REVIEW OF LITERATURE
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1. HISTORICAL BACKGROUND OF DIABETES

The story of diabetes mellitus - its discovery, description and treatment is a remarkable chronicle covering 3,500 years of medical history. Looking at this ailment over the edges makes one fact very clear: the incidence of diabetes has increased dramatically from an uncommon ailment during the period of antiquity to a world wide epidemic expected to affect 300 million people by the year 2025.

The story of diabetes unfolds during the period of antiquity, where we begin to see the earliest descriptions of symptoms of diabetes. Across the Nile from Luxor, on the West Bank sits the Necropolis of Ancient Thebes. It was here in the vicinity of Thebes that German Egyptologist Georg Ebers acquired his famous papyrus in 1872. Named for him, the Ebers Papyrus is one of the most famous documents relating to the ancient practice of medicine (written about 1550 BC). The first reference to diabetes is attributed to the Ebers Papyrus, which mentions the remedies for the excessive urination (polyuria).

Although the Greek Physician Hippocrates, "The father of medicine," did not specifically mention diabetes in his writings, there are accounts in the Hippocratic writings that are consistent with signs and symptoms of diabetes. There are references to excessive urinary flow with wasting of the body. Disciple of Hippocrates—Galen, the most influential medical writer of all times, discussed diabetes in a number of his works. He described the condition as rare. He referred to the ailment as "diarrhea of the urine" and "the thirsty disease."

Aretaeus, a contemporary of Galen, provided the first accurate description of the symptoms of diabetes. He was first to use the term "Diabetes,"
derived from the Greek word for “siphon.” Aretaeus classic description begins “Diabetes is a wonderful affection, not very frequent among men, being a melting down of flesh and limbs into urine...”\footnote{32, 34, 35}.

The ancient Hindus were the first to coin the term “honey urine” a thousand years before the first Europeans recognized it in patients with diabetes. The Hindu physicians Charaka, Sushruta and Vagbhata described polyuria and glycosuria. They noted the attraction of flies and ants to the urine of those affected by this ailment. Sushruta described diabetes (madhumeha) as a disease characterized by passage of large amount of urine, sweet in taste, hence the name- “Madhumeha”- honey like urine. He goes on to say that diabetes primarily affects obese people who are sedentary and emphasized the role of physical activity in amelioration of diabetes.\footnote{34, 36}

It was Thomas Willis’s observation in 1674 and Matthew Dobson’s experiments in 1776 that conclusively established the diagnosis of diabetes in the presence of sugar in urine and blood. Willis referred to diabetes as “pissing evil” and noted that in patients with diabetes “the urine is wonderfully sweet, as if it is imbued with honey or sugar”. He claimed that diabetes was primarily a disease of blood and not the kidneys. Willis proposed that the sweetness first appeared in the blood and later was found in the urine.\footnote{37}

Dobson provided experimental evidence that people with diabetes eliminate sugar in their urine. He gently heated two quarts of urine to dryness. The remaining residue was a whitish cake, which Dobson wrote, “was granulated and broke easily between the fingers; it smelled sweet like brown sugar, neither it could be distinguished from sugar, except that the sweetness left a slight sense of coolness on the palate.” Dobson detailed his findings in a paper presented to the medical society of London in 1776.\footnote{38} Prior to presentation of his findings, Dobson consulted with
William Cullen, one of the Britain's foremost clinicians, consultants and educators.

**It was Cullen who was the first to distinguish between Diabetes Mellitus & Diabetes Insipidus.** In his classification (1769), for the first time a distinction between diabetes (mellitus) with the urine of "the smell, colour and flavor of honey," and diabetes (insipidus) with limpid but not sweet urine was made. It was Cullen who added the descriptive adjective "Mellitus," from the Latin word for "Honey." Cullen wrote Dobson, "You have done something in putting it beyond any doubt by your experiments...I have only to add that I wish you would examine both by taste and evaporation what might be called the urina potus or that copious limpid urine which runs in some people after their drinking largely of water or watery liquors."  

The experimental period in the history of diabetes began in first half of the 19th century with the experiments of Claude Bernard. Bernard discovered that the liver releases a substance that affects blood sugar levels. **In 1857, he isolated a starch-like substance that he called "glycogen,"** which was the precursor of glucose, "the internal secretion" of the liver. This observation established the liver's role as a vital organ in diabetes.  

**Paul Langerhan's most famous histological findings,** the pancreatic islets, were presented in his doctoral dissertation at the University of Berlin in 1869. Langerhans acknowledged that he did not know the function of these ductless cells, which were later named as "Islets of Langerhans," in his honor by the French histologist Laguesse. At the close of 19th century Oscar Minkowski demonstrated conclusively that removal of the pancreas from a dog results in production of a fatal diabetes. **This was a turning point in determining the endocrine function of pancreas.**

The discovery and isolation of insulin at the University of Toronto in 1921-1922, was one of the greatest events in the history of medicine. Insulin therapy would soon commute the death sentence associated with the
diagnosis of type I DM. Despite initial rebuffs by JJR Macleod, professor of physiology at the University of Toronto, the persistent Frederick Banting was finally allowed to begin his research in Macleod’s laboratory in May 1921. Banting was assigned laboratory space, research animals and 22 years old research assistant named Charles Herbert Best. Later, Macleod recruited a young biochemist, James Bertram Collip, to assist Banting and Best in obtaining pancreatic extract.

An article written by Moses Barron in 1920 stimulated Banting’s research interest. Barron described a rare case of a pancreatic stone that blocked the pancreatic duct. The blockage resulted into degeneration of glandular cells but not the islets cells. Banting wrote the following words in his research notebook: “Diabetus Ligate pancreatic ducts of dog. Keep dogs alive till acini degenerate leaving islets. Try to isolate the internal secretion of these to relieve glycosurea.” The spelling errors ‘Diabetus’ and “Glycosurea” are Banting’s. It goes to show that brilliance and success are not necessarily tied to spelling proficiency.43-45

Banting and Best are the figures which history has most closely associated with the discovery of insulin. Yet the 1923 Nobel Prize in Medicine was not awarded to Banting and Best, but to Banting and Macleod. In an attempt to remedy this injustice Banting, publicly acknowledged Best’s role in isolation of insulin and shared the monitory prize with him. Macleod agreed to do the same with Collip.

In 1926, John Jacob Abel purified insulin and isolated its crystalline structure.46 In 1958, Frederick Sanger was awarded the Nobel Prize in chemistry for his work on the structure of proteins, specially that of insulin. It was Sanger who was first to discover the exact amino acid sequence of the protein - Insulin.47 The pig has played an important role in the history of diabetes as the source of insulin and life for people with diabetes. For many years beef/pork insulin was the only source of insulin. Human insulin became available in early 1980s and was the first
commercial product developed by recombinant DNA technology. The origin of the term “Insulin” is from the Latin word for “Island: Insula.”

It soon became apparent, however, insulin did not cure diabetes. As people began to live longer, they experienced the complications that had not previously been seen. Elliot P. Joslin noted that “The era of coma as the central problem of diabetes has given way to the era of complications. People with diabetes are at increased risk of developing serious complications including blindness, kidney failure, heart disease, stroke and amputations.” Abundant evidence show that people with diabetes are at high risk for CVD: coronary disease, stroke, peripheral arterial disease and cardiomyopathy.

In 1934, Joslin wrote a paper, “The menace of diabetic Gangrene,” published in the New England Journal of Medicine. Joslin noted that with the introduction of insulin, mortality from diabetic coma had fallen significantly from 60 percent to 5 percent. Yet, deaths from diabetic gangrene (of the foot and leg) had risen significantly. Joslin firmly believed that gangrene and amputations were preventable. He wrote “Consequently it has been forced upon me that gangrene is not Heaven sent but earth born.” His remedy was a team approach to diabetes care, which included patient education in foot care, medical nutrition therapy, exercise, prompt treatment of foot infection, and whenever necessary, specialized surgical care.

The message to “know more about diabetes” is important for all people. However it is most critical for those who are at high risk for diabetes and not yet been diagnosed. This message is also important for health maintenance organizations and hospitals. In 1998 report, the congressionally mandated Diabetes Research Working Group recognized the great urgency and extraordinary opportunities facing us today in diabetes research. Mapping of the human genome marks a new era in medical research, paving the way for treatment and cure of many serious
In the 21st century we are witnessing an exciting new chapter being written in the history of diabetes with regards to its pathogenesis, relation with inflammation, cytokines, acute phase reactants, oxidative stress and lipid peroxidation with newer modalities of treatment. There is a genuine optimism in our search for cure.

2. GLOBAL SCENARIO

Diabetes mellitus has become a major non communicable disease worldwide and is associated with enormous personal, social and economical burden. The prevalence of diabetes is rapidly rising all over the globe with alarming rate.49 The estimated number of adults with diabetes in 2007 was 246 million of these 80 percent live in developing countries, the largest number in Indian subcontinent and in China.1 Approximately 85-95 percent of all cases are type II DM and the world wide explosion of this disorder is a major health care burden. It is estimated that nearly 380 million adults worldwide will have diabetes by 2025.3

The world wide prevalence of diabetes, for all age groups, was estimated to be 2.8 percent in year 2000 and is predicted to be 4.4 percent by the year 2030.50 The diabetes epidemic relates particularly to type II DM and is taking place in both developed and developing countries predominantly due to the changing demography and increased longevity.2,51 In developing countries, demographic changes leading to type II DM include decreasing birth rate and lower mortality from infectious diseases.52

Several reports have proved that the epidemic explosion of type II DM has been observed in populations which have changed from a traditional to a modern life style as shown in many developing nations and few of the middle East Arab states53 as well as in disadvantaged minorities in the developed countries e.g. Australian aboriginal and Torres Strait Islanders,54 migrant Asian Indians, Chinese and in native Africans and Mexican
Americans. In the Pacific island of Nauru, where diabetes was virtually unknown 50 years ago, it is present in approximately 40 percent of adults.

The number of people with diabetes will increase by 42 percent (from 51 to 72 million) in industrialized countries between 1995 and 2025 and it is projected to increase by 170 percent (from 84 to 228 millions) in industrializing countries. Increasing urbanization and industrialization are the chief reasons for the rapid increase in prevalence of type II DM. It is estimated that 20 percent of the current diabetic population of the world resides in South East Asia region. The number of diabetic persons in the countries of this region are likely to triple by year 2025, increasing from the present estimates. Thus South East Asia region will bear the maximum global burden of the disease in the initial decades of the 21st century.

Table 1. List of countries with highest number of estimated cases of Diabetes for year 2000 and 2030.

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Country</th>
<th>Population with Diabetes (millions)</th>
<th>Country</th>
<th>Population with Diabetes (millions)</th>
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<tr>
<td>1</td>
<td>India</td>
<td>31.7</td>
<td>India</td>
<td>79.4</td>
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<td>China</td>
<td>20.8</td>
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<td>42.3</td>
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<td>5</td>
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<tr>
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<td>Bangladesh</td>
<td>3.2</td>
<td>Bangladesh</td>
<td>6.7</td>
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</table>
3. INDIAN SCENARIO

India is expected to experience the largest increase in type II DM cases by year 2030. Diabetes in urban Indians is reaching epidemic scale.\textsuperscript{59-65} The prevalence of type II DM in Asian Indians ranges from 2.7 percent in rural areas to 14 percent in urban areas and up to 16-22 percent in migrant Indians living in Europe, USA, Africa and Fiji.\textsuperscript{66-68} This increase is of great concern because of the high morbidity and mortality and the cost associated with the treatment of complications of diabetes mellitus.\textsuperscript{69,70}

The first authentic data on prevalence of diabetes in India came from a multicentre study conducted by the Indian Council of Medical Research (ICMR) in the early 1970s reporting that the prevalence was 2.1 percent in urban population and 1.5 percent in the rural population while in those above 40 years of age, the prevalence was 5 percent in urban population and 2.8 percent in rural areas.\textsuperscript{71} The results of recent studies showed escalating prevalence even within the Indian subcontinent rising to
In the periurban population the prevalence is found to be midway between the rural and urban populations (5.9 percent). In a study conducted in rural and urban populations of North India (Moradabad) the prevalence of diabetes was 2.8 percent and 6.0 percent respectively. In a cross sectional population survey conducted in Kashmir valley it was observed that 1.89 percent of the general population have known diabetes, 4.25 percent have undiagnosed diabetes and 8.09 percent have impaired glucose tolerance, making the total load of impaired glucose tolerance, 14.23 percent. The Chennai urban population study (CUPS) which looked at the prevalence of diabetes in two socioeconomic classes in Chennai, postulated the overall prevalence was 12 percent in the population aged above 20 years. The middle income group had significantly higher prevalence of type II DM compared to the lower income group (age standardized prevalence rates of 12.4 percent and 6.4 percent respectively). Meanwhile, a study from Manipur reported prevalence of 4 percent in population above 15 years of age.
Figure 2. Recent population based studies showing the prevalence of type II DM in different parts of India. In the recent National Urban Diabetes Survey (NUDS) in year 2000, population based study was conducted in six metropolitan cities across India. The prevalence of diabetes was found to be 13.5 percent among Chennai residents, in Bangalore 12.4 percent, Hyderabad 16.6 percent, Kolkata 11.7 percent, New Delhi 11.6 percent and in Mumbai 9.3 percent. Thus it is clear that in last two decades, there has been a marked increase in prevalence of diabetes among urban Indians.

The Prevalence of diabetes India study (PODIS) survey reports a low prevalence rate when compared to other previous studies (5.6 percent) but the sampling criteria and population size were different.
4. DEFINITION:

Diabetes mellitus is a metabolic syndrome, clinically characterized by polyuria, polyphagia, polydipsia, hyperglycemia and glycosuria due to absolute or relative deficiency of hormone insulin (either by action or by secretion or both, resulting in type I or type II DM respectively) that controls the metabolism of carbohydrate, protein, fat and electrolytes.

Once regarded as a single disease entity, diabetes is now seen as a heterogeneous group of diseases, characterized by a state of chronic hyperglycemia, resulting from a diversity of etiologies, environmental and genetic, acting jointly. The underlying cause of diabetes is the defective production or action of insulin, a hormone that controls glucose, fat and amino acid metabolism. Characteristically diabetes is a long term disease with variable clinical manifestations and progression. Chronic hyperglycemia, from whatever cause, leads to a number of complications: cardiovascular, renal, neurological, ocular and others such as inter-current infections.58
Diabetes mellitus comprise a group of common metabolic disorders that share the phenotype of hyperglycemia. Depending on the etiology of DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic dysregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with DM and on the health care system.58, 80-82

5. CLASSIFICATION (American Diabetes Association [ADA], 2004):58, 82

Diabetes Mellitus is classified on the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. Two features of the current classification of DM diverge from previous classifications. First, the terms insulin dependent diabetes mellitus (IDDM) and non insulin dependent diabetes mellitus (NIDDM) are obsolete. Since many individuals with type II DM eventually require insulin treatment for control of glycemia, the use of the term NIDDM generated considerable confusion. A second difference is that age is not a criterion in the classification system.

I. Type I DM (β-cell destruction, usually leading to absolute insulin deficiency).
   A. Immune-mediated
   B. Idiopathic

II. Type II DM (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretion defect with insulin resistance).

III. Other specific types of diabetes
   A. General defects of β-cell function characterized by mutations in:
1. [Hepatocyte nuclear transcription factor (HNF) 4 α ] MODY 1
2. (Glucokinase) MODY 2
3. (HNF-1 α ) MODY 3
4. [Insulin promoter factor (IPF) 1] MODY 4
5. (HNF-1 β) MODY 5
6. (Neuro D1) MODY 6
7. Mitochondrial DNA
8. Pro-insulin to insulin conversion

B. **Genetic defects in insulin action**
   1. Type A insulin resistance
   2. Leprechaunism
   3. Rabson-Mendenhall syndrome
   4. Lipodystrophy syndrome

C. **Diseases of the exocrine pancreas** - Pancreatitis, pancreatectomy, neoplasia, cystic fibrosis, hemochromatosis, fibrocalculus pancreatopathy.

