CHAPTER III

REVIEW OF LITERATURE
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William Harvey's classic work became the foundation for all modern research on the heart and cardiovascular medicine. It has been said that Harvey's proof "of the continuous circulation of the blood within a contained system was the seventeenth century's most significant achievement in physiology and medicine." The publication of De Motu Cordis in 1628, William Harvey’s seminal description of the circulation and the function of the heart, set the stage for the physiological era several centuries later. The 19th-century French physiologist Claude Bernard catheterized animals and measured the pressures in the great vessels and cardiac chambers. This experiment led to the first human cardiac catheterization, performed by Werner Forssman in 1929, and resulted in the exploration of cardiac hemodynamics by Andre Frederic Cournand and Dickinson W. Richards. In 1956 these three investigators were awarded the Nobel Prize in Physiology or Medicine.

Our current understanding of the genetic and molecular basis of coronary artery disease, the pathways of discovery, innovation, and therapeutic advancement in cardiovascular science and medicine over the past two centuries have been truly remarkable. We are now utilizing the advantage of scientific opportunities, fueled by the results of rich epidemiologic studies of populations and large, randomized clinical trials.
3.1. Views on lipid metabolism

Lipoprotein changes may influence the development of cardiovascular diseases. Before discussing Coronary atherosclerosis and myocardial infarction, we would like to review the present knowledge about plasma lipoproteins. Some of the very earliest studies of lipoprotein pattern in normal subjects were reported by Russ et al\textsuperscript{32} and Barr et al\textsuperscript{33} in 1951. In those studies they separated the lipoprotein classes using cohn fractionation, subsequently by preparative ultracentrifugation. This method was subsequently refined by various scientists in this field.

By this method the various lipoproteins are separated on the basis of their density and this is reflected in the current nomenclature as;

a) Chylomicrons

b) Very low density lipoproteins (VLDL)

c) Low density lipoproteins (LDL)

d) High density lipoproteins (HDL)

The various classes separated by ultracentrifugation of their physical properties.
3.1.1. Nomenclature and classification of lipoproteins

The human lipoproteins provide an efficient mechanism for transporting water-insoluble lipid moieties in the blood circulatory system. Each lipoprotein is composed of a non-polar lipid core, consisting of cholesterol ester and triglyceride, surrounded by a monolayer membrane of polar lipids (free cholesterol and phospholipids) in combination with specific proteins (apoproteins). The ultracentrifugal and electrophoretic characteristics and fractional composition of the four major human lipoproteins are shown in table 6 and 7.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Diameter (Ao)</th>
<th>Mol.Wt.</th>
<th>Electrophoretic mobility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>1,000-10,000 mm</td>
<td>109</td>
<td>Origin</td>
</tr>
<tr>
<td>Very Low Density Lipoprotein</td>
<td>200 – 750</td>
<td>107</td>
<td>Pre β</td>
</tr>
<tr>
<td>Low Density Lipoprotein</td>
<td>200 -230</td>
<td>2.3 x 106</td>
<td>β</td>
</tr>
<tr>
<td>High Density Lipoprotein 2</td>
<td>80 -120</td>
<td>3.8 x105</td>
<td>α</td>
</tr>
<tr>
<td>High Density Lipoprotein 3</td>
<td>50 -100</td>
<td>1.8 x 103</td>
<td>A</td>
</tr>
</tbody>
</table>
Table 7: Composition of major lipoproteins of human plasma.

<table>
<thead>
<tr>
<th>Composition (%)</th>
<th>Chylomicrons</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>3-7</td>
<td>20-30</td>
<td>51-58</td>
<td>18-25</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>80-95</td>
<td>50-65</td>
<td>4-10</td>
<td>3-7</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>3-6</td>
<td>15-20</td>
<td>18-24</td>
<td>24-32</td>
</tr>
<tr>
<td>Proteins</td>
<td>1-2</td>
<td>6-10</td>
<td>18-22</td>
<td>45-55</td>
</tr>
<tr>
<td>Apoproteins (major)</td>
<td>B-48, C, A-1</td>
<td>B-100, C, E</td>
<td>B-100</td>
<td>A-I, A-II</td>
</tr>
</tbody>
</table>

3.1.1.1. Chylomicrons

The largest and least dense particles, the chylomicrons, are synthesized in the small intestine. Their major functions are the transporting of exogenous (dietary) fat as triglyceride. In man, they are cleared from the circulation within few hours.

They are catabolised, mainly by hydrolysis, under the influence of a triglyceride lipase located at the capillary endothelium in adipose tissues and muscles. Triglyceride lipase [lipoprotein lipase] is often referred to as ‘post heparin lipolytic activity’ [PHLA]. This includes a hepatic lipase, also released by heparin. Several techniques have been developed to dissociate the lipoprotein lipase (LPL) of extrahepatic origin from the hepatic lipase.34
3.1.1.2. Very Low Density Lipoproteins [VLDL]

Human very low density lipoproteins consist of endogenous triglyceride in combination with cholesterol, and are mainly of hepatic origin. VLDL composition depends upon the particle size, the proportion of triglyceride and apoprotein C varies directly, and that of apo B and total protein varies inversely with the size of the VLDL particle. Smaller fractions of VLDL also arise from the intestine.

The mechanisms controlling the synthesis and regulation of VLDL are not completely understood. Depending upon the availability of free fatty acids, the synthesis of VLDL is increased in the presence of excessive carbohydrate and the process is undoubtedly under hormonal regulation.35

VLDL and chylomicrons probably share a common saturable catabolic pathway; however, the clearance of VLDL, is probably less efficient than that of the chylomicrons.36 VLDL remnant formed by partial hydrolysis of VLDL in peripheral tissues results in the cholesterol-enriched intermediate density lipoprotein [LDL], a process in which HDL plays a crucial role, as discussed below.

3.1.1.3. Low Density Lipoproteins [LDL]

LDL in man is considered to be largely a breakdown product of VLDL metabolism. LDL constitutes about 50 % of plasma lipoprotein mass in the
normal human and carries about 75% of circulating cholesterol. Peripheral tissues appear to be a major site for LDL catabolism, although certain techniques estimate some degradation in the liver as well. Experimental depletion of plasma cholesterol leads to a several fold increase in the rate of cholesterol synthesis in several extra hepatic sites. These observations support the hypothesis of a receptor mediated control of LDL regulation.

3.1.1.4. High Density Lipoproteins [HDL]

HDL is the densest lipoprotein because 50% of its mass is protein. It carries about 15–20% of total circulating cholesterol. It is synthesized in the liver, as well as in the human intestinal tract. HDL from the normal human can be subdivided into fractions of density 1.0632 – 1.120 [HDL2a] [HDL2b] and 1.120 to 1.210 [HDL3]. The HDL2 concentrations are generally about twice as high in pre menopausal females as in males and may reflect more accurately the protective role of HDL in atherogenesis. Newly synthesized [nascent] HDL particles carry mainly unesterified cholesterol and undergo a number of critical changes in the periphery.

