CHAPTER II

LITERATURE REVIEW
2.0 LITERATURE REVIEW

Cholesterol is a fat like substance (lipid) that is present in cell membranes and is a precursor of bile acids and steroid hormones. Cholesterol travels in the blood in distinct particles containing both lipid and proteins (lipoproteins). Three major classes of lipoproteins are found in the serum of a fasting individual: low density lipoprotein (LDL), high density lipoproteins (HDL), and very low density lipoproteins (VLDL). Another lipoprotein class, intermediate density lipoprotein (IDL), resides between VLDL and LDL. LDL cholesterol typically makes up 60–70 percent of the total serum cholesterol. LDL is the major atherogenic lipoprotein. HDL cholesterol normally makes up 20–30 percent of the total serum cholesterol. HDL-cholesterol levels are inversely correlated with risk for CHD. Some evidence indicates that HDL protects against the development of atherosclerosis, although a low HDL level often reflects the presence of other atherogenic factors. The VLDL are triglyceride-rich lipoproteins, but contain 10–15 percent of the total serum cholesterol. VLDL are produced by the liver and are precursors of LDL; some forms of VLDL, particularly VLDL remnants, appear to promote atherosclerosis.

2.1 CHOLESTEROL BIOSYNTHESIS

The biosynthesis of cholesterol may be divided into five steps (Mayes PA, 2000):

1. Mevalonate (a six carbon compound is synthesized from acetyl CoA)
2. Isoprenoid units are formed from mevalonate by loss of CO₂
3. Six isoprenoid units condense to form the intermediate squalene
4. Squalene cyclizes to give rise to the parent steroid, Lanosterol.
5. Cholesterol is formed from lanosterol.

Step 1—Biosynthesis of Mevalonate: HMGCoA is formed by the reactions used in mitochondria to synthesize ketone bodies. However, since cholesterol synthesis is extramitochondrial, the two pathways are distinct. Initially, two molecules of acetyl-CoA condense to form acetoacetyl-CoA catalyzed by cytosolic thiolase. Acetoacetyl-CoA condenses with a further molecule of acetyl-CoA catalyzed by HMG-CoA synthase to form HMG-CoA, which is reduced to mevalonate by NADPH catalyzed by HMG-CoA reductase. This is the principal regulatory step in the pathway of
Cholesterol synthesis and is the site of action of the most effective class of cholesterol-lowering drugs, the HMG-CoA reductase inhibitors (statins)

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C} & \quad \text{C} \\
\text{S} & \quad \text{S} \\
\text{CoA} & \quad \text{CoA} \\
2 \text{ Acetyl-CoA} & \quad 2 \text{ Acetyl-CoA}
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{O} & \quad \text{H}_2\text{O} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{C} & \quad \text{C} \\
\text{S} & \quad \text{S} \\
\text{CoA} & \quad \text{CoA} \\
\text{Acetyl-CoA} & \quad \text{Acetyl-CoA}
\end{align*}
\]

\[
\begin{align*}
\text{OOC} & \quad \text{OOC} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{C} & \quad \text{C} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{S} & \quad \text{S} \\
\text{CoA} & \quad \text{CoA} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)

\[
\begin{align*}
\text{Bile acid, cholesterol} & \quad \text{Bile acid, cholesterol} \\
\text{HMG-CoA REDUCTASE} & \quad \text{HMG-CoA REDUCTASE} \\
\Theta & \quad \Theta \\
\text{Mevalonate} & \quad \text{Mevalonate} \\
\text{HMG-CoA REDUCTASE} & \quad \text{HMG-CoA REDUCTASE} \\
\Theta & \quad \Theta \\
\text{Mevalonate} & \quad \text{Mevalonate}
\end{align*}
\]

\[
\begin{align*}
\text{OOC} & \quad \text{OOC} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{C} & \quad \text{C} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

Fig 2.1: Biosynthesis of mevalonate.

Step 2—Formation of Isoprenoid Units: Mevalonate is phosphorylated sequentially by ATP to form several active phosphorylated intermediates. By means of a decarboxylation, the active isoprenoid unit, isopentenyl diphosphate, is formed.
Step 3—Six Isoprenoid Units Form Squalene: This stage involves the condensation of three molecules of isopentenyl diphosphate to form farnesyl diphosphate. This occurs via an isomerization of isopentenyl diphosphate involving a shift of the double bond to form dimethylallyl diphosphate, followed by condensation with another molecule of isopentenyl diphosphate to form the ten-carbon intermediate, geranyl diphosphate. A further condensation with isopentenyl diphosphate forms farnesyl diphosphate. Two molecules of farnesyl diphosphate condense at the diphosphate end in a reaction involving first an elimination of inorganic pyrophosphate to form presqualene diphosphate, followed by a reduction with NADPH with elimination of the remaining inorganic pyrophosphate radical. The resulting compound is squalene.

