2.0 REVIEW OF LITERATURE

2.1 HISTORICAL ASPECTS

Ayurveda (Ayur: Life; Veda: Science) dates back around 5,000 years and is widely considered to be the oldest form of health care in the world. It is not only a medical system but a way of life. It is widely practiced in the Indian subcontinent since the pre-biblical era (30-33) and considered to have peaked between 2500-500 BC. Charak Samhita and Sushrut Samhita (500-100 BC) are the two main Ayurvedic classics, wherein more than 700 plants along with their classification, pharmacological and therapeutic properties have been described. Ayurveda during course of ancient times developed as perfect scientific system and it is evident as it is divided into eight major disciplines known as Ashtanga Ayurveda. It is important to note that these specialisations or super specialisations were in existence and practiced by experts hundreds of years before the emergence of modern Anatomy, physiology and contemporary medicinal system. In ayurveda, heart disease particularly CVD was probably referenced as related to natural aging process (Swabhavabalakrut), psychologically affected (Adhyatmika) or due to bad luck, due to bad deeds of previous lives (Daivabalakruta) (34).

Starting in olden times, from Hippocrates, Galen and the middle ages, emerges some picture of heart disease in antiquity. Cardiac diseases were described as Morbus Cardiacus. Morbus Cardiacus was applied both to cardiac and gastric symptoms, and this diagnostic difficulty has persisted until when Huchard described a pseudo-gastralgic form of angina pectoris. The onset of knowledge on coronary heart disease (CHD) cannot be precisely dated. Leonardo da Vinci (1452–1519) and Andreas Vesal (1514–1564) were among the first to describe coronary arteries. Modern cardiology started with description of closed circulation by Willam Harvey, who also described separate coronary circulation in 1628 (Exercitatio anatomica de motu cordis et sanguinis in animalibus). Harvey showed that the blood moves within a closed circuit from the right ventricle through the lung into the aorta, the peripheral vessels and back into the lung. Capillaries were described in 1674 by the anatomist Malpighi by means of strong lenses and microscopical improvements performed by van Leeuwenhoek.

Friedrich Hoffmann (1660–1742) conceptualized that the cause of CHD lies in the reduced passage of blood within the coronary arteries. The cardiologist William Heberden
(1710–1801) was the first to describe angina as the disease in his publication “Some account of a disorder in the breast”. He was, of course, unaware of underlying technical details, yet his work dealt with one of the most important diseases of the century. Laennec also worked with angina pectoris; however, he thought that the course of the disease was harmless. Many English, German, and Italian physicians, however, believed that angina pectoris was a disease of organic order caused by obstruction of the coronary circulation. During second half of the 19th and beginning of the 20th century cardiologist William Osler (1849–1919) worked extensively on angina pectoris appeared in the paper “Lectures on angina pectoris and allied states”. Osler depicted the coronary vessels at great length, as well as coronary sclerosis, embolies, thrombi and cardiac sudden death. The American cardiologist James B. Herrick (1861–1954) made an important contribution to the analysis of coronary sclerosis in the paper “Clinical features of certain obstructions of the coronary arteries”. He concluded in 1912 that “a slow, gradual narrowing of coronary vessels is a possible cause, permitting the heart to adapt to the new conditions, and that a severe obstruction of a vessel must not necessarily lead to death”. He coined the term “heart attack” and described the electrocardiographic changes after ligation of the coronary vessels. The first coronary heart catheterization was performed in 1929 by Werner Forssmann in his famous “self-experiment”. The lung specialist André Cournand together with the cardiology pediatrician Dickinson Richards successfully repeated the experiment of Forssmann in 1941. Mason Sones (1918–1985), a cardiology pediatrician inadvertently injected contrast material in right coronary artery while performing an angiogram on a 26-year-old man without any damage to myocardium. Mason Sones immediately grasped the important gave idea of selective coronary angiography, that is, by injecting smaller amounts of contrast medium into the relevant coronary vessel. This was a breakthrough, and the technique became a routine procedure in the Cleveland Clinic in 1959 (35).

The concept of risk factors for CVD was identified from the landmark FHS, which is the foundation for all heart disease risk assessments today. The FHS is a population-based prospective family study that began in Framingham in 1948 with the recruitment of the Original Cohort (36). In 1971, children of the Original Cohort, called the Offspring cohort, were enrolled (37). Finally, in 2002, the grandchildren of the Original cohort were enrolled (the Third Generation) (38), making the FHS the longest running family-based
study in history. For the past 62 years, investigators at the FHS have collected data related to CVD and its risk factors. Owing to the long duration of follow-up and the rich and carefully-collected phenotypic data, the FHS is an ideal place to study the evolution of CVD risk factors (39).

Subsequently many studies were conducted in various populations including MRFIT study (23), ARIC study (24) and The Strong Heart Study (40) to estimate cardiovascular disease mortality and morbidity rates and the prevalence of known and suspected cardiovascular disease risk factors. ARIC study was a prospective study to investigate the etiology of atherosclerosis and its clinical sequelae and variation in cardiovascular risk factors, medical care, and disease by race, sex, place, and time in four US communities in 4,000 adults aged 45-64 years, which were examined twice, three years apart. The study aimed to investigate atherosclerosis by direct observation of the disease and by use of modern biochemistry. Following these studies came many interventions studies (41,42) for the treatment and prevention of CVD including MRFIT study. The MRFIT was a randomized clinical study to test whether a special-intervention program aimed at reducing serum cholesterol levels, BP and cigarette smoking would prevent CHD in middle-aged men during 7 years of follow-up. The Strong Heart Study consisted of 12 tribes in three geographic areas: an area near Phoenix, Arizona, the southwestern area of Oklahoma, and the Aberdeen area of North and South Dakota. The study includes three components. The first is a mortality survey to estimate cardiovascular disease mortality rates for 1984–1988 among tribal members aged 35–74 years, and the second is a morbidity survey to estimate incidence of both first and first or recurrent hospitalized myocardial infarction and stroke (cerebrovascular disease) among tribal members aged 45–74 years in 1984–1988, and the third is a clinical examination of 4,500 tribal members aged 45–74 years in order to estimate the prevalence of cardiovascular disease and its associations with risk factors (40).

2.2 DEFINITION AND PATHOGENESIS

CVD is a term that refers to more than one disease of the circulatory system including the heart and blood vessels affecting the brain, lungs, kidneys or other parts of the body (43,44). There are various types of cardiovascular disease are highlighted below (45,46).
Ischemic heart disease (IHD) is the most common type of cardiovascular disease in the world. It is a condition in which there is an inadequate supply of blood and oxygen to a portion of the myocardium; it typically occurs when there is an imbalance between myocardial oxygen supply and demand. The most common cause of myocardial ischemia is atherosclerotic disease of an epicardial coronary artery (or arteries) sufficient to cause a regional reduction in myocardial blood flow and inadequate perfusion of the myocardium supplied by the involved coronary artery.

Manifestation of CAD depends on degree and rapidity of development of obstruction of one or more of the coronary arteries. A partial blockage of one or more of the coronary arteries can result in a lack of enough oxygenated blood (ischemia) during exercise thus causing symptoms such as angina (chest pain) and dyspnea (shortness of breath) related to exertion. A complete blockage of an artery causes necrosis (damage to the tissues) or a myocardial infarction, commonly known as a heart attack.

Patients with ischemic heart disease fall into two large groups: patients with chronic coronary artery disease (CAD) who most commonly present with stable angina and patients with acute coronary syndromes (ACSs). The latter group, in turn, is composed of patients with acute myocardial infarction (MI) with ST-segment elevation on their presenting electrocardiogram and those with unstable angina and non-ST-segment elevation MI.

Angina can be classified as either stable or unstable:

- **Stable angina** follows a regular pattern. Physical activity or emotional stress can often trigger angina pain. Stable angina pectoris is characterized by chest or arm discomfort that may not be described as pain but is reproducibly associated with physical exertion or stress and is relieved within 5–10 min by rest and/or sublingual nitroglycerin. Stable angina can usually be controlled with medication or by stopping the physical activity.

- **Unstable angina** is less predictable. Unstable angina pain can happen any time, even when someone is asleep. Unstable angina is defined as angina pectoris or equivalent ischemic discomfort with at least one of three features: (1) it occurs at rest (or with minimal exertion), usually lasting >10 min; (2) it is severe and of new onset (i.e., within the prior 4–6 weeks); and/or (3) it occurs with a crescendo pattern (i.e., distinctly more severe, prolonged, or frequent than previously). Unstable angina is
most commonly caused by a reduction in oxygen supply and/or by an increase in myocardial oxygen demand superimposed on an atherosclerotic coronary plaque, with varying degrees of obstruction.

**Cerebrovascular disease** Cerebrovascular diseases include some of the most common and devastating disorders: ischemic stroke, hemorrhagic stroke, and cerebrovascular anomalies such as intracranial aneurysms and arteriovenous malformations (AVMs). It refers to a problem with the circulation in the blood vessels of the brain. A blockage with effects lasting less than 24 hours is referred to as a transient ischemic attack. A complete blockage with long-term effects is referred to as a cerebrovascular thrombosis or accident or a stroke.

**Peripheral vascular disease**: Peripheral arterial disease (PAD) is defined as a clinical disorder in which there is a stenosis or occlusion in the aorta or arteries of the limbs. Patients with this disease typically complain of pain in their calves especially when walking.

**Heart failure** is a clinical syndrome that occurs in patients who, because of an inherited or acquired abnormality of cardiac structure and/or function, develop a constellation of clinical symptoms (dyspnea and fatigue) and signs (edema and rales) that lead to frequent hospitalizations, a poor quality of life, and a shortened life expectancy. It occurs when the pumping action of the heart cannot provide enough blood to the rest of the body according to its requirement. This happens as a result of damage to the heart muscle due to ischemia, or from excessive consumption of alcohol, or cardiomyopathy. Patients with heart failure usually suffer from shortness of breath and swelling of the legs.

**Rheumatic heart disease** was once common in developing and underdeveloped countries. The disease results from an abnormal autoimmune response to a group A streptococcal infection in a genetically susceptible host. Acute rheumatic fever--the precursor to rheumatic heart disease--can affect different organs and lead to irreversible valve damage and heart failure. This disease begins with a bacterial infection in childhood, affecting joints and heart valves. Symptoms of the heart disease appear many years later. Other infections can occur in the inner tissues of the heart including the valves (endocarditis) and the outer tissue overlying the heart (pericarditis).
Congenital heart diseases are commonly related to abnormalities of the structure of the heart arising because of a birth defect. It is generally the result of aberrant embryonic development of a normal structure or failure of such a structure to progress beyond an early stage of embryonic or fetal development. These anatomical defects can be as simple as a small hole in one of the inside walls of the heart or they can be very complex, affecting the blood flows through the heart and lungs. Malformations are due to complex multifactorial genetic and environmental causes. Recognized chromosomal aberrations and mutations of single genes account for <10% of all cardiac malformations. Some congenital heart problems result in death while other causes disability to varying degrees and are treated by surgery later in life.

Among above described CVD, today with improvement in hygiene and environment and early detection of congenital heart disease, CAD has become synonymous with CVD in adults. The most common cause of CAD is atherosclerosis. Atherosclerosis starts early in life and fatty streaks has been demonstrated in as early as ten years in autopsies (47). Injury or infection can disrupt normal endothelial function and initiate formation of atherosclerotic lesions known as fatty streaks. Fatty streaks typically consist of macrophages and T cells embedded in a thin layer of lipids on the arterial wall (48-51). Macrophages engulf lipids, becoming activated foam cells that release an array of chemoattractant molecules, cytokines, and growth factors. More lymphocytes are attracted to the lesion and, in turn, add to the pool of effector molecules that expand and perpetuate the inflammatory response. As this cycle is repeated, the plaque develops a fatty core covered by a fibrous matrix that stabilizes the structure (49). Although the possible events that can initiate fatty streak formation remain controversial, low density lipoprotein (LDL) cholesterol modified by oxidation, glycation, and association with proteoglycans and immune complexes, can become trapped in the arterial wall, injuring the endothelium and vascular smooth muscle (52,53). Once trapped, LDL particles become progressively more oxidized, form lipid peroxides, and facilitate accumulation of cholesterol esters. Also, modified LDL cholesterol is chemotactic for circulating monocytes and stimulates the proliferation of macrophages already in the lesion (49). Inflammatory mediators increase the binding of LDL cholesterol to endothelial cells and vascular smooth muscle cells that have migrated into the lesion (54). As the plaque becomes thicker, the arterial wall responds by “remodeling,” that is,
gradually dilating to maintain the diameter of the vessel lumen. Eventually, macrophages may be stimulated to release metalloproteinases that degrade the fibrous cap and render the plaque vulnerable to rupture (55,56). Although several types of plaque can result in serious coronary events, retrospective analyses have demonstrated that 70% of all fatal acute myocardial infarctions and sudden coronary deaths are attributable to plaque rupture (57) or plaque erosion (58). This observation is not surprising because plaque destabilization is often accompanied by release of prothrombotic factors. However, a recently developed consensus document emphasizes that all types of atherosclerotic plaques can result in coronary events and sudden death (57). Vulnerable plaques are defined as thrombosis-prone or at risk of rapid progression and exhibit some combination of the following: active inflammation, thinning cap with a large lipid core, endothelial denudation with superficial platelet aggregation, fissures, or greater than 90% stenosis. An accumulating body of evidence suggests that atherosclerotic progression results from microinflammation mediated by proinflammatory cytokines. The observation that monocytes and T lymphocytes are present at all stages of plaque development is consistent with active inflammation. Chronic low-level inflammation increases atherosclerotic plaque deposition in animal models.

As the plaque burden increases, the atherosclerotic mass tends to stay external to the lumen, which allows the diameter of the lumen to be maintained; this is known as the Glagov effect, or positive remodeling. As plaque encroaches into the lumen, the coronary artery diameter decreases. Myocardial ischemia results from a discordant ratio of coronary blood supply to myocardial oxygen consumption. Luminal narrowing of more than 65 to 75 percent may result in transient ischemia and angina. Acute coronary syndromes result from ulceration or erosion of the fibrous cap, with subsequent intraluminal thrombosis (1).

2.3 MAGNITUDE OF PROBLEM

In the last century most populations has witnessed the most striking improvements in health in history. Life expectancy at birth has increased from an average of 46 years to 66 years in 1950 to 1998 respectively. The health status and disease profile of human have historically been related to their level of social organization and economic development. Due to industrialization, the major causes of death and disability have shifted from a
predominance of nutritional deficiencies and infectious diseases, to NCDs such as CVD, cancer, and diabetes. This shift has been termed as “the epidemiologic transition” (59).

Global Burden of Disease Study and in the World Health Report 1999 estimated that high burdens of NCDs contributed to 59% of global mortality (31.7 million deaths) and 43% of the global burden of disease in 1998. Several NCDs such as CVD, cancers, diabetes, and chronic obstructive pulmonary disease contributed to about 50% of global mortality which are related to common lifestyle determinants; diet, tobacco consumption and physical activity (60). In terms of disability adjusted life year loss (DALY loss), Cardiovascular disease is responsible for 10% of DALYs lost in low- and middle-income countries, and 18% in high income countries (59). It has been estimated that 9.2 million productive years were lost secondary to overall CVD in Indian adults, which contributed to the economic decline and as CVD rates will increase, this estimate will increases to 17.9 million by 2030 (61).

India was the second highest contributor to world growth in 2006 and it is one of the fastest growing economies of the world with an average GDP growth of 8.6% for the last 3 years. India’s growth is estimated to fall by 1% of gross domestic product because of the combined economic impact of CVD, stroke, and diabetes (62,63). There is dramatic shift in the diet and living behaviours of individuals, families and communities due to rapid economic growth, rural urban migration, urbanization, globalization and aggressive marketing. Accordingly, adverse dietary changes, sedentary activity, increasing tobacco use with subsequent changes in the CVD risk factors are accumulating at earlier stages and at great speed compared to other countries (17). India is estimated to have lost 8.7 billion international dollars in 2005 and will increase to 54 billion international dollars by 2015 because of CVD, stroke, and diabetes (35,63).

2.3.1 Global

According to WHO 16.7 million people around the globe die of CVD each year which is more than 29 percent of all deaths worldwide (64,65). Today, 80 percent of the burden is in low and middle-income countries affecting all age groups. By 2020 CVD will become the leading cause of both death and disability worldwide. CVD only will kill five times as many people as HIV/AIDS in these countries (63). In United Kingdom (UK) in 2008, CVD accounted for more than 191,000 deaths. Premature deaths from CVD were
more than 50,000 (66). Every seven minutes, a Canadian life is lost due to CVD which accounts for more deaths than any other disease. CVD mortality in 2000 showed 34 percent of male deaths and 36 percent of female deaths which costs the Canadian economy about $18.4 billion annually. The number of deaths due to CVD has increased as the number of elderly Canadians has been increasing. This trend is anticipated to persist for the next 15 years (67).

The number of years of productive life lost to CVD will have increased in 2030 compared to 2000 by 20, 30, 64, 57 and 95 percent in United States, Portugal, Brazil, China and India respectively. The increase in South Africa is 28 percent, comparable to Portugal and greater than that for the United States (68). In the Hoorn Study, the metabolic syndrome is associated with an approximate two-fold increased risk of incident CV morbidity and mortality in a European population (69). In a study of over 90,000 people with an acute vascular event in Oxfordshire, UK in 2002-05, 42 percent were coronary vascular, 9 percent were peripheral vascular; 45 percent were cerebrovascular (70). In United States in 2000, CVD was listed as primary or contributing cause for 39% of all deaths or 1 of every 2.5 among 2,400,000 deaths from all causes and claims more lives each year than the next five leading causes combined: cancer, influenza, COPD, accidents and diabetes mellitus (DM). Almost 150,000 CVD deaths occur in persons under 65 ages each year. CVD at 53% leads all other causes of CVD deaths (71). The high rates of acute vascular events and the steep rise in event rates with age in all territories have suggestions for prevention strategies, clinical trial design, and the targeting of funds for research and service provision.

Similarly, statistics from developing countries from western world like Colombia, Costa Rica, El Salvador, Guatemala, and Mexico also show increasing mortality due to CVD when comparing figures from the late 1960s with the mid 1980s (72). In Mexico among males there was a 5% rise in mortality attributable to CAD compared with a rise of 153% for El Salvador. Decrease in mortality from CAD has been reported from few developed countries e.g. Argentina, Chile, Uruguay, and Venezuela. Among other continents, data from Africa indicate that CAD is still relatively uncommon. However, it is estimated that 15% of deaths in Venezuela are attributable to CAD (73). In 1990 Sub-Saharan Africa CVD incidence rates were reported to be 60 per 100,000, with major
etiologies as cardiomyopathies, HTN, and rheumatic heart disease. A report from Nigeria found that the majority of sudden cardiac deaths were due to hypertensive heart disease, whereas CAD accounted for only 2 of the 50 cases, both of which occurred in upper social class urban dwellers. In South Africa’s population, CAD accounts for only 6% of CVD, and CAD mortality estimates are reported to be as low as 0.2% (74,75). Records from a Soweto, South African hospital showed only 36 patients with CAD in 1994 (76). However, CAD was the major cause of death amongst South African whites and South Africans of Asian descent (165.3 and 101.2 per 100,000 populations, respectively) (77).

2.3.2 Asians

Cardiovascular disease is the leading cause of death accounting for 29% of all deaths in 2005 in India, according to the WHO. In Indian subcontinent, the huge burden of CVD is the result of the large population and high prevalence of CVD risk factors. Moreover, sudden increase in the prevalence of traditional risk factors is expected to cause the increase in deaths and disability from CVD. This steep rise in CVD risk factor burden is further contributed by the rapid increase in the proportion of urban inhabitants (currently at 30% with a projected rise to 43% in 2021) (78). Urbanization encourages the development of dyslipidemia, HTN and dysglycaemia as it is characterized by a marked increase in the intake of high energy foods, psychosocial stress and decrease in physical activity (59,79).

There was a rise in the prevalence of CVD in the early half of twentieth century and a subsequent decline was noticed in the latter half, in the industrialized countries. However, the situation is reversed in India and other developing countries with a steady growth in prevalence of CVD (80). In the year 1975-96, Padmavati reported the prevalence of CAD to be 1.0% to 7.9 % in subjects above 20 years of age (81) and Ramachandran observed rise in prevalence to 14.3% with increasing age above 40 years (82). The Chennai Urban Population Study (CUPS) carried out in 1262 individuals showed the prevalence of CAD to be 11% in subjects with > 20 years of age, while the age-adjusted prevalence rate was 9.0%. The prevalence of CAD in urban Indians is fast approaching the figures reported in migrant Indians. Thus the prevalence of CAD is ten times higher in India compared to 40 years ago (83).

An epidemiological study done in the state of Jammu and Kashmir, where the population is undergoing lifestyle changes due to unusual stressful situation and disturbed
internal environment to assess the prevalence of CVD in both rural and urban areas of the valley, the overall prevalence of CVD was 7.54%, rural prevalence was 6.70% and urban prevalence was 8.37%. Prevalence of CVD was higher in males, 7.88% and slightly lower in females, 6.63% (85). In urban population in North India, CVD prevalence increased from 1% in 1960 to nearly 10% in 1994 and this increase was associated with a steep increase in major risk factors for CVD in the Asian Indian population (78).

The prevalence of CVD (clinical and ECG criteria) was 3.4% in males and 3.7% in females in Rajasthan, India in randomly selected villages. In this rural population, prevalence of CVD was found to be higher than in previously reported studies. Certain traditional risk factors were more common in villages as age, smoking, and HTN were found to be determinant of CVD (85). In Kerala state, a field survey was done in the rural population which indicated a lower prevalence of CVD in rural Thiruvananthapurum when compared to urban centres in India (86). The overall prevalence of CVD in urban population of Delhi, based on clinical history was 31.9 per 1000 adults and prevalence rate based on both clinical history and ECG criteria was 96.7/1000 adults. In both sexes the number of patients with CVD increased with advancing age. HTN, Obesity, diabetes and family history had the strongest association with CVD (87).

In a cross-sectional survey in Siliguri, West Bengal IHD was present in, 11.6% and HTN in 47.2% in subjects older than 40 years of age. Male had a higher (13.5%) prevalence of IHD than females (9.4%). IHD was significantly associated with HTN and smoking (88). In urban Goa, a community based cross-sectional study revealed prevalence of CHD to be 132/1000 population. The prevalence was significantly associated with family history, smoking, obesity, DM, HTN, total cholesterol (TC), LDL cholesterol and high density lipoprotein (HDL) cholesterol (89). Prevalence of CVD is reported to have doubled in rural and urban populations for the last 20 years. There are also regional differences in prevalence of CVD India. In the south of India, CVD prevalence rate of 7.4% in rural and 13.9% in urban dwellers were higher than in the north where 3.0 and 9.7% were reported for rural and urban dwellers, respectively (90). It is obvious from above data that India has a major and increasing CVD problem. Asian men had greater CVD mortality than Europeans, both in with and the without diabetes categories at baseline. The rate of CVD mortality in 1990 at 10.8 per 100 000 is nearly double that of China at 5.7 per 100
000 in similar year. South Asian men had double CVD mortality than of European men adjusted for age, smoking, and cholesterol. CVD mortality remained significantly higher in South Asian men in multivariable models also when that adjusted for conventional risk factors and diabetes and/or impaired glucose regulation, insulin resistance, or the metabolic syndrome. These data confirm that neither conventional risk factors, nor insulin resistance parameters or metabolic syndrome criteria as currently defined can account for this excess risk (91).

2.4 RISK FACTORS (TRADITIONAL)

2.4.1 Age

Aging is a complicated event, and in the process there is a loss in general function that impairs ability. Epidemiological studies have demonstrated that the process of aging is the major risk factor for CVD. The reason for this is, aging occurs while an individual is responding to various stressors by changing patterns of gene expression (genetic traits) on a background of environmental factors and disease states. Aging have effects at the tissue, organ, molecular, cellular and system levels as contributing to the altered function of the organism (92). With respect to the cardiovascular system, it is known that the changes that occur with age are modulated by other systems in the body like, functional changes in the autonomic nervous system during aging affect the overall function of the cardiovascular system. Similarly, changes in the endocrine system can have an important impact on cardiovascular function. With age, testosterone levels decreases and alters the distribution of contractile proteins in the heart. Age-related changes in the cardiovascular system differ in male and female subjects (93).

Prevalence of CVD and associated risk factors increases with increasing age. Prevalence of CVD increased with age and most of the patients (68.3%) were ≥65 years old (94). In a western population-based cohort study the outcome of acute myocardial infarction (AMI), stroke, or death, age at which population enters the high-risk category was 62.5 years and 68.9 years in male and female respectively. The transition to a high-risk category occurred at younger age in men and women with diabetes with mean difference of 14-6 years compared to those without diabetes (95).

Increase in prevalence of CVD with age occurs because of increasing prevalence of CVD risk factors with age. Within the general population there is increasing trend towards
increased BP with age (96). Prevalence of type 2 diabetes and the metabolic syndrome also increase with age and are associated with a high risk for adverse cardiovascular events (97). Large population studies have confirmed that diabetes is related with excess mortality in the elderly even in those 85 years and older and CVD increased with age in diabetic subjects (98,99).

An Indian study investigated the age and sex variation in the prevalence of CVD risk factors among the people of Asian Indian origin among 682 participants aged 25–85 years. Significant differences for age, body mass index (BMI), systolic BP (SBP), diastolic BP (DBP), TC, triglyceride (TG), LDL, VLDL, and TC:HDL and TG:HDL ratios across the groups was found and there were significant gender-specific group differences for obesity, high BP, high TC, high TG, and high fasting blood glucose (FBG). Age irrespective of sex alter CVD risk factors and require prevention as early as middle age (100).

Several pathophysiological mechanisms have been postulated to explain age related increase in prevalence of CVD like arterial stiffening, and endothelial dysfunction.

**Increased Wall Thickening and Arterial Stiffening**

During aging wall thickening and dilation are the major structural changes that occur in the large elastic arteries (101-103). The wall thickening involves both the tunica intima and the tunica media. Due to this remodeling, there is a reduction in arterial compliance with an increase in vessel stiffness. In aging, factors that contribute to the increased wall thickening and stiffening include increased calcification, reduced elastin and collagen. Extracellular matrix increases and becomes mostly rich in glucosaminoglycans (104).

**Endothelial Dysfunction**

The stiffening of large arteries during the aging process can be attributed to a reduction in endothelial function, which opposes contraction of the underlying vascular smooth muscle (105-111). In the vasculature of aging animals and humans, reductions in many of the components of these NO-dependent cell signaling pathways have been observed. Aging causes reduction in the amount of nitric oxide (NO) produced by the endothelial cells which is produced from L-arginine by endothelial NO synthase (eNOS). Endothelial NO synthase enzyme is constitutively active and regulated by the intracellular
concentration of calcium (Ca2+) and inhibited competitively by analogs of L-arginine such as *N*o-nitro-L-arginine. Another inhibitor of eNOS is asymmetric dimethylarginine, which is increased in older individuals and may serve as an additional mechanism to lower NO production by the endothelium (107). It has been demonstrated that the bioavailability of NO is reduced with aging, in addition to a reduction in the production of NO by the endothelial cells. This reduction is mainly the result of an increase in oxidative stress during aging (105,110,112). The dilator activity of NO is removed so vasoconstriction is promoted. Endothelium-derived NO diffuses to the smooth muscle cells and binds to smooth muscle cell-soluble guanylate cyclase, causing an increase in cyclic guanosine 3’5’ monophosphate (cGMP) and the consequent activation of cGMP-dependent protein kinase. NO/cGMP-dependent protein kinase signaling has been proposed to decrease intracellular Ca2+ concentration via the inhibition of L-type Ca2+ channels.

### 2.4.2 Sex

Prevalence of CVD is 2-3 times higher in males compared to females (113). The onset of symptomatic CVD is typically about 10 years earlier in men. Most of the risk factors were more favorable in women, but with the increasing age, sex difference in risk factor levels diminished. Differences in HDL cholesterol and smoking can explain nearly half of the difference in CVD risk between men and women. Nearly one third of the risk in males can be explained by differences in serum TC level, BP, BMI, and diabetes. The age-related increase in CVD incidence and mortality in both sexes was associated with an increase in risk factor levels but to a larger extent in women (113). Also in Edinberg Heart Study, men had less favorable heart disease risk factors than women. Men had significantly less favorable levels of cigarette smoking, dietary fiber, vitamin C, blood viscosity, uric acid, HDL cholesterol, and TGs than women (114). Only physical activity, alcohol intake and lower levels of fibrinogen were detected as favorable cardioprotective factors in men than women. Levels of common CVD risk factors do not explain the gender gap however; the sex difference in incidence rates persists in studies adjusted for risk factor differences (115) or stratified by level of risk factor (116). Renfrew-Paisley Study from Scotland observed lower absolute risk of CVD for any given level of risk factor in females though both had similar relative risk for a similar dose of each CVD risk factor (116). CVD incidence in women increases rapidly at menopause. In a study, men and women underwent
BP monitoring; men had higher BP than premenopausal female counterparts. However, BP increased in women after menopause, to levels even higher than those observed in men (117). Moreover, menopausal transition, leads to an increase in the prevalence of the metabolic syndrome, hyperinsulinemia, dyslipidemia, HTN and elevated body weight (118-119). With increasing age this difference in HDL particle size decreased, with an associated increase in cardiovascular risk (120). An additional CVD risk factor in women increase after menopause is elevated apolipoprotein(a) levels (121).

In women, endogenous female sex hormones, especially estrogens, are cardioprotective. These hormones work via multiple mechanisms: decreased LDL, increased HDL, and release of vasodilators such as NO and prostacyclin (PGI2) from vessel walls, which inhibits vascular constriction, lowering of BP as well as decreased platelet aggregation (122). Differences in body fat distribution, plasma lipoprotein levels, and indices of plasma glucose-insulin homeostasis explain the lower prevalence of CVD in premenopausal women, in comparison to age-matched men (123,124). Additionally, estrogen seems to contribute to glucose homeostasis via increased glucose transport into the cell (125). Women normally have a higher plasma HDL levels and lower plasma insulin, TG levels and apolipoprotein B levels which have been associated with abdominal visceral adipose tissue (126). Gender differences in the regulation of physiological mechanisms are directly influenced by genetic polymorphisms, at the cellular and biochemical level. In some studies CVD is linked with variations in the nuclear hormone family of ER genes, including ER- α gene (ESR1) and ER- β gene (ESR2) (127). These receptors function as ligand-dependent transcription factors and prevail in smooth muscle and vascular endothelial cells (128). Postmenopausal women are at increased risk of CVD who carry a particular ESR1 variant, independent of known CVR factors (129) but this association has not been observed in men (130).

2.4.3 Family history

Family history of a disease is important and an independent predictor of incidence of future disease, because it defines the relatively small subset of families in the population that account for the most cases. A positive family history of disease captures the underlying complexities of gene–gene and gene–environment interactions by identifying families with combinations of risk factors that lead to disease expression. The upper portion of the family
history distribution explains a larger fraction of CVD in the population than can be explained by extreme values of other risk factors (e.g., BP and cholesterol). Family history is a useful tool for identifying most prevalent cases of CVD and also identifies the relatively small subset of families in the population at highest risk for CVD who may benefit most from targeted screening and intensive intervention. Family history may be used to combine population-wide health promotion and risk-reduction efforts with a high-risk, targeted approach to help reduce the burden of CVD (131).

CVD risk can be assessed by using the, information about smoking, alcohol consumption, exercise, weight, HTN, and diabetes in multiple family members and can be correlated with incidence of CVD in the family (131). Family history should be considered positive if any first degree male had evidence of CVD before the age of 55 years or female before the age of 65 years according to current guideline (132). Family history has been used successfully to evaluate risk of CVD in the high school–based Health Family Tree Study in Utah. Family history of early-onset disease was much more predictive of early CVD in unaffected family members than was family history without respect to age. Older persons were at no more risk for CVD than the general population unless they had at least two family members who had been diagnosed with CVD. A similar pattern of risks was observed in families with a positive family history of HTN (133). Because some diseases appear to share certain environmental risk factors and common pathophysiologic pathways, a family history of one of these diseases may be relevant to assessing risk of the others. For example, families with a history of CVD are also more likely to have a history of HTN or diabetes (134). Throughout the United States, many community based programs screen for chronic diseases or risk factors. Most of these programs target only one disease (e.g., CVD or diabetes) or one risk factor (e.g., cholesterol or glucose) at a time. However, because an estimated 45% of families have a positive family history of one or more common chronic diseases (135), taking a family history can capture information about many diseases and risk factors simultaneously.

Familial tendencies in multiple CVD risk factors are observable early in life. As part of a total community study of CVD risk factor variables in children (ages 5-17 years), parental histories of heart attacks, HTN, diabetes, and stroke were obtained for each of 4,074 participating children. A family history of high BP was reported by 26.5% of all
children, diabetes by 7.1%, heart attacks by 6.8%, and stroke by 2.1%. The data indicated that Children of parents with CVD or manifested risk factors clearly differ in their own risk factor profiles from children of parents without these conditions and familial tendencies are observable in early in life (136). Cardiovascular morbidity and mortality in a family should be given importance when considering an individual for preventive measures. A study demonstrates that parents of middle-aged men with a pronounced cardiovascular risk profile show increased cardiovascular morbidity and mortality. Also, the number of parents who had died was increased in this group. However, whether or not a positive family history is an independent risk factor remains to be proven (137). In diabetic population, FH of CVD doubled the risk of CVD, and had synergistic effect in combination with other risk factors. In a large-scale multicenter-based diabetic population, among 3611 diabetic patients, it was found that family history of HTN was significantly associated with presence of HTN and obesity. Synergistic effects of family history of CVD in combination with HTN or aging on increasing CVD were found. Family history of diabetes, HTN, CVD and of stroke was significantly associated with family history of each disease, indicating clustering of family history. Clustering of family history may indicate interrelation of genetic predisposition (138).

2.4.4 Hypertension

Hypertension is a major risk factor for CVD, it is estimated that there were 118 million hypertensives in India in 2000; this number is projected to almost double to 214 million in 2025 (79,139). The attributable risk in India due to HTN is 16% for ischaemic heart disease, 24% for acute myocardial infarction, 21% for peripheral vascular disease, and 29% for stroke (140). In most urban populations of developing nations, have greater prevalence rates of HTN compared with rural populations (141-143). In an urban population of India, rates of hypertensives were (>160/95 mm Hg) were found to be 44% of CAD patients and 22% of CAD-free patients (87). In rural settings, 40% of urban and 12-17% of adults are suffering from HTN. Overall, 26.4% of the adult population in 2000 had HTN (26.6% of men and 26.1% of women), and 29.2% were projected to have this condition by 2025 (29.0% of men and 29.5% of women. The estimated total number of adults with HTN in 2000 was 972 million; 333 million in economically developed countries.
and 639 million in economically developing countries. The number of adults with HTN in 2025 was predicted to increase by about 60% to a total of 1.56 billion (139).