3.1.2. Apoproteins

The major apoprotein constituents of human lipoprotein are being extensively studied. The two major proteins in HDL, A-I and A-II, exist in a ratio of 3:1. Apo A-1 activates lecithin cholesterol acyltransferase [LCAT], the key enzyme involved in the formation of HDL cholesterol esters by
catalyzing in the reaction “lecithin + cholesterol → cholesterol ester + lysolecithin.” There are actually two forms of Apolipoprotein B: Apo B-100 and Apo B-48. Laboratory tests typically measure only apo B-100, which is often reported simply as apo B or apolipoprotein B. Apo protein B-100 is the major protein of LDL and also comprises 20 – 35 % of total protein content of VLDL. Apolipoprotein B helps structural integrity to complexes and directs transport of water insoluble lipids. Apo B is recognized by receptors found on the surface of many of the cells. Apo B-100 is responsible for the recognition by its receptors. Among the laboratory methods that currently exist for determination of LDL, apo B is the most sensitive. It is broadly equivalent to LDL because each LDL particle, independent of density, contains exactly one apo B and the vast majority of apo B is carried on LDL particle. In this way, apo B is not affected by heterogeneity of particle cholesterol content. Both apo B and LDL cholesterol were equally associated with coronary artery calcification (CAC). But Apo B, not LDL cholesterol was associated with CAC scores in type 2 diabetes. This was true despite relatively high correlations of these two lipid parameters in diabetic subjects. The Apo B-48, synthesized by the intestine, is the one contained in chylomicrons and chylomicron – remnants. Apo B-48 is recognized by a distinct remnant receptor in liver. Apo protein C is a family of three well – characterised subunits, Apo C-I, Apo C-II, and Apo C-III, based on the migration on polyacrylamide gel electrophoresis. Apo C constitutes about 10 – 50 % of
VLDL protein and about 5 % of HDL protein. Apo C – II has been shown to be a physiologic stimulator of lipoprotein lipase, but not of hepatic lipase. In contrast, Apo C- II might be an inhibitor of the lipoprotein lipase. With the availability of techniques for measuring these apoprotein constituents, newer mechanisms of hyperlipidemia are being revealed. For example, it has been proposed that a deficiency of Apo C-II, or a relative excess of apo C-III, might account for a resistance to the lipoprotein lipase activation and thus result in certain states of endogenous hypertriglyceridemia. A severe deficiency of apo C-II has also been described in patients with marked hypertriglyceridemia, the deficiency was shown to be transmitted as a familial trait.

3.1.3. Lipoprotein (a) [Lp (a)]

Lp (a) is a cholesterol-ester rich complex composed of an LDL-like particle to which is attached a large, highly glycosylated protein. Lp (a) has two alleles, 11 phenotypes and 19 genotypes, depending on the number of repetitive domains. The smaller isoforms are associated with higher Lp (a) concentrations. Lp (a), when present, is attached to apo B by a disulphide bond. It has significant homology with plasminogen. So, it interferes with plasminogen activation and impairs fibrinolysis. This leads to unopposed intravascular thrombosis and possible myocardial infarction. In 40 % population, there is no detectable level of Lp (a) in serum.
Childhood levels of Lp(a) are a better predictor and marker for future CAD in young adult life than any other lipoproteins.\textsuperscript{43} Although the relationship of Lp(a) to CAD is continuous and graded, a level of 15-20 mg/dL is now considered the threshold.\textsuperscript{44} Enas \textit{et al}\textsuperscript{45} were the first to report high levels of Lp(a) in Asian Indians. Subsequent studies have reported elevated Lp(a) levels in Asian Indians in the U.S.,\textsuperscript{46} Canada,\textsuperscript{47} Singapore,\textsuperscript{48} U.K.,\textsuperscript{49} and India.\textsuperscript{50} Numerous case control and angiographic studies have shown Lp(a) to be a powerful risk factor for CAD among Asian Indians.\textsuperscript{51} The Lp(a) levels in Asian Indian newborns are significantly higher than in Chinese in Singapore and the differences in Lp(a) levels in cord blood parallel the 3 to 4-fold differences in adult CAD mortality between these two populations, observed over the past 40 years.\textsuperscript{52}

3.2. Coronary artery disease

Warren’s description of angina pectoris\textsuperscript{53} is very much useful for medical community today. The angina means “infection of the throat” and pectus means “chest”. Both of the words were derived from Latin. Caleb H.Parry speculated that Syncope Anginosa was related to coronary artery calcification, occurring predominantly in men at about 50 years of age and rarely in women and children.\textsuperscript{54} Medical knowledge in 18th and 19th centuries was grounded in clinical observation and anatomical dissection.
Cardiovascular signs emerged in physiological era of the late 19th and 20th centuries, first in Europe and subsequently in North America.

Heberden was described the angina in 1772. Then, it took about a century for pathologist to focus their attention on the coronary arteries and describe the thrombotic occlusions in addition to calcification. However, for decades thereafter, these observations were not related to the symptoms of myocardial ischemia, which had become well known to physicians. Near the end of 19th century, cardiovascular physiologists noted that occlusion of a coronary artery in the dog caused “quivering” of the ventricles and were rapidly fatal. Ludving Hectoen, a pathologist, concluded that myocardial infarction is caused by coronary thrombosis “secondary to sclerotic changes in the coronaries”. Obrastzov WP et al described five patients with the clinical picture of acute myocardial infarction, which was confirmed at postmortem examination.

The important developments in the 1960s radically changed the understanding and management of acute myocardial infarction, which struck down and killed or greatly impaired apparently healthy men in their 40s and 50s, during their most productive years. One of the first acts of National Heart Institute, later renamed the Heart, Lung and Blood Institute (NHLBI), and was establish the Framingham Heart Study in 1948, which involved the close collaboration of professionals from clinical cardiology, biostatistics, and
epidemiology. Their goal was to understand how heart disease developed by studying the life styles of the residents of Framingham, Massachusetts. The first description of their findings, “Factors of Risk in Development of Coronary Heart Disease,”\textsuperscript{57} indicated that elevation in cholesterol level and blood pressure were associated with an increased incidence of ischemic heart disease and acute myocardial infarction. The study also showed a high frequency of myocardial infarction among women, which often occurred later in life than it did man.

The ability to access vascular and cardiac tissue rapidly led to the development of animal models of vascular disease, as well as clinical studies in humans. Two lines of investigation in the 1970s and 1980s forged the field of vascular biology: the observations that thrombotic occlusion of a ruptured or eroded atherosclerotic plaque led to acute myocardial infarction\textsuperscript{58} and that nitric oxide was a physiological dilator of blood vessels, a discovery for which Furchgott\textsuperscript{59}, Ignarro\textsuperscript{60}, and Murad\textsuperscript{61} received the 1998 Nobel Prize in Physiology or Medicine.\textsuperscript{62} We now understand that atherosclerosis is a chronic inflammation of arteries, which develops over decades in response to the biologic effects of risk factors.\textsuperscript{63}

Atherogenesis begins as a qualitative change to intact endothelial cells; when subjected to oxidative, hemodynamic, or biochemical stimuli (from smoking, hypertension, or dyslipidemia) and inflammatory factors, they
change their permeability to promote the entry and retention of blood-borne monocytes and cholesterol-containing LDL particles. Inflammation and biochemical modifications ensue, causing endothelial and smooth-muscle cells to proliferate, produce extracellular matrix molecules, and form a fibrous cap over the developing atheromatous plaque. Plaques lead to clinical symptoms by producing flow-limiting stenoses (causing stable angina) or by provoking thrombi that interrupt blood flow on either a temporary basis (causing unstable angina) or a permanent one (causing myocardial infarction). Physical disruption (rupture) of the plaque exposes procoagulant material within the core of the plaque to coagulation proteins and platelets, triggering thrombosis.64

A remarkable victory for patients with coronary artery disease came when the LDL-cholesterol pathway was delineated65 and the use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), discovered by Akira Endo, 66 was developed to lower LDL-cholesterol levels. Brown and Goldstein’s discovery of the LDL-receptor pathway, 65 for which they are awarded Nobel Prize, provided a genetic cause for myocardial infarction in persons with familial hypercholesterolemia and introduced three general concepts to cell biology: receptor-mediated endocytosis, receptor recycling, and feedback regulation of receptors. This last concept is the mechanism, by which statins selectively lower LDL-
cholesterol levels in plasma, reducing the risk of myocardial infarction and prolonging life, as shown in multiple, definitive clinical trials.\textsuperscript{67,68}

However, statin therapy does not eliminate cardiovascular risk.\textsuperscript{69,70} Levels of high-density lipoprotein (HDL) cholesterol correlate inversely with cardiovascular risk, but despite considerable improvements in our understanding of HDL cholesterol and its metabolism, none of the pharmacologic agents that raise HDL cholesterol that have been tested so far have had a significant effect on cardiovascular morbidity and mortality, and many agents that raise HDL cholesterol levels and have other anti-inflammatory and antiatherosclerotic effects are under clinical trial.\textsuperscript{71}

3.3. Aspartate amino Transferase, Creatine Kinase-MB, Cardiac Troponins as Biochemical markers of myocardial injury

High concentrations of the marker in the myocardium, with relatively low concentrations in non cardiac tissue, will ensure cardiac specificity. It is important to consider the tissue distribution of a potential marker in pathological states as well as in normal physiological conditions.

