CHAPTER 1
1. INTRODUCTION
Cardiovascular diseases (CVDs), currently the leading cause of death and illness in developed countries, will soon become the pre-eminent health problem worldwide.\(^1\) CVDs are the major causes of death in adults in most developed and many developing countries, and are now the commonest cause of death worldwide.\(^2\) It has been found that mortality and morbidity from Coronary Artery Disease (CAD) remain high in spite of traditional diagnostic and intervention methods aimed at identifying persons at risk.\(^3\) These disorders also lead to substantial morbidity and disability and are the leading source of the rising cost of health care.

1.1 FACTS ABOUT CARDIOVASCULAR DISEASE
More than 40% of all deaths in the U.S. are from CVDs, and a person has a greater chance of dying from heart disease than from cancer, AIDS, diabetes and accidents combined. One in 5 men and women has some form of CVDs. If all forms of major CVDs were eliminated, life expectancy would rise by almost 7 years.\(^4\) CVD claimed 39.4 percent of all deaths or 1 in every 2.5 deaths in the United States in 2000. CVD was about 60% of “total mentioned mortality.” This means that of over 2,400,000 deaths from all causes, CVD was listed as a primary or contributing cause in about 1,415,000 death certificates.

According to the Framingham Heart Study, dyslipidemia, which can range from hypercholesterolemia to hypoapolipoproteinemia, is one of many modifiable major risk factors for CAD. Hypertension, diabetes mellitus and tobacco smoking are the other major risk factors. More specifically, dyslipidemia can lead to the development of coronary atherosclerosis, which can be further accelerated in the presence of multiple risk factors.\(^5\)

A decade ago, the treatment of hypercholesterolemia and hypertension was expected to eliminate CAD by the end of the 20\(^{th}\) century. Lately, however, this optimistic prediction needed revision. CVDs are expected to be the main cause of death globally within the next 15 years owing to a rapidly increasing prevalence in developing countries and Eastern Europe and the rising incidence of obesity and diabetes in the western world. Since 1900, CVD has been the No. 1 killer in the United States. Every year nearly 2,600 Americans die of CVD each day, an average of 1 death every 33 seconds. CVD claims more lives each year than the next 5 leading causes of death combined, which are
cancer, chronic lower respiratory diseases, accidents, diabetes mellitus and pneumonia. Almost 150,000 Americans under age of 65 years are killed by CVD each year.\textsuperscript{6} In comparison amongst UK and South Asians, mortality from coronary heart disease (CHD) is approximately 40\% higher, and admission rates with myocardial infarction in one study were 2-fold higher when compared with white Europeans. The increase in CHD risk is evident in each of the major South Asian subgroups and is most striking in young males, amongst whom CHD mortality is at least two-fold higher than in their white European counterparts.

The risk of CAD in Indians is 3-4 times higher than White Americans, 6-times higher than Chinese, and 20-times higher than Japanese.\textsuperscript{7} Indians are prone as a community to CAD at a much younger age.\textsuperscript{9} The disease pattern is severe and diffuse. Premature CAD is defined as cardiac event occurring before the age of 55 in men and 65 in women. In its severe form, it is defined as CAD occurring below the age of 40 years. CAD is affecting Indians 5-10 years earlier than other communities. Indians also show higher incidence of hospitalization, morbidity, and mortality than other ethnic groups.\textsuperscript{9} This global phenomenon of prematurity and severity suggests that the disease starts at an early age and has a malignant and progressive course.\textsuperscript{10}

The Global Burden of Diseases (GBD) study reported the estimated mortality from CHD in India at 1.6 million in the year 2005. Hospital statistics reveal that 20-25\% of all medical admissions are due to CHD. The economic costs of CHD are poorly understood. It is calculated that India annually spends about Rs. 100 billion as direct costs of treatment.\textsuperscript{11} It is reported that mortality from CVDs was projected to decline in developed countries from 1970 to 2015 while it was projected to almost double in the developing countries.\textsuperscript{12}

\textbf{1.2 HYPERLIPIDEMIA}

According to American Heart Association, the major cause for the CAD is hyperlipidemia. \textit{Hyperlipidemia} is defined as an excess of fatty substances called lipids, largely cholesterol and triglycerides, in the blood. It is also called \textit{hyperlipoproteinemia} because these fatty substances travel in the blood along with proteins. This is the only way that these fatty substances can remain dissolved while in circulation. Hyperlipidemia, in general, can be divided into two subcategories:

\begin{itemize}
  \item \textit{Hypercholesterolemia}, in which there is a high level of cholesterol.
\end{itemize}
Hypertriglyceridemia, in which there is a high level of triglycerides, the most common form of fat.

1.2.1 Description of hyperlipidemia

The fat-protein complexes in the blood are called lipoproteins. Lipoproteins have been separated based on their electrophoretic mobility mainly into four classes: High density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons. Excess LDL cholesterol (LDL-C) contributes to the blockage of arteries, which eventually leads to heart attack. Population studies have clearly shown that the higher the level of LDL-C, the greater the risk of heart disease. This is true in men and women, in different racial and ethnic groups, and in all adult age groups. Hence, LDL-C has been labeled the “bad” cholesterol. In contrast, the lower the level of HDL-C, the greater the risk of coronary heart disease. As a result, HDL-C is commonly referred to as the “good” cholesterol. Low HDL-C levels are typically accompanied by an increase in blood triglyceride levels. Studies have shown that high triglyceride levels are associated with an increased risk of CHD.

1.2.2 Classification of plasma lipid levels

National Cholesterol Education Program (NCEP) Adult Treatment Panel III provides the following guidelines for plasma lipid levels. 15

<table>
<thead>
<tr>
<th>1.2.2.1 Total cholesterol</th>
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<tbody>
<tr>
<td>&lt;200 mg/dl</td>
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<tr>
<td>200-239 mg/dl</td>
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<tr>
<td>≥240 mg/dl</td>
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<table>
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<tr>
<th>1.2.2.2 HDL-cholesterol</th>
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<td>&lt;40 mg/dl</td>
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<tr>
<td>&gt; 60 mg/dl</td>
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<td>&lt;70 mg/dl</td>
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<tr>
<td>&lt;100 mg/dl</td>
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<tr>
<td>100-129 mg/dl</td>
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<tr>
<td>130-159 mg/dl</td>
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1.2.2.4 Triglycerides

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<tbody>
<tr>
<td>160-189 mg/dl</td>
<td>High</td>
</tr>
<tr>
<td>≥ 190 mg/dl</td>
<td>Very high</td>
</tr>
</tbody>
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1.3 Atherosclerosis

The word atherosclerosis is of Greek origin. “Athero” means gruel, focal accumulation of lipids and “sclerosis” means hardening, thickening of arterial intima. Atherosclerosis is a disease of blood vessels characterized by accumulation of lipids in the innermost layers of the large and medium sized arteries, resulting in narrowing of passageway, loss of elasticity and weakening of arterial wall. This causes blockade of arteries i.e. ischemia and prevents oxygen-rich blood to the heart leading to sudden cardiac arrest.

Atherosclerotic plaque consists of cells, connective-tissue elements, lipids, and debris. Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular endothelial and smooth muscle cells. The atheroma is preceded by a fatty streak, an accumulation of lipid-laden cells beneath the endothelium. Most of these cells in the fatty streak are macrophages, together with some T cells. Fatty streaks are prevalent in young people, never cause symptoms, and may progress to atheroma or eventually disappear.

Atherosclerosis lesion has been observed to arise at region of the vessel wall exhibiting endothelial activation. Possible causes of such activation comprise elevated and modified LDL, free radicals arising during oxidative stress and cigarette smoking, hypertension, diabetes mellitus, genetic alterations, hyperactive monocytes/platelets, chronic infections such as by microorganisms like herpes, *Chlamydia pneumonia* infection or some unrecognized factors.

1.3.1 Risk factors for atherosclerosis

Several risk factors are involved in pathogenesis of atherosclerosis.
1.3.1.1 Non modifiable risk factors

a. Age
Age is the strong risk factor for the development of CAD. For persons living in most industrialized countries, cholesterol and triglycerides (TG) levels increase through middle age. In men, mean levels of total cholesterol (TC) increase until about age 50, then plateau, and then decrease starting at about age 70. In women, they increase more gradually up to age 65 to 69, then decrease. At about age 55 to 60, women have higher TC levels than men do.

b. Gender
Men traditionally have a higher incidence of CAD. TG levels progressively increase from birth through adulthood. The rate of increase is greater in men than in women. TG levels increase until age 55 in men and until about age 70 in women, then decrease gradually. In men, mean levels of HDL-C decrease at puberty, increase at about age 45, and then levels off at age 50 to 59.

