promising approaches.

**Cardiovascular Diseases**

Cardiovascular diseases (CVDs) have been established as leading cause of death worldwide. Out of total worlds’ CVD deaths 80% comprise from low-and middle-income countries like India. The prevalence of CVD is equal in either sex.\textsuperscript{33}

Various types of disorders (Including heart and vessels) comprising CVDs are:\textsuperscript{267}

- 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.

CVD is the leading cause of death throughout the world as compared to other diseases. Data from 2012 revealed the death of 17.5 million (6.7 million include stroke and 7.4 million include CHD) due to CVD which solely accounts for 31% of all death from the world.\textsuperscript{267} It is predicted that in India 2.6 million will die due to CHD (constitute 54.1% of all CVD) by 2020 and almost half of the young and middle aged individuals (30-69 years) are suspected to suffer from CVD.\textsuperscript{267, 268}

Framingham risk score (proposed by the the Framingham Heart Study) is widely used for estimation and classification of 10-year risk of coronary vascular disease or myocardial infarction.\textsuperscript{269, 270} Various risk factors have been identified, out of which some are modifiable and some non-modifiable. There are more than 300 risk factors for CVDs being used. Some of the commonly used are hypertension, hypercholesterolemia, smoking, tobacco chewing, diabetes mellitus and obesity. For the improvement of risk assessment some of the emerging factors being widely used now are assessment like positive family history, lipoprotein (a), serum homocysteine, coronary artery calcium score, hs-CRP etc.\textsuperscript{271}

The association of obesity, insulin resistance and inflammation in patients with MetS which has been illustrated by number of previous studies.\textsuperscript{38, 39, 43, 44, 45} Uncontrolled and untreated DM, obesity and undetected insulin resistance may aggravate the development of MetS in such individuals. This study intends to analyze the impact of inflammation by measuring hs-CRP and the correlation of inflammation, obesity and IR in patients of MetS. Besides, as MetS is also one of the significant risk factors for the development of CVD, screening for CVD risk using hs-CRP in patients with MetS was also done in the present study. The outcome of this study will highlight the interrelationship and interdependence of obesity, insulin resistance and inflammation in patients with MetS and help us get further insights into the mechanism and implications of this otherwise complex disorder.