The first account of the use of a biochemical marker in the study of myocardial injury was published by La Due and colleagues in the journal Science in 1954 and the so called marker was AST.\textsuperscript{72} Chinski et al (1956) and walkers and Little John (1958) have produced evidence that myocardial ischemia causing angina can give rise to transient elevation in the individuals
“base line” serum transaminase level but not to a level above that accepted as the upper limit of normal range.\textsuperscript{73}

In 1965 Meyers and coworkers reviewed 157 cases of myocardial infarction confirmed by autopsy and compared serum glutamate oxaloacetate transaminase activity with electro cardio graphic alteration.\textsuperscript{74} Later on total lactate dehydrogenase (LDH) and total creatine kinase (CK) activity were considered as markers of myocardial injury. Actually a Clinical perspective on biochemical markers for myocardial necrosis evolved during the 1980s and 1990s. Subsequently, the measurement of LDH isoenzymes and more cardiac-specific enzymes (CK-MB) became available. LDH isoenzymes and CK-MB traditionally were measured by labor-intensive electrophoretic techniques. During the 1990s, electrophoretic methods were replaced by CK-MB mass assays using automated immunodiagnostic instruments that could perform testing faster, more frequently, and at lower cost than older methods. Mass assays for CK-MB became the standard of care for cardiac marker testing in the mid-1990s. Subsequently, markers of myocardial injury, including CKMB isoforms, myoglobin, and cTnT and cTnI have become available on automated commercial instruments. Cardiac marker panels also are available in rapid-format, point-of-care testing (POCT) technologies that can produce a qualitative or quantitative result in as little time as 15 minutes.\textsuperscript{75} The development of assays for new marker proteins has contributed to a greater understanding of the pathophysiology of the disease spectrum of acute
coronary syndromes, helped in their definition and assisted in cardiac risk stratification.

Serum total CK activity and CK-MB concentration rise in parallel following myocardial injury, starting to increase 4±6 hours after injury, reaching peak serum concentrations after 12±24 h and returning to baseline after 48±72 h.\textsuperscript{76} Serum CK-MB is considerably more specific for myocardial damage than is serum total CK, which may be elevated in many conditions where skeletal muscle is damaged. The diagnostic specificity of serum CK-MB for the detection of MI has been reported to be very close to 100\%,\textsuperscript{77} while that of CK is only approximately 70\%.\textsuperscript{78} The M-subunit of creatine kinase was found to exist in plasma in multiple forms, despite the single form of MM or MB found in tissue.\textsuperscript{79} Three forms of the MM isoenzyme and two forms of the MB isoenzyme were subsequently identified and purified from plasma.\textsuperscript{80} The tissue form of CK-MB is designated CK-MB2. The removal of the lysine residue from the carboxy terminus of the single M-subunit, which is catalysed by the action of carboxypeptidase-N and gives rise to the CK-MB1 isoform. Removal of the lysine residue, which is positively charged, leaves a more negatively charged isoform, providing a basis for separation of the isoforms by electrophoresis.

In normal plasma, CK-MB isoforms exist with each other in equilibrium, in a 1:1 ratio. Release of tissue CK-MB2 increases its proportion
in plasma; a change in the ratio of CK-MB2: CK-MB1 from 1:1 to 2:1 can be detected using high-voltage gel electrophoresis, even though there is no significant change in the plasma concentration of CKMB. Significant changes in the ratio of the two isoforms in plasma can be detected between 2 and 4 h after myocardial injury. Systematic prospective studies have confirmed CK-MB isoforms as an early marker of myocardial injury, and established a CK-MB2:CK-MB1 ratio above 1.5:1 as a diagnostic criterion.

Creatine kinase (CK) and the MB isoenzyme (CK-MB) have been accepted as the best biochemical assays for AMI. However, a lack of cardiac specificity (producing false positives) and a narrow time-window, limit their clinical utility. The ongoing search for better biochemical markers of myocardial necrosis has led to the development of assays for cardiac-specific troponin proteins. This initiates a new era in serum cardiac markers. Muscle cells contain large amount of contractile proteins. These are composed of overlapping thick and thin filaments, which slide past each other, results in muscle contraction. The thick filament is composed primarily of myosin, which contains the adenosine triphosphatase (ATPase) activity and forms cross-bridges with actin. The thin filament consists of actin, tropomyosin and the troponin complex. The troponin regulatory complex consists of troponin C (TnC), which binds Ca to initiate contraction; troponin I (TnI), which inhibits the myosin ATPase, thus blocking myosin
movement; and troponin T (TnT), which binds to tropomyosin and stabilizes the complex on the actin filament.\textsuperscript{86}

Although the nomenclature is similar, it should be emphasized that the three troponin proteins have distinct biochemical and genetic characteristics. The troponin complex regulates the contraction of striated muscle (both cardiac and skeletal); smooth muscle does not contain troponin proteins and contraction is initiated by Ca\textsuperscript{2+}/calmodulin. Three genes code for TnT – one each in cardiac muscle, fast skeletal muscle and slow skeletal muscle.\textsuperscript{87} Therefore, the amino acid sequence of cardiac TnT differs from that found in skeletal muscle. An analogous situation exists for TnI.\textsuperscript{88} Cardiac TnT is not present in normal adult skeletal muscle.\textsuperscript{89} However, small amounts are expressed transiently in human skeletal muscle during fetal development and during muscle regeneration following injury.\textsuperscript{90} Cardiac TnI is not expressed in skeletal muscle at any time.

Cardiac troponin is not present in the blood of healthy individuals. Several studies demonstrated that increased serum levels of TnT or TnI have a high sensitivity for the detection of MI. It has been proposed that finding increased serum concentrations of TnT\textsuperscript{91} and TnI\textsuperscript{92} is a more efficient indicator of myocardial cell necrosis than measurement of CK-MB. The early sensitivity of troponin for the prediction of AMI is relatively low, \textsuperscript{93} approximately equivalent to CKMB mass assays.\textsuperscript{94} TnT and TnI are increased
in the blood for at least 5 days after AMI.\textsuperscript{95,96} Adams JE \textit{et al} \textsuperscript{92} suggest that the troponin proteins are significantly better than CKMB in identifying AMI in patients with skeletal muscle injury. Since CK-MB is present in normal skeletal muscle, skeletal muscle trauma (including strenuous physical exertion) may increase serum CK-MB levels.\textsuperscript{97}

Cummins P \textit{et al}\textsuperscript{98} and Collinson PO \textit{et al}\textsuperscript{99} reported that neither TnT nor TnI levels are increased by physical training, indicating that prolonged exertion does not produce cardiac muscle damage. Several reports have documented that approximately 30–40\% of patients with unstable angina have increased TnT concentrations.\textsuperscript{100} TnI was demonstrated to be a sensitive and specific indicator of perioperative AMI.\textsuperscript{101} Sustained release of troponin-T is a marker of permanent myocardial injury. It is released considerably longer than cytosolic CKMB.\textsuperscript{102}

Zurich SW \textit{et al}\textsuperscript{103} found using a single troponin T determination, that 46\% of patients with confirmed MI had an abnormal cTnT and normal CKMB initially. They were concluded that an initial troponin-T determination drawn at the time of the patients’ presentation is a powerful diagnostic tool for a rapid diagnosis rather than serial CKMB determination. Chinnapu Reddy et al found, in 25\% of patients who had AMI had CKMB levels not significantly different from controls.\textsuperscript{104}
3.4. Fibrinogen and Atherogenesis