1.3.1.2 Modifiable risk factors

a. Cigarette smoking
Smokers in their thirties and forties have heart attack rate five times higher than their nonsmoking counterparts. Cigarette smoking may be directly responsible for at least 20% of all deaths from heart disease or about 120,000 deaths annually.

b. Sedentary life style
People who are sedentary are almost as twice likely to suffer from heart attacks as people who exercise regularly. Inactive patients tend to have higher serum lipid levels and they have more LDL levels. Regular moderate aerobic exercise increase HDL/LDL ratio, increase caloric expenditures, benefits the heart by reducing rate, by increasing its efficiency, and by enhancing the development of communication between the right and left coronary arteries.

c. Alcohol
The effect of alcohol on heart disease varies depending on consumption. Evidence strongly suggests that light to moderate alcohol consumption (one or two drinks a day) protects the heart, even in people with type 2 diabetes. The benefits are strongest in people at high risk for heart disease and may be small in those with low risk. Large amount of alcohol can raise blood pressure, trigger irregular heartbeats and damage the heart muscle.
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d. Stress and psychological factors
Incidences of stress have been associated with a higher risk for serious cardiac events, such as heart rhythm abnormalities and heart attacks, and even death from such events in people with heart disease. In one 30 years study, men who were clinically depressed had a greater risk for heart disease and heart attack than men who were not depressed. The more severe the depression, the more dangerous to the health.

e. Obesity and Overweight
Obesity is related to hypertension, diabetes, abnormal cholesterol levels, and lack of exercise, all conditions contributing to heart attack risk. Obesity in children is a greater risk for future heart trouble than a family history of heart disease. People who are overweight in middle age may still not completely reduce their risk for CAD later in life, even if they lose excess weight. Abdominal obesity poses a particular risk.

f. Diet
Diet is also considered to be one of the major risk factors for cardiac events as intake food materials like fried foods, junk foods and high fat diet may cause increase in the levels of the total cholesterol. It is generally recommended that consumption of foods rich in soluble fibers and use of monosaturated fats like olive oil are good for health.

g. Hypertension
Elevated blood pressure (above 160/90) is important risk factor for atherosclerosis. After age of 45, hypertension is a greater risk factor than hyperlipidemia. Blood pressure of 160/90 mmHg or above is associated with a 5 times greater risk of developing atherosclerosis and myocardial infarction. Hypertension induces vascular resistance. This is mediated though increased Ca++ either by reduced nitric oxide (NO) synthase or by excess production of reactive oxygen species (ROS), which inhibit NO production and may lead to endothelial injury which trigger atherosclerotic events. High blood pressure increases the heart's workload, causing the heart to enlarge and weaken over time.

h. Diabetes mellitus and insulin resistance
Heart attacks account for 60% and strokes for 25% of all deaths in diabetics. Hyperglycemia contributes to interactions between endothelial functions producing abnormal responses of acetylcholine and increased production of Ca++, all of which contribute to release of endothelial vasoconstriction agents such as acetylcholine and endothelin-1. Hyperglycemia also accelerates the generation of free radical mediated LDL oxidation. Furthermore, available glucose can bind covalently to proteins by a process called glycation. This process increases the production of free radicals causing
glycoxidation and glycative stresses within the cell, which raises the quality of glycated LDL and atherogenic potential of LDL.

1.3.1.3 Emerging risk factors

a. Homocysteine
Abnormally high blood levels of the amino acid homocysteine are strongly linked to an increased risk of coronary diseases and stroke. Homocysteine may harm the lining of the arteries and reduce blood flow. An excessive level occurs with deficiencies of vitamins B₆, B₁₂ and folic acid. Some experts believe that high levels of homocysteine are only indicators, not causes of heart disease. However, studies are noting a strong association between homocysteine and heart disease.

b. Infectious agents
Some microorganisms and viruses have been suspected to trigger the inflammation and damage in the arteries that contributes to heart disease. The primary suspect has been *Chlamydia pneumonia* (non-bacterial organism that causes mild pneumonia in young adults). This is based on the following:

- High levels of antibodies against *Chlamydia pneumonia* have been associated with a higher risk for heart events.
- Pneumonia has been detected in plaques in the arteries of patients of heart disease.
- Animals incubated with organisms have developed hardening of the arteries.

c. Fibrinogen
Fibrinogen is the precursor of fibrin that influences blood viscosity, flow and coagulation. Raised levels promote platelet aggregation, fibrin and thrombus formation, which stimulates cell proliferation and plaque formation. These processes may also be involved in elevated levels of factor VII and plasminogen activator, which act as predictors of atherosclerosis.

d. Lipoprotein(a)
Lipoprotein(a) is a cholesterol rich lipoprotein with lipid content and size similar to LDL. Protein content of lipoprotein(a) is apoB100 (same as LDL) and apolipoprotein(a). A high plasma level of lipoprotein(a) is associated with increased risk of CAD, myocardial infarction, and angina pectoris. Elevated levels or Lp(a) (>33 mg/dl) is considered as a potential risk factor for CAD. Apolipoprotein(a) (Apo-a) is structurally related to plasminogen and competes with plasminogen and inhibits fibrinolysis and acceleration of atherogenesis. Lipoprotein(a) decreases plasmin synthesis leading to consecutive
decrease in active form of triglycerides that leads to increase proliferation and migration of smooth muscle cells (SMCs) into intima. Lp(a) is a competitive inhibitor of tissue plasminogen activator, inhibits tissue factor pathway and stimulates interleukin VIII. Other effects of lipoprotein(a) includes chemotactic activity on myocytes. It causes increase in expression of vascular cell adhesion molecule (VCAM) and E selectin. All participate in the acceleration of the atherogenic process.

e. C-reactive protein

C-reactive protein (CRP) is an acute phase protein level, which increases during infection and inflammation. It is a marker of systemic inflammation that predicts future risk of atherosclerosis. The precise biological function of CRP is not fully understood. It binds to a variety of substances including phosphocholine and recognizes several pathogens as well as phospholipid constituents of damaged cells. It activates complement system and increases activity of phagocytic cells. It initiates the elimination of targeted cells by its interaction with both the cellular and humoral effector systems. Elevated levels of CRP strongly predict future heart attacks in patients with existing heart disease, particularly unstable angina. Some studies have suggested that the protein itself may directly play a role in damage to heart muscles.

1.4 DIAGNOSIS

Diagnostic procedures required to measure the extent and severity of any CAD are as follows:

- **Electrocardiogram (ECG)**

  In this test, leads with wires (electrodes) are attached to the skin surface to measure electrical impulses given off by the heart. This test can show evidence of a previous heart attack or one that is in progress. Readings taken continuously over a period of 24 hours or longer may help detect silent ischemia. This technique is called ambulatory electrocardiography monitoring, or Holter monitoring. Recorded abnormalities may show evidences of inadequate blood supply to the heart.

- **Stress test**

  Stress tests help to measure whether heart is getting adequate blood supply. They may be used to evaluate symptoms such as chest pain or shortness of breath during exertion. During an exercise stress test, patient has to walk on a treadmill or a pedal of a stationary bike while an ECG records your heart's response to an increasing workload.
Nuclear scan
This test also helps in identifying problems in blood flow to the heart. Trace amounts of radioactive material, such as thallium or a compound known as Cardiolite, are injected into the blood stream. Special cameras can detect areas in the heart that receive inadequate less blood flow.

Echocardiogram
This test uses sound waves to produce an image of the heart. An echocardiogram can help identify an area of the heart that has been damaged from lack of blood supply.

Coronary angiography (arteriography)
It shows specific sites of narrowing in coronary arteries. A small tube (catheter) is inserted into an artery in arm or groin and threaded to the heart. A dye is injected into the catheter. As the dye flows through coronary arteries, narrow areas and blockages can be seen with the help of X-rays.

Blood tests
These tests include kidney and thyroid functions to check cholesterol levels and the presence of anemia. Occasionally, CRP levels are also monitored which gives indications about future risk for CVDs as follows:

<table>
<thead>
<tr>
<th>CRP</th>
<th>Risk for cardiovascular Disease</th>
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<tbody>
<tr>
<td>&lt; 1.0 mg/L</td>
<td>Low</td>
</tr>
<tr>
<td>1.0 -2.9 mg/L</td>
<td>Intermediate</td>
</tr>
<tr>
<td>&gt; 3.0 mg/L</td>
<td>High</td>
</tr>
</tbody>
</table>

Chest X-ray
It gives information about the size of the heart and presence of any fluid build up around the heart and lungs.