Treatment of arterial HTN reduces the incidence of CAD by 14% within 5 years (144) and in middle age, each 20mmHg reduction in SBP approximately halved the risk of stroke, IHD and vascular mortality. In individuals aged over 80 years the risk reduction for stroke, IHD and other vascular disorders was around 30%. The absolute risk reduction is greater in the older age groups, in spite of this apparently diminished benefit of lower BP. Interestingly, observational studies indicate that, low BP may also be associated with an increased risk of death and CVD in the elderly, in addition to high BP (145).

There are important pathophysiologic links between HTN and CVD which might explain the increased risk of CVD. Atherosclerosis is aggravated by arterial HTN. HTN is associated to metabolic disorders, such as dyslipidemia, insulin resistance with hyperinsulinemia and these are additional risk factors for atherosclerosis (146). Increase of transmural pressure in arterial vessels, with an increase in mechanical stress and endothelial permeability leads to deposition of lipids and the formation of the atherosclerotic plaque. Furthermore, there is endothelial dysfunction, remodelling of coronary arteries and increased resistance at microvascular level, contributing to a decrease of coronary reserve (147). Cardiac renin-angiotensin-aldosterone system, endothelial growth factor, platelet derived growth factors, atrial natriuretic peptide, and endothelin among other substances may be involved (148). Due to pressure overload there is increased wall stress which may stimulate perivascular fibroblasts proliferation and extracellular matrix proteins by these cells. It has been suggested that acute coronary syndromes might be favoured by an increased flow velocity and shear stress which could contribute to plaque disruption (149). Many studies have focused on related mechanisms which included a defect in the gene NO synthase, increased symmetrical dimethyl arginine, enhanced local participation of the renin-angiotensin system, or conversely, diminished participation of the local bradykinin-kinin system. Angiotensin II inhibits NO synthesis and that bradykinin promotes local nitric acid synthesis in the endothelium (150,151). There is impaired synthesis of NO from its amino acid precursor L-arginine by the endothelium of the coronary vasculature as well as by the myocytic endothelium in HTN (152,153). There is increasing evidence that HTN, like hyperlipidemia, induces oxidative stress in the arterial wall. It has been suggested that
superoxide anions might trigger the development of HTN by inactivating endothelium-derived NO (154).

2.4.5 Diabetes Mellitus

The incidence and prevalence of diabetes is increasing worldwide, due to adoption of the atherogenic, high-fat “Western” diet. At least 10.3 million Americans have DM and another 5.4 million are estimated to have undiagnosed diabetes. The 199 country analysis, which included 2.7 million individuals, estimated that the number of adults with diabetes has doubled within the past three decades—up from 153 million in 1980 to 347 million in 2008. 66 million people had diabetes in 2011 and by 2030 this will have risen to 552 million. The number of people with type 2 diabetes is increasing in every country. 80% of people with diabetes live in low- and middle-income countries. The greatest numbers of people with diabetes are between 40 to 59 years of age. 183 million people (50%) with diabetes are undiagnosed. Diabetes caused 4.6 million deaths in 2011. Diabetes caused at least 465 billion dollars (US) in healthcare expenditures in 2011 and 11% of total healthcare expenditures in adults (20-79 years) (156).

Approximately 90% of patients with diabetes have the type 2 variety. The onset of type 2 diabetes usually precedes clinical diagnosis by several years (155). An increasing prevalence of type 2 diabetes cannot be separated from the rising prevalence of obesity and physical inactivity in our society. An estimated 97 million adults in the United States are overweight or obese (157) and 75% of adult Americans have minimal physical activity or daily exercise (158). Both excess body fat and physical inactivity predispose to type 2 diabetes. The growing ethnic diversity contributes to the increasing prevalence of type 2 diabetes in the United States (159). Several ethnic groups are susceptible to type 2 diabetes: Hispanics, blacks, Native Americans, and Asians (especially South Asians) (160-163).

DM and impaired glucose tolerance are significant risk factors for CAD and are prevalent throughout much of the developing world. Close to 50% of the global diabetic population is found within China, where the prevalence of the disease was 1% as of the early 1990s. In Hong Kong, Singapore, and Taiwan show prevalence rates as high as 10% for type 2 DM in those aged >40 years (164). In the Eastern Mediterranean, 17 million people are diabetic and a similar number have impaired glucose tolerance (15). While frank
diabetes may be relatively rare; the frequency of impaired glucose tolerance may be as high as 9% in some populations in Sub-Saharan Africa, (e.g., in Tanzania) (165). Individuals with DM are recognized as being at high risk for developing CAD. The Indian subcontinent has a higher prevalence of DM compared to other region in the world, which is 2-3 times the reported prevalence in Western countries (166). Data from health examination surveys and epidemiological studies showed that Age-standardised adult diabetes prevalence was 9·8% in men and 9·2% in women in 2008, up from 8·3% and 7·5% in 1980. The number of people with diabetes increased from 153 million in 1980 to 347 million in 2008 (156). The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. The prevalence of diabetes is higher in men than women. The urban population in developing countries is projected to double between 2000 and 2030 (167). Approximately 285 million people worldwide (6.6%) in the 20–79 year age group will have diabetes in 2010 and by 2030, 438 million people (7.8%) of the adult population, is expected to have diabetes.(IDF 2009) In India alone, an estimated 19.3 million people had diabetes in 1995, and this is expected to almost triple to 57.2 million in 2025 (168). The International Diabetes Federation (IDF) estimates the total number of people in India with diabetes to be around 50.8 million in 2010, rising to 87.0 million by 2030 (169). CVDs are listed as the cause of death in 65% of persons with diabetes (170). In India, the prevalence of diabetes is estimated at 6% to 8% in urban settings compared with 2% to 3% in rural areas (59,171). The Indian Council of Medical Research (ICMR) estimates that the prevalence of diabetes is 3.8 per cent in rural areas when compared with 11.8 per cent in urban areas. This might be a substantial underestimate, according to some preliminary cross sectional survey data from Prospective Urban Rural Epidemiologic (PURE) study (172). Prevalence of DM is higher among Indians who migrate to industrialized nations (59). Up to 19% of Indians in Britain and 22% of Indians in South Africa have diabetes (173,174). This phenomenon may reflect the impact of any diet changes associated with migration, and, may be a harbinger of the impact of globalization of diet. For diabetes, the attributable risk is 9% for acute myocardial infarction, 4% for stroke, 2% for neuropathy and 32% for cataract among Indians (175).
They threaten to become more common in the context of increasing exposure to Western-influenced diets. In patients having IGT and Diabetes, prevalence of CAD was higher as compared with normal glucose tolerance group in CUPS No.5 study (83). Americans over 50 years of age without metabolic syndrome regardless of diabetes status had the lowest CHD prevalence (8.7% without diabetes, 7.5% with diabetes). Those with metabolic syndrome without diabetes had higher CHD prevalence (13.9%) and those with both metabolic syndrome and diabetes had the highest prevalence of CHD (19.2%) compared with those with neither (176).

A large body of epidemiological and pathological data documents that diabetes is an independent risk factor for CVD in both men and women (177-179). Women with diabetes seem to lose most of their inherent protection against developing CVD (117,180). To make matters worse, when patients with diabetes develop clinical CVD, they sustain a worse prognosis for survival than do CVD patients without diabetes (181-183). Moreover, myocardial ischemia due to coronary atherosclerosis commonly occurs without symptoms in patients with diabetes (184). As a result, multivessel atherosclerosis often is present before ischemic symptoms occur and before treatment is instituted. A delayed recognition of various forms of CVD undoubtedly worsens the prognosis for survival for many diabetic patients (160). These considerations have convinced the Scientific Advisory and Coordinating Committee of the American Heart Association that DM deserves to be designated a major risk factor for CVD. This formal designation commits the AHA to a greater emphasis on diabetes as a risk factor in its scientific and educational programs. Both type 1 diabetes and type 2 diabetes are independent risk factors for CVD (177-179).

Atherosclerosis is the major threat to the macrovasculature for patients with and without diabetes. Clinically, dyslipidemia is highly correlated with atherosclerosis, and up to 97% of patients with diabetes are dyslipidemic (185). Hypertriglyceridemia in diabetes occurs, in part, because insulin action regulates lipid flux. Insulin promotes the activity of the enzyme lipoprotein lipase, which mediates free fatty acid uptake into adipose tissue (storage) and also suppresses the activity of the enzyme hormone-sensitive lipase, resulting in decreased release of free fatty acids into the circulation (186). Hypertriglyceridemia can lead to increased production of the small, dense form of LDL and to decreased HDL transport of cholesterol back to the liver (187). In addition to the characteristic pattern of
increased TGs and decreased HDL cholesterol found in the plasma of patients with diabetes, abnormalities are seen in the structure of the lipoprotein particles. In diabetes, the predominant form of LDL cholesterol is the small, dense form. Small LDL particles are more atherogenic than large LDL particles because they can more easily penetrate and form stronger attachments to the arterial wall, and they are more susceptible to oxidation. Because less cholesterol is carried in the core of small LDL particles than in the core of large particles, subjects with predominantly small LDL particles have higher numbers of particles at comparable LDL cholesterol levels (188).

There is increasing interest in the role of oxidative stress in type 2 diabetes. In diabetes, the levels of a commonly used marker for oxidative stress, plasma oxidized LDL, were shown to be elevated. Oxidized LDL is pro-atherogenic because once the particles become oxidized they acquire new properties that are recognized by the immune system as “foreign.” Thus, oxidized LDL produces several abnormal biological responses, such as attracting leukocytes to the intima of the vessel, improving the ability of the leukocytes to ingest lipids and differentiate into foam cells, and stimulating the proliferation of leukocytes, endothelial cells, and smooth muscle cells, (189) all of which are steps in the formation of atherosclerotic plaque. In patients with diabetes, LDL particles can also become glycated, in a process similar to the glycation of the protein hemoglobin (measured in the hemoglobin A1c [A1C] assay). Glycation of LDL lengthens its half-life (190) and therefore increases the ability of the LDL to promote atherogenesis. Glycation of HDL shortens its half-life and renders it less protective against atherosclerosis (193). When these mechanisms are defective, the process of atherosclerosis is accelerated. Therefore, both insulin deficiency and insulin resistance promote dyslipidemia accompanied by increased oxidation, glycosylation, and TG enrichment of lipoproteins. In addition, endothelial dysfunction is present, and all of these factors contribute to the increase in atherogenicity, and thus macrovascular disease, found in patients with diabetes (192).

Diabetes contributes to defects in the autonomic nervous system, the endothelium, and local metabolism, all of which can result in microvascular disease. Diabetic autonomic neuropathy (DAN) is associated with impaired autoregulation of blood flow in a variety of vascular beds, including the skin and the heart (193,194). Patients with DAN have increased rates of sudden cardiac death as well as a higher cardiovascular mortality rate.
These patients have been found to lack the normal cardiac flow reserve that is activated under conditions of increased demand for myocardial perfusion, (195) which may partially explain the high mortality rate in this population.

In addition to the dysregulation of vascular tone caused by DAN, subjects with diabetes have been found to have decreased bioavailability of NO, a potent vasodilator, as well as increased secretion of the vasoconstrictor endothelin-1. This resulting state of vasoconstriction has been found in subjects with the metabolic syndrome as well as those with diabetes (196). Diabetes decreases NO bioavailability because of either insulin deficiency or defective insulin signaling (insulin resistance) in endothelial cells (197). Hyperglycemia also acutely inhibits the production of NO in arterial endothelial cells (198).

Different molecular mechanisms associated with hyperglycemia have been identified including increased glucose flux through the polyol pathway (197), formation of advanced glycation end products (AGE), activation of protein kinase C (PKC), increased glucose flux through the hexosamine pathway, and activation of the 12/15-lipoxygenase pathway. All these mechanisms finally lead to increased superoxide formation (197-201).

Diabetes has long been considered a state of chronic, low-level inflammation (202) and there is evidence to suggest that this immune activation may precede insulin resistance in diabetic and pre-diabetic states and ultimately may be the factor that initially increases cardiovascular risk in these disease processes (203). Inflammation is a normal response to tissue injury or pathogen exposure and is a critical factor in the body’s ability to heal itself or to fight off infection. The inflammatory response involves the activation of leukocytes (white blood cells) and is mediated, in part, by a family of cytokines and chemokines. Pro-inflammatory cytokines can enhance the production of reactive oxygen species (ROS). The term ROS refers to a subset of molecules called “free radicals.” This term refers to any molecule that contains an unpaired electron in the outer orbital. This unpaired electron makes the molecule highly reactive, seeking to either donate an electron to another compound or take up protons from another compound to obtain a stable electron pair (204). This high reactivity leads to the formation of bonds between the ROS and other compounds, altering the structure and function of the tissue. Because of the reactive propensity of these molecules, ROS can directly damage a number of cell components,
such as plasma membranes and organelles. Although inflammation is beneficial, if this response is chronically activated it can have a detrimental effect.

2.4.6 Dyslipidemia

Dyslipidemia is widely established as an independent risk factor for cardiovascular disease (205). Low HDL cholesterol and hypertriglyceridemia have been found to be independently related to myocardial infarction/stroke (206). Additionally, a combination of high fasting glucose and low HDL cholesterol were shown to have primary predictive ability for CHD (207). The role of HDL cholesterol values, as an important modifiable stroke risk factor, was further supported in the study of Sacco and colleagues (208).

Dyslipidemia is a primary, major risk factor for CAD and may even be a prerequisite for CAD, occurring before other major risk factors come into play. The ICMR surveillance project reported a prevalence of dyslipidemia of 37.5 per cent among adults aged 15-64 yr, with an even higher prevalence of dyslipidemia (62%) among young male industrial workers. The INTERHEART investigators reported that the prevalence of dyslipidemia was higher among study participants living in the five South Asian countries (45%) compared with participants from the other 47 countries represented in the study (35%) (209). The impact of dyslipidemia on the burden of CVD has been otherwise understudied at a population level in native South Asians, despite its large contribution to CVD in other world populations (79).

Given the high prevalence of CAD among Indians, several studies on cardiovascular risk factors in native Indians highlighted differences in lipid levels for CAD compared to Western studies. Achari and Thakur (210) in a large retrospective study on 5748 CAD patients and 8103 healthy normals; reported higher serum cholesterol levels, LDL cholesterol levels and TC to HDL ratio among the CAD subjects compared to normals. They also showed that there is a lack of association of serum TGs levels with CAD. Similarly, Burman et al, observed high LDL cholesterol levels and TC/HDL cholesterol ratio; and Lp(a) levels were higher in CAD patients compared to controls but there was no significant difference in serum TG levels (211). It was noted in CUPS Study that LDL cholesterol and age were risk factors for CAD but serum TG levels did not come out as an independent variable (83). Another large clinic-based study in type 2 diabetic subjects on 17,855 looked for the association of isolated hypercholesterolemia and isolated
hypertriglyceridemia with CAD (212). The prevalence of CAD was significantly higher among patients with isolated hypercholesterolemia, isolated high LDL and isolated low HDL cholesterol compared with normalipidemic individuals, but not in those with isolated hypertriglyceridemia. There are differences in lipid associations with CAD between native and migrant Indians. Serum TG levels have been consistently found to be associated with CAD in migrant Indians. In native Indians, LDL cholesterol and TC/HDL cholesterol ratio appears to be more important. One factor which is common to all Indians is a low HDL cholesterol level (213). In the face of low HDL cholesterol levels, even moderate elevation of LDL cholesterol appears to be sufficient to produce an atherogenic profile. The role of TGs cannot be completely ruled out as the link between hypertriglyceridemia and CAD has been shown in several studies. Moreover, increase in TG levels is associated with low HDL cholesterol and with small dense LDL molecules. In a study conducted in USA there was increase in prevalence of small dense LDL in migrant Asian Indians (214). Prevalence of elevated LDL cholesterol defined by the NCEP guidelines levels is only 38.8% among CAD subjects in the study done by Achari (210). This suggests that either the cut-off used for elevated LDL cholesterol is not appropriate among Indians or that more than 60% of the CAD is not explained by elevated LDL cholesterol levels. In another clinic study done on 17,855 type 2 diabetic patients a similar finding was observed, the prevalence of myocardial infarction was 2.9% in subjects with LDL levels below 100 mg/dl compared to 3.61% in subjects with LDL above 100 mg/dl (212). This shows that in Indians, even those with LDL below the cut-off of NCEP have a high risk for CAD suggesting that aggressive lipid lowering is justified in Indians.

The dyslipidemia may be caused by a combination of overproduction of very low density lipoprotein (VLDL), apo B-100, decreased catabolism of apo B containing particles, and increased catabolism of HDL-apo A-I particles. These abnormalities may be the consequence of a metabolic effect of insulin resistance. Although the underlying mechanisms for this pattern are not fully understood, a cascade of events has been proposed. Macrophages incorporate TG-rich remnants and become foam cells, forming the central pathology of atherosclerosis. Foam cells play a major role in plaque development by accumulating and oxidizing LDL-C within the atherosclerotic vessel wall. Further, the accumulation of foam cells eventually results in the formation of a necrotic core within the
atherosclerotic plaque. If the fibrous cap of the plaque ruptures, then a thrombus is created when the contents of the necrotic core enter the lumen (215).

2.4.7 Smoking

Tobacco is the second largest contributor to the burden of disease, both globally and in each region of the world, and is one of the largest causes of premature death and disability (216). Almost half (48%) of the acute myocardial infarctions, 22% of the strokes and 14.8% of the ischaemic heart disease have been attributed to tobacco. At the beginning of the 21st century, the threat that tobacco poses to health globally is greater than ever before (217-220). Today, the tobacco epidemic kills more than 4 million around the world annually and at least 400,000 people in the US. Projected estimates are 10 million deaths a year worldwide by the year 2015. Of the estimated 1.2 billion currently living smokers, 80% live in poor or middle income countries, 500 million will eventually die of smoking-related diseases, especially cancer and cardiovascular disease. During the 20th century, 100 million fatalities attributed to tobacco. With the current global trend in tobacco use, this figure may reach one billion in the 21st century. Many tobacco deaths can be prevented if current smokers quit, but in many countries, especially poorer ones, quitting is rare (221). If smoking-related morbidity and health care costs are considered, the current and future global burden of the tobacco epidemic can be better estimated (222,223).

In 2002, a national survey of tobacco use reported that the Indian subcontinent, had an alarming rate of current tobacco use of 56 per cent among Indian men aged 12-60 yr which is second to China in both the production and consumption of tobacco products. Reddy and colleagues also observed in a survey of sixth and eighth graders attending school in an urban setting that the prevalence of tobacco use (any history of use or current use) was 2-3 times higher among sixth graders compared with eighth graders (224), suggesting a concerning new wave of smoking among India’s youth may cause serious future public health consequences for the Indian subcontinent (79).

Voluntary changes in tobacco consumption go together with both healthy and unhealthy changes in biological risk factors for CVD. Data of the Amsterdam Growth and Health Longitudinal Study (AGAHLS) found that in sexes, trends for a reduction in BP, body weight, and waist hip ratio (WHR) and a rise in the TC/HDL-C ratio with increasing tobacco consumption. Opposite trends were found with reducing tobacco consumption. In
women, body weight, WHR, and waist circumference reduced significantly and independently with increasing tobacco consumption and increased significantly with decreasing tobacco consumption (225).

There are several potential mechanisms by which smoking may increase the risk of CVD. Several aspects of vascular damage have been identified by smoking which include decreased coronary blood flow and myocardial oxygen delivery; adverse effects on lipids, BP, and insulin resistance; and decreased activity of endothelial NO systems (226). The endothelium is responsible for maintaining vessel integrity and controls vascular tone and inflammatory processes. Endothelial damage can lead to reduced capacity for dilation and increased vessel contraction, prothrombotic and pro-inflammatory states, and cell proliferation in the arterial wall. In both active and passive smokers, the production of endothelial NO is reduced, which mediates endothelium-dependent vasodilatation in response to hemodynamic changes (227). Furthermore, free radicals in the smoke have been shown to cause morphological and biochemical damage to the endothelium (228). In young males, cigarette smoking is associated with the appearance of high-risk vulnerable plaques, such as non-calcified plaques, in vessels with a preserved lumen. In a Japanese imaging study, multislice computed tomography was used to detect non-calcified plaques in 242 consecutive subjects. It was found that smoking-induced blood vessel injury may increase the risk of non-calcified plaques incidence in young males. Exposure to cigarette smoke in both active and passive smokers increases arterial wall stiffness, possibly as a result of the changes in endothelium (229,230). Data from the Bogalusa Heart Study showed that even among healthy young adults, smoking causes deleterious effects on arterial wall dynamics, lowering compliance in small arteries and increasing systemic vascular resistance (231).

Smoking increases the progression of atherosclerosis (226), with more pronounced plaque development in the coronary and carotid arteries and in the abdominal aorta (179). Up-regulation of collagenase I, a matrix metalloproteinase, was observed in human artery smooth muscle cells in response to increased nicotine concentrations in vitro (232). Matrix metalloproteinases are thought to weaken the arterial wall, contributing to destabilization and rupture of existing atherosclerotic plaques. Population studies have shown that tobacco smoking is associated with elevated levels of OxLDL-C which is a risk factor for CVD.
Free oxygen radicals derived from cigarette smoke lead to increased oxidative stress, increasing levels of OxLDL-C and depressing NO production. In a study of patients undergoing carotid endarterectomy, the correlation between levels of oxidized OxLDL-C and plaque morphology was examined. Levels of OxLDL-C were significantly higher in macrophage-rich plaques, compared with macrophage poor plaques. Compared with control subjects; patients with macrophage-rich plaques had higher plasma levels of OxLDL-C. The investigators concluded that high plasma and plaque levels of OxLDL-C are correlated with vulnerability of atherosclerotic plaques to rupture (235).

Serum TC and TG are both increased by smoking, while levels of HDL-Cholesterol are decreased and levels of LDL-Cholesterol are increased (236) Lipid peroxidation is also increased in smokers (237), leading to a more rapid uptake of LDL-C by macrophages. These macrophages subsequently develop into foam cells, the predominant cells in an atherosclerotic lesion. Smoking activates platelets, increasing the risk of thrombus formation and leading to damage to the lining of the arteries, facilitating the development of atherosclerosis. Levels of platelet activation are higher in smokers than non-smokers (226). Thromboxane, a marker of platelet activation, is increased in smokers but rapidly declines upon cessation, indicating that the increase is likely to be a direct result of smoking (238).

Smoking may also lead to mitochondrial damage, resulting in decreased energy production in the heart muscle (239) and mitochondrial DNA damage (240). Smoking also appears to increase other risk factors of CVD, such as type 2 diabetes (241), along with CVD risk factors, such as Hcy, hsCRP, and fibrinogen (markers of inflammation) (237). The inflammatory response is an essential component in the initiation and evolution of atherosclerosis. Several studies have indicated that cigarette smoking causes about a 20% to 25% increase in the peripheral blood leukocyte count (242). In vivo, cigarette smoking is associated with an increased level of multiple inflammatory markers including C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) in both male and female smokers (243-246). 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 (244,247). Cigarette smoking also causes activation of proatherogenic molecules leading to
alteration in cell-cell interactions. Therefore, smoking cessation and the control of HTN should take precedence over all other risk factors in order to lower the incidence of NCPs (248).

2.4.8 Physical Inactivity

Physical activity has been associated with general physical and mental well being. Physical activity means combination of the duration, intensity, or frequency of activity. Thus, defining the amount of physical activity required for health benefits. Most of the societies recommend a minimum of 30 minutes of moderate- to vigorous intensity activity on “most days” of the week (249,250). Prevalence of leisure time physical activity was substantially lower among South Asians (6.1% of control arm patients) compared with the rest of the world (21.6%) in the INTERHEART study (79). Prevalence of physical activity is less than the levels recommended for enhancing health is high in developing countries (17 to 91%) from developed countries (4 to 84%). In developed countries, physical inactivity is associated with considerable economic burden, with 1.5-3.0% of total direct healthcare costs being accounted for by physical inactivity. There is little information on the effectiveness and cost-effectiveness of physical activity-enhancement strategies in developing countries. The WHO has signaled a shift from the treatment of illness to promotion of health, with an emphasis on changing modifiable health-risk factors, including smoking, unhealthy diets and physical inactivity, the question, especially for developing countries (251).

There are gender differences in physical activity. Many investigators have reviewed the literature on the association of physical activity with cardiovascular disease in women. Epidemiological studies revealed that overall levels of physical activity are lower in men as compared with women and because women are engaged in different types of activities than men (252). The relation between physical activity and CVD in women was highlighted by study among 1,564 women from University of Pennsylvania alumnae (mean age, 45.5 years), initially free of CVD, from 1962 until 1993. An interaction between BMI and physical activity on CVD risk was observed (p = 0.023) Walking 210 blocks/day (approximately 6 miles (9.7 km)/week) was associated with a 33% decreased risk of CVD (253). Physical activity is associated with improvement in several cardio-metabolic risk factors and is associated with a lower risk of CVD mortality. A hospital-based case control
study from two urban centers in India suggested that daily moderate intensity physical activity (e.g., the equivalent of briskly walking 35-40 min per day) is associated with a 55 per cent lower risk for CVD (79,254). The extent to which metabolic risk factors mediate the association between physical activity and CVD mortality was evaluated in Third National Health and Nutrition Examination Survey with follow-up of 13.4 ± 3.9 years. They concluded that physical activity was associated with a lower risk of CVD mortality independent of traditional and inflammatory risk factors. The results suggest that physical activity may protect against CVD mortality regardless of the presence of metabolic risk factors (255). Another study (Health Professionals’ Follow-up Study) conducted in 2803 men (> 30 years) with DM; physical activity was associated with reduced risk of CVD, cardiovascular death, and total mortality (256).

Among 23 observational studies describing the association of the amount of leisure time or occupational activity on CVD, only 8 studies failed to observe a dose response association between physical activity and CVD mortality. Some studies that did not report an inverse graded association, did report threshold effects or U-shaped associations, indicating elevated risks of mortality at both the lowest and the highest levels of activity. The complete absence of moderate to vigorous physical activity is associated with a higher incidence of cardiovascular morbidity and mortality (257). In 2002, the Health Professionals Follow-Up Study reported an inverse association between exercise intensity and incidence of CVD in 44 452 men who were followed from 1986 to 1998 (258). Despite the consistent inverse association observed across studies, it is still not possible to determine a summary “minimum dose” required to prevent CVD because of the variety of instruments used to quantify physical activity.

The protective effect of physical activity may be mediated, at least in part, by controlling various known risk factors for CVD. The biological mechanisms which are associated with physical activity are reductions in plasma fibrinogen and platelet activity, as well as elevations in plasma tissue plasminogen activator activity and HDL concentrations (259-262). Regular exercise seems to cause similar favourable changes in the lipoprotein profile, such as decrease in TG, increase of total HDL-C, HDL3-C, apo A-I and apo B and enhancement of the lipoprotein lipase activity (263-265). Thus, one of the
possible biological mechanisms by which physical activity and exercise reduce the risk of stroke could be the serum lipid modification.

Physical activity is also known to reduce thrombotic potential through enhanced fibrinolytic activity and reduced platelet adhesiveness. Even regular leisure-time activity is associated with reductions in several haemostatic and inflammatory markers, including fibrinogen, viscosity, platelet count, white-blood cell count, CRP, coagulation factors (VIII, IX and vWF) and fibrinolytic variables (fibrin and D-dimer) (265,266). The benefit of physical activity on cardiovascular disease may, at least in part, be mediated through these factors (265,266).

Another recent study concerning the possible effects of regular physical activity on vessel behaviour showed that exercise training significantly enhanced the responses to acetylcholine and flow-mediated dilation, and that even short-term exercise training improved endothelium-dependent NO-mediated vascular function (267). Similarly, in CAD patients, regular physical activity has been shown to improve endothelial function by increasing phosphorylation of endothelial NO synthase (eNOS), improving agonist-mediated endothelium-dependent vasodilator capacity (268). The bioavailability of endothelium-derived NO reflects its rates of production and inactivation by superoxide ($O_2^-$), a reactive species dismutated by extracellular superoxide dismutase (ecSOD). Treadmill exercise training was found to increase eNOS and ecSOD expression (269), as well as to improve endothelium-dependent vasorelaxation and determinants of NO bioavailability (270), and therefore it could be considered as another possible risk reduction mechanism in humans.

2.4.9 Ethnicity

Ethnicity is an important factor, because people from some ethnic background are more susceptible to premature CVD than others. There are differences in genetic makeup as well as differences in cultural and social practices between ethnic groups. Risk factors for CVD also differ such as raised cholesterol, hypertension, obesity and diabetes. These differences in ethnic groups increase the probability of CVD also differences. For the migrants, for virtually all population living in the western world, CVD is the main cause of death. The study of ethnic differences in CVD has provided valuable etiological clues, not just for ethnic minority groups but also for the majority population. Migrants of South
Asian descent worldwide have elevated risks of morbidity and mortality from ischaemic heart disease (213). In the UK, mortality from IHD in both South Asian men and women is 1.5 times that of the general population; and African Caribbeans have a significantly lower risk compared to the majority population. These ethnic differences are greatest in the youngest age groups. The traditional risk factors, such as smoking, BP, obesity, and cholesterol vary substantially between subgroups of South Asians that in some cases, levels are equivalent to, or lower than, a comparable European population whereas —levels of glucose intolerance, central obesity, fasting TG, and insulin are uniformly elevated compared to Europeans. This has lead to recognize potential importance of insulin resistance in the etiology of CHD in South Asians. Attention has been paid to associated inflammation, infection, haemostatic factors, and endothelial dysfunction. CRP concentrations are elevated in South Asians (271). CRP is highly correlated with fasting and post-load insulin concentration, and independently predicts the risk of CVD. Plasminogen activator inhibitor-1 (PAI-1) and Hcy concentrations are also raised in South Asians. There is also impairment of endothelial function. These all factors may contribute to the elevation in CVD risk, either directly or indirectly from their associations with insulin resistance. Other factors, which may also account for the ethnic susceptibility to CVD include lipoprotein Lp(a), which is substantially raised in South Asians compared to Europeans, concentrations of which is largely genetically determined (272).

2.4.10 Obesity

Obesity is becoming a global epidemic (273,274). In the past 10 years, there is dramatic increase in obesity in both children and adults in the United States (275,277). Obesity is an increasingly prevalent metabolic disorder affecting not only the US population but also that of the developing world. It is estimated from the third National Health and Nutrition Examination Survey (NHANES III) (1988-1991) that 33% of the US population is obese, compared with 25% in NHANES II (1976-1980) (278). Obesity is associated with a number of comorbidities, including several forms of heart disease. Although heredity can explain 30% to 70% of cases of obesity, there is substantial contribution from environmental changes to the increasing prevalence of obesity because the gene pool has remained stable over the same interval. Diets high in fat and calories (279) and a reduced expenditure of energy in the form of physical activity (280) are the
most likely explanations. In developing countries CVD and associated risk factors are seen at a lesser degree of excess weight, suggesting that relative weight may be as important as absolute adiposity.

The definition of obesity, or being overweight (281), remains controversial. Previously in the United States, mortality data provided by the Metropolitan Life Insurance Company historically have been used to define obesity (282). These data relate to mortality only, which were arbitrary and not independently related to obesity-related mortality or comorbidities. BMI has been a better measure of adiposity (283,284). BMI is defined as weight in kilograms divided by height in meters squared (kg/m²). Unfortunately, a BMI-based definition fails to take body fat distribution into account. Substantial evidence now indicates that an increased waist circumference, or waist-to-hip ratio, predicts comorbidities and mortality from obesity better compared to BMI (285,286).

Epidemic of obesity can be traced from the end of the century with the use of the BMI (276,287). The initial alarm bell was sounded in 1994 by the National Center for Health Statistics when they reported that from 1988–1994 (NHANES III) to NHANES 1999–2000, the prevalence of overweight in adults increased from 55.9% to 64.5%. During that same period, the prevalence of obesity increased from 22.9% to 30.5% (276,277,288). This sudden, unanticipated jump in the prevalence of obesity led the American Heart Association (AHA) to call for action to curb the consequences of this epidemic (289,290).

India, obesity is emerging as an important health problem particularly in urban areas, paradoxically co-existing with undernutrition. Almost 30-65% of adult urban Indians are either overweight or obese or have abdominal obesity. The rising prevalence of overweight and obesity in India has a direct correlation with the increasing prevalence of obesity-related co-morbidities; HTN, metabolic syndrome, dyslipidemia, type 2 DM, and CVD. Recent consensus guideline from India suggested obesity over 25 kg/m², though not accepted worldwide (291). The currently recommended cut-offs of BMI recommended by World Health Organization include 18.5 - 24.9 kg/m² for normal, 25.0 - 29.9 for overweight and >30 kg/m² for obesity (292).

Obesity is associated with numerous comorbidities such as CVD, type 2 diabetes, HTN, and certain cancers. In fact, obesity is an independent risk factor for CVD (293,294) and CVD risks have been documented in obese children (287,295). A 30% increase in all-
cause mortality was also seen in female and male subjects (275). Another study, after 55 years of follow-up, reported an excess mortality rate among male but not female subjects who were overweight in the US reference population as compared with those who were lean (296). Thus, obesity is associated with an increased risk of morbidity and mortality and is associated with reduced life expectancy (297,303). The estimated years of life lost as the result of obesity differ among races and between genders, but it was estimated that the optimal BMI for adults age 18 to 85 years is 23 to 25 for whites and 23 to 30 for blacks (304). Though similar figures are not available for Indian population; Indians have similar degree of fat at BMI of 22; which Caucasians have at BMI of 25 (291). Prospective studies that have reported follow-up data over >2 decades, such as FHS, the Manitoba Study, and the Harvard School of Public Health Nurses Study, have documented that obesity is an independent predictor of clinical CVD.(299,305-307) On the other hand, in patients with known CVD or after acute myocardial infarction, overall obesity as assessed by BMI is inversely related to mortality(308,309).

Abdominal obesity appears to be an independent predictor of all-cause and cardiovascular mortality and morbidity in men and perhaps also in women. Insulin resistance and truncal obesity account for the twofold excess incidence of diabetes in Indian Asian and African Caribbean women, but not men who were first-generation migrants. Compared with Europeans, age-adjusted subhazard ratios for men and women, respectively, were 2.88 and 1.91 in Indian Asians, and 2.23 and 2.51 in African Caribbeans (310). Obesity is the most important factor associated with insulin resistance. Increase of 1/3 over ideal body weight decreases insulin sensitivity by 40% (311). It is important to note that all obese individuals are not insulin resistant. Central obesity is frequently associated with insulin resistance in Asian Indians settled in other countries (312-314) and in India (315,316) Obesity is associated with both fatty streaks and raised lesions in the coronary arteries. Black subjects had more extensive fatty streaks than did white subjects in all arterial segments, and men had more extensive raised lesions compared to women (317). Importantly, when BMI and abdominal skin fold thickness were simultaneously considered in men, a BMI ≥30 kg/m² was associated with coronary lesions only among individuals with a abdominal skin fold thickness (≥17 mm), which reinforces the concept that central fat distribution is more important than total fat as a risk factor for atherosclerosis (317). The
preferential deposition of fat centrally after the menopause may explain in part why the risk for CVD events increases 10 to 20 years later in women than men (318-320).