An association between hemostatic parameters and cardiovascular death was reported by Meade and colleagues in 1980.\textsuperscript{105} They found that persons who died from coronary heart disease (CHD) had higher plasma fibrinogen levels. They also showed that within 5 years after the start of the study, the association of cardiovascular mortality with fibrinogen levels was independent of established CHD risk factors and stronger than the association with serum cholesterol. Lowe et al reported that levels of fibrinogen were higher in patients with two or three stenosed coronary arteries than in those with a single stenosed artery or no stenosis.\textsuperscript{106} In 1984 Wilhelmsen et al reported on the synergistic effect of fibrinogen levels and blood pressure on stroke and suggested that high plasma fibrinogen is a risk factor for myocardial infarction and stroke.\textsuperscript{107}

Stone MC et al\textsuperscript{108} suggest that high plasma fibrinogen levels are an important coronary risk factor and should be included in profiles used to identify those at high risk of heart attacks. Many other prospective studies have given a momentum to these observations and strengthened their clinical relevance.\textsuperscript{109} Fibrinogen and its naturally occurring derivative, fibrin, are involved in mechanisms such as platelet aggregation, blood rheology, and endothelial cell injury, which are thought to play a key role in thrombosis and atherosclerosis.\textsuperscript{12}
Cook et al\textsuperscript{110} reported that, there are a number of mechanisms by which fibrinogen and its metabolites appear to cause endothelial damage and dysfunction. Many human atherosclerotic lesions, showing no evidence of fissure or ulceration, can contain a large amount of fibrin, which may either be in the form of mural thrombus on the intact surface of the plaque, in layers within the fibrous cap, in the lipid-rich core, or diffusely distributed throughout the plaque. This phenomenon may be compounded by the decrease in arterial intimal fibrinolytic activity and plasminogen concentration observed in cardiovascular disease.\textsuperscript{111}

It has been proposed that once in the arterial intima, fibrin stimulates cell proliferation by providing a scaffold along which cells migrate, and by binding fibronectin, which stimulates cell migration and adhesion.\textsuperscript{112} Fibrin degradation products, which are present in the intima, may stimulate mitogenesis and collagen synthesis, attract leukocytes, and alter endothelial permeability and vascular tone. In the advanced plaque, fibrin itself may be involved in the tight binding of LDL and accumulation of lipid, resulting in the lipid core of atherosclerotic lesions.\textsuperscript{111}

According to several epidemiological studies, the risk of developing a cardiovascular event such as IHD or stroke is higher in subjects with increased level of fibrinogen.\textsuperscript{113} They also suggest that reducing fibrinogen
levels in patients with high baseline levels and coronary disease may be beneficial.\textsuperscript{113}

The Caerphilly and Speedwell collaborative heart disease Studies\textsuperscript{114} were based on combined cohort of middle aged man in general population. After the follow up study they proved the role of fibrinogen, viscosity and white blood cell count in ischemic heart disease.

In the Framingham Study,\textsuperscript{115} the risk of developing cardiovascular disease was significantly related to plasma fibrinogen levels. According to this study, in both sexes, the risk of cardiovascular and stroke was increased progressively in relation to antecedent fibrinogen values over the 1.8–4.5 g/l range.

In the Munster Heart Study,\textsuperscript{116} plasma fibrinogen, lipid parameters, factor VIIc, and blood pressure, were measured in 2781 healthy men aged 40–65 years. After 8 years of follow-up, 130 coronary events were observed, and the mean plasma fibrinogen level of the ‘event group’ exceeded that of the non-event group by 0.32 g/l. The incidence of coronary events among men within the upper tertile of plasma fibrinogen concentration was threefold higher than among men within the lower tertile. When fibrinogen and LDL concentration were considered together, there was a graded and dramatic eightfold increase in 8-year risk among men with both fibrinogen and LDL
cholesterol in the higher tertiles, when compared to men with both of these parameters in lower tertile.

Thompson SG et al\textsuperscript{117} reported that, plasma fibrinogen was a strong and independent risk factor for MI and sudden death, particularly in patients with pre-existing coronary artery disease, along with plasma von Willebrand factor (vWF) antigen (a marker of endothelial damage), and tissue plasminogen activator antigen (a marker of thrombolytic activity).

Ellison M et al\textsuperscript{118} suggests that cigarette smoking is strongly associated with increased plasma fibrinogen levels, and the adverse cardiovascular effects of smoking may partly be mediated through an increase in plasma fibrinogen levels. Indeed, each cigarette smoked per day increases mean plasma fibrinogen by 0.35 g/l. Similar data are available from epidemiological studies. In the Framingham study, plasma fibrinogen values were significantly higher in smokers than in non-smokers.

The multiple regression analysis of Hisataka Sakakibara et al\textsuperscript{119} has shown that plasma fibrinogen levels are correlated with conventional cardiovascular risk factors even after adjusting for the CRP levels. Persons with cardiovascular risk factors tended to have higher fibrinogen levels, suggesting that all elevated plasma fibrinogen concentration in those with risk factors may further increase the risk of the development of atherothrombosis and subsequent cardiovascular disease through the blood coagulation system.
Mohammad Shojaie et al\textsuperscript{120} reported that fibrinogen as a risk factor for premature coronary artery disease in Iranian men.

3.5. Homocysteine and endothelial dysfunction

Kilmer S Mc Cully\textsuperscript{121} first linked elevated plasma homocysteine with vascular disease in 1969. Homocysteinuria is a rare autosomal recessive condition marked by major alterations in the activity of MTHFR (Methylene tetrahydro folic acid reductase), CbS (cystathionine beta synthase), and an enzyme implicated in vitamin B12 metabolism, resulting in markedly increased homocysteine levels. Kilmer S Mc Cully and Bruce D Ragsdale\textsuperscript{122} suggested that homocysteinemia produced accelerated arteriosclerosis in the children with cystathionine synthase deficiency by altering the normal fibrillar structure of arterial wall proteoglycan molecules and that a similar process may occur in individual without enzyme deficiency.

Wilcken et al\textsuperscript{123} found higher homocysteine levels due to abnormalities in methionine metabolism in coronary artery disease patients compared to healthy controls. Verhhoef P et al\textsuperscript{10} reported that for every 10\% elevation of homocysteine, there was nearly the same (10\%) rise in the risk of developing coronary artery disease. Many researchers raised questions regarding the role of homocysteine in the development of CVD. A meta-analysis which included 57 studies found low correlations between
homocysteine concentrations and coronary heart and cerebrovascular disease.\textsuperscript{124}

Endothelial dysfunction is generally considered to be the earliest manifestation of vascular disease. In vitro studies indicate that homocysteine may have a harmful effect on endothelial cells, increase coagulability, and have a proliferative effect on smooth muscle cells.\textsuperscript{125,126} Nitric oxide is the important mediator of endothelial, platelet, and smooth muscle function, which reacts with homocysteine to form S-nitroso-homocysteine, counteracting the adverse vascular effects of homocysteine, including endothelial dysfunction, vasoconstriction, and platelet aggregation.\textsuperscript{127} Further evidence for the interaction of homocysteine with nitric oxide in endothelial dysfunction is the stimulation of endothelial nitric oxide production in aortic endothelial cells by increased endothelial nitric oxide synthase by homocysteine.\textsuperscript{128} In addition, homocysteine decreases nitric oxide production by decreasing transcription of the mRNA for glutathione peroxidase in aortic endothelial cells.\textsuperscript{129}

These experiments suggest that increased oxidative stress induced by homocysteine promotes endothelial dysfunction by decreased availability and activity of nitric oxide. Human studies show that hyperhomocysteinemia is associated with impaired endothelium-dependent vasodilation, presumably by decreased bioavailability of nitric oxide.\textsuperscript{130} Additional evidence for
endothelial dysfunction in hyperhomocysteinemia is the observation of endothelial hyperplasia, swelling and vacuolization of endothelial cells, and fibrin deposition in cerebral arterioles in patients with homocystinuria.121

