INTRODUCTION

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1. INTRODUCTION

1.1 EPIDEMIOLOGY OF MYOCARDIAL INFARCTION AND CHD

1.1.1 The standardized mortality ratio (SMR) for coronary heart disease (CHD) among Indians (people from the Indian subcontinent namely India, Pakistan, Bangladesh, Nepal, Bhutan and Sri Lanka) living in Great Britain, (Pedoe et al, 1975; Marmot et al, 1984) in South Africa (Cosnett, 1957; Walker, 1963) and in the West Indies (Miller et al, 1982) has been found to exceed those observed for the populations of European and African origins.

1.1.1.1 Epidemiological data on CHD in India is scanty and the few reports are mutually contradictory. Dhar et al (1978) reported that the incidence of cardiovascular disease during two periods 1965-1967 and 1973-1975 was 31.1% and 26.1% respectively and between the sexes, the incidence was more in males.

1.1.1.2 Pinto et al (1970) conducted a survey in Bombay (India), which showed a prevalence of 29 cases in 1000 persons aged over 30 years. In Chandigarh, the prevalence rate was 24 in 1000 among those above the age of 40. This study clearly indicated that the proportion of middle aged patients with IHD is fairly high in India.

1.1.1.3 Sapru (1984) estimated that about 21.89 million people in India at present were victims of cardiovascular disease and to this pool approximately 1.12 million are likely to be added every year. Senthl Nathan (1979) observed that IHD may be considered as common as in the developed countries.
1.1.1.4 According to an epidemiological survey in three urban centres in India, namely, Delhi, Agra and Coonoor (Padmavathi, 1962) it has been shown that the high income groups had an incidence rate of 5 to 6%. The low income group had 0 to 1% of the total population. Another survey in Uttar Pradesh (Balla and Tandon, 1962) found an incidence in general population of 1.77%, 0.46% and 0% in higher, middle and lower socioeconomic groups respectively.

1.1.1.5 There is a large difference in the incidence of IHD between South Indians and North Indians (Padmavathi, 1962; Malhotra, 1967). Men from South India have seven times greater mortality rate from IHD than their counterparts from North India (Malhotra, 1967a). Even though the mortality rate due to IHD in Indian male population (between the age of 18 and 55 years) is 59 in 100,000 (average for the country), the southern region alone recorded a mortality rate of 135 in 100,000 (Malhotra, 1967). This is very close to the mortality rate of 150 in 100,000 in Federal Republic of Germany, Australia and Poland in the age group of 45 to 55 years reported in the WHO survey (Turner, 1980).

1.1.2 Coronary heart disease is the number one killer in the developed countries and is emerging as a major health problem in the developing countries (Gotto, 1987) and acute myocardial infarction is the presenting symptom in the terminal stage. Morris (1981) has calculated and stated that perhaps 50% of men in their forties, fifties and sixties develop myocardial infarction and that in half of these cases it may be regarded as either very or potentially dangerous. The incidence of myocardial infarction is higher in males than females, and the incidence increases with age for both sexes (Castelli and Anderson, 1986).
1.1.2.1 Castelli (1984) states that by the age of 60 years, "every 5th man and every 17th women will develop some form of CHD". The mortality rate due to AMI was more than 50% in U.S.A. (Gotto, 1986), 33% in U.K. (Turner, 1978) and about 47% of all deaths in USSR (Klimov, 1976). In Australia, Israel and Japan the mortality rates were found to be 290,210 and 250 respectively per 100,000 in the WHO study (Turner, 1980).

1.1.3 Incidence of MI and MI mortality in humans vary with time, their culture and social and dietary heterogeneity. For example, mortality from IHD in England and Wales have been studied identifying the country of birth of the diseased. During 1979-83 mortality from ischaemic heart disease was highest in men and women born in the Indian subcontinent (Standardised mortality ratio 136 and 146 respectively). Young men of India suffer greatest excess (313 out of 100,000 at age 20-29). Other groups observed with raised mortality included Irish, Scottish, and Polish born emigrants. Those born in the Carribean, the old Commonwealth, West Europe, and the United States have low death rates (Balarajan, 1991).

1.1.3.1 Japanese men who emmigrated to Hawai or California and changed their dietary habits (Westernisation, implying increased consumption of total fat, saturated fatty acids and cholesterol) died of myocardial infarction and heart disease more frequently than their counterparts in their native country (Marmot et al, 1975). Two-fold and three-fold increases in the mortality rates have been observed for Japanese men who have migrated to Hawai and Calitonia respectively.
1.1.4 Many risk factors for the development of acute myocardial infarction (AMI) have been identified and incorporation of dietary modifications in the last two decades have changed the mortality rates.

1.1.4.1 Martin et al (1989) have given their comments on 8 prospective studies including their own study which has attempted to document the incidence of myocardial infarction and the result of intervention methods in reducing IHD. The studies were conducted in Finland (Koskenvuo et al, 1985; Pohjola et al, 1985), Western Australia (Martin et al, 1989), United States (Eleveback et al, 1981; Gillum et al, 1983; Goldberg et al, 1986) and New Zealand (Beaglehole et al, 1984) each covering a wide range of population. All these studies show a net decline in the incidence of myocardial infarction for both sexes over the respective period of study. The decline in the incidence of myocardial infarction range from 0.9% to 2.5% in males and from 0.7% to 4.1% in females annually. These declines correspond closely to reduction in mortality from IHD observed in the same countries over the same period (Havlik and Feinleib, 1979; Beaglehole et al, 1981; Koskenvuo et al, 1985; National Heart Foundation of Australia, 1987).

1.1.4.2 With intervention, incidence of myocardial infarction and mortality from IHD fell faster in females than in males. It also fell faster in younger people than in older people (Martin et al, 1989).

1.1.4.3 Martin et al (1989) have proposed the following causes for the decline in the mortality from IHD:

a. Reduced exposure to one or several risk factors for acute myocardial infarction.
b. Increased use of therapies that reduce the frequency of acute myocardial infarction.

c. Improved education of patients reduce serum cholesterol (by diet restriction) blood pressure and cigarette smoking. Since the 1960's, there have been substantial decreases in the apparent consumption of meat, milk, egg and butter and substantial increases in the consumption of polyunsaturated fats in Australia (Australian Bureau of Statistics, 1987) and in New Zealand (Jakson and Beaglehole, 1987). Moreover, it has been estimated that consumption of cholesterol fell by 18% (Cashel, 1983). Hence, they conclude that this decrease could explain 40% of the decline in MI mortality between 1968 and 1980 in New Zealand.

1.2 RISK FACTORS IN ACUTE MYOCARDIAL INFARCTION

1.2.1 The Framingham study played a major role in delineating and confirming factors associated with development of CHD (Kannel et al, 1976; Dawbar, 1980). Several other investigations have made significant contributions to our understanding of these factors. They include the Stockholm Prospective Study (Botliger and Carlson, 1980), the Tromso Heart Study (Thelle et al, 1976), the Belgian Heart Disease Prevention Project (Kortnitzer et al, 1980), the Multiple Risk Factor Intervention Trial (MRFIT, 1982), the Oslo Risk Reduction Study (Hjermann et al, 1981) etc. The risk factors often interact, posing an even greater threat to patients who have more than one risk factors (Kaplan, 1987).

1.2.1.1 Epidemiological studies have led to the recognition of as many as 246 risk factors, some major, some minor, some reversible and some irreversible. They may be classified into (a) non-modifiable risk factors, (b) modifiable risk factors and
(c) miscellaneous risk factors; Non modifiable and modifiable risk factors are known to be primary and miscellaneous risk factors to be secondary (Hopkins and Williams, 1981).

1.2.2 There is consistent evidence relating the incidence of myocardial infarction to high level of serum cholesterol and consumption of diet rich in saturated fats (Shekelle et al, 1981 and several others). A number of national and international commissions are unanimous in advocating a reduction in the level of saturated fat consumption.

