Review of Literature
REVIEW OF LITERATURE

Diabetes epidemiology

Type-2 diabetes is a global health problem of enormous magnitude that is calculated to have affected 5-7% of the world’s population. The prevalence of T2DM varies considerably throughout the world, ranging from <1% in certain populations of developing countries (e.g. rural Melanasians in Papua New Guinea and rural Chinese) to over 50% in the celebrated example of the Pima Indians of Arizona. Generally, there is higher predominance of T2D in urban than in rural areas. Overall, the prevalence of diabetes is expected to increase worldwide from 366 million to 552 million between 2011 and 2030 and 90% of these will have T2D. The developing world will suffer the most with a predicted 170% increase in cases that will mainly affect the 45-64 years age group. By contrast, the diabetic population in developed countries is said to increase by only 40% and particularly among those aged >65 years.

Once considered primarily a risk factor for heart disease, diabetes has now become a high profile public health concern due to the escalating epidemic of diabetes in older people and the emergence of T2D in children. It is clear from epidemiological studies that diabetes risk manifestation, natural history and even the criteria for definition and diagnosis of diabetes itself may vary considerably by population. T2DM accounts for 8-45% of new diabetes cases in paediatric urban diabetes centers. A high prevalence of Impaired glucose tolerance (IGT) (upto 30%) in many populations indicates an accumulation of a susceptible group.

The number of people with diabetes worldwide is set to double in the next 20 years as a result of increasing obesity and longevity, physical inactivity and urbanisation. In a study conducted by Wild et al., data on diabetes prevalence by age and sex from a limited number of countries were extrapolated to all 191 World Health Organisation (WHO) member states. Urban and rural populations were considered separately for developing countries. They found...
a prevalence of 2.8% in 2000 and estimated it to rise to 4.4% in 2030 even if levels of obesity remain constant. The greatest relative increases were projected to occur in the Middle East countries, Sub Saharan Africa and India. They estimated that greatest absolute increase in people with diabetes will be in India.

The Indian scenario:

India currently harbors approximately 61 million diabetes patients and was the diabetes capital of the world until recently. According to the latest IDF release it is now China which has the largest number of diabetics in the world. Over the past 30 years, the status of diabetes has changed from being considered a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. The first study of National Epidemiological Survey conducted by ICMR between 1972 and 1975 screened about 35,000 individuals above 14 years using 50 g glucose and a capillary blood glucose level >170 mg/dL was used to diagnose diabetes. The prevalence was 2.1% in urban population, 1.5% in the rural population and in those >40 years it was 5% and 2.8% in urban and rural populations respectively. Subsequent studies showed a rising trend. At the same time, reports from different parts of the world showed that migrant Asian Indians had a higher prevalence of diabetes than other ethnic groups. There has been a fivefold increase in India (from 2.3% to 12.1%) between 1972 and 2000.

The National Urban Diabetes Survey (NUDS), a population based study was conducted in six metropolitan cities across India and recruited 11,216 subjects aged 20 years and above representative of all socioeconomic strata. An OGTT was done using capillary glucose and diabetes was defined using the WHO criteria. The study reported that the age standardized prevalence of T2D was 12.1% with slight male preponderance. The study also revealed that the prevalence of diabetes in the southern part of India is higher – 13.5% in Chennai, 12.4% in Bangalore and 16.6% in Hyderabad compared to 11.7% in Kolkata (Eastern), 11.6% in New Delhi (Northern) and 9.3% in Mumbai (Western). The study also suggested that there was a 14% pool of IGT
subjects at high risk of conversion to diabetes. In Chennai, Bangalore, Hyderabad and Mumbai, IGT subjects exceeded diabetics.

A very high prevalence of 16.3% was reported from Thiruvananthapuram in Kerala State in 1999 and 8.2% from Guwahati in the same year. A cross sectional population survey in the Kashmir valley in 2000 put the prevalence of known diabetes among adults aged >40 years at 1.9%. A study conducted in Western India showed age standardized prevalence of 8.6% in urban population. A more recent study reported a high prevalence of 9.3% in rural Maharashtra. The ‘Amrita Diabetes and Endocrine Population Survey’ (ADEPS), a community based cross-sectional survey conducted in urban areas of Ernakulam district of Kerala has revealed a high prevalence of 19.5%.

Although in rural India the prevalence of diabetes is much lower than in the urban population, even here the prevalence rates are rapidly rising. Increasing prevalence of T2D in children, teenagers and adolescents is a new and alarming facet of the epidemic of diabetes. Though there are a few data on T2D in children and adolescents in India, it is reasonable to believe that this is a phenomenon waiting to declare itself in a large measure in India also.
Causes and risk factors for type 2 diabetes

Type 2 diabetes is a very heterogeneous syndrome with many possible causes. It is the result of the interaction of environmental factors with a genetic susceptibility and the relative contributions of the two can differ considerably even among individuals with similar clinical phenotype.

Risk factors

- Genetic
  - Socio economic status
  - Diet
  - Physical Activity
  - Malnutrition in early life
  - Asian Indian phenotype

- Environmental

Genetic factors:

Genetic susceptibility has been shown to play a role in most types of diabetes though the genes involved and pathogenesis are different. There are a few varieties of diabetes for which single gene defect is responsible and are called monogenic diabetes. These include varieties of Maturity Onset Disease of the Young (MODY), Maternally Inherited Diabetes and Deafness (MIDD) etc. T2D has polygenic inheritance unlike Mendelian disorders. The strength of the genetic component accounts for 40-80% of the total disease susceptibility in T2D. T2D is highly concordant (60-90%) in monozygotic twins but less so in nonidentical twins (17-37%). The risk of developing T2D increases strikingly if there is a family history of the disease especially among first degree relatives.

During evolution, a major stressor has been the shortage of food and the resulting depletion of the body's energy stores. It was first suggested by Neel in 1962 that the evolutionary responses to this stressor may have been the selection of “THRIFTY GENES” which favour energy storage as triglycerides in adipose tissue. Expression of these genes would be selected in populations living in a precarious environment and this selection process may have operated throughout most of human history. By contrast, human
beings are poorly adapted when forced relatively suddenly into a modern environment with novel stresses of over-nutrition and physical inactivity. Thus, individuals with a thrifty genotype which defends metabolic economy in times of famine may be most at risk when exposed to a westernised lifestyle.  

These polymorphisms may be localised in the coding or regulatory parts of the genes and are present in patients of T2DM as well as in healthy populations, although with different frequencies. So far, genes like Calpain 10, PPARγ, KCJN 11, Adiponectin, IRS-1 and insulin have been implicated in the pathogenesis of T2DM. Genomewide linkage studies of presumed polygenic T2D populations indicate that loci on chromosomes 1q, 5q, 8p, 10q, 12q and 20q contain susceptibility genes. Mutation analyses of selected candidate susceptibility genes in various populations have also identified the widespread Pro12Ala variant of the PPAR and the common Glu23Lys variant of the ATP-sensitive potassium channel and Kir 6.2(KCJN 11). These variants may contribute significantly to the risk of T2D conferring IR of liver, muscle and fat (Pro12Ala) and a relative insulin secretory deficiency (Glu23Lys).  

Indian patients have been demonstrated to have some peculiarities. Pro12Ala polymorphisms in PPARγ genes which are protective against diabetes do not appear to offer protection among Indians. The Thr394Thr(G→A) polymorphism PGC-1 has been strongly associated with diabetes as well as body fat, which is not reported in other ethnic groups. Gly1057Asp polymorphism of IRS-2 gene predisposes Indians to diabetes particularly in presence of obesity. No single major locus explains the inheritance of T2D. The disease is caused by the interaction of multiple genes with environmental factors.

Environmental factors:

India has witnessed an epidemiological transition of increased urbanisation. Socio-economic development over the last 40-50 years has resulted in a dramatic change in life style from traditional to modern, leading to physical
inactivity due to technological advancement, affluence leading to consumption of diets rich in fat, sugar and calories and high level of mental stress.\textsuperscript{51} This has been demonstrated in the Chennai Urban Population Study (CUPS) where prevalence of diabetes was higher among middle income groups and urban dwellers than in low socio-economic groups and rural population.\textsuperscript{51}

Increase in the prevalence of T2D may also result due to migration i.e., a movement from one environment to another, either external or internal, which brings with it, marked social and cultural changes. Migration from rural areas to urban slums led to obesity, glucose intolerance and dyslipidemia.\textsuperscript{52} Many epidemiological studies on diabetes in migrant population mostly in people originating from developing countries and settled in developed countries have reported a higher prevalence of diabetes.\textsuperscript{53}

The ‘fast food culture’ which has overwhelmed our cities and towns is also a major contributor to the diabetes epidemic. Sedentarism is another driver of the diabetes epidemic. Over the last few decades, a huge number of working population has shifted from manual labour associated with the agricultural sector to physically less demanding office jobs. Children and youth are highly addicted to television and computer games that prevents any outdoor physical activity.\textsuperscript{4}

The natural genetic predisposition combined with a sedentary life style has led to a concurrent epidemic of obesity affecting all age groups. The intimate relationship between diabetes and obesity has given rise to the term ‘diabesity’ to characterise the association of these two disorders.\textsuperscript{6} Having a BMI of $>35 \text{ kg/m}^2$ increases the risk of developing diabetes over a 10 year period by a staggering 80 fold as compared with individuals with BMI $<22 \text{ kg/m}^2$.\textsuperscript{49} Data from the long term prospective Study of North American Nurses\textsuperscript{54} show that life style factors account for 90\% of the excess susceptibility to T2D and that obesity is the most important of these. Obesity (especially abdominal and visceral) is associated with insulin resistance (IR) and fat is presumed to secrete potentially diabetogenic factors that can act on distant tissues like liver and muscle and can interfere with glucose metabolism in them.\textsuperscript{19}
Malnutrition in utero and during the first year of life has been associated with subsequent development of diabetes in some studies but not in others. The thrifty phenotype hypotheses suggests that specific nutritional defects in fetal and early infant life predispose to T2D, by compromising the development and function of the β-cells and possibly by inducing IR.

The Asian Indian phenotype:

Asian Indians have always been at high risk of diabetes and CAD. They have a typical phenotype with high percentage body fat at low BMI, less muscle, more fat, truncal obesity, classical lipid triad, hypertension and T2D compared to Caucasians and a higher risk at low waist circumference. Asian Indians have a highly atherogenic lipid profile with high levels of small dense low density lipoproteins. The diet is rich in carbohydrates and low in proteins despite the cultural diversity across India. Modernisation has affected both urban and rural Indians causing them to lead a sedentary life style. Higher percentage body fat at low BMI, central obesity with low waist circumference, highly atherogenic lipid profile, atherogenic diet and sedentary life style are peculiarities of Indians that predispose them to higher risk of CAD. All these environmental factors add on to the genetic susceptibility of Asian Indians.
Role of family history

Type 2 diabetes shows a clear familial aggregation but does not segregate in a classical Mendelian fashion.\textsuperscript{51} In western populations, it has been demonstrated that risk for T2D among offspring for single diabetic parent was 3.5 fold higher and for those with two diabetic parents was 6 fold higher compared with offspring without parental history of diabetes.\textsuperscript{59}

A strong familial aggregation of diabetes is observed among Asian Indians with high prevalence among the first degree relatives and vertical transmission through two or more generations. A comparative study of migrant Indians and Europeans conducted in the United Kingdom (UK) by Mohan \textit{et al}\textsuperscript{44} in the 1980’s showed that 10% of Asian Indian diabetic patients had both parents with diabetes compared to only 1% of European diabetic patients. In the CUPS study from Chennai, participants belonging to high socio-economic status and who had a positive family history of diabetes had 5 times higher prevalence of glucose intolerance compared to participants from low socio-economic status and no family history.\textsuperscript{60}

Faerch \textit{et al}\textsuperscript{61} describing the natural history of insulin sensitivity and insulin secretion during the progression from normal glucose tolerance to the prediabetic states, showed abnormalities in insulin secretion and insulin sensitivity at least 5 years before the development of IFG, IGT and IFG and IGT. The proportion of men and individuals with a family history (FH) of diabetes was highest in the groups who later developed IFG and IFG/IGT.

Although FH has been established as a risk factor for diabetes development, after the initial diagnosis, no weightage is given to this factor. The subjects with FH as such are exposed for a longer duration to the diabetes and its complications. Gong \textit{et al}\textsuperscript{46} studied glycemic control in people with and without history of diabetes. They report that participants with positive FH were younger, had longer duration of diabetes, higher HbA\textsubscript{1c} values (HbA\textsubscript{1c} increased with number of affected individuals in the family) indicating that genetic factors may have contributed significantly to higher glucose levels present in those with positive histories giving these individuals more severe
cases of diabetes. A strong association between HbA\textsubscript{1c} and FH was shown even with adjustments for age, sex and BMI indicating that these were not factors that significantly influenced the relationship. However, duration of diabetes caused the association to become no longer significant. This study adds further evidence of genetics contributing not only to diabetes incidence but also to increased severity of disease.

It has been well established that FH of T2DM has been found to be associated with an increased risk of developing the disease and diabetes in turn is a risk factor for CHD.\textsuperscript{29,60,62} Scheuner et al\textsuperscript{63} demonstrated that FH of diabetes was a risk factor for CHD in non-diabetes individuals, with an Odds Ratio of 1.3, 95%CI:1.1,1.7. Subjects with a FH also were shown to have higher blood pressure, increased carotid artery intima media thickness, IR and were also prone to the development of metabolic syndrome.\textsuperscript{29,30,64-66}

Long term studies with young onset T2DM (18-44 years) compared to those diagnosed later have found a greater genetic predisposition in them. Also, relatives of young onset DM patients are at higher risk of developing diabetes than the risk to relatives of those diagnosed later (>45 years).\textsuperscript{67-69} Hillier and Pedula\textsuperscript{70} studied the characteristics of an adult population with newly diagnosed T2D in relation to obesity and age of onset and found raised BMI, HbA\textsubscript{1c}, random plasma glucose (RPG), diastolic blood pressure (DBP) and total cholesterol to high density lipoprotein (TC/HDL) and lower HDL in the patients with early onset diabetes (age of diagnosis <45 years). Those with late onset (>45 years) had higher chances of hypertension and raised low density lipoprotein (LDL). The metabolic and clinical profile differed in early onset compared to late onset incident T2DM with worsening glycemia and obesity in the early onset patients. The Odds increased by 6% for every one kg/m\textsuperscript{2} increase in BMI. This in turn affected lipid levels, A\textsubscript{1C}, systolic blood pressure (SBP) and diastolic blood pressure (DBP). IR was present at diagnosis in most of the young adults. Kim et al\textsuperscript{71} also report higher maximal BMI, FPG, post prandial glucose (PPG), A\textsubscript{1C} and homeostasis model assessment- insulin resistance (Homa-IR). Older subjects with diabetes had higher incidence of HTN and postprandial C-peptide. No difference was found
in TC, triglyceride (Tg), HDL and LDL. In a small group in whom microalbumin was estimated, it was more frequently detected in the younger population. They opined that among persons with FH, diabetes is known to occur earlier with obesity, genetic predisposition to β-cell dysfunction and IR playing important roles.

Similar findings were reported in young non-Caucasians. This study had 21 affected child-parent pairs where the child was diagnosed at a younger age as compared to the parents' age of onset. The offspring were diagnosed at an alarming 21 years on average earlier than their parents' age of onset. They also had poorer glycemic control. Several other studies also have demonstrated that subsequent generations present with diabetes at an earlier age with longer duration, greater severity and worse complications. Early abnormalities in CHD risk factors have been reported in relatives of subjects with T2DM even when these subjects are in the prediabetes or normoglycemic range.

As a natural process of aging, the subjects without a FH of diabetes also demonstrate adverse changes in body fat compositions, plasma glucose levels, lipids, β-cell function, blood pressure (BP) and inflammatory markers although to a much lesser degree than in those with a FH.

Accepting that familial history is an important risk factor for developing T2DM, while studying the inheritance pattern it was observed that T2DM patients are more likely to have diabetic mother than diabetic father. However, this inheritance pattern has not been reported uniformly, especially in South Asians. Lee et al in their study of Hongkong Chinese subjects found paternal history of diabetes to be associated with younger age of onset. In a study from southern Brazil, maternal transmission was homogeneous over all age groups in 396 patients with 62% in the youngest group (30-42 years) and 44% in the oldest group (52-86 years) compared to 26% and 12% with fathers. They also observed that the frequency of patients who reported an affected mother and/or father decreased as the age at T2DM diagnosis was later which indicated that the severity of DM and the age at onset are genetically
determined and that environmental factors are more important when onset of DM occurs later in life.

Racial and ethnic differences also cannot be ignored in the susceptibility to diabetes and the underlying pathogenic mechanisms. Consensus does not exist regarding the metabolic changes occurring during the progression from normoglycemia to prediabetes and further to frank diabetes. A FH of diabetes was associated with an insignificant increase in insulin level in normoglycemic subjects but frank hyperinsulinemia was seen only in subjects with IGT in a study from Finland. Indian studies reported significant hyperinsulinemia and decreased insulin sensitivity even in the normoglycemic subjects. While certain studies report severe dysfunction and accumulation of cardiovascular risk factors in the younger group, certain others have shown it only in the older age group.