D. **Endocrinopathies** - Acromegaly, Cushing's syndrome, Glucagonoma, Pheochromocytoma, Hyperthyroidism, Somatostatinoma, Aldosteronoma

E. **Drug or chemical induced** - Vacor, pentamidine, nicotinic acid glucocorticoids, thyroid hormone, diazoxide, β-adrenergic agonists, thiazides, phenytoin, α-interferon, protease inhibitors, clozapine, β-blockers.

F. **Infections** - Congenital rubella, cytomegalovirus, Coxsackie.


IV. Gestational diabetes mellitus (GDM)

6. PATHOGENESIS OF TYPE II DM:

Insulin resistance and abnormal insulin secretion are central to the development of type II DM. Most studies support that the primary defect is the insulin resistance which precedes insulin secretion defects and diabetes develops only if the insulin secretion becomes inadequate.\textsuperscript{80}

Earlier it was known as NIDDM. It constitutes about 85 percent of all cases of diabetes in developed countries and the majority of cases in some developing countries, especially those with a high prevalence of diabetes.\textsuperscript{81,82}

![Diagram of Etiopathogenesis of type II DM](image)

Figure 4. Etiopathogenesis of type II DM\textsuperscript{58}
6.1 Risk factors for type II DM \textsuperscript{58, 82}

The term risk factor is used by different authors with at least 2 meanings:

- An attribute or exposure that is significantly associated with the development of a disease.
- A determinant that can be modified by intervention thereby reducing the possibility of occurrence of a disease or other specified outcomes

Risk factors may characterize the individual, the family, the group, the community or the environment. The main risk factors independently associated with diabetes are:

**Non modifiable risk factors:**

1. Age
2. Family History of Diabetes

**Modifiable risk factors:**

1. Socioeconomic status
2. Alcohol consumption
3. Lack of physical activity
4. Lack of exercise
5. Dietary habits
6. Obesity

6.2 Etiology of type II DM \textsuperscript{58, 82}

The occurrence of type II DM is generally ascribed to primary predisposing factors, viz. genes and an adverse intrauterine environment; and secondary or tertiary precipitating factors such as obesity, low physical activity, increasing age, smoking, glucose and lipid toxicity. Both genes and environment play a role in the development of diabetes. According to the commonly accepted 'fetal origins' theory of adult disease, pro-diabetic genes (several possible) and fetal environment (mainly maternal
hyperglycemia or maternal and fetal under nutrition) lead to changes in fetal growth and metabolism and lead to 'programming' of possible future events viz, the pre-diabetic state and the diabetic state. The rapidity and extent to which the individual so programmed will progress is influenced by three possible factors, viz:

1. Over nutrition and physical inactivity.

2. Unmasking of the latent 'programmed' insulin resistance either at puberty or later and/or

3. Decreased pancreatic beta-cell function (due to genetic or environmental programming).

6.3 Pathophysiology of type II DM

Type II DM patients have detectable levels of circulating insulin. On the basis of oral glucose tolerance testing the essential elements of type II DM can be divided into 4 distinct groups; those with normal glucose tolerance, chemical diabetes (called impaired glucose tolerance), diabetes with minimal fasting hyperglycemia (fasting plasma glucose <140 mg/dl), and diabetes mellitus in association with overt fasting hyperglycemia (fasting plasma glucose >140 mg/dl). In patients with the highest levels of plasma insulin (impaired glucose tolerance group) there was also elevated plasma glucose. This indicates that these individuals are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus the level of insulin declines indicating that patients with type II DM have decreased insulin secretion.

Additional studies have subsequently demonstrated that both insulin resistance and insulin deficiency is common in the average type II DM patients. Many experts conclude that insulin resistance is the primary cause of type II DM; however, others contend that insulin deficiency is the primary cause because a moderate degree of insulin resistance is not
sufficient to cause type II DM. As indicated above, most patients with the common form of type II DM have both defects.\textsuperscript{58,82-84}

Type II DM is a multiple organ disease. The affected organs and systems include pancreas impaired insulin secretion; skeletal muscle insulin resistance; liver increased hepatic glucose output; adipose tissue increased lipolysis; gut decreased GLP-1 secretion; and possibly the kidney, the brain and other organs/systems. \textbf{Pathophysiological mechanisms leading to diabetes can involve inappropriate secretion of insulin; insulin insensitivity of liver, muscle and fat; and combined defects.}\textsuperscript{58,84-87}

7. CRITERIA FOR THE DIAGNOSIS OF DIABETES MELLITUS:\textsuperscript{58,88}

1. Symptoms of diabetes plus casual plasma glucose concentration \(\geq 200\) mg/dl (11.1mol/L). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

   Or

2. Fasting plasma glucose \(\geq 126\) mg/dl (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hr.

   Or

3. Two hour post load glucose \(\geq 200\) mg/dl (11.1 mmol/L) during an Oral Glucose Tolerance Test (OGTT). The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 gram anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine clinical use.
8. GLYCOSYLATED HEMOGLOBIN (HbA1c):

8.1 Introduction & History

At the present time, HbA1c is the most commonly measured method of assessing chronic glycemia in clinical practice. It has been used as a surrogate marker of long term glycemic control in patients with DM for more than 30 years, both in clinical practice and in countless studies and trials. Glycation of Hemoglobin occurs following exposure to glucose involving a 2 stage process within the erythrocyte. The first follows a transient rise in glucose leading to a reversible formation of an aldimine. Following prolonged exposure, an Amadori rearrangement takes place forming an irreversible ketoamine (fig.5). This effect is permanent until the destruction of the Hemoglobin.

![Figure 5. Conversion of hemoglobin to HbA1c](image-url)
The value of the $\text{HbA}_{1c}$ measurement is dependent on the amount of circulating glucose and that of Hemoglobin. The value of the $\text{HbA}_{1c}$ (which are expressed as a percentage of total Hemoglobin) gives a time-weighted indication of the average glucose over the lifespan of the red cell. HbA$_{1c}$ was first described in late 1960s, when Rahbar and his colleagues from the University of Tehran discovered a 'diabetic Hemoglobin component' on the electrophoresis of 2 patients with DM. This component was later found to be identical to the $\text{HbA}_{1c}$. The clinical utility of $\text{HbA}_{1c}$ as a measurement of glycemic control was initially proposed by Trivelli and colleagues in 1971 who suggested a possible relationship between mean blood glucose, long term diabetic complications and $\text{HbA}_{1c}$ values. This theory was later supported by numerous studies showing that the increased proportions of $\text{HbA}_{1c}$ in DM could reliably measure the glycemic control over the preceding 6-8 weeks. $\text{HbA}_{1c}$ was introduced into clinical use in the 1980s and subsequently has become a cornerstone of clinical practice.

8.2 $\text{HbA}_{1c}$ role in diagnosis and prognosis of Diabetes

$\text{HbA}_{1c}$ reflects average plasma glucose over the previous eight to 12 weeks period. It can be performed at any time of the day and does not require any special preparation such as fasting. These properties have made it the preferred test for assessing glycemic control in people with diabetes. More recently, there has been substantial interest in using it as a diagnostic test for diabetes and as a screening test for persons at high risk of diabetes. Owing in large part to the inconvenience of measuring fasting plasma glucose levels or performing an OGTT, and day-to-day variability in glucose, an alternative to glucose measurement for the diagnosis of diabetes has long been sought. $\text{HbA}_{1c}$ has now been recommended by an International Committee and by the ADA as a means to diagnose diabetes. Although $\text{HbA}_{1c}$ gives equal or almost equal sensitivity and specificity to a fasting or post-load glucose measurement as a predictor of
prevalent retinopathy, it is not available in many parts of the world and in general, it is not known which is better for predicting microvascular complications. Also, many people identified as having diabetes based on HbA1c will not have diabetes by direct glucose measurement and vice versa.\textsuperscript{102}

The relationship between HbA1c and prevalent retinopathy is similar to that of plasma glucose. This relationship was originally reported in the Pima Indians\textsuperscript{103} and has also been observed in several other populations including Egyptians,\textsuperscript{104} the National Health and Nutrition Examination Survey [NHANES] study in the USA,\textsuperscript{105} in Japanese.\textsuperscript{106} Overall, the performance of HbA1c has been similar to that of fasting or 2-hour plasma glucose. For all three measures of glycemia, the value above which the prevalence of retinopathy begins to rise rapidly has differed to some extent between studies. It is unclear whether HbA1c or blood glucose is better for predicting the development of retinopathy, but a recent report from Australia has shown that a model including HbA1c for predicting incident retinopathy is as good as or possibly better than one including fasting plasma glucose.\textsuperscript{107}

The use of HbA1c can avoid the problem of day-to-day variability of glucose values, and importantly it avoids the need for the person to fast and to have preceding dietary preparations. These advantages have implications for early identification and treatment which have been strongly advocated in recent years. However, HbA1c may be affected by a variety of genetic, haematologic and illness-related factors.\textsuperscript{108}

Some of the factors that influence HbA1c and its measurement\textsuperscript{108}

1. Erythropoiesis

Increased HbA1c : iron or vitamin B\textsubscript{12} deficiency, decreased erythropoiesis.
Decreased HbA1c : administration of erythropoietin, iron or vitamin B\textsubscript{12}, reticulocytosis, chronic Liver disease
2. Altered Hemoglobin
Genetic or chemical alterations in Hemoglobin: Hemoglobinopathies, HbF, methemoglobin, may increase or decrease HbA1c.

3. Glycation
Increased HbA1c: alcoholism, chronic renal failure, decreased intraerythrocyte pH.
Decreased HbA1c: aspirin, vitamin C and E, certain Hemoglobinopathies, increased intra-erythrocyte pH.
Variable HbA1c: genetic determinants.

4. Erythrocyte destruction
Increased HbA1c: increased erythrocyte life span: Splenectomy.
Decreased HbA1c: decreased erythrocyte life span: Hemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone.

5. Assays
Increased HbA1c: hyperbilirubinemia, carbamylated Hemoglobin, alcoholism, large doses of aspirin, chronic opiate use.
Decreased HbA1c: hypertriglyceridemia.
Variable HbA1c: Hemoglobinopathies.

A further major factor concern is the cost and availability of HbA1c assays in many countries. Also, the situation in several of these countries will be exacerbated by high prevalence of conditions such as Hemoglobinopathies, which affect HbA1c measurement, as discussed earlier. A report published in 2009 by an International Expert Committee (IEC) on the role of HbA1c in the diagnosis of diabetes recommended that HbA1c can be used to diagnose diabetes and that the diagnosis can be made if the HbA1c level is $\geq 6.5$ percent. Diagnosis should be confirmed with a repeat HbA1c test, unless clinical symptoms and plasma glucose levels $>11.1$ mmol/L (200 mg/dl) are present in which case further testing is not required. Levels of HbA1c just below 6.5 percent may indicate the presence...
of intermediate hyperglycemia. The precise lower cut-off point for this has yet to be defined, although the ADA has suggested 5.7–6.4 percent as the high risk range. While recognizing the continuum of risk that may be captured by the HbA1c assay, the International Expert Committee (IEC) recommended that persons with a HbA1c level between 6.0 and 6.5 percent were at particularly high risk and might be considered for diabetes prevention interventions. Long term prospective studies are required in all major ethnic groups to establish more precisely the glucose and HbA1c levels predictive of microvascular and macrovascular complications.

The diagnosis of diabetes in an asymptomatic person should not be made on the basis of a single abnormal plasma glucose or HbA1c value. At least one additional HbA1c or plasma glucose test result with a value in the diabetic range is required, either fasting, from a random (casual) sample, or from the OGTT. It is advisable to use one test or the other but if both glucose and HbA1c are measured and both are “diagnostic” then the diagnosis is made. If only one is abnormal then a further abnormal test result, using the same method, is required to confirm the diagnosis.

At the present time, the HbA1c is used worldwide as the marker of long term glycemic control and also a therapeutic target in the prevention and delay of the development of hyperglycemic complications. After reviewing the various studies related to glycated Hemoglobin, WHO in year 2011 has proposed HbA1c as a diagnostic tool for DM and as a screening test in identifying patients at future risk of developing the condition.

WHO Recommendation (2011)

HbA1c can be used as a diagnostic test for diabetes provided that stringent quality assurance tests are in place and assays are standardised to criteria aligned to the international reference values, and there are no conditions present which preclude its accurate measurement. An HbA1c of 6.5
percent is recommended as the cut off point for diagnosing Diabetes. A value of less than 6.5 percent does not exclude diabetes diagnosed using glucose tests.\textsuperscript{102}

Situations where HbA\textsubscript{1c} is not appropriate for diagnosis of diabetes:\textsuperscript{102}

- Acute Lymphoblastic Leukemia (ALL) children and young people
- Patients of any age suspected of having Type I DM
- Patients with symptoms of diabetes for less than 2 months
- Patients at high diabetes risk who are acutely ill (e.g. those requiring hospital admission)
- Patients taking medication that may cause rapid glucose rise e.g. steroids, antipsychotics
- Patients with acute pancreatic damage, including pancreatic surgery
- In pregnancy
- Presence of genetic, haematologic and illness-related factors that influence HbA\textsubscript{1c} and its measurement.

8.3 Glycemic control and relation to dyslipidemia, inflammation and oxidative stress

DCCT\textsuperscript{113} and UKPD\textsuperscript{114} studies have shown the impact of blood glucose control on reducing risk of retinopathy and nephropathy. Control of glucose levels as well as blood pressure, and cholesterol levels can delay or prevent the macrovascular and microvascular complications of diabetes.\textsuperscript{115-119} According to the ADA Guidelines 2007, the value of HbA\textsubscript{1c} should be kept below 7 percent in all diabetics.\textsuperscript{120} According to the same guidelines, HbA\textsubscript{1c} is now referred to as A\textsubscript{1c}. Values greater than 7 percent indicate an increased chance of progression to diabetic complications, especially microvascular ones. The ADA has also recommended that the lowering of HbA\textsubscript{1c} reduces the risk of microvascular and neuropathic complications and possibly, macrovascular complications.\textsuperscript{120}
HbA$_{1c}$ levels have been shown to be significantly higher in type II diabetics with proteinuria (nephropathy) compared to diabetics without proteinuria with the highest HbA$_{1c}$ in macroalbuminuria group of type II diabetics. Direct positive correlation of HbA$_{1c}$ values to serum creatinine levels with higher values has been found in the type II diabetic nephropathy (DN) patients. 

Serum cholesterol levels are shown to be significantly higher in the patients with macroproteinuria and proteinuria without retinopathy compared with non-proteinuric groups in diabetes. HbA$_{1c}$ shows direct and significant correlations with cholesterol, triglycerides and Low Density Lipoprotein (LDL) and inverse correlation with HDL. The levels of cholesterol, triglycerides and LDL have been shown to be higher along with HbA$_{1c}$ in diabetes patients with nephropathy compared to without nephropathy in studies by various authors.