According to “response to injury hypothesis,”131 the factor leading to inflammation in atherosclerosis is considered to be endothelial dysfunction and intimal damage from low-density lipoprotein (LDL), oxidized LDL, hyperhomocysteinemia, hypertension and elevated angiotensin II, and infectious organisms such as Herpesvirus and Chlamydia pneumoniae. A number of observations appear to contradict the “response to injury hypothesis.” The concept that LDL cholesterol causes endothelial dysfunction and intimal damage is contradicted by the observation that there is no association between LDL levels in blood and the degree of endothelial dysfunction.132 The concept that intimal damage causes influx of LDL cholesterol is contradicted by the observation that atherosclerotic plaques in hyperhomocysteinemia caused by inborn errors of methionine metabolism in children contain no lipid deposition despite pronounced intimal damage.121 A study of 194 consecutive autopsies showed that two-thirds of individuals with severe atherosclerosis had no elevation of blood cholesterol concentration and no evidence of renal failure, hypertension, or diabetes.133 Hypercholesterolemia is not a risk factor in women of any age or in men over 50 years old, even though most cardiovascular deaths occur in subjects over 65.134 These studies cast doubt on the ability of hypercholesterolemia to cause
intimal damage and trigger the inflammatory reaction in human atherosclerosis. In the late 19th and early 20th centuries, many investigators suspected micro-organisms or toxic factors related to infection as the cause of atherosclerotic plaques. However, most contemporary experimental models failed to support this view. Instead, investigators focused on dietary protein or dietary cholesterol as pathogenic factors, leading to the lipid/cholesterol hypothesis and the homocysteine theory of arteriosclerosis.¹³⁵

Homocysteine thiolactone, the reactive cyclic anhydride of homocysteine, reacts with free amino groups of proteins to form peptide-bound homocysteine groups, a process called thiolation because of the introduction of a free sulfhydryl group.¹³⁶ When increased concentrations of homocysteine thiolactone react with human LDL, the resulting homocysteinylated LDL becomes aggregated and susceptible to precipitation in vitro.¹³⁷ The homocysteinylated LDL aggregates are phagocytosed by cultured human macrophages, forming foam cells with greatly increased cytoplasmic cholesterol and cholesterol esters.¹³⁷ Foam cells are generally considered to be an important initiating factor in formation of atherosclerotic plaques, and rupture of vulnerable plaques leads to hemorrhage and occlusive thrombosis.¹³⁸ Another factor leading to endothelial dysfunction is ascorbate deficiency, which leads to intimal lipid deposition along elastica interna in aortas of scorbutic guinea pigs endothelial dysfunction in human subjects with atherosclerosis is reversed by intravenous infusion of ascorbate, potentially
ameliorating the early stages of atherogenesis.\textsuperscript{139} Papatheodorou \textit{L et al}\textsuperscript{140} have summarized the extensive literature on vascular oxidant stress in hyperhomocysteinemia and the key role of glutathione peroxidase in modifying endothelial dysfunction from oxidant stress.\textsuperscript{141}

### 3.6. C-reactive protein as a predictor of myocardial infarction

William Tillett and Thomas Francis\textsuperscript{142} of the Rockefeller University discovered the C-reactive protein (CRP) in 1930. They described a serologic fraction, or “fraction C,” that could be isolated from patients with pneumococcal infection that was distinct from previously known capsular polysaccharide and nucleoprotein fractions detectable by specific antibody response.

A decade later, Oswald Avery\textsuperscript{143} and Maclyn McCarty\textsuperscript{144} described CRP as an “acute-phase reactant” that was increased in serum of patients suffering from a spectrum of inflammatory stimuli, including myocarditis and the inflammation associated with rheumatic fever. Early clues that this inflammatory biomarker might be linked to atherothrombosis are evident in 2 case reports presented by Gunnar Lofstrom\textsuperscript{145} from the State Bacteriologic Laboratory in Stockholm in 1943, in which increases in CRP following acute myocardial infarction are described.

In the mid 1950s, case series presented by Irving Kroop\textsuperscript{146} and others indicated that CRP concentrations consistently increase after coronary
ischemia and myocardial necrosis, data that was clinically important, as diagnostic tools for acute coronary syndrome did not yet include creatinine kinase or troponin. Later on, the work of many researchers had identified CRP as a hepatically derived, nonglycosylated, circulating pentraxin composed of 5 identical subunits arranged with pentameric symmetry that had characteristic calcium-dependent binding to specific ligands, including binding to LDL cholesterol.\textsuperscript{147,148} They and other investigators further demonstrated that the bulk of circulating CRP is produced by hepatocytes largely under regulatory control of inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor-\(\alpha\); that the plasma half-life of CRP is approximately 19 h under basal and stress conditions; and thus that the plasma concentration is largely determined by synthetic rate.\textsuperscript{149}

Numerous studies have confirmed that high-sensitivity CRP (hsCRP) in healthy volunteers predicts cardiovascular events. In men and women, hsCRP seems to be an additive risk factor for coronary artery disease to an elevated total cholesterol level (\(>75\text{th}\) percentile) and a total cholesterol/high-density lipoprotein ratio.\textsuperscript{150} Also, there was the same report from the Women’s Health Study.\textsuperscript{151}

Smoking is one of the established risk factor for coronary artery disease. CRP levels also are increased in smokers, and this confers greater cardiovascular risk. It is proved by several trials such as the European
Concerted Action on Thrombosis trial\textsuperscript{152}, the Monitoring Trends and Determinants in Cardiovascular Disease study\textsuperscript{153}, and the Multiple Risk Factor Intervention Trial.\textsuperscript{154} In the Cardiovascular Health Study,\textsuperscript{155} CRP levels strongly correlated with smoking status.

In the Physicians Health Study, the increased cardiovascular risk attributable to increased CRP remained significant even when adjusted for smoking status.\textsuperscript{154} In the Helsinki Heart Study,\textsuperscript{155} Cigarette smokers with an hsCRP level in the highest quartile had a risk of 8.6, while smokers with a low hsCRP level had a cardiovascular disease relative risk of 1.6. In a report of the Insulin Resistance and Atherosclerosis Study Festa \textit{et al},\textsuperscript{156} showed that hsCRP was positively correlated with body mass index, waist circumference, blood pressure, and levels of triglycerides, cholesterol, LDL-cholesterol, plasma glucose, and fasting insulin, and inversely correlated with the high-density lipoprotein cholesterol level and the insulin sensitivity index.

This has been confirmed by numerous groups\textsuperscript{157, 158} and there seems to be a clear relationship between a number of metabolic disorders (dyslipidemia, upper body adiposity, insulin resistance, hypertension) and increasing hsCRP levels. It is believed that the proinflammatory cytokines in the metabolic syndrome derive from adipose tissue. In the British Regional Heart Study, underestimation of CVD risk due to time-related variability in
risk factors was similar for CRP and systolic blood pressure (but slightly lower than for total cholesterol).

The genetic studies of CRP have been conflicting, and links between genotype, phenotype, and vascular risk as they associate with CRP remain inconclusive. For example, in the Copenhagen City Heart Study, Jeppe Zacho and Borge Nordestgaard provided cross-sectional data that strongly affirm the role of hsCRP as a potent biomarker of vascular risk but were unable to link specific polymorphism within the CRP gene with that risk despite associations between the polymorphisms evaluated and plasma hsCRP concentrations. On this basis, some have concluded that CRP is thus only a biomarker of risk, and not a causal participant in the atherothrombotic process.

3.7. Body mass index and waist-to-hip ratio as a measure of obesity

Obesity is a worldwide epidemic. Its prevalence among children, adolescents, and adults has increased markedly. As a measure of obesity Thias Coutinho et al was used the variables like Body mass index (BMI), waist-to-hip ratio (WHR) and Waist Circumferanc (WC). WHR and WC are unified variable for central obesity. Body mass index was defined as the weight (in kilograms) divided by the square of height (in meters). Abnormal WC and WHR were defined with clinically acceptable standard cutoffs. High WC was defined by National Cholesterol Education Program Adult Treatment Program.
Panel III cutoffs of >88 cm for women and >102 cm for men, high WHR was defined on the basis of World Health Organization criteria as 0.85 for women and 0.90 for men. As per WHO reports BMI is a simple measure used to characterize a person as being undernourished, normal or overweight (preobese and obese).