Thus, normoglycemic first degree relatives of T2D patients serve as an excellent cohort to study the natural history and progress of diabetes.
Pathophysiology and biochemical basis of T2DM

Diabetes Mellitus is a group of metabolic diseases characterised by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.\textsuperscript{94}

Several pathogenic processes are involved in the development of diabetes. They range from autoimmune destruction of the $\beta$-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficiency of action of insulin on target tissues. Impairment of insulin secretion and defects in insulin action frequently co-exist in the same patient and it is often unclear which abnormality, if either alone is the primary cause of hyperglycemia.\textsuperscript{94}

In T2DM, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues but without clinical symptoms may be present for a long period of time before diabetes is detected.\textsuperscript{95,96} The degree of hyperglycemia may change over time depending on the extent of the underlying disease process. The spectrum ranges from presence of the disease but not enough progression to cause hyperglycemia to IFG and/or IGT to frank diabetes. The severity of the metabolic abnormality can progress, regress or remain unchanged.\textsuperscript{94} Thus, the degree of hyperglycemia reflects the severity of underlying metabolic process. The T2DM which accounts for $\sim 90$-$95\%$ of those with diabetes, previously referred to as non-insulin dependent diabetes mellitus (NIDDM), type 2 diabetes or adult onset diabetes encompasses individuals who have insulin resistance and usually a relative rather than absolute insulin deficiency. At least initially and often throughout their life time, they do not need insulin treatment to survive.\textsuperscript{94} Most patients with this form of diabetes are obese and obesity itself causes some degree of insulin resistance. If not obese, by traditional weight criteria, they may have an increased percentage of abdominal fat.\textsuperscript{97,98} Because
glucose is a continuous variable, the use of thresholds to make a diagnosis is somewhat arbitrary. The term ‘prediabetes’ has become established and implies plasma glucose concentrations above the normal and below the threshold for diabetes criteria (FPG ≥100 mg/dL but <126 mg/dL and 2h values in the oral glucose tolerance test ≥140 mg/dL but <200 mg/dL). This intermediate group of subjects with IFG or/and IGT are referred to as having ‘prediabetes’ indicating the relatively high risk for development of diabetes in these patients.99

Individuals with IFG have a 20-30% chance of developing diabetes over the next 5-10 years and the risk is even greater if they have combined IFG and IGT. IFG and IGT are also associated with increased risk of cardiovascular events.100 However, little is known about the rate of progression and the characteristics of such progression.

Nichols et al101 studied the progression of prediabetes to overt disease and observed that 8.1% of subjects with initial fasting glucose between 100 and 109 mg/dL and 24.3% of subjects with initial fasting glucose of 110-125 mg/dL developed diabetes over an average of 29 months. A steeper rate of increasing fasting glucose, higher BMI, blood pressure and triglycerides and lower HDL cholesterol, predicted diabetes development. It was concluded by the Baltimore Longitudinal Study of Aging102 that fasting and post-challenge hyperglycemia may represent phenotypes with distinct natural histories in the evolution of T2DM.

A hallmark of T2D is a decline in the β-cell function which is said to begin as early as 12 years before diagnosis and continues throughout the disease process in association with progressively increasing hyperglycemia despite treatment.61,103

Pathophysiology of β-cell failure:

Pancreatic β-cells normally respond to IR by increasing their output of insulin to meet the needs of the tissues. Development of T2D essentially stems from a failure of the β-cell to adequately compensate for insulin resistance. The β-
cell dysfunction progresses over time and is well advanced by the time a person's plasma glucose level is in the diabetic range and continues to worsen after diabetes develops.\(^{104}\)

Genetic predisposition to β-cell failure has been identified in a subtype of the disease characterised by diagnosis at <25 years of age, β-cell dysfunction and autosomal dominant mode of inheritance and heterozygous mutations in β-cell transcription factors.\(^{105,106}\)

**Mechanism of decline in insulin secretion:**

Normal β-cell adaptation to insulin secretions can occur through increased insulin secretion from each β-cell and/or an increase in β-cell mass. In insulin resistant subjects or subjects with T2D, there is inadequate insulin secretion from each β-cell or an inadequate β-cell mass for the levels of prevailing insulin sensitivity.\(^{107,108}\) Insulin secretion is in response to levels of plasma glucose. β-cells maintain their responsiveness in the face of insulin resistance through increased insulin secretion in response to meals as well as through a chronic response by increasing β-cell mass.\(^{99}\) Normal-weight and obese individuals maintain a normal and similar 24 hour glucose response to meals. However, the groups differ in their mean insulin secretion which is significantly higher in obese subjects than in their normal-weight counterparts. In addition, insulin secretion in obese subjects fails to return to baseline between meals.\(^{99}\)

Two acquired defects have been implicated with regards to impaired insulin secretions – ‘glucotoxicity’ whereby β-cells become sensitised to the presence of glucose and ‘lipotoxicity’, whereby accumulated fatty acids and their metabolic products deleteriously affect β-cells.\(^{109}\) In glucotoxicity, chronic hyperglycemia depletes insulin secretory granules from β-cells, lessening the amount of insulin available to be released in response to new glucose stimuli. Lowering plasma glucose levels permits regranulation of β-cells and a better acute insulin response. In lipotoxicity, prolonged increases in free fatty acid (FFA) levels adversely affect the conversion of proinsulin to insulin and
eventually affect insulin secretion. Fatty infiltration of pancreatic islets may also contribute to β-cell dysfunction.\textsuperscript{110} Concepts of gluco and lipotoxicity remain hypotheses and attempts are on to elicit the exact mechanism of β-cell function and dysfunction.

\textbf{β-cell mass deficits:}

Although β-cell function is paramount, decreasing β-cell mass is an important factor in progress of T2D. β-cell mass is increased by neogenesis as well as replication and hypertrophy. These activities are counter-balanced by apoptosis and necrosis, thereby maintaining a balance in β-cell mass. In individuals who are obese or insulin resistant, the number of islet and β-cells in the presence of increased insulin demand increase with some degree of hypertrophy.\textsuperscript{111}

While there was an \approx 50\% increase in relative β-cell volume in obese subjects without diabetes, those with IFG or T2DM had 40\% and 63\% deficits in relative β-cell volume. These findings suggest that a decreased number of β-cells, rather than a decreased volume of individual cells causes β-cell functional decrease. Thus an \approx 50\% deficit in β-cell mass can cause alterations in the glucose mediated insulin secretion and insulin action in humans with IFG and IGT supporting a mechanistic role of a deficit in β-cell mass in the evolution of IFG/IGT and diabetes.\textsuperscript{112,113}

That β-cell mass and functions deteriorate progressively in association with increasing hyperglycemia despite treatment is evident from monotherapy failures. Because HbA\textsubscript{1C} increases by \approx 1\% every 2 years even with most therapies, patients with diabetes require repeated and vigorous intervention. Hence, younger patients with diabetes and those with weight gain are more likely to have diabetes progression and need aggressive management.\textsuperscript{99}

\textbf{Biochemical and molecular basis of complications:}

T2DM is associated with an increased risk of premature atherosclerosis. CAD, CVD and peripheral vascular disease are the cause of death in 75-80\%
of adult diabetic subjects. Although the conviction that hyperglycemia plays a role in the pathogenesis of cardiovascular complications in diabetic patients has waned and waxed in recent years, both prospective studies on the relationship between plasma glucose and cardiovascular events and clinical trials of intensive glucose control have found a link between high glucose levels and cardiovascular diseases without any apparent threshold.\textsuperscript{114}

Many studies have shown that diabetes and hyperglycemia increase oxidative stress.\textsuperscript{115,116} Hyperglycemia induced superoxide formation is brought about by increases in polyolpathway flux, advanced glycation end products (AGEs) formation, Protein Kinase C (PKC) activity, hexosamine pathway flux and mutations in mitochondrial DNA.\textsuperscript{117} The pancreatic islets is among the least well endowed tissues in terms of intrinsic antioxidant enzyme expression including superoxide dismutase (SOD)-1, SOD-2, catalase and glutathione peroxidase.\textsuperscript{118} Several antioxidant drugs have been evaluated as protectors against beta cell oxidative stress. The oral hypoglycemic agents, metformin and troglitazone have antioxidant properties. The sulphonylurea glicazide is known to protect beta cells from hydrogen-peroxide damage. New low molecular mass compounds that act as superoxide dismutase (SOD) or catalase mimetics and glutathione are under evaluation for their potential role in preventing the development and progression of diabetic complications.\textsuperscript{116}
Insulin Sensitivity and Insulin Resistance

Insulin is an essential peptide hormone whose metabolic actions maintain whole body glucose homeostasis and promote efficient glucose utilization, including increased glucose disposal in skeletal muscle and adipose tissue and inhibition of gluconeogenesis. The brain, pancreatic β-cells, heart and vascular endothelium are the other targets sensitive to insulin action other than skeletal muscle, adipose tissue and liver. Insulin has concentration dependent saturable actions to increase whole body glucose disposal. The maximal effect of insulin defines ‘insulin responsiveness’, whereas the insulin concentration required for a half-maximal response defines ‘insulin sensitivity’ (ISn).\(^{119}\)

Insulin Resistance (IR) is defined as decreased sensitivity or responsiveness to metabolic actions of insulin. The concept of IR was proposed as early as 1936 to describe diabetic patients requiring high doses of insulin. IR plays a major pathophysiological role in type 2 diabetes and is tightly associated with major public health problems, including obesity, hypertension (HTN), coronary artery disease, dyslipidemias and a cluster of metabolic and cardiovascular abnormalities that define the MetS.\(^{120}\)

At the cellular level, insulin action involves a complex network of molecules. Following insulin binding to the α-subunit of its receptor, the tyrosine kinase in the β-subunit undergoes autophosphorylation and activation. This results in phosphorylation of several intracellular substrates on tyrosine residues, the best characterized of which is insulin receptor substrate-1 (IRS-1). This is a cytoplasmic protein with multiple tyrosine phosphorylation sites which, following insulin stimulation, serve as docking sites for intracellular molecules that contain specific recognition domains, termed SH\(_2\) domain. Some of the SH\(_2\) domain containing molecules are enzymes such as phosphotidyl-inositol-3-OH kinase (PI3k) and are directly activated by docking; others are adaptors which link insulin signaling to the Ras pathway and families of serine-threonine kinases termed the raf, MAP and S\(_6\) kinases.\(^{121}\)
The exact locus of IR is poorly understood. Defects in insulin receptor, genetic variations in the sequence of IRS-1, α-2-HS-glycoprotein an inhibitor of the insulin-receptor tyrosine kinase (said to inhibit insulin stimulated IRS-1 phosphorylation, PI3k activation and insulin stimulated DNA synthesis), membrane glycoprotein PC-1-inhibitor of insulin receptor kinase, Rad, a member of the Ras/GTPase super family as an intracellular inhibitor of glucose-transporter (GLUT) translocation and a decrease in insulin receptor kinase activity by Tumor Necrosis Factor-α (TNF-α) have been implicated.\textsuperscript{121,122}

Shulman GI,\textsuperscript{123} using nuclear magnetic spectroscopic (NMR) studies provides evidence that defective muscle glycogen synthesis plays a major role in causing IR in patients with T2DM. He hypothesized that either decreased glucose transport activity or decreased glycogen synthase activity could be responsible. Further experiments were in support of the former. Thus, a predominant role of glucose transport and control of insulin stimulated muscle glycogen synthesis was suggested as the rate controlling step. He also reported that studies undertaken in insulin resistant lean normoglycemic offspring of parents with T2DM showed a 50% reduction in the rate of insulin stimulated whole body glucose metabolism, mainly due to decreases in rates of muscle glycogen synthesis. Defective muscle glycogen synthesis therefore, is found even before the onset of diabetes.

In a cross-sectional study of young normal-weight offspring of T2DM patients, Shulman and his team\textsuperscript{123} found an inverse relation between fasting plasma fatty acid concentrations and insulin sensitivity consistent with a previously held hypothesis, thus describing the role of increased plasma free-fatty acids in the development of IR. In a series of further studies where subjects were maintained in euglycemic, hyperinsulinemic conditions with either low or high plasma fatty acids, a reduction of approximately 50% in muscle glycogen synthesis and whole body glucose oxidation was found in high plasma fatty acids group. An interference of fatty acids with a very early step in stimulation of GLUT 4 activity was proposed, probably through alterations in upstream insulin signaling events resulting in decreased GLUT 4 translocation to the
plasma membrane. This hypothesis was supported by the finding that elevations in plasma fatty acid concentrations abolished IRS-1-associated PI3 kinase activity resulting in decreased activation of glucose transport and other downstream events. Agreeing with the hypothesis suggested by previous animal experiments, the finding by his team suggest that IR develops in obesity and T2DM because of alterations in the partitioning of fat between the adipocyte and muscle or liver. This change leads to the intracellular accumulation of Tgs and fatty acyl CoAs, in these insulin responsive tissues which leads to acquired insulin signaling defects and IR. These data suggest that increases in plasma fatty acid concentrations initially induce IR by inhibiting glucose transport or phosphorylation activity and that the reduction in muscle glycogen synthesis and glucose oxidation follows.\textsuperscript{124}

**Measures of Isn and IR:**

The hyperinsulinemic Euglycemic Glucose clamp technique is widely accepted as the reference standard in determining ISn/IR. This method directly measures whole body glucose disposal at a given level of insulinemia under steady state conditions. The main limitations are that, it is time consuming, labour intensive, expensive and requires an experienced operator. Moreover, the clamp study utilizes steady state insulin levels that may be supraphysiological which results in reversal of the normal portal to peripheral insulin gradient. Thus, it may not accurately reflect insulin action and glucose dynamics under physiological conditions as an oral meal or oral glucose load may determine.\textsuperscript{119}

The other method that directly measures ISn is the ‘Insulin suppression test’. Indirect measures include ‘minimal model analysis of frequently sampled intravenous glucose tolerance test’ (FSIVGTT), Oral glucose tolerance test (OGTT) and Meal tolerance test. Information derived from OGTT, meal tolerance test and FSIVGTT are used in indexes like Matsuda index, Stumvoll index, Gutt index, Avignon index, Belfiore index and Oral glucose insulin sensitivity index. Fasting insulin levels, Glucose to insulin ratio, Homeostasis model assessment, Quantitative insulin sensitivity check index (QUICKI) are surrogate indices for ISn/IR.\textsuperscript{125}
Homeostasis model assessment (Homa):

Developed in 1985 and further updated, this model assumes a feedback loop between the liver and β-cell i.e. glucose concentrations are regulated by insulin dependent hepatic glucose production (HGP), whereas insulin levels depend on the pancreatic β-cell response to glucose concentrations. Thus, deficient β-cell function reflects a diminished response of β-cell to glucose-stimulated insulin secretion. Likewise, IR is reflected by diminished suppressive effect of insulin on HGP. HOMA-IR describes this glucose-insulin homeostasis by a set of empirically derived nonlinear equations. The model predicts fasting steady-state levels of plasma glucose and insulin sensitivity.

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\text{Homa-IR} = \frac{\text{fasting insulin (µU/mL)} \times \text{fasting glucose (mmol/L)}}{22.5}
\]

The constant denominator 22.5 is a normalizing factor which is the product of normal fasting plasma insulin of 5 µU/mL and normal fasting plasma glucose of 4.5 mmol/L typical of a normal healthy individual. The denominator is 405 when glucose is expressed as mg/dL (4.5 mmol/L corresponding to 81 mg/dL). In an individual with normal insulin sensitivity, Homa-IR = 1. Homa-IR has a reasonable linear correlation with glucose clamp and minimal model estimates of ISn/IR. It is used extensively in large epidemiological studies, prospective clinical trials and clinical research studies. Homa-IR correlated significantly with whole body insulin action in both nondiabetic and T2D patients. The prediction of IR by Homa in IGT subjects however was not satisfactory. Log transformed Homa-IR can be used to get a better linear correlation when data is skewed. Homa-IR or Log Homa-IR can be used for evaluation of IR in individuals with glucose tolerance, mild to moderate diabetes and other insulin resistant conditions. However, it may be inappropriate in subjects with severely impaired or absent β-cell function.