Diabetic complications in target organs arise from chronic elevations of glucose. The pathogenic effect of high glucose, possibly in concert with fatty acids, is mediated to a significant extent via oxidative stress, carbonylic stress and inflammation. Oxidative stress causes insulin resistance, β-cell dysfunction and late diabetic complications. The carbonylic stress is reflected by the high values of glyoxal and methylglyoxal (the main dicarbonyls). These compounds are the major precursor of advanced glycation end-products (AGE) implicated in the development of diabetic complications. Present evidence supports the notion that atherosclerosis develops in parallel with type II DM, with both conditions sharing the common antecedent of activated innate immunity, but like hyperglycemia and possibly some other manifestations of type II DM such as obesity, macroangiopathy, once present, would presumably further enhance inflammation. Obesity was strongly related to elevated circulating levels of inflammatory markers, mainly CRP, in several cross-sectional studies in the general population and type II
Because the acute-phase response and cytokinemia are so closely related to insulin resistance, the relationship with hyperglycemia is not unexpected. **Lowering of blood glucose levels in type II DM patients is accompanied by reduced levels of inflammation markers.**¹³⁰ Diabetic patients with nephropathy have higher plasma ceruloplasmin, plasma uric acid and higher glycated hemoglobin compared to diabetics without nephropathy.¹³¹ Because of a positive correlation between serum high sensitivity C Reactive Protein (hs-CRP) and HbA₁c, inflammation, insulin resistance and hyperglycemia jointly contribute to the cardiovascular risk in type II DM men.²⁰,¹³³

Hyperglycemia induces the overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation. A significant difference in the mean plasma concentration of total antioxidant status is observed in diabetes patients.¹³¹ The findings of different studies suggest that diabetes is an altered metabolic state of oxidation-reduction and that it is convenient to give therapeutic interventions with antioxidants. Known sequelae of hyperglycemia such as cellular damage, increased extra cellular matrix production and vascular dysfunction have all been implicated in the pathogenesis of vascular disease in type I and type II DM.¹³⁵⁻¹⁴¹ Mechanisms involved in the increased oxidative stress in diabetes include not only oxygen free radical generation due to non-enzymatic glycosylation (glycation), auto-oxidation of glycation products, but also changes in the tissue content and activity of antioxidant defense systems. Increased levels of the products of oxidative damage to lipids have been detected in serum of diabetic patients, and their presence correlates with the development of complications.¹³⁷,¹⁴¹⁻¹⁴⁶ The increased glucose levels induces diabetes, the overproduction of oxygen free radicals and consequently increases the protein oxidation and lipid oxidation. Plasma MDA and protein carbonyl group (PCG) levels are significantly higher, while SOD is lower which would indicate that free radical mediated oxidative damage of lipids and proteins is produced in
Increase in lipid peroxidation and oxidative stress in diabetes indicate a positive correlation between the degree of hyperglycemia and oxidative stress. There are two proposed mechanisms for the observed inverse association between vitamin C and HbA1c: competition of the ascorbic acid and dehydro ascorbic acid with glucose for the reaction with the protein amino group, thereby inhibiting glycation or the anti-oxidant properties of vitamin C.

8.4 Advanced Glycation end products

Other hyperglycemia dependent metabolic abnormalities that may play role in the development of DN include formation of advanced glycation end products (AGEs) and polyols. Studies of the mechanisms connecting hyperglycemia with the complications of long term diabetes have provided a large body of evidence for the involvement of non enzymatic glycosylation process.

Non enzymatic glycosylation is a pathobiochemical process by which glucose is covalently bound to protein amino groups through a series of chemical reaction explained by Maillard. Maillard reactions are complex and their final products are heterogenous structures collectively called as AGEs. Proteins of many types are affected by this process, and the levels of tissue and circulating AGEs have been shown to correlate with microalbuminuria in diabetic patients. In a study of low and high molecular weight AGEs in subjects with and without diabetes, AGE content in arterial wall collagen was four folds in diabetics. Diabetic patients with end stage renal disease (ESRD) have twice as much tissue AGE as patients without renal disease. Circulating AGEs have been reported to be higher in diabetics compared to non diabetic individuals and their levels correlated directly with creatinine levels. Hyperglycemia accelerates the formation of non enzymatic glycosylation products which accumulate in vascular tissues. There are number of sites where non enzymatic protein glycosylation can affect the key processes in atherogenesis and
vascular remodeling. Indeed a close association has been noted between the accumulation of increased levels of AGEs and vascular disease.\textsuperscript{158}

AGEs alter the function of intracellular proteins and modify plasma proteins in a way that enables them to bind to AGE receptors on the surface of endothelial cells and macrophages, inducing oxidative stress and changes in gene transcription.\textsuperscript{143} In addition, AGEs modify extracellular matrix proteins which changes vessel permeability and remodeling capacity.\textsuperscript{143}

The functional role of AGEs in diabetic complications has been demonstrated by the use of AGE inhibitors (for example aminoguanidine and pyridoxamine) in diabetic animal models of nephropathy and retinopathy.\textsuperscript{159-161}

9. DIABETES AND INFLAMMATION:

Chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of type II DM. Since this hypothesis was first proposed in 1997 and 1998,\textsuperscript{162,163} numerous studies have shown that circulating markers of inflammation, acute-phase reactants, or interleukin-6 (IL-6) (the major cytokine mediator of the acute-phase response) are strong predictors of the development of type II diabetes.\textsuperscript{164-170}

Several cross-sectional studies in non diabetic subjects or the general population,\textsuperscript{171-179} or in individuals with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG),\textsuperscript{180-183} have confirmed that acute phase reactants such as CRP (and sometimes the cytokines IL-6 and Tumour necrosis factor-alpha [TNF-\(\alpha\)]) are positively correlated with measures of insulin resistance/plasma insulin concentration, Body Mass Index (BMI)/waist circumference, and circulating triglyceride and negatively correlated with HDL cholesterol concentration.

Additional cross-sectional studies in newly diagnosed\textsuperscript{182} or established type II diabetic patients\textsuperscript{18,184-189} have confirmed that inflammatory and acute-phase markers such as CRP, IL-6, TNF-\(\alpha\), nitric oxide, ceruloplasmin, ferritin, sialic acid and others are elevated in these subjects compared
with nondiabetic control subjects. The inflammatory marker, serum sialic acid, is cross-sectionally related to CHD in type II DM\textsuperscript{190} and also predicts future cardiovascular mortality in type II DM, independent of baseline atherosclerosis.\textsuperscript{191} Low-grade chronic inflammation, as reflected by elevated circulating levels of inflammatory cytokines, may promote insulin resistance in liver, skeletal muscle, and vascular endothelium,\textsuperscript{192,193} ultimately leading to the clinical expression of both type II DM and CVD.\textsuperscript{194} These studies suggest that activation of the innate immune system is likely to be at least one of the long-postulated\textsuperscript{195} common antecedents of both atherosclerosis and type II DM.\textsuperscript{191} Because the acute-phase response and cytokinemia are so closely related to insulin resistance, the relationship with hyperglycemia is not unexpected. Lowering of blood glucose levels in type II DM patients is accompanied by reduced levels of inflammation markers.\textsuperscript{130}

Figure 6. Several factors such as altered nutrition, inactivity, age, fetal metabolic programming, and genetic propensity are known activators of the innate immune system. Cytokine production leads to insulin resistance (possibly impaired insulin secretion), type II diabetes, and other components of the metabolic syndrome, such as dyslipidemia. Activated innate immunity is a possible common antecedent of both type II DM and atherosclerosis.\textsuperscript{130}
9.1 Acute Phase Proteins

In addition to local effects in inflammation, there is a systemic reaction known as the acute-phase response, best characterized by pronounced changes in the concentration of certain circulating proteins and other substances, called acute-phase reactants (APP).\textsuperscript{196-198} APP usually increase in concentration, with examples being CRP, complement, serum amyloid A, α 1-acid glycoprotein, haptoglobin, ceruloplasmin, sialic acid and fibrinogen, but some such as albumin are negative acute-phase reactants that decrease in concentration. The APP are mostly synthesized in the liver, but they can also be synthesized by adipocytes, fibroblasts and endothelial cells.\textsuperscript{199} The production is stimulated by cytokines of the innate immune response-mainly IL-6 and TNF-α (Fig.7).\textsuperscript{23} In general, the APP limit injury or aid healing.\textsuperscript{23}

APP changes are only part of a large number of systemic manifestations, distant from inflammatory sites that replace normal homeostasis during inflammatory states. **APP changes are not limited to acute illness, but persist during a great variety of chronic inflammatory states as well, constituting a semantically paradoxical chronic acute-phase response.** A change of approximately 25 percent in plasma concentration has been suggested as the definition of an APP.\textsuperscript{200} Changes in plasma protein concentrations largely result from alterations in synthesis by hepatocytes in response to circulating inflammation associated cytokines. While other cells, including macrophages, fibroblasts, epithelial cells and adipocytes can also produce APPs, it is unlikely that synthesis at these sites contributes significantly to plasma concentrations.\textsuperscript{201}
Psychological stress

Figure 7. The components of the innate immune system. Sentinel cells such as the macrophage detect potential environmental threats from infection, chemicals, and foods by PRRs that activate signaling pathways and release proinflammatory cytokines (IL-6 and TNF-α). Known PRRs include TLR-4, which senses bacterial LPS and the receptor for AGEs. Cytokines stimulate acute phase protein production from the liver and also act on the brain to release adrenocorticotropic hormone (and thereby cortisol from the adrenal gland) and activate the sympathetic nervous system with the release of catecholamines. Psychological stress can cause an acute-phase response via innervation of cytokine-producing cells and via activation of the sympathetic nervous system and adrenergic receptors on macrophages. Central cytokine-induced "sickness behavior" includes lethargy, sleep changes, and depression. The innate immune system also controls the adaptive (acquired) immune system via costimulatory molecule expression that is necessary for antigen presentation. (PRR - pattern recognition receptor, TLR- Tyrosine like receptor, LPS - Lipopolysaccharide, RAGE- Receptor for AGE, ACTH – Adrenocorticotropic hormone, SAA- serum amyloid A)\textsuperscript{130}

Positive and Negative Acute-phase Proteins-

As indicated above, circulating levels of plasma proteins can increase (positive APPs) or decrease (negative APPs) during the acute-phase response. Changes in different proteins occur at different rates and to different degrees. Rapidity of change of plasma APP concentrations generally parallels magnitude of change. Ceruloplasmin and the complement components C3 and C4 exhibit relatively modest acute-phase behaviour (typically about 50 percent increases). Concentrations of haptoglobin, α1-acid glycoprotein, α-1 protease inhibitor, α1-antichymotrypsin and fibrinogen ordinarily increase about 2–5-fold. The two major APPs in humans, CRP and serum amyloid A (SAA) protein, are normally present in only trace amounts, but may exhibit dramatic increase
(1000-fold or more) in individuals with severe infections. In contrast, plasma concentrations of negative APPs such as albumin, transferrin, transthyretin, α-2 HS glycoprotein, α-fetoprotein, T4-binding protein globulin, insulin-like growth factor I and coagulation factor XII, typically decrease during the acute-phase response.\textsuperscript{201}

APPs can be classified into different categories based on their functions. Examples of these categories include the following-

1. Members of the complement system: complement factors C3, C4, C9, factor B, C-1 inhibitor, binding protein and mannose-binding lectin.

2. Members of the coagulation and fibrinolytic systems: plasminogen, tissue plasminogen activator, urokinase, protein S, vitronectin and plasminogen activator inhibitor1.

3. Antiproteases: α-1 protease inhibitor, α1-antichymotrypsin, pancreatic secretory trypsin inhibitor and inter-α1-trypsin inhibitors.


Some positive APPs can play a role as modulators of the inflammatory response. These include secreted phospholipase A2, lipopolysaccharide (LPS)-binding protein, and interleukin 1 receptor antagonist (IL-1Ra).\textsuperscript{202}

Finally, some APPs cannot be easily classified functionally because their function is still not completely clarified or cannot be included in one typical category. These include CRP, SAA, α1-acid glycoprotein, fibronectin, angiotensinogen and ferritin.\textsuperscript{201}

Two decades ago, Crook et al showed that, in comparison with nondiabetic subjects, circulating concentrations of commonly recognized acute-phase reactants were increased in type II but not type I diabetic patients who were matched for age, sex, glycemic control, and the absence of tissue complications.\textsuperscript{203} These acute-phase reactants included CRP, serum amyloid A, α1-acid glycoprotein, and sialic acid (the latter is an integrated measure of the acute-phase response because many of the APP are glycoproteins with sialic acid as the terminal sugar of the oligosaccharide
chain). Serum levels of acute-phase reactants (including cortisol) and the cytokine mediator of the acute phase response, IL-6, showed a graded increase with increasing features of the metabolic syndrome in type II diabetic and non diabetic subjects, i.e. obesity, coronary heart disease, hypertension, hyper-triglyceridemia, and low levels of HDL.  

9.1.1 C Reactive Protein

C-reactive protein is a protein found in the blood, the levels of which rise in response to inflammation (i.e. CRP is an APP). Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via the C1Q complex. CRP is synthesized by the liver in response to factors released by fat cells (adipocytes). It is a member of the pentraxin family of proteins. It is not related to C-peptide or protein C. C-reactive protein was the first pattern recognition receptor (PRR) to be identified. CRP was so named because it was first discovered as a substance in the serum of patients with acute inflammation that reacted with the capsular (C) polysaccharide of pneumococcus. Discovered by Tillett and Francis in 1930, it was initially thought that CRP might be a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer, however, discovery of hepatic synthesis demonstrated that it is a native protein. CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of inflammatory cytokines such as IL-6 and TNF-α, which are produced predominantly by macrophages as well as adipocytes. CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin mediated phagocytosis), which express a receptor for CRP. It is also
believed to play another important role in innate immunity, as an early defense system against infections.

In the past decade, it has become widely accepted that inflammation plays a key role in the pathogenesis of CVD. CRP, an APP and marker of chronic, low-grade inflammation, is a reliable predictor of CVD.\(^{213}\) Data from prospective, epidemiologic studies revealed a significant association between CRP and future CHD risk in apparently healthy subjects.\(^{214}\) Similarly, high plasma CRP has been shown to be an independent risk factor for CHD deaths in type II DM patients.\(^{215}\) It is perceived that chronic low-grade inflammation as evidenced by elevated hs-CRP might potentially be a cause underlying the etiology and manifestation of type II DM, although the exact mechanisms are still not well understood.\(^{165,216}\)

**Hyperglycemia is an associated factor to the increase of serum CRP levels, in uncontrolled type II diabetic subjects.**\(^{217}\) Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with BMI, family history of DM, smoking and other factors.\(^{165}\) In people with diabetes, CRP levels in highest tertile ( > 0.28 mg/dl) were associated with a 2 fold increase in cardiovascular mortality after adjusting for age, sex and glucose tolerance tests.\(^{218-220}\) Hypertensive patients with type II DM had higher levels of hs-CRP, a circulating inflammatory marker, than normal subjects. This finding suggests that patients with two associated diseases have a more active inflammatory state.\(^{221,222}\) More than 20 large prospective trials have shown that the inflammatory biomarker hs-CRP is an independent predictor of future cardiovascular events plus it predicts risk of incident hypertension and diabetes.\(^{223}\) In type I and type II DM, Hb A\(_{1c}\) significantly correlates with hs-CRP levels and future cardiovascular risk. Also, hs-CRP levels increase with the stage of beta-cell dysfunction and insulin resistance.\(^{220}\) In few studies serum glycated albumin and hs-CRP levels were significantly
elevated and were independent predictors of coronary artery disease (CAD) in patients with type II DM and CAD.\textsuperscript{21,224}

9.1.2 Sialic Acid

Sialic acids comprise of N-or O-acyl derivatives of 9- carbon sugar neuraminic acid (5- amino- 3, 5-dideoxy- D-glycero-D-galacto-non-2-ulosonic acid). Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids. In human beings it is present in body fluids (blood plasma, breast milk, gallbladder excretions, synovial fluid, sweat, gastric juices and urine) and tissues (erythrocytes, leucocytes, platelets, salivary glands, throat, stomach, cervix, colon, cartilage etc).\textsuperscript{225} In blood plasma a large quantity of sialic acids are present in orosomucoid, fibrinogen, haptoglobin, ceruloplasmin, $\alpha_1$-antitrypsin, complement proteins and transferrin.\textsuperscript{226,227} It is also present as constituent of membrane glycoproteins of erythrocytes, leucocytes and platelets. About 80 percent of sialic acid in human serum is N-acetyleneuraminic acid (Neu5Ac; NANA) and approximately 20 percent is N-acetyl-9-o-L-lactoylneuraminicacid.\textsuperscript{228} Low amount of N-acetyl-9-o-acetyleneuraminicacid, have also been shown to be present in human blood serum.\textsuperscript{229} Similarly, in tissues the major sialic acid is NANA. The other small fractions are 2-deoxy-2, 3-didehydro-D-N-acetyleneuraminic acid, N-glycoloneuraminicacid, N-acetyl-7,9-di-0-acetyleneuraminicacid, N-acetyl-8,9-di-0-acetyleneuraminicacid etc, the role of which is yet to be defined. Most of the research papers reported about NANA, the most abundant sialic acid. The structure, occurrence and general functions of sialic acids have been extensively reviewed.\textsuperscript{230-235} Some important functions attributed to sialic acids are

1) Sialic acids contribute significantly to the overall negative charge of cell surface and glycoproteins. The negative charge contributes to cell to cell repulsion (anti adhesion effect), functioning stability and
survival of glycoproteins in blood circulation and cell-to-matrix interactions.