Step 4—Formation of Lanosterol: Squalene has a structure that closely resembles the steroid nucleus. Before ring closure occurs, squalene is converted to squalene 2,3-epoxide by a mixed-function oxidase in the endoplasmic reticulum, squalene epoxidase. The methyl group on C₁₄ is transferred to C₁₃ and that on C₈ to C₁₄ as cyclization occurs, catalyzed by oxidosqualene lanosterol cyclase.

Step 5—Formation of Cholesterol: The formation of cholesterol from lanosterol takes place in the membranes of the endoplasmic reticulum and involves changes in the steroid nucleus and side chain. The methyl group on C₁₄ is oxidized to CO₂ to form 14-desmethyl lanosterol. Likewise, two more methyl groups on C₄ are removed to produce zymosterol. The double bond at C₄–C₇ is subsequently moved to C₅–C₆ in two steps, forming desmosterol. Finally, the double bond of the side chain is reduced, producing cholesterol (Mayes, 2001).
Fig 2.2: Biosynthesis of cholesterol.

The numbered positions are those of the steroid nucleus and the open and solid circles indicate the fate of each of the carbons in the acetyl moiety of acetyl-CoA.
2.2 PLASMA LIPOPROTEIN METABOLISM

Lipoproteins are spherical particles made up of hundreds of lipid and protein molecules. They are smaller than red blood cells and visible only by electron microscopy. However, when the larger, triglyceride-rich lipoproteins are present in high concentration, plasma can appear turbid or milky to the naked eye. The major lipids of the lipoproteins are cholesterol, triglycerides, and phospholipids. Triglycerides and the esterified form of cholesterol (cholesteryl esters) are nonpolar lipids that are insoluble in aqueous environments (hydrophobic) and comprise the core of the lipoproteins. Phospholipids and a small quantity of free (unesterified) cholesterol, which are soluble in both lipid and aqueous environments (amphipathic), cover the surface of the particles, where they act as the interface between the plasma and core components. A family of proteins, the apolipoproteins, also occupies the surface of the lipoproteins. The apoproteins are very important since they provide structural stability to the lipoproteins, and a number of apoproteins function as ligands in lipoprotein-receptor interactions or are cofactors in enzymatic processes that regulate lipoprotein metabolism. In all spherical lipoproteins, the most water-insoluble lipids (cholesteryl esters and triglycerides) are core components, and the more polar, water-soluble components (apolipoproteins, phospholipids, and unesterified cholesterol) are located on the surface. These apolipoproteins include apolipoprotein (apo) A-I, apoA-II, apoA-IV, apoB-100, apoB-48, apoC-I, apoC-II, apoC-III, apoE, and apo (a). Except for apo (a), the lipid-binding regions of all apoproteins contain structural features called amphipathic helices that interact with the polar, hydrophilic lipids (such as surface phospholipids) and with the aqueous plasma environment in which the lipoproteins circulate. Differences in the non-lipid-binding regions are responsible for the functional specificities of the apolipoproteins. Lipoproteins have been classified on the basis of their densities into five major classes: (1) chylomicrons, (2) VLDL, (3) IDL, (4) LDL, and (5) HDL (Mahley RW and Bersot TP, 2001).

Chylomicrons

Chylomicrons are synthesized from the fatty acids of dietary triglycerides and cholesterol absorbed from the small intestine by epithelial cells. Triglyceride synthesis is regulated by diacylglycerol transferase, an enzyme that regulates triglyceride synthesis in many tissues (Mahley RW and Bersot TP, 2001). After they
are synthesized in the endoplasmic reticulum, triglycerides are transferred by microsomal triglyceride transfer protein (MTP) to the site where newly synthesized apoB-48 is available to form chylomicrons. Dietary cholesterol is esterified by one of two forms of the enzyme acyl coenzyme A: cholesterol acyltransferase (ACAT). This enzyme, ACAT-2, is found in the intestine and in the liver, where cellular free cholesterol is esterified before triglyceride-rich lipoproteins [chylomicrons and VLDL] are assembled. In the intestine, ACAT-2 regulates the absorption of dietary cholesterol, and it may be a potential pharmacological target for reducing blood cholesterol levels (Cases S et al., 1998). A second ACAT enzyme, ACAT-1, is expressed in macrophages, including foam cells, adrenocortical cells, and skin sebaceous glands. Although ACAT-1 esterifies cholesterol and promotes foam-cell development, ACAT-1 knockout mice do not have a reduced susceptibility for developing atherosclerosis (Accad M et al., 2000).