BMI and WC are measures of obesity that can be useful in identifying individuals with these risk factors (321). Mechanisms which relate obesity with an increase in BP are related to changes in cardiac output, peripheral vascular resistance, endothelial dysfunction, insulin resistance, sympathetic nervous system, substances released from adipocytes (IL-6, TNF-α, and so forth), and sleep apnea. As individuals become obese and their adipocytes enlarge, the adipose tissue undergoes molecular and cellular alterations that subsequently affect systemic metabolism. Macrophage numbers in adipose tissue also increase with obesity (322), apparently acting as scavengers of apoptotic adipocytes. It also has been reported that there is a marked increase in these scavengers in obese subjects (323). Macrophage accumulation and the subsequent local inflammation are believed to result in numerous metabolic dysfunctions that accompany obesity, including systemic inflammation and atherosclerosis. Several proinflammatory factors are produced in adipose tissue as obesity increases. When compared to lean individuals, adipose tissue in obese individuals shows higher expression of proinflammatory proteins, including TNF-α and IL-6 (324,325).

Obesity is associated with abnormal endothelial function (326). The reduction in endothelial function is the result of a decrease in NO. Decreased NO in obesity may be related to an increase in oxidative stress (327) or may result from pro-inflammatory cytokines. In the FHS, BMI was highly associated with systemic oxidative stress, as determined by creatinine-indexed urinary 8-epi-PGF$_2$α levels (328). A decrease in the function of NO would result in vasoconstriction and an increase in vascular resistance that may predispose to CVD risk factors such as HTN. The adipocyte acts as an endocrine organ, and plays a substantial role in the pathogenesis and complications of obesity (329,330). Increase in obesity is related to increase in production of TNF-α and IL-6 and other cytokines. A strong correlation exists between obesity and IL-6 and CRP levels (331). IL-6 is a pro-inflammatory cytokine that, among many other things, stimulates the production of CRP from the liver. Thus, obesity is somewhat similar to a low-grade systemic inflammation. Low-grade inflammation may play a role in increasing BP (332). Increases in systolic and diastolic BPs, pulse pressure, and mean arterial pressure were
significantly associated with levels of IL-6, whereas systolic BP, pulse pressure, and mean arterial pressure were associated with levels of soluble intercellular adhesion molecule-1. Elevated plasma IL-6 levels were significantly associated with systolic and diastolic BP in women, whereas in men, IL-6 was associated with fasting insulin and fasting insulin resistance index (332). This increased synthesis may interfere with the action of insulin to suppress lipolysis; if so, this would represent insulin resistance of adipose tissue. CRP may play a role in the development of leptin resistance, which is important because endogenous hyperleptinemia does not reduce appetite or increase energy expenditure. Increased concentrations of both CRP and leptin were associated with insulin resistance and an increased risk of major CV events (333).

2.5 RISK FACTORS (NON-TRADITIONAL)

2.5.1 Insulin Resistance

Insulin resistance is a multifaceted syndrome that can express itself in many ways, depending on a particular individual's genetic background. The term Metabolic syndrome have been initially defined, in a formal way, by the Swedish physician Eskil Kylin. In 1923, Dr Kylin reported on a metabolic disorder characterized by HTN, hyperglycemia, and hyperuricemia (336). More recently, in 1988, Gerald Reaven and his colleagues identified a clustering of risk factors that they coined Syndrome X (335). Reaven was among the first to suggest that insulin resistance and obesity were directly linked to this clustering of metabolic risk factors that predispose an individual to cardiovascular CVD and type 2 DM. Insulin resistance is a common disorder in the general population, within any given age-group, i.e., young, middle aged, and elderly. Although it is recognized that insulin resistance increases the risk for type 2 diabetes, it is not well known that it increases the risk for CVD (336-338). There are number of factors which increase the risk for insulin resistance, including genetic predisposition, obesity and inactivity, aging, medications, polycystic ovary syndrome, and rare disorders such as partial lipodystrophy (339). Insulin resistance is a statistical predictor of type 2 DM, where it is found in more than 80% of subjects. About 20% of subjects with isolated high BP or isolated hyperuricemia are insulin resistant, at least half of subjects with isolated overweight or obesity are insulin resistant, and as many as 80% of subjects with isolated hypertriglyceridemia and/or isolated low
HDL cholesterol are insulin resistant. Moreover, 20-25% of apparently healthy individuals have insulin resistance (340,341).

Obesity drives the development of insulin resistance, which in turn promotes the development of CVD (339) through several pathophysiologic mechanisms; Glucose intolerance and hyperglycemia facilitate the accelerated formation of advanced glycation end products (AGEs), which interact with AGE-binding receptors to promote atherosclerosis, through changes in the function of endothelial, macrophage, and smooth muscle cells. Increased small, dense LDL cholesterol particles, apolipoprotein (apo)-B, decreased HDL cholesterol, and hypertriglyceridemia characterizes the dyslipidemia of visceral obesity and insulin resistance, and contribute to CVD (339,342,343). Insulin resistance decreases production of NO, responsible for normal vasodilatory response and endothelial function (339,343). Insulin resistance impairs thrombolysis by increasing the levels of PAI-1(339,344-347). Insulin resistance itself is a proinflammatory state characterized by elevated levels of inflammatory markers because a growing body of data suggests that adipose tissue is an inflammatory milieu that directly produces inflammatory mediators of CVD (348).

Insulin resistance is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells (349). Insulin resistance in fat cells reduces the effects of insulin. Increased flux of stored lipids in these cells elevates free fatty acids in the blood plasma. Insulin resistance in muscle cells reduces glucose uptake, whereas insulin resistance in liver cells results in impaired glycogen synthesis and a failure to suppress glucose production. Elevated blood fatty acid, reduced muscle glucose uptake, and increased liver glucose production all contribute to elevated blood glucose levels (350). High plasma levels of insulin and glucose due to insulin resistance are believed to be the origin of metabolic syndrome and type 2 diabetes, including its complications. In the young group, the most insulin-sensitive individual uses glucose at a rate that is four times that of the most insulin-resistant person. To compensate for this defect in insulin-mediated glucose metabolism, the beta-cell must increases its secretion of insulin. This is an adaptive process in that the hyperinsulinemia preventing the development of glucose intolerance and frank DM. In most people, the increase in plasma insulin concentration probably has little or no consequence. However, in genetically
predisposed individuals, the hyperinsulinemia may have important clinical implication (351).

Insulin resistance and hyperinsulinemia are also associated with an atherogenic plasma lipid profile. Elevated plasma insulin concentration increases VLDL synthesis, leading to hypertriglyceridemia. Elimination of lipid and apolipoproteins from the VLDL particle leads to formation of intermediate-density and LDLs, which are atherogenic. Insulin is known to be atherogenic because it enhances cholesterol transport into arteriolar smooth muscle cells and increases endogenous lipid synthesis by these cells. It also stimulates the proliferation of arteriolar smooth muscle cells, increases collagen synthesis in the vascular wall, formation of lipid plaques, and stimulates the production of various growth factors. The atherosclerotic plaque is affected by the plasma insulin concentration which is characterized by excessive amounts of lipid and collagen, foam macrophages, and proliferated smooth muscle cells (352). In a study, Cruz et al. (353) demonstrated that insulin infusion into one femoral artery of the dog resulted in marked proliferation and the accumulation of cholesterol and fatty acids on the insulin-infused side. Consequent studies have shown that adding insulin markedly stimulated the proliferation of cultured smooth muscle cells (354-356). In both vivo and in vitro after the addition of insulin, enhanced LDL-receptor activity and increased cholesterol and TG synthesis have been demonstrated in arterial smooth muscle cells, fibroblasts, and mononuclear cells (357-362). The effect of insulin on vascular smooth muscle cells increases lipid synthesis by its stimulatory action on the lipogenic enzymes glucose-6-phosphate dehydrogenase, malic enzyme, and 3-hydroxyacyl-CoA dehydrogenase (363-364). Hyperinsulinemia has been shown to inhibit the reabsorption of plaques once formed and promote the development of the atherosclerotic plaque (365-366). Insulin and insulin like growth factors augments the collagen synthesis which is an integral component of the atherosclerotic lesion (367,368). (270,271). Insulin itself is a growth-promoting substance and it stimulates various other growth factors, including IGFI, which cause cell proliferation and thereby contribute to the atherosclerotic process (369-377).

Insulin resistance when present with HTN it adds significantly to the overall risk for cardiovascular disease (340). Insulin resistance of essential HTN correlates directly with the severity of HTN. The association of insulin resistance and essential hypertension can be
due to four mechanisms: Na+ retention, sympathetic nervous system overactivity, disturbed membrane ion transport, and proliferation of vascular smooth muscle cells. Abnormalities in vasodilation and blood flow have been suggested to provide a link between HTN and insulin resistance (378). The link between endothelial dysfunction and the HTN of the insulin resistance syndrome is appealing because of the possibility that defective vasodilation. Insulin or hyperinsulinaemia may induce endothelial dysfunction in insulin-resistant individuals by interfering with generation of vasodilatory and vasoconstrictive substances such as NO and endothelin-1.

Ferrannini E et al (1991) surveyed 2,930 subjects, and reported prevalence rates for obesity, Type 2 DM, impaired glucose tolerance, HTN, hypertriglyceridaemia, and hypercholesterolaemia 54.3, 9.3, 11.1, 9.8, 10.3 and 9.2%, respectively in Mexican Americans population. The prevalence of each of these conditions in its isolated form was 29.0% for obesity, 1.3% for type 2 diabetes, 1.8% for impaired glucose tolerance, 1.5% for HTN, 1.0% for hypertriglyceridaemia, and 1.7% for hypercholesterolaemia. In this population fasting and post-glucose hyperinsulinaemia was associated with higher BMI, waist-hip ratio (WHR), fasting and post-glucose glycaemia, systolic and diastolic BP, serum TGs, TC levels, and with lower HDL-cholesterol concentrations. It was concluded that insulin sensitivity, glucose tolerance, BP, body fat mass and distribution, and serum lipids were metabolically interrelated and an insulin resistance syndrome underlies each and all of the six disorders carrying an increased risk of CAD (379).

Zavaroni I et al (1999) evaluated the ability of hyperinsulinemia to predict the development of glucose intolerance, HTN, and CHD in a previously healthy population. The study population (647 individuals) consisted of the subjects evaluated in 1981 and divided into quartiles on the basis of the plasma insulin response to a glucose challenge. The results indicated that the 25% of the population with the highest insulin response had significant increased in the incidence of impaired glucose tolerance (IGT) or type 2 diabetes (eightfold), HTN (twofold), or CHD (threelfold). Hyperinsulinemia predicted the three clinical endpoints independent of differences in age, gender, or BMI. The development of CHD was predicted by plasma TG and mean arterial BP in multiple logistic regression analysis (380).
Han TS et al (2002) performed an 8-year follow-up study in 628 non-Hispanic whites and 1340 Mexican Americans with age 25 to 64 years, from the second cohort of the San Antonio Heart Study. High WHR and fasting insulin levels were found to be significant predictors of developing metabolic syndrome. High anthropometric indices remained significant predictors of metabolic syndrome after adjusting for fasting insulin. Waist circumference, BMI, and insulin had similar areas under the receiver operating characteristic curves (0.74 to 0.76). Of subjects who had a combination of high BMI (>or =30 kg/m²) and high waist circumference, 32% developed metabolic syndrome, compared with 10% of subjects with both low BMI and low waist circumference (381).

Hanley AJ et al (2002) investigated the relationship of the HOMA-IR and insulin levels, with risk of nonfatal and fatal CVD over the 8-year follow-up of the San Antonio Heart Study. Logistic regression analysis indicated that risk of a CVD event increased across quintiles of HOMA-IR after adjustment for age, sex, and ethnicity. Additional adjustment for LDL, TG, HDL, systolic BP, smoking, alcohol consumption, exercise, and waist circumference (WC) only modestly reduced the magnitude of these associations. There was similar direction of the relationship between insulin concentration and incident CVD (382).

Piche ME et al (2005) examined 112 postmenopausal women not receiving hormone therapy. Body fat distribution was measured by computed tomography, and insulin sensitivity was determined by a euglycemic-hyperinsulinemic clamp. hs-CRP and IL-6 were significantly associated with anthropometric and metabolic variables, including visceral and subcutaneous adipose tissue, systolic and diastolic BP, TGs, HDL cholesterol, and insulin sensitivity (p<0.05). Women with greater hs-CRP concentrations showed deterioration in their metabolic risk profiles, including abdominal obesity, greater TG and lower HDL cholesterol concentrations, and lower insulin sensitivity compared with women with lower hsCRP levels. These results suggested that increased visceral adipose tissue levels appear to be a determinant covariable of the association between high hsCRP concentrations and alteration in the metabolic profile (383).

Saely CH et al (2005) investigated the impact of the metabolic syndrome and insulin resistance on the incidence of vascular events. Both the metabolic syndrome and insulin resistance predicted vascular events after controlling for non-metabolic syndrome...
risk factors. After additional adjustment for insulin resistance, the metabolic syndrome remained significantly predictive of vascular events and conversely, insulin resistance remained significantly predictive of vascular events despite adjustment for the metabolic syndrome. Additional adjustment for the presence of type 2 diabetes revealed that both the metabolic syndrome and homeostasis model assessment of insulin resistance significantly predicted vascular events independent from diabetes status. Both the metabolic syndrome and insulin resistance are strong and mutually independent predictors of vascular risk among angiographed coronary patients (384).

Mojiminiyi OA et al (2007) evaluated the determinants and associations of plasma adiponectin in relation to the metabolic syndrome in patients with Type 2 diabetes. Adiponectin, leptin, hs-CRP, fasting plasma insulin, glucose, glycated hemoglobin and full lipid profile was done. Adiponectin levels were inversely correlated with age, indices of obesity, IR and hs-CRP. Overweight/obese and non-obese insulin-sensitive patients had significantly higher adiponectin levels than those with IR despite similar BMI and waist circumference. Adiponectin showed stepwise decrease with increasing number of the criteria for diagnosis of the metabolic syndrome. In patients with Type 2 diabetes, adiponectin concentrations were closely related to IR and the components of the metabolic syndrome (385).

Ajjan R et al (2007) investigated ethnic differences in insulin resistance and non-traditional cardiovascular risk factors in relation to the [International Diabetes Federation (IDF)] definition of the metabolic syndrome. In 245 healthy South Asians and 245 age and sex-matched Caucasians, 95 (39%) South Asian and 50 (20%) Caucasian subjects had the metabolic syndrome. In South Asian subjects, HOMA-IR, CRP, and PAI-1 were significantly higher in subjects with the metabolic syndrome. In contrast, in Caucasian individuals there was no difference in HOMA-IR and only CRP, and PAI-1 were higher in subjects with the metabolic syndrome. They concluded that metabolic syndrome was associated with insulin resistance in the South Asian but not the Caucasian population (386).

Esteghamati et al (2008) determined the association of insulin resistance (HOMA-IR) and HTN in a total of 2047 diabetic and non-diabetic individuals with or without HTN, with age range of 30-75 years. Hypertensive patients had significantly higher HOMA-IR
than age, sex, and waist girth-adjusted normotensive individuals in both non-diabetic and diabetic groups. Multivariate logistic regression analysis showed that after adjustment for age, sex, waist girth, BMI, TG, TC, FBG, and C-peptide, HOMA-IR was a significant independent predictor of HTN in all subjects and in diabetic and non-diabetic subjects separately (387).

You T et al (2008) examined the relationship of metabolic syndrome to several adipokines in a cross-sectional analysis among 1914 individuals aged 70-79 years without cardiovascular disease or type 2 DM. Both the presence of metabolic syndrome and the number of metabolic syndrome components were associated with higher levels of leptin, PAI-1, IL-6, TNF-alpha, and CRP and with lower levels of adiponectin (388).

Park K et al (2009) studied the cross-sectional and longitudinal relationships between CRP and insulin resistance in a population-based, prospective observational study, Coronary Artery Risk Development in Young Adults (CARDIA) study, during 1992-2006. CRP showed a significant positive association with insulin resistance. The gradient of HOMA-IR across CRP was attenuated but remained statistically significant after controlling for body fat measurements, and was little changed by further adjustment for oxidative stress markers [F(2)-isoprostanes and oxidised LDL]. There were consistent increments in the levels of HOMA-IR with increasing concentrations of CRP over time. In contrast, higher HOMA-IR did not predict future increases in CRP. Although a substantial portion of this association was explained by obesity, CRP was independently related to concurrent and future insulin resistance (389).

Esteghamati A et al (2009) determined the association between physical activity and insulin resistance in Iranian adults. The data of the third national Surveillance of Risk Factors of Non-Communicable Diseases (SuRFNCD-2007) in Iran were used. HOMA-IR values significantly increased from the high category to the moderate and low categories of physical activity. After adjustment for age, area of residence, smoking, and BMI (BMI), total physical activity, duration of intensity of activity, and the time spent on sedentary behaviors were significantly correlated to HOMA-IR. The prevalence of physical inactivity increased linearly with increasing HOMA-IR quintiles. They suggested that encouraging physical activity may help to prevent insulin resistance and its adverse consequences (390).
Chou HH et al (2010) conducted a cross-sectional study to investigate whether the association between insulin resistance and CRP levels is independent of abdominal obesity in a nondiabetic Taiwanese population (300 men and 274 women). The CRP levels were categorized into quartiles from the lowest to the highest concentrations. BP, fasting glucose level, TGs level, waist circumference, and HOMA-IR were all found to be significantly higher in Q3 and Q4 than in Q1 and Q2. HOMA-IR was significantly associated with CRP levels in both sexes in either obese or non-obese populations. Multiple linear regression analysis adjusting for age, smoking, components of metabolic syndrome, and waist circumference showed that the association between HOMA-IR and CRP levels remained significant in both men and women. This study shows that insulin resistance is strongly associated with CRP levels independent of abdominal obesity in nondiabetic Taiwanese population (391).

Bertoluci MC et al (2010) examined 131 patients (57.0 +/- 10 years-old, 51.5% men) in a cross-sectional study and were classified with or without CAD. Prevalence of CAD was 56.7%, HOMA-IR (HOMA-IR: 3.19 (1.70-5.62) vs. 2.33 (1.44-4.06), p = 0.015) and TG/HDL index were higher in the subjects with CAD compared to those without CAD. Increased HOMA-IR, TG/HDL and their product were positively associated with angiographic CAD (392).

Garg MK et al (2011) studied beta-cell function, as assessed by HOMA model, in subjects with metabolic syndrome. Clinical evaluation included anthropometry, body fat analysis by bioimpedance, biochemical, and insulin measurement. Subjects with metabolic syndrome had more HOMA-IR than controls (3.35 ± 3.14 vs. 1.76 ± 0.53, P = 0.029) and secreted less insulin (HOMA-S) than controls (66.80 ± 69.66 vs. 144.27 ± 101.61, P = 0.0003), although plasma insulin levels were comparable in both groups (10.7 ± 10.2 vs. 8.2 ± 2.38, P = 0.44). HOMA-IR and HOMA-S were related with number of metabolic abnormalities. HOMA-IR was positively associated with BMI, WHR, body fat mass, and percent body fat. HOMA-S was negatively associated with WHR, fasting plasma glucose and TC and positively with basal metabolic rate. Percent body fat was an independent predictor of HOMA-IR and WHR of HOMA-S in multiple regression analysis. Subjects with MS have increased IR and decreased insulin secretion compared with healthy controls (393).
Kim J et al (2011) investigated the association between objectively measured physical activity and metabolic syndrome in middle-aged Japanese individuals (179 men and 304 women, aged 30 and 64 years). Participants were divided into two groups using the Japanese criteria for metabolic syndrome as those with metabolic syndrome or pre-metabolic syndrome, and those without metabolic syndrome The results of this cross-sectional study indicate that the Exercise and Physical Activity is inversely associated with the prevalence of metabolic syndrome in men (394).

Indulekha K et al (2011) assessed levels of hs-CRP, TNF-α, IL-6, and VCAM-1 in South Indian subjects with and without metabolic syndrome and among metabolic syndrome subjects with and without insulin resistance. From the population-based Chennai Urban Rural Epidemiology Study, 334 subjects with metabolic syndrome and 342 subjects without metabolic syndrome were selected. Subjects with metabolic syndrome had significantly higher levels of inflammatory markers compared to those without metabolic syndrome: hs-CRP (2.57 vs 2.19 mg/liter) (p < .05), TNF-α (4.47 vs 3.89 pg/ml) (p < .05), IL-6 (16.22 vs 10.96 pg/ml) (p < .05). In the total study subjects, hs-CRP (r = 0.089, p = .047), TNF-α (r = 0.113, p = .040), IL-6 (r = 0.176, p = .042) were significantly correlated with metabolic syndrome. With increasing quartiles of insulin resistance, mean levels of hs-CRP and TNF-α increased linearly. Metabolic syndrome subjects with insulin resistance had higher levels of hs-CRP and TNF-α compared to metabolic syndrome subjects without insulin resistance. In Asian Indians, inflammatory cytokines hs-CRP, TNF-α and IL-6 are elevated in subjects with metabolic syndrome while hs-CRP and TNF-α are further elevated in those with metabolic syndrome and insulin resistance (395).

Sandeep S et al (2011) assessed the association of IR with cardiovascular risk factors in subjects with normal glucose tolerance in Asian Indians. This cross-sectional study recruited subjects from the Chennai Urban Rural Epidemiology Study in which 1550 subjects with normal glucose tolerance were included. HOMA-IR was found to be significantly associated with systolic BP (beta = 0.100, p < 0.001), diastolic pressure (beta = 0.094, p < 0.001), TC (beta = 0.068, p = 0.005), serum TGs (beta = 0.105, p < 0.001), LDL cholesterol (beta = 0.118, p < 0.005), and HDL cholesterol (beta = -0.060, p < 0.001) even after adjusting age, gender and BMI. Subjects with family history of type 2 diabetes had significantly higher HOMA-IR compared to those without family history. Subjects
with heavy grade activity had significantly lower HOMA-IR values compared to the light grade activity. Subjects with generalized obesity and abdominal obesity had significantly higher HOMA-IR which remained statistically significant even after adjusting for age and gender. There was a linear increase in the mean values of HOMA IR with increase in number of components of metabolic syndrome. Among Asian Indians who are known to have high risk of premature CAD and diabetes, a significant association exists between insulin resistance and cardiovascular risk factors even among subjects with normal glucose tolerance (396).

Færch K et al (2012) examined whether associations between hyperglycemia and CVD risk were explained by underlying insulin resistance. The study was done in 60 middle-aged individuals without diabetes and the associations of fasting plasma glucose, 2-hour post oral glucose plasma glucose, insulin sensitivity and body fat percentage with CVD risk were studied. Both fasting and 2-hour plasma glucose levels were associated with higher Framingham risk score and insulin sensitivity with lower Framingham risk score. However, association between fasting and 2-hour glucose with Framingham risk score after adjustment for insulin sensitivity was interdependent and disappeared after adjusting for each other. In contrast, insulin sensitivity was strongly associated with Framingham risk score after adjusting for both glucose levels. Hence, they concluded that the association between plasma glucose levels and CVD risk is mainly explained by insulin resistance (397).

An X et al (2012) estimated the relationship between insulin resistance and progression of coronary atherosclerotic plaque in 366 patients who have undergone coronary angiogram and were subsequently found to have coronary atherosclerotic plaques or normal angiograms in China; and were followed for 1-year for the progression of the coronary lesions. The modified Gensini score was adopted for assessing coronary lesions and HOMA-IR was measured. The Gensini score between both visits was significantly elevated in the higher insulin resistant group. Multivariate logistic binomial regression analysis revealed that insulin resistance (HOMA-IR > 3.4583) was an independent predictor for coronary arterial plaque progression. HOMA-IR remained an independent predictor for atherosclerosis plaque progression when participants were divided into diabetic and non-diabetic population also (398).
Marques-Vidal P et al (2012) assessed the associations between diabetes, insulin resistance (using HOMA-IR) and metabolic syndrome with the inflammatory markers hs-CRP, interleukin-1beta (IL-1β), IL-6 and TNF-α. IL-6, TNF-α, and hs-CRP were significantly and positively correlated with fasting plasma glucose, insulin and HOMA-IR. Participants with diabetes had higher IL-6, TNF-α and hs-CRP levels than participants without diabetes; this difference persisted for hs-CRP after multivariate adjustment. Participants with metabolic syndrome had increased IL-6, TNF-α and hs-CRP levels; these differences persisted after multivariate adjustment. Participants in the highest quartile of HOMA-IR had increased IL-6, TNF-α and hs-CRP levels; these differences persisted for TNF-α and hs-CRP after multivariate adjustment. Subjects with diabetes, metabolic syndrome and increased insulin resistance, present with increased levels of IL6, TNF-α and hs-CRP, while no association was found with IL-1β (399).

Gotoh S et al (2012) studied 2,356 Japanese individuals aged 40 to 79 years with a 75 g oral glucose tolerance test and followed them for 14 years. They estimated insulin resistance by homeostasis model assessment (HOMA-IR). During follow-up, 260 subjects developed CVD. The age- and sex-adjusted hazard ratios of CVD significantly decreased with increasing HOMA-IR levels. After adjustment for age, sex, serum TC, electrocardiogram abnormalities, proteinuria, smoking habits, alcohol intake, and regular exercise, the risk of CVD was significantly higher in the fifth quintile of HOMA-IR values compared with the first quintile (HOMA-IR Q5: HR= 1.55 [1.05-2.29]). They concluded that elevated HOMA-IR levels were a significant risk factor for stroke, but not for CHD and suggested that insulin resistance significantly increases the risk of incident CVD through metabolic syndrome in Japanese (400).

Nakagomi A et al (2012) studied the effects of insulin resistance associated with hyperinsulinemia on the long-term prognosis in patients with vasospastic angina (VSA) in a total of 265 selected patients with VSA and 56 control subjects with atypical chest pain. During the median follow-up period of 90.0 months, thirty-one patients developed cardiac events, including 6 sudden cardiac deaths and 25 readmissions for acute coronary syndrome. The data indicated that insulin resistance associated with compensatory hyperinsulinemia increases the risk of cardiac events in VSA patients (401).
2.5.2 Vitamin B12 and homocysteine Status

Homocysteine is a sulphhydryl containing amino acid produced by demethylation of methionine which is an essential amino acid (402). Methylation of Hcy, which is catalyzed by methionine synthetase, produces methionine. Methionine synthetase needs vitamin B12 as a co-factor. Hcy can also metabolise to cystathionine through the action of the cystathionine-B-Synthetase (CBS) enzyme. In humans folic acid provides the methyl essential for the reactions to take place, while vitamin B12 acts as a coenzyme (403,404). Therefore, deficiency of folic acid and vitamin B12 cause reduction in methylene tetrahydrofolate reductase (MTHFR) activity, leading to decrease in methionine synthesis and Hcy accumulation. Mild or moderate cases of hyperhomocysteinemia (Hcy > 15 µmol/lit), which is commonly prevalent in the general population is due to nutritional deficiency, while severe hyperhomocysteinemia (Hcy>100 µmol/lit), usually due to metabolic disorders of methionine metabolism (405-407). Other conditions which can also cause hyperhomocysteinemia are polymorphism in the coding gene of MTHFR, consumption of folate antagonists such as carbamazepine and methotrexate and disorders of Hcy metabolism during hypothyroidism and renal failure (408-409). Several studies demonstrated that high plasma level of Hcy acts as an independent risk factor of atherosclerosis and CAD. In the first landmark study by Wilcken and Wilcken in 1976, they showed that CAD had a correlation with higher levels of Hcy (410). It has been demonstrated that in the presence of traditional risk factors, Hcy may play a permissive role in endothelial damage. On the contrary sufficient data is not available regarding the role of hyperhomocysteinemia in development of premature CAD (411-412). The evidence suggests that high levels of Hcy may damage coronary arteries or make it easier for blood clotting cells called platelets to clump together and form a clot. Hyperhomocysteinaemia was associated with an increased risk of atherosclerotic disease. In a meta-analysis of 27 observational studies which included about 4,000 subjects, analysis suggested that an increase in basal total plasma Hcy levels of 5 µmol/L was associated with 60% and 80% increased risk of CHD in men and women, respectively (413). Subsequent meta-analysis of prospective observational studies of first events demonstrated an association between hyperhomocysteinaemia and elevated risk of CVD. An increase in plasma Hcy levels by 25% was associated with 11% and 19% excess risk for IHD and stroke, respectively, after
correction for other cardiovascular risk factors (414). Many observational studies have provided support for an association between hyperhomocysteinaemia and atherosclerotic vascular disease and the largest of these, the European Concerted Action Project, which included 750 subjects with arterial vascular disease and 800 controls, showed that an increase in plasma Hcy levels was an independent risk factor for CVD. Subjects with total Hcy levels > 80th percentile had a 2.2-fold increased risk for CVD compared with those with Hcy levels < 80th percentile. Several studies have reported a statistically significant positive association between elevated Hcy and CHD (415-418) and stroke (418-419). Other studies failed to demonstrate a significant association between plasma Hcy and CHD. In a nested case-control study (Physicians’ Health Study) including 333 male patients and 333 controls (from a total population of 14,916 male patients) followed up for a mean of 7.5 years, failed to demonstrate any significant association between elevated Hcy and risk for myocardial infarction and CHD death (420). Similarly, the Atherosclerosis Risk in Communities Study cohort (421), the Multiple Risk Factor Intervention Trial cohort (422), and the North Karelia Project (423) failed to show any significant association between elevated Hcy levels and risk of major coronary events or stroke (424-426). Taken together, evidence from case-control studies as well as prospective studies supports an association between elevated plasma Hcy levels and increased cardiovascular risk (427). Adequate concentrations of folate, vitamin B12, or vitamin B6 may decrease the circulating level of Hcy. However, whether lowering Hcy levels is associated with any significant decrease in vascular events by administration of folate and vitamins B6 and B12 in populations at risk remains the subject of ongoing controversy. Folate and vitamin B12 treatment improved insulin resistance and endothelial dysfunction, along with decreasing Hcy levels, in patients with metabolic syndrome (26).

**Metabolic pathway**

Hcy is not obtained from the diet (428) but it is biosynthesized from methionine via a multi-step process. Hcy is metabolised via two major pathways: remethylation and transsulfuration. In the remethylation cycle, homocysteine is recovered by the attainment of a methyl group in a reaction catalyzed by methionine synthase. Vitamin B12 (cobalamin) is an essential cofactor for methionine synthase, N5-methyl-tetrahydrofolate is the methyl donor, and N5,N10-methylenetetrahydrofolatereductase functions as a catalyst in the
remethylation process. When methionine is in excess, Hcy is metabolised via the transsulfuration pathway, resulting in the production of cystathionine. This process requires vitamin B6 as a cofactor, and subsequently leads to formation of cysteine. Any excess cysteine is oxidized to taurine or sulphates or eliminated from the body. However, when there are low methionine levels, Hcy is mainly metabolised via a methionine-conserving pathway. In most tissues, there is remethylation of Hcy to methionine, a process which requires methyltetrahydrofolate (from folic acid) and vitamin B12 as cofactors. These two pathways are coordinated by S-adenosylmethionine (SAM), which is the source of methyl groups for all methylation reactions within the cell. S-adenosylhomocysteine (SAH), which is the by-product of these methylation reactions, is rehydrolysed to regenerate Hcy, which is again available to start a new cycle of methyl-group transfer. Thus, high levels of Hcy are associated with reduced methylation potential, whereas folate and vitamin B12 increase this potential. As a result of changes in dietary intake of methionine there occur changes in the concentration of methionine in the body, which particularly, affect the rate of SAM synthesis, as well as the metabolism of Hcy (429,430).

Mechanism

Following McCully’s and Wilcken’s pioneering observation some 30 years ago (431), many studies have confirmed that hyperhomocysteinemia is an important and independent risk factor for a variety of cardiovascular diseases (410,432-436). A number of possible mechanisms have been proposed to explain the atherogenic actions of homocystenine, which include vascular endothelial dysfunction (435,437-438), direct cytotoxic effects to vascular endothelial cells (439), diminished release of NO (440) and increased production of ROS in vascular endothelial cells (439), stimulation of the low-density lipoprotein oxidation (441,442), promotion of platelet activation and enhanced coagulability (443) and increased proliferation of vascular smooth muscle cells (445).

Reduced Hcy generates ROS by autoxidation in the presence of transition metals. Evidence that such a phenomenon may be occurring in the tissues may be gained from studies in cultured endothelial cells. Incubation of cultured porcine aortic endothelial cells with reduced Hcy led to a marked increase in superoxide production. Furthermore, an extended incubation was associated with a significant increase in the intracellular activity of superoxide dismutase and a concomitant reduction in superoxide production. This
suggests that endothelial cells can respond to Hcy-induced oxidative insult by upregulating antioxidant systems (445).

Studies suggest that the atherogenic susceptibility associated with hyperhomocysteinemia results from endothelial dysfunction and injury, subsequently platelet activation and thrombus formation (443,446,447). It is proposed that Hcy-induced endothelial injury exposes the sub-endothelial matrix, which in turn leads to platelet activation (443,446). Similarly Celermajer et al (448) have demonstrated impaired endothelium-dependent vasodilation, and others have shown that impaired endothelial anticoagulant function in young patients with hyperhomocysteinemia and peripheral vascular disease (437,440). It has been recently shown that Hcy (but not cysteine) suppresses the expression of cellular glutathione peroxidase by endothelial cells, which promotes lipid peroxidation by the ROS elaborated during the oxidation of Hcy (440).

Although the exact mechanism of endothelial dysfunction is not known but there is growing evidence that Hcy exerts its effects by promoting oxidative damage. Hcy is rapidly auto-oxidized to form homocystine, mixed disulfides, and Hcy thiolactone when added to plasma (449,450). During auto-oxidation of Hcy, potent ROS, including superoxide and hydrogen peroxide are produced which has been implicated in the vascular toxicity of hyperhomocysteinemia (451). There are evidences that Hcy induces endothelial-cell injury in vitro is largely due to the generation of hydrogen peroxide (439,452,453). Harker et al (446) have proposed that Hcy-induced endothelial cell injury mediated by hydrogen peroxide exposes the underlying matrix and smooth-muscle cells, which in turn proliferate and promote the activation of platelets and leukocytes. Hcy auto-oxidises and produces other cytotoxic ROS which includes the superoxide anion radical and hydroxyl radical (454,455). Superoxide-dependent formation of the hydroxyl radicals initiates lipid peroxidation, which affects endothelial plasma membrane and lipoprotein particles (456,457).

Although the precise molecular mechanism is unknown, Hcy causes endothelial dysfunction at several levels. Hcy enhances the activities of factor XII77 and factor V78 and depresses the activation of protein, thereby altering the normal antithrombotic phenotype of the endothelium (458,459). It also inhibits the expression of thrombomodulin induced expression of tissue factor, and suppresses the expression of heparan sulfate by the
endothelium (460-462). All of these ultimately facilitate the formation of thrombin and create a prothrombotic environment.