(2) Due to the shielding effect, sialylated glycans protect parts of a glycoprotein from proteolytic attacks.

(3) Membrane sialic acids assist in cell to cell recognition and interaction and serving as chemical messengers in tissues and body fluids.

(4) It serves as a component of cell surface receptors. (e.g., insulin receptor), and is positively associated with most of the serum acute phase reactants.236

Serum Sialic Acid

Sporadic reports on serum sialic acid in CVD were published in 70s and 80s but the real interest in serum sialic acid in CVD grew only after the report of Lindberg et al.237 who showed that serum sialic acid is a strong predictor of cardiovascular mortality and may also reflect the existence or activity of an atherosclerotic process. Later on, many studies came up showing that serum sialic acids were elevated in CVD.238-245 Serum sialic acid levels correlated with carotid atherosclerosis, independently of major cardiovascular risk factors.246 It was also found to be raised in patients with NIDDM who were designated as a group with a markedly increased frequency of CHD, stroke and peripheral vascular diseases compared to nondiabetics.247 Serum sialic acid is considered as a marker of innate immunity and activated innate immunity is a risk factor for CVD in type II DM.191 Most recently, in a 17 year-follow up study serum sialic acid has been proposed to be a long - term predictor of CHD events in adults, especially in women.248

Serum total sialic acid (TSA) has been correlated with serum lipids.249,250 It was found to be higher in groups with high serum triglycerides or cholesterol and significantly lower in a group with high HDL cholesterol. Crook et al.251 showed that serum TSA significantly correlated with
systolic BP, fasting serum cholesterol and triglycerides and BMI in females. In males serum TSA significantly positively correlated with fasting serum cholesterol and triglycerides concentration and correlated inversely with hip to waist ratio. **Serum TSA is a newly established potential risk factor for the development of macro and microvascular complications of diabetes.**

Masuda et al.\textsuperscript{253} have shown that serum TSA reflects the status of blood glucose control and the progression of ischemic disease of the lower extremities in NIDDM. Zahedi et al.\textsuperscript{254} have found that it increased post prandially giving further insight as to why it is considered to be a cardiovascular risk factor. Serum TSA has shown positive correlations with blood platelet count, plasma fibrinogen, D-dimer, thrombin-antithrombin III complex and plasma alpha 2-plasmin inhibitor complex in type II DM.\textsuperscript{255}

### 9.1.3 Ceruloplasmin

Ceruloplasmin (Cp) is a ferroxidase enzyme that in humans is encoded by the \textit{CP} gene.\textsuperscript{256,258} **Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism.** It was first described in 1948.\textsuperscript{259} It is an enzyme synthesized in the liver containing 6 atoms of copper in its structure. Ceruloplasmin carries about 70 percent of the total copper in human plasma while albumin carries about 15 percent. The rest is accounted for by macroglobulins.

It is secreted into the plasma as a 2-glycoprotein.\textsuperscript{260,261} Although its precise biological roles are unknown, it may be related to angiogenesis,\textsuperscript{262} copper transport,\textsuperscript{263} iron metabolism,\textsuperscript{264} and antioxidant defense.\textsuperscript{265} **Ceruloplasmin exhibits a copper-dependent oxidase activity, which is associated with possible oxidation of ferrous iron (Fe\textsuperscript{2+}) into ferric iron (Fe\textsuperscript{3+}), therefore assisting in its transport in the plasma in association with transferrin, which can carry iron only in the ferric state.** Like any other
plasma protein, levels drop in patients with hepatic disease due to reduced synthesizing capabilities.

**Decreased levels**

- Wilson disease (a rare copper storage disease)
- Menkes disease (Menke kinky hair syndrome) (very rare)
- Overdose of Vitamin C
- Copper deficiency
- Hereditary ceruloplasmin deficiency (HCD)

**Elevated levels**

- Pregnancy
- Oral Contraceptive Pill use
- Lymphoma
- Acute and chronic inflammation (it is an acute-phase reactant)
- Rheumatoid Arthritis
- Angina
- Alzheimer's disease
- Schizophrenia
- Obsessive-compulsive disorder

HCD is an autosomal recessive disease characterized by neurological abnormalities such as progressive cerebral degeneration, complete Cp deficiency, and excessive storage of iron in the systemic organs, such as liver and brain. In many HCD cases, type II DM is the first symptom, and 5–20 years later at ages 40–60 years, the neurological abnormalities occur.

**Ceruloplasmin is an APP.** The change in the plasma concentration is due largely to change in the production by hepatocytes. The magnitude of the increase may be about 50 percent for ceruloplasmin. In general, the APP limit injury or aid healing. It is known that ceruloplasmin has antioxidant properties because of its ferroxidase activity. Alternatively, ceruloplasmin
is thought to be a scavenger of ROS\textsuperscript{272} and plays an important role in nitrosothiol formation, which may contribute to its potent antioxidant activities.\textsuperscript{273} Recently \textbf{unexpected, prooxidant effects of plasma ceruloplasmin were demonstrated by some authors}. An increase in serum ceruloplasmin in type II DM could generate excess oxidized LDL, which causes atherosclerosis.\textsuperscript{274}

These observations support the hypothesis that copper bound at specific sites on protein surfaces can cause oxidative damage to macromolecules in their environment.\textsuperscript{275} Ceruloplasmin could also cause vascular injury by generating free radicals, such as hydrogen peroxide (H$_2$O$_2$), in the course of oxidization of serum homocysteine.\textsuperscript{276}

An increase in serum Cp levels has also been reported in type II DM.\textsuperscript{277-280} However, it has been reported that blood HbA$_1c$ levels, duration of type II DM, patient age, and the presence or absence of diabetes complications are not major factors influencing its increase.\textsuperscript{277-281}

\textbf{10. LIPIDS IN DIABETES:}

\textbf{10.1 Introduction}

Several studies have revealed an excess morbidity and mortality from CVD in type I and type II diabetic patients.\textsuperscript{282,283} Thus, international treatment guidelines consider diabetes mellitus a high-risk condition for development of CVD, and treatment with cardio protective agents such as lipid lowering agents, aspirin, and angiotensin converting enzyme inhibitors are highly recommended for many diabetic patients.\textsuperscript{284,285}

Epidemiologic studies have demonstrated that \textbf{DM is an independent risk factor for CVD} and it amplifies the effects of other common risk factors, such as smoking, hypertension and hypercholesterolemia.\textsuperscript{286,287} The mortality associated with a coronary event in diabetics is significantly higher than the mortality in nondiabetic individuals.\textsuperscript{288} The increased risk of atherosclerosis in DM consists of multiple factors. Diabetes related
changes in plasma lipid levels are among the key factors that are amenable to intervention. The spectrum of dyslipidemia in diabetes mellitus can include all the various types of dyslipidemia identified in the general population. However, one phenotype is particularly common in DM, which is attributed mostly to insulin resistance and insulin deficiency. The characteristic features of this phenotype are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL cholesterol particles.

In the Framingham Heart Study, both men and women with diabetes had an increased prevalence of hypertriglyceridemia and low HDL cholesterol levels, but their total cholesterol and LDL cholesterol levels did not differ from those of their non diabetic counterparts. A similar pattern of altered plasma lipid profile was observed in the UKPDS. In this study, total cholesterol levels of those with diabetes mellitus and control individuals did not differ. However, women with type II DM had markedly higher LDL cholesterol levels than women who were not diabetic. The plasma triglyceride levels of patients with type II DM were substantially increased, whereas HDL cholesterol levels were markedly reduced in both men and women with DM compared with the non diabetic controls.

10.2 Pathophysiology of Diabetic Dyslipidemia

The precise pathogenesis of diabetic dyslipidemia is unknown. Various studies suggest that insulin resistance has a central role in the development of this condition. The main cause of the three cardinal features of diabetic dyslipidemia is the increased free fatty-acid release from insulin-resistant fat cells. The increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production, which in turn stimulates the secretion of apolipoprotein B (ApoB) and very low density lipoprotein (VLDL) cholesterol. The impaired ability of insulin to inhibit free fatty-acid release leads to enhanced hepatic VLDL cholesterol production.
which correlates with the degree of hepatic fat accumulation.\textsuperscript{297} Hyperinsulinemia is also associated with low HDL cholesterol levels.\textsuperscript{298,299}

The increased number of VLDL cholesterol particles and increased plasma triglyceride levels decrease the level of HDL cholesterol and increase the concentration of small dense LDL cholesterol particles via several processes: VLDL-transported triglyceride is exchanged for HDL-transported cholesteryl ester through the action of the cholesteryl ester transfer protein (CETP), which results in increased amounts of both atherogenic cholesterol-rich VLDL remnant particles and triglyceride-rich, cholesterol-depleted HDL particles. The triglyceride enriched HDL is subsequently hydrolyzed by hepatic lipase or lipoprotein lipase. Apolipoprotein A-I (Apo A-I) dissociates from the reduced-size HDL, which is filtered by the renal glomeruli and degraded in renal tubular cells.\textsuperscript{299,300} The increased concentration of small dense LDL-cholesterol particles is explained by a similar lipid exchange. Increased levels of VLDL-transported triglyceride enable CETP to promote the transfer of triglyceride into LDL in exchange for LDL-transported cholesteryl ester. The triglyceride-rich LDL undergoes hydrolysis by hepatic lipase or lipoprotein lipase, which results in lipid-depleted small dense LDL particles. The relative importance of the above lipid exchange pathway in individuals with low HDL cholesterol levels who do not have increased VLDL cholesterol production or hyper triglyceridemia is not known. In these patients, inability of insulin to upregulate the Apo A-I production (owing to insulin resistance) might contribute to low HDL cholesterol levels.\textsuperscript{300} Furthermore, insulin resistance and low HDL levels might have a common mediator; for example, TNF-\(\alpha\). \textbf{TNF-\(\alpha\) is implicated in obesity-related insulin resistance and is known to lower serum HDL cholesterol levels.}\textsuperscript{300,301} In addition, several key enzymes that are involved in HDL cholesterol metabolism are altered in people with insulin resistance.\textsuperscript{302,303} Insulin resistance is associated with a decreased ratio of lipoprotein lipase to hepatic lipase in heparin-treated plasma, which contributes to the low HDL-cholesterol level seen in such individuals.\textsuperscript{299}
In insulin resistance, the esterification of cholesterol (mediated by lecithin-cholesterol acyl transferase) is either modestly increased or unaltered, whereas CETP activity is increased.

CETP depletes HDL of its cholesteryl ester and its increased activity contributes to the lowering of HDL cholesterol levels. Plasma CETP mass is a determinant of cholesteryl ester transfer, and has an increased effect in individuals with high triglyceride levels. In addition, adiponectin might have a direct role on HDL cholesterol catabolism. Kinetic studies show a strong negative correlation between adiponectin level and the Apo A-I fractional clearance rate, which can explain the positive correlation between the levels of HDL cholesterol and adiponectin. This positive correlation occurs independently of obesity, insulin resistance and the triglyceride content of HDL cholesterol particles. The high triglyceride and low HDL cholesterol levels might occur in familial and sporadic syndromes (e.g. familial combined hyperlipidemia and familial hypertriglyceridemia), but only combined hyperlipidemia seems to be generally associated with increased cardiovascular risk. Overall, insulin resistance seems to contribute either directly or indirectly to the triad of plasma lipid abnormalities of diabetes mellitus, namely hypertriglyceridemia, low HDL-cholesterol levels and high small dense LDL-cholesterol levels.

Low HDL cholesterol and increased triglyceride levels might also contribute to the increased risk of CVD found in patients with diabetes mellitus. Emerging data suggest that hypertriglyceridemia, in conjunction with increased small dense LDL cholesterol and low HDL cholesterol levels, is an important contributor to accelerated atherosclerosis in DM and insulin-resistant conditions. However, the association between hypertriglyceridemia and the increased risk of CVD is not as strong as that between LDL cholesterol level and CVD risk. Patients with elevated triglyceride levels might have accompanying dyslipidemias that increase
the risk for CVD (e.g. familial combined hyperlipidemia or low HDL level).\textsuperscript{306}

10.3 Dyslipidemia in Diabetic Nephropathy

Diabetic nephropathy (DN) is associated with an altered lipid profile characterised by elevated triglyceride rich lipoproteins, in particular VLDL, but also LDL and thus, plasma triglycerides are high.\textsuperscript{307} The levels of HDL are low as a secondary phenomenon.\textsuperscript{307} Elevated plasma concentrations of Apo-B, Apolipoprotein C-III and lipoprotein (a) have also been reported.\textsuperscript{307,308} However, there still seems to be uncertainty on the underlying mechanisms, but changes in lipoprotein lipase (LPL) and hepatic lipase (HL) have been suggested. An increased HL-activity and a reduced post heparin plasma LPL/HL ratio have been reported.\textsuperscript{308} These multiple lipoprotein alterations become more accentuated with declining renal function and increasing urinary albumin excretion.\textsuperscript{309} When compared with non-diabetic patients having renal failure, the lipid abnormalities are more marked in DN, probably reflecting an additional effect of the diabetic state and, in particular, the level of glycemia and the relative insulin deficiency.\textsuperscript{310} The lipid disorders seen in chronic renal failure resemble those seen in the metabolic syndrome and it has also been speculated that the insulin resistance seen in uremic patients may also be associated with some of the observed lipid disorders.\textsuperscript{311}

The total and LDL cholesterol levels usually are normal or slightly elevated in renal failure and the diameter of LDL particles has been reported to be smaller, i.e. small dense LDL, in patients with both incipient and overt DN.\textsuperscript{312} Small dense LDL are more readily oxidized and glycosylated and they are also more deleterious to vessel walls than normal, larger LDL particles.\textsuperscript{312} LDL cholesterol seems to have a similar effect on glomerular mesangial cells as on endothelial cells. Mesangial cells are closely related to vascular smooth muscle cells and possess binding sites for LDL and oxidized LDL. They help recruit macrophages and then
secrete proliferative factors inducing glomerulosclerosis, i.e. a process similar to the role of endothelial cells in the process of atherosclerosis.\textsuperscript{313}

Another factor that may reduce insulin sensitivity is an elevated level of non-esterified fatty acid (NEFA).\textsuperscript{314} However, NEFA levels are not generally elevated in chronic renal failure (CRF)\textsuperscript{315} and the anti-lipolytic effects of insulin in uremic patients are comparable with those in healthy subjects.\textsuperscript{316}

\section*{11. OXIDANTS AND ANTIOXIDANTS IN DIABETES:}

\subsection*{11.1 Free Radicals}

\subsubsection*{11.1.1 Basic concept of free radicals}

In 1956, Denham Harman, father of the free radical theory postulated that free radicals produced during aerobic respiration cause cumulative oxidative damage, resulting in aging and death. Free radicals are generally considered harmful byproducts of oxidative metabolism,\textsuperscript{317} causing molecular damage in living systems. This concept has implications in numerous biological phenomena such as cellular aging, mutagenesis, inflammation, and other pathologies. Furthermore, it has been suggested that free radicals are implicated in the process in part for the development of diabetic microangiopathy and macroangiopathy,\textsuperscript{318} and excessive free radical production has been reported in diabetics with CRF treated by haemodialysis.\textsuperscript{319} Consequently, free radical mechanisms have been implicated in the pathogenesis of tissue damage in diabetes.\textsuperscript{135,142,317,320} The term "free radical" can be defined as any atoms or molecules that contain an unpaired electron in its outer obit that can exist independently.\textsuperscript{135,321} As a result, they can be highly reactive, although this varies from radical to radical, reacting locally to accept or donate electrons to other molecules to achieve a more stable state. Ground state O$_2$ (\textsuperscript{3}O$_2$) has two unpaired electrons each located in a different antibonding orbital. An oxidizing agent, such as O$_2$ is effective at absorbing electrons from the molecule it oxidizes.\textsuperscript{135} The collective terms reactive oxygen species
(ROS) or active oxygen species have been applied for a variety of free radicals and non-radicals intermediates.\textsuperscript{321}