Chylomicrons are the largest of the plasma lipoproteins and are the only lipoproteins that float to the top of a tube of plasma that has been allowed to stand undisturbed for 12 hours. The buoyancy of chylomicrons reflects their 98% to 99% fat content, of which 85% is dietary triglyceride. In chylomicrons, the ratio of triglycerides to cholesterol is 10 or greater. In normolipidemic individuals, chylomicrons are present in plasma for 3 to 6 hours after a fat-containing meal has been ingested. After a fast of 10 to 12 hours, no chylomicrons remain. The apolipoproteins of chylomicrons include some (apoB-48, apoA-I, and apoA-IV) that are synthesized by intestinal epithelial cells and others (apoC-I, apoC-II, and apoC-III) acquired from HDL after chylomicrons have been secreted into the lymph and enter the plasma. The apoB-48 of chylomicrons is one of two forms of apoB present in lipoproteins. ApoB-48, synthesized only by intestinal epithelial cells, is unique to chylomicrons, whereas apoB-100 is synthesized by the liver and incorporated into VLDL and IDL and LDL, which are products of VLDL catabolism. The apparent molecular weight of apoB-48 is 48% that of apoB-100, which accounts for the name "apoB-48." (Innerarity TL et al., 1996). ApoB-48 does not contain the portion of the sequence of apoB-100 that allows apoB-100 to bind to the LDL receptor, so apoB-48 appears to function primarily as a structural component of chylomicrons (Mahley RW and Bersot TP, 2001).
After gaining entry to the circulation via the thoracic duct, chylomicrons are metabolized initially at the capillary luminal surface of tissues that synthesize lipoprotein lipase (LPL), a triglyceride hydrolase. These tissues include adipose tissue, skeletal and cardiac muscle, and breast tissue of lactating women. As the triglycerides are hydrolyzed by LPL, the resulting free fatty acids are taken up and utilized by the adjacent tissues. The interaction of chylomicrons and LPL requires apoC-II as an absolute cofactor that mediates the interaction of LPL and chylomicron triglycerides. The absence of functional LPL or functional apoC-II prevents the hydrolysis of triglycerides in chylomicrons and results in severe hypertriglyceridemia and pancreatitis during childhood or even infancy (chylomiconemia syndrome). The concentration of chylomicrons can be controlled only by reducing dietary fat consumption. There is no current therapeutic approach that will enhance chylomicron catabolism except for insulin replacement in patients with type I diabetes mellitus (Mahley RW and Bersot TP, 2001).

Chylomicon Remnants
After LPL-mediated removal of much of the dietary triglycerides, the chylomicron remnants, which still contain all of the dietary cholesterol, detach from the capillary surface and within minutes are removed from the circulation by the liver in a multi-step process mediated by apoE (Mahley RW and Ji ZS, 1999). First, the remnants are sequestered by the interaction of apoE with heparan sulfate proteoglycans on the surface of hepatocytes and are processed by hepatic lipase (HL), which further reduces the remnant triglyceride content. Next, apoE mediates remnant uptake by interacting with the hepatic LDL receptor or the LDL receptor–related protein (LRP). The LRP is a receptor with multiple functions that recognizes a variety of ligands—including apoE, HL, and LPL—and several ligands unrelated to lipid metabolism. In plasma lipid metabolism, the LRP is important because it is the backup receptor responsible for the uptake of apoE-enriched remnants of chylomicrons and VLDL. Cell-surface heparan sulfate proteoglycans facilitate the interaction of apoE-containing remnant lipoproteins with the LRP, which mediates uptake by hepatocytes. Inherited absence of either functional HL (very rare) or functional apoE impedes remnant clearance by the LDL receptor and the LRP, resulting in a hyperlipidemia characterized by an increase of triglyceride- and cholesterol-rich remnant lipoproteins in the plasma (type III hyperlipoproteinemia) (Mahley RW and Bersot TP, 2001).
Chylomicron remnants are not precursors of LDL. However, during the initial hydrolysis of chylomicron triglycerides by LPL, apoA-I and phospholipids are shed from the surface of chylomicrons and remain in the plasma. This is one mechanism by which nascent (precursor) HDL are generated (Mahley RW and Bersot TP, 2001).

Very-Low-Density Lipoproteins
VLDL are produced in the liver and are synthesized when triglyceride production is stimulated by an increased flux of free fatty acids or by increased de novo synthesis of fatty acids by the liver. The VLDL are 400 to 1000 Å in diameter and are large enough to cause plasma turbidity, but VLDL particles, unlike chylomicrons, do not float spontaneously to the top of a tube of plasma that is allowed to stand undisturbed for 12 hours (Mahley RW and Bersot TP, 2001).

Apo B-100, apoE, and apoC-I, C-II, and C-III are synthesized constitutively by the liver and incorporated into VLDL. If triglycerides are not available to form VLDL, the newly synthesized apoB-100 is degraded by hepatocytes. Triglycerides are synthesized in the endoplasmic reticulum and, along with other lipid constituents, are transferred by MTP to the site in the endoplasmic reticulum where newly synthesized apoB-100 is available to form nascent (precursor) VLDL. The nascent VLDL incorporate small amounts of apoE and the C apoproteins within the liver before secretion, but most of these apoproteins are acquired from plasma HDL after the VLDL are secreted by the liver. Without MTP, hepatic triglycerides cannot be transferred to apoB-100. As a consequence, patients with dysfunctional MTP fail to make any of the apoB-containing lipoproteins (VLDL, IDL, or LDL). MTP also plays a key role in the synthesis of chylomicrons in the intestine, and mutations of MTP that result in the inability of triglycerides to be transferred to either apoB-100 in the liver or apoB-48 in the intestine prevent VLDL and chylomicron production and cause the genetic disorder abetalipoproteinemia (Mahley RW and Bersot TP, 2001).