Hcy also adversely affects the production of endothelial-derived NO. It has been previously shown that normal endothelial cells detoxify Hcy by releasing NO. NO combines with Hcy to form S-nitroso-Hcy in the presence of oxygen. Nitrosation of the sulfhydryl group of Hcy inhibits sulfhydryl-dependent generation of hydrogen peroxide. S-nitroso-Hcy is also a potent platelet inhibitor and vasodilator (463). A long-term exposure to hyperhomocysteinemia damages the endothelium sufficiently to limit NO production. Impaired production of NO by endothelial cells leaves the endothelium susceptible to Hcy-mediated oxidative injury (464). Hcy may also decrease the bioavailability of NO by impairing its synthesis (451,457). Hcy promotes lipid peroxidation, which may decrease the expression of endothelial, NO synthase and directly decreases NO (442,465,466).

Hcy is also a potent mitogen for vascular smooth-muscle cells. Harker et al (467), have demonstrated that exposure to Hcy leads to a marked increase in vascular smooth-muscle proliferation in vitro. Similar results were obtained by Tsai et al (444,468). Hcy increases NO production in vascular smooth muscle cells by activating the transcription factor NF-κB (469). Since NF-κB/rel activity is essential for the proliferation of vascular smooth-muscle cells (470), it may contribute to the mitogenic effect of homocysteine.

Hcy directly damages the vascular matrix by affecting the biochemical and biosynthetic functions of vascular cells. Hcy thiolactone combines with low-density lipoprotein to form aggregates that are taken up by intimal macrophages and incorporated into foam cells within nascent atheromatous plaques (471). McCully et al has suggested that Hcy thiolactone also impairs oxidative phosphorylation in mitochondria and promote the proliferation and fibrosis of smooth muscles (472-474). This Hcy-induced disturbance in oxidative metabolism also leads to overproduction of oxidative radicals that subsequently induce intimal injury, activate elastase, and increase calcium deposition (473,474). Possibly sulfur group of Hcy thiolactone is incorporated into phosphoadenosine phosphosulfate, which ultimately leads to the formation of sulfated glycosaminoglycans in the matrix (474). Hcy increases the formation of highly atherogenic oxycholesterols, increases lipid peroxidation, and increases the oxidation of LDL.
Stampfer MJ et al (1992) assessed prospectively the risk of CHD associated with elevated plasma levels of Hcy in 14,916 male physicians (40 to 84 years), with no prior vascular disease and were followed up for 5 years. Hcy levels were measured in 271 men who subsequently developed MI and compared with controls who were matched by age and smoking. Homocysteine levels were higher in cases than in controls (11.1± 4.0 vs. 10.5± 2.8 nmol/mL; P =0.03). This study suggested that moderately high levels of plasma Hcy were associated with subsequent risk of MI independent of other coronary risk factors. Because high levels can often be easily treated with vitamin supplements, Hcy may be an independent, modifiable risk factor (416).

Selhub J et al (1993) described the distribution of plasma Hcy concentrations and analyzed the relationship between Hcy level and intake of vitamins and serum levels of vitamins in an elderly population. A total of 1160 adult survivors, aged 67 to 96 years, from the original FHS cohort were enrolled. Hcy levels were positively correlated with age and exhibited a strong inverse association with plasma folate. Hcy demonstrated inverse associations with plasma vitamin B12, intakes of folate and vitamin B6, but not vitamin B12. Prevalence of high Hcy (> 14 mumol/L) was 29.3% and was greatest among subjects with low folate status. The results indicated a strong association between Hcy concentration and folate, vitamin B12, and vitamin B6 status, as well as age. They concluded that a substantial majority of the cases of high Hcy in this older population can be attributed to vitamin status (407).

Alfthan G et al (1994) studied the relation of serum total Hcy and lipoprotein(a) with the incidence of atherosclerotic disease among 7424 men and women, aged 40-64 years, free of atherosclerotic disease at baseline in 1977. During the 9-year follow-up, 134 male and 131 female cases with CVD were identified. The mean serum Hcy concentration of male cases and controls were 9.99µmol/l and 9.82µmol/l at baseline; and that of female cases and controls were 9.58µmol/l and 9.24µmol/l, respectively. The results of this study did not support the hypothesis that serum Hcy is risk factors for atherosclerotic disease. The lack of association between serum Hcy and atherosclerotic disease may be due to the exceptionally low gene frequency predisposing to homocysteinemia in Finland (423).

Arnesen E et al (1995) conducted a nested case-control study among the 21 826 subjects, aged 12–61 years, who were surveyed in the municipality of Tromsø, Norway.
Among those free from MI at the screening, 123 later developed CVD. Four controls were selected for each case. Level of Hcy was higher in cases than in controls (12.7 ± 4.7 versus 11.3 ± 3.7 µmol/l (mean ± SD); P = 0.002). Adjusting for possible confounders reduced the relative risk to 1.32. There was no threshold level above which serum Hcy is associated with CHD events. In the general population serum total Hcy is an independent risk factor for CHD with no threshold level (415).

P H Whincup et al (1999) examined the relation between total Hcy and major CHD events. A nested case control study carried out within the British regional heart study, in men aged 40–59 years. Serum total Hcy concentrations were analysed in baseline samples from 386 cases that had a myocardial infarct during 12.8 years of follow up and from 454 controls. Geometric mean of serum total Hcy was slightly higher in cases (14.2 µmol/l) than in controls (13.5 µmol/ l), with a proportional difference of 5.5%. Serum total Hcy among control subjects varied between towns and was correlated with town standardized mortality ratios for CHD (r = 0.43, p = 0.08). It was concluded that serum total Hcy is prospectively related to increased coronary risk and may also be related to geographical variation in coronary risk within Britain. These results strengthened the case for trials of total Hcy reduction with folate (475).

Al-Obaidi MK et al (2000) studied whether the amount of myocardial damage during acute coronary syndromes (ACS) is related to the plasma Hcy concentration. Consecutive patients presenting with acute myocardial infarction (MI) (n = 205) and unstable angina pectoris (UAP) (n =185) were studied. Elevated Hcy levels were associated with a higher risk of ischemic myocardial injury in patients presenting with ACS (476).

Meigs et al (2001) examined relationships between Hcy levels and features of insulin resistance syndrome in subjects aged 28-82 years (mean age 54 years). In this study, 12.3% had hyperinsulinemia, and 15.9% had two or more of the insulin resistance syndrome phenotypes. Adjusted mean Hcy levels were higher comparing those with hyperinsulinemia (9.8 mmol/l) and those without (9.4 mmol/l, P <0.04) and were higher among subjects with two or more IRS phenotypes (9.9 mmol/l) compared with those with 1 or no phenotype (9.3 mmol/l, P < 0.003). Hyperhomocysteinemia is associated with hyperinsulinemia and may partially account for increased risk of CVD associated with insulin resistance. Because hyperhomocysteinemia also reflect endothelial injury, these
observations also supported the hypothesis that endothelial dysfunction is associated with expression of the insulin resistance syndrome (477).

Godsland et al (2001) measured total Hcy in samples from 100 male participants in the second follow-up of the Heart Disease and Diabetes Risk Indicators in a Screened Cohort Study. In univariate correlation, Hcy concentrations were unrelated to insulin sensitivity or to components of the metabolic syndrome, including fasting serum TGs, high density lipoprotein cholesterol, high density lipoprotein subfraction-2 cholesterol, BP, uric acid, systolic BP, or BMI. These findings strengthened the possibility that in healthy humans, Hcy metabolism is not substantially affected by insulin action (478).

Ng KC et al (2002) determined whether hyperhomocysteinaemia is a risk factor for acute myocardial infarction in a Southeast Asian population comprising different ethnic groups and its association with traditional risk factors, plasma vitamin B12 and folate levels. This was a case-control study comprising 168 acute myocardial infarction patients and 141 controls with a median age of 55 years (range, 27 to 77 years), living in Singapore. It was found that the odds of having acute myocardial infarction was higher for subjects with HTN, smoking habit, lower plasma folate and vitamin B12 levels and non-Chinese ethnic group. Plasma Hcy level was not significantly associated with acute myocardial infarction. The baseline levels of plasma total Hcy in both acute myocardial infarction patients and controls were higher than other studies (median values between 12 and 14µmol/L). Plasma total Hcy levels were not associated with acute myocardial infarction but low plasma levels of folate and vitamin B12 were independently associated (402).

Agulló-Ortuño et al (2002) studied Hcy and other biochemical parameters in a group of 57 type 1 and 32 type 2 diabetic patients and 54 control subject. It was studied whether plasma homocysteinaemia was related to macroangiopathy, nephropathy, retinopathy and neuropathy. Patients with DM had higher Hcy than control subjects (11.7±5.4 vs. 10.1±2.4 µmol/l, p<0.05). In the studied groups with complications, significant differences between normohomocysteinaemic type 1 diabetic patients and those considered hyperhomocysteinaemic was found. A relationship was found between high Hcy levels and prevalence of macroangiopathy, retinopathy and nephropathy in the type 1 diabetic patients, which was not been observed in the type 2 diabetic patients of the study (479).
Lim HS et al (2002) described plasma Hcy levels and their relationship to plasma folate and vitamin B12 status in 195 middle income group Korean adults. The mean plasma Hcy levels of males (11.18±3.88 µmol/L) were significantly higher than that of females (9.20 ±2.65 µmol/L). The incidence of hyperhomocysteinemia in males (10.1%), was significantly higher than the 2.1% in females. As age increased, plasma Hcy levels tended to be higher in females. Sex differences in plasma Hcy levels disappeared in subjects over fifty. On the other hand, both plasma folate (6.47±3.06 vs 7.96 ±3.55 ng/mL, p < 0.01) and vitamin B12 levels (537.0±222.0 vs. 664.1± 309.8ng/mL, p < 0.01) were significantly lower in males than in females. A plasma folate deficiency (< 3.0 ng/mL) was found in 6.1% of males and 2.1% of females and vitamin B12 deficiency (< 150 pg/mL) was detected in 2.0% and 1.0%, respectively. Plasma Hcy levels were related with inversely plasma concentrations of folate (r = -0.37249, p < 0.001) as well as vitamin B12 (r = -0.22560, p < 0.01). Plasma levels of Hcy and the prevalence of hyperhomocysteinemia in Korean adults are similar to findings in the West. The results indicated that male adults may be in worse condition for CVD than females. And improving folate and vitamin B12 status may reduce plasma Hcy level, which may be more important in males (480).

Misra et al (2002) studied serum levels of Hcy in subjects living in an urban slum of North India and healthy subjects from urban nonslum area. Group I consisted of 46 subjects (22 males and 24 females) living in an urban slum, while group II consisted of healthy subjects (n = 26, 13 males and 13 females) living in the adjacent non-slum area. Sex-adjusted serum levels of Hcy were high, though statistically comparable, in both the groups (group I: 20.8±5.9 and group II: 23.2±5.9µmol/L). Overall, hyperhomocysteinemia was recorded in 84% of the subjects. Low intakes of folic acid and vitamin B12, and hyperhomocysteinemia, in both the healthy population living in urban slums and adjacent urban non-slum areas, were important observations for the prevention of nutritional and cardiovascular diseases in the Indian subcontinent (481).

Shai I et al (2004) examined 32,826 women from the Nurses’ Health Study who provided blood samples in 1989–1990, 237 CHD events were documented during 8 years of follow-up. It was found that Plasma Hcy was inversely associated with blood levels of folate (r = −0.3, P < 0.0001) and B12 (r = −0.2, P < 0.0001) and with dietary intake of folate (r = −0.1, P < 0.01) and B2 vitamin (r = −0.1, P = 0.01). Hcy was positively
associated with soluble tumor necrosis receptor (sTNF-R) 1 and 2. In a multivariate model adjusted for age, smoking, BMI, parental history, HTN, diabetes, postmenopausal hormone use, physical activity and alcohol intake, the relative risk of CHD between the extreme quartiles of Hcy was 1.66. Hcy was found to be an independent risk predictor of CHD and modestly associated with TNF-receptors (482).

Setola et al (2004) performed a double-blind, parallel, identical placebo–drug, randomized study in 50 patients for 2 months. Folate treatment significantly decreased Hcy levels by 27.8%. A significant decrement was observed for insulin levels accompanied by a 27% reduction in the homeostasis model assessment levels. A positive relationship was found between the decrement of Hcy and insulin levels. In parallel, endothelial dysfunction significantly improved in the treated Group. On the contrary, in group treated with placebo, no changes were shown in any of the variables. It was concluded that folate and vitamin B12 treatment improved insulin resistance and endothelial dysfunction, along with decreasing Hcy levels, in patients with metabolic syndrome, suggesting that folic acid has several beneficial effects on cardiovascular disease risk factors (26).

Iqbal MP et al (2005) investigated the possible correlation between deficiency of vitamins B6, B12 or folic acid and hyperhomocysteinemia in Pakistani patients with acute myocardial infarction. A case-control study was carried out involving 224 acute myocardial infarction patients (age 30-70 years; 55 females and 169 males) and 126 normal healthy subjects (age 31-70 years; 35 females and 91 males). Mean serum B12 concentration in acute myocardial infarction patients was found to be significantly lower than the mean for controls (241±185 pg/ml vs 608±341 pg/ml; p < 0.001). Mean serum folate level in patients was also found to be lower than controls; however, the differences were not statistically significant. Mean plasma Hcy level in acute myocardial infarction cases (18±8.36 micromol/l) was higher than the mean level in controls (16.4±4.9 micromol/l), but not to a significant extent. Mean plasma Hcy levels in smokers were found to be significantly higher in both cases and controls. Similarly, mean serum folate levels in smokers (compared to nonsmokers) were significantly lower in both cases and controls. Substantial nutritional deficiencies of these three vitamins along with mild hyperhomocysteinemia, perhaps through interplay with the classical cardiovascular risk factors (highly prevalent in
this population), could be further aggravating the risk of CAD in the Pakistani population (483).

Rudy A et al (2005) evaluated the levels of Hcy in patients with type 2 diabetes in respect to the diabetes treatment as well as the presence of diabetic complications. The investigation was carried out in 64 patients with type 2 diabetes and in 18 healthy subjects from the control group. Hcy concentration was significantly higher in the group of patients with diabetes in comparison to the control group. Diabetic patients had significantly lower concentrations of folic acid and HDL cholesterol together with higher levels of systolic BP. In the group of patients with diabetes no differences in Hcy levels were found in respect to diabetes treatment. Diabetic patients with CAD had significantly higher Hcy concentration in comparison to the group with diabetes without history of CAD. Hcy levels correlated significantly with incidence of ischaemic heart disease (r = 0.44, p = 0.001). Negative correlation was noticed in HDL concentrations (r = -0.30, p = 0.013) and the levels of folic acid (r = -0.30, p = 0.008). Results suggested that hyperhomocysteinemia in diabetic patients may contribute to the development of chronic complications (484).

Chen KJ et al (2005) investigated the relationship between Hcy (Hcy) and B vitamins status in the Taiwanese elderly population. The study sample was taken from the Elderly Nutrition and Health Survey in Taiwan (1999-2000) (Elderly NAHSIT) and included 1094 males and 1135 females aged 65-90 years. The results showed that average plasma Hcy was 13.3+/−0.6 µmol/ L for males and 10.6+/−0.7 µmol/L for females. The average plasma Hcy levels of males were significantly higher than those of females, and significantly increased with age. The prevalence of hyperhomocysteinemia was 23.4% for elderly males and 11.2% for elderly females, and this also increased with age. Further analysis suggested that folate, vitamin B6 or B12 insufficiency were associated with hyperhomocysteinemia in both sexes, while vitamin B2 insufficiency was significantly associated only in males. In elderly persons with adequate folate, vitamin B6, and B12 status, there was no significant association between vitamin B2 and hyperhomocysteinemia. This association occurred only in those who had concurrent poor folate, vitamin B6, or B12 status. The strength of the association between vitamin B12 insufficiency and hyperhomocysteinemia was not affected by simultaneous vitamin B2 or B6 insufficiency, but increased about 3-fold when combined with folate. This suggested that poor folate and
vitamin B12 status has a synergistic effect on the risk of hyperhomocysteinemia in the elderly, as did a poor folate and vitamin B6 status. Therefore, maintaining adequate vitamin B12 status and avoiding multiple B vitamin insufficiency, especially that of folate and vitamin B12 or B6, should be emphasized as an important measure for reducing plasma Hcy levels among elderly Taiwanese (485).

de Luis DA et al (2005) analyzed the relationship between total Hcy and the body composition, other cardiovascular risk factors and chronic complications in type 2 diabetic patients. In this cross-sectional study, a total of 155 patients with DM (90 females/65 males) were enrolled consecutively. Patients were divided in two groups (Group I: Hcy> or =15 µmol/l; Group II: Hcy<15 µmol/l). The prevalence of CHD in the total group was 5.8% without statistical differences between groups. Concerning macrovascular complications, only peripheral vascular disease prevalence was higher in Group I. No correlation was detected among Hcy and anthropometric parameters (BMI, weight, percentage of fat mass, fat mass, and tricipital skinfold). Elevated levels of fibrinogen, lipoprotein (a), microalbuminuria, and BP were detected in Group I. The study showed that elevation of plasma Hcy levels in type 2 diabetic patients was associated with a higher prevalence of peripheral arteriopathy (486).

Banu S et al (2005) studied the association of serum Hcy with acute myocardial infarction and chronic IHD patients in Bangladesh population and took seventy subjects. Out of them 20 were of Acute MI, 20 were Chronic IHD and 30 were age and sex matched healthy controls. Mean Hcy level of acute myocardial infarction were 21.16±4.56 (µmol/l), 27.55±10.40 (µmol/l) and that of control was 13.03±10.51 (µmol/l). Serum Hcy was significantly higher in both cases than control. But insignificant difference was found between acute myocardial infarction vs CHD (487).

Saeed Sadeghian et al (2006) assessed the role of hyperhomocysteinemia, folate and Vitamin B12 deficiency in the development of premature CAD among 294 individuals (225 males and 69 females) less than 45 years of age from Tehran. The mean age of participants was 39.9 +/- 4.3 years. Compared to the control group, the level of Hcy of the male participants was significantly higher than female (14.9±1.2 versus 20.3±1.9 µmol/lit, P = 0.01). Mean plasma level of folic acid and vitamin B12 in the study group were 6.3±0.2 and 282.5 ±9.1 pmol/L respectively. Folate and vitamin B12 deficiency was present in 10.7%
and 26.6% of the study group respectively. Logistic regression analysis for evaluating independent CAD risk factors showed hyperhomocysteinemia as an independent risk factor for premature CAD in males. It was concluded that hyperhomocysteinemia is an independent risk factor for CAD in young patients (below 45 years old), especially in men and vitamin B12 deficiency is a preventable cause of hyperhomocysteinemia (468).

Yajnik CS, et al (2006) investigated prevalence and associations of low vitamin B12 concentration and hyperhomocysteinemia in rural and urban Indian men living in and around Pune, Maharashtra. Middle-aged men (149 rural, 142 slum and 150 urban middle-class residents, mean age 39 y were studied. Median plasma B12 concentration was low (110 pmol/L): Overall, 67% of men had low vitamin B12 concentration (<150 pmol/L) and 58% had hyperhomocysteinemia (>15 µmol/L). Of the urban middle class, 81% had low vitamin B12 concentration and 79% had hyperhomocysteinemia. Low vitamin B12 concentration contributed 28% to the risk of hyperhomocysteinemia (population attributable risk) while low red cell folate contributed 2%. Vegetarians had 4.4 times higher risk of low vitamin B12 concentrations and 3.0 times higher risk of hyperhomocysteinemia compared to those who ate non-vegetarian foods frequently. Urban middle-class residence was an additional independent risk factor of hyperhomocysteinemia, compared to rural men. They concluded that low vitamin B12 concentration and hyperhomocysteinemia were common in Indian men, particularly in vegetarians and urban middle class residents (489).

Bazzano LA et al (2006) evaluated the effects of folic acid supplementation on risk of cardiovascular diseases and all-cause mortality in randomized controlled trials among persons with preexisting cardiovascular or renal disease. Studies included data from 16 958 participants with preexisting vascular disease were analyzed. The overall relative risks (95% confidence intervals) of outcomes for patients treated with folic acid supplementation compared with controls were 0.95 (0.88-1.03) for cardiovascular diseases, 1.04 (0.92-1.17) for CHD, 0.86 (0.71-1.04) for stroke, and 0.96 (0.88-1.04) for all-cause mortality. Folic acid supplementation has not been shown to reduce risk of cardiovascular diseases or all-cause mortality among participants with prior history of vascular disease (490).

Anan F et al (2007) evaluated that Hcy correlates with aortic stiffness and insulin resistance in type 2 diabetic patients. The study consisted of 40 Japanese patients with type 2 DM and high Hcy levels and a control group of 45 age-matched patients with normal Hcy
levels. BMI values, waist circumferences, and the waist-to-hip ratios were larger in the high-Hcy group than in the normal-Hcy group. Fasting plasma glucose and insulin concentrations and the HOMA index were higher in the high-Hcy group than in the normal-Hcy group. In conclusion, the results indicated that the elevated level of Hcy in Japanese patients with type 2 DM is characterized by increased aortic stiffness and insulin resistance, and the HOMA index are independent predictors of Hcy (491).

Yajnik CS et al (2007) studied the effect of oral vitamin B12 (500 microg) and/or 100 g cooked green leafy vegetables every alternate day in a 2x2 factorial design over a 6-week period. High-dose per oral vitamin B12 supplementation significantly reduced plasma Hcy within 2 weeks but did not achieve normal plasma Hcy concentration even after 6 weeks. People in India have a high prevalence of low vitamin B12 status and high plasma total Hcy concentrations (492).

Martijn G.H et al (2007) assessed whether vitamin B12 deficiency or hyperhomocysteinaemia is associated with recurrent cardiovascular events. In the follow-up period of 810 person-years, 48 (21%) of the patients experienced a nonfatal recurrent cardiovascular event and another 14 (7%) died of a cardiovascular cause. Among those with ischaemic heart disease at discharge, no difference in survival was found between the patients with a low (<250 pmol/l) or a high vitamin B12 level. In patients with hyperhomocysteinaemia (>16 μmol/l), an increased risk of a recurrent cardiovascular event in comparison to those with normal plasma Hcy levels was proven. In conclusion, high plasma Hcy concentration, but not a low serum vitamin B12 concentration, increased the risk of cardiovascular morbidity and mortality in patients with ischaemic heart disease (493).

Ebbing M et al (2008) assessed the effect of treatment with folic acid and vitamin B12 in patients with CAD or aortic valve stenosis. Randomized, double-blind controlled trial was conducted in the university hospitals in western Norway in 1999-2006. A total of 3096 adult participants undergoing coronary angiography (20.5% female; mean age, 61.7 years) were randomized. Mean plasma total Hcy concentration was reduced by 30% after 1 year of treatment in the groups receiving folic acid and vitamin B12. The trial did not find an effect of treatment with folic acid/vitamin B12 or vitamin B6 on total mortality or
cardiovascular events and findings do not support the use of B vitamins as secondary prevention in patients with CAD (494).

Albert CM et al (2008) tested whether a combination of folic acid, vitamin B6, and vitamin B12 lowers risk of CVD among high-risk women with and without CVD. 5442 women who were US health professionals, aged 42 years or older, with either a history of CVD or 3 or more coronary risk factors, were enrolled in the trial, to receive a combination pill containing folic acid, vitamin B6, and vitamin B12 or a matching placebo. After 7.3 years of treatment and follow-up, a combination pill of folic acid, vitamin B6, and vitamin B12 did not reduce total cardiovascular events among high-risk women, despite significant Hcy lowering (495).

Kaya C et al (2009) investigated whether polycystic ovarian syndrome patients have lower or higher vitamin B12, folate and Hcy concentrations when compared with healthy, age and BMI matched controls, and, examined associations between vitamin B12, folate, Hcy, insulin resistance and obesity in polycystic ovarian syndrome patients. Hcy concentrations and homeostasis model assessment index were higher, whereas concentrations of vitamin B12 were lower in polycystic ovarian syndrome patients with insulin resistance compared with those without insulin resistance. Serum vitamin B12 concentrations were significantly lower in obese polycystic ovarian syndrome women in comparison with obese control women. Fasting insulin, insulin resistance and Hcy are independent determinants of serum vitamin B12 concentrations in polycystic ovarian syndrome patients. Insulin resistance, obesity, and elevated Hcy were associated with lower serum vitamin B12 concentrations in polycystic ovarian syndrome patients (496).

Bhagwat V R et al (2009) examined the relationship of hyperhomocysteinaemia with lipid profile and antioxidants in patients with ischaemic heart disease from rural areas in Maharashtra, India. Total Hcy levels were significantly higher by almost three times more than the controls. There was a definite inverse relationship between total Hcy, thiobarbituric acid reactive substances and antioxidants in the patients. The levels of folic acid and vitamin B12 were 3–4 times higher in the patients compared to the controls. There was a poor correlation between the total Hcy and vitamin levels in the patients. Blood Hcy was found to be very important biomarker of cardiovascular diseases and must be evaluated
along with other risk factors. There was a higher prevalence of hyperhomocysteinaemia in rural Indian patients (497).

Deshmukh US et al (2010) studied the effect of physiological doses of B12 and folic acid on plasma total Hcy concentration. From 119 families in the Pune Maternal Nutrition Study, 300 individuals were randomized in the placebo-controlled, double-blind, 2 x 3 factorial trial. There was no interaction between B12 and folic acid in relation to Hcy concentration change and their effects were analyzed separately. Daily oral supplementation with physiological doses of B12 is an effective community intervention to reduce Hcy. Folic acid (200 microg per day) showed no additional benefit, neither had any unfavorable effects (498).

Gammon CS et al (2012) described the vitamin B12 status of predominantly overweight/obese women of South Asian origin living in Auckland and correlated serum vitamin B12 and vegetarian status with IR as part of the larger Surya Study. This was a cross-sectional study of 135 women at least 20 y of age. Mean serum vitamin B12 was 227pmol/L (95% CI, 210-245), serum folate was 19.1nmol/L (18.0-20.2), and HOMA-IR was 1.24 (1.13-1.36). Non-vegetarians had higher serum vitamin B12 levels (257 pmol/L, 235-281) than vegetarians (181pmol/L, 159-207). Vitamin B12 deficiency (<150 pmol/L) in vegetarians was 24% versus 9% in non-vegetarians. Non-vegetarians had increased BMI (25.9 kg/m², 25.0-26.9, versus 23.9 kg/m², 22.6-25.3), waist circumference (81 ± 10.1 versus 75.8 ± 9.88 cm), and HOMA-IR levels (1.30, 1.17-1.46, versus 1.00, 0.83-1.22). No correlation was found between serum vitamin B12 and HOMA-IR. There was a significant positive correlation between non-vegetarian status and IR, which disappeared after controlling for BMI. The study population had a low serum vitamin B12 status, especially if vegetarian (499).

Al-Maskari MY et al (2012) examined the status of folate and vitamin B12 in relation to serum Hcy and oxidative stress indices in patients with type 2 DM. This case-control study involved 100 Omani adults (50 patients newly diagnosed with type 2 DM and 50 age- and gender-matched healthy controls). Low serum levels of folate, B12, and hyperhomocysteinemia were prevalent in patients with type 2 diabetes compared with controls. Oxidative stress was evident in patients with type 2 diabetes. The low intake of
folate and B12 was associated with low serum levels of these two nutrients and hyperhomocysteinemia in Omani adults with type 2 diabetes (500).

Vazquez-Pedrazuela et al (2012) established the prevalence of vitamin B12 and folic acid deficiency in the population of 65 years and over in semi-urban and rural area, investigated the risk factors, and determined factors of this deficiency in this population. This cross-sectional study was conducted in the Geriatrics Outpatients between 2008 and 2010. Vitamin B12 deficiency was found in 16.5% of the sample, and no folic acid deficiency. A strong association was found with vitamin B12 deficiency and cardiac and cerebrovascular diseases, vascular risk factors. There was a higher prevalence of vitamin B12 deficiency in the elderly population in the area of Medina del Campo compared to that found in the literature, but not so with the isolated deficiency of folic acid (501).

Kang JY et al (2012) examined whether serum Hcy levels correlated with CVD depending on the presence or absence of metabolic syndrome in Korean men. In this case-control study, 138 CVD and 290 non-CVD age-matched control subjects were included. The subjects were divided into four subgroups: 34 CVD/metabolic syndrome, 104 CVD, 77 metabolic syndrome, and 213 normal subgroups. The mean Hcy was significantly higher, whereas HDL and intake of vitamin B1 and B2 were lower in the CVD group than non-CVD group. When compared to the control group, subjects with CVD/ metabolic syndrome, CVD and metabolic syndrome exhibited high Hcy levels, with the highest observed in the CVD/ metabolic syndrome subgroup. The results of the study showed that the presence of metabolic syndrome needs to be considered when using Hcy levels for predicting CVD (502).

Lietava J et al (2012) investigated in crosssectional population study in high risk age 35-75 years and found that, there was very high prevalence of classic as well as newer risk factors and risk markers both in IHD patients and in controls. Increased Hcy (Hcy >15 µmo/l for males and Hcy >13 µmo/l for females) was found in 32.9 % of patients and 13.6 % of controls. Comparison of regulating vitamins levels between IHD patients and controls demonstrated similar prevalence. Homocysteine Slovakia study found very high prevalence of hyperhomocysteinemia in patients with stable IHD (503).
2.5.3 Magnesium

Magnesium is one of the most abundant intracellular ions with an essential role in fundamental biological reactions, deficiency of which provoke biochemical and symptomatic alterations in the human (504,505). Magnesium is the second predominant component in the intracellular compartment. It is an important regulator of the cellular processes, co-factor of more than 300 essential metabolic reactions which are related to the synthesis of tissue constituents, growth and thermogenesis, and with the activity of tyrosine kinase, in the metabolism of glucose (506). The homeostasis of magnesium depends on the amount of ingestion, the efficiency of absorption and the intestinal and renal excretion. The magnesium level in the body is regulated by the action of parathormone, calcitonin, vitamin D, glucagon, antidiuretic hormone, aldosterone and sexual steroids. The insulin is also involved in the transport of magnesium through the cellular membrane and in the intracellular supply (504,507). Magnesium influences the activity of enzymes by binding to ligands such as ATP in ATP-requiring enzymes, binding to the active site of the enzyme, e.g. enolase, pyruvate kinase, pyrophosphatase, causing a conformational change during the catalytic process eg Na-K-ATPase, promoting the aggregation of multi-enzyme complexes eg aldehyde dehydrogenase, or a mixture of the above mechanisms, eg F1-ATPase (508). Magnesium helps to maintain a low resting intracellular free calcium ion concentration which is important in many cellular functions, by competing with calcium for membrane binding sites and by stimulating calcium sequestration by sarcoplasmic reticulum. Magnesium has important effects on the cardiovascular system. It affects myocardial contractility by influencing the electrical activity of myocardial cells, the intracellular calcium concentration and the specialised conducting system of the heart (509). Magnesium may also affect the vascular smooth muscle tone and biological processes such as cellular energy metabolism, cell replication, and protein synthesis (509).

Body content and distribution of magnesium

The normal adult human body contains approximately 1,000 mmols of magnesium (22-26g) (504). Sixty percent of the magnesium is present in bone, of which 30% is exchangeable and functions as a reservoir to stabilize the serum concentration. Twenty percent is present in skeletal muscle, 19% in other soft tissues and less than 1% in the extracellular fluid (510-512). In normal adults, total serum magnesium ranges between 1.6
and 2.6 mg/dl, approximately 20% is protein bound, 65% is ionized and the rest is complexed with various anions such as phosphate and citrate (513,510). Free ionized magnesium constitutes only 0.5-5% of the total cellular magnesium; the remainder is bound to anionic compounds such as ATP, ADP, citrate, proteins, RNA and DNA or is sequestered within mitochondria and endoplasmic reticulum. The concentration of free magnesium within the cell is about 0.5mmol/L (510,511). The recommended daily allowance (RDA) for magnesium in adults is 350 mg/day (514). The daily requirement is higher in pregnancy, lactation and following debilitating illness. Recent dietary surveys show that the average intake in many western countries is less than the RDA (504).

The specific clinical manifestations of hypomagnesemia are difficult to be diagnosed, due to the frequent associations of this deficiency with hypokalemia, hypocalcemia and metabolic alkalosis. Patients with hypomagnesemia can present cardiovascular alterations, ischemic cardiac insufficiency, vascular complications of DM and HTN. Neurological, hormonal, renal, gastrointestinal and muscular dysfunction also has been associated to hypomagnesemia (515,516). Hypomagnesemia has been related to insulin resistance, and when it is chronic leads to macro and microvascular complications of diabetes (517-520).

Magnesium is an essential element that has numerous biological functions in the cardiovascular system. Magnesium regulates contractile proteins, alters transmembrane transport of calcium, sodium and potassium; controls metabolic regulation of energy-dependent cytoplasmic and mitochondrial pathways; regulates oxidative-phosphorylation processes, and affects DNA and protein synthesis (521,522). By serving as a cofactor in the sodium-potassium ATPase pump, magnesium deficiency can lead to increased intracellular sodium and calcium concentrations, which lead to increase reactivity of arteries to vasoconstrictor agents, attenuate responses to vasodilators, promote vasoconstriction and increase peripheral resistance, leading to increased BP. Magnesium is also important for the activity of the extracellular enzymes lecithin-cholesterol acyl transferase and lipoprotein lipase (523). Serum magnesium has been linked to atherosclerosis, myocardial infarction, HTN and cardiac failure (524). Epidemiologic evidence linking magnesium deficiency to IHD and sudden death has been investigated for many years. People living in areas with magnesium-low water have more heart disease than those living in areas with magnesium-
rich water. The marginal difference in magnesium intake occurs from the water supply (525). Therefore, there is increased cardiovascular mortality in people living in magnesium-low water areas because the total intake is insufficient. This is referred to as the "water story" and is supported by numerous studies showing an inverse relationship between cardiovascular mortality and water hardness (526,527). An independent role of magnesium in IHD has been provided by a study of South Africa in whites that reported an inverse relationship between deaths from IHD and the magnesium content of drinking water. Magnesium levels in the drinking water of 12 South African districts and deaths due to IHD were assessed in white residents in the South Africa and a significant negative correlation was found between these two variables (528). An increased incidence of sudden death associated with IHD has been found in some areas in South Africa where soil and drinking water lack magnesium. It was demonstrated experimentally that reduction of the plasma magnesium level is associated with arterial spasm (529). The mortality rates for acute myocardial infarction and IHD of white males and females in South Africa were noted to be much higher than those in the USA, Australia, England and Wales when individuals in the 15 to 64 year age group are considered. A number of studies carried out in several Asian countries have shown negative correlations between coronary mortality and the presence of trace elements in water supplies. A cross-sectional survey was conducted in 20 randomly selected streets in North India to determine the association of magnesium with risk of CAD. The results suggest that magnesium intake and serum magnesium were inversely correlated with CAD. The odds ratio for dietary magnesium intake indicates a higher prevalence of CAD at lower intakes of magnesium in both rural (0.67) and urban (0.72) subjects. Multivariate regression analysis showed that serum and dietary magnesium, but not HTN, were significantly associated with CAD (530,531).