Electron beam computerized tomography (EBCT)
This test, also called as ultra fast CT scan, can detect calcium within plaques that narrow coronary arteries. Most, but not all, plaques contain some calcium. If a substantial amount of calcium is identified, CAD is likely.
1.5 PATHOPHYSIOLOGY OF ATHEROSCLEROSIS

A normal artery has 3 distinct layers:

**Intima**

*Intima* is the innermost layer and is composed of a single layer of endothelial cells on the luminal surface. Endothelial cells of the intima have a number of important functions: forming a nonthrombotic, nonadherent surface; acting as a semipermeable membrane; synthesizing and releasing chemical mediators; maintaining the basement membrane; and modifying lipoproteins as they cross into the artery wall.

**Media**

The middle layer, called as *media*, is a tube of vascular smooth muscle cells (VSMCs) and their extracellular matrix. VSMCs of the media contract and relax to alter the lumen diameter of the vessel in response to a variety of circulating and local stimuli, regulating vascular tone, blood flow, and blood pressure. This is caused by the production of a number of vasoactive substances, including prostaglandins, endothelin, and nitric oxide (NO).

**Adventia**

The outermost protective layer, called as *adventitia*, is made up of loose connective tissue that holds the blood vessels and nerves that the artery supplies.

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![Fig. 1](image-url)

**(a)** Anatomy of a normal artery **(b)** cross-section of arterial wall
Atherosclerotic lesions (atheromata) are asymmetric focal thickenings of the innermost layer of the artery, the intima. In the center of an atheroma, foam cells and extracellular lipid droplets form a core region, which is surrounded by a cap of smooth-muscle cells and a collagen-rich matrix. T cells, macrophages, and mast cells infiltrate the lesion and are particularly abundant in the shoulder region where the atheroma grows. Many of the immune cells exhibit signs of activation and produce inflammatory cytokines. Myocardial infarction occurs when the atheromatous process prevents blood flow through the coronary artery. It was previously thought that progressive luminal narrowing from continued growth of smooth-muscle cells in the plaque was the main cause of infarction. Angiographic studies have, however, identified culprit lesions that do not cause marked stenosis, and it is now evident that the activation of plaque rather than stenosis precipitates ischemia and infarction. Coronary spasm may be involved to some extent, but most cases of infarction are due to the formation of an occluding thrombus on the surface of the plaque.

There are two major causes of coronary thrombosis: plaque rupture and endothelial erosion. Plaque rupture, which is detectable in 60 to 70 percent of cases, is dangerous because it exposes prothrombotic material from the core of the plaque phospholipids, tissue factor, and platelet-adhesive matrix molecules to the blood. Ruptures preferentially occur where the fibrous cap is thin and partly destroyed. At these sites, activated immune cells are abundant. They produce numerous inflammatory molecules and proteolytic enzymes that can weaken the cap and activate cells in the core, transforming the stable plaque into a vulnerable, unstable structure that can rupture, induce a thrombus, and elicit an acute coronary syndrome. As the atherosclerotic lesion progresses, it may rupture exposing the rough surface and collagen to blood platelets leading to its activation, aggregation, adhesion and ultimately resulting in thrombus formation. Atheromas produce three negative effects:

- Narrowing of lumen of arteries causing decrease in blood flow (ischemia)
- Thrombosis and subsequent embolism (thromboembolism)
- Ballooning of the blood vessels by weakening the walls; resulting in an “aneurysm”.

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1.5.1 Steps involved in progression of atherosclerosis

Atherosclerosis is usually preceded by mainly following steps:

A. Endothelial Dysfunction

Primary causes of endothelial dysfunction are:

a. Elevated and modified LDL
b. Free radical and Reactive Oxygen Species (ROS)
c. Elevated plasma homocysteine
d. Hypertension
e. Diabetes mellitus
f. Genetic alterations
g. Infections by herpes virus, Chlamydia pneumonia

Endothelial dysfunction involves a series of early changes that precede lesion formation. The changes include greater permeability of lipoproteins, up regulation of leukocyte, endothelial adhesion molecules and migration of leukocytes into the artery wall. During the initiation, LDL accumulates in the subendothelial extracellular space within the arterial wall.

Local vascular cells mildly oxidize the LDL accumulated in the subendothelial extracellular space to a specific form known as minimally modified LDL (MMLDL), which is able to stimulate the recruitment of monocytes and eventual deposition of macrophages which upon further oxidation eventually becomes oxidized LDL. Specific adhesion molecules such as Von Willebrand factor, the selectins, and VCAM-1, expressed on the surface of activated vascular endothelial cells, mediate leukocyte adhesion. Once adherent, the mononuclear cells enter the artery wall directed by chemoattractant chemokines such as monocyte chemoattractant protein-1 (MCP-1). LDL particles trapped in the intima are prone to progressing oxidation, rendering them recognizable by macrophage scavenger receptors and thus targets for internalization by these cells. Upon extensive uptake of modified LDL via scavenger receptors (CD36 and SR-A), macrophages are ultimately turned into foam cells. Such cells form the earliest visible lesion, the fatty streak.
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Fig. 2 Endothelial dysfunction

B. Fatty-streak Formation

Fatty-streak formation occurs early with filtration by lipid-laden monocytes and macrophages along with T lymphocytes. Later on, lesions include smooth muscle cells. A complicated series of steps is involved, including smooth muscle migration, T-cell activation, foam cell formation, and platelet adherence and aggregation.

a) Smooth-muscle migration: Stimulated by platelet-derived growth factor, fibroblast growth factor-2, and transforming growth factor-β.

b) T-cell activation: Mediated by tumor necrosis factor-α, interleukin-2, and granulocyte-macrophage colony-stimulating factor.

c) Foam-cell formation: Mediated by oxidized low-density lipoprotein, macrophage colony-stimulating factor, tumor necrosis factor-α, and interleukin-1.

d) Platelet adherence and aggregation: Stimulated by integrins, P-selectin, fibrin, thromboxane A₂, tissue factor.
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As fatty streaks progress to intermediate and advanced lesions, they tend to form a fibrous cap that creates a barrier between lesion and the lumen. This represents a type of healing or fibrous response to the injury. The fibrous cap covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core. These lesions expand at their shoulders by means of continued leukocyte adhesion and entry caused by the same factors. The principle factors associated with macrophage accumulation include macrophage colony-stimulating factor, monocyte chemotactic protein-1, and oxidized low-density lipoprotein. The necrotic core represents the results of apoptosis and necrosis, increased proteolytic activity, and lipid accumulation. This core contains many lipid-rich macrophage foam cells derived from circulating monocytes. As these monocytes pass through the arterial wall, they produce tissue factor, a potent coagulant. This factor, when exposed to the blood, promotes coagulation and contributes to the thrombogenicity of plaques. Development of a strong fibrous cap favors atherosclerotic
plaque stability and protects tissue factor and other matrix elements in the core from making contact with the lumen and promoting thrombus formation.

D. Unstable Fibrous Plaques
Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap. Thinning of the fibrous cap is apparently due to the continuing influx and activation of macrophages, which release metalloproteinases (collagenase, elastase, stromelysin) and other proteolytic enzymes at these sites. These enzymes cause degradation of the matrix, which can lead to hemorrhage from the lumen of the artery. This causes thrombus formation and occlusion of the artery. Weakening of arterial walls by atherosclerotic plaque accumulation may result in aneurysm.

Fig. 4 Complete plaque formation
The atherosclerotic lesions could be classified as types I to VI, which range from minimal intimal change to changes associated with clinical manifestations (Table 1).

**Table 1: Stary's classification of atherosclerotic lesions**

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
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<tbody>
<tr>
<td>Type I</td>
<td>Isolated macrophage foam cells, no tissue injury</td>
</tr>
<tr>
<td>Type II</td>
<td>Fatty streak, foam cells lipid-laden smooth muscle cells under an intact endothelium</td>
</tr>
<tr>
<td>Type III</td>
<td>Type II lesions with increased extra cellular lipid and small lipid pool, microscopic evidence of tissue injury (preatheroma)</td>
</tr>
<tr>
<td>Type IV</td>
<td>Extensive lipid core, massive structural injury (atheroma)</td>
</tr>
<tr>
<td>Type V</td>
<td>Increased smooth muscle and collagen (fibroatheroma)</td>
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<tr>
<td></td>
<td>Va, multiple lipid core; Vb, calcific; Vc, fibrotic</td>
</tr>
<tr>
<td>Type VI</td>
<td>Thrombosis or hematoma</td>
</tr>
<tr>
<td></td>
<td>Vla, disruption of surface; Vlb, hematoma; Vlc, thrombosis</td>
</tr>
</tbody>
</table>

1.5.2 Lipoproteins, Lipid peroxidation and Atherosclerosis

1.5.2.1 LDL and atherosclerosis

Cholesterol, being a hydrophobic molecule, is unable to travel as such in the aqueous plasma medium and lipoproteins serve as carrier molecules to transport cholesterol in various tissues of the body. Fig. 5 represents a schematic diagram of a plasma lipoprotein.