### 11.1.2 Patho-physiology of free radicals

Free radicals are formed in large amounts as an unavoidable byproduct of many biochemical processes and in some instances, deliberately, such as in activated neutrophils. In addition, free radicals can be generated in the body in response to electromagnetic radiation from the environment and acquired directly as oxidizing pollutants such as ozone and nitrogen dioxide.\textsuperscript{321} However, most of the free radicals in biological systems are oxygen-derived free radicals. The complete reduction of oxygen to $\text{H}_2\text{O}$ requires four steps and the generation of several free radicals and $\text{H}_2\text{O}_2$, which in itself is not a free radical. $\text{H}_2\text{O}_2$ is however, considered a ROS because of its ability to generate highly reactive hydroxyl free radicals through interactions with reactive transition metals.\textsuperscript{322,323} The complete reduction of oxygen can be explained in the following equations (1 to 4).

\begin{align}
(1) \quad & \text{O}_2 + e^{-} \rightarrow \text{O}_2^- \quad \text{superoxide radical} \\
(2) \quad & \text{O}_2^- + \text{H}_2\text{O} \rightarrow \text{HO}_2^+ + \text{OH}^- \quad \text{hydroperoxyl radical} \\
(3) \quad & \text{HO}_2^+ + e^- + \text{H} \rightarrow \text{H}_2\text{O}_2 \quad \text{hydrogen peroxide} \\
(4) \quad & \text{H}_2\text{O}_2 + e^- \rightarrow \cdot\text{OH} + \text{OH}^- \quad \text{hydroxyl radical}
\end{align}

Each of these oxygen-derived intermediates are considered highly reactive species because their electron configurations allow for the attraction of electrons from other molecules, resulting in the formation of other free radicals that are capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation, DNA damage, and protein degradation during oxidatively stressful events. Although all the intermediates are potentially reactive, the intermediates vary in their biological importance. The superoxide radical is the leading oxygen-derived free radical and, unlike the other oxygen derived intermediates, can lead to the formation of additional ROS.\textsuperscript{324} Furthermore, the protonation of superoxide anion radicals results in the formation of perhydroxyl radical
(HO₂⁻), a much more aggressive radical than the superoxide radical itself. In addition, superoxide acts as a Bronsted base in aqueous solutions to shift the acid-base equilibrium to form a hydroxyl (·OH) radical thereby forming hydrogen peroxide in acidic environments. H₂O₂, although not a free radical by definition is a biologically important oxidant because of its ability to generate the hydroxyl radical, an extremely potent radical species. Furthermore, because of its nonionized and low charge state, H₂O₂ is able to diffuse through hydrophobic membranes, as observed with the leakage of H₂O₂ from mitochondria. Hydroxyl radicals are formed not only by the reduction of H₂O₂ but also through the interaction between H₂O₂ and the reduced forms of metal ions, i.e., copper and iron. The ability of the hydroxyl radical to remove or add hydrogen molecules to the unsaturated hydrogen bonds of organic lipids makes it potentially one of the most reactive oxidants in biological systems. Furthermore, the hydroxyl radical is the most powerful oxidant that can readily attack polyunsaturated fatty acids to initiate lipid peroxidation. Consequently, the most toxic oxygen free radical involved in pathological processes is the hydroxyl radical (·OH). In biological systems, ·OH derives from the less toxic superoxide anion radical (O₂⁻) and H₂O₂ via the Haber-Weiss and Fenton reactions (5 & 6).

$$\text{(5) } \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \text{OH}^- + \text{O}_2$$

$$\text{Haber-Weiss Reaction}$$

$$\text{(6) } \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \cdot\text{OH} + \text{OH}^- + \text{Fe}^{3+}$$

$$\text{Fenton Reaction}$$

Figure. 8 Damage produced by ROS
11.2 Antioxidant defense systems

Antioxidants are defined as any substance that when present at low concentrations, compared with those of the oxidative substrate considerably delays or inhibits oxidation of the substrate.\textsuperscript{135,321,322} Antioxidants can act at many different stages in an oxidative sequence including removing oxygen or decreasing local oxygen concentrations, removing catalytic metal ions, removing key ROS such as oxygen and \( \text{H}_2\text{O}_2 \), scavenging initiating free radicals, breaking the chain of an initiated sequence, and quenching or scavenging singlet oxygen species.\textsuperscript{135,322}

Furthermore, a variety of antioxidant defense systems operates, including enzymatic and non-enzymatic antioxidants.\textsuperscript{321} Enzymatic antioxidants directly involved in the detoxification of ROS are SOD and hydroxyperoxidases such as catalase (CAT) and glutathione peroxidase (GSHPx), a selenium-containing enzyme glutathione (GSH).\textsuperscript{322,321} Cells have formidable defense mechanisms against oxidative damage of which some may not be readily recognizable as antioxidants.\textsuperscript{322} Enzyme such as SOD rapidly promote the dismutation of superoxide into \( \text{H}_2\text{O}_2 \) and oxygen at a rate considerably faster than it occurs uncatalyzed. Two different SOD are found in mammalian tissue, namely a Cu/Zn-containing enzyme which is found in the cytoplasm of most cells, and a further Mn-containing enzyme present within the mitochondrial compartment.\textsuperscript{326,322} Both enzymes catalyze the same reaction as shown below.

\[
2 \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2
\]

\( \text{H}_2\text{O}_2 \), a product of the dismutation reaction, can be destroyed by two enzymes, CAT and GSHPx. Glutathione peroxidase can metabolise \( \text{H}_2\text{O}_2 \), generated by SOD, by oxidizing the tripeptide glutathione into its oxidized from (GSSG). In addition, CAT transforms \( \text{H}_2\text{O}_2 \) into water and oxygen\textsuperscript{322} as shown below.

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O}
\]
\[
2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]
Apart from these endogenous antioxidants, an important source of antioxidants is in the diet, which contains numerous compounds exhibiting antioxidant activity. The most prominent dietary antioxidants are tocopherols, the fat-soluble vitamin (vitamin E), ascorbate water-soluble vitamin (vitamin C) and carotenoids. Furthermore, other antioxidants such as albumin and other proteins including ceruloplasmin and transferin also protect against oxidative injury by binding the transition metals Fe$^{2+}$ and Cu$^{+2}$ thereby preventing generation of the hydroxyl radical via the Fenton reaction.\textsuperscript{322}

![Figure 9. The Balance between Oxidants and Antioxidants]\textsuperscript{325}

### 11.3 Oxidative stress

As previously described, oxygen-derived free radicals are constantly formed in the body during normal metabolic processes. When free radical formation is greatly increased, or protective antioxidant mechanisms compromised, a state of oxidative stress will result. If oxidative stress persists, it will eventually lead to molecular damage and tissue injury.\textsuperscript{327} Consequently, oxidative stress has been defined as a disturbance in the balance between the production of free radicals (ROS) and antioxidant defenses, which may lead to tissue injury.\textsuperscript{321} Subjects with diabetes may be especially prone to oxidative stress, which enhances the development and progression of diabetic micro and macrovascular complications.\textsuperscript{328,329}

Animal and human studies and in vitro experiments all suggest a role of
oxidative stress, via an increased formation of free radicals in the pathophysiology of diabetic microvascular complications\textsuperscript{328,329} such as nephropathy and retinopathy.

\textbf{Figure 10. Role of oxidative stress in diabetes}\textsuperscript{327}

\subsection{11.4 Free radical-mediated oxidative tissue damage}

The human body has a multiplicity of different antioxidant defense mechanisms.\textsuperscript{330} If the defensive processes are overwhelmed, free radicals can then become highly destructive to cells and tissues. During oxidative stress, the prooxidant-antioxidant balance is tipped in favour of the former, and this may be due to exogenous sources of free radicals or other endogenous stresses.\textsuperscript{326} However, \textbf{oxidative stress can produce major interrelated derangements of cell metabolism, including DNA damage, protein damage and peroxidation of lipids}.\textsuperscript{330} The relative importance of damage to different molecule as targets in producing cell injury or death by improving oxidative stress depends on duration, degree of stress underlying mechanism and the nature of the system stressed.\textsuperscript{330}
11.5 Lipid peroxidation

The free radical oxidation of polyunsaturated fatty acid (PUFA) in biological systems is known as lipid peroxidation. A PUFA contains two or more double bonds, and the presence of an increasing number of double bonds in fatty acids makes it more susceptible to oxidative damage by free radicals and peroxidation. Both monounsaturated and saturated fatty acids are much less reactive and do not usually participate in lipid peroxidation. In the non-enzymatic lipid peroxidation process, the addition of oxygen yields a lipid peroxyl radical, which is considered a hallmark of peroxidising lipids. Lipid peroxidation consists of mainly three processes, namely initiation, propagation and termination (reactions 7-10).

(7) \( X' + RH \rightarrow R' + XH \)  
Initiation:

(8) \( R' + O_2 \rightarrow ROO' \)  
Propagation:

(9) \( ROO' + RH \rightarrow ROOH + R' \)

(10) \( ROO' + ROO' \rightarrow [ROOOOR] \rightarrow NRP \)  
Termination:

Lipid peroxidation can be initiated by any primary free radicals ('OH, \( O_2^- \)) of sufficient reactivity to substitute an allylic hydrogen atom from a reactive methylene group of PUFA side-chains. In the initiation step, polyunsaturated lipids (RH) may form alkyl radicals (R') which react very rapidly with oxygen to form peroxyl radicals (ROO'). In the propagation step, a chain reaction with more lipids produces hydroperoxides (ROOH), i.e. primary oxidation products. Propagation reactions can repeat themselves many times. Thus, an initial event triggering lipid peroxidation can be amplified with the availability of oxygen and PUFA side chains. Consequently, the accumulation of hydroperoxides and their subsequent decomposition to alkoxy and peroxyl radicals can accelerate the chain reaction with PUFA leading to oxidative damage in cells, membranes and lipoproteins. Under such conditions where lipid peroxidation is continuously initiated, a termination reaction limits the extent of lipid
peroxidation, yielding non-radical products (NRP), and destroying two radicals at the same time.\textsuperscript{331} In contrast, the potential consequences of the peroxidation of membrane lipids include loss of PUFA; loss of decreased lipid fluidity, altered membrane permeability, effects on membrane-associated enzymes, altered iron transport, release of material from subcellular compartments and the generation of cytotoxic metabolites of lipid hydroperoxide.\textsuperscript{326} Furthermore, cleavage of the carbon bonds during lipid peroxidation reactions results in the formation of aldehyde products such as cytotoxic alkanals and alkenals, as well as alkanes. The breakdown products of lipid peroxidation, for example alkanals such as MDA, and hydroxyl alkenals such as 4-hydroxynonenal (HNE), have all demonstrated cytotoxic properties.\textsuperscript{326} \textbf{Measuring MDA can help to estimate oxidative stress.}

\section*{11.5.1 Mechanism of antioxidant actions on lipid peroxidation}

In order to prevent overload of free radicals and peroxides, biological systems possess sophisticated antioxidant defensive mechanisms, which operate both in the intra and extracellular aqueous phases, and also in membranes.\textsuperscript{326} In extracellular fluids, numerous antioxidants are present; they can either prevent initiation or intercept lipid peroxyl radicals involved in the propagation phase. In human plasma there are abundant binding proteins present in order to prevent metal-induced catalysis. In addition, cell membranes and lipoproteins contain lipophilic antioxidants, which are able to react with lipid peroxyl radicals, eventually terminating the chain reaction.\textsuperscript{331}

\[ \text{LOO'} + \text{TocOH} \rightarrow \text{LOOH} + \text{TocO'} \]
\[ \text{TocO'} + \text{Ascorbate} \rightarrow \text{TocOH} + \text{Ascorbate radical'} \]
\[ \text{TocO'} + \text{LH} \rightarrow \text{L'} + \text{TocOH} \]

The tocopherol (Toc) radical (TocO'), located in lipid membranes, can be reduced to TocOH by ascorbate, located in the aqueous phase. It is probable that physiological homeostasis requires a balance presence of
antioxidants located in both aqueous and lipid phase. There are also synergistic interactions between the tripeptide GSH and vitamin E (α-tocopherol), which may involve membrane-bound enzymes. It has been demonstrated that under specific experimental conditions in vitro, in the absence of water-soluble antioxidants, TocO' can abstract it from an adjacent fatty acid and therefore act as a pro-oxidant. Consequently, removal of lipid hydroperoxides is an essential mechanism for preventing such reformation of free radicals.326,331

11.6 The role of free radicals and oxidative stress in the pathogenesis of diabetes

There is emerging evidence suggesting that subjects with diabetes have concomitant increased free radicals production and depletion of cellular antioxidant defense systems. It is well established that alloxan and streptozotocin induced diabetic animals become hyperglycemic as the result of destruction of β-cells of the pancreas by free radicals.317 It is probable that in certain genotypes, glycation and glycoxidation lead to an increased susceptibility to oxidative stress than in other genotypes. This would be the genotypes in which β-cell destruction leads to the development of type II DM.329 Pancreatic β-cell are especially vulnerable to oxidative stress, probably because of their low free radical scavenging enzyme capacity reflected in low SOD, CAT and GSHPx activities. Recent studies have reported a direct link between the imbalance of oxidative stress and antioxidants leading to impaired glucose uptake. Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals.332,333

Increased oxidative stress, in addition to antioxidant depletion, leads to decreased glucose uptake were also observed in muscle cells. Furthermore, depletion of antioxidants accompanied by decreased glucose uptake has also been observed in subjects with type II DM.334 These observations lead
to the hypothesis that the imbalance of free radicals and antioxidants is an important pathogenic factor affecting insulin-signaling pathways. However, clinical and experimental studies have demonstrated that supplementation with antioxidants such as vitamin E and α-lipoic acid stimulate glucose uptake through activation of the insulin-signaling pathway and provide protective effects to diabetic state.