The medical risks due to obesity have been shown to be linked more to the abdominal distribution of fat. This was measured by the (WHR) and, giving importance to waist circumference rather than BMI. Waist circumference has been found to be a better predictor of visceral adipose tissue than WHR. Pais P, Pogue J, Gerstein H et al had postulated that markers of central obesity (especially the waist-to-hip ratio) would be more strongly related to the risk of myocardial infarction than BMI. But Shan Kuan Zhu et al reported that waist circumference is more closely linked to CAD risk factors than BMI.

Sunita Simon Kurpad et al observed in an Indian study that Waist circumference correlates better with body mass index than waist-to-hip ratio. The prevalence of abdominal obesity using waist circumference is higher than that with waist-to-hip ratio. Thias Coutinho et al observed that Central obesity is directly associated with higher mortality in individuals with CAD, whereas the opposite is observed with BMI. The effect of central obesity on mortality is observed even in subjects with normal BMI. Their data shows
WC and WHR to be more reliable than BMI in stratifying mortality risk in CAD patients, and WC and/or WHR should be documented in individuals with CAD and normal BMI for better risk stratification and therapeutic considerations. But, research studies on cardiovascular diseases, diabetes and obesity in Asian Indians reported that WHR is a risk factor and not waist circumference.\textsuperscript{171}

So the risk due to overweight and obesity have become increasingly common worldwide, at least 1.1 billion adults are overweight and 312 million are obese, when overweight and obesity are defined conventionally as having a body mass index (BMI) of $>25 \text{ kg/m}^2$ and $>30 \text{ kg/m}^2$, respectively.\textsuperscript{172} In the general population, overweight and obesity are associated with increased risk of developing cardiovascular disease,\textsuperscript{173} and thus it is not surprising that in cohorts of patients with prevalent ischemic heart disease or acute coronary events, well over 50\% are overweight or obese.\textsuperscript{174}

3.8. Diet and risk of cardiovascular disease

The science of diet and chronic disease is relatively young, spanning perhaps only half a century.\textsuperscript{175} Dietary factors that may contribute to a high IHD risk in India include low intakes of vitamin B-6 and folate\textsuperscript{176,177} and high intakes of trans fatty acids, which have been associated with risk in studies conducted in the West.\textsuperscript{14} The Indian Heart Study, a randomized controlled trial of IHD patients, advised patients in the experimental group to eat a diet rich
in fruit, vegetables, whole grains, and nuts rich in α-linolenic acid. The Lyon Diet Heart Study, a secondary prevention trial among patients with a first MI, promoted a diet rich in α-linolenic acid, with higher intakes of bread, fruit and vegetables, fish, and rapeseed (canola) oil and less meat, butter, and cream. The inverse association that we observed between intake of green leafy vegetables and risk of IHD could be explained by the protective effects of folate. Low folic acid intake is associated with increased plasma homocysteine concentrations and elevated risk of IHD. Elevated homocysteine concentrations may contribute to the higher IHD rates among Asian Indians living abroad than among Europeans.

In parts of India, trans fats from hydrogenated vegetable oil in the form of vanaspati are consumed in greater quantity than in the United States. In contrast, in North India, the most commonly used oil in cooking is mustard oil. Mustard oil (Brassica juncea), like canola oil, is produced from rapeseed, a member of the crucifer family that is rich in α-linolenic acid (18:3), which may reduce the risk of IHD. More the consumption mustard oil, less the risk of IHD may support a beneficial effect of α-linolenic acid which reduces the adhesion-aggregation tendency of blood platelets, which should decrease the risk of thrombosis and consequent MI. Mustard oil is also a source of erucic acid, a long chain mono unsaturated fatty acid (MUFA). Ghafoorunissa et al has been suggested that erucic acid in
mustard oil may counterbalance the beneficial effects of linolenic acid by increasing serum LDL-cholesterol and triacylglycerol concentrations.\textsuperscript{186}

The Indian Experiment of Infarct Survival Study assessed the effects of treatment with fish oil and mustard oil, both of which are high in omega-3 fatty acids, among patients with suspected acute MI.\textsuperscript{187} In vitro studies have been conducted to assess the effects of long-chain fatty acids on leukocyte-endothelial interactions that play a role in atherogenesis and inflammation. These interactions are mediated importantly by factors that regulate expression of leukocyte adhesion molecules.\textsuperscript{188}

Fish and other seafood are a good source of long-chain omega-3 polyunsaturated fatty acids (PUFAs), which include eicosapentaenoic acid (EPA; 20:5 omega-3) and docosahexaenoic acid (DHA; 22:6 omega-3). In humans, EPA and especially DHA are synthesized in low amounts (<5%) from their plant-derived precursor, \(\alpha\)-linolenic acid (18:3 omega-3).\textsuperscript{189} Thus, tissue levels of EPA plus DHA are strongly influenced by their direct dietary consumption. Average EPA plus DHA contents of different seafood species vary by >10-fold. Fatty (oily) fish such as anchovies, herring, farmed and wild salmon, sardines, trout, and white tuna tend to have the highest concentrations.\textsuperscript{190} In vitro and animal experiments suggest that fish oil has direct antiarrhythmic effects,\textsuperscript{191} but trials to establish direct antiarrhythmic effects in patients with preexisting arrhythmias have been inconsistent.\textsuperscript{192,193}
In human trials, fish oil lowers triglyceride levels,\textsuperscript{194} systolic and diastolic BP,\textsuperscript{195} and resting heart rate.\textsuperscript{196} Observational evidence suggests that fish or fish oil consumption may also reduce inflammation, improve endothelial function, normalize heart rate variability, improve myocardial relaxation and efficiency, and, at high doses, limit platelet aggregation. Seafood-derived omega-3 PUFAs has strong inverse relations with CHD mortality.\textsuperscript{197}

In meta-analyses of prospective cohort studies, total red meat consumption was associated with overall non significant trends toward higher risk of CHD and DM.\textsuperscript{198} When different types of meat were evaluated systematically, consumption of processed meats but not unprocessed red meats was associated with higher incidence of CHD and DM.\textsuperscript{199} In one observational analysis, both unprocessed and processed meat consumption were associated with higher CHD risk when such consumption replaced foods with cardio metabolic benefits, such as low-fat dairy, nuts, and fish.\textsuperscript{200}

Alcohol use has been related to both beneficial and adverse cardiovascular outcomes. Keefe JH et al\textsuperscript{201} reports that light to moderate alcohol consumption (up to 1 drink daily for women and 1 or 2 drinks daily for men) is associated with cardio protective benefits, whereas increasingly excessive consumption results in proportional worsening of outcomes. Alcohol consumption confers cardiovascular protection predominately through improvements in insulin sensitivity and high-density lipoprotein
cholesterol. Moderate alcohol intake, increased the level of HDL and its subfraction, and decreases the risk of myocardial infarction. McElduff P et al reported that the frequent consumption of an alcoholic beverage decreased the risk of MI. Mukamal and colleagues found that beer and spirits, the predominant alcohol beverages in their study population, significantly reduced the risk of MI. In contrast, wine, which was consumed less, showed no significant protective effect on MI.

Habitual heavy alcohol intake is cardiotoxic, causing a large portion of nonischemic dilated cardiomyopathies in many nations. The ensuing ventricular dysfunction is often irreversible, even when alcohol consumption is stopped; continued drinking in such patients is associated with high mortality. Both acute binges and higher habitual intake of alcohol have also been associated with higher risk of atrial fibrillation. Roerecke M and Rehm J reported recently that a cardioprotective association between alcohol use and ischaemic heart disease cannot be assumed for all drinkers, even at low levels of intake. More evidence on the overall benefit–risk ratio of average alcohol consumption in relation to ischaemic heart disease and other diseases is needed in order to inform the general public or physicians about safe or low-risk drinking levels.