Fig. 5 Schematic diagram of a plasma lipoprotein; ○- neutral core, ⬇️- phospholipid molecule with polar head, ⬇️- cholesterol, □- apoprotein, ⬇️- coat (shell), □- cholesteryl ester, ⬇️- triacyl glycerol
High cholesterol levels, more specifically, the low density lipoprotein LDL moiety, are well-recognized risk factors for CAD.\textsuperscript{23,24} The protective properties and significance of HDL are difficult to comprehend without a clear understanding of the role of LDL-C. Oxidized LDL is a major precursor of atherogenesis. Unlike LDL, HDL protects against atherogenesis through two mechanisms. First, HDL mediates the removal of excess cholesterol from peripheral tissues, such as blood vessels, and moves it back to the liver through a process known as Reverse Cholesterol Transport.\textsuperscript{25} Once cholesterol is in the liver, it can be excreted from the body in bile. Therefore, higher levels of HDL allow excretion of excess cholesterol. Second, HDL impedes oxidation of LDL.\textsuperscript{26} The Framingham Heart Study showed low HDL levels to be an independent risk factor for CAD, which showed 10% increase in CAD for each 4 mg/dl decrease in HDL.\textsuperscript{27} LDL becomes atherogenic when they are modified by oxidation. This process leads to foam-cell formation in arterial lesion. Oxidized LDL has a plethora of components that are not present in native LDL. Their presence and quantity depend on the nature, type and extent of oxidation. Lipids oxidize to form oxidized fatty acids which are the major components formed during the early phase of oxidation.

Fig. 6 Oxidation of LDL-C and foam cell formation
Modified LDL

A. Native LDL
It contains intact apoB-100 and no lipid peroxide or aldehydes. It is rich in polyunsaturated fatty acids (PUFA) and antioxidants. It is cleared by LDL receptor mediated uptake.

B. Seeded LDL
It contains intact apoB-100, enriched in PUFA and antioxidants. It is cleared by LDL receptor mediated uptake. Peroxidized lipid and other proteins generated in peripheral tissues including liver and intestine might become associated with the circulating LDL. It might have an increased propensity to undergo further oxidation. Since particle itself has not reacted with an oxidant, there is no loss of PUFA and antioxidants.

C. Mildly oxidized LDL
It contains intact apoB-100, decreased amounts of PUFA and antioxidants. Some of the lipids of LDL themselves might be oxidized. It is biologically active and cleared by LDL receptor mediated uptake. This de novo oxidation of intrinsic LDL lipid might occur under a wide variety of normal and pathological conditions contributed by xanthine oxidase, peroxynitrite and myeloperoxidase.

D. Extensively oxidized LDL
It contains degraded and cross-linked apoB-100. There is enormously decreased amount of PUFA and antioxidants. There is extensive oxidation of lipids of LDL and presence of massive amounts of lipids peroxides and non-lipid such as lysophosphatidylcholine. There is presence of degraded lipids such as core lipid aldehydes. Such LDL particles are recognized by the CD30 receptors.

1.5.2.2 Atherogenic effect of oxidized – LDL (Ox-LDL)
Most of the atherogenic effects of Ox-LDL are derived from the oxidized lipid components. The biologically active lipids of Ox-LDL include both esterified and unesterified peroxidation lipids, lysophosphatidylcholine, cholesterol oxidation products and aldehydes derived from breakdown of both esterified and unesterified oxidized lipids bound to fragmented apoB-100. These products are abundant in mildly or moderately Ox-LDL and have longer half-life. These components may initiate or promote atherogenesis. The induction of adhesion molecule for monocytes at the endothelial cell surface and the chemotactic process itself are affected by oxidized fatty acids and
oxidized phospholipids, respectively. Lysophosphatidylcholine is chemotactic to both monocytes and T-lymphocytes.

1.5.2.3 HDL and atherosclerosis
HDL is predominantly composed of phospholipids and apolipoproteins, with 20% of the mass consisting of the cholesteryl esters. Apolipoprotein A-I (70%) and apolipoprotein A-II (20%) account for most of the protein of HDL, with small amounts of apolipoprotein C, D and E. HDL is derived from two major pathways: a) direct secretion from the liver and intestine and b) formation from excess surface components produced during hydrolysis of chylomicrons and VLDL by lipoprotein lipase. Intact HDL are probably not secreted or formed directly, but HDL are first found as discoidal phospholipids apolipoprotein bilayers that acquire unesterified cholesterol efflux from peripheral cells. The unesterified cholesterol is then converted to cholesteryl esters in the HDL core by the plasma enzyme lecithin cholesterol acyl transferase (LCAT), which is activated by apolipoprotein A-I. The cholesteryl ester in HDL can then be transferred to "acceptor" lipoproteins, for example chylomicron remnants, VLDL and LDL by a process requiring lipid transfer proteins called cholesteryl ester transfer protein (CETP). Thus, HDL plays an important role in transporting cholesterol from peripheral tissues to the liver in a process of "reverse cholesterol transport".28

1.6 TREATMENT APPROACHES
If atherosclerosis leads to symptoms, some symptoms such as angina pectoris can be treated. Non-pharmaceutical means are usually the first method of treatment, such as cessation of smoking and regular exercise. If these methods do not work, medicines are usually the next step in treating CVDs. However, medicines are criticized for their expense, patented control and occasional undesired effects. Studies around the world indicate that fat and cholesterol are not the only nutrition concerns that relate to an attempt to prevent the heart disease, but a low fat, high fibre diet rich in unrefined complex carbohydrates, helps to lower the risk of heart disease and improve the heart health.29 Furthermore, fat rich in omega-3 fatty acids protects heart function.30,31

Lipid lowering therapies have been the most traditional, interesting treatment for most medicinal chemists, pharmacologists and physicians. These traditional therapies include HDL-elevating niacin, fibrates and the current statin drugs. Niacin (7) is recommended in many forms of primary hyperlipidemia and atherosclerosis. In order to eliminate the side
effects associated with niacin, the more potent and safe analog acipimox was developed and successfully launched.\textsuperscript{32}

Fibrates (1-3) and bile acids sequestrants (5,6) have also been widely used, particularly for hypertriglyceridemia and those patients with high LDL-C levels. The antioxidants vitamin E and probucol are already obsolete. Currently, the blockbuster molecules, hydroxymethylglutaryl coenzyme A (HMG-CoA) inhibitors, statins, are the best sellers among drugs in all therapeutic categories with or without ezetimibe (a cholesterol absorption inhibitor).

1.6.1 Current hypolipidemic agents
Clinical studies have exploited the advantages and discovered therapeutic niches for existing hypolipidemic drugs.\textsuperscript{33}

1.6.1.1 Fibric acid derivatives
The fibric acids are employed primarily for the treatment of combined hypertriglyceridemia (HT) and hypercholesterolemia (HC).\textsuperscript{34} Although no clear structural definition of these compounds appears to exist, the most potent possess an \( \alpha \)-dimethylacetic acid moiety attached by a 0-7 carbon spacer to a phenoxy moiety which may be further substituted. Clofibrate (1), gemfibrozil (2), fenofibrate (3) are commonly used fibrates.

\[
\text{(1)} \quad \text{(2)} \quad \text{(3)}
\]

Fibric acids are thought to lower plasma triglyceride concentrations by stimulating the catabolism of triglyceride-rich lipoproteins (VLDL). This is exerted through an increased activity of the enzyme lipoprotein lipase, responsible for triglyceride hydrolysis in VLDL.\textsuperscript{35}
More active fibrates have been developed, most notably beclobrate (4), which is about nine times more potent than gemfibrozil.\(^{36}\)

\[\text{Chemical structure of beclobrate} (4)\]

1.6.1.2 Bile acid sequestrants
Bile acid sequestrants are high molecular weight cationic ion exchange resins, which bind anionic bile acids in the intestine, thereby preventing their reabsorption in the liver. The consequence of the bile acid loss is a compensatory increase in cell-surface LDL receptors in the liver. This results in an enhanced uptake of LDL from plasma, with a reduction of LDL-C levels.\(^{37}\) Cholestyramine (5) and colestipol (6) and are the usually prescribed drugs in this category.