Chronic Hyperglycemia

AGE formation
Glucose autoxidation
Glucosamine
Oxidative phosphorylation

\[
\text{ROS} \quad \text{Oxidative stress}
\]

Low capacity of antioxidant enzymes
- Superoxide dismutase
- Catalase
- Glutathione peroxidase

β-cell dysfunction

Figure 11. Free radicals in pathogenesis of diabetes

11.6.1 Biochemical pathways of oxidative stress in diabetic complications

Although the underlying patho-mechanisms remain incompletely understood, it can be postulated that oxidative stress due to chronic hyperglycemia may play a significant role in the pathogenesis of diabetic nephropathy, retinopathy and neuropathy. Several biochemical pathways have emerged as being predominant potential pathophysiological mechanisms of oxidative stress that can be associated with hyperglycemia in diabetes mellitus. Furthermore, diabetes associated oxidative stress is probably a result of both an increased production of plasma free radical concentrations and a significant reduction in antioxidant defense mechanisms.
A) Advanced glycation end products (AGEs) pathway-

Reducing sugars such as glucose react non-enzymatically with amino groups in proteins and initiate glycation, the early stage of the Maillard reaction. This process begins with the conversion of a reversible Schiff base Amadori adducts. The occurrence of this class of non-enzymatic glycosylated adducts in vivo was established after chemical structural analysis of the minor Hemoglobin species, HbA1c.\textsuperscript{337} HbA1c is formed in the erythrocytes by a non-enzymatic reaction between glucose and Hemoglobin. The aldehyde group of the glucose and a free amino group of the Hemoglobin first react to form a Schiffs base (aldimine, unstable form). Subsequently a stable ketoamine is formed in a reaction known as Amadori rearrangement.\textsuperscript{337} The increase in the ambient circulating levels of HbA1c, in diabetes is due to a shift in the equilibrium between the serum glucose concentration and the amount of protein-bound Amadori products. Since Hemoglobin has a half-life of sixty days and Amadori product formation reaches equilibrium over 28 days, significant elevations of HbA1c can be measured during periods of prolonged hyperglycemia.\textsuperscript{338}

Furthermore, in the intermediate stage of the Maillard reaction, the Amadori products can then undergo further rearrangement, oxidation, multiple dehydration and polymerization resulting in the formation of AGEs.\textsuperscript{337} AGEs are irreversibly formed, and there accumulation has been demonstrated with aging, atherosclerosis, and diabetes, especially associated with long-lived proteins such as collagens, lens crystallins, and nerve proteins.\textsuperscript{337, 339} It has been suggested that the formation of AGEs not only modifies protein properties, but also induces biological damage in vivo.\textsuperscript{340} AGEs deposited in the arterial wall, could themselves generate free radicals capable of oxidizing vascular wall lipids and accelerate atherogenesis in hyperglycemic diabetics.\textsuperscript{340-343}

The molecular structures of some AGEs have been identified as N\textsuperscript{ε} carboxymethyllysine, pentosidines and pyrraline. In the presence of molecular oxygen, the formation of these products from sugars is catalyzed
by transition metal ions via glycoxidation, which oxidizes Amadori products to N\textsuperscript{ε}-carboxymethyllysine, and the autoxidation of glucose, which produces superoxide radical anions, H\textsubscript{2}O\textsubscript{2} and α-ketoaldehydes contribute to the generation of free radicals.\textsuperscript{344} Several cell associated binding proteins for AGEs have been identified, including receptor for AGEs (RAGE).\textsuperscript{345} The best characterised AGE receptor is RAGE, which is multiligand member of the immunoglobulin superfamily.\textsuperscript{346} The RAGE receptor probably acts as a scavenger and mediates intracellular signaling. Recently in vitro studies have demonstrated that AGE-RAGE binding on macrophages leads to free radical mediated oxidative stress and activation of the transcription factor NF-κB.\textsuperscript{346,347}

B) Polyol pathway-

Hyperglycemia associated with increased glucose metabolism can lead to the accumulation of sorbitol.\textsuperscript{348} Intracellular formation of sorbitol from glucose catalysed by aldose reductase (AR) is the first enzyme in the polyol pathway. In a hyperglycemic situation increased intracellular glucose results in its enzymatic conversion to the polyalcohol sorbitol, with concomitant decrease in NADPH. In the polyol pathway, sorbitol is then oxidized to fructose by the enzyme sorbitol dehydrogenase, with a stoichiometric reduction of NAD\textsuperscript{+}.\textsuperscript{348} Organ dysfunction in diabetes caused by the increased flux of glucose through the polyol pathway has been linked to the hyperglycemia-induced increase in the NADH/NAD\textsuperscript{+} ratio, which is associated with the de novo synthesis of diacylglycerol (DAG) and the downstream stimulation of protein kinase C (PKC) activity. Disordered cellular metabolism or depletion of myo-inositol uptake, increased osmotic pressure and diminished Na\textsuperscript{+}K\textsuperscript{+}-ATPase activity may also be contributory factors, for example the reduced glomerular hyperfiltration observed in an animal model of type I DM.\textsuperscript{349} Furthermore, recently it has been suggested that oxidation of sorbitol by NAD\textsuperscript{+} increases the cytosolic NADH/NAD\textsuperscript{+} ratio, thereby inhibiting activity of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and subsequent increased concentrations of triose phosphates. Considerable evidence also implicates activation of the sorbitol pathway by glucose as a component in
the pathogenesis of diabetic complications, for example, in lens cataract formation or peripheral neuropathy.\textsuperscript{350-352}

Elevation of triose phosphate concentrations could increase formation of AGEs, and DAG thus activating PKC.\textsuperscript{143} The activation of PKC, through which hyperglycemia stimulates extracellular matrix (ECM) production, is presumably due to increased de novo synthesis of DAG. The increase in the NADH/NAD\textsuperscript{+} ratio that results from the increased activity of the polyol pathway favours this process. Increased de novo synthesis of DAG activates PKC both in cultured vascular, retinal and glomeruli cells in diabetic animals.\textsuperscript{353} However, increased de novo synthesis of DAG\textsuperscript{353} and the effect of hyperglycemia on PKC activation probably reflects increased dihydroxy-acetone phosphate concentrations, resulting from inhibition of GADPH by free radicals. Hyperglycemia may also activate PKC isoforms (PKC-\(\alpha\) and PKC-\(\beta\)) indirectly through both ligation of AGE receptors and increased activity of the polyol pathway, presumably by increasing production of free radicals\textsuperscript{143} thereby enhancing oxidative stress.

![Figure 12. Biochemical pathways of oxidative stress in diabetes\textsuperscript{329}](image-url)
11.7 Biochemical marker of free radical mediated oxidative stress: Malondialdehyde (MDA)

Malondialdehyde is the organic compound with the formula CH\(_2\) (CHO)\(_2\). The structure of this species is more complex than this formula suggests. MDA mainly exists in the enol form.\(^{354}\)

\[
\text{CH}_2\text{(CHO)}_2 \rightarrow \text{HOCH=CH-CHO}
\]

MDA is a ROS, and as such is assayed in vivo as a bio-marker of oxidative stress.\(^{35}\) ROS degrade polyunsaturated lipids, forming MDA.\(^{356}\) This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to AGE.\(^{357}\) The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism.\(^{358,359}\)

MDA reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts, the primary one being M\(_{1}\)G, which is mutagenic.\(^{360}\) The guanidine group of arginine residues condense with MDA to give 2-aminopyrimidines. Human ALDH1A1 aldehyde dehydrogenase is capable of oxidising MDA.

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from PUFA, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, of which the most abundant is MDA.\(^{355}\) Therefore; measurement of MDA is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in both humans and model systems. MDA reacts readily with amino groups on proteins and other biomolecules to form a variety of adducts, including cross-linked products. MDA also forms adducts with DNA bases that are mutagenic and possibly carcinogenic.\(^{359}\) DNA-protein cross-links are another result of the reaction.
between DNA and MDA. MDA in the thiobarbituric acid (TBA) reacting substances assay is a widely used biomarker for lipid peroxidation but is generally regarded as a less specific marker of lipid peroxidation since other compounds, like sugars and amino acids, also can react with TBA in vivo.\(^{361}\)

Corneas of patients suffering from keratoconus and bullous keratopathy have increased levels of MDA, according to one study.\(^{362}\) MDA also can be found in tissue sections of joints from patients with osteoarthritis.\(^{363}\) The oxidative stress in DM is greatly increased due to prolonged exposure to glycemia and impairment of the oxidant/antioxidant balance. Lipids are among the primary targets of oxidative stress.\(^{364}\) Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of DM.\(^{365,366}\) MDA is a major player in LDL modification and is a product of the peroxidation of arachidonic, eicosapentaenoic and docosahexaenoic acids.\(^{367}\) Oxidised-LDL (ox-LDL) results from the interactions between aldehydes such as MDA and lysine residues in apoB-100 of LDL.\(^{368}\) The pathologic effects of ox-LDL include the induction of atherosclerosis (by stimulating monocyte infiltration and smooth muscle cell migration and proliferation),\(^{369,370}\) atherothrombosis (by inducing endothelial cell apoptosis),\(^{371}\) and plaque erosion (by impairing the endothelial anticoagulant balance).\(^{372}\) Although microvascular and macrovascular complications of DM are known to increase with DM duration,\(^{373,374}\) the association between DM duration and MDA levels in type II DM remains controversial.

### 11.8 Antioxidant Defense Mechanism

#### 11.8.1 Superoxide dismutase (SOD)

Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen.
The SOD-catalysed dismutation of superoxide may be written with the following half-reactions:

- \( \text{M}^{(n+1)+} \cdot \text{SOD} + \text{O}_2^- \rightarrow \text{M}^{n+} \cdot \text{SOD} + \text{O}_2 \)
- \( \text{M}^n \cdot \text{SOD} + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{M}^{(n+1)+} \cdot \text{SOD} + \text{H}_2\text{O}_2. \)

Where \( \text{M} = \text{Cu} \) (\( n=1 \)); \( \text{Mn} \) (\( n=2 \)); \( \text{Fe} \) (\( n=2 \)); \( \text{Ni} \) (\( n=2 \)).

In this reaction the oxidation state of the metal cation oscillates between \( n \) and \( n+1 \).

Irwin Fridovich and Joe McCord first discovered and established their superoxide dismutase activity. However, SOD's were previously known as a group of metallo-proteins with unknown function. For example, CuZn-SOD was known as erythrocuprein and as the veterinary anti-inflammatory drug "Orgotein". Likewise, Brewer (1967) identified a protein that later became known as superoxide dismutase as an indophenol oxidase by protein analysis of starch gels using the phenazine-tetrazolium technique. Three isoforms of SOD have been identified thus far in humans. These include the copper interaction with superoxide, zinc SOD (Cu Zn SOD), which is present in the cytoplasm, manganese SOD (Mn SOD), which is found in the mitochondria, and extracellular SOD (ECSOD), which exists in the interstitial fluid, plasma, lymph and synovial fluid.

SOD out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is spin forbidden. In biological systems, this means its main reactions are with itself (dismutation) or with another biological radical such as nitric oxide (NO) or a metal. The superoxide anion radical (\( \text{O}_2^- \)) spontaneously dismutes to \( \text{O}_2 \) and \( \text{H}_2\text{O}_2 \) quite rapidly. SOD is necessary because superoxide reacts with sensitive and critical cellular targets. For example, it reacts with the NO radical, and makes toxic peroxynitrite. Even at the sub nanomolar concentrations achieved by the high concentrations of SOD
within cells, superoxide inactivates the citric acid cycle enzyme aconitase, which can poison energy metabolism, and releases potentially toxic iron. Aconitase is one of several iron-sulfur containing dehydratases in metabolic pathways shown to be inactivated by superoxide. Superoxide is one of the main ROS in the cell. Consequently, SOD serves a key antioxidant role.

**Role in disease**

Mutations in the first SOD enzyme (SOD1) can cause familial amyotrophic lateral sclerosis (ALS, a form of motor neuron disease). The other two isoforms of SOD have not been linked to any human diseases. Mutations in SOD1 can cause familial ALS by a mechanism that is presently not understood, but not due to loss of enzymatic activity or a decrease in the conformational stability of the SOD1 protein. Over expression of SOD1 has been linked to the neural disorders seen in Down syndrome. Genetic polymorphisms in SOD enzymes and their altered expressions and activities are associated with oxidative DNA damage and subsequently the individual’s risk of cancer susceptibility. In recent years it has become more apparent that in mice the extracellular superoxide dismutase (SOD3, ECSOD) is critical in the development of hypertension. In other studies, diminished SOD3 activity was linked to lung diseases such as Acute Respiratory Distress Syndrome (ARDS) or Chronic obstructive pulmonary disease (COPD).

Implication of oxidative stress in the pathogenesis of diabetes is suggested, not only by oxygen free-radical generation, but also due to non enzymatic protein glycosylation, autooxidation of glucose, impaired GSH metabolism, alteration in antioxidant enzymes, lipid peroxides formation and decreased ascorbic acid levels. In red blood cells from patients with diabetes, an increased amount of the glycated form of SOD accompanied by a lower activity of this enzyme is been found.
Hyperglycemia may also result in increased production of the reactive oxygen species within numerous biochemical pathways that have the potential to initiate adverse changes in endothelial function. EC-SOD is a secretory glycoprotein with an affinity for heparan-like substances, and it is the principal enzymatic scavenger of superoxide in the extracellular space. It has been shown that 99 percent of the enzyme is bound to heparan sulfate proteoglycans in vascular walls and to a lesser extent within the interstitium, and 1 percent is contained within the circulation in equilibrium between the plasma phase and the glycocalyx of the endothelium.

Several studies, including human and experimental have reported significant depletion of GSHPx in diabetics associated with enhanced lipid peroxidation as a result of decreased scavenging of free radicals. In uncontrolled diabetes, the level of SOD, the enzyme responsible for inactivating the superoxide radical, along with the levels of the antioxidants vitamin E and α-lipoic acid are decreased.

Decreased activity of antioxidants also occurs in diabetic kidney disease. Researchers have shown that increased glucose is associated with a lack of increase in antioxidant enzymes such as GSHPx, CAT and Cu,Zn SOD, suggesting a problem in the antioxidant system as the increased oxidant stress caused by diabetes should lead to increased activity of these enzymes. Others have shown that overexpression of Cu,Zn SOD has significant beneficial effects in preventing the development of diabetic kidney disease in animals; thus, impaired antioxidant function also plays a role in the development of diabetic kidney disease.

11.8.2 Vitamin C

Vitamin C or L-ascorbic acid or L-ascorbate is an essential nutrient for humans. In living organisms ascorbate acts as an antioxidant by protecting the body against oxidative stress. It is also a cofactor in at least eight
enzymatic reactions including several collagen synthesis reactions that, when dysfunctional, cause the most severe symptoms of scurvy.\textsuperscript{396} When L-ascorbate, which is a strong reducing agent, carries out its reducing function, it is converted to its oxidized form, L-dehydroascorbate.\textsuperscript{397} L-dehydroascorbate can then be reduced back to the active L-ascorbate form in the body by enzymes and GSH.\textsuperscript{397} During this process semi dehydroascorbic acid radical is formed. Ascorbate free radical reacts poorly with oxygen, and thus will not create a superoxide. Instead two semi dehydroascorbate radicals will react and form one ascorbate and one dehydroascorbate. With the help of GSH, dehydroxyascorbate is converted back to ascorbate.\textsuperscript{398} The presence of GSH is crucial since it spares ascorbate and improves antioxidant capacity of blood,\textsuperscript{397} reduce dehydroascorbic acid to ascorbate.\textsuperscript{399,400} Ascorbic acid performs numerous physiological functions in the human body. These functions include the synthesis of collagen, carnitine, and neuro-transmitters; the synthesis and catabolism of tyrosine; and the metabolism of microsome.\textsuperscript{397} During biosynthesis ascorbate acts as a reducing agent, donating electrons and preventing oxidation to keep iron and copper atoms in their reduced states.

Ascorbic acid is well known for its antioxidant property. Ascorbate is a powerful reducing agent capable of rapidly scavenging a number of ROS such as superoxide, \( \text{H}_2\text{O}_2 \) and singlet oxygen. Ascorbic acid is absorbed in the body by both active transport and simple diffusion. The amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly activity, acting as a reducing agent to reverse oxidation in liquids. Oxidative stress has an impact on CVD, hypertension, chronic inflammatory diseases, diabetes,\textsuperscript{401-404} as well as on critically ill patients and individuals with severe burns.\textsuperscript{405} Individuals experiencing oxidative stress have ascorbate blood levels lower than healthy individual.\textsuperscript{406}
Diabetes is an important etiopathological factor in oxidative stress. As a result of lipid and protein oxidation, the levels of SOD, GSH-Px and CAT increase in kidneys. Various studies have reported protective effects of antioxidants such as Vitamin C against oxidative damage of diabetes. The level of Vitamin C in plasma and renal tissues is significantly reduced in diabetic patients. Decrease in Vitamin C level causes hyperlipidemia and hypertension. Some studies showed that certain fruits and vegetables and that Vitamins C and E are important to prevent or alleviate the complications of diabetes mellitus and have complication reducing effects. Vitamins C and E not only reduce the risk of thrombo-embolism in patients with diabetes-related hypertension but also exert favorable effects on wound healing. Vitamins C and E can be used as antioxidants separately or in combination. Both vitamins act synergistically.

There have been multiple trials using vitamin E, vitamin C, and other antioxidant drugs in animals. Animal studies using these compounds have been shown to be effective in reducing the development of diabetic kidney disease. It’s hard to translate these animal results to humans as many trials in mice and rats are very effective in the particular animal but often ineffective in humans. This may be due to short duration of animal studies, dose differences between animals and humans, and different pathophysiologic processes between animals and humans. Human studies with antioxidants for DN are limited and have had variable results.