Plasma VLDL are then catabolized by LPL in the capillary beds in a process similar to the lipolytic processing described for chylomicrons. When triglyceride hydrolysis is nearly complete, the VLDL remnants, usually termed IDL, are released from the capillary endothelium and reenter the circulation. ApoB-100–containing small VLDL
and IDL (VLDL remnants), which have a half-life of less than 30 minutes, have two potential fates. About 40% to 60% are bound by LDL receptors or the LRP, which recognizes ligands (apoB-100 and apoE) on the remnants, and are cleared from the plasma primarily by the liver. The remainder of the IDL are further acted upon by LPL and HL—which remove additional triglycerides, C apoproteins, and apoE—and are converted to plasma LDL. Virtually all LDL particles in the plasma are derived from VLDL (Mahley RW and Bersot TP, 2001).

ApoE plays a major role in the metabolism of triglyceride-rich lipoproteins (chylomicrons, chylomicron remnants, VLDL, and IDL) and has a number of major functions related to the binding and uptake of plasma lipoproteins and to the redistribution of lipids locally among cells. About half of the apoE in the plasma of fasting subjects is associated with triglyceride-rich lipoproteins, and the other half is a constituent of HDL. ApoE controls the catabolism of the apoE-containing lipoproteins by mediating their binding to cell-surface heparan sulfate proteoglycans (especially in the liver) and to LDL receptors and the LRP (Mahley and Ji ZS, 1999).

About three-fourths of the apoE in plasma is synthesized by the liver and the remainder by a variety of tissues. The brain is the second most abundant site of apoE mRNA synthesis, which occurs primarily in astrocytes. ApoE also is synthesized by macrophages, where it appears to play a role in modulating cholesterol accumulation. In transgenic mice, over expression of apoE by macrophages inhibits hypercholesterolemia-induced atherogenesis (Bellosta S et al., 1995 and Hasty AH et al., 1999). There are three commonly occurring alleles of the apoE gene (designated 2, 3, and 4) that occur with a frequency of 8%, 77%, and 15%, respectively. These alleles code for the three major forms of apoE: E2, E3, and E4. Consequently, there are three homozygous apoE phenotypes (E2/2, E3/3, and E4/4) and three heterozygous phenotypes (E2/3, E2/4, and E3/4). Approximately 60% of the population is homozygous for apoE3. Single amino acid substitutions result from the genetic polymorphisms in the apoE gene. ApoE2, with a cysteine at residue 158, differs from apoE3, which has arginine at this site. ApoE3, with a cysteine at residue 112, differs from apoE4, which has arginine at this site. These single amino differences affect both receptor binding and lipid binding of the three-apoE isoforms. Both apoE3 and apoE4 can bind to the LDL receptor, but apoE2 binds much less
effectively and, as a consequence, causes the remnant lipoprotein dyslipidemia of type III hyperlipoproteinemia. ApoE2 and apoE3 bind preferentially to the phospholipids of HDL, whereas apoE4 binds preferentially to VLDL triglycerides (Mahley RW and Bersot TP, 2001).

**Low-Density Lipoproteins**

The LDL particles arising from the catabolism of IDL have a half-life of 1.5 to 2 days, which accounts for the higher plasma concentration of LDL than of VLDL and IDL. In subjects without hypertriglyceridemia, two-thirds of plasma cholesterol is found in the LDL. Plasma clearance of LDL particles is mediated primarily by LDL receptors; a small component is mediated by nonreceptor clearance mechanisms. Defective or absent LDL receptors cause high levels of plasma LDL and familial hypercholesterolemia (Brown MS and Goldstein JL, 1986). ApoB-100, the only apoprotein of LDL, is the ligand that binds LDL to its receptor. The liver expresses a large complement of LDL receptors and removes 75% of all LDL from the plasma (Dietschy JM et al., 1993). Consequently, manipulation of hepatic LDL receptor expression is a most effective way to modulate plasma LDL and cholesterol levels. Thyroxine and estrogen enhance LDL receptor gene expression, which explains the LDL-cholesterol-lowering effects of these hormones (Windler EE et al., 1980; and Wiseman SA et al., 1993).

The most effective dietary (decreased consumption of saturated fat and cholesterol) and pharmacological (treatment with statins) treatments of hypercholesterolemia act by enhancing hepatic LDL receptor expression (Bilheimer DW et al., 1983; Woollett LA and Dietschy JM, 1994). Regulation of LDL receptor expression is part of a complex process by which cells regulate their free cholesterol content. This regulatory process is mediated by transcription factors called sterol regulatory binding element proteins (SREBPs). SREBPs enhance LDL receptor expression when cellular cholesterol content is reduced. LDL become atherogenic when they are modified by oxidation (Steinberg D, 1997). This process leads to foam-cell formation in arterial lesions (Nakata A et al., 1999; Dhaliwal BS and Steinbrecher UP, 1999).