\[\text{Chemical structure of cholestyramine} (5)\]

\[\text{Chemical structure of colestipol} (6)\]

1.6.1.3 Nicotinates
Nicotinic acid (niacin, 7) was first prescribed for the treatment of hypercholesterolemia over 25 years ago and continued to be utilized despite of its well-known side effects.\(^{38}\) The hypolipidemic effects of niacin require larger doses than are required for its vitamin effects. Niacin is the best agent available for increasing HDL-C (increments of 30-40%); it also lowers triglycerides by 35-45% (as effectively as fibrates and the more potent statins) and reduces LDL-C levels by 20-30%.\(^{39-41}\) In adipose tissue, niacin inhibits the lipolysis of triglycerides by hormone-sensitive lipase, which reduces transport of free fatty acids to the liver and decreases hepatic triglyceride synthesis. Niacin may also inhibit a rate-limiting enzyme of triglyceride synthesis, diacyl-glycerol acyltransferase.\(^{42}\) Efforts
to dissect out untoward side effects have evolved the new analog acipimox (8), which is effective at lower doses with fewer side effects.\(^4^3\)

![Chemical Structures](https://via.placeholder.com/150)

### 1.6.1.4 Antioxidants

It is now widely accepted that oxidative modification of LDL accentuates its atherogenicity by a variety of mechanisms\(^4^4\) and that agents acting to inhibit this process may retard the atherosclerotic process.\(^4^5,4^6\) This view is supported by studies with the antioxidants vitamin E (9)\(^4^7\) and probucol (10), which clearly demonstrated their variety of antiatherogenic effects, which is supported by epidemiological data linking CHD with susceptibility of LDL to oxidation.\(^4^8\) The search for more potent antioxidants has primarily uncovered compounds similar to probucol or vitamin E, i.e. lipophilic hindered phenols.

![Chemical Structures](https://via.placeholder.com/150)

Noteworthy are the spirocycle (11), which was 10-100 times as potent as probucol against LDL oxidation by Cu\(^{2+}\) or endothelial cells\(^4^9\) and the tocopherol analog (12), which reduced serum cholesterol in a dose-dependent manner in mice.\(^5^0\) A 71% reduction in arterial lesion area in cholesterol-fed rabbits was observed upon administration of N,N'-diphenyl-p-phenylenediamine (13), accompanied by significant protection of the LDL from
Phenothiazine (14) was approximately ten times more potent than probucol at blocking Cu^{2+} catalysed oxidation of LDL. 

1.6.1.5 HMG-CoA Reductase inhibitors

The clinical success of the statins has led to newer synthetic molecules, which are more potent or equipotent on a milligram basis compared with synthetic simvastatin (15). All the statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme catalyzing rate-limiting step in cholesterol biosynthesis. The reduction of intrahepatic cholesterol stimulates synthesis of apo B/E (LDL) receptors in the liver, thereby enhancing removal of LDL and VLDL remnant particles from circulation and further lowering LDL. They also modestly increase HDL and lower triglyceride levels as VLDL remnant particles are cleared by newly synthesized apo B/E (LDL) receptors. Pravastatin (16), fluvastatin (17), atorvastatin (18) and rosuvastatin (19) are some of most effective and tolerated statins.
Clinical trials have documented the efficacy and safety of statins in reducing fatal and nonfatal coronary events, strokes and total mortality. Statins are also effective when
given in combination with other drugs such as colestipol, cholestyramine and fibrates. Triple therapy with resins, niacin and statins can reduce LDL-C by up to 70\%.

**Pleiotropic effect of statins**
Along with lipid lowering effect of statins, relevant additional properties that targets platelet and endothelial dysfunction, leukocyte adhesion, macrophage stimulation, inflammatory and procoagulant response, SMC transformation that impair progression of atherosclerosis and to stabilize the atherosclerotic plaque have also been observed.

**Side effects of statins**
Statins have been shown to deplete Coenzyme Q10. Coenzyme Q10 (Ubiquinone, CoQ10) is a natural, fat-soluble nutrient present in virtually every cell of the body. CoQ10 is the coenzyme for mitochondrial enzyme complexes involved in oxidative phosphorylation in the production of ATP. Statins used to treat elevated blood cholesterol levels by blocking cholesterol biosynthesis also block CoQ10 biosynthesis. The resulting lowering of blood CoQ10 level is due to the partially shared biosynthetic pathway of CoQ10 and cholesterol (Fig. 7).

A deficiency of CoQ10 in the blood and the heart muscle has been documented in congestive heart failure (CHF). The second fundamental property of CoQ10 involves its antioxidant (free radical scavenging) functions. Concurrent inhibition of CoQ10 is the prime reason for statin-induced rare but potentially severe side effects. Frequent side effects are no doubt a major reason why up to 75\% of people taking statins discontinue their use. Myopathy and rhabdomyolysis are the most frequent side effects caused by statins.
Fig. 7: The Mevalonic Acid Pathway of Cholesterol Biosynthesis
1.6.1.6 Cholesterol absorption inhibitors

Ezetimibe (20) is the first compound approved for lowering total and LDL-C levels that inhibits cholesterol absorption by enterocytes in the small intestine.

\[
\text{OH} \quad \text{OH} \\
\text{F} \quad \text{F} \\
(20)
\]

Ezetimibe inhibits a specific transport process in jejunal enterocytes, which take up cholesterol from the lumen. The putative transport protein is Niemann-pick C1 Like 1 (NPC1L1).\textsuperscript{75,76} Ezetimibe does not affect intestinal triglyceride absorption. Statins, which inhibit cholesterol biosynthesis, increase intestinal cholesterol absorption.\textsuperscript{77} Ezetimibe, which inhibits intestinal cholesterol absorption, enhances cholesterol biosynthesis by as much as 3.5 times in experimental animals.\textsuperscript{78} Dual therapy with these two classes of drugs prevents the enhanced cholesterol synthesis induced by ezetimibe and the increase in cholesterol absorption induced by statins. This combination provides additive reductions in LDL-C levels irrespective of the statin employed.\textsuperscript{79-81}

1.6.2 Novel approaches in the treatment of hyperlipidemia

The undisputed success of current therapy for hypercholesterolemia, particularly the statins, has not deterred interest in discovering novel methods to reduce serum cholesterol or retard the progression of atherosclerosis. The majority of work has focused on inhibiting cholesterol biosynthesis at points other than HMG CoA reductase (HR) and on altering the absorption of dietary cholesterol. Following are newer approaches for the treatment of hyperlipidemia and atherosclerosis.

1.6.2.1 HMG-CoA synthase (HS) inhibitors

HS catalyses the cholesterol biosynthetic step just prior to the reduction of HMG CoA (Fig. 7). Reports have shown L-659,699 (21) as a potential HS inhibitor.\textsuperscript{82} Structure Activity Relationship (SAR) work on 21 established that manipulation of the lipophilic side
Introduction

chain of activity, but the β-lactone can be substituted with an N-p-tosyl β-lactam (22). Subsequent analogous work identified 23 as one of the most potent of these tosyl lactams with an IC₅₀ value of 2.1 nM. A second class of inhibitors was isolated from fermentation broths; the most active compound 24 had a modest IC₅₀ value of 0.18 μM.

1.6.2.2 Squalene synthase (SS) inhibitors

The majority of efforts have focused on inhibition of SS, the first committed step in the biosynthesis of cholesterol (Fig. 7). The substrate for the enzyme, farnesyl pyrophosphate (FPP), is water soluble and easily metabolized, thereby potentially avoiding harmful effects due to accumulation of the substrate during inhibition. A potent inhibitor of SS is BMS-188,494 (25), which is a prodrug having the ability to lower cholesterol in rats after oral administration, a model insensitive to lipid lowering with statins.
SAR studies within a series of stable FPP analogs resulted in the potent competitive SS inhibitor 26 ($IC_{50} = 0.05 \mu M$), which proved to be two orders of magnitude more active than the closely-related compound 27, illustrating the importance of the ether oxygen for efficient binding to the enzyme.\(^{89}\)

\[
\begin{align*}
X &= \text{OCH}_2 \\
X &= \text{CH}_2\text{CH}_2
\end{align*}
\]

Azabicyclo[2.2.2]octane (28) inhibits rat liver microsomal SS with an $IC_{50}$ of 11 nM in the presence of pyrophosphate\(^ {90}\), but it belongs to series of compounds also reported to be 5-HT\(_3\) antagonists, causing doubt on its clinical potential.