1.2.2.1 The expert panel on National Cholesterol Education Programme (NCEP, 1984) identify three dietary habits which contribute significantly to elevated plasma cholesterol. They are (a) high intake of saturated fats (b) a relatively high intake of food rich in cholesterol and (c) a high caloric intake that exceeds body requirements, commonly causing obesity. The panel also suggests that if these three factors could be controlled, it would be possible to reduce the mortality from MI to a great extent. Alternatively, the panel recommends a high intake (eg. 15 to 25 g/day) of soluble fibre which has been reported to lower the plasma cholesterol level by 5 to 15% (NCEP, 1984).

1.2.3 Hypertension is regarded a major risk factor for stroke, congestive heart failure and MI (Castelli and Anderson, 1986). Several other epidemiological studies such as Hypertension Detection and Follow up Programme (Taylor, 1977; HDFP, 1978) have shown that high blood pressure occurs in about 20-30% of the adult population and 60% of these adults are unaware that they have high blood pressure. Framingham study has revealed (Kannel, 1983) that elevated pressure
accelerates both atherosclerosis and arteriosclerosis, and in hypertensives, occlusions and ruptures in the aorta and coronary arteries occur 20 years earlier than in normotensives.

1.2.3.1 The relationship among the three major risk factors - hypertension, elevated serum cholesterol and cigarette smoking and the development of CHD have received great attention over the last five years (Gotto, 1987) in the formulation of preventive measures.

1.2.3.2 Dugani (1989) has observed that in hypertensives, complications like heart attack is 3 times, stroke 7 times and congestive cardiac failure 4 times more frequent when compared to normotensive people. Hypertension becomes an atherogenic factor of greater clinical significance when it is associated with elevated plasma lipid levels (Levy and Lern, 1986).

1.2.4 Several studies demonstrate a strong positive association between cigarette smoking and the risk of cardiovascular disease morbidity and mortality (Libow and Schlant, 1982; Rose et al, 1982). Cigarette smoking leads to a change in the vascular endothelium or platelets which in turn adversely affects the balance between vasoactive substances derived from endothelium or platelets with subsequent spasm of the infarct-related artery (Khalilullah and Gambhir, 1986). Several reports have demonstrated an inverse relationship between smoking and HDL-cholesterol (Garrison et al, 1978; Criqui et al, 1980; Stamtord et al, 1984). This was confirmed in the recent studies made by Moffatt (1988) and Dwyer et al (1988) wherein they were also able to raise HDLc levels significantly as a consequence of cessation of smoking.
1.2.4.1 Arownow et al (1974) reported that cigarette smoking causes an increase in resting heart rate and an increase in resting systolic and diastolic blood pressure, thereby increasing the myocardial oxygen demand.

1.2.5 Castelli and Anderson (1986) identify an increase in cholesterol concentration markedly in cholesteryl esters. Rifai (1986) reported that serum lipid levels show the highest correlation with the development of myocardial infarction.

1.2.5.1 St. Clair et al (1968) postulated that the development of atherosclerotic lesions are accompanied by a stimulation of several metabolic processes of which one is the synthesis of complex lipids. Many studies have confirmed that free and esterified cholesterol accumulates in the aorta, coronary arteries and cerebral vessels and the rate of accumulation varies among individuals (Zilversmit, 1979). Cholesterol is a pivotal element in the aetiology of MI (Castelli and Anderson, 1986).

1.2.6 Triacylglycerol is a major risk factor for the development of myocardial infarction (Carlson et al, 1979). Several prospective studies address higher serum triglyceride levels as a risk factor for MI (Gordon et al, 1977; Hulley et al, 1980; Camhein et al, 1986). European investigators have reported that raised level of triglyceride may predispose to coronary artery disease (Carlson et al, 1972; Cambien et al, 1986; Brieier et al, 1989). This has not been confirmed in US studies (Hulley et al, 1980), although a recent review of the Framingham data suggests that elevated triacylglycerols are an independent myocardial infarction risk factor in men and women with low levels of HDL cholesterol (Castelli, 1986).
1.2.6.1 In his review, Austin (1989) reported that elevated plasma triglycerides has been found to be a univariable risk factor for IHD in many epidemiological studies. Hence, this increased risk is not independent of other lipid and lipoprotein levels. Calabresi et al (1990) reported that there is clear negative correlation between HDL\textsubscript{2} levels and triglyceridemia in the case of healthy people but not in coronary patients. By contrast, triglyceridemia is negatively correlated with HDL\textsubscript{3}, both in healthy subjects and in IHD at all triglyceride levels.

1.2.7 Portman (1970) reports that total phospholipids are increased in atherosclerotic arteries both in humans and in experimental animals. Increases in the sphingomyelin fraction occur in both cases (McCandless and Zilversmit, 1956; Adams and Bayliss, 1963).

1.2.7.1 Bovet et al (1989) reported elevated levels of serum phospholipids and their subtractions in cardiovascular disease. But they are not better predictors of CHD than other lipoprotein levels.

1.2.8 Free fatty acids in plasma are elevated in atherosclerosis. The increase in free fatty acids and the induction of atherosclerosis have been demonstrated by Abdulla et al (1967). Free fatty acids are released from adipose tissue and are taken up by the liver and peripheral tissues and esterified or oxidised. The product of beta oxidation of fatty acids, acyl CoA is considered to be the major energy source for the myocardium and their levels are increased in older patients with myocardial infarction than young normal subjects (Wisneski et al, 1987).
1.2.9 Friedman (1969) identifies the coronary prone personality TYPE A behaviour pattern and describes a style of life activity characterised by some or all of the following: competitiveness, intense striving for achievement, easily provoked hostility, a sense of urgency about doing things quickly and being punctual, impatient, abrupt and rapid speech and gestures and concentration upon self selected goals. People who are relaxed, unhurried, less easily provoked and who have more smoothly modulated speech and gesture patterns are defined as TYPE B (Jenkins, 1979).

1.2.9.1 Since 1964, several epidemiological studies have shown the positive association of TYPE A behaviour with the development of IHD (Rosenman et al, 1964; De Backer et al, 1983 and several others). The TYPE A pattern has been shown to have the same strength as standard risk factors like serum cholesterol, cigarette smoking and hypertension (Shekelle et al, 1976).

1.2.10 Non-Modifiable risk factors

1.2.10.1 Atherosclerotic complications are a major cause of morbidity and mortality among the elderly (Kreisberg and Kasim, 1987). A strong and consistent association of age with atherosclerotic lesions has been reported repeatedly. Since lipoprotein concentrations increase with age (Kreisberg and Kasim, 1987), it is important to determine whether such changes contribute to the aging of the vascular system or whether they are parallel but unrelated processes. Nevertheless, in the Framingham study (Castelli et al, 1981) the strong association between cholesterol and atherosclerotic diseases observed in younger individuals weakens the possibility of aging contributing to atherosclerotic disease. Based on these observations, it may be
appropriate to state that mere aging cannot independently produce, but may increase the susceptibility to and or accelerate cardiovascular complications.

1.2.10.2 The risk of CHD is greater for the male sex than in the females (Fulwood et al, 1986). The rates of MI are 3 to 4 times higher in men than in women in the middle decades of life and roughly 2 times higher in the elderly (Lerner and Kannel, 1986; Feinliep and Gillum, 1986). The lower incidence of IHD in women is because of the protective effect of estrogen, differences in blood lipids, hematocrit and lesser cigarette smoking.

1.2.10.3 Genetic predisposition to atherosclerosis is a non-modifiable risk factor. The importance of family history in coronary heart disease leads to differences of opinion (Conroy et al, 1985; Friedlander et al, 1985). Apart from the inheritance of predisposition to the atherosclerotic process, other aggravating factors and conditions such as unhealthy diet and sedentary habits are observed singly, as well as in unison, in families. This aggregation of risk factors is the principle cause of the high frequency of CHD in certain families (Mulcahy 1987). Simons et al (1988) reported that familial aggregation of IHD may be partially mediated by a reduced HDLc concentration.