The association of magnesium deficiency with dyslipidemia has been suggested in some studies (532-537). Studies in rats have shown that magnesium deficiency produces hypertriglyceridemia (538,539), hypercholesterolemia (538-541), increased LDL (539), and reduced HDL (537) through reduced TG clearance, (542) diminished activity of lecithin cholesterol acetyltransferase (LCAT) (538) and lipoprotein lipase, and increased activity of HMG-COA reductase (543). The association between hypomagnesemia and hypertriglyceridemia has been confirmed in studies of pigs (544). However, this association
has not been as clear in human studies. Population studies have shown positive (545), inverse (533), or no (546) correlation between serum magnesium and cholesterol levels. Magnesium deficiency has been linked to the pathogenesis of another risk factor, HTN (547,548). Magnesium deficiency is associated with increased free radical-dependent oxidative tissue damage (549,550). However, magnesium depletion makes cells more sensitive to oxidative stress (551).

Joffres MR et al (1987) investigated the associations between BP and intakes of dietary variables in 615 men of Japanese living in Hawaii who had no history of cardiovascular disease or treated HTN. Magnesium showed inverse associations with BP in univariate and multivariate analysis. They suggested that foods such as vegetables, fruits, whole grains, and low-fat dairy items are major sources of nutrients that may be protective against HTN (552).

Ma J et al (1995) examined the relationships of serum and dietary magnesium (Mg) with prevalent CVD, HTN, DM, fasting insulin, and average carotid intimal-medial wall thickness in population based cross sectional study namely ARIC Study, which included 15,248 participants, aged 45-64 years. The results showed that serum magnesium levels and dietary magnesium intake were both lower in blacks than whites. Mean serum magnesium levels were significantly lower in participants with prevalent CVD, HTN, and diabetes than in those free of these diseases. In participants without CVD, serum magnesium levels were also inversely associated with fasting serum insulin, glucose, systolic BP and smoking. Dietary magnesium intake was inversely associated with fasting serum insulin, plasma high density lipoprotein-cholesterol, systolic and diastolic BP. In conclusion, low serum and dietary magnesium may be related to the etiologies of CVD, HTN, diabetes, and atherosclerosis (553).

Van Leer EM et al (1995) studied the relation between BP and dietary calcium, potassium and magnesium and the combined effect of these minerals on BP in 20,921 Dutch men and women aged 20-59 years. An inverse association was observed between BP and dietary magnesium in both men and women. Men and women who consumed a diet with intakes in the upper tertiles of the entire mineral had a lower SBP and DBP compared to those who had intakes in the lower tertiles (554).
Zargar AH et al (1998) evaluated the role of magnesium in 83 patients with non-insulin dependent DM (40 men and 43 women), with a mean duration of diabetes of 3.9±3.6 years. Thirty healthy non-diabetic subjects were studied for comparative analysis. Subjects were subdivided into obese and non-obese. Plasma magnesium levels were comparable between diabetic and non-diabetic subjects. Age, sex, duration and control of diabetes did not influence magnesium concentrations. It was concluded that magnesium levels are not altered in DM (555).

Peacock JM et al (1999) examine the relationship of serum and dietary magnesium with incident HTN. The setting was the Atherosclerosis Risk in Communities (ARIC) Study, which included a biracial cohort, aged 45-64 years, from four U.S. communities. This analysis included 7731 participants (4190 women and 3541 men) free of HTN at baseline and followed six years. Fasting serum magnesium was measured, and usual dietary intake was assessed with a food frequency questionnaire. No association between dietary magnesium intake and incident HTN. These associations were attenuated after the addition of baseline systolic BP to the models. The study suggests that low magnesium may play a modest role in the development of HTN (556).

Guerrero-Romero F et al (2000) assessed the relationship between serum magnesium and HDL-cholesterol concentration adjusted by serum glucose values. Thirty (60.0%) controlled diabetic subjects, 58 (52.7%) non-controlled diabetic patients, 21 (52.5%) subjects with IFG, and 39 (20.5%) healthy volunteers had serum magnesium levels ≤1.7 mg/l. Serum HDL-cholesterol value showed significant graded increase with serum magnesium levels irrespective of glucose values. Results of this study suggest that hypomagnesemia decrease HDL-cholesterol by glycemia independent pathway (557).

Guerrero-Romero F et al (2002) analyzed the serum magnesium concentration in individuals with metabolic syndrome in a cross-sectional population-based study to compare 192 individuals with metabolic syndrome and 384 disorder-free control subjects, matched by age and gender was performed. Low serum magnesium levels were identified in 126 (65.6%) and 19 (4.9%) individuals with and without metabolic syndrome. The mean serum magnesium level among subjects with MS was 1.8±0.3 mg/dl, and among control subjects 2.2±0.2 mg/dl, p<0.00001. There was a strong independent relationship between low serum magnesium levels and MS. Among the components of metabolic syndrome,
dyslipidemia and BP were strongly related to low serum magnesium levels. This study reveals a strong relationship between decreased serum magnesium and metabolic syndrome (558).

Abbott RD et al (2003) examined the relation between dietary magnesium intake and future risk of CHD in 7,172 men in the Honolulu Heart Program. In 30 years of follow-up, 1,431 incident cases of CHD were identified. Within 15 years after dietary assessment, the age-adjusted incidence decreased significantly from 7.3 to 4.0 per 1,000 person-years in the lowest (50.3 to 186 mg/day) versus highest (340 to 1,183 mg/day) quintiles of magnesium intake. When adjustments were made for age and other nutrients, there was a 1.7 to 2.1 fold excess in the risk of CHD in the lowest versus highest quintiles. The excess risk ranged from 1.5 to 1.8 fold after further adjustment for other cardiovascular risk factors. Associations between dietary magnesium and coronary events occurring after 15 years of follow-up were modest. It was concluded that the intake of dietary magnesium is associated with a reduced risk of CHD (559).

Wälti MK et al (2003) compared plasma magnesium concentrations of type 2 diabetics and healthy controls in Switzerland. Mean plasma magnesium concentrations of the diabetics and controls were 0.77±0.08 and 0.83±0.07 mmol/L, respectively. Plasma magnesium concentrations were below the normal reference range in 37.6% of the diabetic patients and 10.9% of the control subjects. Plasma magnesium was not correlated with glycemic control as measured by HbA1c. Lower plasma magnesium concentrations and poor magnesium status are common in type 2 diabetics in Zurich, Switzerland (560).

Al-Delaimy WK et al (2004) assessed the relationship between magnesium intake and risk of CHD among men. During 12 years of follow-up (414,285 person-years), they documented 1,449 cases of total CHD (1,021 non-fatal myocardial infarction cases, and 428 fatal CHD). The age-adjusted relative risk of developing CHD in the highest quintile (median intake = 457 mg/day) compared with the lowest quintile (median intake = 269 mg/day) was 0.73 (95% CI 0.62-0.87, p for trend <0.0001). After controlling for standard CHD risk factors and dietary factors, the RR for developing CHD among men in the highest total magnesium intake quintile compared with those in the lowest was 0.82 (95% CI 0.65-1.05, p for trend = 0.08). They suggested that intake of magnesium may have a modest inverse association with risk of CHD among men (561).
Wang JL et al (2005) investigated the magnesium status and association with diabetes in the elderly Taiwanese. Average magnesium intake was 250 mg in men and 216 mg in women, which is equivalent to 68-70% of relevant Taiwanese Dietary Reference Intakes. The mean plasma magnesium concentration was 2.19 mg/dl in men and 2.20 mg/dl in women. The prevalence of plasma magnesium level of <1.7 mg/dl was 0.7-0.9% in the elderly, and that of <1.94 mg/dl was 8.0-9.1%. Elderly vegetarians had a significantly lower magnesium intake than ovo-lacto vegetarians and non-vegetarians. Diabetic men and women had significantly higher blood glucose levels than non-diabetics. The risk of diabetes was elevated 3.25 times at plasma magnesium levels 2.09 mg/dl. There was an inverse association between plasma magnesium concentration and the prevalence of diabetes. However, no association was found between diabetes and low dietary magnesium. Taiwanese elderly persons had suboptimal levels of dietary magnesium intake, which although may be sufficient to avoid overt magnesium deficiency, may not be sufficient to reduce the risk of diabetes in the elderly (562).

Corica F, et al (2006) evaluated circulating serum ionized magnesium concentrations in patients with type 2 DM, and investigated its relationship with the components of the metabolic syndrome. In univariate analysis, serum ionized-magnesium levels were significantly reduced in patients with low HDL cholesterol, high TGs values, high waist circumference, high BP. Hypomagnesemia was highly prevalent in study population (N = 143, 49.3%). After adjusting for potential confounders, plasma TGs was independently associated with hypomagnesemia (563).

Song Y et al (2007) examined whether magnesium intake is related to inflammatory and endothelial markers in a cross-sectional study of 657 women from the Nurses' Health Study cohort, who were aged 43-69 y and free of cardiovascular disease, cancer, and DM when blood was drawn in 1989 and 1990. Plasma concentrations of CRP, IL-6, soluble tumor necrosis factor alpha receptor 2 (sTNF-R2), E-selectin, soluble intercellular adhesion molecule 1 (sICAM-1), and soluble VCAM-1 were measured. In age-adjusted linear regression analyses, magnesium intake was inversely associated with plasma concentrations of CRP (P for linear trend = 0.003). After further adjustment for physical activity, smoking status, alcohol use, postmenopausal hormone use, and BMI, dietary magnesium intake remained inversely associated with CRP and E-selectin. Magnesium intake from diet was
modestly and inversely associated with some but not all markers of systematic inflammation and endothelial dysfunction in apparently healthy women (564).

Larsson SC et al (2007) did meta-analysis of seven cohort studies to assess the association between magnesium intake and risk of type 2 DM. All but one study found an inverse relation between magnesium intake and risk of type 2 diabetes, and in four studies the association was statistically significant. The overall relative risk for a 100 mg/day increase in magnesium intake was 0.85 (95% CI, 0.79-0.92). Results were similar for intake of dietary magnesium (RR, 0.86; 95% CI, 0.77-0.95) and total magnesium (RR, 0.83; 95% CI, 0.77-0.89). Magnesium intake was inversely associated with incidence of type 2 DM. This finding suggests that increased consumption of magnesium-rich foods such as whole grains, beans, nuts, and green leafy vegetables may reduce the risk of type 2 DM (565).

Sharma A et al (2007) evaluated the relationship between serum magnesium and course of DM and also to find out, if there is any relation between serum magnesium and various complications of DM. A cross-sectional study was conducted to examine the relationship between serum magnesium in 50 type-1 and type-2 diabetic patients with or without complications and 40 normal healthy persons. Serum magnesium levels in diabetic population was significantly low (1.93±0.282 meq/l) in comparison to control (2.25±0.429 meq/l). It was statistically significant (+3.84; p<0.005). Duration of diabetes and serum magnesium was inversely related. Poor glycaemic control was associated with hypomagnesaemia (-2.623; p<0.05). There was strong association between hypomagnesaemia and obesity (1.878±0.326) and HTN (1.75±0.071) and it was statistically significantly (p < 0.005, 0.042, 0.000 respectively) (566).

Akizawa Y et al (2008) conducted a population survey focusing on the relationship between dietary magnesium intake and serum magnesium level. The subjects were 62 individuals from Fukui Prefecture who participated in the 1998 National Nutrition Survey. The mean daily magnesium intake was 322±132, 323±163, and 322±147 mg/day for men, women, and the entire group, respectively. The mean serum magnesium concentration was 20.69±2.83, 20.69±2.88, and 20.69±2.83 ppm for men, women, and the entire group, respectively. The daily magnesium intake correlated with serum magnesium concentration (567).
Nasri H et al (2008) investigated whether and how serum magnesium concentrations influence the serum lipids in DM patients. The mean patients' age was 63±10 years. The mean length of time they were diabetic was 7.4±5.8 years (median: 6 years). The mean serum magnesium was 2±0.4 mg/dl (median: 1.99 mg/dl). In the study significant inverse correlations of serum magnesium with serum cholesterol and LDL as well as non significant correlations of serum magnesium with serum HDL, TG and with serum A1C were seen. Moreover a significant inverse correlation of serum magnesium with ages of the patients was also found (568).

Randell EW et al (2008) examined whether the relationships between serum magnesium levels and biochemical and anthropometric risk factors for DM and metabolic syndrome are also present in the general adult population. Serum magnesium positively correlated with age, TC, HDL-cholesterol, LDL-cholesterol and TG levels. Serum magnesium negatively correlated with HOMA-beta. The results indicate that Serum magnesium levels positively correlate with TC and possibly all lipoproteins in a large adult study population which suggests that variation of Smagnesium with serum lipid levels may be different in healthy individuals compared with those with DM (569).

Masood N et al (2009) assessed serum magnesium level in type-2 diabetic patients and the effect of age, gender, glycemic control and duration of diabetes on these trace elements in comparison with those of control subjects. There were 42 diabetic patients and 42 age matched non-diabetic (control) subjects included in this study. No significant difference was found in serum magnesium level with mean of 22.67±24.5 mg/dL in diabetic patients as compared to controls (18.3±3.4 mg/dL, p = 0.26). There was no association of age, gender, glycemic status and duration of diabetes on the serum concentration of these trace elements in type-2 diabetic patients (570).

Reffelmann T et al (2011) analyzed all-cause mortality and cardiovascular mortality in relationship to serum magnesium concentrations at baseline by Cox proportional hazard model in (Study of Health in Pomerania) SHIP (n=4203, exclusion of subjects with magnesium supplementation). The median duration of mortality follow-up was 10.1 years (25th percentile: 9.4 years, 75th percentile: 10.8 years; 38,075 person-years). During the follow-up, 417 deaths occurred. Mortality in subjects with magnesium ≤0.73 mmol/l was significantly higher for all-cause deaths (10.95 death per 1000 person years), and
cardiovascular deaths (3.44 deaths per 1000 person years) in comparison to higher magnesium concentrations (1.45 deaths from all-cause per 1000 person years, 1.53 deaths from cardiovascular cause per 1000 person years). This association remained statistically significant after adjustment for multiple cardiovascular risk factors, including arterial HTN, and antihypertensive therapy including diuretics. Low serum magnesium levels were associated with higher all-cause mortality and cardiovascular mortality (571).

Stevanovic S et al (2011) assessed the relationship between dietary magnesium intake and the risk of CHD in a case-control study which included 290 randomly selected cases (mean age 59.98±10.03 years) with first event of an acute coronary syndrome and 290 selected controls paired by sex, age and region (mean age 59.43±10.10 years). Subjects with CHD had significantly lower intake of foods containing high levels of magnesium like whole grain, legumes and nuts. Lower dietary magnesium intake was found to be positively associated with risk of CHD. The findings suggests that dietary intake of magnesium was associated with reduced risk of CHD among Serbian population (572).

Zhang W et al (2012) investigated the relationship between dietary magnesium intake and mortality from cardiovascular disease in a population-based sample of Asian adults. Reported findings were based on dietary magnesium intake in 58,615 healthy Japanese aged 40-79 years, in the Japan Collaborative Cohort (JACC) Study. During the median 14.7-year follow-up, 2690 deaths from cardiovascular disease, comprising 1227 deaths from strokes and 557 deaths from CHD were documented. Dietary magnesium intake was inversely associated with mortality from hemorrhagic stroke in men and with mortality from total and ischemic strokes, CHD, heart failure and total cardiovascular disease in women. The multivariable hazard ratio for the highest vs. the lowest quintiles of magnesium intake after adjustment for cardiovascular risk factor and sodium intake was P for trend = 0.005 for CHD, 0.50 (0.28-0.87), P for trend = 0.002 for heart failure and 0.64 (0.51-0.80), P for trend < 0.001 for total cardiovascular disease in women. The adjustment for calcium and potassium intakes attenuated these associations. They concluded that dietary magnesium intake was associated with reduced mortality from cardiovascular disease in Japanese, especially for women (573).
2.5.4 Inflammatory markers and adipokine

Inflammation is basic defensive response of human body to any tissue injury, which consists of dolor, rubor, pallor, colour and loss of function. Inflammation involves immune system of body involving neutrophils, lymphocyte and mononuclear cells. These cells secrete various chemicals known as cytokines also named as chemokines (574). Past decades have witnessed spurt in research activity which has shown that inflammation plays a key role in CAD and other manifestations of atherosclerosis. Immune cells dominate early atherosclerotic lesions, their effector molecules accelerate progression of the lesions, and activation of inflammation can elicit acute coronary syndromes (575). The inflammatory process associated with atherosclerosis lead to increased blood levels of inflammatory cytokines and other acute-phase reactants. Levels of CRP and IL-6 are elevated in patients with unstable angina and myocardial infarction, with high levels predict worse prognosis (576-578). The levels of other inflammatory markers are also elevated in these patients, including fibrinogen, interleukin-7, interleukin-8, soluble CD40 ligand, and the C reactive protein- related protein pentraxin 3. (579-582) Levels of CRP are elevated in patients with unstable angina, dependent on coronary thrombosis of atherosclerotic plaques, but not in variant angina caused by vasospasm (583). Therefore, elevated CRP levels in patients with acute coronary syndromes likely reflect inflammation in the coronary artery rather than in the ischemic myocardium (583). Activated and inflammatory T cells are increased in the blood of patients with acute coronary syndromes (584,585). Collectively, these findings suggest that inflammation and immune activation in coronary arteries may initiate acute coronary syndromes, with circulating levels of inflammatory markers reflecting the clinical course of the condition. The balance between inflammatory and anti-inflammatory activity may also control the progression of atherosclerosis. Inflammation and related cytokines also interact with various metabolic factors which are related to atherosclerotic process in several ways. They contribute to lipid deposition in the artery, initiating new rounds of immune-cell recruitment. Furthermore, the adipose tissue of patients with the metabolic syndrome and obesity produces inflammatory cytokines, particularly TNF-α and IL-6. (586,587) “Adipokines”-cytokines of the adipose tissue, including leptin, adiponectin, and resistin— may also influence inflammatory responses throughout the organism (586). Finally, molecules generated during lipid peroxidation in atherosclerotic disease can induce
protective as well as inflammatory reactions, for instance, by binding to nuclear receptors that control inflammatory genes (588,589).

CRP levels varies considerably in patients with ACS and may be indicative of coronary instability, and may be pathogenetic involved in causation of ACS. Elevated CRP (>3 mg/L) is found in >10% of normals, in >20% of patients with chronic stable or variant angina, but in >65% of patients with unstable angina, Braunwald class IIIb, and in >90% of patients with acute infarction preceded by unstable angina, but in >50% of those in whom the infarction was totally unheralded.(576,590,591) The absence of elevated CRP in >30% of patients with severe unstable angina and in >50% of those with acute MI not preceded by unstable angina suggests an important heterogeneity of the role of inflammatory triggers of the clinical syndromes of coronary instability (592). Individuals may vary in their response to inflammatory stimuli. The increase in CRP and IL-6 observed in response to the vascular trauma caused by coronary angioplasty, or by uncomplicated cardiac catheterization, and after acute infarction (593) correlates linearly with baseline CRP and IL-6 levels. In vitro, the IL-6 production by isolated monocytes from unstable patients with elevated CRP and IL-6 significantly exceeds that produced by monocytes from patients with normal values (590). These individual differences in the degree of response to given inflammatory stimuli may have a genetic basis. For example, certain haplotypes in the IL-1/IL-1 receptor agonist gene complex correlate with heightened inflammatory responses and incidence of ACS (594).

2.5.4.1 Cytokines

Cytokines are small cell-signaling protein molecules that are secreted by numerous cells involved in intercellular communication. Cytokines belong to varied class of molecules such as proteins, peptides, or glycoproteins. The term "cytokine" encompasses a large and diverse family of chemical regulators produced by cells of diverse embryological origin (595). The term "cytokine" has also been used to refer to the immunomodulating agents, such as interleukins and interferons. Tumour necrosis factor, IL-6 and CRP levels are most studied cytokines in cardiovascular disease.

TNF-α is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. It is produced chiefly by activated macrophages, although it can be produced by other cell types as well like CD4+.
lymphocytes and natural killer cells. The primary role of TNF is in the regulation of immune cells. TNF, being an endogenous pyrogen, is able to induce fever, apoptotic cell death, sepsis (through IL1 & IL6 production), cachexia, inflammation, and to inhibit tumorigenesis and viral replication. Dysregulation of TNF production has been implicated in a variety of human diseases, including Alzheimer's disease (596), cancer (597), major depression (598), and inflammatory bowel disease (IBD) (599).

IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. IL-6 is also a "myokine," a cytokine produced from muscle, and is elevated in response to muscle contraction (600). It is significantly elevated with exercise, and precedes the appearance of other cytokines in the circulation. During exercise, it is thought to act in a hormone-like manner to mobilize extracellular substrates and/or augment substrate delivery (601). Additionally, osteoblasts secrete IL-6 to stimulate osteoclast formation. Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6's role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1ra and IL-10. Adipose tissue can also synthesize cytokines such as TNF-α and IL-6. In this way obesity itself promotes inflammation and potentiates atherogenesis independent of effects on insulin resistance or lipoproteins (602-604).

CRP is a member of the class of acute-phase reactants as its levels rise dramatically during inflammatory processes. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages (605) as well as adipocytes (606). The acute phase response develops in a wide range of acute and chronic inflammatory conditions like bacterial, viral, or fungal infections; rheumatic and other inflammatory diseases; malignancy; and tissue injury or necrosis. These conditions cause release of interleukin-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver. CRP binds to phosphocholine on microbes and damaged cells and enhances phagocytosis by macrophages. Thus, CRP participates in the clearance of necrotic and apoptotic cells. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, 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. CRP rises up to 50,000-fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause).

Vaddi K et al (1994) investigated cytokine production (TNF-\(\alpha\) and interferon-gamma) by mononuclear leukocytes in patients with IHD (10 patients with stable angina pectoris, and 10 patients with unstable angina pectoris) and 8 control subjects. Secretion of both TNF-alpha and interferon-gamma increased progressively over 48 hours, and it was consistently higher (\(P < .02\)) in patients compared with control subjects. A similar increase in cytokine secretion was observed in patients with stable or unstable angina pectoris. In addition, there was no relation between the severity of CAD by angiography and cytokine secretion. It was concluded that increased cytokine secretion in IHD may play a role in superoxide radical generation, endothelial injury, deposition and activation of cellular elements on the vessel wall, and possibly in the progression of atherosclerosis.\(^{(607)}\)

de Maat MP et al (1996) estimated the inflammatory markers in 34 patients with severe CAD and 30 healthy controls comparable for age and smoking habits. Smoking appeared to increase the CRP levels, while both CAD and smoking seemed to affect the IL6 levels. Results indicate that both smoking and CAD induce an inflammatory condition but that the increase of plasma levels of different inflammatory markers is complex. Although the acute phase reaction is the main regulatory mechanism of fibrinogen, the increase of fibrinogen in the group of CAD patients could not be fully explained by increased inflammation.\(^{(608)}\)

Mendall MA et al (1997) determined whether serum concentrations of the cytokines TNF-\(\alpha\) and IL-6, which regulate C reactive protein, are associated with cardiovascular risk factors and prevalent CHD in 198 men aged 50 to 69 years who were part of a random population sample drawn from south London. Serum TNF-\(\alpha\) concentration was positively related to BMI. IL-6 concentrations were positively associated with smoking and age. TNF-\(\alpha\) was associated with increased IL-6 and TGs, and reduced high density lipoprotein cholesterol. IL-6 was associated with raised fibrinogen, and TGs. ECG abnormalities were independently associated with increases in IL-6 and TNF alpha, each by approximately
50% (P < 0.05 for TNF alpha, P < 0.1 for IL-6). The corresponding increases in men with an abnormal ECG or symptomatic CHD were 28% for TNF alpha and 36% for IL-6 (P = 0.14 for TNF alpha and P < 0.05 for IL-6). This study confirmed that many of the phenomena with which C reactive protein is associated, are also associated with serum levels of cytokine, which may be the mechanism (609).

Roubenoff R et al (1998) determined the association among aging, inflammation, and cytokine production by peripheral blood mononuclear cells. Production of IL-6 (p < .00001) was higher in the elderly subjects than in the control group. IL-6 production increased with increasing CRP. However, no difference was found in the production of TNF-α between the young and elderly groups, regardless of CRP status. IL-6 population correlated with TNF-α production (r = .25, p < .0001). Production of IL-6 but not TNF-α was increased in the elderly compared to healthy, young subjects. The increase in IL-6 also correlated with increased production of CRP, a marker of inflammation (610).

Cavusoglu Y et al (2001) tested whether CRP, fibrinogen and antithrombin-III are associated with angiographic CAD, history of myocardial infarction and extensive atherosclerotic involvement. CRP was higher in patients with CAD (0.95±1.31, n = 180, vs. 0.39±0.61 mg/dl, n = 39, P < 0.0001) and than in control subjects. The patients who developed unstable angina had higher CRP levels than the patients with stable CAD (2.07±2.38, n = 7, vs. 0.80±1.13 mg/dl, n = 173, P < 0.001). Results indicated that CRP was elevated in patients with CAD and a history of MI. Elevated levels of CRP at the time of hospital admission is a predictive value for future ischemic events (611).

Pradhan AD et al (2001) determined whether elevated levels of the inflammatory markers; IL-6 and CRP are associated with development of type 2 DM in healthy middle-aged women from a nationwide cohort of 27628 women. Over a 4-year follow-up period, 188 women who developed DM who were defined as cases and 362 disease-free controls matched by age and fasting status were selected from same population. Baseline levels of IL-6 and CRP were significantly higher among cases than among controls. The relative risks of future DM for women in the highest vs lowest quartile of these inflammatory markers were 7.5 for IL-6. Positive associations persisted after adjustment for BMI, family history of diabetes, smoking, exercise, use of alcohol, and hormone replacement therapy.
Elevated levels of CRP and IL-6 predicted the development of type 2 DM. These data supported a possible role for inflammation in diabetogenesis (612).

Madsen T et al (2001) studied the effect of marine n-3 PUFA on CRP levels in 269 patients of coronary artery disease. All patients filled out a food questionnaire regarding fish intake. CRP was significantly higher in patients with significant coronary stenosis than in those with no significant angiographic changes (p <0.001), but the CRP levels were not associated with the number of diseased vessels. Subjects with CRP levels in the lower quartile had a significantly higher content of docosahexaenoic acid (DHA) in granulocytes than subjects with CRP levels in the upper quartile (p = 0.02), and in a multivariate linear regression analysis, DHA was independently correlated to CRP ($R^2 = 0.179$; p = 0.003). The inverse correlation between CRP and DHA may reflect an anti-inflammatory effect of DHA in patients with stable coronary artery disease and suggest a novel mechanism by which fish consumption may decrease the risk of coronary artery disease (613).

Fang L et al (2004) evaluated the significance of inflammatory markers as novel risk factors for CAD in 170 angiographically defined CAD patients and 177 healthy control subjects in the Chinese population in Singapore. High-sensitivity CRP, soluble cellular adhesion molecules including VCAM-1, ICAM-1, P-selectin and E-selectin and white blood cell (WBC) count were measured. The levels of hs-CRP were higher in CAD patients than in control subjects. Patients with unstable angina or myocardial infarction had higher levels of hs-CRP than those with stable angina or atypical chest pain (all P<0.05). Study suggested that inflammatory markers, including hs-CRP and WBC count, together with sP-selectin and sVCAM-1, could serve as markers of atherogenesis in Chinese patients with CAD, with potential diagnostic and therapeutic implications (614).

Pai JK et al (2004) examined plasma levels of sTNF-R1, sTNF-R2, IL-6, and CRP as markers of risk for CHD among women participating in the Nurses' Health Study and men participating in the Health Professionals Follow-up Study in nested case-control analyses. Levels of IL-6 and CRP were significantly related to an increased risk of CHD in both sexes, whereas high levels of soluble TNF-α receptors were significant only among women. Elevated levels of inflammatory markers, particularly CRP, indicate an increased risk of CHD. Although plasma lipid levels were more strongly associated with an increased
risk than were inflammatory markers, the level of CRP remained a significant contributor to the prediction of CHD (615).

Rutter MK et al (2004) assessed the relations of CRP to the metabolic syndrome in a cross sectional study among 3037 subjects (1681 women; mean age, 54 years). In persons with metabolic syndrome, age-adjusted CRP levels were higher in women than men (7.8 versus 4.6 mg/L; P<0.0001). Metabolic syndrome and baseline CRP were individually related to CVD events. Greater risk of CVD persisted for metabolic syndrome and CRP even after adjustment in a model including age, sex, metabolic syndrome and CRP. Elevated CRP levels were related to insulin resistance and the presence of the metabolic syndrome, especially in women. Both CRP and metabolic syndrome were independent predictors of new CVD events (616).

Hu FB et al (2004) conducted a prospective, nested, case-control study of inflammatory markers as predictors of type 2 diabetes among 32,826 women who provided blood samples in 1989 through 1990 in the Nurses’ Health Study. Baseline levels of TNF-α receptor 2, IL-6, and CRP were significantly higher among case than control subjects. After adjusting for BMI and other lifestyle factors, all three biomarkers significantly predicted diabetes risk. In a multivariate model including the three biomarkers, only CRP levels were significantly associated with risk of diabetes. These data supported the role of inflammation in the pathogenesis of type 2 diabetes. Elevated CRP levels were a strong independent predictor of type 2 DM and may mediate associations of TNF-alphaR2 and IL-6 with type 2 diabetes (617).

Bo M et al (2004) investigated the relationships between body fat, CRP levels and metabolic variables in healthy, non-obese sons of patients affected by metabolic syndrome. CRP levels were associated with total and regional body fat, the anthropometric index of weight, age, and with some metabolic alterations (HDL-cholesterol and TG levels, systolic BP, and fasting insulin and LDL-cholesterol levels). The associations between total body fat and the metabolic variables did not change after adjustment for CRP levels. Total body fat was the best predictor of CRP levels (618).

Carey AL et al (2004) carried out two of euglycaemic-hyperinsulinaemic clamp experiments. Skeletal muscle mRNA expression and plasma concentrations of IL-6 and TNFalpha were examined in patients with Type 2 diabetes subjects, matched for age and
young healthy control subjects. A strong positive correlation (r=0.85; p<0.00001) was observed between basal plasma IL-6 and BMI. Conversely, a negative relationship (r=-0.345; p<0.05) was found between basal plasma TNF-α and BMI, although this was not significant when corrected for BMI. When corrected for BMI, no relationship was observed between either basal plasma IL-6 or TNF-α. These data showed that the increased circulating IL-6 concentrations seen in patients with Type 2 diabetes are strongly related to fat mass and not insulin responsiveness, and suggest that neither IL-6 nor TNF-α are indicative of insulin resistance (619).

Yip HK et al (2005) conducted a prospective cohort study in 128 consecutive patients, including unstable angina pectoris patients (class I: n = 59; combined class II and III: n = 16), stable angina pectoris patients (n = 53) undergoing elective coronary stenting and 40 healthy volunteers. Blood samples for hs-CRP were obtained before coronary angiography. The circulating levels of these three inflammatory markers were substantially higher in patients than in healthy volunteers. Additionally, circulating levels of hs-CRP and the WBC count were significantly higher in patients with unstable angina pectoris than in patients with stable angina pectoris. Multiple stepwise logistic regression analysis showed that only hs-CRP level was independently associated with unstable angina pectoris (P = 0.0002). It was concluded that circulating levels of hs-CRP was significantly increased in patients with angina pectoris. The circulating level of hs-CRP was strongly associated with the clinical setting of unstable angina pectoris (620).

Tuomisto K et al (2006) analyzed the associations of CRP, IL-6 and TNF-α with incident CHD events, CVD events, and total mortality. A random population sample, including men and women aged 25-64 years was examined in Finland in 1992. The sample size was 7,927 and 6,051 (76%) participated. The cohort was followed up until the end of 2001. After adjustment for conventional CVD risk factors, CRP showed a significant association with CHD risk in men. This association remained significant after further adjustment for TNFalpha. TNFalpha also was a significant predictor of CHD among men, but the association was nonlinear (HR=2.21, 1.18-4.14 comparing the three upper quartiles to the first quartile). Further adjustment for CRP did not change this association substantially. CRP and TNF-α, both predicted all CVD events and total mortality among men. Among women the findings were nonsignificant. In conclusion, CRP and TNFalpha
were significant, independent predictors of CHD and CVD events and total mortality among men. These findings provide further support to the important role of inflammation in the pathogenesis of CVD (621).

Ye X et al (2007) evaluated the distributions of CRP and its association with metabolic syndrome in middle-aged and older Chinese people (age 50 to 70 years) in a population-based cross-sectional survey among 1,458 men and 1,831 women in 2005 in Beijing and Shanghai. The prevalence of metabolic syndrome progressively increased with elevated CRP levels. In the highest quartile of CRP levels (>1.50 mg/l), the risk for metabolic syndrome was substantially higher compared with that in the lowest quartile of CRP levels after adjustment for age, gender, geographic location, lifestyle factors, educational attainment, and family history of chronic diseases. This association was observed in both obese and non-obese participants. The overall plasma level of CRP was low but highly associated with the metabolic syndrome among the middle-aged and elderly Chinese population (622).

Dupuy AM et al (2007) assessed the relationship of CRP levels to components of metabolic syndrome in apparently healthy elderly subjects living in the South of France. Subjects were grouped into three categories based on the 75th and 25th percentiles of CRP, corresponding to 3.05 and 0.82, respectively. Metabolic Syndrome, which had a prevalence of 31%, was significantly associated with elevated CRP levels. Among metabolic syndrome components, the strongest positive association with the highest quartile of CRP was with waist circumference in both sexes. Each component of the metabolic syndrome was significantly associated with high CRP values in elderly women only. In men, smoking was significantly associated with high CRP levels. In women, the association observed in univariate analysis with fasting glucose or HTN did not reach statistical significance in the multivariate analysis, while only a weak association could be observed with lipid parameters such as TGs and high-density lipoprotein cholesterol (623).

Kim ES et al (2007) investigated the association among adiposity, insulin resistance, and inflammatory markers hs-CRP, IL-6, and TNF-α and adiponectin and to study the effects of exercise training on adiposity, insulin resistance, and inflammatory markers among obese male Korean adolescents. The study demonstrated higher insulin resistance, TC, LDL-C levels, TG, and inflammatory markers and lower adiponectin and
HDL-C in obese Korean male adolescents. Six weeks of increased physical activity improved body composition, insulin sensitivity, and adiponectin levels in obese Korean male adolescents without changes in TNF-α, IL-6, and hs-CRP (624).