The fasting steady state insulin and glucose concentrations can also be used to determine β-cell function (i.e. glucose-stimulated insulin secretion) as Homa%β.

\[
\text{Homa%β} = \frac{20 \times \text{Fasting insulin (µU/L)}}{\text{Fasting glucose (mmol/L)} - 3.5}
\]
IR has been suggested to constitute one of the primary and key pathogenic factors for the development of glucose intolerance and T2DM. The potential role of impaired β-cell function in the deterioration of glucose tolerance has been supported by cross-sectional studies in FDRs of individuals with T2D, in subjects with impaired glucose tolerance and in women with a history of gestational diabetes or polycystic ovary syndrome. Prospective studies have demonstrated that IR is the best predictor of whether or not an individual will become diabetic. The presence of IR has been demonstrated 10-20 years before the onset of diabetes. Peripheral insulin sensitivity is decreased at relatively low glucose levels within the normal range of FPG and 2-hr PG among Finnish men with MetS. In the same study, raised fasting insulin and first phase insulin release were seen in increasing glucose intolerant individuals which was not found in those with increasing fasting glucose. However, in a study by Faerch et al transition from normal glucose tolerance to isolated impaired fasting glucose was also associated with reduced insulin secretion, further supported by Lin et al.

IR is frequently attributed to obesity, more so with visceral fat. Visceral adiposity is also a predictor of future IR. It is shown that obesity and IR are inherited. The Asian Indians have a greater degree of central obesity, increased visceral fat, higher plasma insulin levels and IR.

The MetS is also termed Insulin Resistance Syndrome. IR is the common pathology seen in the components of MetS i.e. obesity, dyslipidemia, dysglycemia and hypertension. Again, the major determinant of MetS itself is said to be visceral adiposity. Among nondiabetic Asian Indians, visceral, but not subcutaneous fat was significantly associated with MetS and IR.

The abnormalities associated with IR in nondiabetic individuals include hyperinsulinemia, borderline glucose intolerance, dyslipidemia and hypertension. The pathophysiological states associated with IR include enhanced postprandial lipemia, small dense LDL particles, high uric acid levels, enhanced renal sodium retention and decreased urinary clearance of uric acid, higher resting heart rate, dysfibrinolysis and polycystic ovarian
syndrome (PCOS). Thus, the concept of IR has evolved from its role in the pathogenesis of T2D to attain a greater role.\textsuperscript{120,133,135}

Recently, inflammation and inflammatory cytokines have been postulated to be important pathogenetic factors in the development of IR. Whether these molecules play a causative role or simply act as markers of the acute phase reaction is debatable. A study from North India by Ahmad \textit{et al}\textsuperscript{136} showed an association of CRP and fibrinogen with IR but another study from Italy by Muscari \textit{et al}\textsuperscript{137} could not find an association of IR with CRP. CRP was associated with obesity. Thus variations in correlations can be expected due to the ethnic differences.\textsuperscript{138-140}
Glycated haemoglobin

Glycation is the non-enzymatic addition of a sugar residue to the amino groups of proteins. Human adult haemoglobin (Hb) usually consists of 97% HbA, 2.5% HbA₂ and 0.5% HbF. HbA is again made up of minor haemoglobins HbA₁a, HbA₁b and HbA₁c, collectively referred to as HbA₁. HbA₁c is the major factor constituting approximately 80% of HbA₁. HbA₁c is formed by the condensation of glucose with the N-terminal valine residue of each β-chain of HbA to form an unstable Schiff base (aldimine, preHbA₁c). The Schiff base may either dissociate or undergo an Amadori rearrangement to form a stable ketoamine.

Formation of glycated Hb is essentially irreversible and the level depends on both the life span of the red blood cell and the plasma glucose concentration. Because the rate of formation of HbA₁c is directly proportional to the concentration of glucose in the blood, the glycated haemoglobin concentration represents the integrated values of glucose over the preceding 6-8 weeks.¹⁴¹

Glycated Hb occurs over the entire 120-day life span of the red blood cell, but within these 120 days, recent glycemia has the largest influence on the HbA₁c value. A patient in stable control will have 50% of HbA₁c formed in the month prior to sampling, 25% in the month before that and the remaining 25% in months 2-4. Normoglycemic subjects have values between 4 and 6%.¹⁴²

HbA₁c was accepted as a useful tool to objectively assess the prior glycemic control of patients with diabetes.¹⁴³ The clinical use of HbA₁c was strengthened by the reports of the ‘Diabetes Control and Complications Trial’ (DCCT) where a linear relationship between mean plasma glucose (MPG) was established (r=0.82). More recently, initial reports from ‘the mean blood glucose study’ for which patients were recruited during 2006-2007 found a correlation coefficient of 0.91.¹⁴⁴

From the DCCT where 1441 patients participated for an average of 6.5 years, it was possible to make 26056 comparisons between HbA₁c and a full 7 point
plasma glucose profile. The linear relation found has since been used as the most accurate guide of glycemic control:

\[ \text{Mean plasma glucose} = 1.98 \times \text{HbA}_{1c} - 4.29 \]

The results of the DCCT\textsuperscript{145} and United Kingdom Prospective Diabetes Study (UKPDS)\textsuperscript{146} that aimed to establish the effect of intensive glycemic control on the development of microvascular complications, show a 76% reduction in developing retinopathy, 54% proteinuria and 60% clinical neuropathy in DCCT and about a 25% overall reduction in the UKPDS.

More importantly, however, was the finding that subsequent follow up of the DCCT intensive therapy group in the ‘Epidemiology of diabetes interventions and complications study’ revealed an increase in \( \text{A}_{1c} \) to 8% from an average 7.3% during the earlier trial. Despite this, the benefits of improved glycemic control were maintained in the long run. This observation that ‘glycemia from several years previously influences subsequent long term complications risk’, has since been dubbed ‘metabolic memory’ and has reinforced the importance of good glycemic control as soon as possible after the diagnosis of diabetes.\textsuperscript{147}

Macrovascular complications also were largely contained in the intensive therapy group. HbA\textsubscript{1c} has shown to predict macrovascular events in several other studies.\textsuperscript{114,148}

Both fasting glucose concentrations and post prandial glucose concentrations contribute to HbA\textsubscript{1c}. But studies have revealed that the relative contributions of the two vary over the range of HbA\textsubscript{1c}. For values >10%, the major contribution is by FPG and for values <7%, PPG is the major contributor. Therefore, in a diabetes patient with HbA\textsubscript{1c} <7%, although the control appears good, it could still be possible to have PPG values beyond the acceptable range.\textsuperscript{149}

The use of HbA\textsubscript{1c} in screening and diagnosis of diabetes has been debated for a long time.\textsuperscript{150-153} HbA\textsubscript{1c} can be estimated at any point of time unlike FPG, for which the patient needs to fast for a minimum of 8 hours and oral glucose
tolerance test which is very time consuming, labour intensive and not suitable for large population screenings. Another advantage with A₁c testing is that capillary blood samples are suitable and sample stability is better than for blood glucose. The specificity and sensitivity of the test, however is different when applied to normoglycemic individuals, prediabetics and diabetes patients. The sensitivity was only about 50% in detecting impaired glucose tolerance.¹⁵⁴,¹⁵⁵ Several studies have advocated the use of A₁c as a screening tool. The discussions however did not conclude on a single opinion mainly because of the difficulties in the assay methods and standardizations used.

With the rising diabetes epidemic and the alarming increase in CHD events even in A₁c values <7% (mainly attributed to PPG values), the reference values for HbA₁c has been revised from time to time. Also, revisions in the FPG values had to be accommodated. The current expected normal value is <5.6% corresponding to FPG ≤100 mg/dL and PPG ≤140 mg/dL. Values between 5.6% and 6.1% represent prediabetes and A₁c >6.1% indicates diabetes.³⁷
Prediabetes

Prediabetes is a condition in which blood glucose levels are elevated above the normal range but do not satisfy the criteria for the diagnosis of diabetes mellitus.\textsuperscript{156}

Prediabetes has been first described by the World Health Organisation (WHO) in 1980 as impaired glucose tolerance (IGT).\textsuperscript{157} In order to avoid the rather cumbersome and time consuming measurement of 2 hour post glucose load concentrations, the ADA proposed in 1997\textsuperscript{158} to identify prediabetes as impaired fasting glucose (IFG), which relies on one fasting measurement only. In 2003, the ADA lowered the cut off point for IFG from 6.1 to 5.6 mmol/L (110 mg/dL to 100 mg/dL) and advocated this to be used to screen the population.\textsuperscript{159} This resulted in missing a large population having IGT which was associated with cardiovascular events.

Plasma glucose cut off for prediabetes - The dilemma:

That prediabetes is a risk factor for diabetes and CVD is well established. Because of the ease of the measurement, FPG is the preferred test in the screening and diagnosis of diabetes. When ADA lowered the threshold of IFG from 110-125 mg/dL to 100-125 mg/dL, it generated an international controversy as regards the specificity and sensitivity of such values in detecting prediabetes. WHO defines IFG as glucose levels >110 and <126 mg/dL. The 2 hr post glucose load level has remained the same through the years as >140 and <199 mg/dL.\textsuperscript{94}

Nichols \textit{et al}\textsuperscript{81}, who followed 46,578 subjects with fasting glucose <100 mg/dL until they developed diabetes, died or left the health plan, presented their findings using Cox regression analysis. The diabetes risk increased from about 85 mg/dL and there was a 49% greater risk of developing diabetes at plasma glucose levels of 90-94 mg/dL. Another study in young Israeli men found significantly greater risk at the level of 87-90 mg/dL.\textsuperscript{160}

A recent study from India by Somannavar \textit{et al}\textsuperscript{61}, where random capillary blood glucose measurements were analysed in 1,03,878 people from
diabetes screening camps, a cut point of 140 mg/dL by ADA criteria and 143 mg/dL by WHO criteria identified people with diabetes. For IGT it was 119 mg/dL and for IFG 118 mg/dL and 113 mg/dL by WHO and ADA definitions respectively. They suggested that all Asian Indians with a random capillary blood glucose level >110 mg/dL are candidates for extensive workup to detect prediabetes/diabetes.

Consensus does not exist regarding the contributions of IFG, IGT or both to the risk of T2DM and CVD. 2 hr glucose was found to be a better predictor of T2D and CVD in some studies; fasting glucose was implicated in some and still others found both FPG and PPG strong predictors.

In evidence of the fact that plasma glucose levels may be maintained within normal limits by hyperinsulinaemia early in the course of the disease, high risk subjects were identified by 1 hour glucose levels. Bardini et al demonstrated a fivefold risk of developing diabetes in normal glucose tolerance (NGT) subjects with 1 hour plasma glucose >155 mg/dL and considered this a new marker for CV risk.

The current ADA criteria for Prediabetes is, FPG ≥100 mg/dL but <126 mg/dL or 2 hr value ≥140 mg/dL but <200 mg/dL or both IFG and IGT.94

All forms of diabetes pass through a stage of IGT and/or IFG. These categories are a part of the natural history of diabetes. The prediabetic states are characterized by different degrees of insulin sensitivity, insulin secretion and hepatic glucose output as well as secretion of glucagon and incretin hormones. Many individuals with IGT are euglycemic in their daily lives and manifest with hyperglycemia only when challenged with the oral glucose load. The main features of IFG/IGT94 are:

i) A stage in the natural history of disordered glucose metabolism.
ii) Can lead to any type of diabetes.
iii) Increased risk of progression to diabetes.
iv) Increased risk of CVD.
v) Little or no risk of microvascular disease.
vi) Some patients may revert to normoglycemia.
In India, the prevalence of ‘prediabetes’ as estimated by epidemiological studies was much higher than incident diabetes, putting a large population at risk of progression to diabetes. The NUDS results indicate that the prevalence of IGT was higher than that of T2D in 4 out of 6 cities studied. The prevalence of IGT was 16.8% in Chennai, 14.9% in Bengaluru, 29.8% in Hyderabad, 10% in Kolkata, 10.8% in Mumbai and 8.6% in New Delhi. The ADEPS conducted in Kerala showed that 11.2% of the subjects had either IFG or IGT. The ‘Prevalence of Diabetes in India Study’ (PODIS) reported that the prevalence of IGT was significantly high in both rural and urban populations.

As in T2D, the pathogenesis of prediabetes is linked to relative insulin deficiency and tissue insulin resistance causing abnormal blood glucose levels despite secondary hyperinsulinaemia. It is suggested that IFG is related to hepatic insulin resistance resulting in fasting hyperglycemia and IGT is associated predominantly to skeletal muscle insulin resistance. Prediabetes is associated with obesity, higher waist circumference, physical inactivity, increasing age and the metabolic syndrome.

The clinical risk factors for prediabetes have been described as those for T2D. Screening specifically for prediabetes is not recommended, but occurs in association with screening for diabetes. Accordingly, if in screening for T2D, a formal laboratory FPG measurement is between 100 to 125 mg/dL then a formal 75 gm OGTT should be performed to exclude diabetes. To overcome the difficulty and the unpleasantness of OGTT, several studies have endorsed the use of HbA1c either alone or in conjunction with FPG to diagnose diabetes. As there are no standard measurement practices and reporting units, HbA1c was not recommended for use until recently. However, the latest guidelines from ADA include HbA1c as a screening tool for diabetes/prediabetes.

Life style interventions like weight loss, exercise, changes in dietary habits and certain pharmacological agents have been demonstrated to prevent or delay the development of diabetes in prediabetics, particularly IGT patients. Whether preventive interventions in IFG have effects is not known.
Clinical significance of prediabetes:

1. Diabetes risk:

Given the natural history of prediabetes, about 3-10% people per year develop diabetes. Data are particularly sustained for IGT. The combination of IFG and IGT confers a greater risk of diabetes than either category alone. Overall, prediabetes confers about a six fold increased risk of diabetes compared with NGT.

Most of the progression studies also reported a significant percentage (upto 38%) of reversion to normoglycemia. It can thus be safely assumed that a quarter of the subjects with prediabetes may revert long term to having normal glucose tolerance, a quarter of them may remain unchanged in the prediabetic stage and after a protracted follow up, about 50% of the people with IGT/IFG or both will develop diabetes.

2. Cardiovascular disease risk:

Prediabetics have an increased risk of developing cardiovascular disease and cardiovascular and all cause mortality than people with NGT. There is a 2-3 fold increased risk of prospective cardiovascular events which is most marked in younger adults with prediabetes. An atherogenic lipid profile and hypertension, the frequent accomplices conferred a higher CVD risk in prediabetes. These observations led to the ‘ticking clock hypothesis’ wherein the elevated CVD risk among diabetes patients may be due to long standing atherogenic risk profile than to hyperglycemia per se. Several studies have indicated that IGT rather than IFG patients have a greater risk of CVD. Faeh et al found graded relationships between impaired glucose regulation categories and both major CVD risk factors and carotid/femoral intima media thickness. A more recent report also demonstrates that both IFG and IGT are risk factors for subsequent development of CVD. When other known CVD risk factors like hypertension, smoking and lipid abnormalities were adjusted for statistically, IGT remained an independent CVD risk factor.
3. Other complications of diabetes:
While CV risk is well studied in prediabetes, reports on other micro and macro vascular implications are scarce. The Diabetes Prevention Programme (DPP) study group found retinopathy consistent with diabetic retinopathy in 7.9% of prediabetic patients (n=302). Microalbuminuria, an early indicator of diabetic nephropathy has also been noted at subdiabetic levels of hyperglycemia in the Framingham offspring study. Altered lipids and lipoproteins metabolism that are found in diabetes/prediabetes along with hypertension contribute to arteriosclerosis. Other than the major blood vessels, arteriosclerosis can also be expected in the peripheral vessels resulting in peripheral vascular diseases. Neuropathy was found in prediabetics by Papanas et al.182

4. Associations with the metabolic syndrome (MetS):
The metabolic syndrome refers to a clustering of CVD risk factors and diabetes susceptibility in an individual. People with MetS have about a two-fold increased risk of developing diabetes and CVD, compared with those without the syndrome. Increased waist circumference, increased Tg, decreased HDL, increased blood pressure and increased plasma glucose concentrations constitute the components of the MetS, all standalone CVD risk factors. Many adults who have prediabetes frequently also have MetS, increasing the possibility of subjects with MetS clusters developing overt diabetes and CVD. Three quarters of patients with dysglycemia had MetS in a report from Turkey. It was also suggested that MetS cases with normal FPG should undergo OGTT as 8.1% of the cases with normoglycemia had IGT in that study. In fact, opinions exist that there is an overlap between MetS and prediabetes/T2D and that MetS need not be considered a separate clinical entity. MetS has been described as a prediabetic state that embodies factors implicated in CV risk including dyslipidaemia, hypertension, IGT or T2D, insulin resistance and abdominal obesity.183
5. Insulin and prediabetes:

Resistance to insulin action was found to have developed at least five years before demonstrable IGT in the Inter99 study.\textsuperscript{61} That IGT is associated with insulin resistance has been shown by several studies. \(\beta\)-cell dysfunction and decreased insulin secretions are the hallmarks of IFG. Laakso \textit{et al}\textsuperscript{184} reported higher insulin resistance in IFG also. In the early stages, prediabetes is associated with hyperinsulinaemia which maintains normal plasma glucose levels. Progressive \(\beta\)-cell failure, relative or absolute deficiency in insulin secretion and increasing peripheral insulin resistance determine the conversion of prediabetes to frank diabetes.\textsuperscript{99,100,120,128-130}

As the dilemma for prediabetes threshold value continues, what is clear is the understanding that the disease process begins long before clinical hyperglycemia is demonstrated. It is estimated that the pathology begins 10-12 years before the onset of clinical diabetes. Focus on detecting people with prediabetes and delivering effective lifestyle interventions to them is an immediate and difficult challenge.
Metabolic syndrome

The metabolic syndrome (MetS) comprises a constellation of metabolic abnormalities that occur together more often than expected by chance. The triad of hyperglycemia, hypertension and hyperuricemia was described as early as 1929. A major milestone in the history of this syndrome occurred in 1988, when Gerard Reaven proposed the concept of syndrome X, which he described as the co-occurrence of resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased very low density lipoprotein (VLDL) triglyceride, decreased high density lipoprotein cholesterol (HDL-C) and hypertension. This paper stimulated renewed interest in the syndrome. In 1998, the WHO proposed a formal definition of the MetS. Three years later the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) proposed its definition of the metabolic syndrome.