A dietary pattern with lower fruit and vegetable intakes in women, and a pattern characterized by higher consumption of red meat and alcohol
(and lower of dairy products and vegetables) in both sexes, were associated with an increased risk of AMI and adverse cardiovascular risk profiles. These findings highlight the importance of sustained recommendations for fruit and vegetable intake and cautious guidance on consumption of alcoholic beverages, which clusters with less healthy dietary patterns of men and women. The Mediterranean diet is a modern nutritional recommendation inspired by the traditional dietary patterns of southern Italy, Greece, Spain, Turkey, Iran, and Israel. The principal aspects of this diet include high olive oil consumption, high consumption of legumes, high consumption of unrefined cereals, high consumption of fruits, high consumption of vegetables, moderate consumption of dairy products (mostly as cheese and yogurt), moderate to high consumption of fish, low consumption of meat and meat products, and moderate wine consumption. It is a cardio protective diet pattern and UNESCO recognized this diet pattern as an Intangible Cultural Heritage of Italy, Greece, Spain and Morocco.207

3.9. Diabetes, dyslipidemia and myocardial infarction

Diabetic subjects are more likely to experience a myocardial infarction and have worse outcomes compared to non-diabetic subjects. The underlying pathophysiology of the atherosclerotic process is not significantly different in diabetic subjects, but the prothrombotic and procoagulant state with which diabetes is associated is thought to contribute to the higher incidence of and
worse prognosis after myocardial infarction. Ciruzzi M et al reported the independent association of acute myocardial infarction with total cholesterol, hypertension, smoking and diabetes mellitus in a study conducted in Latin American countries such as Argentina, Cuba, Mexico and Venizula. Ming Wei et al conducted their study in Mexican Americans and reported that diabetes, cigarette smoking, high cholesterol, and hypertension are important predictors of both all-cause and cardiovascular disease deaths in Mexican American population.

Diabetes mellitus is a major risk factor for coronary artery disease and is associated with a higher incidence of myocardial infarction (MI) and sudden death. Morbidity, mortality and reinfarction rate are higher following MI in diabetic with one-year mortality in this population as high as 50%. Hyperglycemia plays a specific role in atherosclerosis progression in patients with diabetes and Impaired glucose tolerance (IGT). Several risk factors have been proposed to explain the increased risk of cardiovascular disease with diabetes. They include: hyperglycemia, dyslipidemia, accelerated formation of advanced glycation end-products (AGEs), increased oxidative stress, and genetic factors. It is difficult to precisely establish the elements leading to diabetes-accelerated atherosclerosis by means of epidemiological studies because all these factors coexist in diabetic patients. Renard C and Van Obberghen E were used diabetic animal models that reproduce exacerbation of atherosclerosis.
In patients with diabetes and IGT, the hyperglycemia is usually associated with other coronary risk factors, such as dyslipidemia, hypertension, and obesity. These factors are also known to cause endothelial dysfunction\textsuperscript{216} which is the initial step in atherosclerosis and occurs in patients with chronic hyperglycemia.\textsuperscript{217} In hyperglycemia patients, oral glucose loading rapidly suppresses endothelial-dependent vasodilatation through increase in the production of oxygen-derived free radicals.\textsuperscript{218} In the presence of vascular risk factors, vascular endothelial cells undergo phenotypic changes resulting in decreased nitric oxide bioactivity, thereby promoting vasoconstriction, vascular inflammation, endothelial-mesenchymal transition, and thrombosis.\textsuperscript{219} Coronary risk factors are associated with impaired vasomotor function, and individuals with abnormal vasodilator function have increased cardiovascular event rates.\textsuperscript{220} Obesity and diabetes, along with the associated dyslipidemia and insulin resistance, have been linked to impaired vasodilator responses in humans\textsuperscript{221} and animal models.\textsuperscript{222}

One of the important mechanisms responsible for the accelerated atherosclerosis in diabetes is the nonenzymatic reaction between glucose and proteins or lipoproteins in arterial walls, collectively known as Maillard, or browning reaction. Glucose forms chemically reversible early glycosylation products with reactive amino groups of circulating or vessel wall proteins, which subsequently rearrange to form the more stable early glycosylation products. Equilibrium levels of vessel wall proteins and stable early
glycosylation products (the best known of which is hemoglobin A1C) are reached in hours and weeks, respectively.\textsuperscript{223} Some of the early glycosylation products on long-lived proteins (e.g. vessel wall collagen) continue to undergo complex series of chemical rearrangement to form advanced glycosylation end products (AGEs). Once formed, AGE-protein adducts are stable and virtually irreversible. AGEs accumulate continuously on long-lived vessel wall proteins with aging and at an accelerated rate in diabetes. The degree of nonenzymatic glycation is determined mainly by the glucose concentration and time of exposure.\textsuperscript{223}

Glycosylation of proteins and lipoproteins can interfere with their normal function by disrupting molecular conformation, alter enzymatic activity, reduce degradative capacity, and interfere with receptor recognition. Thus, changes in the normal physiology of proteins that are relevant to atherogenesis, may promote atherosclerosis in diabetic individuals. Perhaps the most studied example is interference of the normal physiology of the low-density lipoprotein (LDL) particle. The glycosylation process occurs both on the apoprotein B\textsuperscript{224} and phospholipid\textsuperscript{225} components of LDL, leading to both functional alternations in LDL clearance and increased susceptibility to oxidative modifications. Clinical studies have shown an increased level of AGEs on LDL obtained from diabetics compared with normal individuals.\textsuperscript{226}
The glycated LDL are poorly recognized by the specific LDL receptor and are preferentially recognized by a nonspecific (scavenger) receptor present on human macrophages. Because LDL glycosylation enhances its uptake by human aortic intimal cells and monocyte-derived macrophages with stimulation of foam cells formation, the recognition of glycated LDL by the scavenger receptor pathway is thought to promote intracellular accumulation of cholesteryl esters and promote atherosclerosis.

Another atherogenic effect of glycosylation is to confer increased susceptibility of LDL to oxidative modification. Oxidation reactions occur normally during glycation can oxidize the amine-containing phospholipids component of LDL, independently of transition metals or exogenous free radical-generating systems. Advanced glycosylation of an amine-containing phospholipids component of LDL is accompanied by progressive oxidative modification of unsaturated fatty acid residues. LDL oxidation following AGE-LDL formation occurs in direct proportion to glucose concentration and can be inhibited by the AGE formation inhibitor aminoguanidine. Thus, glycation confers increased susceptibility of LDL to oxidative modification, which is considered a critical step in its atherogenicity.

The presence of the AGE receptor (RAGE), a member of the immunoglobulin superfamily of receptors, has been demonstrated in all cells relevant to the atherosclerotic process including monocyte-derived...
macrophages, endothelial cells, and smooth muscle cells AGE interaction with RAGE on endothelial cells results in the induction of oxidative stress and consequently of the transcription factor NF-κB$^{229}$ and VCAM-1.$^{230}$

In addition, engagement of AGEs with their specific receptors results in reduced endothelial barrier function with increased permeability of endothelial cell monolayers. $^{231}$ Thus, the interaction of AGEs with RAGE-bearing endothelial cells can mediate initiating events in atherogenesis. For example, increased endothelial permeability can lead to increased lipid entry into the subendothelium. Enhancement of adhesive interactions of monocytes with the endothelial surface can subsequently result in transendothelial migration. Monocyte-macrophage interaction with AGEs results also in the production of mediators such as interleukin-1, tumor necrosis factor-α, platelet-derived growth factor, and insulin growth factor-I, which have a pivotal role in the pathogenesis of atherosclerosis.$^{231}$

3.10. Hypertension and atherosclerosis

Hypertension is the chief cause of atherosclerosis and it may double or quadruple the risk CVD. It also pulse greater strain on the heart by forcing it to pump harder against a high pressure. Heart muscle that has to work harder is more likely to fail, especially if the arteries supplying the heart muscle have also been damaged by atherosclerosis. People with high blood pressure are more likely to develop coronary artery disease because high blood pressure
puts added force against the artery walls. Over time, this extra pressure can
damage the arteries. These injured arteries are more likely to become
narrowed and hardened by fatty deposits. Damaged arteries cannot deliver
enough oxygen to other parts of the body. For this reason, high blood
pressure can harm the brain and kidneys.