1.2.11 Several miscellaneous or secondary risk factors have been identified. Diabetic nephropathy develops in 30-40% of all diabetic patients and end stage renal failure is the leading cause of death followed by cardiovascular disease (Borch-Johnsen and Kreiner, 1987) in insulin dependent (TYPE I) diabetics. The risk of atherosclerotic complications is markedly increased in both insulin dependent
(IDDM) and noninsulin dependent diabetes mellitus (NIDDM) (Pyorala and Laakso, 1983; Pyorala et al, 1987).

1.2.11.1 Clinical complications of atherosclerosis remain a major cause of mortality in most developed countries. Epidemiologic data supported by three large prospective surveys initiated independently in the late sixties, have suggested a role for hyperinsulinaemia as an independent risk factor for coronary heart diseases (Welborn and Wearne, 1979; Pyorala et al. 1985; Fontbonne et al, 1991).

1.2.11.2 Obesity is associated with an increased risk of myocardial infarction (Bjorntorp, 1985; Hubert, 1986). It has been suggested that the relationship is mainly mediated by the association of obesity with other cardiovascular risk factors such as hypertension, diabetes mellitus and lipoprotein abnormalities (Grundy et al, 1979; Borkan et al, 1986; Bertiie et al, 1988).

1.2.11.3 Women using oral contraceptives show a consistently higher risk for CHD than non users (Mann and Inman, 1975; Beral, 1976; Jick et al, 1978). Numerous surveys have established that oral contraceptives produce hyperlipidaemia. Wallace et al (1979) explained that oral contraceptive usage leads to increase in LDL cholesterol and triglyceride levels in circulation which in turn increase the mortality from myocardial infarction.

1.3 PLASMA LIPOPROTEINS, APOLIPOPROTEINS AND MI

1.3.1 Lipoproteins transport lipids in plasma and exist as micelle consisting of an outer surface coat of specific apoproteins and polar lipids (unesterified
cholesterol, phospholipids) and an inner core of non-polar lipids (cholesteryl ester and triglycerides) (Schaefer et al, 1978; Kostner, 1983).

1.3.1.1 The major functions of plasma lipoproteins are (i) to transport endogenously synthesized and exogenous glycerol to sites of utilisation and storage, and (ii) to transport cholesterol, an essential structural component of all membranes from the sites of absorption and synthesis to the sites of catabolism and excretion (Miller, 1979).

1.3.2 Lipoproteins are classified into five major classes according to their physical and chemical properties (Table 1.1) and are named according to their densities or electrophoretic mobility, they are classified as the following (i) chylomicrons, (ii) very low density lipoprotein (VLDL), (iii) intermediate density lipoproteins (IDL), (iv) low density lipoproteins (LDL) and (v) high density lipoproteins (HDL) (Chapman et al, 1981). High density lipoproteins can be divided further into two density subpopulations, HDL2 and HDL3 (Eyre et al, 1981). Levy (1981) observed that these subpopulations of HDL, namely HDL2 and HDL3 differ in their metabolic roles and chemical significance.

1.3.2.1 Lipoproteins differ in density because they consist of different proportions of triglycerides, cholesterol and phospholipids in addition to apolipoproteins. The highest concentration of plasma triglycerides is present in VLDL in the fasting state and in chylomicron in the non-fasting state. LDL cholesterol carries 70% of total plasma cholesterol, while HDL contains the lowest amount of plasma triglycerides and about 20% of plasma cholesterol (Kreisberg, 1983; Stein, 1986).
Table 1.1 Operational Classification of plasma lipoproteins and composition and characteristics of plasma lipoproteins (Gotto and Jackson, 1977; Levy and Rifkind, 1980)

<table>
<thead>
<tr>
<th>Lipoprotein Classes</th>
<th>Density (g/ml)</th>
<th>Electrophoretic Mobility</th>
<th>Molecular Weight</th>
<th>Composition %</th>
<th>Lipid protein ratio</th>
<th>Apolipoprotein Major</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYL.</td>
<td>0.95</td>
<td>Origin</td>
<td>0.4-30x10⁶</td>
<td>99</td>
<td>5</td>
<td>90</td>
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<td>VLDL</td>
<td>0.95X1.006</td>
<td>pre-B</td>
<td>5.1x10⁶</td>
<td>90</td>
<td>13</td>
<td>65</td>
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<tr>
<td>LDL</td>
<td>1.006X1.063</td>
<td>B</td>
<td>2.7-4.8x10⁶</td>
<td>80</td>
<td>55</td>
<td>5</td>
</tr>
<tr>
<td>HDL</td>
<td>1.063-1.21</td>
<td>α</td>
<td>1.8-3.6x10⁶</td>
<td>50</td>
<td>18</td>
<td>2</td>
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13.3 Each class of lipoprotein differ from the other in the variety of apolipoproteins. LDL is unique in that it carries a single type of apolipoprotein namely apo B (Table 1.1). Apolipoproteins A are the major proteins in HDL, Apolipoprotein C and E are present in various proportions in both HDL and VLDL lipoproteins. The major apolipoproteins A, B, C and E consists of two or more immunologically distinct proteins (Table 1.2) (Mahley et al, 1984; Stein, 1986).

13.4 Castelli and Anderson (1986) have observed that although plasma cholesterol has been proved to be an excellent predictor of CHD, more accurate predictor of CHD are serum lipoprotein measurements, including low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoproteins (HDL); LDL and VLDL promote atherogenicity. HDL cholesterol (HDL$_c$) is an independent predictor of CHD and an increase in this protective cholesterol fraction is associated with a reduction in the incidence of CHD (Lern, 1987). Further analysis has shown that the cholesterol content of HDL$_2$ subtraction correlated even better than that of HDL or HDL$_3$ with increased risk of MI (Eder and Gidez, 1982).

13.5 The inverse correlation between HDL bound cholesterol and IHD has been first reported by Miller and Miller (1975) and is repeatedly seen in several other populations also. Miller (1984) concludes that HDL appears to act like a biological vacuum cleaner, sucking up excess cholesterol in the blood stream for eventual elimination through the liver. Because of this anti-atherogenic effect, HDL is termed "good or protective cholesterol". The power of low HDL levels to predict clinical coronary events is probably as strong as that of high LDL levels (Aro et al, 1986).
Table 1.2 Characteristics of Human major plasma Apolipoproteins (Rifai, 1986)

<table>
<thead>
<tr>
<th>Apoprotein</th>
<th>Molecular weight</th>
<th>Plasma concentrations mg/dl (mean &amp; S.D.)</th>
<th>Function</th>
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<tr>
<td>A1</td>
<td>28,000</td>
<td>121 ± 24</td>
<td>Activates LCAT</td>
</tr>
<tr>
<td>AII</td>
<td>17,000</td>
<td>37 ± 9</td>
<td>Inhibit LCAT? Activities hepatic lipase?</td>
</tr>
<tr>
<td>AIV</td>
<td>44,000 - 46,000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B-48</td>
<td>-</td>
<td>-</td>
<td>Cholesterol clearance</td>
</tr>
<tr>
<td>B-100</td>
<td>6,605</td>
<td>98 ± 20</td>
<td>Cholesterol clearance</td>
</tr>
<tr>
<td>CI</td>
<td>8,824</td>
<td>7 ± 2</td>
<td>Activate LCAT?</td>
</tr>
<tr>
<td>CII</td>
<td>3,50,000 - 5,50,000</td>
<td>3.71 ± 1.8</td>
<td>Activate LPL</td>
</tr>
<tr>
<td>CIII</td>
<td>8,750</td>
<td>13 ± 5</td>
<td>Inhibits LPL? Activates LCAT?</td>
</tr>
<tr>
<td>E</td>
<td>34,000</td>
<td>4 ± 1</td>
<td>Cholesterol clearance</td>
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</table>
1.3.5.1 The subfractions of HDL, namely HDL₂ and HDL₃ play a meaningful role in the control of atherogenesis (Pilger et al, 1983). HDL₂ (in contrast to HDL₃) is influenced by sex (Anderson et al, 1978), physical activity (Wood and Haskell, 1979), hormones (Krauss et al, 1979) and pharmacological agents (Shepherd et al, 1979). Women have high levels of HDL and HDL₂ and develop atherosclerotic lesions more slowly than men (Anderson et al, 1979). Administration of isocaloric high fat diet in men is followed by lowering of HDL₂ bound cholesterol and HDL₂/HDL₃ ratio (Shanmugasundaram et al, 1986).