Definition of the MetS:
Several efforts have been made to develop standard definitions to determine the prevalence of this syndrome. The three major definitions that have been proposed by WHO, ATP III and IDF are shown below. Apart from these major three, a few other groups also have given the criteria for the diagnosis of MetS. Over the years, all these definitions have been modified based on epidemiological and clinical study outcomes.
### Definitions of metabolic syndrome:

**WHO definition**

Diabetes or impaired fasting glycemia or impaired glucose tolerance or insulin resistance (under hyperinsulinemic euglycemic conditions, glucose uptake in lowest 25%) plus two or more of the following:

1. Obesity: BMI > 30 kg/m² or WHR > 0.9 (male) or > 0.85 (female)
2. Dyslipidemia: Tg ≥ 1.7 mmol/L or HDL < 0.9 (male) or < 1.0 (female) mmol/L
3. Hypertension: BP ≥ 140/90 mmHg
4. Microalbuminuria: Albumin excretion ≥ 20 µg/min

**ATP III Definition**

Three or more of the following:

1. Central obesity: WC > 102 cm (male) or > 88 cm (female)
2. Hypertriglyceridemia: Tg ≥ 1.7 mmol/L
3. Low HDL-C: < 1.0 mmol/L (male) or < 1.3 mmol/L (female)
4. Hypertension: BP ≥ 135/85 mmHg or medication
5. Fasting plasma glucose ≥ 6.1 mmol/L

**IDF definition**

Presence of central obesity (ethnic specific cut points) plus any two of the following:

1. Hypertriglyceridemia: Tg ≥ 1.7 mmol/L or specific treatment for lipid abnormality
2. Low HDL-C: < 1.0 mmol/L (male) or < 1.3 mmol/L (female) or specific treatment
3. Hypertension: BP ≥ 130/85 mmHg or medication or specific treatment
4. Fasting plasma glucose ≥ 5.6 mmol/L

Although all definitions include a measure of abdominal obesity, elevated blood pressure, dyslipidemia and hyperglycemia, the exact measures and cut points used to define the elevations differ among the definitions. The NCEP-ATP III definition is perhaps the most straightforward to implement because the 5 criteria are clearly defined and easy to measure. The WHO definition requires an oral glucose tolerance test (OGTT) to be performed so that hyperglycemia can be defined using WHO criteria; Microalbuminuria is not commonly measured in medical practice and there are no standard procedures for measuring IR. The IDF has one of the measurements i.e. abdominal obesity, a must plus 2 other factors. This definition also has separate cut points of WC for Asians.

The Asian Indians have a typical phenotype with higher measure of abdominal fat compared to BMI, greater degree of insulin resistance and
strong genetic susceptibility to T2DM and CVD. The modified ATP III criteria for Asian Indians South Asian Modified-NCEP\textsuperscript{190} (SAM-NCEP) is better suited for the Asian Indians in detecting MetS and is defined as any three or more of the following five parameters.

- WC ≥ 90 cm in men, ≥ 80 cm in women
- BP ≥ 130/85 mmHg
- Tg ≥ 150 mg/dL
- HDL ≤ 40 mg/dL in men, ≤ 50 mg/dL in women
- FPG ≥ 100 mg/dL.

Prevalence of MetS:

Several studies across the globe have estimated the prevalence of the MetS based on any of the above definitions often with slight modifications in one or more components. These estimates ranged from 4.6% among white women in the ‘Atherosclerosis Risk In Communities’ study to 29.4 % among men in the ‘Framingham Offspring Study’. While it is difficult to compare the prevalence rates when different definitions have been used, some studies have compared MetS using different definitions in the same study. These studies find that the results vary only slightly from each other.\textsuperscript{191}

A few studies have compared prevalence estimates of the MetS using the definitions of the NCEP-ATP III and WHO. Results from National Health and Nutrition Examination Survey III (NAHANES III) conducted from 1988-1994 showed that the prevalence of the MetS was similar using both definitions. The age adjusted prevalence of the syndrome was 23.9% by the ATP III definition and 25.1% by WHO definition.\textsuperscript{192} Among Pima Indians who did not have diabetes, the prevalence of the MetS was 31% under both definitions,\textsuperscript{193} while in the Framingham offspring study, it was, among men 26.9% and 30.3% by ATP III and WHO definitions respectively, and among women 21.4% and 18.1% by ATP III and WHO definitions respectively. In India, a study on south Indians put the estimate of MetS (%) at 23.2, 18.3 and 25.8 according to WHO, ATP III and IDF definitions respectively.\textsuperscript{185} Major differences have also been noted in the two Indian studies, which differed in the definition of obesity. One study by Ramachandran \textit{et al}\textsuperscript{194} used obesity
criteria that were suitable for Indians, while the other by Gupta et al[^1] used the standard ATP III definition of obesity. Both studies used population based samples within the same age range but reported prevalence of 13% in Jaipur[^1] and 41% in Chennai. Although it appears that this difference could be because of the differing obesity criteria, in reality both study groups had almost similar obesity prevalence (31% Vs 33%) despite the different definitions. Large difference was also observed between the two studies for the prevalence of elevated triglycerides (46% Vs 30%), hypertension (55% Vs 39%) and elevated fasting plasma glucose (27% Vs 5%), each of which was reported as having used the same cut points specified in ATP III criteria. Therefore, ethnic differences prevail in the prevalence of both individual factors that constitute the MetS and the MetS itself.^[22,195^]

A consistent finding is the observation that the prevalence of the MetS across the globe is highly age dependent. Other factors that contribute would be lifestyle influences, genetic factors and sex. Prospective studies evaluating the ability of the various definitions of the MetS to predict future cardiovascular disease and other disease end points will be valuable in determining which definition of the MetS is most useful.^[185^]

The aetiopathogenesis of MetS:

The deadly quartet of central obesity, HTN, dyslipidemia (high Tg and decreased HDL) and glucose intolerance called the MetS is now a well recognized entity where each component of the syndrome conveys increased CVD risk and is more powerful in combination. The features of MetS may be present for upto 10 years before detection of glycemic disorders.^[134^]

There is increasing evidence that IR is the fundamental defect linking individual components of the metabolic syndrome, although the strength of association of insulin resistance to these components is variable in different populations and even within populations. IR is not synonymous with MetS although ‘IR syndrome’ was also a name proposed for MetS. IR is a physiological change in insulin action manifesting as resistance to insulin-mediated glucose disposal. It leads to compensatory hyperinsulinemia and
puts the individual at risk of developing one or more of the abnormalities listed below.¹⁹⁵

**Abnormalities associated with insulin resistance/hyperinsulinemia:**

- Some degree of glucose intolerance
  - Impaired fasting glucose
  - Impaired glucose tolerance
- Abnormal uric acid metabolism
  - Plasma uric acid concentration
  - Renal uric acid clearance
- Dyslipidaemia
  - Triglycerides
  - HDL-C
  - LDL-particle diameter
  - Post-prandial lipaemia
- Haemodynamic
  - Sympathetic nervous system activity
  - Renal sodium retention
  - Blood pressure (= 50% of patients with hypertension are insulin resistant)
- Haemostatic
  - Plasma activator inhibitor-1
  - Fibrinogen
- Endothelial dysfunction
  - Mononuclear cell adhesion
  - Plasma concentration of cellular adhesion molecules
  - Plasma concentration of asymmetric dimethyl arginine
  - Endothelial-dependent vasodilatation
- Reproductive
  - Polycystic ovary syndrome

**Obesity and MetS:**

Obesity has become a major problem of the world and the increase in obesity precisely parallels the increase in the prevalence of diabetes. Obesity increases the risk of CVD and has been strongly associated with insulin resistance in normoglycemic persons also.¹³⁹

Along with more and more adults becoming obese, obesity is also striking at a much younger age leading to a high number of obese children and adolescents.¹⁹⁶ Although adipocytes are specifically designed to store energy in the form of Tgs, evidence has accumulated that in response to adipocyte hypertrophy during development of obesity, adipose tissue function is compromised. Obesity also provokes structural and metabolic alterations in other organs including skeletal muscle and liver and is a major risk factor for the development of fatty liver.²² Metabolic abnormalities that often accompany obesity include hypertension, impaired glucose tolerance, IR leading to hyperinsulinemia and dyslipidemia. Collectively these abnormalities have
been clustered into the MetS.\textsuperscript{7,134,197} It is also shown by several studies that not all obese individuals have insulin resistance and MetS and conversely not all MetS patients have general obesity. This has given birth to terminologies like metabolically obese normal-weight individuals (to mean subjects with normal weight and with features of the MetS) and metabolically healthy but obese to mean obese individuals without MetS.\textsuperscript{7,58,198}

The association of obesity with the IR syndrome and CVD risk is not only related to the degree of obesity but seems to be critically dependent on body fat distribution. Individuals with greater degree of central adiposity develop this syndrome more frequently than do those with a peripheral body fat distribution.\textsuperscript{97,199}

Thus, it is the visceral or the abdominal fat which is the metabolically adversely active organ. Various measures of obesity namely BMI, WC, WHR and more recently magnetic resonance imaging of the intra-abdominal fat area have been used to define abdominal and whole body fat in the clinical and research settings. All these indices have been shown to correlate well with each other also. Still, WC, a measure of abdominal/visceral obesity is known to detect persons who may be having IR as in the case of the Asian Indians.\textsuperscript{92} The body composition of South Asians in general and Indians specifically is conducive to development of the MetS. They have high percentage of body fat, abdominal obesity at normal or below normal BMI, insulin resistance, hyperinsulinemia and low muscle mass.\textsuperscript{93,200}

Genetic propensity to develop dyslipidemia, obesity and diabetes has been shown in South Asians. Polymorphism of ‘hepatic glucokinase promoter’ gene is correlated to hepatic IR in Asian Indians. The Ala54thr polymorphism in the ‘fatty acid binding protein 2 (FABP2)’ gene as well as T-455C and C-482T polymorphisms in ‘apolipoprotein C-III (APOC3)’ gene promoter were associated with the MetS in South Indians.\textsuperscript{201}

Investigations in white Caucasians have shown that lipid accumulation in skeletal muscle i.e. intramyocellular lipids (IMCL) could be a marker of IR, A few investigations have been done on IMCL in South Asians. No relation was
found between IMCL and IR in South Asians in UK and Asian Indians in India, but IMCL related to excess body fat and abdominal obesity. Further studies are needed on this for conclusive proofs.22

The Non-alcoholic Fatty Liver disease (NAFLD) is a consequence of increased abdominal adiposity. It is also associated with diabetes, hypertriglyceridemia and IR. All these frequently coexist and all these are highly prevalent in urban Asian Indians. Enzymes of the gluconeogenic pathway are activated in both obese and nonobese NAFLD patients indicating increased future risk of developing T2DM.22

Excess fat, as long as it is inside adipocytes, does not cause deleterious effect on health. Leptin is known to cause fat to deposit primarily in adipocytes. Leptin deficiency or resistance leads to tissue deposition of fat. This ectopic deposition of fat (triacylglycerols) particularly in viscera causes IR.134

Genetic factors and racial susceptibility play an important role in human fat distribution and are responsible for 70% of the variation in intra-abdominal fat mass.132 Other important determinant is sex, with males being typified by central and females by peripheral fat distribution.6

Central obesity and IR is being increasingly noted in children and adolescents also. Similar to development of diabetes, MetS also has been observed to be present in subclinical form for at least 5-8 years before manifesting. Losing weight of 10% or more has a favorable outcome in terms of risks associated with MetS. The epidemiological evidence linking central obesity with diabetes and MetS is unequivocal. Based on the present evidence, the following explanations are possible for the role of adiposity in diabetes and MetS.123

(i) Excess adipose tissue lysis leads to increased plasma concentrations of free fatty acids (FFAs). There is strong evidence to say that FFAs in excess can cause IR. There is intracellular accumulation of Tgs and fatty acid metabolites (viz. fatty acid CoAs, diacylglycerol and ceramides) in the insulin responsive tissues which interfere with insulin signaling resulting in signaling defects.124
(ii) In recent years, it has become clear that obesity is also a state of heightened inflammation. Adipose tissue mass and expression of the pro-inflammation gene, tumor necrosis factor α (TNFα) are strongly related. Several inflammation markers found in obese individuals appear to originate from adipose tissue.\textsuperscript{139,140,169,202-204}

Despite the pathogenic mechanisms discussed, several ethnic populations do not display obesity levels as described for Caucasians but are still at high risk of developing metabolic syndrome. Conversely, even morbidly obese individuals may not exhibit other constituents of the MetS. Hence, MetS needs to be defined, diagnosed and managed with respect to susceptible populations.

**Lipid abnormalities and MetS:**

Increased plasma Tgs and reduced HDL-C are key features of the MetS. Although elevated LDL-C is not an integral characteristic, very often, an increase in the proportion of small dense LDL particles is noted.\textsuperscript{205}

**HDL-C**

HDLs are the smallest and densest of the plasma lipoproteins. An inverse relation between the level of HDL-C and the risk of developing premature CHD has been a consistent finding in many prospective population studies. It has been concluded from these studies that for every 1 mg/dL increase in HDL-C, the CHD risk is reduced by 2-5%. The ability of HDL to protect against atherosclerosis lies in its function of promoting the efflux of cholesterol from the cells of the peripheral tissues. This minimizes the accumulation of cholesterol in the periphery and of particularly the formation of foam cells in the artery walls. HDLs also function as antioxidants. The major proteins of HDLs, apoA-I and apoA-II and other proteins such as paraoxonase that co-transport with HDL in plasma are well known to have antioxidant properties. HDL also can prevent oxidative modification of small dense LDL and can inhibit the expression of adhesion molecules in endothelial cells reducing the recruitment of monocytes into the artery wall and subsequent development of atheromatous plaque.\textsuperscript{206}
An HDL-C concentration of $\leq 40$ mg/dL in males and $\leq 50$ mg/dL in females is one of the features of MetS. Each of the other features of the MetS viz. increasing Tg, hypertension and IR are associated with decreasing HDL. HDL in subjects with the MetS tend to be smaller and denser than normal, predominantly in people with Tg levels more than 150 mg/dL.\textsuperscript{206}

Low HDL concentration in MetS is a consequence of an increased rate of HDL catabolism, possibly secondary to triglyceride enrichment of particles and this in turn reflects the enhanced activity of the enzyme, Cholesterol Ester Transport Protein (CETP). CETP promotes the transfer of cholesterol esters from HDL to Tg rich lipoproteins in exchange for Tg, to generate HDLs that are depleted of cholesteryl esters and enriched in Tg. Hydrolysis of the Tg-rich HDL by hepatic lipase leads to a reduction in volume of the particle core, a consequent decrease in the particle size and a dissociation of the lipid free/lipid-poor apoA-I from the HDL surface.\textsuperscript{207,208}

This explains why HDL concentration is often low in hypertriglyceridemia. IR proportionately increases with increasing ratio of Tg/HDL.\textsuperscript{22,169} In the MetS, the low HDL concentrations cannot oppose the prevailing proatherogenic forces viz. deposition of Tg-rich lipoproteins to form foam cells, increase in small dense LDL and increased susceptibility of small dense LDL to oxidation and decreased endothelial nitric oxide production and increased expression of endothelial adhesion of cell proteins and chemokines. HDL has the capacity to negate all the above proatherogenic changes seen in MetS. Thus, subjects with low HDL in MetS are at high risk of CHD.\textsuperscript{134}

Low HDL-C is an inherent part of the clinical presentation of MetS and T2D and the prevalence of diabetes or MetS is particularly high among subjects with low HDL-C.\textsuperscript{209} Plasma HDL-C concentration was found to decrease progressively with increasing BMI in the PROCAM study and with increasing Tg in the Framingham Heart Study. HDL-C was a strong predictor of CHD in patients with the metabolic syndrome and T2D in the PROCAM study.\textsuperscript{210}
Triglycerides (Tg)

The other dyslipidemic feature forming a constituent of the MetS is increased concentrations of Tg. Excess adiposity and IR foster increased hepatic production of very low density lipoprotein (VLDL) particles and reduced intravascular catabolism and plasma clearance of VLDL and intestinally derived chylomicron particles. A major determinant of increased VLDL secretion in the MetS is higher hepatic Tg content, derived in part from increasing Free fatty acid (FFA) acid delivery from adipose tissue and return of Tg-rich lipoproteins remnants to the liver. Increased flux of FFA from the periphery stimulates hepatic Tg synthesis which in turn promotes the assembly and secretion of Tg containing VLDL as well as the apoB production in the liver. IR and compensatory hyperinsulinemia directly promote the increased hepatic secretion of VLDL particles.\(^{211}\)

In the presence of IR, the visceral adipocyte is more sensitive to the metabolic effects of lipolytic hormones, glucocorticoids and catecholamines. This hormonal lipolytic activity produces an increased release of FFAs into the portal system, which serves as hepatic substrate to assemble Tg and Tg–rich VLDLs. IR also leads to increased production of apoB, the major protein of VLDL.\(^{210}\)

Insulin resistant state results in increased levels of apoCIII, an inhibitor of endothelial bound lipoprotein lipase (LPL).\(^{205,211}\) The delayed postprandial clearance of diet derived lipids in individuals with the MetS can result from reduced intravascular catabolism of chylomicrons and competition with VLDL for LPL activity.\(^{205}\)

The net-effect change in Tg-rich lipoprotein metabolism is an increase in plasma transport and prolonged plasma residence of these lipoproteins and their potentially atherogenic catabolic products. This in turn leads to increased levels of intermediate density lipoproteins (IDL), the immediate metabolic precursors of LDL.\(^{205}\)

Prospective population studies and meta-analysis of such studies found that an increase in plasma Tg was associated independently with CAD. An
increase of 89 mg/dL was associated with an increased CAD risk of 14% in men and 37% in women after adjustment for LDL-C, HDL-C and other confounding variables. Recent studies have emphasized the role of postprandial Tg concentrations in CAD and myocardial infarction. Combined measurement of plasma Tg and HDL-C expressed as Tg/HDL-C is found to be a strong index of risk of CAD. In the PROCAM study, a strong inverse correlation was demonstrated between fasting insulin levels and HDL-C and this was invariably associated with increased Tg levels. Hence, it was proposed that the Tg/HDL-C ratio most likely reflects the state of IR and is strongly associated with CAD.