12. CHRONIC COMPLICATIONS OF DIABETES MELLITUS:

12.1 Introduction

Before Banting and Best discovered insulin in 1921 the only therapy for diabetes was diet and more than 80 percent of patient died within the first ten years of type I DM. The most common cause of death was ketoacidosis. The first injection of insulin for treatment of juvenile diabetes was given in February 1922 and insulin therapy for general use
was introduced a few years later. After the introduction of insulin treatment life expectancy increased and instead the problem with chronic complications evolved, CVD and renal failure becoming the major causes of death among patients with diabetes. Today, patients with diabetes still have an excess morbidity and mortality when compared with the general population, the major causes still being CVD and CRF.

12.2 Complications in type II DM

There is sufficient evidence to indicate that microvascular and macrovascular diabetic complications are more common among persons with type II DM than type I DM. The development and progression of chronic complications in type II DM are known to be related to certain factors such as glycemic control, increased age, longer duration of diabetes, less physical activity, history of smoking, hypertension and obesity. Micro and macrovascular lesions can involve various organs and tissues resulting in significant morbidity and mortality. Studies have shown DN to be the leading cause of ESRD. Data collected by United State Renal Data system (USRDS) in 2001 on ESRD including DN has shown that incidence rates of treated ESRD have raised worldwide. The prevalence rates are also increasing with the highest being in Japan, Taiwan, and USA. (1400-1640 per million population [pmp]) with lowest in Pakistan and Bangladesh (45-58pmp). Similarly diabetic retinopathy is an important cause of blindness.

The development of complications of diabetes varies in different ethnic groups. The frequency of ESRD is four fold higher in blacks and native American population compared to the white population. Several studies from India showed a high prevalence of vascular complications in type II DM. A comprehensive study from Pakistan has shown high figures of complications. This emphasizes the need of adoption of strict measures of prevention and early detection of diabetic complications.
Lesions in chronic complications of Diabetes Mellitus

Microvascular

Eye Disease
Retinopathy (nonproliferative / proliferative)
Macular edema.

Neuropathy
Sensory and motor (mono- and polyneuropathy)
Autonomic

Nephropathy

Macrovascular
Coronary artery disease
Peripheral vascular disease
Cerebrovascular disease

Other
Gastrointestinal (Gastroparesis, diarrhea)
Genitourinary (Uropathy / Sexual dysfunction)
Dermatologic
Infections
Cataracts
Glaucoma

The classical long-term complications are thus retinopathy, nephropathy, neuropathy, all of which are considered to be microvascular complications and microangiopathy. These complications affect quality of life and/or life expectancy. Retinopathy
may lead to severe retinal bleeding and has previously been the most common cause of blindness among young adults. DN can progress to renal failure and need for renal replacement therapy. A symmetric peripheral loss of sensibility and motor nerve function in the lower extremities are commonly early signs of neuropathy and increase the risk of developing foot ulcers. The combination of lower extremity arterial disease and neuropathy may contribute to an increased risk for gangrene and amputation. Autonomic neuropathy may lead to alterations in gastrointestinal, cardiovascular and urogenital function. Hyperglycemia is a common risk factor for all these complications but there are also other risk factors, some of which seem to have organ specific effects. 

12.3 Biochemical basis of diabetic complications

Large prospective clinical studies demonstrated a strong relationship between glycemic control and the development of microvascular complications in both type I and type II DM. Furthermore, hyperglycemia and insulin resistance both play important roles in the pathogenesis of macrovascular disease. As a consequence of its underlying microvascular pathology, diabetes is a leading cause of blindness (retinopathy), ESRD and a variety of debilitating neuropathies.

The 'glucose hypothesis' attributed the microvascular and neuropathic complications of diabetes to chronic hyperglycemia and it postulates that hyperglycemia precipitates these complications. Clinical and epidemiological data from human studies suggest that the magnitude and duration of hyperglycemia in diabetes are strongly associated with the severity of microvascular complications but genetic and environmental factors may also be associated with the development and progression of these complications.

Diabetic retinopathy primarily affects the retinal blood vessels, which may progress through different stages of retinopathy including background, preproliferative, proliferative, advanced and maculopathy according to the
features demonstrated on ophthalmoscopy. Cataract is also a common cause of blindness in diabetics due to non-enzymatic glycation of lens proteins and the accumulation of sorbitol in the diabetic lens as a consequence of increased polyol pathway activity. The clinical manifestations of neuropathy in subjects with type I and type II DM can be severe. A peripheral, symmetric sensorimotor neuropathy is the most common form of diabetic neuropathy that affects the long nerves, whereas other forms include cranial and peripheral motor neuropathies and autonomic neuropathy, which are asymptomatic. As a consequence of hyperglycemia, the late complications of diabetes represent in large part, microvascular dysfunction and diabetic-specific complications in the retina, glomerulus and vasa nervorum have similar pathophysiological features.

At an early stage in the course of diabetes, intracellular hyperglycemia is associated with abnormalities in blood flow and increased vascular permeability. This reflects decreased activity of vasodilators such as nitric oxide, increased activity of vasoconstrictors, for example angiotensin II, and endothelin-1, and elaboration of permeability factors such as vascular endothelial growth factor. An important link between the polyol pathway and non-enzymatic gyration is supported by the ability of aldose reductase inhibitors to stimulate the action of amino guanidine (a drug used on controlling AGEs formation) and the fact that both increased polyol pathway activity and non-enzymatic gyration process increase the rate of free radical production. This may ultimately lead to compound endothelial dysfunction. Furthermore, micro vascular cell loss occurs, in part because of apoptosis, and there is progressive capillary occlusion caused by both ECM overproduction induced by growth factor including transforming growth factor-β (TGF-β). In contrast, hyperglycemia may change the function of endothelial and neuronal cells resulting in edema, ischemia and hypoxia-induced revascularization in the retina, essential
matrix expansion and glomerulosclerosis in the kidney and multifocal axonal degeneration in peripheral nerves.\textsuperscript{143}

In diabetic macrovascular disease, it has been postulated that both hyperglycemia and insulin resistance may play a significant role. In diabetic arteries, endothelial dysfunction may involve both insulin resistance and hyperglycaemia. Furthermore, in diabetic dyslipidemia, hyperglycemia increases atherogenic cholesterol-enriched Apo B-containing remnant particles by reducing expression of the heparan sulphate proteoglycan on hepatocytes \textsuperscript{442} and the associations of atherosclerosis and atherosclerosis risk factors with glycemia have been demonstrated over a wide range of glucose tolerance, from normal to diabetic.\textsuperscript{143}

In contrast, the UKPDS \textsuperscript{114} and the DCCT \textsuperscript{113} research groups emphasized that diabetic complications are common, frequently present by the time of diagnosis, and significantly affect the quality of life. Therefore, the preclinical stages of retinopathy, nephropathy and neuropathy might also be appropriate targets for screening and diagnosis\textsuperscript{440} of diabetic complications. However, it is now well established that intensive therapy to improve glycemic control reduces the risk of microvascular complications in general and diabetic nephropathy in particular.\textsuperscript{113,114}

13. DIABETIC NEPHROPATHY:

Diabetes mellitus is the commonest systemic disease involving kidney. Since the advent of insulin therapy and improved survival of subjects with diabetes, nephropathy has proved to be an important consequence of mortality. DN plays a significant role as one cause of ESRD. DN is a clinical syndrome characterized by persistent albuminuria, arterial blood pressure elevation, a relentless decline in glomerular filtration rate (GFR), and an associated high risk of cardiovascular morbidity and mortality.\textsuperscript{443} This major life-threatening complication develops in approximately 35
percent of subjects with type I DM.\textsuperscript{444} The prevalence in type II DM is higher than type I DM; this form of diabetes now contributes to at least 50 percent \textsuperscript{445} of those with DN who develop ESRD and require dialysis or transplantation for survival.

\begin{center}
\textbf{Figure 13. Biochemical basis of complications in diabetes}\textsuperscript{143}
\end{center}
13.1 Definition of nephropathy

Clinical DN is defined as the development of persistent proteinuria and hypertension, and it is preceded by incipient DN, characterized by persistent microalbuminuria. Once clinical DN has developed, progression is difficult to prevent by improved metabolic control or through the use of antihypertensive therapy.446-448

13.2 An Overview of Nephropathy in Type II DM

Prevalence of nephropathy in type II DM varies in different population groups i.e., fairly low incidence in Caucasians and a very high incidence in Pima Indians.449 However, data suggests that the renal risk is currently equivalent in the two types of diabetes. Evidence in support of this hypothesis includes the observations in one report that the time to proteinuria from the onset of diabetes and the time to ESRD from the onset of proteinuria were similar in type I and type II DM.450 Some of the most robust data relating to the development of DN in a population of predominantly white patients with type II was reported from the UKPDS.451 With respect to the development and progression of nephropathy among over 5000 type II diabetics enrolled in UKPDS, the following results were reported.451 At ten years following diagnosis, the prevalence of microalbuminuria, macroalbuminuria, and either an elevated plasma creatinine concentration (defined as 175 μmol/L [2.0 mg/dL]) or requirement for renal replacement therapy was 25, 5, and 0.8 percent, respectively. The yearly rate of progression from diagnosis to microalbuminuria, from microalbuminuria to macroalbuminuria, and from macroalbuminuria to an elevated plasma creatinine concentration or renal replacement therapy was 2.0, 2.8, and 2.3 percent. Based upon a statistical model, an estimation of the median time spent in each stage without progression to another nephropathy stage was 19, 11, and 10 years for those with no nephropathy, microalbuminuria, and macroalbuminuria, respectively. As with type I DM, some patients with microalbuminuria due to type II DM, particularly those with good glycemic control, experience regression of microalbuminuria.452
Moreover the fact that many newly diagnosed type II diabetic subjects already suffer from chronic complications of diabetes at the time of diagnosis,\textsuperscript{453} indicates that there may be a delay in diagnosis, in addition that the pre-diabetic condition is harmful to human health also. Thus, type II DM represents only the “tip of the iceberg” of long existing metabolic disturbances with deleterious effects on the vascular system, tissues and organs.\textsuperscript{454} (About one third of type II diabetics will eventually have progressive deterioration of renal function.\textsuperscript{455} In the "Chennai Urban Rural Epidemiology Study," by R Unnikrishnan\textsuperscript{456} the prevalence of overt nephropathy and microalbuminuria was 2.2 and 26.9 percent. The estimated overall incidence rate of chronic kidney disease and ESRD in India is currently 800 pmp and 150-200 pmp, respectively.\textsuperscript{457} DN is a public health concern of increasing proportions. It has become the most common single cause of ESRD in India and all over the world.\textsuperscript{458,459}

The first clinical sign of renal dysfunction in patients with diabetes is generally, microalbuminuria (a sign of endothelial dysfunction that is not necessarily confined to the kidney). The degree of microalbuminuria determines the progression of DN. It may reflect the renal manifestation of a global vascular dysfunction.\textsuperscript{460} Microalbuminuria is also a marker of inflammation and an independent risk factor for cardiovascular mortality.\textsuperscript{461}

**13.3 Clinical stages, structural changes and development of diabetic nephropathy**

The natural history of DN has been relatively well defined in type I DM. In type II DM, the process remains unclear with regard to the time of onset of disease or the presence of other factors such as hypertension, age or race. However, clinical investigators have been able to classify the development of ESRD based on the onset and duration of the disease. The classifications of ESRD were introduced by Mogensen \textit{et al},\textsuperscript{462} and divided into five stages as described below.
In stage 1, at the onset of the diabetes, there is glomerular hyperfiltration with hyperperfusion, renal hypertrophy and glomerular capillary hypertension associated with high blood glucose concentrations. At this stage urinary albumin excretion may be normal or slightly elevated.

Stage II is a silent phase that follows hyperfiltration and is associated with subtle morphological changes including thickening of the glomerular basement membrane (GBM), glomerular hypertrophy, mesangial expansion, and modest expansion of the tubulointerstitium.

After 7-15 years, with diabetic patients who develop stage III or incipient nephropathy this can be detected clinically by the presence of microalbuminuria. It occurs in 2-4 percent of cases per year and this is associated with poor glycemic control and high levels within the normal range of urine albumin excretion. Abnormal urinary albumin excretion cannot be detected by conventional or dipstick methods (semi-quantitative) but are measurable using sensitive techniques such as quantitative immunoassay. Studies in Type II DM have suggested that in the transition from normoalbuminuria to microalbuminuria, there is modest rise in blood pressure (systolic/diastolic) of about 3mm of Hg per year.

Stage IV is characterised by the presence of overt nephropathy, with dipstick-positive proteinuria. GFR falls steadily, by about 12ml/min. a year, and clinically, measurement of plasma creatinine and microalbuminuria are used to monitor renal function and to indicate the decline of GFR. Hypertension when present in patients at this stage is usually associated with the presence of >500 mg urinary total protein/24 hr. However, histological glomerular lesions, found in most long-term diabetics with nephropathy, may include thickened glomerular capillaries, mesangial expansion, intercapillary nodules of glomerulosclerosis of the afferent and efferent arterioles and the presence of glomerular microaneurysms. The mesangial cells of one or more of the glomerular segments produce excessive amounts of a cellular matrix to form spherical nodules known as Kimmelstiel-Wilson nodules. The development of
oedema is one of the earliest clinical features of renal impairment, often associated with anemia and a rather non-specific decline in general health.

**Stage V is end stage renal failure**, with the presence of severe mesangial expansion, uremia, hypertension, and serum creatinine concentration of more than 400 µmol/L.\(^{462}\)

### 13.4 Pathogenesis

There appear to be different pathogenetic processes leading to the pathologic mechanisms in DN. Glomerulosclerosis, for example, may result from intraglomerular hypertension induced by renal vasodilatation, or from ischemic injury induced by hyaline narrowing of the vessels supplying the glomeruli.\(^{465}\)

#### i) Glomerular hyperfiltration

The role of glomerular hypertension and hyperfiltration in DN is reinforced by the apparent benefits of blockade of the renin-angiotensin system. Antagonizing the profibrotic effects of angiotensin II may also be a significant factor in benefits observed with these agents.\(^{465}\) Support for such profibrotic elements underlying DN is provided by findings in an animal model of DN.\(^{466}\) Transient renin-angiotensin system blockade (for seven weeks) in prediabetic rats reduced proteinuria and improved glomerular structure.

#### ii) Hyperglycemia and AGEs

Hyperglycemia may directly induce mesangial expansion and injury, perhaps in part via increased matrix production or glycosylation of matrix proteins. In vitro studies have demonstrated that hyperglycemia stimulates mesangial cell matrix production, and mesangial cell apoptosis.\(^{467,468}\) Glycosylation of tissue proteins also may contribute to the development of DN and other microvascular complications. In chronic hyperglycemia, some of the excess glucose combines with free amino acids on circulating or tissue proteins. This non-enzymatic process initially forms reversible early glycosylation products and later irreversible AGEs. Circulating AGE
levels are increased in diabetics, particularly those with renal insufficiency, since AGEs are normally excreted in the urine. The net effect is tissue accumulation of AGEs, in part by cross linking with collagen, which can contribute to the associated renal and microvascular complications.

iii) Prorenin

Prorenin binds to a specific tissue receptor which promotes activation of the mitogen-activated protein kinases (MAPK). A possible pathogenic role for prorenin in the development of DN was suggested by an experimental model (streptozotocin diabetes in mice) in which prolonged prorenin receptor blockade abolished MAPK activation and prevented the development of nephropathy despite an unaltered increase in angiotensin II activity.

iv) Cytokines

Activation of cytokines, profibrotic elements, inflammation, and vascular growth factors (vascular endothelial growth factor, VEGF) may be involved in the matrix accumulation in DN. Hyperglycemia stimulates increased VEGF expression (a mediator of endothelial injury in human diabetes). A potentially pathogenic role for VEGF in DN is supported by the observation that VEGF blockade improves albuminuria in an experimental model of DN. Hyperglycemia also increases the expression of TGF-β in the glomeruli and of matrix proteins specifically stimulated by this cytokine. The combination of an anti-TGF-β antibody plus an angiotensin converting enzyme (ACE) inhibitor completely normalized proteinuria in rats with diabetic nephropathy; proteinuria was only partially lessened with an ACE inhibitor alone. Glomerulosclerosis and tubulointerstitial injury were also improved.

v) Nephrin Expression

The renal expression of nephrin may be impaired in DN. Congenital mutations in nephrin, a transmembrane protein expressed by podocytes, result in severe congenital nephritic syndrome.
13.5 Risk Factors

Studies in patients who have or do not have clinically evident DN have identified a number of factors as being associated with increased risk of renal involvement. 476

1) Genetic susceptibility

Genetic susceptibility may be an important determinant of both the incidence and severity of diabetic nephropathy. 477 The likelihood of developing diabetic nephropathy is markedly increased in patients with a diabetic sibling or parent who has DN. One report evaluated Pima Indian families in which two successive generations had type II DM. 478 The likelihood of the offspring developing overt proteinuria was 14 percent if neither parent had proteinuria, 23 percent if one parent had proteinuria, and 46 percent if both parents had proteinuria.