1.3.5.2 In 1971, Alaupovic has suggested that apolipoproteins should also be considered in the evaluation of lipoprotein disorders. Kreisberg (1983) and Thanhauser et al (1984) reported that the apolipoprotein B levels were elevated and apo A levels were decreased in individuals with coronary heart disease. It has been proposed that the concentrations of apo B and apo A are better predictors of IHD than LDL and HDL respectively (Maciejko et al, 1983; Kukita et al, 1985). Later studies have shown that in IHD, differences in serum levels of apo A and apo B are parallel to those in the cholesterol levels of HDL and LDL respectively.

1.4 METABOLISM OF LIPOPROTEINS

1.4.1 Lipoprotein metabolism can be divided conceptually into exogenous and endogenous systems that transport lipids of dietary and hepatic origin respectively (Cooper, 1985). Lipoproteins efficiently transport triglycerides and cholesterol esters before they can be used by cells. Triglycerides and cholesterol esters must be hydrolysed to liberate fatty acids and free cholesterol (unesterified) respectively.
Through interactions with enzymes and cell surface receptors, the apolipoproteins direct each lipoprotein to its site of metabolism (Brown et al., 1981; Cooper, 1985).

1.4.2 Exogenous System

In the intestine, triglyceride and cholesterol from the diet are absorbed into the lymphatics and are incorporated into chylomicrons. The chylomicrons then enter the blood at the thoracic duct and most of the triglyceride is hydrolysed by lipoprotein lipase (LPL), an enzyme which is bound to the capillary endothelium. The product of hydrolysis is free fatty acids which enter the adjacent muscles where they are used for energy, or adipose cells where they are connected to triglyceride and stored as energy reservoirs (Glomset, 1980; Brown et al., 1981).

1.4.2.1 During circulation chylomicrons loses about 96% of its mass (mostly triglycerides), together with A and C apolipoproteins which are taken up by the nascent HDL in circulation (Cooper, 1985) (Fig. 1.1).

1.4.2.2 A smaller, cholesterol rich chylomicron remnant particle results in which apo E, apo-B48 and cholesterol oleates are retained. The remnants are carried to the liver where they bind to apo-B-E-receptors on the cell surface, followed by internalisation and subsequent degradation by the lysosomal enzymes (Brown et al., 1981; Levy 1981).
1.43 Endogenous system

When dietary cholesterol is insufficient, the liver synthesises its own cholesterol by increasing the activity of the rate limiting enzyme HMG-CoA reductase (Brown et al, 1981) (Fig. 1.2).

1.43.1 VLDL is subjected to the LPL on the endothelial cell surface and is broken down to FFA, apolipoproteins and the cholesterol rich lipoprotein LDL, with the intermediate formation of IDL (Intermediate density lipoproteins). VLDL remnants are cleared by the liver and IDL is ultimately converted to LDL (Eisenberg and Levy, 1975; Grundy, 1986 and several others). The details of the conversion of VLDL to LDL are not fully understood, but they involve the progressive hydrolysis of VLDL triglycerides, enrichment of cholesteryl esters and loss of all the apolipoproteins except apo B-100.

1.43.2 It has been suggested that hepatic lipase, which has both lipase and phospholipase activity, may play a role in the conversion of IDL to LDL (Kinnunen et al, 1983).

1.44. LDL particles formed by the catabolism of VLDL are composed of cholesteryl esters, cholesterol and phospholipids making up 75% by weight (Skipski, 1972). LDL cholesterol is delivered to the tissues primarily via LDL receptor (apo B,E-receptor) mediated endocytosis (Goldstein and Brown, 1983). These receptors regulate cholesterol metabolism in a number of tissues and prevent excessive accumulation of cholesterol in the cells. After reacting with a receptor, an LDL
Endogenous system of Lipoprotein Metabolism pathway (Rifai, 1986)
particle becomes internalised, then undergoes lysosomal hydrolysis of the LDL-component, resulting in the intracellular release of cholesterol (Goldstein et al, 1975).

1.4.4.1 HDL is formed essentially in the plasma by the lipolysis of chylomicrons (Schaefer et al, 1978). During the hydrolysis of chylomicrons, discoidal HDL particles are formed, which consists of lipid bilayer membrane containing apo-A-1 (Schaefer et al, 1978). The discoidal particles contain free cholesterol only and no esterified cholesterol. These particles are converted into the circulating form of HDL, through esterification of the cholesterol by the action of the enzyme lecithin cholesterol acyl transferase (LCAT) (Hamilton, 1978).

1.4.4.2 Cholesterol bound to HDL is antiatherogenic and is linked to the transport of cholesterol from peripheral cells to the liver for eventual clearance and was elaborated by several workers. In his review, Miller (1984) describes that this process, known as reverse cholesterol transport, involves 4 basic steps — cellular efflux of free cholesterol to HDL, esterification by LCAT, transport of esterified cholesterol from HDL to lower density lipoproteins and finally, uptake by the liver. An essential component of this pathway is a lipid transfer protein known as cholesteryl ester transfer protein (CETP) or lipid transfer protein (LTP) (Tall, 1986). CETP has been shown to promote the transfer/exchange of cholesteryl ester and triglycerides between lipoproteins (Morton and Zilversmit, 1983).

1.4.5 Eisenberg (1984) observed that the HDL particles, which are said to carry the protective form of cholesterol, can become larger or smaller when and if lipid and protein molecules are added or deleted from them and can exist as HDL₂ and HDL₃.
1.4.5.1 The conversion of HDL3 to HDL2 is a two step process: (Fig. 1.3), lipolysis of triglyceride rich lipoproteins the surface coat of intact lipoproteins, and cell membranes supply HDL3 with phospholipids, free cholesterol and apolipoproteins (Patsch et al, 1978). These molecules accumulate in existing HDL3 particles, which are subsequently converted to cholesterol ester rich HDL2 by the action of LCAT (Gotto, 1983).

1.4.5.2 HDL2 can carry twice as many cholesterol molecules per unit of apolipoproteins as compared with HDL3 (Gotto, 1983). Hence HDL2 can be viewed as a doubly efficient vehicle for the transfer of cholesterol from peripheral tissues back to the liver (i.e.) the reverse cholesterol transport (Patsch et al, 1984).

1.4.6 The conversion of HDL2 to HDL3 as shown in Fig. 1.4 is a two-step process (Eisenberg, 1984). The step depends on the lipid transfer reaction (CET) and replacement of part of the HDL2 cholesteryl ester molecules by triacylglycerols. In the second step, hydrolysis of the transferred triacylglycerols by hepatic lipase and lipoprotein lipase results in the formation of HDL3 with lower size and density (Eisenberg, 1984).

1.4.6.1 A new cholesterol rich lipoprotein "Lipoprotein a" (Lp (a)) has been identified as a risk factor for coronary atherosclerosis (Kostner, 1981). This is synthesised in the liver and corresponds in protein and lipid composition to the LDL to which an additional protein, apoprotein (a), is bound via a disulfide bridge (Morrisset et al, 1987) and bears a strong resemblance to plasminogen (Machlin et al, 1987). Lp (a) catabolism is associated with LDL, and Utermann et al (1981) observed that in familial hypercholesterolaemic heterozygous subjects with defective
Fig. 1.3 | HDL$_3$ $\rightarrow$ HDL$_2$ CONVERSION

(adapted from EISENBERG, 1984)
Fig. 1.4  \( \text{HDL}_2 \rightarrow \text{HDL}_3 \) CONVERSION

(adapted from EISENBERG, 1984)
LDL receptors, Lp (a) is elevated. Lp (a) levels above 20 to 30 mg/dl increase the risk of arteriosclerosis by two to three times (Kostner, 1981 and several others).