Although studies like UKPDS have demonstrated significant association of low HDL-C with CVD risk, A study from Japan did not find HDL-C levels significant risk factor for CVD.

Low density lipoprotein (LDL) is not a constituent of the MetS features. But increased Tg and decreased HDL concentrations are closely related to LDL concentrations. In MetS cases, the LDL-C levels are usually within normal limits or mildly elevated. However, the LDL particle is often of abnormal composition. A predominance of small dense LDL is the key element contributing to atherogenic dyslipidemia of MetS. It has been found that small dense LDL is not seen until plasma Tg levels exceed 1.5 mmol/L (133 mg/dL).

The Tg-rich LDL which is formed during the lipoprotein cascade VLDL → IDL → LDL) is acted upon by LPL. A large proportion of LDL particles with changed apoB conformation is produced. These particles fail to bind efficiently to LDL receptors and so have a prolonged residence time in circulation. Increased action of CETP further replaces cholesteryl esters by Tg in HDL and LDL particles. Tg-rich LDL is a good substrate for hepatic lipase that finally generates small dense LDL which is shown to be associated with cardiovascular risk as small dense LDL have proatherogenic properties such as, (a) reduced LDL receptor-mediated clearance, (b) increased arterial wall retention, (c) increased susceptibility to oxidation.
Prospective studies have shown that the incidence of and mortality from CAD among Asian Indians are at least two-fold higher than among whites, even when fully adjusted for the high rates of IR, MetS, DM and socio-economic status. This is attributed to the Asian dyslipidemia characterized by high serum levels of apolipoprotein B, Tg, lipoprotein(a), high LDL, low levels of apolipoprotein A1 and HDL-C. They also have high ratios of total cholesterol (TC)/HDL, Tg/HDL and apo B/apo A1. Among the sub-fractions of HDL, Asian Indians have a preponderance of small, dense, dysfunctional HDL particles that are associated with less efficient reverse cholesterol transport and less protection against CAD. The level of HDL 2b, the most protective component of HDL is low in >90% of Asian Indians.\textsuperscript{190}

Obesity/visceral adiposity is closely related to dyslipidemia. BMI associated with diabetes, hyperytension and dyslipidemia among participants of the Tehran lipid and glucose study.\textsuperscript{198} Tg and HDL correlated significantly with visceral fat in healthy Asian Indians\textsuperscript{7,213} and others.\textsuperscript{84} In a study from south India, abnormality in the lipid levels were observed in the absence of gross central obesity.\textsuperscript{214} Visceral fat was associated with insulin resistance, cardiovascular risk factors and MetS in nondiabetic south Indians.\textsuperscript{92}

**Blood pressure (BP):**

A blood pressure of $\geq 130/85$ mmHg is one of the components of MetS. Essential hypertension is a condition characterized by multiple metabolic disturbances. Obesity, IGT, hyperinsulinemia, low HDL-C and high Tgs are more frequently found in patients with hypertension than in normotensive subjects. Natali and Ferrannini\textsuperscript{24} in their discussion on HTN, insulin resistance and the MetS suggest that HTN is probably the component of the MetS that is most loosely connected with the others because it is found in isolation only within ethnic groups in which its prevalence is high. Their speculation is that two types of HTN exist, one with a strong genetic background (largely independent of the MetS) and the other whose clinical manifestation needs the milieu of the MetS.
There is evidence that patients with essential HTN are insulin resistant and hyperinsulinemic. Normotensive first degree relatives of patients with essential HTN are relatively insulin resistant and hyperinsulinemic as compared to age match control group without a family history of HTN. Hyperinsulinemia as a surrogate estimate of insulin resistance has been shown in population based studies to predict the eventual development of essential HTN in children, adolescents and adults. These findings provide substantial support that insulin resistance/hyperinsulinemia plays a role in the pathogenesis of essential HTN. Jeppesen et al showed from the Copehagen male study that CVD risk was not increased in patients with HTN in the absence of high Tg and low HDL-C. High Tg, low HDL-C and Tg/HDL-C ratio are markers of IR.

The relation between IR and essential HTN is complex and much remains to be clarified. Insulin increases renal sodium retention while increasing free water clearance. IR is also associated with increased sympathetic nervous system activity and stimulation of vascular smooth muscle growth. The other compounding factor in the insulin-hypertension link is obesity. Blood pressure is directly correlated with body weight. But results of the Coronary Artery Risk Development In young Adults (CARDIA) study show a weight independent association between fasting insulin concentration and HTN.

Despite the IR/hyperinsulinemic basis, not more than 50% of patients with essential HTN are insulin resistant but this subset is at greatest risk of CVD. Previous studies have shown that even within the normal range of BP, prediabetic individuals have higher BP 3-16 years before diagnosis compared with individuals who remain non-diabetic. Thus, HTN may also be an indicator of the pathogenesis of type 2 diabetes. Golden et al conducted a prospective cohort study of white male medical students to longitudinally assess BP from young adulthood through middle age who went on to develop diabetes and found that BP elevations precede the development of T2D by 20-25 years and concluded that higher BP in the prediabetic state may contribute to the presence of vascular disease at the time of diagnosis of T2D. MetS has also been associated with early vascular
alterations such as increased arterial stiffness and endothelial dysfunction. These independent predictors of CVD morbidity and mortality can be attributed at least in part to the blood pressure in the MetS cases. Ghiadoni et al. studied the vascular alterations in normotensive MetS subjects at risk of diabetes mellitus and found that among the components of the MetS, only blood pressure significantly affected carotid-femoral pulse wave velocity, whereas blood pressure and fasting glucose influenced flow mediated dilation. Since dysglycemia is an important component of MetS comprising of mild hyperglycemia of the prediabetes state or severe of overt diabetes, raised BP can be expected to be a companion of dysglycemia especially in the insulin resistant and dyslipidemic MetS subjects.\(^{23}\)

**Fasting plasma glucose (FPG):**

In 1999, when WHO proposed the working definition for MetS, impaired glucose regulation or diabetes and insulin resistance were included as mandatory and any two of the other components. In 2001, when NCEP ATP III guidelines were formulated, fasting plasma glucose was one of the five components of MetS. IR results in interference of upstream insulin signaling events resulting in acquired insulin signaling defects. In skeletal muscle, it leads to impaired GLUT4 translocation to the cell membrane and results in resistance to insulin stimulated glucose uptake. In liver, there is resistance to insulin mediated suppression of hepatic glucose production. Concommittant increased plasma free fatty acid level leads to impaired insulin secretion by β-cells probably via increased intra cellular expression of uncoupling protein 2, all contributing to hyperglycemia.\(^{134}\)

Most patients with diabetes have MetS with estimated prevalence of 69.9% for Whites, 64.4% for Blacks and 62.4% for Mexican Americans. The values for Asian Indians range from 31.6% to 49.2% in various studies using different MetS criteria.\(^{219}\) In the Framingham Offspring Study of 2,848 adult men and women who did not have diabetes or CVD at their baseline examination it was found that 12.5% of women and 21.4% of men had MetS according to the modified NCEP ATP III criteria. When these patients were re-examined 8 years later the percentages had increased to 23.6% and 33.9% respectively.
After 11 years it was found that MetS criteria increased the risk for developing diabetes 6-fold, regardless of the degree of IR. MetS is also known to have excellent predictive accuracy for future development of T2D. In the Diabetes Prevention Programme Study, 53% of subjects met the ATP III criteria for MetS at base line. Approximately 60% of those who initially did not meet the criteria did meet them after four years. On the basis of these data it is suggested that people with IFG or IGT be screened for other metabolic risk factors and treated appropriately. The CVD risk in MetS cases can be largely due to the inclusion of T2DM patients in the defining criteria. Several studies have impressed that T2D in itself should be treated as equivalent to an episode of myocardial infarction. Thus, some authors feel that inclusion of diabetes patients in MetS to assess CVD risk is questionable. However, most recent criteria of MetS considers FPG over 100 mg/dL as one of the components, encompassing the prediabetes group.

Many studies have reported that as the fasting glucose levels increase beyond 90 mg/dL, the coronary risk escalates. On the other hand, there is accumulating evidence to say that impaired glucose tolerance carries greater coronary risk as compared to IFG. Gupta et al studied fasting glucose and cardiovascular risk factor in an urban Indian population and found a significant positive correlation of fasting glucose with BMI, SBP, Total and LDL Cholesterol and triglycerides. They concluded that FPG <75 mg/dL carries the least risk and incremental increases over this value proportionately increases coronary risk. Uzunulu and Oguz from Turkey report that development of MetS is independent of the level of glycemia. Their study compared normoglycemic and dysglycemic individuals with MetS.

Whether the use of impaired fasting glucose provides an effective way to identify the presence of insulin resistance or predict risk of CVD is not clear. The results of the DECODE study showed that post glucose challenge plasma glucose concentration were superior to fasting values in predicting risk of CVD. Also, IR is very commonly associated with IGT and proposed to be the ‘common soil’ that connects all components of the MetS. The use of
IFG alone for the diagnosis of MetS may thus leave out a large proportion of insulin resistant IGT subjects who are at risk of CVD.

FH of diabetes and the MetS:

The first degree relatives of T2D patients have a strong genetic susceptibility to the development of T2D often earlier than their relatives. CVD risk is also high in such individuals. Studies conducted in FDRs for the prevalence of MetS have shown that these individuals develop MetS in larger numbers and to a more severe degree than those without a FH of diabetes. Among nondiabetic individuals MetS predicts the development of T2D. The MetS is a constellation of metabolic risk factors contributing to CVD risk. The nondiabetic FDRs have been shown to have dysmetabolism and altered homeostasis resulting in raised WC, BP, atherogenic lipids and plasma glucose levels before pathological hyperglycemia sets in, thus rendering the FDRs highly prone to the risk of T2D, MetS and CVD. Currently, hyperglycemia of the diabetes range is not excluded from the MetS definitions. Hence, it can be expected that practically every diabetic individual is also a candidate for MetS. In fact it has been suggested that the diagnosis of T2D should be considered equivalent to an episode of CV event.

The hyperinsulinaemia/IR pathology which has been strongly implicated as the basis of all the components of MetS also does not completely explain the etiopathogenesis. While it is found that BP has the weakest association with IR and increasing plasma glucose the strongest, the rest of the components have been associated to varying degrees depending on the population in whom the MetS is studied. However, in a recent study by Sung et al elevated fasting insulin predicted the incidence of MetS in a 5-year follow up study. Racial/ethnic and gender differences are also observed in the prevalence and predictability of the MetS components. Although the understanding of epidemiology, etiology and pathogenesis of MetS has greatly advanced by almost a decades’ research, several questions still remain unanswered. Conversely, with the advancement in understanding, there are researchers questioning the very existence of the ‘MetS’ entity. This is in view of the risk involved with
each individual component. While clustering of three or more components as in the MetS, definitely synergises the risk, waiting for the diagnosis of MetS may adversely affect the asymptomatic individuals with one or two components and deprive them of clinical attention.

Over the past decade several propositions of the MetS criteria have come up. The existing ones (like the NCEP ATP III) have undergone modifications depending on the findings of population based and case control studies on MetS. Also, based on their predictive value for detecting MetS, several other indices are proposed to be included as constituents of the MetS syndrome in ethnic groups.\textsuperscript{22,212} Recently, there is a drive to include pro-inflammatory and pro-coagulation markers in the definitions which may see the light in the near future.\textsuperscript{187} The figure below explains the various interactions leading to diabetes, CVD and MetS.

![Diagram explaining the various interactions leading to diabetes, CVD and MetS.](image)

Complex interactions of genetic, perinatal, nutritional and other acquired factors in development of insulin resistance, type 2 diabetes and coronary heart disease in South Asians. T2DM: type 2 diabetes mellitus; CRP, C-reactive protein; CHD: coronary heart disease; dotted lines represent weak relationships.
Proinflammatory markers

a) Interleukin-6 (IL-6):

Low molecular weight protein mediators involved in cell growth, inflammation, immunity, differentiation, and repair are generically termed ‘cytokines’. These include interleukins, interferons, growth factors and colony stimulation factors and are collectively called cytokines.\(^{228}\)

Most cytokines are not constitutively produced, but are produced after activation of the cells. Cytokines are chiefly involved in local effects. Some do have systemic effects, IL-6 being the most notable. The major physiological role of cytokines however is short-range, within a few cell diameters. Production after activation is short-lived, usually for only a few days. Typically, cytokines are effective in the picogram (pg) to nanogram (ng) ml\(^{-1}\) range.\(^{228}\)

Cytokines have very potent effects and it is important that their action be limited to avoid the pathogenic effects of cytokine overdose.\(^{229}\)

IL-6 is a multifunctional cytokine produced by various cells. IL-6 has a molecular weight of about 21kDa with 212 amino acids. Although many cells (e.g., fibroblasts, stromal cells, keratinocytes, endothelial cells etc.) outside the immune system make IL-6, within the immune system the major source is macrophages. IL-6 production \textit{in vitro} can be triggered by a variety of stimuli including antigens, viruses, bacteria, TNF, IL-1, IL-2, IL-3, colony stimulating factors (CSFs), platelet derived growth factor and lipopolysaccharides (LPS). \textit{In vivo} administration of TNF, IL-1, IL-2 or LPS have been shown to elevate circulating levels of IL-6.\(^{230,231}\)

IL-6 is a major inducer of terminal differentiation of B-cell growth. IL-6 causes the B-cells transformed by Epstein-Barr virus (EBV) to produce Immunoglobulin (Ig), induces IgA secretion by Peyer’s patch, enhances the secretion of IgM, IgG and IgA and stimulates the specific antibody response to antigen \textit{in vitro} and \textit{in vivo}. In relation to T cells, IL-6 has
been shown to play an important role in the T cell response to alloantigen and the growth and differentiation of haemopoietic stem cells.\textsuperscript{230,231}

IL-6 plays a major role in the acute phase response elicited by tissue trauma. IL-6 elicits the synthesis of acute phase proteins by hepatocytes both \textit{in vivo} and \textit{in vitro}. IL-1 and TNF induce IL-6 production and act synergistically with it in eliciting the response. Both IL-1 and IL-6 indirectly affect the acute phase response by triggering the production of Adrenocortico trophic hormone (ACTH), which stimulates the production of glucocorticoids. In turn glucocorticoids will negatively control the levels of IL-1 and IL-6, such that a feedback loop is completed and the acute phase response subsides.\textsuperscript{230}

b) Cortisol:

Cortisol is the major glucocorticoid synthesized from cholesterol in the zona fasciculata and reticularis of the adrenal cortex. The influence of glucocorticoids on carbohydrate metabolism includes promotion of gluconeogenesis, deposition of liver glycogen, and a reduction in glucose utilization. Increased gluconeogenesis is primarily due to the stimulation of protein catabolism. In addition, glucocorticoids inhibit amino acid uptake and protein synthesis in peripheral tissues (muscle, skin, bone). These steroids also affect fat metabolism. When present in excess, glucocorticoids cause a central distribution of fat in the face, neck, and trunk. Glucocorticoids are used therapeutically to treat inflammatory conditions such as rheumatoid arthritis. Closely related to the anti-inflammatory actions of glucocorticoids are their immunosuppressive actions.\textsuperscript{232}

Cytokines such as IL-1, IL-2 and IL-6, TNF, interferon-γ, and granulocyte-macrophage colony stimulating factor that mediate both acute and chronic phases of inflammation are inhibited by glucocorticoids as part of the anti-inflammatory and immunosuppressive effects of these steroids. The cytokines IL-1, IL-6 and TNF also stimulate the hypothalamic-pituitary-adrenal axis as part of the link that exists between the neuroendocrine-
immune axis. Activated immunocompetent cells produce cytokines that suppress the immune response partly through glucocorticoids and the hypothalamo-pituitary-adrenal axis (HPA axis).