A group of fungal metabolites was recently isolated that exhibits extremely potent inhibition of SS.\(^ {91}\) Due to their virtually simultaneous discovery by two different groups, they are known collectively as either zaragozic acids or squalestatins. Zaragozic acid C (29) has an $IC_{50}$ value of 9 nM against rat liver SS.\(^ {92}\) Administration of the closely related analog squalestatin 1 (30) subcutaneously to mice results in inhibition of hepatic cholesterol synthesis with an $ED_{50}$ of 0.2 mg/kg.\(^ {93,94}\)
Lipid lowering properties of TAK-475 (31) have been examined. Other novel SS inhibitors with potential hypocholesterolemic properties include RPR-107393 (32), ER-28448 (33) and ER-27856 (34).
1.6.2.3 Squalene epoxidase (SE) inhibitors
This enzyme catalyses another rate-determining step in cholesterol synthesis, viz. squalene epoxide from squalene (Fig. 7). Trinorsqualene alcohol (35) was one of the first squalenomimetics to effectively inhibit SE (IC$_{50}$ = 4 µM). The 1,1-difluorosqualene (36) is orally active in mice, as indicated by dose dependent reductions in hepatic cholesterol synthesis. Roughly equivalent in vitro potency is seen with cyclopropylamine (37) against rat hepatic SE.

\[
\text{(35) } R = \text{CH}_2\text{OH} \quad \text{(36) } R = \text{CH} = \text{CHF}_2 \quad \text{(37) } R = \text{H}_2\text{C} - \text{NH}
\]

NB-598 (38), having an IC$_{50}$ of 0.75 nM against HepG2 SE, effectively reduces serum cholesterol level and increases serum squalene in dogs following oral administration.

\[
\text{(38) } R_1 = \text{Et}, R_2 = \text{Ph}
\]

1.6.2.4 Oxidosqualene lanosterol cyclase (SLC) inhibitors
The physiological substrate for SLC, 2,3-oxidosqualene, has served as the template for a number of synthetic inhibitors (Fig. 7). Strong inhibition is obtained even with an amide such as azadecalin (39) (IC$_{50}$ = 0.7 µM) comparable to the older bicyclic amine (40). Roughly equivalent activity is observed with the monocyclic analog (41) also. The piperidine sulfone (42) retains a significant hepatic SLC inhibition (IC$_{50}$ = 5 µM) within a structure markedly simpler than the squalene analogs.
Another new SLC inhibitor, Ro48-8.071 (43) has shown effective lowering of plasma cholesterol in hamsters and squirrel monkeys when compared to simvastatin.\textsuperscript{108}

1.6.2.5 Lanosterol 14\(\alpha\)-demethylase (LDM) inhibitors
Inhibition of the cytochrome P-450 enzyme LDM has the potential to attenuate cholesterol biosynthesis by a dual mechanism - direct blockage of the conversion of lanosterol to cholesterol (Fig. 7) and indirect inhibition via a putative feedback mechanism whereby the accumulation of oxylanosterol intermediates (44 and 45) regulates the expression of HR.\textsuperscript{109} The ethenyl lanosterol (46) is reported to function as an irreversible inhibitor of rat liver LDM\textsuperscript{110} while the epoxide (47) is a competitive inhibitor with a \(K_i\) of 0.6 \(\mu\text{M}\).\textsuperscript{111} Lanosterols bearing substitution at the 15-carbon have been targeted due to their expected resistance to elimination of the 14-methyl group as formic acid, the final transformation induced by LDM. The oxime (48) is a modest inhibitor of LDM (\(IC_{50} = 55 \mu\text{M}\)), but is an effective oral hypocholesterolemic agent in hamsters.\textsuperscript{112}
1.6.2.6 Acyl-coenzyme A: cholesterol acyltransferase (ACAT) inhibitors

One possible therapy of current interest is prevention of the absorption of dietary cholesterol by inhibiting the enzyme ACAT, which catalyses the intracellular esterification of cholesterol with fatty acids. It is now widely accepted that ACAT plays a key role in both the absorption of dietary cholesterol and in the accumulation of cholesterol within arterial tissue, two processes that are intimately involved in the atherogenic process.\textsuperscript{113} Current research is strongly focused on developing inhibitors that act directly at the artery to determine if lesion development can be retarded.\textsuperscript{114} ACAT inhibitors have the potential to function as antiatherogenic as well as hypocholesterolemic agents.\textsuperscript{115-117} Cl-976 (49) decreases foam cell formation and cholesterol ester content in mechanically-induced arterial lesions in micropigs at a dose which does not alter plasma LDL-C or HDL-C levels.\textsuperscript{118} KF-17828 (50) is reported to accelerate the regression of hypercholesterolemia in previously cholesterol-fed hamsters, implying a systemic effect more profound than simple withdrawal of dietary cholesterol.\textsuperscript{119} Cl-1011 (Avasimibe) (51) has distinguished itself from all other agents by demonstrating cholesterol lowering in non-cholesterol fed animal models, a finding that may hold clinical significance as no other ACAT inhibitor has shown efficacy in this model.\textsuperscript{120}
Some of the most effective members of this class, which are currently in clinical development, are Cl-976 (49), DuP-128 (52), RP-70676 (53), YM17E (54), and 447C88 (55). An extensive review on selective ACAT inhibitors as promising antihyperlipidemic, antiatherosclerotic and anti-alzheimer drugs has been published.

1.6.2.7 ATP citrate lyase inhibitors
Mammalian ATP-citrate lyase is the main enzyme responsible for the supply of acetyl-CoA for synthetic pathways. The enzyme is present in most tissues but particularly in
those with an active *de novo* synthesis of fatty acids such as adipose tissue and liver, especially during conditions of carbohydrate surplus. ATP-citrate lyase is the only enzyme shared by the synthetic pathways of fatty acid and cholesterol synthesis. Due to this unique position, it has been proposed that inhibition of this enzyme may be more efficacious in correcting mixed hyperlipidemia than statins. A series of 2-substituted butanedioic acids have been designed and synthesized as inhibitors of the enzyme among which 56-59 are the most potent compounds.

\[
\begin{align*}
(56) & \quad X = \text{HOOC-} \quad (n = 6) \\
(57) & \quad X = \text{HOOC-} \quad (n = 6) \\
(58) & \quad X = \text{HOOC-} \quad (n = 6) \\
(59) & \quad X = \text{HOOC-} \quad (n = 7)
\end{align*}
\]

Efforts to discover more potent analogs acting through this mechanism have uncovered compounds SB-201076 (60) (Ki = 1 \(\mu\)M) and its \(\gamma\)-lactone prodrug SB-204990 (61).

\[
\begin{align*}
(60) \\
(61)
\end{align*}
\]

1.6.2.8 Cholesterol absorption inhibitors
In addition to inhibiting cholesterol biosynthesis, reducing dietary cholesterol intake by inhibiting absorption at the intestinal wall exists as an alternative method of reducing LDL-C. Several agents, which inhibit cholesterol absorption and reduce LDL-C in animal models through an unknown mechanism of action, have been reported to possess modest clinical efficacy. The synthetic plant saponin derivative pamaqueside (CP-148,623) (62) inhibited cholesterol absorption by 35-40% in normolipidemic individuals with a resulting 10-12% decrease in LDL-C at 300 mg twice daily. SAR studies indicated that modifications at the 4’- and 6’- positions of the sugar moiety of 62 resulted in analogs, exemplified by CP-242,184 (63), which are 50-100 times more potent in the cholesterol-fed hamster model.

1.6.2.9. Cholesteryl ester transfer protein (CETP) inhibitors

1.6.2.9.1. HDL, Reverse cholesterol transport and CETP

Reverse cholesterol Transport (RCT) involves the removal of excess free cholesterol from peripheral organs, specifically the macrophages in the arterial wall and its delivery to the liver for ultimate elimination through the biliary system into the gut and feces. The process of RCT begins with the interaction of lipid-poor ApoA-I with ATP binding cassette transporter A-1 (ABCA-1) on macrophages wherein free cholesterol and phospholipids from the macrophage are transferred to the lipid-poor ApoA-I. Lipidated ApoA-I is then able to remodel itself when lecithin cholesterol acyl transferase (LCAT) converts free cholesterol to cholesterol ester creating discoid small HDL3 particles, further
accumulation of cholesterol and cholesterol ester converts HDL3 to larger more spherical HDL2 particles.\textsuperscript{135} Cholesterol ester from HDL is then transferred to LDL/VLDL particles in exchange for triglycerides by a glycoprotein enzyme, cholesterol ester transfer protein (CETP).\textsuperscript{136} Cholesterol ester transferred to VLDL and LDL particles is delivered to the liver via the LDL-receptor pathway. HDL cholesterol ester may also be taken up by the liver through selective uptake via the Scavenger Receptor B-1 (SRB-1) pathway.\textsuperscript{137} RCT process, along with role CETP is depicted in Fig. 8.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig8.png}
\caption{The role of HDL and CETP in reverse cholesterol transport}
\end{figure}