1.5 THE HYPERLIPOPROTEINAEMIAS AND MI

1.5.1 Hyperlipoproteinaemia is a common abnormality of plasma lipids and goes unnoticed in young and the middle aged. They have been first classified by Fredrickson et al (1967). Six different types of hyperlipoproteinaemias have been identified and the latest classification is by Gotto (1987). They are described below:

1.5.2 Type I hyperlipoproteinaemia (fat induced hyperlipaemia) is characterised by markedly lipaemic or creamy plasma. This is a rare disorder caused by deficiency of apo C II, a cofactor required for lipoprotein lipase activity (Breckenridge et al, 1978). In addition, deficiency of lipoprotein lipase itself has been reported by Nikkila (1982). This condition leads to the accumulation of dietary triglycerides in the plasma in the form of chylomicrons. Triglyceride concentration is increased and there is a typical wide chylomicron band on electrophoresis of fasting plasma. This is an inherited recessive disease and is characterised by eruptive xanthomata, lipaemia retinalis, heptosplenomegaly and often, pancreatitis.

1.5.3 Type IIa hyperlipoproteinaemia (Familial hypercholesterolemia, hyper beta lipoproteinaemia) is a common form and is inherited as an autosomal dominant, but may appear secondary to hypothyroidism, nephrotic syndrome, obstructive jaundice, multiple myeloma, acute porphyria or idiopathic hypercalcaemia (Brown and Goldstein, 1976). Triglyceride concentrations are normal while total cholesterol and LDL concentrations are always increased. Familial hypercholesterolemia is due
to a defect in the LDL (apo B,E) receptors (Goldstein and Brown, 1982). As a consequence of impaired receptor function, LDL accumulates in the plasma ultimately leading to premature atherosclerosis.

1.5.3.1 Type IIb hyperlipoproteinaemia which is less common. There is also hypertriglyceridemia and hyper prebeta-lipoproteinaemia. The condition is characterised by atherosclerosis and premature ischaemic heart disease associated with tendinous and tuberous xanthomata and often corneal arcus. Such subjects are usually heterozygous.

1.5.4 Type III hyperlipoproteinaemia resembles Type IIb, HLP; While both cholesterol and triglyceride concentrations are increased in plasma there is a broad indistinct beta lipoprotein band often merging with the chylomicrons. It is probably an autosomal recessive disease and does not become manifested, until later in life. Xanthomata occurs, consisting mainly of triglyceride in tendons and particularly as orange red lesions in the palm or creases. In these subjects there is a very high incidence of peripheral vascular disease as well as of ischaemic heart disease (Brown et al, 1983).

1.5.4.1 Dysbeta-lipoproteinaemia appears to result from the confluence of two factors: (1) the presence of apo E2/E2 phenotype for apo E; and (2) presumably another gene associated with over-production of VLDL (Brown et al, 1983). Dysbeta-lipoproteinaemia results from a slow clearance of the VLDL remnants because apoprotein E2 has a decreased affinity for the LDL (apo B,E.) receptor (Brown et al, 1983).
1.5.5 Type IV hyperprebetalipoproteinaemia (endogeneous hyperlipaemia, carbohydrate induced lipaemia) is a very common hyperlipoproteinaemia which may also occur as secondary to lipodystrophy, diabetes, gout, pancreatitis, pregnancy and estrogen administration. Primary type IV HLP is an autosomal dominant disease and is characterised by an increase in the prebeta lipoprotein (VLDL). Triglyceride is increased and is carried in the VLDL fraction. This pattern results from an over-production of VLDL in liver with a subsequent over production of apo-B in plasma (Teng et al, 1986).

1.5.5.1 Patients with this disorder also have a defect in the clearance of postprandial lipoproteins following a fat load (Genest et al, 1986). The defect is accompanied by a decrease in HDL₂ in HDL (Kwiterovich and Sniderman, 1983).

1.5.6 Type V hyperlipoproteinaemia, the least common form is characterised by excessive chylomicrons and prebeta lipoproteins (VLDL). The plasma is lipaemic and on standing separates into an upper chylomicon layer containing prebeta lipoproteins. The condition is not associated with primary vascular disease but obesity, with attacks of pancreatitis, eruptive xanthomata, lipaemia retinalis, hyperuricemia and impaired glucose tolerance are common. In this type, apo CII is normal and lipoprotein lipase activity is either normal or low but not absent.

1.5.6.1 While cholesterol, triglycerides, lipoprotein cholesterol and apolipoproteins can be assayed by a variety of methods, phenotyping the patients with HLP has to take into account the values of each parameter obtained in the healthy population and delineates clearly a boundary or a cut off point for identifying
elevated or depressed values. Immarino (1975) provided a schedule for such a diagnostic classification.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Lipid cases</td>
<td>&lt;120</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Normal</td>
<td>121-260</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Type I</td>
<td>&lt;260</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>Type II a borderline</td>
<td>261-299</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Overt</td>
<td>&gt;300</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Type II</td>
<td>261-299</td>
<td>151-350</td>
</tr>
<tr>
<td>IIb borderline</td>
<td>&gt;300</td>
<td>151-300</td>
</tr>
<tr>
<td>Overt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type III</td>
<td>351-500</td>
<td>351-500</td>
</tr>
<tr>
<td>Type IV</td>
<td>&lt;250</td>
<td>151-200</td>
</tr>
<tr>
<td>borderline</td>
<td>&lt;260</td>
<td>201-999</td>
</tr>
<tr>
<td>Overt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type V</td>
<td>&lt;300</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

Based on this criteria, Virmani (1982) from our laboratory classified 165 cases of IHD and observed that 26% of the cases showed no hyperlipoproteinemia, 18.8% and 9.7% showed borderline and overt Type Ila HLP; 11.5 and 10.3% had Type IIb HLP; and 12 and 11.5% showed Type IV HLP; Type Ila, IIb and IV HLP's appear with the same frequency in the case of IHD studied in Madras.

1.6 USE OF BLOOD CELLS FOR METABOLIC PROFILING OF INTERNAL ORGAN LIPID DISORDERS

1.6.1. Animal models of IHD and MI have provided significant data on the metabolic and structural alteration in the liver, blood vessels and the cardiac muscles.
While extrapolating these findings to men, bulk of the studies are made on the blood plasma. Erythrocytes have been used in the assessment of antioxidant studies. Other works are largely based on studies of mixed leucocyte population, buffycoat preparation (Boyd, 1933; Marks et al, 1960; Malamos et al, 1962; Finke et al, 1966) or of abnormal cells such as leukemic leucocytes (Boyd, 1933).

1.6.2 The use of peripheral leucocytes in the study of congenital or chromosomal abnormalities had become the accepted method in the fifties, followed by the use of this cell population for the diagnosis of inherited enzyme deficiencies and heterozygous carriers. In our laboratory, leucocytes have been used for the assay of the enzymes responsible for cholesterol esterification (ACAT also called cholesterol ester synthetase or CES) and the lysosomal cholesterol ester hydrolase, CEH.

1.6.2.1 Shanmugasundaram et al (1986) reported a lowering of CEH/CES ratio with concomitant increase in cholesterol in the leucocytes of healthy volunteers after consuming high fat diet with increased esterifying activity. When CEH/CES is lowered, cholesterol will be predominantly in its ester form (as in LDL) and can lead to the development and progression of atherosclerosis. It may be inferred from these observations that leucocyte CEH and CES assays would help in measuring the potential for cholesterol ester accumulation in the metabolic state of the organism.

1.6.3 Cholesterol exists in the body in two forms, namely, free cholesterol and as cholesteryl esters. Free cholesterol is the functionally important form of the sterol. For the purpose of storage and transport, cholesterol is attached to long chain fatty acids to form cholesteryl esters (Skipski, 1972). Normal tissues are capable of
hydrolysis and synthesis of cholesteryl esters by three reactions each catalysed by distinct class of enzymes.