Oren H et al (2007) determined whether new inflammatory biomarkers have roles in atherosclerosis, the authors measured the levels of CRP, macrophage colony stimulating factor, and IL-3 in patients with chronic stable CHD and in healthy controls. Mean plasma CRP, macrophage colony stimulating factor, and IL-3 concentrations in patients with chronic stable CHD were significantly higher than those in controls (8.2 vs 4.6 mg/L, 195.3 vs 28.9 pg/mL, 173 vs 118 ng/mL). These findings suggested that these are new inflammatory biomarkers that may have important roles in the development of atherosclerotic lesions (625).

Sukhija R et al (2007) measured hs-CRP, IL-6, and TNF-α in 249 patients who were admitted with acute chest pain and underwent coronary angiography. The relation between serum levels of inflammatory markers and angiographic severity of CAD was analysed. A follow-up at 6 months was conducted to assess major adverse cardiovascular events, defined as a cumulative of myocardial infarction, all-cause death, or coronary revascularization. After adjusting for conventional CAD risk factors (age, gender, diabetes, HTN, smoking, and hypercholesterolemia), there was no association between inflammatory markers (hs-CRP, IL-6, and TNF-α) and angiographic severity of CAD. There was a significant positive correlation between age, male gender, DM, and hypercholesterolemia with atherosclerotic burden determined by angiography. There was no significant positive association between major adverse cardiovascular events and hs-CRP, IL-6, or TNF-α level in unadjusted and adjusted models. In conclusion, in patients hospitalized with chest pain, no association of serum levels of hs-CRP, IL-6, or TNF-α with coronary atherosclerotic burden or major adverse cardiovascular events were found at 6 months after adjustment for traditional CAD risk factors (626).

Habib SS et al (2008) determined the serum levels of the circulating acute-phase reactant hsCRP in 107 Saudi patients with chronic stable CAD and 33 healthy, age and BMI-matched individuals. TC (Control 4.41±0.57 vs CAD 4.28±1.40, p = 0.8394) and LDL levels (Control 2.70±0.52 vs CAD 2.71±1.20, p = 0.7963) did not differ significantly between the two groups. While there were significant differences in TG (Control 1.13±0.47
vs CAD 1.84±1.10, p = 0.0135) and HDL levels (Control 1.06±0.30 vs CAD 0.71±0.25, p = 0.0000). hsCRP levels were significantly higher in patients with CAD (5.0±4.4) compared to healthy individuals (2.7±2.7, p = 0.0166). It was suggested that Saudi patients with stable chronic CAD have higher hsCRP levels compared to healthy individuals and the prevalence of undesirable risk levels of hsCRP is also higher in CAD patients (627).

Irfan G et al (2008) examined 100 patients admitted with acute coronary syndrome and assessed the relationship between preprocedural hs-CRP concentration and coronary angiographic lesions. Mean age 59.26±11.04, 64% male and 36% female. The mean value of hs-CRP was 4.26±1.42 mg/dl with highest values in patients eccentric/irregular lesion and patients having macroscopic thrombus (p = 0.01). Among patients with acute coronary syndrome increased levels of hs-CRP correlates with specific high risk coronary artery lesions (628).

Souza JR et al (2008) evaluated the inflammatory response in patients with diabetes and acute coronary events. Higher serum IL-6 levels were found in diabetic or non-diabetic patients with ACS than in the group with chronic CHD. On the other hand, diabetic patients with ACS had higher CRP levels in comparison with the other groups. Serum IL-18 levels were not significantly different among the patients studied (629).

Gotsman I et al (2008) determined the relationship between inflammatory markers and the angiographic severity of CAD. Inflammatory markers included CRP, fibrinogen, serum cytokines (interleukin-1 beta, IL-1 receptor antagonist, IL-6, IL-8, IL-10 and TNF-α). It was concluded that TNF-α and IL-6 are significant predictors of the severity of coronary artery disease. This association is likely an indicator of the chronic inflammatory burden and an important marker of increased atherosclerosis risk (630).

Crandall MA et al (2009) determined hs-CRP level in patients according to atrial fibrillation (AF) status. A total of 2,340 patients were studied (64±12 years). Comorbid diseases included CAD 1,438 (61%), HTN 1,309 (56%), DM 433 (19%), congestive heart failure 345 (15%), and a prior stroke 43 (2%). The hs-CRP level was significantly higher in patients with AF (n = 238) compared to those without (14.0 mg/L vs 9.1 mg/L, P < 0.001). The presence of AF was associated with higher hs-CRP level across all scores (medians [mg/L], 0: 2.22 vs 1.98, P = 0.83, 1: 3.85 vs 2.86, P = 0.057, 2: 4.96 vs 3.29, P = 0.021, 3:
6.29 vs 3.17, P = 0.09, 4-5: 4.82 vs 4.50, P = 0.87). Risks factors associated with AF were associated with higher hs-CRP in an incremental manner (631).

Iso H et al (2009) conducted a nested case-control study as part of the Japan Collaborative Cohort Study in 39,242 subjects (40-79 years) who provided serum samples at baseline between 1988 and 1990. Median hs-CRP levels for controls were 0.40 mg/L for men and 0.41 mg/L for women. Hs-CRP levels were positively associated with risks of mortality from stroke, CHD, and total cardiovascular disease for men. For women, positive associations with hs-CRP levels were weaker, reaching statistical significance only for total cardiovascular disease. The positive association with total cardiovascular disease did not vary according to sex, age, smoking status, or BMI. Higher serum hs-CRP levels were associated with higher mortality from cardiovascular disease in Japanese (632).

Huffman FG et al (2009) investigated metabolic syndrome and its association with hs-CRP levels in Cuban Americans. The study included 161 nondiabetic Cuban Americans (55 men and 106 women) aged ≥30 years living in South Florida. The odds of having elevated hs-CRP levels were approximately 4 times higher in participants with metabolic syndrome than in those without it. Mean log hs-CRP increased as number of components of metabolic syndrome increased. Of the components of metabolic syndrome, only abdominal obesity was significantly associated with elevated hs-CRP (633).

Nordestgaard BG et al (2009) evaluated human studies to see whether elevated plasma levels of CRP cause CVD and suggested that elevated CRP per se does not cause CVD; however, inflammation per se possibly contributes to CVD. Elevated CRP levels more likely is a marker for the extent of atherosclerosis or for the inflammatory activity and vulnerability of atherosclerotic plaques, and thus simply an innocent bystander in CVD (634).

Khan SA et al (2010) correlated levels of TNF-α and other co-variates (BMI; abnormal lipids; blood sugar; microalbuminuria) and different grades of BP in essential hypertensive patients. Sixty newly detected patients of Essential HTN were recruited in the study. It comprised of 36 males and 24 females. TNF-α level were consistently found to be elevated in both the stages of HTN. Though females in the study had slightly higher mean values of TNF- alpha than the males, the difference was not statistically significant. No significant correlation was observed between levels of TNF-α and stage of HTN. Using
Pearson’s Correlation coefficient, TNF-alpha levels were found to significantly correlate with LDL and BMI (635).

Goswami B et al (2010) determined a possible inter-relationship between inflammation and dyslipidaemia, which are important risk factors for CAD in the atherosclerosis-prone North Indian male population. The study groups comprised 150 clinically patients with acute myocardial infarction, and 150 healthy controls. Apolipoprotein-AI, apolipoprotein-B (Apo-B) and CRP levels were estimated in the study. The patients with AMI showed highly significant elevations in the levels of total serum cholesterol, TGs, LDL cholesterol, Apo-B and a significant decline in HDL cholesterol, compared with healthy controls. Significantly elevated serum levels of inflammatory markers, TNF-α and CRP were seen in patients with acute myocardial infarction, compared to the control subjects. A significantly positive correlation of TNF-α was observed with lipoprotein(a) in patients with CAD. The data suggested a possible interplay between inflammation and dyslipidaemia in the pathogenesis of CAD in the Indian context. This insight into the aetiopathogenesis of CAD will prove highly beneficial for devising better preventive measures and pharmacological interventions for CAD (636).

Monteiro CM et al (2010) examined the major determinants of coronary disease severity, including those coronary risk factors associated with metabolic syndrome, during the early period after an acute coronary episode. Subjects of both genders aged 30 to 75 years (N = 116) were included if they had suffered a recent acute coronary syndrome (acute myocardial infarction or unstable angina pectoris requiring hospitalization) and if they had metabolic syndrome diagnosed and hsCRP was analysed. The severity of coronary disease was correlated (Spearman's or Pearson's coefficient) with gender (r = 0.291, P = 0.008), age (r = 0.218, P = 0.048), hsCRP (r = 0.256, P = 0.020). After multiple linear regression, only male gender (P = 0.046) and hsCRP (P = 0.012) remained independently associated (637).

Shahid HS et al (2011) compared hsCRP and lipoprotein(a) levels between diabetic and non-diabetic patients with CAD in 103 individuals and 30 healthy individuals matched for age and BMI, in Riyadh. They were analyzed for TC, TGs, LDL and High density lipoprotein, Lp(a) and hsCRP. Both groups of CAD without and with DM had significantly higher hsCRP (0.52±0.71, 0.82±0.78 mg/dl respectively) when compared with healthy
control subjects (hsCRP=0.27±0.21) [p<0.05]. Lipoprotein(a) levels between the two CAD groups were non significant. hsCRP levels were significantly high in CAD with DM compared to those without DM. They concluded that elevated lipoprotein(a) and hsCRP levels are associated specifically with angiographically defined CAD. However, hsCRP elevation but not Lp(a) is also associated with CAD in type 2 DM. Measurement of hsCRP and Lp(a) may be considered optional markers for better prediction of cardiovascular risk (638).

Syvänen K et al (2011) investigated correlation between hsCRP and ankle brachial index in a cardiovascular risk population in men and women aged 45 to 70 years from a rural town Harjavalta, Finland. Smokers had higher hsCRP (mean 2.2 mg/L) than nonsmokers (mean 1.8 mL/L). hsCRP in women was higher than in men (mean 2.0 mg/L versus 1.8 mg/L). hsCRP correlated with BMI (r=0.208,p<0.0001) and waist circumference (r=0.325,p<0.0001). hsCRP was correlated to the measures of obesity (waist circumference and BMI), indicating its role as a marker of adipose tissue-driven inflammation (639).

Nishida H et al (2011) assessed the predictive power of interleukin (IL)-6 for future CV events. In 121 Japanese patients with multiple CV risk factors and/or disease, serum concentrations of IL-6 and hs-CRP were measured, follow-up periods was 2.9 years (mean). The serum level of IL-6, but not hs-CRP, was significantly higher in patients who had CV events than in event-free subjects (3.9±2.6 and 3.0±2.2 pg/mL, P=0.04). When the patients were divided into three groups by tertiles of basal levels of IL-6 (<1.85, 1.85-3.77, and ≥3.77 pg/mL), cumulative event-free rates decreased according to the increase in basal IL-6 levels (65%, 50%, and 19% in the lowest, middle, and highest tertiles of IL-6, respectively; log-rank test, P=0.002). The highest tertile of IL-6 was only an independent determinant for the morbidity in the multivariate analysis (hazard ratio 2.80 vs. lowest tertile, P=0.006). These findings indicate that IL-6 is a powerful independent predictor of future CV events in high-risk Japanese patients, suggesting its predictive value is superior to that of hs-CRP (640).

Swardfager W et al (2012) performed a meta-analysis of changes in inflammatory biomarkers over the course of exercise interventions in patients with CAD. Twenty-three studies were included. It was concluded that exercise training was associated with reduced
inflammatory activity in patients with CAD. Higher baseline CRP and adverse baseline lipid profiles predicted greater reductions in CRP (641).

Sarrafzadegan N et al (2012) compared the serum levels of IL-6, E-selectin, and trans-fatty acids (TFA) between those with stable (93 patients called control group) and unstable angina pectoris (89 patients called case group). Patients with stable angina had significantly higher levels of TC (187±3.7 vs 171.6±4.2 mg/dl; p=0.009), LDL (104.8±2.4 vs 95.4±2.7; p=0.017) compared to those with ACS. Serum levels of IL-6 were found to be significantly higher in those with stable angina compared to ACS. In the study, inflammation measured by IL-6 and E-selectin were not found to play an important role in progression of IHD from stable angina to unstable angina or myocardial infarction, which is contrary to previous studies (642).

Yaseen F et al (2012) measured serum resistin, IL-6 levels and lipid profile in nondiabetic controls, diabetics, and IHD subjects with and without diabetes. In this cross-sectional study, 147 subjects (36 were controls and 111 were cases), aged between 40 and 70 years were enrolled. Serum IL-6 levels increased significantly in diabetics (73.1±2.57 ng/ml) and nondiabetics with IHD (66.2±2.08 ng/ml) compared with diabetics and nondiabetic controls. Positive correlations were found between IL-6 and resistin, and a significant positive correlation was shown in IHD groups (r = 0.659; p = 0.001). It suggested that there was a possible role of IL-6 in inflammatory processes, especially in atherosclerosis (643).

Niu W et al (2012) investigated whether the association between circulating IL-6 levels and the risk for CAD is robust and perhaps even causal by a meta-analysis implementing Mendelian randomization approach with IL-6 gene G-174C polymorphism as an instrument. Data were available from 19 articles encompassing 9417 CAD patients and 15982 controls. Findings provided strong evidence on the causal association of circulating IL-6 levels with the development of CAD in White populations (644).

Young D et al (2012) reported that elevated levels of the inflammatory marker, CRP, were cross-sectionally associated with traditional CVD risk factors after lifestyle intervention. CRP change was negatively associated with TG change in men (p = 0.003) and women (p = 0.05), positively associated with change in systolic BP in men (p = 0.01), but was not associated with changes in the other risk factors. Dietary and/or physical
activity induced changes in CRP may be largely independent of traditional CVD risk factors in persons with dyslipidemia (645).

Yang T et al (2012) examined the association between CRP concentration, and the metabolic syndrome in a sample of Chinese adults in Taiwan. A cross-sectional analysis was performed of data from 4234 subjects [mean age, 47.1 ±18.2 years; 46.4 % males] who participated in a population-based survey on prevalences of HTN, hyperglycemia, and hyperlipidemia in Taiwan. Prevalence of metabolic syndrome was 22.1 %. A significantly progressive increase in the prevalence of metabolic syndrome across quartiles of CRP was observed. There was a strong stepwise increase in CRP levels as the number of components of the metabolic syndrome increased (646).

Aydin M et al (2012) investigated the relation between the body fat composition, metabolic syndrome, and the hs-CRP plasma levels. Total 246 consecutive Turkish subjects with DM, insulin resistance or metabolic syndrome, were included into the study. The hs-CRP levels increased in parallel with the body weight in Turkish subjects. This increase was significant especially in the women. The waist circumference, BMI, and body composition variables (visceral fat level, total body fat, and total body muscle mass) were significant correlates of the hs-CRP. The waist circumference and BMI were independent predictors of the hs-CRP in Turkish adults (647).

2.5.4.2 Adipokines

The adipokines or adipocytokines (Greek adipo-, fat; cyto-, cell; and -kinos, movement) are cytokines (cell-to-cell signaling proteins) secreted by adipose tissue. Adiponectin was first characterised in 2007 in mice as a transcript overexpressed in preadipocytes (648) differentiating into adipocytes (648,649). Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism (650). Adiponectin is secreted from adipose tissue (651) into the bloodstream and is very abundant in plasma relative to many hormones. Levels of the hormone are inversely correlated with body fat percentage in adults (652), while the association in infants and young children is less clear. The hormone plays a role in the suppression of the metabolic derangements that may result in type 2 DM (652), obesity, atherosclerosis (650), non-alcoholic fatty liver disease and an independent risk factor for metabolic syndrome (653). Plasma concentrations reveal a sexual dimorphism, with
females having higher levels than males. Levels of adiponectin are reduced in diabetics compared to non-diabetics. Weight reduction significantly increases circulating levels (654). Adiponectin automatically self-associates into larger structures. Initially, three adiponectin molecules bind together to form a homotrimer, then trimers continue to self-associate and form hexamers or dodecamers. Like the plasma concentration, the relative levels of the higher-order structures are sexually dimorphic, where females have increased proportions of the high-molecular weight forms. Recent studies showed that the high-molecular weight form may be the most biologically active form regarding glucose homeostasis (655). High-molecular-weight adiponectin was further found to be associated with a lower risk of diabetes with similar magnitude of association as total adiponectin (656).

Shand BI et al (2003) investigated the relationship between plasma adiponectin and a range of anthropometric, glycaemic, lipid and inflammatory parameters in overweight and obese subjects expressing characteristics of the metabolic syndrome. Plasma adiponectin concentration was higher in females than males (median 10.3 vs. 7.1 µg/ml, p < 0.001) despite being matched for BMI. In both genders, adiponectin levels were inversely related to BMI, waist circumference, percentage body fat, insulin resistance and the fasting plasma concentration of leptin. A direct correlation in both sexes was found between adiponectin levels and high-density lipoprotein (HDL)-cholesterol, apolipoprotein A1 and age. An association between reduced adiponectin and increased high-sensitivity plasma CRP concentration was observed only in female subjects and was independent of anthropometric variables. It was observed that adiponectin levels increase with age differs from the majority of other studies and may simply reflect the demographics of the population studied. The study showed that adiponectin is an important molecular link between obesity, insulin resistance and atherogenic lipoproteins (657).

Patel DA et al (2006) examined in a biracial (black-white) community-based sample of 1153 individuals (mean age, 36.2 years; 70% white, 43% male) who participated in the Bogalusa Heart Study. Adiponectin levels were inversely associated with insulin resistance which was more pronounced at higher level of visceral adiposity. Furthermore, adiponectin levels decreased with increasing number of metabolic syndrome risk factors. Moreover, adiponectin levels were low among those with positive parental histories of CHD, HTN,
and type 2 DM, considered as surrogate measures of risk. These findings, by showing an inverse association of adiponectin with insulin resistance, visceral adiposity, and related metabolic syndrome, and also with positive parental histories of CHD, HTN, and type 2 DM, underscore the value of adiponectin in CV and type 2 DM risk assessments in young adults (658).

Otsuka F et al (2007) investigated whether plasma adiponectin levels correlate with IGT and CAD in non-diabetic men. Glucose intolerance was evaluated by an oral glucose tolerance test and plasma adiponectin levels were measured in 232 non-diabetic men who underwent coronary angiography. Patients with IGT had significantly lower adiponectin levels than those with normal glucose tolerance (4.47 vs 5.85 µg/ml, p=0.003). Multiple logistic regression analysis demonstrated adiponectin independently correlated with the presence of CAD. Hypoadiponectinemia was associated with IGT and CAD in non-diabetic men, suggesting that the adiponectin level can provide valuable information regarding the risk of CAD even in non-diabetic subjects (659).

Bahceci M et al (2007) evaluated adipocyte volume and its relationship with TNF-α, IL-6, adiponectin and hsCRP levels. Patients were divided into 4 groups; lean healthy controls [BMI: 24.2±1.4 kg/m2], non-diabetic obese patients (30.2±2.9), obese (30.1±3.2) and non-obese (22.2±1.5) type 2 diabetic patients. Mean TNF-α, hs-CRP and IL-6 levels were higher in obese diabetic patients than in control subjects, obese non-diabetic and non-obese diabetic patients. Mean IL-6 levels of diabetic and non-diabetic obese patients were higher than control subjects. Mean adiponectin levels of control subjects were higher than non-diabetic obese, non-obese diabetic and obese-diabetic subjects. Mean adiponectin levels of obese diabetic patients were lower than non-diabetic obese subjects. It was concluded that mean hs-CRP levels were higher in diabetic patients whether they were obese or not (660).

You T et al (2008) examined the relationship of metabolic syndrome to several adipokines and the role of total and visceral adiposity in influencing this relationship in older adults. A cross-sectional analysis was conducted including 1914 individuals aged 70-79 years without cardiovascular disease or type 2 DM. Both the presence of metabolic syndrome and the number of metabolic syndrome components were associated with higher levels of leptin, PAI-1, IL-6, TNF-α, and CRP and with lower levels of adiponectin. Levels
of leptin, PAI-1, IL-6, and TNF-α were higher, and adiponectin was lower, in persons with, compared to those without, metabolic syndrome within each tertile of percent body fat. The metabolic syndrome was associated with adipokines in older adults across a wide range of adiposity, including in those with low or normal overall fatness (388).

Kappelle PJ et al (2012) examined in a community-based prospective case-control study, in 103 non-diabetic men who developed a first cardiovascular event (cases) and 106 male control subjects with a median follow-up of 3.0 and 10.5 years, respectively. It was found that the difference in adiponectin was not significant (p=0.10). Age-adjusted incident CVD was associated with plasma adiponectin. The relationships of incident CVD with plasma adiponectin (p=0.073) lost statistical significance after additional adjustment for smoking, waist circumference, HTN, microalbuminuria, the TC/HDL-C ratio, hs-CRP and HOMA-IR (661).

Garg MK et al (2012) studied the adipokines - adiponectin and plasminogen activator inhibitor-1 in subjects with metabolic syndrome. Subjects with metabolic syndrome had lower adiponectin (4.01 ± 2.24 vs. 8.7 ± 1.77μg/ml, P < 0.0001) and higher PAI-1 (53.85 ± 16.45 vs. 17.35 ± 4.45 ng/ml, P < 0.0001) levels than controls. Both were related with the number of metabolic abnormalities. Adiponectin was negatively and PAI-1 was positively associated with BMI, WHR, body fat mass, percent body fat, and all the parameters of metabolic syndrome, except HDL where the pattern reversed. WHR and TGs were independent predictors of adipokines in multiple regression analysis (662).

2.5.5 Dietary Factors

In the past two decades, understanding of the nutrients and foods, likely to promote cardiac health has grown substantially, because of the studies showing association between metabolic effects of various nutrients and foods and molecular mechanisms of atherosclerosis. Dietary patterns that give emphasis to whole grain foods, legumes, vegetables, fruits and that limit red meat, full-fat dairy products, food and beverages high in added sugars are associated with decreased risk of a variety of chronic disease (663). Adherence to healthy lifestyle practices, including healthy diets, has been found to be associated with 83% reduction in the rate of coronary disease, 91% reduction in diabetes in women, and 71% reduction in colon cancer in men (664). Despite this growing evidence, ‘Western’ diets have been practiced unfavourably. Adults and youth consumes vegetable
and fruit below recommended levels, only 24.5% of adults and 21.4% of youth consume at least five servings each day, whereas consumption of refined grains and food high in added sugars are rising. In developed countries, Low consumption of fruit and vegetables along with physical inactivity, are now among the top 10 causes of mortality (665).

In most patients, overnutrition or inappropriate diet are thought to lead to chronically elevated glucose, insulin, and free fatty acid (FFA) concentrations in the blood. Both excessive carbohydrate and fat intake could play a role in increasing blood glucose, FFA, and insulin concentrations, independently or together. These dietary conditions contribute to resistance to insulin-stimulated glucose uptake at the level of the adipose tissue and skeletal muscle as well as resistance to the insulin-mediated suppression of TG hydrolysis in adipose tissue (666,667). Increased glucose concentrations in the blood lead to increased glucose uptake by the liver, which is not insulin dependent. Insulin-mediated stimulation of de novo lipogenesis leads to an increased conversion of glucose to fatty acids (668). The increased concentrations of both glucose and FFA in the blood contribute to excessive accumulation of neutral lipids in the liver (669).

Macronutrient intake may produce oxidative stress and inflammatory responses. Glucose ingestion in normal subjects is associated with increased superoxide generation in leukocytes and mononuclear cells, and raised amount and activity of nuclear factor-kB (NF-kB), a transcriptional factor regulating the activity of at least 125 genes, most of which are pro-inflammatory (670). Glucose ingestion increases transcription of two pro-inflammatory transcription factors, activating protein-1 (AP-1) and Egr. The AP-1 regulates the transcription of matrix metalloproteinases and the Egr modulates the transcription of tissue factor and plasminogen activator inhibitor-1. A mixed meal from a fast-food chain has also been shown to induce activation of NF-kB associated with the generation of ROS (ROS) by mononuclear cells. There is an increased generation of ROS and raised circulating levels of inflammatory cytokines, such as TNF-α, IL-6, and interleukin 18 (IL-18) after oral or intravenous glucose challenges, in both normal subjects and patients with type 2 DM (671-673). A single high-fat meal in normal subjects produces endothelial activation, evidenced by increased concentrations of the adhesion molecules VCAM-1, ICAM-1, and IL-6 and TNF-α (674). Moreover, the same high-fat meal (675) may increase
the circulating levels of IL-18, a pro-inflammatory cytokine supposed to be involved in plaque destabilization.

Oxidative stress and inflammation may reduce insulin sensitivity. Both FFA and inflammatory markers have been shown to predict type 2 DM independent of known risk factors (676,677). Both FFA and TNF-α activates inhibitor K kinase b (IKKb) in adipocytes and hepatocytes, which can then increase the serine phosphorylation of insulin receptor substrate-1(IRS-1), with consequent reduction in insulin-dependent tyrosine phosphorylation of IRS-1, and ultimately glucose transport (678). IKKb is a serine kinase that controls the activation of NF-kB, a transcription factor associated with inflammation. IRS-1 may be directly phosphorylated by IKKb at serine residues, representing a novel class of substrates for IKKb (679). Similar result has been shown by a study (680) in which hepatic expression of the IkappaBalpha super-repressor, which reduces IKKb activity, reversed the phenotype of wild-type mice fed a high-fat diet, indicating that lipid accumulation in the liver leads to subacute hepatic ‘inflammation’ through NF-kB activation and downstream cytokine production. This causes insulin resistance both locally in liver and systemically. IL-6 circulates in plasma at high concentrations and is associated with insulin resistance in men and in obese or hyperandrogenic women (681). Circulating IL-6 levels and insulin sensitivity relationships seem to occur in parallel to increases in plasma FFA. In contrast to IL-6 and TNF-α, adiponectin mRNA is reduced in adipose tissue from patients with type 2 diabetes (682). Although the precise mechanism by which a low adiponectin production contributes to insulin resistance has not been fully elucidated, there is evidence that adiponectin decreases circulating FFA levels by increasing fatty acid oxidation by skeletal muscle (683). It is also associated with insulin sensitizing, anti-inflammatory, and antiatherogenic properties (682,684).

Marshall JA et al (1997) determined associations of dietary factors with fasting serum insulin concentrations. In longitudinal data analysis, lower age, female gender, Hispanic ethnicity, higher BMI, higher waist circumference, and no vigorous activity were significantly related to higher fasting insulin concentrations. High total and saturated fat intake were associated with higher fasting insulin concentrations after adjusting for age, sex, ethnicity, BMI, waist circumference, total energy intake and physical activity. Dietary fiber and starch intake were inversely associated with fasting insulin concentrations. There
was no association between fasting insulin concentrations and intake of monounsaturated fat, polyunsaturated fat, sucrose, glucose and fructose. Associations were similar in men and women and for active and inactive subjects, though associations of fiber and starch intake with insulin concentrations were strongest in lean subjects (685).

Steffen LM et al (2005) evaluated associations of dietary intake with the 15-year incidence of HTN. Plant food intake (whole grains, refined grains, fruit, vegetables, nuts, or legumes) was inversely related to HTN after adjustment for age, sex, race, center, energy intake, cardiovascular disease risk factors, and other potential confounding factors. Dairy intake was not related to HTN and positive relations for HTN were observed across increasing quintiles of meat intake. In subgroup analyses, risk of HTN was positively associated with red and processed meat intake, whereas it was inversely associated with intakes of whole grain, fruit, nuts, and milk. These findings were consistent with a beneficial effect of plant food intake and an adverse effect of meat intake on BP (686).

Millen BE et al (2006) examined the relation between dietary quality and incident Metabolic syndrome in adult women and identified foci for preventive nutrition interventions. This was a prospective study of 300 healthy women (aged 30-69 y) in the Framingham Offspring-Spouse study who were free of metabolic syndrome risk factors at baseline. Women with higher nutritional risk profiles consumed more dietary lipids (total, saturated, and monounsaturated fats) and alcohol and less fiber and micronutrients; they had higher cigarette use and waist circumferences. Compared with women with the lowest nutritional risk, those in the highest tertile had a 2- to 3-fold risk of the development of abdominal obesity and overall Metabolic syndrome during 12 y of follow-up (687).

2.5.5.1 Carbohydrates

Carbohydrates are class of organic that contains either an aldehyde moiety (polyhydroxyaldehydes) or a ketone moiety (polyhydroxyketones). Monosaccharides (simple sugars) can be classified according to the number of carbon atoms they contain. Carbohydrates with an aldehyde as their most oxidized functional group are called aldoses, whereas those with a keto group as their most oxidized functional group are called ketoses. Monosaccharides can be linked by glycosidic bonds to create larger structures. Disaccharides contain two monosaccharide units; oligosaccharides contain from three to about ten monosaccharide units, whereas polysaccharides contain more than ten
monosaccharide units, and can be hundreds of sugar units in length. The presence of the hydroxyl groups allow carbohydrates to interact with the aqueous environment and to participate in hydrogen bonding, both within and between chains. Derivatives of the carbohydrates can contain nitrogens, phosphates and sulfur compounds. Carbohydrates also can combine with lipid to form glycolipids or with protein to form glycoproteins (688).

Early studies in humans had shown that alteration in relative amount of carbohydrate and fat leads to change in the effect on insulin action. In 1935, Himsworth found that as dietary carbohydrate increased, the ability of insulin to lower blood glucose was improved. That is a low fat, high carbohydrate diet was associated with an improvement in insulin’s ability to stimulate glucose disposal. This general finding has been seen in animal studies and more recent human studies as summarized in the excellent review by Daly et al (689). Swinburn et al compared the effects of a high carbohydrate diet with a lower carbohydrate diet in Pima Indians by using the euglycemic, hyperinsulinemic clamp method (689). In this study, fasting insulin and glucose levels were improved despite no change in insulin action with change in dietary carbohydrate Contrary to this, other studies revealed no relation between dietary carbohydrate, insulin action and onset of diabetes. However, in the San Luis Valley study, no relationship was found between dietary carbohydrate and hyperinsulinemia or the onset of frank diabetes (690). Three other studies, the Health Professionals Follow-Up Study (691), the Nurses Health Study (692) and the Iowa Women’s Health Study (693), examined the relationship between diet composition and the onset of diabetes in large populations of men and women. These studies also failed to show a relationship between total carbohydrate intake and development of diabetes. Most recently, Swinburn et al (694) demonstrated in a prospective study that a low fat (26% of energy), high carbohydrate (54% of energy) diet was associated with improved glucose tolerance and reduced progression to diabetes in individuals with impaired glucose tolerance. These data supports the idea that high carbohydrate diets do not adversely affect insulin sensitivity and may be beneficial for insulin sensitivity. On the contrary, high intakes of dietary fat, particularly saturated fat, do appear in some of these studies to be associated with a decline in insulin sensitivity (695).

An inverse association has been demonstrated between serum HDL and diet glyemic index in British men and women, low diet glyemic index was associated with
increased HDL cholesterol. Since low HDL cholesterol is a feature of the metabolic syndrome, it was suggested that the relationship between glyemic index and HDL was due to the effect of a low-glyemic index diet in improving insulin sensitivity. Fasting HDL cholesterol was significantly lower after the high-carbohydrate/high-GI diet than after either of the other two diets (696) after one month. Willett et al has shown that in both men and women, a high glycaemic load is associated with increased risk of developing diabetes (691,692), by altering sensitivity, and plasma FFA concentrations. Elevated serum FFA concentrations are associated with diabetes and insulin resistance (697-699). Free fatty acids raise plasma glucose by reducing insulin-stimulated glucose uptake, increasing hepatic glucose output, and reducing glucose-induced insulin secretion (700,701). Free fatty acids in plasma are derived from adipose tissue, when the supply of carbohydrate as a fuel is exhausted (e.g. after an overnight fast), and release from chylomicrons under the influence of lipoprotein lipase. FFA are elevated under fasting conditions, indicating that adipose tissue fatty acids are being released and used as fuel by muscle.

In normal subjects after an oral glucose load the rise in plasma insulin rapidly suppresses plasma FFA. However, the high rise in insulin causes the blood glucose to undershoot, which is followed by a rebound in plasma FFA (702). In another study, consistent with the older literature, there was a marked and significant improvement in oral glucose tolerance and a significant reduction in fasting plasma glucose on the high- versus the low-carbohydrate diet. Although this was not accompanied by any significant change in insulin sensitivity, there were significant improvements in glucose effectiveness (the ability of glucose to stimulate its own removal) and in pancreatic responsiveness (plasma insulin response after intravenous glucose injection). The metabolic syndrome is related to defects in both insulin sensitivity and b-cell function. It suggested that dietary carbohydrate may have more important effects on pancreatic function, or on a combination of factors, than insulin sensitivity alone (702,703).

Liu S et al (1999) evaluated whether high whole-grain intake reduces risk of CHD in women. During 729472 person-years of follow-up, 761 cases developed CHD. After adjustment for age and smoking, increased whole-grain intake was associated with decreased risk of CHD. The inverse relation between whole-grain intake and CHD risk was even stronger in the subgroup of never smokers. The lower risk associated with higher
whole-grain intake was not fully explained by its contribution to intakes of dietary fiber, folate, vitamin B-6, and vitamin E. Increased intake of whole grains may protect against CHD (704).

Meyer KA et al (2000) examined the relations of baseline intake of carbohydrates, dietary fiber, dietary magnesium, and carbohydrate-rich foods and the glycemic index with incidence of diabetes. This was a prospective cohort study of 35988 older Iowa women initially free of diabetes. During 6 years of follow-up, 1141 incident cases of diabetes were reported. Total grain, whole-grain, total dietary fiber, cereal fiber, and dietary magnesium intakes showed strong inverse associations with incidence of diabetes after adjustment for potential nondietary confounding variables. Intakes of total carbohydrates, refined grains, fruit and vegetables, and soluble fiber and the glycemic index were unrelated to diabetes risk. These data support a protective role for grains (particularly whole grains), cereal fiber, and dietary magnesium in the development of diabetes in older women (693).

Marques-Lopes I et al (2001) assessed the postprandial metabolic changes and the fractional hepatic de novo lipogenesis induced by a high-carbohydrate, low-fat meal in lean and overweight young men. After intake of the high-carbohydrate meal, the overweight men had hyperinsulinemia and higher fatty acid and triacylglycerol concentrations than the lean men. The overweight group showed greater energy expenditure, whereas there was no significant difference in carbohydrate oxidation between the groups. De novo lipogenesis was significantly higher before and after meal intake in the overweight men and was positively associated with fasting serum glucose and insulin concentrations. Furthermore, postprandial de novo lipogenesis was positively correlated with body fat mass, energy expenditure, and triacylglycerol (705).