1.6.2.9.2. CETP deficiency, HDL-C levels and CVDs
The crucial role of CETP in determining HDL-C levels was first identified in Japan where several cases of familial and marked elevations in HDL-C levels were shown to have heterozygous or homozygous loss-of-function mutations in the CETP gene.\textsuperscript{138} In a study of 201 patients with HDL-C> 100 mg/dl, 12 individuals were found to have atherosclerotic disease of which 10 were heterozygous for CETP deficiency.\textsuperscript{139} Additional observational studies have also suggested a relationship between elevated CETP activity and increased cardiovascular risk. In the REGRESS study, CETP levels were studied in 674 men with CHD who were randomized to pravastatin versus placebo.\textsuperscript{140} Men in the highest quartile for CETP concentrations had significantly greater progression of disease over two years as compared to men in the lowest quartile. The association between high
CETP levels and progression of disease as assessed by carotid intima-medial thickness was seen in 281 patients with familial hypercholesterolemia.\textsuperscript{141} An increase in fatal and nonfatal CHD was seen in participants in the EPIC-Norfolk Population Study with high CETP levels and low HDL-C levels.\textsuperscript{142} This increased risk was enhanced in those with elevated triglycerides above the median level as also observed in animal models.

**1.6.2.9.3. Is CETP proatherogenic or antiatherogenic (atheroprotective)?**

In view of the inverse relationship between CETP activity and HDL-C levels, it was hypothesized that pharmacological CETP inhibition could be atheroprotective through increase in HDL-C levels and enhanced RCT. In mice, which are normally CETP-deficient, overexpression of human CETP resulted in a 76\% reduction in HDL-C levels.\textsuperscript{143} Similarly, LDL receptor-knockout mice overexpressing the CETP gene develop more early atherosclerotic lesions than controls.\textsuperscript{144} These findings support a proatherogenic effect of increased CETP activity. By contrast, in a mouse model of hypertriglyceridemia from Apo C-III overexpression, CETP overexpression reduced lesions thus suggesting that increased CETP activity might be atheroprotective in the setting of elevated triglycerides.\textsuperscript{145} Similar atheroprotective effects of CETP overexpression were recently reported in transgenic mice deficient in SRB-1.\textsuperscript{146} Rabbits have high levels of CETP activity and thus provide a model for evaluating the effect of CETP inhibition on atherosclerosis. Increases in HDL-C levels and subsequent reductions in plaque have been seen in rabbits given CETP inhibitors.\textsuperscript{147} Despite these inconsistencies, continued development of CETP inhibition for HDL-C raising effect progressed to eventual testing in humans. Human studies of CETP inhibition have involved the evaluation of an anti-CETP vaccine and two oral agents, JTT-705 (64) and torcetrapib (65).\textsuperscript{148-150}
The anti-CETP vaccine was shown to produce anti-CETP antibodies in 53% of individuals who received two injections but the effect on HDL-C was unimpressive. Two orally effective CETP inhibitors have been evaluated in human clinical trials and have shown significant decreases in CETP activity and increases in HDL-C. The CETP inhibitor JTT-705 showed a dose-dependent decrease in CETP activity (37%) and increase in HDL-C levels (34%) as well as a small decrease in LDL levels (7%) at the highest 900 mg dose. JTT-705 has also been studied in combination with pravastatin in 155 subjects with LDL-C > 160 mg. Patients were on pravastatin 40 mg daily at baseline and were randomized to receive placebo, 300 or 600 mg of JTT-705. In the patients receiving 600 mg of JTT-705, there was a 30% reduction in CETP activity, a 28% increase in HDL-C and a 5% decrease in LDL-C. Similarly, in a phase I study, there was also a dose-dependent decrease in CETP activity and associated changes in lipids with the CETP inhibitor torcetrapib. HDL-C levels increased 91% at the 120 mg twice-daily dose as compared to a 16% elevation in HDL-C with the 10 mg daily dose (Fig. 9). The LDL-C also showed significant differences with a 9% increase at the lower dose and a 43% decrease at the 120 mg twice-daily dosing schedule. Additionally at the higher dose, Apo A-1 and Apo E increased by 27 and 66%, respectively, and Apo B decreased by 26%. These CETP inhibitors provide partial CETP inhibition, thus leading to production of HDL-C with higher free cholesterol/cholesterol ester ratio than seen in individuals with complete CETP deficiency.

Fig. 9: Dose-dependent increase in HDL-C with CETP inhibitor torcetrapib
Torcetrapib at a 60 mg dose (increasing HDL-C by 50–60%) in combination with atorvastatin was the first CETP inhibitor to advance all the way to Phase III clinical trials. However, this large Phase III multicenter trial involving the CETP inhibitor torcetrapib was stopped early because of a 61% increase in adverse cardiovascular events compared to control.\textsuperscript{156} The Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial was prematurely stopped on 2\textsuperscript{nd} December 2006 by the Data Safety Monitoring Board because of a 61% relative excess of the endpoints of death, myocardial infarction, angina, heart failure and revascularization in patients receiving torcetrapib. Previous studies had suggested a modest increase in blood pressure with torcetrapib.\textsuperscript{157} More detailed results of the ILLUMINATE trial were recently published.\textsuperscript{158} Contrary to expectation, overall mortality was increased in torcetrapib-treated patients with an increase in cardiovascular mortality as well mortality from infection and cancer (Table 2).\textsuperscript{158}

\textbf{Table 2: Summary of the major results of the ILLUMINATE trial}

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atorvastatin</th>
<th>Atorvastatin + Torcetrapib</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C % change</td>
<td>+1.8 ± 14.0</td>
<td>+72.1 ± 34.7</td>
</tr>
<tr>
<td>LDL-C % change</td>
<td>+3.0 ± 23.7</td>
<td>-24.9 ± 28.5</td>
</tr>
<tr>
<td>Apo A-1 % change</td>
<td>+1.3 ± 18.6</td>
<td>+25.3 ± 24.4</td>
</tr>
<tr>
<td>CRP absolute change</td>
<td>0</td>
<td>+0.04</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>+0.9 ± 11.5</td>
<td>+5.4 ± 13.2</td>
</tr>
<tr>
<td>All cause death</td>
<td>59 (0.8%)</td>
<td>93 (1.2%)</td>
</tr>
<tr>
<td>CHD mortality</td>
<td>33 (0.4%)</td>
<td>40 (0.5%)</td>
</tr>
<tr>
<td>Infection mortality</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Cancer mortality</td>
<td>14</td>
<td>24</td>
</tr>
</tbody>
</table>

These adverse outcomes occurred despite large increases in HDL-C and decreases in LDL-C levels with torcetrapib. Torcetrapib use was associated with an average of 5.4 mmHg increase in systolic blood pressure consistent with the results reported from earlier trials.\textsuperscript{159}
1.6.2.9.4. Small molecules CETP inhibitors

Remarkable progress has occurred particularly over the past 10 years such that multiple classes of inhibitors have now been identified that not only inhibit CETP-mediated transfer with at least nanomolar IC\textsubscript{50} values under buffered conditions but also display low micromolar to low nanomolar activity in the presence of human plasma.

a. Thiol-based CETP inhibitors

Crystal structure of human CETP reveals that it is monomeric in nature and contains seven cysteine (Cys) residues, suggesting that at least one of these thiol-containing amino acids is unpaired and available for modification.\textsuperscript{160} Several thiol-modifying inhibitors of CETP have been identified, and their sites of action have been mapped to specific CETP Cys residues.\textsuperscript{152} Several hydrophobic bis[o-amidophenyl]disulfides (66,67) (Japan Tobacco), have been identified as low-micromolar CETP inhibitors in vitro.\textsuperscript{149}

![Chemical structure]

(66) R = CH\textsubscript{3}  (67) R = \alpha\text{-}CH\textsubscript{3}\text{-}cyclohexyl

The most extensively studied and advanced of these sulfur-based inhibitors is represented by the thiol ester (64, JTT-705, Japan Tobacco).\textsuperscript{149} The bulky, highly substituted amido moiety in 64 was optimized starting from 66, where the R-methylcyclohexyl moiety in 67 (IC\textsubscript{50} = 8 \(\mu\)M) was nearly 60-fold more potent in human plasma assays than the original simple acetamide lead (66, IC\textsubscript{50} = 500 \(\mu\)M). Both the ortho orientation of the phenyl ring substituents and the need for the secondary amide in analogues of 66 were shown to be required for activity. Unfortunately, these disulfides were not orally bioavailable, and the corresponding free thiols though equally active, proved to be unstable. The isobutyryl ester analogue (64, JTT-705, IC\textsubscript{50} = 6 \(\mu\)M, human plasma) provided the optimal combination of chemical stability and oral absorption, thus acting as a potential prodrug for the free thiol derivative. Administering rabbits with a single oral dose of JTT-705 at 30 mg/kg inhibited 95% of the CETP in the isolated rabbit
plasma versus the vehicle alone, and this inhibition persisted for up to 9 h. Compound JTT-705 was also shown to have no significant interaction with or inhibitory effects on other lipid transfer proteins (PLTP) or enzymes involved with lipid metabolism such as HMG-CoA reductase or ACAT. Additional SAR studies of JTT-705 have been reported that explored modifications to the central benzene ring and identified the related 4,5-dichloropivaloyl thioester analogue (68, IC<sub>50</sub> = 2 µM, human plasma) having the extended R-(3-methylbutyl)cyclohexyl side chain as a slightly more potent inhibitor than JTT-705, although only in vitro data were disclosed.