1.6.3.1 St. Clair (1976) described the enzyme system for cholesterol esterification in animal tissues.

1. Fatty acyl CoA cholesterol acyltransferase (ACAT)
   Fatty acyl CoA + Cholesterol → Cholesterol ester + CoA SH

2. Cholesterol ester synthetase (CES)
   Fatty acid + cholesterol → Cholesterol ester + H₂O

3. Lecithin cholesterol fatty acyl transferase (LCAT)
   Lecithin + Cholesterol → Cholesterol esters + lysolecithin

1.6.3.2 ACAT catalyses cholesterol ester formation and has been implicated in the development of atherosclerosis (Hashimoto and Dayton, 1977). Lysosomal acid lipase (LAL) appears to inhibit cholesterol ester accumulation balancing with the cholesterol ester synthetase activity (Brecher and Chobanian, 1973). De Duve (1974) proposed that the enzyme (cholesterol ester synthetase) results in intracellular accumulation of cholesteryl ester in arterial smooth muscle cells.

1.6.4 Blood leucocytes play a role in the transport of cholesterol in circulation (Suzuki, 1969) and cholesteryl ester is the major lipid that accumulates in atherosclerosis. Pronounced changes in esterifying activities take place during experimentally induced atherosclerosis in various species (St. Clair, 1976). Therefore, the study of cholesterol esterification and hydrolysis and their balance in leucocytes may reflect the pattern or balance of arterial cholesterol deposition in an individual.
1.6.5 The lipid analysis of the white cells resembles that of plasma whereas in other respects it more closely approximates that of body tissues (Boyd, 1933). The lipid content of normal circulating leucocytes have been investigated (Gottfried, 1967), he has observed that lipid constitutes about 5% of weight of normal human lymphocytes and polymorphonuclear leucocytes and about one third of the total lipid content (by weight) is phospholipid. Another one third consists of neutral lipids, primary triglycerides and free cholesterol. About 10-12% of the total lipid in human leucocytes is free cholesterol (Gottfried, 1967). The cholesterol to phospholipid molar ratio is 0.68 for human lymphocytes and 0.55 for polymorphonuclear leucocytes in contrast to the much higher ratio of 0.94 found in erythrocytes (Jaffee and Gottfried, 1968). It will be interesting to see whether lipid accumulation takes place in leucocytes in ischaemic heart disease.

1.7 LIPID PEROXIDATION IN BLOOD AND IHD

1.7.1 Studies in both animals and humans have shown that there is a close relationship between atherosclerosis and lipid peroxidation (Goto, 1982; Yagi, 1985). Serum lipid peroxides remaining at a high level for long periods are known to be one of the initiating factors for atherosclerosis (Goto, 1982; Yagi, 1985).

1.7.2 Oxidation reactions have long proposed as a pathological event in degenerative processes associated with cell breakdown and aging (Tappel, 1973). Machlin and Bendich (1987) observed that the formation of highly reactive, oxygen containing molecular species (free radicals) is a normal consequence of a variety of essential biochemical reactions.
1.7.2.1 An early event in atherogenesis is the accumulation of lipid laden foam cells in the arterial intima which can progress to fatty streaks and plaques. Henriksen et al (1981), Steinbrecher et al (1984), Heinecke et al (1986) and several others observed that oxidised LDL is quickly taken up by macrophages to give rise to foam cells.

1.7.3 Oxidation of LDL is a free radical process in which the polyunsaturated fatty acids contained in the LDL are degraded by a lipid peroxidation process to a great variety of aldehydes (e.g., Malondialdehyde, 4-hydroxynonenal, propanal, hexane, 2,4-alkadienols and other products) (Esterbauer et al, 1988; Esterbauer et al, 1989). A variety of indirect evidence suggests that these lipid peroxidation products bind to amino acid side chains (e.g., epsilon amino groups of lysine residues) of the apolipoprotein-B and create thereby new epitopes that are no longer recognized by the apo B/E receptor, but by the scavenger receptor (Esterbauer et al, 1988; Steinberg et al, 1989).

1.7.4 Free radicals may arise endogenously from normal metabolic reactions such as superoxide during respiration (or) endogenously as components of tobacco smoke and air pollutants and indirectly through the metabolism of certain solvents, drugs and pesticides as well as through exposure to radiation (Yagi, 1987) Free radicals are highly reactive and cause tissue damage by reacting with polyunsaturated fatty acids in cell membranes, a process known as lipid peroxidation (Machlin and Bendich, 1987) They also react with the nucleotides in DNA, and critical sulfhydryl bonds in proteins (Machlin and Bendich, 1987)
1.7.5 Erythrocyte membranes are rich in polyunsaturated fatty acids and are continuously exposed to high concentrations of oxygen and erythrocyte ghost membranes are oxidized by a free radical chain mechanism (Chiu et al, 1982; Yamamoto et al, 1985). The three main conditions that favour lipid peroxidation are (1) a high degree of unsaturation in the lipid substrate, (2) a rich supply of oxygen and (3) the presence of transition metal like iron as catalysts (Chiu et al, 1982). These requirements are met by the red cell (Chiu et al, 1982).

1.7.5.1 Dormandy (1971) observed that peroxidation of the membrane could alter its permeability and also lead to hemolysis. Peroxidation of unsaturated fatty acids in the erythrocyte membrane is responsible for the increased membrane rigidity and decreased erythrocyte deformability (Palek and Liu, 1979; Lubin and Chiu, 1982). Studies on the peroxidative damage to cellular integrity and membrane permeability have been made by several others with RBCs as model.

1.8. FAILURE OF ANTIOXIDANT DEFENSE SYSTEMS AND MYOCARDIAL INFARCTION

1.8.1 The antioxidant defense systems to combat and control free radical and reactive oxygen radicals have been the subject of study for over a decade and had been reviewed by many authors (Dimascio et al, 1991).

1.8.1.1 The aerobic myocardium is able to handle and survive the continuous oxygen free radical production because of the existence of a delicate balance between cellular systems that generate the various oxidants and those that maintain the antioxidant defense mechanism. The heart of these defense mechanisms are the
enzymes superoxide dismutase, catalase and glutathione peroxidase and scavenger antioxidants as vitamin E, ascorbic acid, carotene, vitamin A, glutathione and other thiol compounds (Diplock and Lucy, 1974; Ferrari et al, 1988).

1.8.1.2 Endogenous defenses against free radical damage of unsaturated fatty acids include antioxidants like glutathione, uric acid, bilirubin and several metallo enzymes like glutathione peroxidase, catalase and superoxide dismutase (Fig. 1.5) (Chiu et al, 1982).

1.8.2 Exogenous peroxidized polyunsaturated fatty acid damage the endothelium and heart muscle cells (Yagi, 1987) and provoke proliferation of smooth muscle cells. This cytotoxicity can be prevented by antioxidant vitamins, vitamin A, vitamin C and vitamin E (Henning et al, 1987). The extent of lipid peroxidation at any given time is the result of the balance between the free radicals generated and the antioxidant protective defense system (Machlin and Bendich, 1987). In atherosclerosis the critical balance between these two opposing forces is lost.