**High-Density Lipoproteins**
HDL is the smallest of the five lipoprotein classes. HDL is rich in protein, containing approximately 50% of lipid and 50% of protein by weight. It contains a hydrophobic lipid core of cholesterol esters and triglycerides surrounded by phospholipids, unesterified cholesterol and apolipoproteins. Apoproteins (Apo)A-I and ApoA-II are the main structural proteins in HDL, accounting for approximately 70% and 20%, respectively, of total HDL protein mass. ApoA-I can exist on its own, and is present in all HDL subclasses, but ApoA-II is present only with ApoA-I. The two main HDL fractions, according to the ultracentrifugation density, are HDL2 and HDL3. HDL2 particles are less dense, having a higher relative amount of cholesterol and phospholipids than HDL3 particles (Tavintharan S et al., 2005).

The metabolism of HDL is complex because of the multiple mechanisms by which HDL particles are modified in the plasma compartment and by which HDL particles are synthesized (Segrest JP et al., 2000; and Tall AR et al., 2000). ApoA-I is the major HDL apoprotein, and its plasma concentration is a more powerful inverse predictor of CHD risk than is the HDL-C level (Maciejko JJ et al., 1983). ApoA-I synthesis is required for normal production of HDL. Mutations in the apoA-I gene that cause HDL deficiency are variable in their clinical expression and often are associated with accelerated atherogenesis. Conversely, over expression of apoA-I in transgenic mice protects against experimentally induced atherogenesis (Plump AS et al., 1994).

Mature HDL can be separated by ultra centrifugation into HDL2 (d = 1.063 to 1.125 g/ml), which are larger, more cholesterol-rich lipoproteins (70 to 100 Å in diameter), and HDL3 (d = 1.125 to 1.21 g/ml), which are smaller particles (50 to 70 Å in diameter). In addition, two major subclasses of mature HDL particles in the plasma can be differentiated by their content of the major HDL apoproteins, apoA-I and apoA-II. Mature HDL particles have electrophoretic mobility. Some migrating HDL particles contain only apoA-I and no apoA-II and are called LpA-I HDL particles. Others contain both apoA-I and apoA-II and are called LpA-I/A-II HDL particles. These two particles usually are separated by electro immunoassay and quantitated by assessment of their apoA-I content. LpA-I particles are larger than LpA-I/A-II and are primarily associated with HDL2. LpA-I/A-II particles are smaller and are primarily associated with HDL3. Patients with reduced HDL-C levels and CHD have lower
levels of LpA-I, but not of LpA-I/A-II, than subjects with normal HDL-C levels (Duriez P and Fruchart JC, 1999). This finding suggests that HDL particles containing apoA-I and apoA-II may not be atheroprotective. In fact, over expression of apoA-II in transgenic mice enhances susceptibility to atherosclerosis (Schultz JR et al., 1993). ApoA-II deficiency is not associated with any apparent deleterious effects in human beings (Deeb SS et al., 1990).

The precursor of most of the plasma HDL is a discoidal particle containing apoA-I and phospholipid, called pre-1 HDL because of its pre-1 electrophoretic mobility. Pre-1 HDL are synthesized by the liver and the intestine, and they also arise when surface phospholipids and apoA-I of chylomicrons and VLDL are lost as the triglycerides of these lipoproteins are hydrolyzed. Phospholipid transfer protein plays an important role in the transfer of phospholipids to HDL (Tall AR et al., 2000).

Discoidal pre-1 HDL can then acquire free (unesterified) cholesterol from the cell membranes of tissues, such as arterial wall macrophages, by an interaction with the class B, type I scavenger receptor (SR-BI), to which the apoA-I of HDL docks, so that free cholesterol can be transferred to or from the HDL particle. SR-BI facilitates the movement of excess free cholesterol from cells with excess cholesterol (e.g., arterial wall foam cells) (Williams DL et al., 1999). In the liver, SR-BI facilitates the uptake of cholesteryl esters from the HDL without internalizing and degrading the lipoproteins.

After free cholesterol is acquired by the pre-1 HDL, it is esterified by lecithin:cholesterol acyltransferase. The newly esterified and nonpolar cholesterol moves into the core of the discoidal HDL. As the cholesteryl ester content increases, the HDL particle becomes spherical and less dense. These newly formed spherical HDL particles (HDL3) further enlarge by accepting more free cholesterol, which is in turn esterified by lecithin: cholesterol acyltransferase. In this way, HDL3 are converted to HDL2, which are larger and less dense than HDL3 (Mahley RW and Bersot TP, 2001).

As the cholesteryl ester content of the HDL2 increases, the cholesteryl esters of these particles begin to be exchanged for triglycerides derived from any of the triglyceride-
containing lipoproteins (chylomicrons, VLDL, remnant lipoproteins, and LDL). This exchange is mediated by the cholesteryl ester transfer protein and, in human beings, accounts for the removal of about two-thirds of the cholesterol associated with HDL. The transferred cholesterol subsequently is metabolized as part of the lipoprotein into which it was transferred. The triglyceride that is transferred into HDL2 is hydrolyzed in the liver by HL, a process that regenerates smaller, spherical HDL3 particles that recirculate and acquire additional free cholesterol from tissues containing excess free cholesterol (Mahley RW and Bersot TP, 2001).