Hu EA et al (2012) summarised evidence on the association between white rice consumption and risk of type 2 diabetes and to quantify the potential dose-response relation. Meta-analysis of prospective cohort studies was done. Studies included were prospective cohort studies that reported risk estimates for type 2 diabetes by rice intake levels. During follow up of 4-22 years, 13,284 subjects developed type 2 diabetes among 352,384 participants. Asian (Chinese and Japanese) populations had much higher white rice consumption than did Western populations. The pooled relative risk was 1.55 (95% confidence interval 1.20 to 2.01) comparing the highest with the lowest category of white
rice intake in Asian populations, whereas the corresponding relative risk was 1.12 (0.94 to 1.33) in Western populations (P for interaction=0.038). In the total population, the dose-response meta-analysis indicated that for each serving per day increment of white rice intake, the relative risk of type 2 diabetes was 1.11 (1.08 to 1.14) (P for linear trend<0.001). Higher consumption of white rice was associated with a significantly increased risk of type 2 diabetes, especially in Asian (Chinese and Japanese) populations (706).

Goff LM et al (2012) reviewed randomised controlled trials (RCTs) of low glyemic index diets on blood lipids. Random effects meta-analyses were performed on twenty-eight RCTs comparing low with high GI diets. Low glyemic index diets significantly reduced total and LDL-cholesterol compared with high glyemic index diets and independently of weight loss. This meta-analysis provided consistent evidence that low GI diets reduce total and LDL-cholesterol and have no effect on HDL-cholesterol or TGs (707).

2.5.5.2 Protein

Proteins consist of amino acids which consists of amine (\(-\text{NH}_2\)) and carboxylic acid (\(-\text{COOH}\)) functional groups, along with a side-chain specific to each amino acid. Amino acids are the basic building blocks of enzymes, hormones, proteins, and body tissues. In proteins, amino acids are joined covalently by peptide bonds, which are amide linkages between the α-carboxyl group of one amino acid and the α-amino group of another. Polypeptides are chains of amino acids. Proteins are made up of one or more polypeptide molecules. One end of every polypeptide, called the amino terminal or N-terminal, has a free amino group. The other end, with its free carboxyl group, is called the carboxyl terminal or C-terminal.

Proteins are complex, organic compounds composed of many amino acids linked together through peptide bonds and cross-linked between chains by sulfhydryl bonds, hydrogen bonds and van der Waals forces. There is a greater diversity of chemical composition in proteins than in any other group of biologically active compounds. The proteins in the various animal and plant cells confer on these tissues their biological specificity.

Proteins can be classified as: Simple proteins conjugated protein or derived proteins. Simple proteins on hydrolysis yield only the amino acids and occasional small carbohydrate compounds e.g. albumin, globulin, glutelin, albuminoid, histones and
protamine. Conjugated proteins are simple proteins combined with some non-protein material in the body eg nucleoproteins, glycoproteins, phosphoproteins, haemoglobins and lecithoproteins. Derived proteins are proteins derived from simple or conjugated proteins by physical or chemical means eg denatured proteins and peptides.

The potential configuration of protein molecules is so complex that many types of protein molecules can be constructed and are found in biological materials with different physical characteristics. Globular proteins are found in blood and tissue fluids in amorphous globular form with very thin or non-existent membranes. Collagenous proteins are found in connective tissue such as skin or cell membranes. Fibrous proteins are found in hair, muscle and connective tissue. Crystalline proteins are exemplified by the lens of the eye and similar tissues. Enzymes are proteins with specific chemical functions and mediate most of the physiological processes of life. Several small polypeptides act as hormones in tissue systems controlling different chemical or physiological processes. Muscle protein is made of several forms of polypeptides that allow muscular contraction and relaxation for physical movement.

Proteins can also be characterized by their chemical reactions. Most proteins are soluble in water, in alcohol, in dilute base or in various concentrations of salt solutions. Proteins have the characteristic coiled structure which is determined by the sequence of amino acids in the primary polypeptide chain and the stereo configuration of the radical groups attached to the alpha carbon of each amino acid. Proteins are heat labile exhibiting various degrees of lability depending upon type of protein, solution and temperature profile. Proteins undergo characteristic bonding with other proteins in the so-called plastein reaction and will combine with free aldehyde and hydroxy groups of carbohydrates to form Maillard type compounds.

Most proteins consist of linear polymers built from series of up to 20 different L-α-amino acids. L-amino acids and their derivatives participate in cellular functions as diverse as nerve transmission and the biosynthesis of porphyrins, purines, pyrimidines, and urea. Peptides perform prominent roles in the neuroendocrine system as hormones, hormone-releasing factors, neuromodulators, or neurotransmitters. Humans lack the capability to synthesize 9 of the 20 common L-amino acids in amounts adequate to support growth or to
maintain health in adults. These are called essential amino acids and the human diet must contain adequate quantities of these amino acids (708,709).

Pasini E et al (2004) investigated the anti-ischemic effects of immediate and long-term supplementation of an amino acid mixture on isolated rats hearts subjected to global ischemia for 30 minutes. Long-term treatment with an amino acid mixture lead to reduction in the diastolic pressure maintained the tissue content of adenosine triphosphate during ischemia; and improved the recovery of pressure at the end of post ischemic reperfusion, reduced the release of creatine kinase and lactate. It was concluded that long-term supplementation of an amino acid mixture reduced myocardial ischemic damage 710).

Scarabelli TM et al (2004) determined whether oral supplementation with mixed amino acids may provide cardioprotection to the rat heart exposed to ischemia-reperfusion through preservation of the energy-producing properties of mitochondria. Sprague-Dawley rats were fed (by enteral route) a liquid diet, with or without mixed essential amino acids for 30 days. Amino acid supplements minimized infarct size and occurrence of cardiomyocyte apoptosis, as assessed by co-localization of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and caspase-3-positive staining. Long-term treatment with amino acids also reduced the proportion of cardiomyocytes exhibiting immunostaining for cleaved caspase-9. The oxygen consumption rate in myocardial skinned bundles was markedly reduced in ischemia-reperfusion control hearts and almost normalized in amino acid-treated hearts. They suggested that oral amino acid supplementation attenuates the extent of ischemia-reperfusion injury in the rat heart, through preservation of the mitochondria-generated production of high-energy phosphates (711).

Scognamiglio R et al (2004) assessed the effects of an oral amino acids mixture (AAM) on myocardial function in patients with type 2 DM which included 65 consecutive patients who had normal resting left ventricular ejection fraction and did not have obstructive CAD. Myocardial dysfunction was easily inducible with isometric exercise in patients with DM who had normal resting LV function and did not have CAD. An increased amino acid supply prevented this phenomenon (712).

Song Y et al (2004) assessed the relation between red meat intake and incidence of type 2 diabetes. Over an average of 8.8 years, evaluated 37,309 participants in the Women’s
Health Study aged ≥ 45 years who were free of cardiovascular disease, cancer, and type 2 diabetes. During 326,876 person-years of follow-up, 1,558 incident cases of type 2 DM were documented. After adjusting for age, BMI, total energy intake, exercise, alcohol intake, cigarette smoking, and family history of diabetes, positive associations between intakes of red meat and processed meat and risk of type 2 DM were found. Comparing women in the highest quintile with those in the lowest quintile, the multivariate-adjusted relative risks (RRs) of type 2 diabetes were 1.28 for red meat (95% CI 1.07-1.53, P < 0.001 for trend) and 1.23 for processed meat intake (1.05-1.45, P = 0.001 for trend). Intakes of TC, animal protein, and heme iron were also significantly associated with a higher risk of type 2 diabetes (713).

Dumontier O et al (2007) evaluated the specific effects of both diets (low protein and low energy) during different periods of gestation and the mechanisms underlying the decreased beta cell mass. Pregnant Wistar rats were fed either a low-protein or a low-energy diet during the last week of gestation or throughout gestation. Fetuses and their pancreases were analysed at days 15 and 21 of gestation. Although both diets reduced the fetal beta cell mass, the cellular mechanisms and the sensitivity windows were different. Early alteration of neogenesis due to elevated corticosterone levels is likely to be responsible for the decreased beta cell mass in low-energy fetuses, whereas impaired beta cell proliferation and islet vascularisation at later stages are implicated in low-protein fetuses (714).

Bernstein AM et al (2010) examined the relation between foods that are major dietary protein sources and incident CHD. This study prospectively followed 84,136 women aged 30 to 55 years in the Nurses’ Health Study. In multivariate analysis including age, smoking, and other risk factors, higher intakes of red meat (excluding processed meat), and high-fat dairy were significantly associated with elevated risk of CHD. Higher intakes of poultry, fish, and nuts were significantly associated with lower risk. These data suggested that high red meat intake increases risk of CHD and that CHD risk may be reduced importantly by shifting sources of protein in the US diet (715).

de Koning L et al (2011) compared the associations of 3 low-carbohydrate diet scores with incident type 2 diabetes in a cohort study in participants from the Health Professionals Follow-Up Study who were free of type 2 diabetes, cardiovascular disease, or
cancer at baseline (n = 40,475) and followed up to 20 years. Type 2 diabetes developed in 2689 cases during follow-up. After adjustments for age, smoking, physical activity, coffee intake, alcohol intake, family history of type 2 diabetes, total energy intake, and BMI, the score for high animal protein and fat was associated with an increased risk of type 2 diabetes. A high score for vegetable protein and fat was not significantly associated with the risk of type 2 diabetes overall but was inversely associated with type 2 diabetes in men aged <65 years (716).

Teunissen-Beekman KF et al (2012) determined whether 4 wk of increased protein intake (~25% compared with ~15% of energy intake that isoenergetically replaces carbohydrate intake) lowers office and daytime BP compared with increased carbohydrate intake in a randomized, double-blind, parallel study compared consumption of 3 × 20 g protein/d with 3 × 20 g maltodextrin/d. Office SBP and DBP were 4.9 ± 1.7 mm Hg (P = 0.005) and 2.7 ± 1.3 mm Hg (P = 0.05) lower, respectively, in the protein group. Daytime SBP was 4.6 ± 1.7 mm Hg lower in the protein group (P = 0.006), whereas daytime DBP did not differ between groups (P = 0.37). Urinary sodium excretion was higher in the maltodextrin group (P = 0.004) (717).

Lagiou P et al (2012) studied the long term consequences of low carbohydrate diets, generally characterised by concomitant increases in protein intake, on cardiovascular health. From a random population sample, 43,396 Swedish women, aged 30-49 years at baseline, completed an extensive dietary questionnaire and were followed-up for an average of 15.7 years. A one tenth decrease in carbohydrate intake or increase in protein intake or a 2 unit increase in the low carbohydrate-high protein score were significantly associated with increasing incidence of cardiovascular disease. Low carbohydrate-high protein diets, used on a regular basis and without consideration of the nature of carbohydrates or the source of proteins, are associated with increased risk of cardiovascular disease (718).

2.5.5.3 Fat

Fats consist of a wide group of compounds that are generally soluble in organic solvents and generally insoluble in water. Chemically, fats are TGs, triesters of glycerol and any of several fatty acids. Fats may be either solid or liquid at room temperature, depending on their structure and composition. Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules that can be extracted from tissues by nonpolar solvents.
Because of their insolubility in aqueous solutions, body lipids are generally found compartmentalized, as in the case of membrane-associated lipids or droplets of triacylglycerol in adipocytes, or transported in plasma in association with protein, as in lipoprotein particles, or on albumin. Lipids are a major source of energy for the body, and they also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and subcellular structures. The lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. Lipids are classified as simple lipids (Esters of fatty acids with various alcohols e.g. fats, oils, waxes), complex lipids (Esters of fatty acids containing groups in addition to an alcohol and a fatty acid e.g. phospholipids, glycolipids, and other complex lipids), and derived lipids (These include fatty acids, glycerol, steroids, other alcohols, fatty aldehydes, ketone bodies, hydrocarbons, lipid-soluble vitamins, and hormones).

Fats can be categorized into saturated fats and unsaturated fats. Unsaturated fats can be further divided into cis fats, which are the most common in nature, and trans-fats, which are rare in nature but present in partially hydrogenated vegetable oils. Fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Fatty acids are usually derived from TGs or phospholipids. Fatty acids that have double bonds are known as unsaturated. Fatty acids without double bonds are known as saturated. They differ in length as well. Fatty acid chains differ by length; Short-chain fatty acids are fatty acids with aliphatic tails of fewer than six carbons (i.e. butyric acid), Medium-chain fatty acids are fatty acids with aliphatic tails of 6–12 carbons, which can form medium-chain TGs, Long-chain fatty acids are fatty acids with aliphatic tails 13 to 21 carbons. Very long chain fatty acids are fatty acids with aliphatic tails longer than 22 carbons.

**Saturated Fatty acids**

Saturated fat is fat that consists of TGs containing only saturated fatty acids. *Saturated* fatty acids have no double bonds between the individual carbon atoms of the fatty acid chain. That is, the chain of carbon atoms is fully "saturated" with hydrogen atoms. There are many kinds of naturally occurring saturated fatty acids, which differ mainly in number of carbon atoms, from 3 carbons (propionic acid) to 36 (hexatriacontanoic acid) (719).
Various fats contain different proportions of saturated and unsaturated fat. Examples of foods containing a high proportion of saturated fat include animal fats such as cream, cheese, butter, and ghee; suet, tallow, lard, and fatty meats; as well as certain vegetable products such as coconut oil, cottonseed oil, palm kernel oil, chocolate, and many prepared foods.

In humans, saturated fat intake increases LDL cholesterol in comparison with all nutrients except trans fats (720). Because saturated fat also increases high-density lipoprotein (HDL) cholesterol, the TC to HDL cholesterol ratio (a risk marker for CVD) is not altered. Changes in dietary saturated fat have been associated with changes in concentrations of larger, more buoyant particles (721). In the context of a lower-carbohydrate diet (26% of total energy), high saturated fat content (15% of energy) provided from dairy products was associated with increased concentrations of large and medium LDL particles, but not small LDL particles, compared with a diet lower in saturated fat (8% of energy) (722).

**Monounsaturated fatty acid**

Monounsaturated fats or MUFAs (Monounsaturated Fatty Acid) are fatty acids that have one double bond in the fatty acid chain and all of the remainder of the carbon atoms in the chain is single-bonded. By contrast, polyunsaturated fatty acids have more than one double bond. Fatty acids are long-chained molecules having an alkyl group at one end and a carboxylic acid group at the other end. Fatty acid viscosity (thickness) and melting temperature increase with decreasing number of double bonds. Therefore, monounsaturated fatty acids have a higher melting point than polyunsaturated fatty acids (more double bonds) and a lower melting point than saturated fatty acids (no double bonds). Monounsaturated fatty acids are liquids at room temperature and semisolid or solid when refrigerated. And if are taken to vacuum, they are destroyed.

MUFAs are a class of fatty acids that are found in foods such as olive oil, nuts, and avocados. The beneficial effects of MUFAs on cardiovascular disease risk and blood lipid profiles have been extensively studied (723). In particular, dietary MUFAs decrease oxidized LDL (724), LDL cholesterol (725), TC, and triacylglycerol concentrations, without the concomitant decrease in HDL typically seen with low-fat diets (726-728). Additionally, the replacement of carbohydrate and saturated fat with MUFAs leads to
reductions in glucose and BP and to an increase in HDL in patients with diabetes (729). A MUFA-rich diet (40% of energy as fat) also decreased VLDL-cholesterol and VLDL triacylglycerol more and was more acceptable to patients with type 2 DM than was a high-carbohydrate diet (28% of energy as fat) (730,669).

**Polyunsaturated fatty acid**

Polyunsaturated fatty acids (PUFAs) are a class of fatty acids that include n-6 and n-3 fatty acids. The n-3 fatty acid, linolenic acid is a precursor for the long-chain products docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). PUFAs have been shown to decrease the risk of heart disease when consumed in lieu of SFAs in both epidemiologic (731,732) and clinical (733) studies. The ratio of n-6 to n-3 fatty acids seems to be important in determining the effect of PUFAs on various lipid and nonlipid indexes. Replacement of n-6 PUFAs with linolenic acid improved peripheral insulin sensitivity and lowered cholesterol concentrations in rats with fructose-induced insulin resistance (734). An approximate dietary intake of 6% n-6 and 1% n-3 fatty acids as percentage of energy has been recommended to maximize the cardiovascular benefits of these essential fatty acids (735).

Omega 3 fatty acids (also called ω-3 fatty acids or n-3 fatty acids) are fats commonly found in marine and plant oils. They are polyunsaturated fatty acids with a double bond (C=C) starting after the third carbon atom from the end of the carbon chain. The fatty acids have two ends—the acid (COOH) end and the methyl (CH3) end. The location of the first double bond is counted from the methyl end, which is also known as the omega (ω) end or the n end.

The health effects of n-3 fatty acids supplementation are controversial. They are considered essential fatty acids, meaning that they cannot be synthesized by the human body but are vital for normal metabolism. Though mammals cannot synthesize n-3 fatty acids, they have a limited ability to form the long-chain n-3 fatty acids including eicosapentaenoic acid (EPA, 20 carbons and 5 double bonds), docosahexaenoic acid (DHA, 22 carbons and 6 double bonds) and α-linolenic acid (ALA, 18 carbons and 3 double bonds).

Omega-3 fatty acids—such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—are found in fish oils. They stimulate the production of NO, which relaxes
vascular smooth muscle. Their actions can counteract the impairment of NO production that is caused by atherosclerotic plaques. In addition, consumption of EPA stimulates the production of prostaglandin I3, an antithrombotic and anti-platelet-aggregating agent similar to prostacyclin. As an anticoagulant, omega-3 fatty acids can increase bleeding time, inhibit platelet adhesiveness, decrease platelet count, and reduce serum thromboxane levels. (736) Omega-3 fatty acids can also blunt the vasopressor effects of angiotensin II and norepinephrine and may reduce BP and the risk of arrhythmia. In one placebo-controlled trial, an average systolic reduction of 5 mm Hg and a mean diastolic decrease of 3 mm Hg was realized in those participants taking DHA. The TG-lowering effect of these fish oil components may be one of many factors that inhibit the progression of atherosclerosis. There are conflicting data from studies regarding the role of omega-3 fatty acids in the reduction of arterial restenosis after coronary angioplasty (737-741).

The effects of n-3 fatty acids on dyslipidemia and insulin resistance have been extensively reviewed (742). Specifically, DHA and EPA induce fatty acid catabolism through the activation of peroxisome-proliferator activated receptor (PPAR) mediated pathways (743) and down-regulate de novo lipogenesis through sterol regulatory element binding protein (SREBP) pathways (744). The beneficial effects of omega-3 fatty acid include decreased plasma triacylglycerol, FFAs, glucose, and insulin; prevention of peripheral insulin resistance; decreased triacylglycerol concentrations, VLDL secretion, and lipogenesis in the liver; decreased lipid concentrations and utilization and storage of glucose in skeletal muscle; and decreased adipocyte cell size and visceral fat content and increased insulin-stimulated glucose transport in the adipose tissue (669).

**Trans-Fatty acids**

Trans-fat is the common name for unsaturated fat with *trans*-isomer (E-isomer) fatty acid(s). Because the term refers to the configuration of a double carbon-carbon bond, trans-fats are sometimes monounsaturated or polyunsaturated, but never saturated. Trans fats do exist in nature but also occur during the processing of polyunsaturated fatty acids in food production.

The consumption of trans-fats increases the risk of CHD-by raising levels of LDL cholesterol and lowering levels of HDL cholesterol. Trans fatty acids occur naturally in foods such as dairy products as a result of bacterial metabolism and in foods such as
margarines as a result of hydrogenation. Trans-Fatty acids consist of multiple isomers that have differential effects on human metabolism. The bacterially derived cis-9, trans-11 conjugated linoleic acid and trans-11 oleic acid typically found in dairy products do not have adverse effects on lipoprotein profiles. Conversely, intake of trans-10, cis-12 conjugated linoleic acid from hydrogenated oils has been found to increase inflammatory markers (745), induce endothelial dysfunction (746), and unfavorably alter the blood lipid profile by increasing the LDL:HDL and TC:HDL ratios (747).

Nappo F et al (2002) compared the effect of a high-fat meal and a high-carbohydrate meal (pizza), with and without antioxidant vitamins, on endothelial activation in healthy subjects and in patients with type 2 DM. Study was done in a randomized, observer-blinded, crossover study in 20 newly diagnosed type 2 diabetic patients and 20 age- and gender-matched healthy subjects. In normal subjects, the high-fat meal increased the plasma levels of TNF-α, IL-6, ICAM-1 and VCAM-1, which were prevented by vitamins. No change in these parameters occurred after pizza ingestion or pizza ingestion with vitamins. In diabetic patients, basal concentrations of glucose, cytokines and adhesion molecules were significantly higher than in non diabetic controls. Both meals significantly increased cytokine and adhesion molecule levels, but the increase was more sustained following the high-fat meal. There was no significant change from baseline when vitamin supplementation accompanied each meal. An oxidative mechanism mediates endothelial activation induced by post-meal hyperlipidemia and hyperglycemia (674).

van Dam RM et al (2002) examined dietary fat and meat intake in relation to risk of type 2 diabetes and prospectively followed 42,504 male participants of the Health Professionals Follow-Up Study who were aged 40-75 years and free of diagnosed diabetes, cardiovascular disease, and cancer in 1986. Intakes of total fat were associated with a higher risk of type 2 diabetes. However, these associations disappeared after additional adjustment for BMI. Intakes of oleic acid, trans-fat, long-chain n-3 fat, and alpha-linolenic acid were not associated with diabetes risk after multivariate adjustment. Linoleic acid was associated with a lower risk of type 2 diabetes in men <65 years of age and in men with a BMI <25 kg/m² but not in older and obese men. Frequent consumption of processed meat was associated with a higher risk for type 2 diabetes. Total and saturated fat intake were
associated with a higher risk of type 2 diabetes, but these associations were not independent of BMI (748).

Lemaitre RN, et al (2003) investigated the association of plasma phospholipid concentrations of DHA, EPA, and alpha-linolenic acid as biomarkers of intake with the risk of incident fatal IHD and incident nonfatal myocardial infarction in older adults. A higher concentration of combined DHA and EPA was associated with a lower risk of fatal IHD, and a higher concentration of alpha-linolenic acid with a tendency to lower risk, after adjustment for risk. In contrast, n-3 polyunsaturated fatty acids were not associated with nonfatal myocardial infarction. Higher combined dietary intake of DHA and EPA, and possibly alpha-linolenic acid, may lower the risk of fatal IHD in older adults (749).

Neri S et al (2005) investigated within and between group changes in various circulating markers of oxidation-reduction balance and endothelial function after a balanced moderate-fat meal with and without antioxidant supplementation in patients with early-stage, untreated type 2 DM; subjects with IGT; and healthy controls. This study showed changes in oxidation-reduction balance, NO bioavailability, and nonthrombogenic endothelial factors after a moderate-fat meal in patients with type 2 diabetes and those with IGT, but these postprandial changes were reverse in all subjects after 15 days of standard antioxidant supplementation (750).

Aeberli I et al (2006) determined whether dietary fat or antioxidant intakes influence circulating TNF-α, IL-6, CRP, and leptin concentrations in overweight children. CRP, IL-6, and leptin increased significantly (P < 0.02) with increasing adiposity, independent of age; TNF-α did not increase. Total dietary fat and the percentage of energy from fat were significant predictors of CRP concentration, independent of BMI. Meat intake was a significant predictor of IL-6 and leptin, independent of BMI (P < 0.05). Intakes of antioxidant vitamins (vitamins E and C and beta-carotene) were significant predictors of leptin (P < 0.05) but not of CRP, IL-6, or TNF-α. Inflammatory markers are elevated in Overweight Swiss children as young as 6 years. Intakes of total fat and antioxidant vitamins are determinants of subclinical inflammation in this age group (751).

Sartika RA (2011) determined the effects of trans-fatty acid intake on blood lipid profile. A prospective cohort study was conducted on 388 workers at an on-shore oil company in East Kalimantan. The mean intake of trans-fatty acid was 0.48% of the total
dietary calories. The high consumption of fried foods is associated with risks of hypertriglyceridemia, high ratio of TC/ HDL-C and dyslipidemia. Every additional one percent of saturated fatty acid intake is associated with an increase in trans-fatty acids amounting to 0.03% of total calories (r = 0.320, p = 0.000). They suggested that a reduction in consumption of fried foods will be of benefit as it will reduce intake of both saturated and trans-fatty acids (752).

de Oliveira Otto MC et al (2012) investigated the association of SF consumption from different food sources and the incidence of CVD events in a multiethnic population. Participants who were 45-84 y old at baseline (n = 5209) were followed from 2000 to 2010. After adjustment for demographics, lifestyle, and dietary confounders, a higher intake of dairy SF was associated with lower CVD risk. After adjustment for demographics, lifestyle, and dietary confounders, a higher intake of dairy SF was associated with lower CVD risk. The substitution of 2% of energy from meat SF with energy from dairy SF was associated with a 25% lower CVD risk. No associations were observed between plant or butter SF and CVD risk, but ranges of intakes were narrow. Associations of saturated fat with health may depend on food-specific fatty acids or other nutrient constituents in foods that contain SF, in addition to SF (753).

Miglio C et al (2012) evaluated the inflammatory and antioxidant response of the body to the acute ingestion of a high-fat meal (HFM). Fifteen healthy overweight subjects were recruited for the study. After HFM consumption, plasma glucose, insulin, uric acid (UA), TGs, TC, thiols (SH), inflammatory cytokines (IL-6 and TNF-α) and dietary antioxidants were measured. The ingestion of HFM induced significant increases in both TG and TC. IL-6 and TNF-α significantly increased postprandially, whereas plasma concentrations of vitamins and carotenoids were not changed by HFM, SH and UA increased. This study indicated that as a consequence of an excess of dietary fat, the body responds through an inflammatory reaction, which is accompanied by an increment of endogenous antioxidant defenses, mediated by UA and SH, but not by vitamins C and E and carotenoids (754).

Young D et al (2012) examined changes in CRP and several CVD risk factors after one-year diet and physical activity interventions to assess whether CRP changed concurrently with other risk factors, or was independent of the traditional risk factors. Data
were analyzed from 143 men and 133 women with dyslipidemia who were randomized to one-year interventions of low-fat diet only, physical activity only, diet plus physical activity, or control. Baseline mean CRP levels were 1.3±1.3 (men) and 1.9±1.8 mg/L (women), with mean changes of -0.11±1.3 and -0.17±1.5 mg/L, respectively. Plasma CRP change was negatively associated with TG change in men and women, positively associated with change in systolic BP in men, but was not associated with changes in the other risk factors. Dietary and/or physical activity induced changes in CRP may be largely independent of traditional CVD risk factors in persons with dyslipidemia (645).

Nagata C et al (2012) examined the relationship between dietary fat intake and all-cause and cause-specific mortality in a Japanese community. A high intake of total fat and PUFA was associated with a decrease in all-cause mortality in men; the HR for the highest compared with the lowest quintile was 0.83 for total fat and 0.77 for PUFA. Both fats were associated with a decrease in mortality from cancer and diseases other than cardiovascular disease. In women, a higher SFA intake was associated with higher all-cause mortality. A favorable effect was suggested for total fat and PUFA intake on mortality in men except for that from cardiovascular disease, whereas increased SFA may be associated with adverse health consequences in women (755).

2.5.5.4 Fiber

Dietary fiber and whole grains contain a unique blend of bioactive components including resistant starches, vitamins, minerals, phytochemicals and antioxidants. Research regarding their potential health benefits has received considerable attention in the last several decades. Epidemiological and clinical studies demonstrate that intake of dietary fiber and whole grain is inversely related to obesity, type 2 diabetes, cancer and CVD (756).

Defining dietary fiber is a divergent process and is dependent on both nutrition and analytical concepts. The most common and accepted definition is based on nutritional physiology. Dietary fiber is defined as food material, particularly plant material, that is not hydrolysed by enzymes secreted by the human digestive tract but that may be digested by microflora in the gut. Plant components that fall within this definition include non-starch polysaccharides such as celluloses, hemi-celluloses, gums and pectins, as well as lignin, resistant dextrins and resistant starches. The types of plant material that are included within
the definitions of Dietary fiber (DF) may be divided into two forms, based on their water solubility. Insoluble dietary fiber which includes celluloses, hemicelluloses and lignin; and soluble dietary fiber which includes β-glucans, pectins, gums, and mucilages. In the simplest form, carbohydrates can be separated into two basic groups based upon their digestibility in the gastrointestinal tract. The first group (i.e., starch, simple sugars, and fructans) is easily hydrolyzed by enzymatic reactions and absorbed in the small intestine, are called non-structural carbohydrates, non-fibrous polysaccharides or simple carbohydrates. The second group which are resistant to digestion are named complex carbohydrates, non-starch polysaccharide or structural carbohydrates (757).

Dietary fiber can be separated into many different fractions. Recent research has begun to isolate these components and determine if increasing their levels in a diet is beneficial to human health. These fractions include arabinoxylan, inulin, pectin, bran, cellulose, β-glucan and resistant starch (758). The mechanisms behind the reported effects of dietary fiber on metabolic health are not well established. It is speculated to be a result of changes in intestinal viscosity, nutrient absorption, rate of passage, production of short chain fatty acids and production of gut hormones (757).

Liu S et al (2002) examined the hypothesis that higher intake of dietary fiber is inversely related to the risk of CVD and myocardial infarction in a large prospective cohort of women. During 230,006 person-years of follow-up, 570 incident cases of CVD were documented, including 177 MIs. After adjustment for age and randomized treatment status, a significant inverse association was observed between dietary fiber intake and CVD risk. Inverse relations were observed between both soluble and insoluble fiber and risk of CVD and MI, and among those who had never smoked and those with BMI <25. A higher intake of dietary fiber was associated with a lower risk of CVD and MI, although the association was not statistically significant after further adjusting for multiple confounding factors (759).

Mozaffarian D et al (2003) determined whether fiber consumption from fruit, vegetable, and cereal sources (including whole grains and bran) is associated with incident CVD in elderly persons. Population-based, multicenter study among 3588 men and women aged 65 years or older and free of known CVD at baseline was done in 1989-1990. During 8.6 years mean follow-up, there were 811 incident CVD events. After adjustment for age,
sex, education, diabetes, ever smoking, pack-years of smoking, daily physical activity, exercise intensity, alcohol intake, and fruit and vegetable fiber consumption, cereal fiber consumption was inversely associated with incident CVD, with 21% lower risk in the highest quintile of intake, compared with the lowest quintile. In similar analyses, neither fruit fiber intake nor vegetable fiber intake were associated with incident CVD. When CVD events were separately evaluated, higher cereal fiber intake was associated with lower risk of total stroke and ischemic stroke and a trend toward lower risk of IHD death. Cereal fiber consumption late in life is associated with lower risk of incident CVD, supporting recommendations for elderly individuals to increase consumption of dietary cereal fiber (760).

Schulze MB et al (2007) examined associations between fiber and magnesium intake and risk of type 2 diabetes and summarized existing prospective studies by meta-analysis. A prospective cohort study of 9702 men and 15365 women was conducted, aged 35 to 65 years who were observed for incident diabetes from 1994 to 2005. During 176117 person-years of follow-up, 844 incident cases of type 2 DM in the European Prospective Investigation into Cancer and Nutrition-Potsdam was observed. Higher cereal fiber intake was inversely associated with diabetes risk, while fruit fiber and vegetable fiber were not significantly associated. Magnesium intake was not related to diabetes risk. They suggested that higher cereal fiber and magnesium intakes may decrease diabetes risk (761).

Eshak ES et al (2010) examined the association between dietary fiber intake and mortality from CVD in a Japanese population in a prospective study of 58,730 Japanese men and women aged 40-79 yrs. Total, insoluble, and soluble dietary fiber intakes were inversely associated with risk of mortality from CHD and total CVD for both men and women. For fiber sources, intakes of fruit and cereal fibers but not vegetable fiber were inversely associated with risk of mortality from CHD. They concluded that dietary intakes of fiber, both insoluble and soluble fibers, and especially fruit and cereal fibers, may reduce risk of mortality from CHD (762).

Kokubo Y et al (2011) also investigated the association between dietary fiber and the risk of CVD, in a Japanese population. An inverse association of total fiber with CVD was observed primarily in non-smokers and not in smokers. Higher total dietary fiber was associated with reduced risk of CVD in Japanese non-smokers (763).
Parikh S et al (2012) studied associations of dietary fiber intake with inflammatory-related biomarkers and measures of total and central adiposity in a sample of 559 adolescents aged 14-18 years. Multiple linear regression analysis revealed that dietary fiber intake was inversely associated with fat mass and serum leptin in males but not in females. In both genders, dietary fiber intake was negatively associated with visceral adipose tissue, plasma CRP, and plasma fibrinogen and positively associated with plasma adiponectin. Their data suggested that greater consumption of dietary fiber is associated with lower visceral adiposity and multiple biomarkers implicated in inflammation (764).

Post RE et al (2012) determined if an increase in dietary fiber affects A1C and FBG in patients with type 2 DM in metanalysis of randomized studies published from January 1, 1980, to December 31, 2010, where dietary fiber intake was used as an intervention. They evaluated HbA1c and/or FBG as an outcome. This metanalysis revealed that an intervention involving fiber supplementation for type 2 DM can reduce FBG and A1C (765).

Ye EQ et al (2012) in a second metanalysis examined longitudinal studies investigating whole-grain and fiber intake in relation to risk of type 2 DM, CVD, weight gain, and metabolic risk factors. Forty five prospective cohort studies and 21 randomized-controlled trials between 1966 and February 2012 were identified. This meta-analysis also provided evidence to support beneficial effects of whole-grain intake on vascular disease prevention (766).

van de Laar RJ et al (2012) investigated whether a lower intake of fiber (and fiber-rich foods) throughout the course of young life (i.e., from adolescence to adulthood) is associated with arterial stiffness in adulthood. This was a longitudinal cohort study among 373 participants in whom dietary intake was assessed between the ages of 13 to 36 y (2-8 repeated measures, median of 5), and arterial stiffness estimates of 3 large arteries were ascertained at age 36 y. After adjustment for sex, height, total energy intake, and other lifestyle variables, subjects with stiffer carotid arteries consumed less fiber (in g/d) during the 24-y study than did those with less stiff carotid arteries. Furthermore, subjects with stiffer carotid arteries were characterized by a lower lifetime consumption of fruit, vegetables, and whole grains. Hence, deleterious associations could be explained to a great extent, by related low fiber intake. Lower lifetime intake of fiber during the course of young age is associated with carotid artery stiffness in adulthood. Promoting consumption
of fiber-rich foods among the young may offer a means to prevent accelerated arterial stiffening in adulthood and related cardiovascular sequelae (767).

Ericson U et al (2012) studied intakes of macronutrients, fiber and protein sources in relation to incident type 2 diabetes. In total, 27,140 individuals, aged 45-74 years, from the population-based Malmö Diet and Cancer cohort. During 12 years of follow-up, 1709 incident type 2 diabetes cases were identified. High protein intake was associated with increased risk of type 2 diabetes. When protein consumption increased by 5% of energy at the expense of carbohydrates increased diabetes risk was observed. Intake of fiber-rich bread and cereals was inversely associated with type 2 diabetes. They concluded that high protein intake is associated with increased risk of type 2 diabetes. Replacing protein with carbohydrates may be favourable, especially if fiber-rich breads and cereals are chosen as carbohydrate sources (768).