1.8.3 Superoxide dismutase (SOD)

The danger from the superoxide anion \((O_2^-)\) in the red cell probably lies in its ability to interact with peroxides to form hydroxyl radicals (Thomas et al, 1978), which causes haemolysis in the red cells. Superoxide dismutase (SOD) catalytically scavenges the superoxide radical and thus provides a first line of defense against free radical damage. SOD catalyses the following reaction:

\[ 2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \]
Fig. 1.5
PROTECTIVE SYSTEMS IN RED CELL AGAINST PEROXIDATIVE REACTIONS
(Adapted from Chiu et al, 1982)

- Glutathione synthetase
- Glutathione peroxidase
- Glutathione reductase
- NADPH
- NADP
- G6P
- HMP Shunt
- Glutathione
- Aminoacids
- Vitamin E
- RH
- Hb Desaturation
- Cell membrane
- Heinz body
- Hemolysis
1.8.4 Glutathione peroxidase (GPx)

Glutathione peroxidase nullifies the threat of hydroperoxides in the red cells. In the presence of reduced glutathione, this enzyme catalyses the reduction of $\text{H}_2\text{O}_2$ and free fatty acid hydroperoxides to water and the corresponding hydroxy fatty acid respectively (Little and O' Brien, 1968; Christopherson, 1969). It catalyses the following reaction:

$$\text{ROOH} + 2\text{GSH} \rightarrow \text{GSSG} + \text{ROH} + \text{H}_2\text{O}$$

1.8.5 Catalase

Hydrogen peroxide which is formed by the flavin linked oxidases is acted upon by catalase (Nicholls and Schonbaum, 1963). This enzyme catalyses the following reaction:

$$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

The decomposition of $\text{H}_2\text{O}_2$ by catalase proceeds at one of the highest rates known for enzymatic reactions (Forman and Fisher, 1981).

1.8.5.1 It has been proposed that in certain diseases including ischaemia, these enzymes may be structurally altered or functionally impaired, thereby allowing the unregulated or over-production of oxygen reactive species (Delmaestro, 1980; Burton, 1985).
1.8.6  Antioxidant Vitamins

1.8.6.1  Vitamin C (Ascorbic acid)

Ascorbic acid is an important component in the scavenging systems for free radicals. Patients with IHD have increased plasma levels of lipid peroxidation product, malondialdehyde, measured as thiobarbituric acid-reactive material (Stringer et al., 1989), i.e., an indicator of increased susceptibility of LDL towards lipid peroxidation, which can be decreased by vitamin C and E. Riemersma et al. (1989) reported that low plasma level of lipid standardized vitamin E and of vitamin C are risk factors in early angina pectoris and that incidence of IHD may be inversely related to plasma vitamin C.

1.8.6.2  Vitamin E (Tocopherol)

Vitamin E is the major lipid soluble antioxidant responsible for protecting the polyunsaturated fatty acids in membranes against lipid peroxidation (Burton et al. 1984; Horwitt, 1986). The vitamin E content of membranes often determines the susceptibility of microsomal membranes, low-density lipoproteins, hepatocytes, or whole organs to damage, by peroxidizing agents such as hydroxyl radical, alkoxyl radicals, peroxyl radicals, singlet oxygen, and perhaps a number of oxygen-metal complex (Cadenas et al. 1984; Quehenberger et al. 1988). These agents not only damage the lipids but produce secondary intermediates, lipid hydroperoxides, which can decompose into alkoxyl and organic peroxyl radicals and thus lead to a chain reaction of lipid peroxidation. Tocopherol protect lipids by scavenging peroxyl radicals without reacting in further chain propagating steps.
1.8.7 Superoxide dismutase and catalase have been used therapeutically with success in lowering the oxidative insult after ischemia (Flohe et al, 1985). A large, multinational epidemiological study suggests that higher dietary intake and blood levels of the antioxidant vitamins and selenium (required for glutathione peroxidase) are associated with reduced risk of mortality from cardiovascular disease (Gey, 1986; Gey et al, 1987).

1.9 FACTORS AFFECTING BLOOD COAGULATION AND HAEMORHEOLOGICAL PROPERTIES AND MYOCARDIAL INFARCTION

1.9.1 The clinical progress of acute cardiovascular disease is strictly related to the concomitant rheological situation (Dormandy, 1983). There is growing evidence that MI is associated with the activation of the clotting system (Huebner et al, 1988). Davis and Thomas (1984) suggested that coronary thrombosis occurs in most patients with acute myocardial infarction. There is also epidemiologic evidence indicating the pathogenic importance of hemostatic function in myocardial infarction (Meade et al, 1986). However, the mechanism of this relationship still remains unclear (Handa et al, 1989).

1.9.2 Levels of plasma fibrinogen have received attention as an independent predisposing factor for coronary heart disease (CHD). These levels show a prognostic significance comparable with that of cigarette smoking, serum cholesterol, hypertension, and other major risk factors in prospective studies (Wilhelmsen et al, 1984; Meade et al, 1986; Kannel et al, 1987). They have consistently shown that individuals with high concentrations of plasma fibrinogen are more likely to have CHD.
1.9.3 Konarska et al (1986) have reported increased levels of major immunoglobulin IgG, IgA and IgM in the circulating immune complexes in hypercholesterolemic patients in the United Kingdom. Klimov et al (1985) found circulating immune complexes in elevated concentrations in coronary heart disease.

1.9.3.1 According to the autoimmune theory of pathogenesis of atherosclerosis, the formation of lipoprotein antibody (LP-Ab) complex is one of the key moments of lesion development in the arteries (Klimov, 1986; Klimov et al, 1987).

1.9.3.2 Klimov et al (1985; 1987; 1988) explained that the lipoprotein antibody autoimmune complex in the human blood may be taken up by macrophages. Such uptake may accelerate the accumulation of cholesteryl esters in macrophages and their transformation into foam cells.

1.9.4 Fibrinogen, factor I of the coagulation cascade has been recognised as a risk factor in the development of acute cardiovascular disease (Wilhelmsen et al, 1984; Balleisen et al, 1985). In the Northwick Park Heart Study, carried out by Meade et al (1986), the association between plasma fibrinogen and MI was stronger than that of cholesterol and MI. Meade et al (1986) also pointed out that the association between smoking and MI may be mediated through the increase in plasma fibrinogen level in smokers.

1.9.5 The atherosclerotic role of fibrinogen is mediated through its promotion of red cell and platelet aggregation (Schmidt-Schonbein et al, 1976; Dimmno et al, 1983), increased rheological stasis (Dormandy, 1983) and amplification of the coagulation cascade at higher concentrations.
1.9.6 Increased platelet mass and reduced platelet survival have been reported in patients with atherosclerosis (Corash et al, 1981; Maritin et al, 1985) or hyperlipoproteinaemia (Harker and Hazzard, 1979).

1.9.6.1 The role that blood platelets play in the pathogenesis of vascular diseases has not been established (Ross, 1986). However, the findings that platelet function, morphology and turnover can be abnormal in hyperlipidemia (or) atherosclerosis suggest that they are either involved in the development of atherosclerosis or adversely affected by some metabolic abnormalities that produce atherosclerosis (Mazoyer et al, 1988).

1.10 Therapeutic Measures in the Prevention of IHD

1.10.1 In the use of hypolipidemic agents three questions are often raised. They are:

1. Can the development of coronary heart disease be prevented?
2. Can life expectancy be increased in the case of clinically manifested coronary atherosclerosis?
3. Are side effects compatible with long term therapy?

1.10.2 Lipid lowering drugs have been extensively used in attempts at the primary and secondary prevention of coronary heart disease together with changes in the life styles and antihypertensive therapy. The WHO collaborative trial in the multifactorial prevention of coronary heart disease (carried by researchers from Belgium, Italy, Poland, Spain and U.K.) included cholesterol lowering dietary advice, cessation of cigarette smoking, weight reduction among the obese, daily exercise for
sedentary participants and hypertensive regimen (WHO, E.C.G. 1974) in nearly 50,000 men of mean age 48.5 years, obtained 7% reduction in CHD death (DeBacker, 1983). In the MRFIT (Multiple Risk Factor Intervention Trial) conducted in USA, coronary prone middle aged men already exposed for decades to environmental and physiological factors accelerating the atherosclerotic process, were recruited and half of them were provided intervention aimed at lowering cholesterol cutting in on smoking and antihypertensive therapy (Stamler, 1985). Based on the findings in these and several other observations, Gotto (1989) has concluded that (1) lowering cholesterol by 1% leads to a 2% decrease in CHD events (death, heart attack) (2) raising HDL, while lowering LDL and total cholesterol results in 34% decrease in CHD events (Helsinki Heart Study) (3) lowering total cholesterol showed 10% reduction in mortality after 15 years follow up (Coronary Drug-Project) (4) 36% fewer deaths by lowering cholesterol and triglycerides (Stockholm Ischaemic Heart Study) and (5) regression of atherosclerosis with simultaneous reduction in LDL and raise in HDL (Cholesterol Lowering Atherosclerosis Study).