HL activity is regulated and modulates HDL cholesterol levels. Both androgens and estrogens affect HL gene expression, but with opposite effects (Haffner SM et al., 1983 and Brinton EA, 1996). Androgens increase HL activity, which accounts for the lower HDL cholesterol values observed in men than in women. Estrogens reduce HL activity, but their impact on HDL cholesterol levels in women is substantially less than that of androgens on HDL cholesterol levels in men. HL appears to have a pivotal role in regulating HDL cholesterol levels, as HL activity is increased in many patients with low HDL cholesterol levels (Mahley RW and Bersot TP, 2001).

HDL are protective lipoproteins that decrease the risk of CHD; thus, high levels of HDL are desirable. This protective effect may result from the participation of HDL in reverse cholesterol transport, the process by which excess cholesterol is acquired from cells and transferred to the liver for excretion. HDL also may inhibit oxidative modification of LDL through the action of paraoxonase, an HDL-associated antioxidant protein (Mahley RW and Bersot TP, 2001).

### 2.3 PRIMARY DISORDERS OF PLASMA LIPOPROTEINS

Table 2.1: Primary disorders of plasma lipoproteins (Mayes PA, 2000)

<table>
<thead>
<tr>
<th>NAME</th>
<th>DEFECT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypolipoproteinemia</td>
<td>No chylomicrons, VLDL, or LDL are formed because of defect in triacylglycerol transfer protein (MTP), which prevents the loading of apo B with lipid</td>
<td>Rare; blood acylglycerols low; intestine and liver accumulate acylglycerols intestinal malabsorption Early death, which can be avoided by administration of large doses of fat soluble vitamins, particularly vitamin E</td>
</tr>
<tr>
<td>Abetalipoproteinemia</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.1: Primary disorders of plasma lipoproteins (Contd....)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Description</th>
<th>Chylomicron formation still occurs; most individuals healthy and long living</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial Hypolipoproteinemia</td>
<td>LDL concentration is 10-60% of normal Truncated apo B series present.</td>
<td>No impairment of chylomicron or VLDL formation No post-beta-lipoprotein but broad beta band on agarose electrophoresis; tendency toward hypertriglyceridemia as a result of absence of apo C-II, causing inactive LPL Low LDL levels Atherosclerosis in the elderly</td>
</tr>
<tr>
<td>Familial alpha-lipoprotein deficiency</td>
<td>Tangier disease Fish-eye disease Apo-A-I deficiencies</td>
<td>All have low or near absence of HDL Treated by reducing fat and increasing complex carbohydrates in diet</td>
</tr>
<tr>
<td>Familial hypercholesterolemia (type II)</td>
<td>Wolman’s disease (cholesteryl ester storage disease)</td>
<td>Deficiency of cholestereryl ester hydrolase in lysosomes of cells such as fibroblasts that normally metabolize LDL</td>
</tr>
<tr>
<td>Familial type III</td>
<td>Hypolipoproteinemia (broad beta disease), remnant removal disease, familial dysbetalipoproteinemia</td>
<td>Deficiency in remnant clearance by the liver is due to abnormality in apo E, which is normally present in 3 isoforms: E3, E4, and E5. Patients only have E5, which does not react with E receptor</td>
</tr>
<tr>
<td>Familial Hypertriglyceridemia (type IV)</td>
<td>Familial (type V) hyperlipoproteinemia</td>
<td>Over production of VLDL often associated with glucose intolerance and hyperinsulinemia, which may be the cause of overproduction Cholesterol levels rise with the VLDL concentration. HDL &amp; LDL tend to be subnormal. This type of pattern is commonly associated with CHD, type II non-insulin dependent diabetes mellitus, obesity, alcoholism, &amp; administration of prostaglandin E, &amp; atherosclerosis in peripheral &amp; coronary arteries</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>Familial Lipoprotein Lipase deficiency (type I)</td>
<td>Hypertriglyceridemia due to (a) Deficiency of LPL or (b) Production of Abnormal LPL or (c) Apo C-II deficiency causing inactive LPL Slow clearance of chylomicrons and VLDL Low levels of HDL and LDL Treat by reducing fat and increasing complex carbohydrates in diet No increased risk of coronary disease</td>
</tr>
<tr>
<td>Familial hypercholesterolemia (type II)</td>
<td>Type IIa: Defective LDL receptors or mutation in the ligand region of apo B-100 Type IIb: Tendency of VLDL to be elevated in addition Reduced rate of LDL clearance leads to elevated LDL levels and hypercholesterolemia, resulting in atherosclerosis and coronary disease</td>
<td></td>
</tr>
<tr>
<td>Familial (type III)</td>
<td>Hypolipoproteinemia (broad beta disease), remnant removal disease, familial dysbetalipoproteinemia</td>
<td>Deficiency in remnant clearance by the liver is due to abnormality in apo E, which is normally present in 3 isoforms: E3, E4, and E5. Patients only have E5, which does not react with E receptor</td>
</tr>
<tr>
<td>Familial Hypertriglyceridemia (type IV)</td>
<td>Familial (type V) hyperlipoproteinemia</td>
<td>Elevated chylomicrons and VLDL Not completely understood, but defects in LPL or apo-II are possible Hypertriglyceridemia and hypercholesterolemia with low HDL and LDL Increased coronary heart disease risk in some patients</td>
</tr>
</tbody>
</table>
2.4 HYPERCHOLESTEROLEMIA AND DYSLIPIDEMIA