2.5.5.5 Micronutrients (Vitamins and Minerals)

Micronutrients are nutrients required by humans and other organisms throughout life in small quantities for many physiological functions, but which the organism itself cannot produce. For humans, they include dietary trace minerals in amounts generally less than 100 mg/day as opposed to macrominerals which are required in larger quantities. The microminerals or trace elements include at least iron, cobalt, chromium, iodine, manganese, selenium, zinc and molybdenum. Micronutrients also include vitamins, which are organic compounds required as nutrients in tiny amounts by an organism.

Rimm EB et al (1998) examined intakes of folate and vitamin B6 in relation to the incidence of nonfatal myocardial infarction and fatal CHD. Risk of CHD was reduced among women who regularly used multiple vitamins, the major source of folate and vitamin B6, and after excluding multiple vitamin users, among those with higher dietary intakes of folate and vitamin B6. In a subgroup analysis, compared with nondrinkers, the inverse association between a high-folate diet and CHD was strongest among women who consumed up to 1 alcoholic beverage per day or more than 1 drink per day. These results suggested that intake of folate and vitamin B6 above the current recommended dietary allowance may be important in the primary prevention of CHD among women (769).

Joffres MR et al (1987) investigated in 615 men of Japanese ancestry living in Hawaii who had no history of cardiovascular disease or treated HTN. Magnesium, calcium,
phosphorus, potassium, fiber, vegetable protein, starch, vitamin C, and vitamin D intakes were inversely associated with BP in univariate and multivariate analyses. Magnesium had the strongest association with BP. These results suggest that foods such as vegetables, fruits, whole grains, and low-fat dairy items are major sources of nutrients that may be protective against HTN (552).

Blom HJ (2000) found that elevated Hcy levels due to mutations can be normalised by administration of folate, but whether folate reduces the risk of cardiovascular disease remains to be established. It was concluded that heterozygosity for cystathionine beta-synthase deficiency is a minor cause of hyperhomocysteinaemia. The current data on mutations in the methylene-tetrahydrofolate reductase gene do not tell us whether elevated plasma Hcy plays a causal role in vascular disease. Low cellular vitamin status may be a possible cause and Hcy may just be a marker for this situation (770).

Folsom AR et al (2003) analyzed the cross-sectional relation of Hcy, plasma and dietary B vitamin levels with multiple markers implicated in inflammation, endothelial dysfunction, or thrombogenesis: CRP, fibrinogen, white blood cell count, ICAM-1, VCAM-1, E-selectin, factor VIII, and von Willebrand factor among 519 healthy middle aged adults in the ARIC Study. No marker was associated significantly with serum Hcy. Contrary to our hypothesis, plasma PLP was not associated with CRP concentration. In ostensibly healthy adults, B-vitamin status is not a strong correlate of circulating levels of inflammatory markers, cellular adhesion molecules, or thrombogenic factors (771).

Lee DH et al (2005) examined associations of CVD mortality with dietary intakes of iron (a possible pro-oxidant), zinc (a possible antioxidant), and alcohol (a disruptor of iron homeostasis) in Postmenopausal women (n = 34 492) aged 55-69 y. They were followed for CVD mortality over 15 yrs. Among women who consumed ≥10 g alcohol/d, after adjustment for CVD risk factors in a model that contained dietary heme iron, nonheme iron, and zinc intakes, dietary heme iron showed a positive association, dietary nonheme iron showed a U-shaped association, and dietary zinc showed an inverse association with CVD mortality. Results suggested that a higher intake of heme iron might be harmful, whereas a higher intake of zinc might be beneficial in relation to CVD mortality in the presence of a trigger that can disturb iron homeostasis, such as alcohol consumption (772).
Alissa EM et al (2005) investigated the dietary intake of vitamin A, C, and vitamin E, and carotenoids, serum concentrations of vitamin E and A and indices of lipid peroxidation were measured in male Saudi patients with established CVD and age-matched controls (130). Serum lipid profiles (TC, TGs, HDL-C, LDL-C), lipoprotein (a), oxidized LDL, and serum lipid peroxide concentrations, DM (P<0.0001), a positive smoking habit (P<0.0001) and HTN (P<0.05) were more prevalent among CVD patients. Levels of dietary vitamin E and A were also significantly higher among cases. Multivariate analysis showed that dietary total fat and vitamin A and the presence of DM were independent coronary risk factors in a non-Caucasian population (773).

Alissa EM et al (2006) measured serum and urine selenium, copper, and zinc; and superoxide dismutase, glutathione peroxidase, and lipid peroxide concentrations in 130 Saudi male subjects with established CVD, and 130 age-matched controls. DM, positive smoking habit (p<0.0001 for both), and HTN (p<0.05) were more prevalent among CVD patients. Urinary copper (p<0.0001) and zinc (p<0.05) were higher among controls. Serum selenium concentrations were lower among CVD patients (p<0.001), and a high proportion (52%) had selenium levels below 79µg/L compared to controls (22%). Measures of trace metals status appear to be associated with the risk of atherosclerosis in a Saudi male population (774).

Kazemi-Bajestani SM et al (2007) investigated the association between serum copper and zinc, and CAD in Iranian subjects undergoing coronary angiography in 114 patients (67 male and 47 female). Male patients had lower serum copper (p<0.05), lower serum zinc (p<0.05), and higher serum zinc/copper ratio (p<0.05) than females. Serum copper and zinc concentrations were significantly lower in the subjects with angiographically defined CAD than those patients with a normal angiogram, although the zinc/copper ratio was higher in these patients (p<0.001). Serum copper (r=-0.303, p<0.001) and zinc (r=-0.250, p<0.01) concentrations were both inversely related to age, and copper was positively associated with fasting serum TGs (r=0.188, p<0.05). Serum zinc and copper concentrations appear to be influenced by several physiological factors including age and gender (775).

Qi L et al (2007) assessed the associations of long-term intakes of dietary iron and red meat with CHD risk among 6,161 women who reported a diagnosis of type 2 diabetes.
After adjustment for age and BMI, high intakes of both heme iron and red meat were associated with a significantly increased risk of fatal, coronary revascularization, and total CHD. Women with the highest intake of heme iron had 50% (6-94%) increased risk of total CHD compared with those with the lowest intake. Further adjustment for other lifestyle and dietary factors did not appreciably change the associations. The positive association between heme iron and red meat intakes and CHD was more evident among postmenopausal women compared with premenopausal women. Data indicated that higher consumption of heme iron and red meat may increase CHD risk among women with type 2 DM (776).

Il'yasova D et al (2008) examined cross-sectional correlations between two inflammatory markers, serum CRP and IL-6, and three oxidative indices, plasma levels of alpha-tocopherol and beta-carotene, and urinary levels of 2,3-dinor-5,6-dihydro-15-F2t-isoprostane (F2-IsoP), in 60 individuals at high risk of cardiovascular disease. No correlation was found between plasma levels of alpha-tocopherol and either of the inflammatory markers. Plasma beta-carotene inversely correlated with IL-6 (r = -0.46, p=0.0002) and CRP (r = -0.41, p = 0.001) (777).

Ghayour-Mobarhan M et al (2008) examined the relationship between dietary macro- and micronutrient intake, serum concentrations of zinc and copper, and markers of inflammation in dyslipidaemic patients (n = 238) with or without established CAD and healthy controls from the local General Hospital in Guildford, UK. Fifty-five of these patients had established CAD and 135 were controls. Dietary protein, total fat, starch, fiber, monounsaturated fat, zinc, and zinc/copper ratio were also significantly higher in the patients compared to controls. Patients with established CAD had significantly higher serum CRP (p < 0.05) and lower serum zinc (p < 0.01) and zinc/copper ratio (p < 0.01) compared to both patients without CAD and healthy controls (778).

Helmersson J et al (2009) investigated the effects of the dietary intake of beta-carotene, alpha-tocopherol and ascorbic acid on in vivo biomarkers of inflammation (PGF2alpha, hsCRP and IL-6 formation) and oxidative stress (F2-isoprostane formation) in 704 participants in the Uppsala Longitudinal Study of Adult Men (ULSAM) at age 70 years and were quantified 7 years later. Intakes of ascorbic acid and alpha-tocopherol were negatively associated with both PGF2alpha, hsCRP, IL-6 and F2-isoprostanes, where
ascorbic acid intake generally was more strongly associated. Dietary intake of beta-carotene was only significantly negatively associated with F2-isoprostanes. The study suggested that the intake of food rich in antioxidants is associated with reduced cyclo-oxygenase and cytokine-mediated inflammation and oxidative stress at 7 years of follow-up. These associations could be linked to the beneficial effects of fruit and vegetables observed on CVD (779).

Ahmad M et al (2009) determined and compared the levels of oxidative stress and iron indices in CHD and healthy individuals. Blood malondialdehyde, iron, total iron-binding capacity, transferrin saturation and ferretin levels were determined in 140 CHD and 100 healthy subjects. Values of blood malondialdehyde, iron, transferrin saturation and ferretin were significantly increased with exception of total iron-binding capacity, which was significantly decreased in CHD patients when compared with normal healthy controls. It was suggested that elevated serum malondialdehyde, iron concentration and body iron stores in patients reveal a possible role of iron indices in the development of coronary atherosclerosis. Therefore, it is suggested by this study that levels of malondialdehyde and biochemical markers of body iron stores can be used as an early investigative tool for assessing the oxidative stress in CHD (780).

Sentürk T et al (2010) investigated whether serum choline levels are increased across the spectrum of CAD manifestations and correlate with the severity of coronary stenosis in 36 patients with acute coronary syndrome, 22 patients with stable angina pectoris, and 18 controls were recruited. Serum choline levels on admission were significantly higher in the entire group of patients with ACS than in controls. Results suggested that serum choline levels are increased in ACS patients. However, there was no clear correlation between levels of choline and the severity and extent of CAD in this patient group (781).

Kataja-Tuomola MK et al (2010) determined whether alpha-tocopherol or beta-carotene supplementation affects diabetic macrovascular complications and total mortality. A total of 29,133 middle-aged male smokers received either vitamin E 50 mg/day or beta-carotene 20 mg/day, or both, or placebo for a median of 6.1 years. At base-line, 1700 men had type 2 DM. Of these men, 662 were diagnosed with first-ever macrovascular complication, and 1142 died during the 19-year follow-up. Neither supplementation
affected the risk of macrovascular complication or total mortality during the intervention period. No essential changes were found in these effects when the follow-up was extended up to 19 years. Alpha-tocopherol or beta-carotene supplementation has no protective effect on macrovascular outcomes or total mortality of diabetic male smokers (782).

Muzáková V et al (2010) investigated the interrelationships between plasma beta-carotene, alpha-tocopherol, level of systemic inflammation and oxidative stress in patients with advanced CAD in 91 cases and 49 controls. Advanced CAD coincided with significantly lower plasma concentrations of high-density lipoprotein (HDL)-cholesterol and beta-carotene as well as with elevated levels of all inflammatory markers, but only with mild increase of oxidative stress. Beta-carotene significantly inversely correlated with IL-6. This inverse correlation could suggest potential protective effect of beta-carotene on atherosclerosis due to the inhibition of inflammatory processes (783).

Kumar J et al (2010) analysed the association of the CBS 844Ins68 polymorphism alone and in combination with methylene-tetrahydrofolate reductase (MTHFR C677T) and choline dehydrogenase (CHDH A119C) polymorphisms (the two polymorphisms recently shown to be associated with levels of Hcy) with Hcy, cysteine, folate and vitamin B(12) in 817 individuals (397 patients with coronary artery disease and 420 controls). The CBS 844Ins68 polymorphism alone or in combination with MTHFR C677T and CHDH A119C polymorphisms was not significantly associated with any of the biochemical variables studied (784).

Abebe W et al (2010) examined the potential impact of Chromium picolinate on blood pressure, vascular reactivity and myocardial ischemia-reperfusion injury in male spontaneously hypertensive rats. Dietary supplementation (as 10 mg chromium/kg diet for six weeks) did not affect blood pressure of the SHR. Chromium treatment was associated with improved coronary flow and recovery of myocardial contractility and relaxation following ischemia-reperfusion insult. It was concluded that, dietary Chromium treatment of SHR alters neither blood pressure nor vascular smooth muscle reactivity but causes enhancement of endothelium-dependent vasorelaxation associated with NO production/release. Additionally, while the treatment does not affect infarct size, it improves functional recovery of the viable portion of the myocardium following ischemia-reperfusion injury (785).
de Oliveira Otto MC et al (2011) investigated associations of heme iron, nonheme iron, zinc, magnesium, \( \beta \)-carotene, vitamin C, and vitamin E with CRP, IL-6, total Hcy, fibrinogen, coronary artery calcium, and common and internal carotid artery intima media thickness in 5,181 participants from the Multi-Ethnic Study of Atherosclerosis, aged 45-84 y and free of diabetes and cardiovascular disease. Dietary nonheme iron and magnesium intakes were inversely associated with Hcy concentrations. Dietary zinc and heme iron were positively associated with CRP. Other tested micronutrient-marker associations were not significant. Dietary nonheme iron and magnesium intakes were inversely associated with Hcy concentrations (786).

Karppi J et al (2011) examined the effect of carotenoids on early atherosclerosis in a population-based study in 1212 elderly men (aged 61-80 years) in Eastern Finland for intima-media thickness of the common carotid artery and plasma carotenoid concentrations. Men in the lowest quartile of intima-media thickness of the common carotid artery had significantly higher concentrations of plasma \( \alpha \)-carotene than men in the highest quartile. Results suggested that high plasma concentrations of \( \alpha \)-carotene may be associated with decreased carotid atherosclerosis in elderly men from eastern Finland (787).

Koh WP et al (2011) examined the associations between plasma concentrations of specific carotenoids and incidence of acute myocardial infarction. The study included 280 incident cases of acute myocardial infarction and 560 matched controls nested within the Singapore Chinese Health Study, a prospective cohort of 63,257 Chinese men and women aged 45-74 years old enrolled in 1993-1998 in Singapore. There was no statistically significant association between other carotenoids or retinol and risk of acute myocardial infarction. High plasma levels of \( \beta \)-cryptoxanthin and lutein were associated with decreased risk of acute myocardial infarction. The findings of the study support a cardio protective role of these two carotenoids in humans (788).

de Oliveira Otto MC et al (2012) investigated associations of hypothesized prooxidative and antioxidative (zinc, magnesium, \( \beta \)-carotene, vitamin C, vitamin E) micronutrients with incident metabolic syndrome, type 2 diabetes, and CVD in the Multi-Ethnic Study of Atherosclerosis. Participants, 45-84 y at baseline (2000-2002), were followed through 2010. Dietary vitamin E intake was inversely associated with incident metabolic syndrome and CVD. Intakes of heme iron and zinc from red meat, but not from
other sources, were positively associated with risk of metabolic syndrome. Dietary intakes of nonheme iron, magnesium, vitamin C, and β-carotene were not associated with risk of metabolic syndrome, type 2 diabetes, or CVD. Nutrients consumed in red meat, or red meat as a whole, may increase risk of metabolic syndrome and CVD (789).

Wójcik OP et al (2012) conducted a study in New York University Women's Health Study to evaluate the association between circulating taurine levels and risk of CHD. Taurine was measured in two yearly pre-diagnostic serum samples of 223 CHD cases and 223 matched controls. Mean serum taurine was positively related to age and dietary intake of poultry, niacin, vitamin B1, fiber and iron, and negatively related to dietary intake of saturated fat. There was no statistically significant association between serum taurine levels and the risk of CHD in the overall study population. There was a significant inverse association between serum taurine and CHD risk among women with high total serum cholesterol but not among those with low total serum cholesterol. The data suggest a possible inverse association of serum taurine with diabetes and HTN risk. Findings suggested that high levels of taurine may be protective against CHD among individuals with high serum cholesterol levels (790).

2.6 Ayurveda (Prakriti)

“Every individual is different form another and hence should be considered as a different entity. As many variations are there in the universe, all are seen in human beings.’
–Charaka Samhita

“It’s far more important to know what person has the disease then what disease the person has”- Hippocrates

Ayurveda is an ancient system of medicine thought to be originated out from India which is widely practiced in the Indian subcontinent since the pre-biblical era (30-33) and influenced the ancient Chinese system of medicine, Unani medicine, and the humoral medicine practiced by Hippocrates of Greece. Ayurveda is often referred to as the "Mother of all healing." The knowledge of Ayurveda is believed to be of Divine origin, being communicated to the saints and sages of India who received its wisdom through deep meditation. Ayurvedic knowledge was subsequently passed down orally through the generations and then written down in the Vedas, the sacred texts of India believed to be the oldest writings in the world. The Vedas which is written in Sanskrit cover a vast number of
subjects from grammar to health care. The Vedas were written approximately 2500 BC or earlier. Current knowledge about Ayurveda is mostly drawn from relatively later written documents, primarily the Charaka Samhita (approximately 1500 BC), the Ashtang Hrdayam (approximately 500 AD), and the Sushrut Samhita (300 - 400 AD). These three classics describe the basic principles and theories from which Ayurveda has evolved. They also contain vast clinical information on the management of a vast number of diseases expanded by later writings and research.

Like modern medicine it has a well developed and accurate diagnosis and prognosis framework for the helping the sick. Ayurveda treats the person on the level of physical body, emotions and mind, all being integrated aspects, which are involved in the pathology of a disease. It focuses on treating the cause rather than just the symptoms. It appears that Modern Medicine is suited well to deal with acute cardiovascular conditions while ayurveda deals with cardiovascular disease in its earlier stages, benefiting from its unique understanding of the pathology of these diseases from an Ayurvedic perspective.

Ayurveda’s primary emphasis is on prevention of disease and preservation and promotion of health. According to Ayurveda, the human body is composed of five Mahabhutas (basic elements that have the properties of space, air, fire, water, and earth) that combine to form Vata, Pitta, and Kapha, the three psychophysiologic principles known as Doshas. Distinct properties and functions have been ascribed to each Dosha. For instance, Vata contributes to manifestation of shape, cell division, signalling, movement, excretion of wastes, cognition and also regulates the activities of Kapha and Pitta. Kapha is responsible for anabolism, growth and maintenance of structure, storage and stability. Pitta is primarily responsible for metabolism, thermo-regulation, energy homeostasis, pigmentation, vision, and host surveillance (791,792). The Gunas are mental qualities. Sattva is the creative influence associated with intelligence, purity, and balance. Rajas is the spur to activity. Tamas is the influence of inertia, which results in stability.

Prakriti is the individual’s psychophysiologic constitution and is determined at the time of birth by the individual’s Dosha proportions. Each individual has a certain ratio of Vata, Pitta, and Kapha which is unique. In the Ayurveda system of medicine, predisposition to a disease as well as selection of a preventive and curative regime is primarily based on phenotypic assessment of a person which includes one's body constitution termed
"Prakriti". Prakriti of an individual is based on the dominance of any single or a combination of two or three Doshas (Tri-Doshas), Vata (V), Pitta (P) and Kapha (K), which are not only genetically determined (Shukra Shonita), but also influenced by season (Rutu), maternal diet and lifestyle (Matur Ahara Vihara), and age of the transmitting parents or menstrual cycle (Kala-Garbhashaya) (793). According to Ayurveda, ideally constitutions are classified into seven varieties namely vataja, pittaja, kaphaja, vata-kaphaja, vata-pittaja, kapha-pittaja and sama prakriti, among these, the first three are considered as extremes (791,794,795).

Individuals with a Vata-predominant Prakriti have a light, thin build; perform activity quickly; have a tendency toward dry skin and constipation; have an aversion to cold weather; have a tendency to worry; and sleep lightly. Individuals with a Pitta-predominant Prakriti are moderate in build; perform activity with medium speed; have an aversion to hot weather; have sharp hunger and digestion; prefer cold foods and drinks; have a tendency toward irritability and short temper; and are excellent speakers. Individuals with a Kapha predominant Prakriti have a solid build and great strength and endurance; are slow and methodical in activity; have oily, smooth skin; have a steady and tranquil personality; sleep long and heavily; have slow digestion and mild hunger; and have a tendency toward greed (791). A state of disease occurs whenever there is a deviation from the normal physical or mental constitution of a human being, this is called as Vikriti. During diagnosis, Ayurveda examines the Prakriti first, and then it examines the diseased state or Vikriti. Ayurveda is interested in the individual, not only the disease.

An individual may have a natural predominance of one or more doshas. Accordingly disorders arise from an augmentation or depletion of doshas or combination of both in conglomerations with dushas manifest disease, whereas maintaining balance of the doshas results in good health. Over time, the natural balance of the doshas in an individual can be disturbed by a number of factors, such as improper diet, poor digestion, day-to-day stress levels and environmental pollution and chemicals (796). Research in the fields of genomics and pharmacogenomics is revealing the possibility of utilizing Prakriti to correlate phenotypes with genotypes in the human population. This would have a significant impact on the field of personalized, predictive medicine. In the recent years, there have been several studies indicating biochemical basis of constitutional types.
described in Ayurveda (789,797-801). CAD has element of cellular proliferation and metabolic abnormalities. Hence, combined abnormality of prakriti will be more prevalent in CAD.

In the body there are also seven Dhatus, which are fundamental principles that support the various bodily tissues; these are Rasa (plasma), Rakta (blood), Mamsa (muscle), Meda (fat), Asthi (bone), Majja (bone marrow), and Shukra (sperm or ovum). There are three metabolic waste products known as Malas, which are Mutra (urine), Purisha (feces), and Sweda (sweat). Energy flow and communication take place through various channels known as Srotas (Bodily Channels). The maintenance of health and disease depends on the functioning of these various constituents. A state of equilibrium in their functioning results in health and disequilibrium leads to disease (802).

2.6.1 Cardiovascular Disease from Ayurvedic Perspective.

Ayurveda classifies Cardiovascular Disease under the following:

<table>
<thead>
<tr>
<th>Adibalapravruta</th>
<th>: Hereditary Cardiovascular Disease e.g. metabolic diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janmabala pravruta</td>
<td>: Congenital Cardiovascular Disease</td>
</tr>
<tr>
<td>Doshabala pravruta</td>
<td>: Vata, Pitta, Kapha, Sannipatika</td>
</tr>
<tr>
<td>Sanghatabala pravruta</td>
<td>: (a) Traumatic. (b) Poisoning</td>
</tr>
<tr>
<td>Kalabala pravruta</td>
<td>: Environmental or seasonal</td>
</tr>
<tr>
<td>Upasargaja</td>
<td>: Infectious diseases e.g. Endocarditis, Myocarditis, Pericarditis</td>
</tr>
<tr>
<td>Swabhavabalakruta</td>
<td>: Natural diseases e.g. ageing, death</td>
</tr>
<tr>
<td>Adhyatmika</td>
<td>: Psychological factors leading to Cardiovascular Disease or hypertension</td>
</tr>
<tr>
<td>Daivabalakruta</td>
<td>: Idiopathic, bad luck due to bad deeds of previous lives.</td>
</tr>
</tbody>
</table>

The diseases of each organ are classified and treated according to the tissue (dhatu) and dosha affected. Embryologically, endocardium is derived from Rasa and Rakta dhatu. Myocardium is derived from muscular tissue (Mamsa) and pericardium is derived from fatty (Meda) and connective tissue. Each of these three layers of the heart can be affected by one or all of the doshas. Looking at the endocardium, if it is a Vata disorder then this will lead to valvular affection like aortic and mitral stenosis and regurgitation. If Pitta is effected then we would include bacterial endocarditis and if Kapha the endocardial
fibroelastosis. It would be treated by strengthening Rasa and Rakta and restoring the balance to the Doshas involved. For diseases of the myocardium, if they involved Vata then atrophy or fibrosis of the heart muscle will be seen, which would result in dilation of the heart. If Pitta is involved then myocarditis will be seen and with Kapha, hypertrophy of heart muscle as well as for example, glycogen storage diseases. The treatment in these cases would look at strengthening the muscular tissue (Mamsa) and treating the affected Doshas. For diseases affecting the coronary arteries; if a Vata disorder, it gives rise to angina pectoris, if Pitta then specific or non-specific arteritis, and Kapha to atherosclerosis changes. For each of the above classifications of Cardiovascular Disease Ayurveda has a different treatment approach (802).

2.6.2 Ayurvedic Pathogenesis (Samprapti)

Causes for Cardiovascular Disease from an Ayurvedic perspective can be classified into:
1. causes which directly act on the heart,
2. causes which are important for maintaining physiological functions of the heart,
3. cardiovascular Disease as a complication of other diseases.

These would be classified as being imbalances of (1) Vata, (2) Pitta, (3) Kapha, (4) Tridoshas (all three doshas), or (5) Parasites, viruses, worms, bacteria (Krumi). The aetiological factors are generally classified as psychological factors, diet, activity, excessive sexual indulgence, suppression of natural urges, alcohol in excess, bacteria, viruses, worms and other toxins, iatrogenic, causes effects of drugs, improper management of disease, abnormal or excess use of emetics, purgatives or enemas, trauma to the heart, complications of other diseases. These will cause abnormal increase or decrease in Vata, Pitta and Kapha and in turn Rasa which enters the heart and gives rise to the Cardiovascular Disease.

In summary, the eight basic elements that maintain the integrity of the cellular structure and functions of the heart are, Rasa, Rakta, Mamsa, Ojas, Prana vata, Vyana vata, Sadhaka pitta and Avalambaka kapha. In Ayurveda the pathogenesis provides insights into the development of the disease process, showing in detail how the doshas when aggravated by certain aetiological factors affect the dhatus and srotas of the body, eventually manifesting in disease. Ayurveda describes the following types of pathogenesis of cardiac disease (802).
Diagrammatic representation of Samprapti of Hridroga
Derangement of Rasa Dhatu

The following can be seen as the interpretation of the Samprapti of Cardiovascular Disease.

1. Vitiation of Doshas
2. Accumulation of Vitiated Doshas and Vitiated Rasa Dhatu in the heart.
3. Development of Obstruction in the heart and impairment of physiological function of the heart.

Thus, due to the impairment of nutrition of the cardiac muscle (by Rasa) the outcome is some sort of Cardiovascular Disease.

Once the condition reaches ‘sthana Sansharaya’ the symptoms of Cardiovascular Disease appear clinically. During this process the deformities in the Rasavaha Srotas are well established and there is an excess flow (atipravritti) or growth (granthi) established in the heart region which will cause the dosha or dhatu to increase or decrease. If the proper treatment of Cardiovascular Disease (Hridroga) is not followed and the causative factors are continued the doshas in the heart become more and more vitiated along with the development of various complications. This condition is called “Bheda Avastha”. In this condition the circulation of Rasa- Rakta may be affected and is also called “Marma-Upaghata”.

2.6.3 General Causes of Hridaya Roga

Among various causes identified to precipitate a heart disease, most appreciable ones as per Ayurveda are vyayama (excessive exercise), tikshna ahara (pungent diet), excessive use of virechana (purging), vasti (medicated enema), or vamana (emesis). Some primary diseases leading to emaciation, excessive worry, stress, fear, and a direct trauma to heart may also lead to a heart disease. For causes described here as a reason to heart disease, most are concerned with water and nutrition depletion from the body either though an excessive loss (vaman, virechana, vasti, excessive exercise, trauma) or a reduced intake (emaciation, stress, fear, worry). All these causes ultimately lead to a depletive state, where the functions of the heart are not adequately met, hence mimicking a heart disease. This is also important to observe that contrary to contemporary understanding of risk factors to cardiac diseases, besides psychological factors, Ayurveda does not mention any other factor in its list of etiology to heart diseases. Of most important among all is the nonobservance of obesity and dietary excess from the list of risk factor to heart disease among descriptions of
Ayurveda. This important observation raises a question against the linking of fundamental understanding of heart disease in both the streams of medicine. This puts further emphasis to our previous connotation of Ayurvedic descriptions for being more functional compared to the modern understanding which is primarily pathological. This fundamental difference to the understanding of etiopathogenesis of heart disease in both the streams of medicine forms the basis to their differential approach which is often contrasting to each other. Contrary to the modern approach of treating heart disease which is marked by a salt, oil, and fat reduction; Ayurvedic medicaments used in this condition are predominantly marked by an excess of salt, oil, and fat.

Recently some studies have tried to integrate modern system of medicine with Ayurveda. Modern medicine have strong genetic base, hence Bhusan et al (2005) have tried to correlate human leucocytic antigen with Prakriti. Seventy six subjects were evaluated for their Prakriti and human leucocyte antigen (HLA) DRB1 types. A reasonable correlation between HLA type and Prakriti type was found (799).

Bhavana Prasher et al (2008) found that among the 33 biochemical parameters, 15 parameters in males and 4 in females, revealed significant differences (p ≤ 0.05), with respect to Prakriti. Notably, the components of the lipid profiles like TGs, TC, VLDL, LDL, LDL/HDL ratio, the common risk factor for cardiovascular diseases was higher in Kapha when compared to Pitta and Vata in males. Additionally, Kapha also had lower levels of HDL when compared to Vata. The levels of serum uric acid, recently considered to be an independent predictor of cardiovascular mortality, were also found to be elevated in Kapha. In addition, GGT, SGPT, and serum Zinc were found to be high in Kapha. Serum prolactin and prothrombin time were high in Vata in comparison to Kapha and/or Pitta (791).

The extreme constitution types revealed differences at gene expression level as well as biochemical levels and also included genes with reported disease involvement. Interestingly, it revealed differences in a significant number of hub and housekeeping genes which if perturbed can have system-wide effects. This study provided a molecular framework for understanding the Indian Traditional System of Medicine, Ayurveda. Identification of genetic variations that underlie differential expression of genes and biochemical end-points, co-relatable to Prakriti phenotypes will further provide a strong
basis for integration of this Ayurveda science with modern genomic approaches for predictive marker discovery and system biology studies. Shilpi Aggarwal et al (2010) used the Ayurvedic concept of Prakriti, which relates to phenotypic differences in normal individuals, including response to external environment as well as susceptibility to diseases, to explore molecular differences between three contrasting Prakriti types: Vata, Pitta, and Kapha. Egl nine homolog 1 (EGLN1) was one among 251 differentially expressed genes between the Prakriti types. A link between high-altitude adaptation and common variations rs479200 (C/T) and rs480902 (T/C) in the EGLN1 gene was reported. Furthermore, the TT genotype of rs479200, which was more frequent in Kapha types and correlated with higher expression of EGLN1, was associated with patients suffering from high-altitude pulmonary edema, whereas it was present at a significantly lower frequency in Pitta and nearly absent in natives of high altitude. Thus, EGLN1 polymorphisms are associated with high-altitude adaptation. This genotype is rare in highlanders but is overrepresented in a subgroup of normal lowlanders discernable by Ayurveda may confer increased risk for high-altitude pulmonary edema.

Ghodke Y et al (2011) hypothesized that different Prakriti may have different drug metabolism rates associated with drug metabolizing enzyme (DME) polymorphism. CYP2C19 (Phase I DME) genotyping in 132 unrelated healthy subjects of either sex by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was done. A significant association between CYP2C19 genotype and major classes of Prakriti types was observed. The extensive metabolizer (EM) genotype was found to be predominant in Pitta Prakriti (91%). Poor metabolizer (PM) genotype was highest (31%) in Kapha Prakriti when compared with Vata (12%) and Pitta Prakriti (9%). Genotype which is typical for PM group was significant in Kapha Prakriti (odds ratio = 3.5, P =0.008). An interesting correlation between CYP2C19 genotypes and Prakriti with fast and slow metabolism being one of the major distinguishing and differentiating characteristics was observed. These observations are likely to have significant impact on phenotype-genotype correlation, drug discovery, pharmacogenomics and personalized medicine.

Tripathi et al (2011) concluded that the modified self-assessment questionnaire that was used in the study measured the dominance of Dosha to a reliable extent and may be
used to quantitatively express the *Dosha* dominance. Though, the study indicates that the basic cardiovascular responses do not significantly vary in accordance with the dual constitutional types of Ayurveda, it suggested that the fall in DBP recorded immediately after performing the isotonic exercise for five minutes varies significantly in relation to *Prakriti* groups and this fall is significant among VK group in comparison to VP and PK groups. This finding is indicative of some kind of positive association of *Pitta* with adrenal medullary hormones, sympathetic activity and/or such other mechanisms that regulate the total peripheral resistance. The study may also be considered as a lead to further investigations as to whether the individuals with a dual constitutional type differ significantly from those with an extreme constitutional type or not. A similar study, if carried out among the individuals belonging to truly extreme constitutional types, may throw some more light in this regard (801).

### 2.7 AIMS AND OBJECTIVES

The aetiology of CVD is multifactorial and there are many risk factors that are associated with cardiovascular disease. Indians have high incidence of cardiovascular disease which cannot be explained by traditional risk factors. Hence, many researchers investigated and reported other novel risk factors to explain the increasing incidence and prevalence of CVD i.e. vitamin B12, Hcy, inflammatory markers, macro and micronutrients and presence of metabolic syndrome (25-29). Most of the risk factors have been evaluated in epidemiological studies; there are very few studies which assessed the correlation of traditional risk factors with newer non-traditional risk factors.

Considering the increasing incidence and acute nature of disorder, necessity of improved risk factor determination for cardiovascular disease is essential. In India there are many type of medical practices like Ayurveda, Homeopathic, Unani etc, all have their own logic and science. However, ayurvedic science in thought to be oldest and had originated in India. Ayurvedic principles like vata, pitta and kapha may be checked to estimate risk factors for CVD. Clinical findings based upon ayurvedic system of vata, pitta and kapha would be useful in conjunction with other biochemical and routine investigations. This kind of integrated approach would certainly lead to identification of more risk factors. There is an urgent need to integrate various medical practices to cope
up this problem. It would help in early diagnosis, preventive management and appropriate therapy in early stages.

With this background we planned a study among subjects with known CAD to evaluate traditional and non traditional risk factors like insulin resistance, inflammatory markers, micronutrients (magnesium, vitamin B12, and folic acid), Hcy, and dietary factors; and their interrelationship and association with “Prakriti” types described in ayurveda system of medicine.

2.7.1 **Hypothesis**

1. Nutritional factors, and its related disorders like vitamin B12 deficiency, hypomagnesaemia are related to CVD risk factors.

2. Prakriti classification of Ayurveda is indicative of risk factors for cardiovascular disease.

2.7.2 **Aim**

To find out the additional risk factors for CVD by using an integrated approach based upon biochemical and ayurvedic principles.

2.7.3 **Objectives**

1. To find the presence of traditional risk factors in subjects with cardiovascular disease.

2. To find association between coronary risk factors and vitamin B12 status in subjects with cardiovascular disease.

3. To find the association between magnesium coronary risk factors and in subjects with cardiovascular disease.

4. To find association between coronary risk factors and inflammatory markers in subjects with cardiovascular disease.

5. To find the association between traditional and non-traditional coronary risk factors and dietary factors.

6. To find differences in coronary risk factors in subjects with or without diabetic and association of insulin resistance with coronary risk factors.

7. To find the association of constitutional type of Ayurveda (prakriti) and coronary risk factors and in subjects with cardiovascular disease.