1.10.3 The mechanism of action of some hypolipidemic drugs was reviewed by Assman (1982) as follows:

1. They competitively inhibit the resorption of cholesterol in the intestinal wall (Sitosterol).
2. Interrupt the extrahepatic circulation of bile acids (Cholestyramine, colestipol).
3. Suppress the synthesis of VLDL and LDL (nicotinic acid, clofibrate, benzaflibrate).
4. Accelerate LDL degradation (Cholestyramine, D-thyrooxine, benzafibrate).

1.10.4 Unfortunately, most lipid lowering drugs or agents available today do not satisfy the above criteria. The coronary drug project (CDP Research group, 1975), a secondary intervention study and report from the committee of principal investigators (1978), a primary intervention study and two longterm studies, have raised the question on the rational for hypolipidemic therapy. In the WHO study, although there was a decline in the incidence of nonfatal myocardial infarction with clofibrate, the overall mortality was higher with use of the medication than in the untreated control group (Report from the committee of principal investigators, 1978).

1.10.4.1 The incidence of cholelithiasis (gall stone formation) is elevated after the longterm administration of clofibrate (Oliver et al, 1978), nicotinic acid (Angelin et al, 1979) and cholestyramine (CDP Research group, 1977). Lithogenecity is considered to be a potential side effect of clofibrate and other fibric acid derivatives.

1.10.4.2 Coronary drug project on the efficacy of clofibrate, niacin, dextrothyroxine and estrogen have found all four to be harmful, although effective to varying degrees in lowering plasma lipids (CDP Research group, 1977; Krol, 1978; Marmot, 1979; La Rosa, 1982). They have also reported that most of these drugs have side effects such as constipation, nausea, vomiting, abdominal discomfort, reduced glucose tolerance, hyperuricemia, disturbance of potency, etc. (La Rosa, 1982).
1.10.4.3  Gemfibrozil, a derivative of clofibrate is found to augment HDL concentration in circulation (Samuel, 1983).

1.10.5  Newer lipid lowering drugs function as HMG Co-A reductase inhibitors and antioxidants.

1.10.5.1  Probucol, another drug inhibits the autoxidation of LDL and serves as an antioxidant in atherosclerosis therapy (Cominacini et al, 1989).

1.10.5.2  Lovastatin and its derivatives interfere with cholesterol biosynthesis by inhibiting the crucial step namely the enzyme HMG-CoA reductase. They have been found to reduce plasma fibrinogen also (Bo et al, 1991).

1.10.6  A number of metal ions play an important roles in reducing vascular complications. According to Yacowitz (1976), calcium forms soaps with dietary fats and excretes them in the faeces.

1.10.6.1  Trace of copper in the diet was found to be antiatherosclerotic (Klevay et al, 1975) and relative copper deficiency is reported in American diets (Klevay et al, 1979).

1.10.7  Anna Pavala Sindhooram (APS) is a lipid lowering drug, satisfying all the requirements of an ideal lipid lowering drug. Anna Pavala Sindhooram is a herbomineral Indian medicine in the form of a health food supplement, developed by Shanmugasundaram et al (1983) for controlling the progress of atherosclerosis. The herbomineral preparation is aimed at clearing the blood vessels of accumulated lipids and associated products and increase the life span, according to the traditional
Indian medicinal (Ayurvedha and Siddha) concepts. The minerals and herbs used in APS are noted for their pharmacological effects namely, stimulating metabolic activity including fat metabolism in liver, cholorective (augmenting bile production), cardiotonic, diuretic, expectorant and laxative properties.

1.10.7.1 Anna Pavala Sindhooram (APS) lowers serum cholesterol and increases the proportion of cholesterol bound to HDL in rabbits (Shanmugasundaram and Marita, 1982) and in humans and chicks (Shanmugasundaram et al, 1983a). The drug has been modified and in this form (modified Anna Pavala Sindhooram, APSm), it has been shown to regress the atherosclerotic lesions in rats (Dhandapani et al, 1984) and to lower the levels of plasma lecithin and aortic sphingomyelin in rabbits during atherosclerosis (Marita and Shanmugasundaram, 1988), the atherosclerotic lesions in rats (Dhandapani et al, 1984) and to lower the levels of plasma lecithin and aortic sphingomyelin in rabbits during atherosclerosis (Marita and Shanmugasundaram, 1988).

1.10.7.2 Modified Anna Pavala Sindhooram (APSm) enhanced faecal elimination of bile acids, cholesterol and other fats (Kareem, 1983), increased lipolytic activities and cholesterol ester hydrolase activities (Marita and Shanmugasundaram, 1982; Parthasarathy and Shanmugasundaram 1983). Srinivas and Shanmugasundaram (1987) observed that administration of APSm for periods upto 4 months prevent the beta agonist (isoproterenol hydrochloride) induced myocardial infarction in rats. In the experimentally induced atherosclerosis in rats, rabbits and chicks, APSm showed no hepato or nephrotoxicity or any other undesirable side effects and was found to be well tolerated.
1.10.7.3 APSm was administered to 25 patients with coronary heart disease with abnormal lipoprotein profile for 30 days in the hospital of orthodox medicine (Allopathy). During APSm therapy, HDL cholesterol increased significantly with significant reduction of VLDL and LDL bound cholesterol and triglycerides were observed (Kancharla, 1987).

1.10.7.4 Modified Anna Pavala Sindhooram was tested for its lipid lowering activity in a double blind cross over design on 30 patients with IHD for a period of 2 months. During therapy, plasma cholesterol was reduced and HDL cholesterol increased significantly. HDL₂ cholesterol increased significantly together with reduction in LDL and VLDL bound cholesterol and serum triglycerides were also observed (Shanmugasundaram et al, 1991). APSm was well tolerated and nephro or hepatotoxicity, gastrointestinal disturbances, anorexia, nausea, and sleeplessness were not reported by any patient.

1.11 SCOPE OF THE PRESENT INVESTIGATION

1.11.1 The investigation described in the subsequent pages may be divided broadly into two parts. The first part is a survey of a variety of biochemical parameters in blood of patients suffering from acute myocardial infarction and is compared with age and sex matched healthy controls. The parameters investigated are lipids, lipoprotein profile, serum enzymes, fibrinogen, platelets, Immunoglobulins lipid peroxidation products and antioxidant enzymes in RBC and leucocyte lipids and cholesterol esterifying agents.
1.11.2 The second part describes the changes during (modified) Anna Pavala Sindhooram therapy administered to 50 patients with a history of ischaemic heart disease. The therapy was followed for a period of 3 years in case of 12 patients, 2 years for 18, 18 months in 27, 12 months in 41 and 6 months in 50 cases. Blood analysis were made before and after 2nd, 4th, 6th, 12th, 18th, 24th, 32nd and 36th month of APSm administration.

1.11.3 Blood analysis in the survey as well as the clinical trials included the following assays.

1.11.3.1 Plasma lipids and lipoprotein profile together with lipid peroxidation, fibrinogen, prothrombin time and serum immunoglobulins were assayed.

1.11.3.2 Lipid peroxidation in the erythrocytes and their membranes were studied along with the antiperoxidative enzymes superoxide dismutase, glutathione peroxidase and catalase. Serum enzymes creatine phosphokinase (CPK), lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and gammaglutamyl transpeptidase (GGTP) were assayed.

1.11.3.3 Cholesterol ester is the major lipid that accumulates in atherosclerosis. Hence the enzymes responsible for cholesterol esterification and its hydrolysis namely, cholesterol ester synthetase and cholesterol ester hydrolase were assayed in the leucocytes.

1.11.3.4 Toxicity in organ systems in the body was studied by assaying blood sugar, urea, hemoglobin and platelet in the blood and serum uric acid.

The observations made are discussed in light of the literature available.