Atherogenesis has been the subject of investigation for more than 300 years, yet understanding of cholesterol-deposition and plaque-formation processes continues to evolve. In 1695, Johann Conrad von Brunner illustrated autopsy-identified changes that he referred to as “hardening” of the aorta and major blood vessels. William Heberden penned a description of angina pectoris based on 20 cases in 1768, then expanded his observations to 100 cases in 1782. The next milestone was Karl Freiherr von Rokitansky’s 1852 description of atheroma pathology, which elucidated the “thrombogenic” or “encrustation” theory of atherosclerosis. A few years later, in 1856, Rudolf Virchow presented the “inflammatory” theory, which held that atherosclerosis is the result of intimal inflammation and subsequent fibrosis. Between 1909 and 1913, A. Ignatowsky, S. Saltykow, and N. N. Anitschkow developed the “lipid” theory of atherosclerosis, which linked dietary cholesterol to elevated circulating cholesterol and, in turn, to subintimal cholesterol deposits. Studies carried out in the developed world have documented a clear relationship between serum cholesterol levels and the risk of coronary heart disease (CHD) (LaRosa JC, 2003).

Coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease accounts for the majority of morbidity and mortality among middle-aged and older adults. Hyperlipidemia (hypercholesterolemia) is a major cause of increased...
atherogenic risk, and both genetic disorders and diets enriched in saturated fat and cholesterol contribute to the elevated lipid levels of our population and many other developed countries around the world. Despite a continuing decline in the incidence of atherosclerosis-related deaths in the past 35 years, deaths from CHD, cerebrovascular disease, and peripheral vascular disease accounted for 30% of the 2.3 million deaths in the United States during 1997. Two-thirds of atherosclerosis deaths were due to CHD. About 85% of CHD deaths occurred in individuals over 65 years of age. Among the 15% dying prematurely (below age 65), 80% died during their first CHD event. Among those dying of sudden cardiac death in 1997, 50% of the men and 63% of the women had been previously asymptomatic (American Heart Association, 1999). These statistics illustrate the importance of identifying and managing risk factors for CHD. The major known risk factors are elevated LDL-C, reduced HDL-C, cigarette smoking, hypertension, type 2 diabetes mellitus, advancing age, and a family history of premature (men < 55 years; women < 65 years) CHD events in a first-degree relative. Observational studies indicate that, when total cholesterol levels are below 160 mg/dl, CHD risk is markedly attenuated, even in the presence of additional risk factors (Grundy SM et al., 1998).

Recognition of hypercholesterolemia as a risk factor has led to the development of drugs that reduce cholesterol levels. These drugs have been used in well-controlled studies of patients with high cholesterol levels caused primarily by elevated levels of LDL. The results of these trials indicate that CHD mortality is reduced by as much as 30% to 40% and that nonfatal events are similarly reduced when hypercholesterolemic patients are treated with moderate doses of hypolipidemic drugs. Clinical trial data support extending the benefit of lipid-lowering therapy to high-risk patients whose major lipid risk factor is a reduced plasma level of HDL even if the LDL cholesterol levels of these patients do not meet the existing threshold values for initiating hypolipidemic drug therapy (The Expert Panel, 1993). In patients with low HDL and average LDL levels, appropriate drug therapy reduced CHD endpoint events by 20% to 35% (Downs JR et al., 1998; Rubins HB et al., 1999). Since 40% of patients with CHD in the United States have low HDL levels, it is of obvious importance to include low-HDL patients in management guidelines for dyslipidemia, even if their LDL levels are in the "normal" range (Rubins HB et al., 1995).
Hypertriglyceridemia (elevated levels of triglycerides), if severe (>1000 mg/dl), requires therapy to prevent pancreatitis. Moderately elevated triglyceride levels (150 to 400 mg/dl) also are of concern because they often occur as part of a syndrome distinguished by insulin resistance, obesity, hypertension, and substantially increased CHD risk. The atherogenic dyslipidemia in patients with this insulin resistance or metabolic syndrome is characterized by moderately elevated triglycerides, low HDL-C levels, and lipid-depleted LDL (sometimes referred to as "small, dense LDL") (Grundy SM, 1998). The metabolic syndrome is common in CHD patients; hence, identification of moderate hypertriglyceridemia in a patient, even if the total cholesterol level is normal, should trigger an evaluation to identify this disorder (National Cholesterol Education Program Expert Panel, 2001).