IL-6 acts on the pituitary to induce ACTH release and directly on the adrenals to produce glucocorticoids. It is known that different cytokines that share gp 130 as a receptor sub-unit, induce serum amyloid A, and potentiate the induction of IL-6 and the activation of the HPA axis by IL-1. Potentiation of acute-phase protein synthesis may represent an important feedback regulatory mechanism of inflammation.

The key peripheral mediators of the stress system are corticotrophin, cortisol, arginine, vasopressin, norepinephrine, epinephrine and IL-6. These are activated in a co-ordinated fashion during acute stress. Chronic activation of the stress system however, is associated with many negative manifestations and sequelae including obesity, MetS, atherosclerosis, loss of bone mineral density and behavioral disorders. Chronic or intermittent stress also causes unfavorable genetic variations that increase both the activity of the HPA axis and the sensitivity of the tissues to glucocorticoids.

Results of the Hoorn study testing whether chronic psychological stress is associated with prevalence of T2D, and visceral obesity by analyzing major stressful life events in 2262 Caucasian population aged 50-74 years show a clear association of chronic psychological stress and development of T2D and visceral obesity. Several other studies also are in favour of these findings.

The cortisol awakening response (CAR) represents a rise in cortisol of 50-75% with peak levels about 30 minutes post wake up. Age, gender, oral contraceptive use, menstrual cycle phase, wake up time and smoking are known to affect CAR. Recently, a link between hippocampal integrity and CAR has been postulated. Literature is available to say that there is a loss of hippocampal volume in T2DM and given the role of hippocampus in HPA axis feedback regulation, HPA axis disturbances in T2DM is
expected. Both unstimulated and dexamethasone suppressed cortisol levels have been shown to be elevated in T2DM. With this background Hannah et al.\textsuperscript{39} studied CAR, hippocampal volume and diurnal salivary cortisol profile in T2DM. They report a smaller MRI based hippocampal volume and a blunting of the CAR relative to controls.

Association of high morning cortisol levels with estimated age related cognitive change in elderly T2DM patients was reported by Rebecca et al.\textsuperscript{240}

Subclinical Cushing’s syndrome has been reported from Japan.\textsuperscript{241} Microadenoma of the pituitary was found and the glucose tolerance and hypertension improved in patients operated for the same. T2DM patients may in fact be suffering from subclinical Cushing’s syndrome and need to be screened for the same.\textsuperscript{242}

Morphological and metabolic similarities exist between Cushing’s syndrome and MetS. Excessive cortisol secretion in Cushing’s syndrome is a classic cause of secondary obesity. Effects of excess cortisol on adipose tissue are complex, with an increase in central (visceral, abdominal, facial and nape of the neck) fat deposition, while peripheral fat is reduced. This may result from opposing effects of glucocorticoids that, on the one hand, increase lipolysis and down-regulate lipoprotein lipase thereby liberating free fatty acids from peripheral fat, but on the other hand, stimulate preadipocyte differentiation and enhance substrate flux in favour of gluconeogenesis and Tg synthesis in central fat. Glucocorticoids act centrally to stimulate appetite and can also cause depression. These also are linked to visceral obesity.\textsuperscript{232}

Higher morning serum cortisol levels independent of body fat and insulin sensitivity were found in 205 overweight Latino MetS subjects aged 8-13 years with a family history of T2DM as reported by Weigensberg et al.\textsuperscript{27} Since IR was not the cause, individual features of MetS were related to cortisol levels. The relationship between cortisol and BP was the
strongest among all the features of MetS followed by cortisol and fasting blood glucose.

Altered endogenous glucocorticoid metabolism, including 11β-hydroxy steroid dehydrogenase type1 (11β-HSD1), which generates active cortisol from cortisone and 5α-reductase which inactivates cortisol has been implicated in the development of IR, IGT and T2DM. The 11β-HSD1 mRNA activity was found to be increased in the adipose tissue of obese patients. Increased adipose tissue 11β-HSD1 expression was associated with glucose intolerance in women. Inhibition of this enzyme may be a promising therapeutic approach in T2DM.

Among prediabetic and diabetic subjects in vivo modification of circulating LDL was found to attenuate its stimulatory effect on adrenal aldosterone and cortisol secretion by Kopprasch et al. They opined that LDL modifications in IGT and T2DM subjects may have significant clinical benefits by counter acting prediabetic and diabetic over activity of the rennin-angiotensin-aldosterone system and enhanced cortisol generation. The involvement of cortisol in the pathogenesis of T2D thus appears complex and much needs to be understood before any further deductions are drawn.

c) Erythrocyte sedimentation rate:

The erythrocyte sedimentation rate (ESR), also called the Biernacki reaction, is the rate at which red blood cells precipitate in a period of 1 hour. It is a common hematology test that is a non-specific measure of inflammation.

The ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen, and those factors resisting sedimentation, namely the negative charge of the erythrocytes (zeta potential). When an inflammatory process is present, the high proportion of fibrinogen in the blood causes red blood cells to stick to each other. The red cells form stacks called ‘rouleaux’, which settle faster. The ESR is increased by any
cause or focus of inflammation. The basal ESR is slightly higher in females.\textsuperscript{245}

Although it is frequently ordered, ESR is of limited use as a screening test in asymptomatic patients. It is useful for diagnosing diseases, such as multiple myeloma, temporal arteritis, polymyalgia, various auto-immune diseases like, systemic lupus erythematosus, rheumatoid arthritis, and chronic kidney diseases. In many of these cases, the ESR may exceed 100 mm 1\textsuperscript{st} hour. The clinical usefulness of ESR is limited to monitoring the response to therapy. The use of the ESR as a screening test in asymptomatic persons is limited by its low sensitivity and specificity.\textsuperscript{245}

The major components of blood viscosity are the blood cell mass (haematocrit) the intrinsic resistance of the plasma to flow and red blood cell aggregability estimated as ESR. ESR measurement by the Westergren method is standardized, accurate, universally available and cheap. Furthermore, when red blood cell physical characteristics are taken into account by adjusting for haematocrit, ESR largely reflects the plasma concentration of acute phase response proteins resulting in a compound index of both viscosity and inflammation. The major determinants of ESR are the concentration of positively charged inflammatory proteins (fibrinogen, immunoglobulin M, \(\alpha_2\)-macroglobins) and the size of the red blood cells (RBCs).\textsuperscript{246}

ESR was found to be an independent correlate of coronary atherosclerosis and the predictor of cardiac death in patients with probable IHD.\textsuperscript{246} Gustavsson and Agardh\textsuperscript{247} found an association of HbA\textsubscript{1c} with CRP, fibrinogen, ESR and white blood cell counts (WBCs) among documented coronary artery disease patients undergoing coronary angioplasty. The relationship was found even in the nondiabetic subjects.

In a study of US adults\textsuperscript{248} a base line determination of leucocyte count and ESR were correlated with diabetes incidence over a period of approximately 20 years. While leucocyte count was significantly
associated with risk of diabetes, CHD, stroke, HTN and cancer, ESR did not show a significant association.

Since C-reactive protein levels in the blood rise more quickly after the inflammatory or infective process begins, ESR is often replaced with C-reactive protein measurement. The combination of the two measurements is likely to improve diagnostic sensitivity and specificity.

d) Total leucocyte count (TLC):

The leucocytes or the white blood cells (WBCs) are capable of motility and defend the body against infection and disease by ingesting foreign materials and cellular debris by destroying infectious agents and cancer cells or by producing antibodies. A healthy adult human has between 4500 and 11000 WBCs per cubic ml of blood. WBC count may increase in response to intense physical exertion, convulsions, strong emotional reactions, pain, pregnancy, infections and inflammations. Although WBCs are found in the circulation, most occur outside the circulation, within tissues, where they fight infections; the few in the blood stream are in transit from one side to another. WBCs are highly differentiated for their specialized function. WBCs are grouped into three major classes – lymphocytes, granulocytes and monocytes, each of which carries out different functions but all derived from a multipotent haematopoietic stem cell in the bone marrow. In addition to eliminating microbes and dead cells, activated leucocytes produce a number of growth factors that aid in repair. However, when strongly activated they may induce tissue damage and prolong inflammation injuring the normal host tissues.\(^{249}\)

A high WBC count has been shown to predict a worsening of insulin action and development of T2D in Pima Indians, whites and African Americans, thus suggesting a role for inflammation in the development of IR and subsequent T2D.\(^{250,251}\) In adult women with genetic predisposition to T2D, WBC count was found to be increased and its main correlates were IR in FH\(^+\) and adiposity in FH\(^-\) individuals.\(^{15}\)
Earlier studies have shown leucocyte count to be positively associated with carotid atherosclerosis, a preclinical atherosclerotic marker and various components of MetS and negatively associated with measures of insulin sensitivity. Leucocytes contribute to blood viscosity, release products that induce plaque rupture and thrombus formation and play a role in endothelial dysfunction. In Asian Indian subjects with normal glucose tolerance, leucocyte count and hsCRP showed an association with most CV risk factors and MetS. Leucocyte count and hsCRP play a major role in linking adiposity to diabetes and CAD. Association between WBC and MetS was reported by Nagasawa et al\textsuperscript{253} in men. A recent study by Oda and Kawai\textsuperscript{254} comparing hsCRP with WBC as inflammatory component of MetS in Japanese found similar associations in men and women but hsCRP was superior to WBC as an inflammatory component.

Vascular endothelial cells are activated by the presence of atherosclerotic risk factors such as HTN, hyperlipidemia and hyperglycemia. Endothelial cells produce intracellular adhesion molecule-1 that causes WBCs to adhere to the vascular wall, after which they can penetrate the vascular endothelium and produce new cytokines and chemokines. Cytokines then activate the WBCs and cells comprising the vascular wall, promoting platelet aggregation and thrombus formation. Pro-inflammatory cytokines are known to increase WBC count\textsuperscript{255} (WBCs have receptors for IL-6).

During an average follow up of 5-7 years, Stranges et al\textsuperscript{18} examined whether novel biomarkers gave additional contribution to the risk prediction of T2D obtained using the traditional risk factors alone. Findings indicate that biomarkers of subclinical inflammation (decreased serum albumin and increased WBC) and endothelial dysfunction (E-selectin) are significant predictors of T2D, independent of traditional risk factors. Moreover, the addition of these emerging risk factors to a basic risk factor model slightly improved the prediction of T2D from 64.6% to 72.6% (p=0.04). They concluded that WBC, a stable, well standardized,
widely available and inexpensive measure of systemic inflammation is an independent predictor of CVD events and all cause mortality.
Acute phase proteins

Acute phase proteins (APPs) are plasma proteins, the synthesis and the circulating concentrations of which are adaptively regulated in response to most forms of inflammation, infection and tissue injury. The name arises from the fact that the first such protein, C-reactive protein (CRP), was originally discovered in serum of patients in the acute phase pneumococcal pneumonia. However, it soon became apparent that increased levels of CRP and certain other plasma proteins occurred during active, tissue damaging disease processes, whether acute or chronic. Nevertheless the term ‘acute phase’ has persisted and is in general use to describe a large group of disparate plasma proteins which share the property of increased production after injury and in disease states. Most acute phase proteins are synthesized in the liver although the genes for some are also expressed in cells and tissues elsewhere. Transcriptional control is the main mechanism for regulation of production but mRNA stability contributes in some cases. A large number of cytokines, including IL-1, IL-6, TNF and various interferons are capable of inducing increased or in some cases decreased, production of various acute phase proteins. Glucocorticoids and steroid sex hormones can play an important permissive role and neural and neuroendocrine influences may be significant in vivo.256

APPs in highest concentration include haptoglobin, alpha₁-antitrypsin, orosomucoid, C3, inter-alpha-trypsin inhibitor, ceruloplasmin, fibrinogen and C-reactive protein. Most perform a specific function, which becomes increasingly important during inflammation. Elevated levels reflect the body’s biochemical preparation to end and repair the process. Many substances such as, complement, kinins, clotting and fibrinolytic factors, glucocorticoids and steroid sex hormones have been proposed as mediators of the inflammatory response. The immune system also may be involved as the initiator of the complement cascade with its resultant production of mediators.256
While an inflammatory process results in increased synthesis of certain proteins by the liver, others often referred to as negative acute phase reactants manifest a decrease. Albumin, transferrin, and the thyroxin-binding prealbumin are examples of negative acute phase reactants.\textsuperscript{257}

The liver responds to a stimulus by increasing acute phase reactant synthesis and therefore, serum levels. The time lag from the insult or the beginning of a process to a detectable change in concentration is different for each protein. CRP increases after only a few hours, while C3 and ceruloplasmin do not increase until several days later. All plasma proteins are under genetic control. Therefore, it is not surprising that synthetic rates in some individuals would be different from those in others.\textsuperscript{257}

CRP and Serum Amyloid A (SAA) are trace constituents of normal plasma and are the most dramatic acute phase reactants increasing very rapidly to peak levels which may be up to 3000 times normal at about 48 hours after an acute event. Persistently high levels may occur in chronic active disease processes, but with effective therapy or spontaneous resolution they fall to normal with a half-time as fast as 24-30 hours. In contrast, all other APPs respond more slowly, taking days to reach their peak values, and also falling much more slowly, reflecting clear and catabolic half-lives of days rather than hours. Furthermore, unlike most other APPs, there seems to be little effect of disease processes on the clearance or catabolism of CRP and SAA. The synthesis rate, which reflects the intensity of the pathology which induced their production, is thus the most important or even sole determinant of their plasma levels. For all these reasons these two APPs are the most useful for clinical purposes in man.\textsuperscript{256,257}

Of the major acute phase reactants, haptoglobin (2-1, 2-2), fibrinogen, and CRP are of the highest molecular weight; others are considerably lower. Low molecular weight APPs may be lost if the plasma membrane is damaged and thus their levels will be falsely lower. Even within a species there is diversity in the acute phase response to different stimuli, to different disease processes and between different individuals.\textsuperscript{257}
a) C-reactive protein (CRP):

CRP, named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae, was the first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage.