Myocardial infarction (MI) is associated with a five- to six fold
increase in the risk of heart failure in hypertensive patients. High blood
pressure levels are associated with increases in circulating levels of
inflammation markers which can reflect vascular inflammatory processes,
suggesting that hypertension is a low-grade inflammatory process. The
vascular inflammation associated with hypertension could be the link between
high blood pressure levels and the atherosclerotic process.

High blood pressure levels are accompanied by increase in oxidative
stress due to both higher reactive oxygen species (ROS) production and
reduced ROS scavenging by antioxidant defence. This situation favours
endothelial function alterations which allow the expression of adhesion
molecules and initiation of fatty streak, the earliest structural change in the
atherosclerotic process. At the same time, this inflammation, allows
endothelial dysfunction since some inflammatory mediators can negatively
affect endothelial cell function. Inflammation, therefore, plays a critical role
in development and in complications of the atherothrombotic process.
Changes in mechanical stress and activation of humoral factors such as the reninangiotensin-aldosterone system can be underlying not only increases in oxidative stress (and consequently endothelial dysfunction) but also the development of the inflammatory process associated with hypertension.\textsuperscript{232} The examination of the Framingham Heart Study by Wolf \textit{et al}\textsuperscript{233} revealed that regardless of smoking status and sex, hypertensive subjects had twice the incidence of stroke.

\textbf{3.11. Smoking and progression of atherosclerosis}

The association between long-term cigarette smoking and coronary artery disease is well established. Furthermore, diabetics who smoke develop more severe cardiovascular diseases early in life. In cigarette smoking, lungs inhale hot smoke because at the time of puff, the temperature at the tip of the cigarette is around 95°C.\textsuperscript{234} The smoke also carries numerous chemicals that adversely affect the elasticity of lungs and the hot smoke raises the lung temperature and in turn raises the core body temperature. The lungs become incapable of performing one of their vital physiological functions that is, cooling or removing the heat from the body.

Karim \textit{et al}\textsuperscript{235} have illustrated that smoking is associated with subclinical atherosclerosis in diabetics and interacts with the duration of diabetes to accentuate atherosclerosis. The association between carotid intima-media thickness and the duration of diabetes increases with both the
frequency and duration of smoking. Unverdorben M et al.\(^8\) stated that smoking enhances platelet aggregability, increases blood viscosity and shifts the prothrombotic and antithrombotic balance towards increased coagulability (e.g., fibrinogen, von Willebrand factor, ICAM-1 and P-selectin). Insulin resistance is higher in smokers compared with nonsmokers, and hemoglobin A1c is dose-dependently elevated, as is homocysteine. Smoke exposure may influence the kinetics of markers with different response to transient or chronic changes in cigarette smoking behavior.

Impairment of vasodilatory function is one of the earliest manifestations of atherosclerotic changes in a vessel. Several studies have demonstrated that both active and passive cigarette smoke exposure were associated with a decrease in vasodilatory function.\(^{236}\) In humans, cigarette smoke exposure impaired endothelium-dependent vasodilation (EDV) in macrovascular beds such as coronary and brachial arteries and in microvascular beds.\(^{237}\)

Nitric oxide (NO), a free radical, is primarily responsible for the vasodilatory function of the endothelium.\(^{238}\) Using cigarette smoke extract (CSE) or isolated components such as nicotine, multiple in vitro studies have found that CS was associated with decreased NO availability.\(^{239}\) NO is also helps to regulate inflammation, leukocyte adhesion, platelet activation, and thrombosis.\(^{238}\) Therefore, an alteration in NO biosynthesis could have both primary and secondary effects on the initiation and progression of atherosclerosis and on thrombotic events.
The inflammatory response is an essential component in the initiation and evolution of atherosclerosis. Several studies have indicated that CS causes about a 20% to 25% increase in the peripheral blood leukocyte count. In vivo, CS is associated with an increased level of multiple inflammatory markers including C-reactive protein, interleukin-6, and tumor necrosis factor alpha in both male and female smokers.\textsuperscript{240} Local recruitment of leukocytes on the surface of endothelial cells is an early event in atherosclerosis. Elevations of various proinflammatory cytokines increase leukocyte-endothelial cell interaction leading to leukocyte recruitment. Indeed, soluble VCAM-1, ICAM-1, E-selectin levels are higher in smokers.\textsuperscript{241} Cigarette smoking also causes activation of proatherogenic molecules leading to alteration in cell-cell interactions. Cigarette smoking extract exposure was associated with a 70% to 90% increase in adherence between human monocytes and HUVECs (human umbilical vein endothelial cells) in culture attributable to the increased expression of adhesion molecules on the surface of both monocytes and HUVECs.\textsuperscript{242} Monocytes isolated from smokers increased expression of the integrin CD 11b/CD 18, which augmented the adhesiveness of the monocytes to HUVECs in culture.\textsuperscript{243} Adams \textit{et al} \textsuperscript{244} exposing human monocytes and HUVECs to smokers' serum, found a significant increase in adhesion between these cells, which was associated with increased expression of ICAM-1 on HUVECs. Thus, CS fuels the fire of inflammation in the blood and at the vessel wall.

Smokers have significantly higher serum cholesterol, triglyceride, and low-density lipoprotein (LDL) levels, but high-density lipoprotein is lower in
smokers than in non-smokers. Cigarette smoking also increases oxidative modification of LDL. Circulating products of lipid peroxidation and autoantibody titers to oxidized LDL are significantly increased in smokers. In 1988, Yakode et al reported that exposure to CSE caused a modification of LDL, which was actively taken up by the macrophages to form foam-cells in culture. Frei et al observed that exposure of human plasma to the gas phase of cigarette smoke caused oxidative modification of plasma LDL. Furthermore, HUVECs isolated from smokers significantly increased oxidative modification of LDL compared with HUVECs isolated from non-smokers. Cigarette smoke extract exposure may also decrease the plasma activity of paraoxonase, an enzyme that protects against LDL oxidation. In a hyperlipidemic rabbit model, injection of CSE accelerated atherosclerosis through oxidative modification of LDL.

Cigarette smoking is associated with an increased incidence of acute MI. Recent data indicate an immediate reduction in thrombotic events with smoking cessation. Pathologic studies of sudden coronary death indicate that CS increased the risk of plaque rupture and acute thrombosis of a lipid-rich, thin-capped atheroma in men; in female smokers, the prevailing mechanism was plaque erosion with superimposed thrombosis. Acute cigarette smoke exposure may also increase coronary artery vascular resistance reducing coronary blood flow. Smoking may also be a risk factor for coronary vasospasm.

Platelets isolated from smokers exhibited an increased stimulated as well as spontaneous aggregation. After exposure to smokers’ serum, platelets
isolated from non-smokers demonstrated hyperaggregability.\textsuperscript{254} Cigarette smoking may decrease availability of platelet-derived NO and decrease platelet sensitivity to exogenous NO, leading to increased activation and adhesion.\textsuperscript{255}

Current smokers have higher fibrinogen levels that correlate with the number of cigarettes smoked. Ex-smokers have fibrinogen levels similar to non-smokers.\textsuperscript{256} Higher red blood cell counts, hematocrits, blood viscosity, and an ongoing inflammatory process potentiate the prothrombotic process associated with smoke exposure.\textsuperscript{257} Human umbilical vein endothelial cells exposed to chronic smoker's serum have significant decreases in both basal and substance-P-stimulated t-PA release in culture with a significant alteration in t-PA/PAI-1 molar ratio.\textsuperscript{258} Similarly, decreased plasma t-PA antigen and activity were observed in smokers in samples isolated from brachial and coronary arteries after pharmacologic stimulation.\textsuperscript{259} Therefore, CS is associated with dysfunctional thrombo-hemostatic mechanism(s) that promote the initiation and/or propagation of thrombus formation and limit its effective dissolution.