![Chemical Structure](attachment:image)

\[\text{(68)}\]

b. 5,6,7,8-Tetrahydroquinolines and related CETP inhibitors

The first work in this area was reported by investigators from Bayer, who have recently described the discovery, key SAR findings, and lead optimization efforts for both their first- and second-generation CETP inhibitors. Starting from an initial series of highly functionalized 5-hydroxymethylpyridine based and benzyl alcohol based leads, the pentasubstituted pyridine (69) was identified as a lead candidate with nanomolar potency (IC<sub>50</sub> = 0.013 µM, buffer). Efforts to optimize metabolic stability of 69 provided unresolved racemic mixtures of related highly substituted secondary and tertiary tetrahydroquinolinols (70, 71, IC<sub>50</sub> = 0.006 µM).
Comparable in vitro potency data have been reported for 72 both as a racemic diastereomer and in its enantiomerically pure form, thus indicating the assay limitations at this potency. In this series, the related 2-isopropyl analogue (73) was about 2-fold less active \( (\text{IC}_{50} = 0.018 \text{ M}, \text{buffer}) \). After further evaluation, 72 was chosen as Bayer’s first clinical candidate.\(^{167}\)

c. 1,2,3,4-Tetrahydroquinolines and related CETP inhibitors

Pfizer has identified a completely distinct but related series of 4-amino-substituted 1,2,3,4-tetrahydroquinoline carbamates where the partial saturation occurs in the heterocyclic ring rather than the carbocyclic ring found in the Bayer leads. Optimization of this series led to an exciting new potent \( (\text{IC}_{50} = 0.05 \text{ M}, \text{human plasma}) \) chiral CETP inhibitor (65, torcetrapib, CP-529,414), for clinical development. The discovery and early optimization of torcetrapib have been reported starting from the initial 6,7-dimethoxy-4-aminotetrahydroquinoline lead (74) that had micromolar potency \( (\text{IC}_{50} = 10 \text{ M}) \) under
buffered conditions and retained its activity in the presence of human plasma (IC\textsubscript{50} = 25 µM).\textsuperscript{168}

\begin{align*}
\text{(74)} & \quad \text{O} \\
\text{H}_3\text{CH}_2\text{CO} & \quad \text{N} \\
\text{H}_3\text{CO} & \quad \text{CH}_3 \\
\text{H}_3\text{CO} & \quad \text{C} \text{OOCCH}_3 \\
\text{COOCCH}_3 & \quad \text{N} \\
& \quad \text{CH}_3 \text{COOCH}_2\text{CH}_3
\end{align*}

\begin{align*}
\text{(75) } \text{R} = \text{H} & \quad \text{(76) } \text{R} = \text{CF}_3 \\
\text{H}_3\text{CO} & \quad \text{N} \\
\text{H}_3\text{CO} & \quad \text{CH}_3 \\
\text{H}_3\text{CO} & \quad \text{C} \text{OOCCH}_3 \\
\text{COOCCH}_3 & \quad \text{N} \\
& \quad \text{CF}_3 \text{R} \quad \text{CH}_3 \text{COOCH}_2\text{CH}_3
\end{align*}

Introduction of one or more trifluoromethyl substituents into the benzylic ring of 74 significantly increased potency. The monotrifluoromethyl analogue (75, IC\textsubscript{50} = 0.5 µM buffer; IC\textsubscript{50} = 1.6 µM, human plasma) was approximately 20-fold more potent than 74, and the 3,5-bistrifluoromethyl derivative (76) was about 100-fold more potent (IC\textsubscript{50} = 0.005 µM buffer; IC\textsubscript{50} = 0.100 µM, human plasma) than 74.\textsuperscript{168}

d. \textit{N,N-Disubstituted trifluoro-3-amino-2-propanol CETP inhibitors}

In contrast to the fused bicyclic templates utilized at Bayer and Pfizer, investigators at Searle/Pharmacia have reported the discovery of an extremely potent acyclic inhibitor class, the trifluoro-3-(tertiary amino)-2-propanols.\textsuperscript{169} The most potent example reported from this series is the chiral R-enantiomer (77, SC-591), which contains an unusual 3-(1,1,2,2-tetrafluoroethoxy)benzylamine substituent.

\begin{align*}
\text{(77)} & \quad \text{O} \\
\text{H} & \quad \text{N} \\
\text{F}_3\text{C} & \quad \text{OH} \\
\text{H} & \quad \text{Cl} \\
\text{O} & \quad \text{CF}_2\text{CF}_2\text{H}
\end{align*}
e. *New exploratory classes*

Early reports in various patent applications indicate that there is a continued strong interest in identifying new structural motifs for CETP inhibitors, although it is still too soon to assess their overall pharmacological significance or clinical potential. In contrast to its first-generation candidate JTT-705, a covalent cysteine modifier of CETP, Japan Tobacco is exploring an alternative acyclic series with likely reversible binding behavior based on novel N,N-disubstituted aminotetrazoles (78, IC₅₀ = 0.08 μM, human plasma) with excellent leadlike activity in human plasma.¹⁷⁰

![Chemical structure of compound 78](image)

Acyclic acylated amino alcohols containing multiple aromatic rings have also been reported by Takeda with potent CETP inhibition properties, as represented by compound 79 (IC₅₀ = 0.008 μM, buffer; IC₅₀ = 0.08 μM, human plasma), in a patent application containing nearly 400 examples marking Takeda’s first entry into this field.¹⁷¹

![Chemical structure of compound 79](image)

Recent patent applications from the Bayer research group disclosed an interest in the chiral highly oxygenated dibenzodioxocin-5-one derivatives (80, IC₅₀ = 0.8 μM, buffer) as a completely new template for CETP inhibition.¹⁷²
Various natural products have activity as CETP inhibitors, and interest in this area continues. Two new total syntheses of the marine natural products (+)-chloropuupehenone (81, IC$_{50}$ = 0.3 µM, buffer) and the related (+)-chloropuupehenol (82, IC$_{50}$ = 31 µM, buffer) have recently been reported$^{173}$, and their individual CETP in vitro inhibition properties have been determined. However, because of their structural complexity, these compounds represent relatively weak inhibitors under buffered conditions compared to many of the other classes described above. No data in the presence of human serum have been reported for either of these compounds.

Xia Y, et al. reported some substitute 1,3,5-triazine derivatives as potential CETP inhibitors.$^{174}$ Among the synthesized compounds, 83 and 84 were found to be the most potent with IC$_{50}$ value of 9 µM and 5 µM, respectively.$^{174}$
A series of novel trifluoropropanols were synthesized and evaluated for its CETP inhibitory activity. Compounds 85 and 86 stood out as most promising leads with submicromolar potency \textit{in vitro} \((\text{IC}_{50} = 0.48 \text{ and } 0.72 \text{ M}, \text{ respectively})\).^{175}

A series of tetrazole and ester substituted tetrahydroquinoxalines were screened for their CETP inhibition potential. R-isomer of compound 87 was found to be extremely potent analog with \text{IC}_{50} \text{ value of } 143 \text{ nM}.^{176}
A series of 1,2,4-triazoles were patented as potential CETP inhibitors. Among the reported compounds, compound 88 stood out as the most potent with IC$_{50}$ value of 2 µM.

![Chemical structure of compound 88](image)

Compound 89 was identified as a hit from a fluorescence-based high-throughput screen of a series of 2-arylbenzoxazoles. Compound 89 displayed IC$_{50}$ value of 0.28 µM in a scintillation proximity assay.

![Chemical structure of compound 89](image)