CRP belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins. The human CRP molecule (M, 115315) is composed of five identical nonglycosylated polypeptide subunits (M, 23027), each containing 206 amino acid residues. The protomers are noncovalently associated in an annular configuration with cyclic pentameric symmetry.258

Human CRP binds with highest affinity to phosphocholine residues, but it also binds to a variety of other autologous and extrinsic ligands, and it aggregates or precipitates the cellular, particulate, or molecular structures bearing these ligands. Autologous ligands include native and modified plasma lipoproteins, damaged cell membranes, a number of different phospholipids and related compounds, small nuclear ribonucleoprotein particles and apoptotic cells. Extrinsic ligands include many glycan, phospholipid and other constituents of microorganisms, such as capsular somatic components of bacteria, fungi and parasites, as well as plant products. When aggregated or bound to macromolecular ligands, human CRP is recognized by C1q and potently activates the classical complement pathway, engaging C3, the main adhesion molecule of the complement system and the terminal membrane attack complex C5-C9. Activation of complement by human CRP may then opsonize and enhance phagocytosis of the various ligands but could also mediate proinflammatory pathophysiological effects. Intriguingly, the spectrum of autologous ligands recognized by CRP overlaps that of anti-phospholipid autoantibodies that are associated with premature cardiovascular disease in autoimmune syndromes.259
In healthy young adult volunteer blood donors, the median concentration of CRP is 0.8 mg/L, the 90th centile is 3.0 mg/L, and the 99th centile is 10 mg/L, but, following an acute phase stimulus, values may increase from less than 50 µg/L to more than 500 mg/L, that is, 10,000-fold. Plasma CRP is produced only by hepatocytes, predominantly under transcriptional control by the cytokine IL-6, although other sites of local CRP synthesis and possibly secretion have been suggested. De novo hepatic synthesis starts very rapidly after a single stimulus with serum concentrations rising above 5 mg/L by about 6 hours and peaking around 48 hours. The plasma half-life of CRP is about 19 hours and is constant under all conditions of health and disease, so that the sole determinant of circulating CRP concentration is the synthesis rate, which thus directly reflects the intensity of the pathological process(es) stimulating CRP production. When the stimulus for increased production completely ceases, the circulating CRP concentration falls rapidly, at almost the rate of plasma CRP clearance. In most, though not all, diseases, the circulating value of CRP reflects ongoing inflammation and/or tissue damage much more accurately than do other laboratory parameters of acute-phase response, such as plasma viscosity and the ESR. Importantly, acute phase CRP values show no diurnal variation and are unaffected by eating. Liver failure impairs CRP production, but no other inter-current pathologies and very few drugs reduce CRP values unless they also affect the underlying pathology providing the acute phase stimulus. The CRP concentration is thus a very useful nonspecific biochemical marker of inflammation. Measurement of which contributes importantly to, (a) screening for organic disease, (b) monitoring of the response to treatment of inflammation and infection, and (c) detection of inter-current infection in immunocompromised individuals, and in a few specific diseases characterized by modest or absent acute phase responses. Indeed, CRP values can never be diagnostic on their own and can only be interpreted at the bedside, in full knowledge of all other clinical and pathological results. However, they can then contribute powerfully to management.\textsuperscript{13,258}
The attention focused on CRP reflects in part the fact that it is an exceptionally stable analyte in serum or plasma, and immunoassays for it are robust, well standardized, reproducible and readily available. The inherent properties of CRP and its behavior may sufficiently explain why it provides closer associations and better predictions than other markers of inflammation.\textsuperscript{258}

Binding of CRP to lipids and to plasma lipoproteins especially LDL and oxidized LDL, activation of complement and stimulation of tissue factor production by monocytes explain the procoagulant effects of CRP.\textsuperscript{25,260} CRP is proatherogenic in endothelial cells. Increased expression of adhesion molecules and modulation of nitric oxide synthesis has been documented, demonstrating a predictive relationship between increased CRP production and future atherothrombotic events, including coronary events, stroke and progression of peripheral arterial disease.\textsuperscript{261} Meta-analysis of all published studies up to the year 2000, comprising a total of 1,953 coronary events showed a relative risk of 2.0 for a future coronary event in subjects with a single initial baseline CRP value in the upper third compared with those in the lower third of the distribution in the general population. Following myocardial infarction, there is a major CRP response, the magnitude of which reflects the extent of myocardial necrosis.\textsuperscript{262}

There is a strong positive association between baseline CRP concentration and BMI. Raised baseline CRP values are also associated with many features of the insulin resistance or MetS up to and including frank diabetes mellitus. This may reflect, in part, the fact that adipocytes are the source of substantial baseline IL-6 production and perhaps also synthesize and secrete some of the baseline CRP itself.\textsuperscript{12,139,261} More generally, these associations raise the possibility that aspects of the inflammatory-markers profile associated with increased atherothrombotic risk in the population at large may not be triggered by inflammation or tissue damage in the classical sense. Rather they may reflect a particular metabolic state that happens also to be proatherogenic and/or to
predispose to atherothrombotic events.\textsuperscript{263} Indeed, CRP production predicts the development of T2D independently of traditional risk factors. In insulin-resistant obese individuals, elevated CRP values fall in parallel with improvements in insulin resistance that are associated with weight loss, but the association between CRP and insulin resistance is independent of body mass.\textsuperscript{25,264}

b) Haptoglobin:

Haptoglobin (Hp) is a $\alpha_2$-sialoglycoprotein with hemoglobin (Hb)-binding capacity. Hp is also a positive acute-phase protein and is characterized by a molecular heterogeneity with three major phenotypes: Hp 1-1, Hp 2-2 and the heterozygous Hp 2-1. Although Hp is found in serum of all mammals, this polymorphism exists only in humans. The geographic distribution of Hp phenotypes has been under a strong genetic pressure.\textsuperscript{265}

Hp consists of two different polypeptide chains, the $\alpha$-chain and the $\beta$-chain. The $\beta$-chain (40 kDa) is heavier than the $\alpha$-chain and is identical in all Hp types. As a glycoprotein, Hp contains N-linked oligosaccharides attached to the $\beta$-chains. These carbohydrate side-chains are characterized by terminal $\alpha_2$-6-linked sialic acid residues. Microheterogeneity of the carbohydrate moiety of Hp has been described. The Hb-binding capacity of Hp is attributed to the Hp $\beta$-chain. The Hp gene is expressed in hepatocytes. Synthesis of Hp is considerably lower in fetal than in adult liver, the result of a difference in transcriptional rate. The hepatic synthesis of Hp is induced by cytokines, such as IL-6, IL-1 and TNF. The physiological half-life of plasma Hp is estimated to be 5.4 days.\textsuperscript{266}

Hp forms a soluble complex with Hb, an oxygen binding tetrameric ($\alpha_2\beta_2$) protein containing a protoporphyrin ring complexed with Fe$^{2+}$ (heme). The binding of Hp with Hb is characterized by a very high affinity ($>10^{10}$ mol$^{-1}$) and stability. After destruction of erythrocytes, free Hb in the circulation passes through the glomerular filter and renal damage may occur. Hp
reduces the loss of Hb and iron because the Hp-Hb complex is not filtered through the glomeruli but is transported to the liver. In physiological conditions, serum Hp is saturated when ~500-1500 mg/L free Hb is present. The Hp-Hb complex is broken down in the parenchymal cells of the liver.\textsuperscript{267}

Hp also has antioxidant properties, bacteriostatic properties, antibody like properties, inhibits nitric oxide and prostaglandin synthesis and activity of cathepsin B. At physiological concentrations in human bile (15 mg/L), Hp is a highly potent promoter of cholesterol crystallization and is potentially important in the formation of gallstones.\textsuperscript{265}

The concentration of Hp in serum decreases after intravascular hemolysis, whether immune (e.g., transfusion reactions), infectious (e.g., malaria), hereditary (e.g., hemoglobinopathy) or mechanical (artificial heart valves, endocarditis). The amplitude of this decrease largely depends on the initial serum Hp concentration. Serum Hp concentrations decrease in malnutrition and chronic liver disease. The nephritic syndrome may be associated with high or low concentrations of serum Hp, depending on the patient’s Hp type and the supervening inflammation. Hp behaves like an acute phase protein, its plasma concentration increasing in response to a variety of stimuli, e.g., infection, neoplasia, pregnancy, trauma, acute myocardial infarction and other inflammatory reactions. Hyperhaptoglobinemia is also observed in inflammatory psychiatric disorders such as major depression. Haptoglobin genotype is an independent risk factor for cardiovascular disease and this relationship is specific for diabetes. Increased haptoglobin expression in the epididymal white adipose tissue was found in obese animal and it was suggested that white adipose tissue could be a source of increase in plasma haptoglobin level observed in obese subjects which contributes to the mild inflammation that accompanies obesity. However, because of the phenotypic variations and differing functions of the phenotypes and ahaptoglobinemia found in certain populations, haptoglobin is not
considered a suitable marker of inflammation unless genotype of the study population is well understood.\textsuperscript{19,265-268}

c) Ceruloplasmin:

Various metal ions play a critical role in enzyme performance. One whose enzyme activity has been well studied is the intensely blue, copper-containing protein, ceruloplasmin. It is monomer of 132 kDa composed almost entirely of three 42-45 kDa domains that are highly homologous to each other and to the three domains that make up the large, active proteolytic subunits of factors V and VIII of the coagulation cascade. Copper and ceruloplasmin levels are low at birth, pregnancy and the administration of contraceptive drugs.\textsuperscript{269}

The physiological function of ceruloplasmin has been a subject of considerable investigation, speculation, and contradiction. Multiple biochemical activities of ceruloplasmin have been described, including copper transport, oxidation of various amines, oxidation of Fe\textsuperscript{2+} to Fe\textsuperscript{3+} by subsequent uptake by transferrin and ferritin, and antioxidant activity against lipid peroxidation. Reported cellular actions of ceruloplasmin include stimulation of cell proliferation.

There is evidence that ceruloplasmin as an antioxidant blocks protein and DNA damage and that it affords protection against free radical initiated cell injury and lysis. Ceruloplasmin has been shown to inhibit the oxidation of tissue homogenates, tissue extracts of lipids, microsomal membranes and vesicles of purified polyunsaturated fatty acids and phospholipids.\textsuperscript{270}

Both ceruloplasmin production and binding of eight atoms of Cu\textsuperscript{2+} to apo-ceruloplasmin occur in the liver. Ceruloplasmin is an APP with a response of intermediate magnitude. Compared with other APPs, the plasma concentration is increased two to three-fold during inflammation, pregnancy, and after traumatic injury, including surgery.\textsuperscript{269}

Ehrenwald \textit{et al}.\textsuperscript{270} investigated the effects of ceruloplasmin on the oxidation of LDL. In contrast to their expectations, highly purified
undegraded human ceruloplasmin enhanced rather than suppressed copper-ion mediated oxidation of LDL. Ceruloplasmin increased the oxidative modification of LDL as measured by thiobarbituric acid-reacting substances by at least 25-fold in 20 hours, and increased electrophoretic mobility, conjugated dienes, and total lipid peroxides. In contrast, ceruloplasmin that was degraded to a complex containing 115 and 19 kDa fragments inhibited cupric ion oxidation of LDL, as did commercial preparations which were also degraded. Ceruloplasmin, depending on the integrity of its structure and its bound copper, can exert a potent oxidant rather than antioxidant action on LDL.

Elevated serum ceruloplasmin levels have been found in patients with cardiovascular disorders including arteriosclerosis, abdominal aneurysms, unstable angina, vasculitis and peripheral artery disease.\textsuperscript{271} Significant higher concentrations of ceruloplasmin have been reported by Awadallah \textit{et al}\textsuperscript{272} in myocardial infarction patients and Shukla \textit{et al} in CVD. Via its copper, ceruloplasmin promotes vasculopathic effects that cause lipid oxidation. Enhanced formation of reactive oxygen species disrupt copper binding to ceruloplasmin, thereby impairing its normal protective function, and the liberated copper in turn may promote oxidative pathology. Summarizing the observations of the above studies, it could be noticed that ceruloplasmin plays a dual role as an antioxidant and as a pro-oxidant. Giurgea \textit{et al}\textsuperscript{273} analysed the dual effect of ceruloplasmin as an oxidant and as an antioxidant through a clear cut-data analysis of the past studies. The conclusion from the review verdicts the pathogenic involvement of ceruloplasmin in CVD, but more studies are required before a firm conclusion can be drawn. Kumar and Sivkanesan\textsuperscript{274} report ceruloplasmin is a coronary risk factor in normolipidemic acute myocardial infarct patients.

d) Fibrinogen:

One of the major plasma proteins, fibrinogen is a soluble glycoprotein found in the plasma with a molecular weight of 340 kDa.\textsuperscript{275} This elongated protein, visible by electron microscopy, is an important component of the
coagulation cascade and the precursor of the fibrin clot. Present at all times of life, its values change little in healthy individuals.

Fibrinogen is composed of three pairs of nonidentical polypeptide chains denoted Ao, BP, and -y. Regulation of fibrinogen gene expression is not fully understood; however, synthesis of the BP chain has been shown to be the rate limiting step in fibrinogen synthesis. Genetic studies in nondiabetic subjects have shown an association between 3-fibrinogen gene polymorphisms and fibrinogen levels. Beta-fibrinogen gene variations may also influence the association between smoking and hyperfibrinogenemia. Polymorphisms of the beta-fibrinogen gene have also been shown to be associated with myocardial infarction, stroke, and peripheral vascular disease in nondiabetic subjects.  

The liver produces fibrinogen in its final form. Fibrinogen is a major determinant of blood viscosity, blood flow and ESR. It plays a vital role in a number of physiological processes in the body including acute phase reactions, atherogenesis and thrombogenesis. Production can be accelerated by hormones and by the development of an inflammatory process. Hence, fibrinogen has been considered a prominent acute phase reactant. The polymerization of soluble ‘dimeric’ fibrinogen to the insoluble fibrillar protein fibrin is the basis for hemostasis. Without adequate amounts of functional fibrinogen, clotting fails. Fibrinogen, described by many workers as a metastable protein on the brink of instability, converts to the thoroughly stable protein fibrin through a series of minor changes. 

Plasma fibrinogen is influenced by many factors. It increases with age, BMI, smoking, diabetes, hypertension, post menopause and is related to fasting serum insulin, LDL-C, lipoprotein(a) and leukocyte count. Female sex, physical inactivity, oral contraceptive use and stress are also associated with raised fibrinogen levels. Consumption of fish and olive oil, male sex, younger age, Caucasians, regular alcohol consumption and hormone replacement therapy are some of the factors associated with lower fibrinogen.
Plasma concentration of fibrinogen increasing with age may be due to a slower rate of disposal of fibrinogen than an increased production rate. Fibrinogen concentration has been positively correlated with BMI, WC and WHR in both sexes.\textsuperscript{278}

Epidemiological studies\textsuperscript{261,263,279,280} in the general population indicate an association between fibrinogen levels and the subsequent development of all the major atherosclerotic cardiovascular events, including myocardial infarction, stroke, and peripheral arterial disease. Fibrinogen may be indirectly associated with vascular disease as a marker of unstable lesions that are undergoing sub-intimal hemorrhage or with potent risk factors such as smoking. In addition, hyperfibrinogenemia may be an indicator of inflammatory vascular changes and endothelial dysfunction. Alternatively, fibrinogen may be directly involved in atherosclerosis and thrombosis. Hyperfibrinogen levels lead to enhanced coagulant activity and are associated with increased blood viscosity. Fibrinogen is also a cofactor in platelet activation and may directly contribute to plaque formation, where it is converted to fibrin and fibrinogen degradation products. Fibrinogen has emerged as an independent risk factor for CVD. Meta-analysis of major epidemiological study reveals that fibrinogen is related to most of the conventional cardiovascular risk factors. It was hypothesized that the relationship of fibrinogen with cardiovascular end points is indirect, merely due to the associations with ‘true’ risk factors. Alternately, it might indicate that other risk factors could mediate through a hyperfibrinogen effect. However, multivariate analysis suggests that fibrinogen represents a mechanism by which various other risk factors lead to CVD.\textsuperscript{277}

Subjects with diabetes and insulin resistance have higher concentrations of fibrinogen which contribute to a more pro-thrombotic fibrin clot structure leading to atherothrombotic cardiovascular events in them. Ambient glucose levels independently affected fibrin clot structure and increasing HbA\textsubscript{1c} was independently associated with tightly cross linked thin-fibred fibrin clots.\textsuperscript{276} Total cholesterol levels have been found to determine fibrin
clot structure, thus linking lipids and fibrinogen. Fibrinogen levels are elevated in patients with type II hyperlipoproteinemia and familial hypercholesterolemia. Levels are positively associated with total cholesterol, triglyceride and LDL and negatively with HDL.\textsuperscript{277}

Fibrinogen levels are higher in patients with essential hypertension than in normotensive controls. Similarly, plasma viscosity is elevated in hypertensive persons and blood pressure readings are positively correlated with this variable. Even when hypertension is mild, fibrinogen levels are higher than in normotensive controls.\textsuperscript{277}

Plasma fibrinogen is a prominent acute phase reactant. The process of inflammation is primarily mediated by its interaction with leukocytes through the surface receptors of the latter termed 'integrin'. Fibrinogen is also a ligand for Intercellular Adhesion Molecule-1 (ICAM-1) and enhances monocyte-endothelial cell interaction.\textsuperscript{278}
Inflammation in diabetes/prediabetes/MetS/normoglycemic FDRs

- Pickup et al.\(^{81}\) proposed that chronic low grade inflammation and activation of innate immune system are closely involved in the pathogenesis of type 2 diabetes, in the year 1997. Since then they and several others have agreed with these findings and this theory has provided a new model in the understanding of the pathogenesis of type 2 diabetes and its associated features.

- The acute phase proteins were studied as markers of inflammation in diabetes. Tan et al.\(^{14}\) in trying to elucidate the mechanism of activation of low grade inflammation in diabetes estimated Advanced Glycation End products (AGEs), CRP and IL-6 and demonstrated that AGEs are independent determinants of CRP levels. They hypothesized that subclinical inflammation in those patients may partly be due to activation of inflammatory response by AGEs.