Figure 14. A schematic view summarizing current hypotheses on the development of diabetic renal disease. Possible interactions between metabolic, hemodynamic, genetic and environmental factors in the initiation and progression of DN. Modified from M Cooper. 489

82
2) Blood pressure

Prospective studies have noted an association between the subsequent development of nephropathy in type II DM and higher systemic pressures.\textsuperscript{479}

3) Glomerular filtration rate

Those patients with glomerular hyperfiltration appear to be at increased risk for diabetic renal disease.\textsuperscript{480} This is particularly true for overt nephropathy if the initial GFR is above 150 ml/min; in comparison, lesser degrees of hyperfiltration may have a slower course, with a lesser risk for microalbuminuria.\textsuperscript{481} The potential importance of intraglomerular hypertension in the pathogenesis of DN may explain why systemic hypertension is an important risk factor for the development of DN. Studies in experimental animals suggest that the diabetic state is associated with impaired renal autoregulation. As a result, raising the systemic pressure does not produce the expected afferent arteriolar vasoconstriction that would minimize transmission of the elevated pressure to the glomerulus.\textsuperscript{482}

4) Glycemic control

DN is more likely to develop in patients with worse glycemic control (higher HbA\textsubscript{1c} levels).\textsuperscript{483}

5) Race

The incidence and severity of DN are increased in blacks (3- to 6-fold compared to Caucasians), Mexican-Americans, and Pima Indians with type II DM.\textsuperscript{484} This observation in such genetically disparate populations suggests a primary role for socioeconomic factors, such as diet, poor control of hyperglycemia, hypertension, and obesity.

6) Obesity

A high BMI has been associated with an increased risk of chronic kidney disease among patients with diabetes.\textsuperscript{485} In addition, diet and weight loss
may reduce proteinuria and improve kidney function among patients with diabetes.\textsuperscript{486, 487}

7) Smoking

Smoking is associated with a variety of adverse effects in patients with diabetes. This includes evidence of increases in albuminuria and the risk of ESRD and of decreased survival once dialysis is begun.\textsuperscript{488}

13.6 Diagnosis of diabetic nephropathy

The diagnosis of DN is only definitively made by renal biopsy, but this is rarely necessary clinically where the diagnosis is based on both the clinical and biochemical abnormalities demonstrated in the kidney, such as the presence of proteinuria, development of a progressive rise in blood pressure, and a progressive and relentless decline in renal function towards end stage renal failure. Elevations of urinary albumin excretion are used to define both the diagnosis of DN and its progression. \textbf{An increase in albumin excretion is taken as the hallmark of DN}.\textsuperscript{447, 490}

14. MICROALBUMINURIA:

14.1 Clinical significance

Microalbuminuria is one of the earliest signs of renal insult in diabetes and is currently the main focus of attention, as it is also associated with increased risk of morbidity and premature death from CVD.\textsuperscript{491} The presence of microproteinuria in general, or microalbuminuria in particular, reflects loss of charge selectivity and an increase in capillary permeability in the kidneys and other organs. Microalbuminuria is a predictor of CRF (overt nephropathy) in DM; its presence here is termed incipient nephropathy.

14.2 Definition

Microalbuminuria, defined as an increased urinary albumin excretion (UAE) detectable only by sensitive immunoassay\textsuperscript{492} expressed either by
time or with reference to creatinine concentration, has been used for many years as a predictor of incipient nephropathy in diabetics.\textsuperscript{447, 490} The 'gold standard' of microalbuminuria measurement is based on the excretion rate of albumin in a timed urine collection, while for more rapid estimations the urinary albumin concentration in an early morning mid stream specimen of urine may be used. In order to correct for variations in body fluid balance, the latter is normally referenced against the urinary creatinine concentration as the albumin: creatinine ratio (ACR) values.

**Classification of albuminuria**

<table>
<thead>
<tr>
<th>Albumin Excretion Rate</th>
<th>Normalalbuminuria</th>
<th>Microalbuminuria</th>
<th>Macroalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mg/24h)</td>
<td>&lt; 30</td>
<td>30-299</td>
<td>≥ 300</td>
</tr>
<tr>
<td>(pg/min)</td>
<td>&lt; 20</td>
<td>20-199</td>
<td>≥ 200</td>
</tr>
<tr>
<td>(mg/l)</td>
<td>&lt; 20</td>
<td>20-199</td>
<td>≥ 200</td>
</tr>
<tr>
<td>ACR</td>
<td>&lt; 30</td>
<td>30-299</td>
<td>≥ 300</td>
</tr>
<tr>
<td>(mg/g creatinine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACR (mg/mmol creatinine)</td>
<td>&lt; 3.5</td>
<td>3.5-35</td>
<td>&gt; 35</td>
</tr>
</tbody>
</table>

Urinary ACR in mg/Gm = \[ \frac{\text{urine albumin (mg/dl)}}{\text{urine creatinine (gm/dl)}} \] = Albumin excretion mg/day

Because of variability in UAE, two of three specimens collected within a 3 to 6 month period should be abnormal before considering a patient to have crossed one of these diagnostic thresholds. Exercise within 24 h, infection, fever, congestive heart failure, marked hyperglycemia, marked hypertension, pyuria, and hematuria may elevate UAE over baseline values.\textsuperscript{453, 493}
14.3 Microalbuminuria and Nephropathy in Type II DM

The first clinical sign of renal dysfunction in patients with diabetes is generally, microalbuminuria (a sign of endothelial dysfunction that is not necessarily confined to the kidney). The degree of microalbuminuria determines the progression of DN. It may reflect the renal manifestation of a global vascular dysfunction. Microalbuminuria is also a marker of inflammation and an independent risk factor for cardiovascular mortality.

Microalbuminuria is often present at the time of diagnosis, either due to insidious nature and asymptomatic initial years of type II DM, or its positive association with insulin resistance, even in non diabetic people. It refers to the excretion of albumin in the urine at a rate that exceeds normal limits but is less than the detection level for traditional dipstick methods. The normal rate of albumin excretion is less than 30 mg/day (20μg/min); persistent albumin excretion between 30 and 299 mg/day (20 to 199 μg/min) is called microalbuminuria. Values ≥ 300 mg/day (≥200 μg/min) are considered to represent overt proteinuria.

Although the 24-hour urine collection was previously the gold standard for the detection of microalbuminuria, it has been suggested that screening can be more simply achieved by a timed urine collection or an early morning specimen to minimize changes in urine volume that occur during the day. The effect of volume can be avoided entirely by calculation of the ACR in an untimed urine specimen. A value above 30 mg/g (or 0.03 mg/mg) suggests that albumin excretion is above 30 mg/day and therefore that microalbuminuria is probably present. With standard units, the comparable value is 3.4 mg of albumin per mmol of creatinine.

Microalbuminuria develops in 2 to 5 percent of patients of type II DM per year. In type II DM, unlike type I DM, microalbuminuria is seldom reversible but, instead, progresses to overt proteinuria in 20 to 40 percent of patients. In 10 to 50 percent of patients with proteinuria, chronic kidney disease develops that ultimately requires
dialysis or transplantation. Forty to 50 percent of patients with type II DM who have microalbuminuria eventually die of CVD, this is three times as high a rate of death from cardiac causes as among patients who have diabetes but have no evidence of renal disease. Thus, microalbuminuria is a major risk factor for renal and cardiovascular events, and the early identification and treatment of patients at increased risk for microalbuminuria may be instrumental to limit the excess renal and CVD associated with type II DM. Attempts at prevention of nephropathy in type II DM have focused on the prevention of microalbuminuria, the earliest clinical hallmark of nephropathy, or its progression to macroalbuminuria.

14.4 Progression

Microalbuminuria may initially be transient in nature, but may become persistent and result in the patient progressing to ESRD if left untreated. Indeed, microalbuminuria may progress to ESRD in 7-10 years after onset of diabetes. In subjects with type I DM, about 80 percent of whom develop persistent microalbuminuria if left untreated, develop overt nephropathy within 10-15 years, accompanied by hypertension. This may eventually lead to end stage renal renal failure within a further 10-20 years without appropriate therapeutic intervention. The higher proportion of individuals with type II DM who develop microalbuminuria and overt nephropathy shortly after the diagnosis of diabetes is probably because diabetes has been present for many years before the diagnosis was made. However, overall 20-40 percent of type II DM with microalbuminuria progress to overt nephropathy which ultimately may lead to ESRD.

14.5 Pathophysiology

The intimate relationship between low-level albumin excretion and vascular permeability makes UAE highly sensitive to the presence of any inflammatory process, including CVD. Almost all filtered albumin is reabsorbed by the proximal tubule via a high-affinity, low-capacity endocytotic mechanism. Since tubular mechanisms for albumin
reabsorption are near saturation, UAE would increase following any increase in tubular load. Glomerular permeability to albumin is dependent on endothelial charge selectivity as well as size selectivity. The negative charge conferred on the glomerular membrane by its constituent glycoproteins plays a role in restricting the permeability of anionic proteins. Loss of glomerular charge selectivity has been found in both diabetic and non-diabetic subjects with microalbuminuria. However, the mechanisms underlying micro-albuminuria still remain to be fully elucidated.510

14.6 Detection

Establishing the diagnosis of microalbuminuria requires the demonstration of a persistent elevation in albumin excretion. Fever, exercise, heart failure, and poor glycemic control are among the factors that can cause transient microalbuminuria.511,512 Although the 24-hour urine collection was previously the gold standard for the detection of microalbuminuria,463,496 it has been suggested that screening can be more simply achieved by a timed urine collection or an early morning specimen to minimize changes in urine volume that occur during the day.463,497 Microalbuminuria is unlikely if the albumin excretion rate is below 20 μg/min in a timed collection or if the urine albumin concentration is less than 20 to 30 mg/L in a random specimen. Higher values (particularly those just above this range) may represent false positive results, and should be confirmed by repeated measurements.493

14.7 Screening

Annual screening for microalbuminuria will allow the identification of those diabetics with either nephropathy or at risk of developing nephropathy. A routine urinalysis should be performed at diagnosis in subjects with type II DM. Conversely, microalbuminuria rarely occurs with short duration of type I DM or before puberty and therefore, screening should begin with puberty and after 5 years disease duration. If the
urinalysis is positive for protein, a quantitative measure is frequently helpful in the development of a treatment regime. Screening for microalbuminuria can be performed by three methods: 1) measurement of the ACR in a random spot urine collection; 2) 24h collection with creatinine, allowing the simultaneous measurement of creatinine clearance; and 3) timed (e.g. 4h or overnight) collection. When obtaining the ACR, it is important to remember that patients with very low body mass may have an abnormally high ratio whereas in patients with high body mass, the ratio may underestimate the presence of microalbuminuria. In these specific patients a timed urine collection may be more appropriate.

14.7.1 Albumin to creatinine ratio

The effect of volume can be avoided entirely by calculation of the ACR in an untimed urine specimen. A value above 30 mg/g (or 0.03 mg/mg) suggests that albumin excretion is above 30 mg/day and therefore that microalbuminuria is probably present. With standard units, the comparable value is 3.4 mg of albumin per mmol of creatinine.

In one report, 24-hour urine collections and random, single-void urine specimens for albumin and creatinine were obtained in 14 normal subjects, 13 with type I DM, and 12 with type II DM. A close correlation was noted between the two measurements and the within-patient variability was very small. A random ACR above 30 mg/g had a sensitivity of 100 percent for the detection of microalbuminuria. Another study of 95 patients with type II DM found an equally high correlation between 24-hour urine albumin excretion and both the urinary albumin concentration and the ACR in the first morning urine; this was true for patients with either microalbuminuria or macroalbuminuria.

14.7.2 Recommendations

Use of the ACR in an untimed urinary sample is now recommended as the preferred screening strategy for all diabetic patients. This test has the following advantages: it does not require early morning or timed
collections, it gives a quantitative result that correlates with the 24-hour urine values over a wide range of protein excretion, it is cheap to perform, and repeat values can be easily obtained to ascertain that microalbuminuria, if present, is persistent. An elevated ratio should be confirmed with at least two additional tests performed over the subsequent 3 to 6 months, with confirmation of the diagnosis requiring at least 2 of 3 positive samples.\textsuperscript{514}

14.7.3 Limitations

There are three important caveats that must be considered to maximize the reliability of this test-

Vigorous exercise can cause a transient increase in albumin excretion.\textsuperscript{515}

As a result; patients should refrain from vigorous exercise in the 24 hours prior to the test. The slope of the relationship between the spot urine and the 24-hour collection varies throughout the day.\textsuperscript{516} The correlation is best if samples are taken in the mid-morning; mid-afternoon specimens are also relatively accurate. The accuracy of the ACR will be diminished if creatinine excretion is substantially different from the expected value; this is particularly important in patients with borderline values. Albumin excretion will be underestimated in a muscular man with a high rate of creatinine excretion and overestimated in a cachectic patient in whom muscle mass and creatinine excretion may be markedly reduced.\textsuperscript{493,517}

15 OVERT NEPHROPATHY AND RENAL FAILURE:

Overt DN is characterised by macroalbuminuria, hypertension and a variable decline (median 12 ml/min/year) in GFR, if left untreated \textsuperscript{518, 450} until GFR <10 ml/min when ESRD evolves. ESRD, independent of its causes, e.g. diabetes, is characterised by several perturbations such as hypertension, accumulation of uremic toxins, hyperkalemia, hyperphosphatemia and anemia due to erythropoietin deficiency. Secondary hyperparathyroidism and alterations in D-vitamin metabolism, together with metabolic acidosis, are considered to be responsible for the osteodystrophy seen in uremic patients. Several risk factors for mortality
among patients with uremia have been identified—age, protein-energy malnutrition and low serum albumin, commonly considered to be an index of malnutrition, appear to be strong predictors of mortality. Uremia due to diabetes is associated with a higher mortality when compared to non-diabetic renal diseases. Many factors may contribute to malnutrition and low serum albumin in uremia and they include low protein and energy intake. Other concomitant diseases such as heart failure and infections, inflammation, catabolic effects of acidosis, reduced physical inactivity and loss of protein and amino acid during dialysis treatment may contribute. Chronic inflammation appears to be involved and aggravate malnutrition and progressive atherosclerotic disease by several pathogenic mechanisms. Available data suggest that inflammation, reflected by high levels of cytokines such as TNF-α and IL-6 play central role in the development of both malnutrition and CVD in ESRD.
STUDY DESIGN

Total Subjects (N=484)

Group A
Control (N=165)

Group B (T2DM)
Diabetics without Nephropathy (N=162)

Group C (T2DMN)
Diabetics with Nephropathy (N=157)

On Basis of Age

Age 21-40 yrs (N=18)
Age 41-60 yrs (N=103)
Age 61-80 yrs (N=44)

On Basis of Gender

Male (N=82)
Female (N=83)

On Basis of Age

Age 21-40 yrs (N=16)
Age 41-60 yrs (N=111)
Age 61-80 yrs (N=35)

On Basis of Gender

Male (N=84)
Female (N=78)

On Basis of Age

Age 21-40 yrs (N=9)
Age 41-60 yrs (N=110)
Age 61-80 yrs (N=38)

On Basis of Gender

Male (N=97)
Female (N=60)

On Basis of Albuminuria

Micro albuminuria (N=127)
Macro albuminuria (N=30)