- A pilot study was conducted at Kasturba Medical College, Mangalore where the acute phase reactants \(\alpha_1\)-antitrypsin, ceruloplasmin, \(\alpha_1\)-acid glycoprotein and fibrinogen were studied in newly diagnosed type 2 as well as type 1 diabetes patients. In comparison with healthy controls, type 1 cases showed significant high concentrations of \(\alpha_1\)-antitrypsin, ceruloplasmin and fibrinogen. The values of all the four proteins studied were significantly elevated in the type 2 cases. Except for ceruloplasmin levels type 2 cases had significantly higher values when compared to the type 1 cases.\(^{282}\)

- Inflammatory markers, insulin resistance and carotid intima media thickness were studied in 81 newly diagnosed type 2 diabetic patients in north India by Ahmad et al.\(^{136}\). Concentrations of inflammatory markers, CRP, fibrinogen and TNF\(\alpha\) were significantly higher in diabetic patients than in control group. CRP correlated with intima media thickness and was higher in diabetic subjects with CHD.
Relation of inflammatory markers to diabetic complications have been reported by Nayak and Roberts\textsuperscript{283} and Akalin et al.\textsuperscript{279}

12330 men and women aged 45-64 years were followed up for a mean of 7 years in the ARIC study.\textsuperscript{251} White cell count, Fibrinogen, albumin, orosomucoid, sialic acid and haptoglobin were estimated in them at baseline visit. After the follow up period, 1335 cases of diabetes were detected. Analysis of their data showed an association for a range of inflammation markers at concentrations lower than that characteristic of acute inflammation. They also proposed that IR leads to, rather than is the consequence of raised concentrations of inflammation mediators.

To determine whether IL-6 and CRP were associated with development of T2DM in healthy middle aged women, 188 women who developed DM over a 4-year follow up period were selected from the Women’s Heart Study and compared with 362 disease free controls. Pradhan et al.\textsuperscript{16} report the positive association of IL-6 and CRP with future DM even after adjustments for BMI, family history of diabetes, smoking, exercise, alcohol use and hormone replacement therapy.

That low grade inflammation is important in the pathogenesis of T2DM was also supported by; the West of Scotland Coronary Prevention Study\textsuperscript{264} (CRP), Ford ES\textsuperscript{248} in US adults (leucocyte count), Nakanishi et al.\textsuperscript{284} among Japanese Americans (CRP), Dalmon et al.\textsuperscript{285} (ceruloplasmin), Engstrom et al.\textsuperscript{286} (C3, C4, fibrinogen, orosomucoid, α\textsubscript{1}antitrypsin, haptoglobin and ceruloplasmin), Stranges et al.\textsuperscript{18} from the Western New York Study (CRP, WBC, e-selectin and albumin), Festa et al.\textsuperscript{287} in the insulin resistance atherosclerosis study (CRP, fibrinogen and plasminogen activator inhibitor-1), Shetty et al.\textsuperscript{288} (resistin, adiponectin, CRP, TNFα, endothelin-1, plasminogen activator inhibitor-1, tissue plasminogen activator and intracellular adhesion molecule). Goldberg RB\textsuperscript{289} reviewed cytokine and cytokine-like inflammation markers, endothelial dysfunction and imbalanced coagulation in the development of diabetes and its complications and concluded that these markers are
predictors of diabetes and increasing morbidity in prediabetic and diabetic subjects.

- Although there are reports on the role of several positive acute phase proteins like ceruloplasmin, Haptoglobin, fibrinogen, α₁-antitrypsin, sialic acid etc and negative acute phase proteins like albumin, and non specific markers of inflammation like WBC and ESR, CRP has come to stay. CRP values show no diurnal variation and are unaffected by eating. Liver failure impairs CRP production, but no other inter-current pathologies, and very few drugs reduce CRP values unless they also affect the underlying pathology providing the acute phase stimulus. It is an exceptionally stable analyte in serum or plasma and that immunoassays for it are robust, well standardized, reproducible and readily available. Hence, it serves as a good measure of inflammation.

- The existence of chronic inflammation and endothelial dysfunction in prediabetic individuals, exhibiting both IFG and IGT was reported by a community based population data from China where 252 prediabetics and 38 newly diagnosed diabetics were studied for serum adiponectin, IL-6, urine albumin to creatinine ratio by Lu et al. and recently by Thompson et al. Gupta and Johnson analysed anthropometry and laboratory measures from healthy disease free obese adults (27 women and 8 men) in a weight loss study. hsCRP and fibrinogen levels were raised among the subjects who had FPG in the prediabetes range (8 women and 3 men). They also had prehypertension. In otherwise healthy disease free obese adults, a higher degree of systemic inflammation is associated with prediabetes and prehypertension. Among the first degree relatives of diabetes patients who had normal or impaired glucose tolerance, inflammatory cytokine concentrations (IL-6, IL-18 and TNFα) were acutely increased by hyperglycemia. An oxidative stress mechanism was proposed and the results also indicated that hyperglycemic spikes affected cytokine concentrations more than continuous hyperglycemia at least in the short term. The results were more pronounced in IGT subjects.
In the Insulin Resistance Atherosclerosis Study, CRP, plasminogen activator inhibitor-1 and fibrinogen were studied in relation to defects in insulin sensitivity and first phase insulin secretion in prediabetic individuals. The proinflammatory state was found predominantly in insulin resistant individuals but not in those with a primary defect in β-cell function. Cardellini et al. found raised IL-6 levels in subjects with impaired glucose tolerance but not in those with IFG in a cohort of Italian Caucasians.

Endothelial dysfunction markers were higher in subjects who develop prediabetes in The Western New York Study. The findings were related to women only. IL-6 and CRP did not show a significant correlation. Gender differences were observed by Saltevo et al. also. Adiponectin concentrations decreased in women relatively more compared to men across individuals with NGT, prediabetes and T2D whereas, inflammatory markers increased relatively more in women.

In partial dissent from the above studies in prediabetes, Muller et al. report increased serum concentrations of IL-6, CRP, Serum Ameloid A and fibrinogen in IGT and T2D subjects. But TNFα and its receptors were not increased in IGT subjects.

Various studies have demonstrated raised proinflammatory proteins, IL-6, TNFα etc in high risk normoglycemic individuals. Gokulakrishnan et al. in the CURES study from Chennai assessed the association of leucocyte count and hsCRP with metabolic abnormalities in NGT subjects. A significant association existed between systemic inflammation and cardiovascular risk factors with increasing tertiles of leucocyte count and with increase in number of metabolic abnormalities constituting the MetS.

WBC count was also measured as a risk factor in glucose tolerant adult women who had first degree family history of T2D. After multivariate analysis these women showed an increase in WBC count and significant
association with age, SBP and HOMA-IR in FH\(^+\) subjects and with age, BMI, fat mass and Tg in FH\(^-\) individuals.\(^{15}\)

- Markers of inflammation (WBC and fibrinogen) were associated with elevated 1 hr PG in subjects with NGT and prediabetes. A 1 hr PG >155 mg/dL was considered a new marker for cardiovascular risk.\(^{167}\) Markers of inflammation (CRP, fibrinogen, albumin, ESR, WBC) were studied by Gustavsson and Agardh\(^{247}\) to correlate the values with HbA\(_{1c}\) in patients with and without diabetes but with known coronary atherosclerosis. Low grade inflammatory activity was found to be increased not only in diabetic patients but also in nondiabetic individuals with A\(_{1c}\) within the normal range.

- Coronary vasomotor function was found to be abnormal in healthy nondiabetic FDRs of T2D by Hirata et al\(^{296}\) raised IL-6, hsCRP and HOMA-IR were thought to be the basis of the coronary dysfunction.

- Among 176 nondiabetic normotensive off spring of T2DM patients, euglycemic hyperinsulinemic clamp study to assess insulin resistance was undertaken by Cardellini et al\(^{29}\). Of the 176 subjects, 145 were glucose tolerant, 18 with IFG and 13 with IGT. Univariate correlations showed that age, BMI, WC, BP, 2 hr post challenge glucose, fasting insulin, Tg, IL-6, fibrinogen and WBC were significantly correlated with carotid intima media thickness whereas, HDL-C and glucose disposal showed a negative correlation. A stepwise multivariate regression analysis associated WC, WBC count, BP and insulin stimulated glucose disposal with intima media thickness.

- Endothelial function as flow mediated dilation (FMD) of the brachial artery, arterial stiffness as carotid femoral pulse wave velocity (PWV), HOMA-IR, plasma levels of hsCRP, TNF\(\alpha\), IL-1\(\beta\), vascular cell adhesion molecule and intracellular adhesion molecule were evaluated in 29 normotensive, normoglycemic FDRs of diabetic subjects and compared with 16 subjects with no parental history of diabetes by Scuteri et al\(^{297}\). There was a 33% reduction in FMD with or without IR and PWV was raised only in FDRs
with IR. These alterations at the young age of 18-42 years were present independent of the presence of the MetS components.

- A cross sectional study of 3594 Japanese men aged 34-69 years evaluated the MetS components as defined by ATP III with measure of obesity as BMI ≥ 25 kg/m² and the association with WBC count. WBC count had a positive association with BMI, BP, Tg, Glucose and Insulin and negative correlation with HDL-C. In multiple linear regression analysis, BMI, HDL-C, SBP, glucose and Tg had a significant independent association with WBC count.²⁵³

- In a similar study from Korea, Kim et al²⁹⁸ found an increase in total WBC, neutrophils and lymphocytes among male MetS subjects only and the counts increased in accordance with the MetS component counts. The female subjects did not show this association. There was no difference in RBC count. Comparison between hsCRP and WBC as inflammatory components of MetS was undertaken by Oda and Kawai²⁵⁴ who found hsCRP superior to WBC. However, since WBC is routinely measured they suggested its use in clinical practice when hsCRP is not available.

- Inflammation in MetS has also been discussed by Das UN,²⁵ and Devaraj et al.²⁶¹ The importance of cortisol and the HPA axis in the activation of the inflammatory cascade has also been described by them. Weigensberg et al²⁷ studied the association of MetS with serum cortisol in over weight Latino youth and found an association between the two independent of body fat and insulin sensitivity. Chronic stress and age-related increases in IL-6 have also been reported by Kiecolt-Glaser et al.⁸⁸ Glucocorticoids are known to induce IR and accumulation of visceral fat. Cortisol and IL-6 can synergistically stimulate APP synthesis in the liver.²³³

- When metformin (850mg bd) was administered short term (90 days) to nondiabetic FDRs of T2DM subjects who had MetS but NGT, improvements were found in the cardiovascular risk profile; lipid profile, FPG, SBP and BMI. No changes were found in the levels of CRP and fibrinogen after the 90 days’ treatment.²⁹⁹
- Relationships between hsCRP, IL-6, adiponectin and oxidative stress in MetS have been recently published by Chen et al. Subjects with MetS had significantly higher concentrations of hsCRP and IL-6 and lower adiponectin level and lower antioxidant enzymes activities (catalase, superoxide dismutase and glutathione peroxidase) than the control subjects. hsCRP, IL-6 and adiponectin were associated with greater risk of MetS.

- Constellation of the MetS features are all individual risk factors for CHD and when they coexist as in the MetS patients, the risk is enhanced. Also the common soil theory linking diabetes, obesity, MetS and CHD further highlights the role of inflammation in CHD beginning with endothelial dysfunction even in the NGT high risk individuals. 1165 people with central obesity but without previous diagnosis of HTN, dyslipidemia, diabetes or CVD aged 20-70 years were assessed for the presence of MetS. Multivariate linear regression to assess which MetS component independently associated with hsCRP showed independent associations of WC and Tg. hsCRP however, had limited capacity to predict the presence of MetS in a population with central obesity.

- To clarify the importance of abdominal obesity in subclinical inflammation, Nishida et al. examined the changes of inflammation markers in clustering of MetS components with or without abdominal obesity. Subjects consisted of 326 apparently healthy Japanese men aged 30-59 years. MetS components (IDF criteria) were assessed and serum levels of hsCRP, IL-6 and adiponectin were examined. hsCRP and IL-6 significantly increased in association with clustering of MetS components with abdominal obesity but not in those without abdominal obesity. Adiponectin did not vary much. They conclude that abdominal obesity may exhibit distinct effect on inflammatory and anti-inflammatory proteins and modulate inflammatory network in MetS.

- Nesto RW discusses obesity as a major component of the MetS and attributes inflammation to visceral fat. The visceral fat is extensively
infiltrated with, by a wide variety of inflammation cells which in turn make a whole host of cytokines which have profound effects on vascular function. Similar results were found by Stienstra et al, Warnberg et al, Mohan et al, Nakamura et al and Corpeleijn et al.

- Insulin resistance that is an effect of obesity is also reported as the cause for elevated levels of inflammatory markers by several workers. Vikram et al however, showed significant association between subclinical inflammation and fasting insulin levels in urban young adult north Indian males.

- The major complication associated with diabetes, prediabetes and MetS is CVD. Subclinical CVD is said to be present before appreciable hyperglycemia sets in. Inflammation markers and markers of endothelial dysfunction have been associated with the prediction of diabetes and CHD. It is often difficult to dissociate inflammation in the two conditions.

- The Atherosclerosis Risk in Communities (ARIC) study group followed 1676 middle aged persons with diabetes but no history of prevalent coronary heart diseases for 8 years and recorded 186 incident CHD events among them. In these patients, other than the traditional risk factors like HTN, smoking, TC level and low HDL-C, CHD was also significantly associated with WHR, HDL₃ cholesterol, apoAI and B, albumin, fibrinogen and von Willebrand factor, factor VIII activity and leucocyte count. After adjustment for traditional risk factors, levels of albumin, fibrinogen, von Willebrand factor, factor VIII activity and leucocyte count remained independently associated with CHD (p < 0.03) and concluded that these were predictors of CHD among persons with diabetes and that the underlying inflammation reaction forms the common antecedents for both diabetes and CHD. That cardiovascular disease risk factors predict the development of type 2 diabetes was shown by The Insulin Resistance Atherosclerosis Study also.
Inflammatory markers like CRP, fibrinogen and IL-6 are associated with lipid and lipoprotein metabolism\textsuperscript{21,177} and with prehypertension\textsuperscript{305} and HTN.\textsuperscript{306} Alterations in BP and lipid metabolism are frequently found with diabetes and prediabetes. These also form the constituents of the MetS.

These studies and several others preclude a need to understand the nuances involved in the inflammatory process in conjunction with hyperglycemia, obesity, dyslipidemia and hypertension both in isolation and in cluster as in MetS.
Screening for diabetes/prediabetes

There is a general agreement on the potential value of screening for diabetes and thus its early diagnosis. This is based on several evidences. Firstly, diabetes is a common and serious condition capable of inflicting much personal, social and economic harm. Moreover, population studies have shown that there are many people with undiagnosed T2D. Secondly, about 20-30% of people with newly diagnosed T2D already have tissue complications such as retinopathy. From the rate of complications with increasing duration of diabetes, it can be estimated that complications begin to develop about 5-6 years before diagnosis and that the actual onset of diabetes may be 10-12 years before clinical diagnosis. Preclinical T2D is also associated with risk factors like hyperglycemia, obesity, hypertension and dislipidemia.61,307

Early treatment of diabetes should therefore delay or prevent the development of complications. Uncertainty prevails regarding the method of screening, the frequency and the setting. Definitive studies on the methods, benefits and risks of screening have yet to be done. A targeted screening programme of those at high risk of T2D is most often suggested. The UKPDS recommended active case-finding every 3 years in those at high risk.146,148

There is no identified best method for testing. Fasting plasma glucose has the advantage of simplicity, speed, acceptability to patients and low cost, and the disadvantage of missing a substantial number of cases, with glucose intolerance. It identifies about half of those with diabetes, depending on the population and their age. The OGTT and 2 hr plasma glucose has been recommended for screening for more than 20 years, but in practice has not been much used. It is more difficult to execute, impractical for large numbers and more expensive, but captures IGT. It has been recommended by the European Diabetes Policy Group that those with IFG or even those with a fasting glucose above the lower value of 5.5mmol/L should have an OGTT to exclude diabetes. From the DECODE study, the European recommendations would mean that about 12% would need an OGTT (in addition to fasting
glucose) and about 18% of those with diabetes would remain unidentified.\textsuperscript{149,179}

The use of HbA\textsubscript{1c} as a diagnostic and screening tool has been discussed. It would diagnose some subjects with IGT and identify additional subjects not recognized by either the fasting or 2 hr criteria. HbA\textsubscript{1c} is predictive of both microvascular and cardiovascular disease. Because of problems with standardization HbA\textsubscript{1c} had not received support globally until recently.\textsuperscript{114,144,150-154}

The diagnosis of diabetes has been simplified and facilitated by the use of new diagnostic criteria, but research is needed into the significance of categories of glucose dysregulation not detected by the fasting glucose level, principally IGT. Thus, plasma glucose may not prove to be the best or only marker for diabetes, and HbA\textsubscript{1c} and genetic susceptibility markers might make a useful combination for screening, if not diagnosis. This need is further emphasized in ethnic populations who show high prevalence and preponderance of diabetes.\textsuperscript{37}

Low grade inflammation is now said to play a major role in the development of prediabetes/diabetes and also in the subsequent complications associated with diabetes. The MetS which is a constellation of several cardiometabolic risk factors is frequently associated with prediabetes/diabetes and each of the constituents is individually associated with inflammation. Hence, whether these markers of inflammation can be used as warning signs to predict diabetes development in an ‘at risk’ group – The FDRs of patients with T2DM, and their role in the metabolic alterations accompanying the progress of diabetes development is the scope of this study.