Hyperlipidemia (elevated levels of triglycerides or cholesterol) and reduced HDL-C levels occur as a consequence of several factors that affect the concentrations of the various plasma lipoproteins. These factors may be lifestyle or behavioral (e.g., diet or exercise), genetic (e.g., mutations in a gene regulating lipoprotein levels), or metabolic conditions (e.g., diabetes mellitus) that influence plasma lipoprotein metabolism. An understanding of these factors requires a brief description of lipoprotein metabolism (Mahley RW and Bersot TP, 2001).

**Coronary Heart Disease in Indians and South Asian (SA)**

The risk of coronary artery disease (CAD) in Indians is 3-4 times higher than White Americans, 6-times higher than Chinese, and 20-times higher than Japanese. Indians are prone as a community to CAD at a much younger age. The disease pattern is severe and diffuse. Premature CAD is defined as cardiac events 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 hospitalisation, morbidity, and mortality than other ethnic groups. This global phenomenon of prematurity and severity suggests that the disease starts at an early age and has a malignant and progressive course. There is a parallel corollary between CAD in Indians and the malignant course of rheumatic fever, rheumatic heart disease with associated severe pulmonary hypertension observed by Indian cardiologists in the sixties. In the
Western population, incidence of CAD in the young is up to 5% as compared to 12-16% in Indians. In some studies from India, the percentage of patients below the age of 45 years suffering from acute myocardial infarction (AMI) is reported as high as 25-40%. In Great Britain the first AMI among Indians at age less than 40 years is reported 10 times higher than local Whites. In Singapore, mortality from CAD below 30 years of age is 10 times higher in Indian than Chinese population of the same age group. Angiographically, Indians have 15 times higher rate of CAD than Chinese and 10 times higher rate than local Malays below the age of 40 years (Rissani HS et al., 2001).

The prevalence of CAD is two-times higher (10%) in urban than in rural India. South Indians have higher prevalence, 7% in rural and 14% in urban areas. The vulnerability of urban Indians to CAD is possibly related to different nutritional, environmental, and life-style factors. The body mass index in urban Indians as compared to rural Indians is 24 Vs 20 in males and 25 Vs 20 in females. Unfortunately, the on-going urbanization of rural India is likely to narrow down these differences. Migration from rural to urban environment and migration from India to industrialized countries is another special risk-factor for our people. Migration is usually associated with stress of seeking and maintaining the new job, stress of coping with the new job-expectations, and stress of competing with the peer-group who is in the organisation longer. New affluence is associated with sedentary life-style and higher consumption of calories, saturated fats, salt, tobacco, and alcohol. These factors contribute to obesity, dyslipidaemia, hypertension, hyperuricaemia, and diabetes mellitus. Therefore, there has to be high index of suspicion for CAD in Indians above the age of thirty years. The risk-factor evaluation must start earlier (Rissam HS et al., 2001).

Disease Burden: There are relatively few mortality studies from India, as there is no uniform completion of death certificates and no centralized death registry for CVD. However, the WHO and the World Bank estimate that deaths attributable to CVD have increased in parallel with the expanding population in India, and that CVD now accounts for a large proportion of disability adjusted life years (DALY) lost. Of all deaths in 1990, approximately 25% were attributable to CVD, compared with 10% from diarrheal diseases, 13% from respiratory infections, and 8% from tuberculosis. SA migrants to the United Kingdom, South Africa, Singapore, and North America
experience 1.5 to 4.0 times higher CHD mortality compared with indigenous populations (Yusuf S et al., 2001)^a.

**Temporal Trends:** In India, the CHD rate is expected to rise in parallel with the increase in life expectancy secondary to increases in per capita income and declining infant mortality. The average life expectancy has increased from 41 years in the years 1951 to 1961, to 61.4 years in the years 1991 to 1996 and is projected to reach 72 years by 2030, which could lead to large increases in CVD prevalence (Yusuf S et al., 2001)^a.

**Risk Factors:** Compared with Europeans, South Asians (in the UK and Canada) do not display high rates of smoking, hypertension, or elevated cholesterol but still have higher rates of CHD. South Asians in the UK and Canada suffer a high prevalence of impaired glucose tolerance (IGT), central obesity, elevated triglycerides, and low HDL cholesterol, and NIDDM at rates 4 to 5 times higher than in Europeans (19% versus 4% by age 55 years). High rates of diabetes has been reported among SAs in the UK (10% to 19%), Trinidad (21%), Fiji (25%), South Africa (22%), Mauritius (20%), and Canada (10%). By contrast, the prevalence of diabetes in rural India is 2% to 3% and approximately 8% in urban areas. In addition, there is increasing evidence that elevations in blood glucose even in the nondiabetic range increases CHD risk among SAs. SAs have elevated levels of Lp(a), a lipoprotein which is genetically mediated and associated with increased atherosclerosis, thrombogenesis, and clinical events. Recent studies have confirmed that SAs also have higher levels of homocysteine, fibrinogen, and plasminogen activator inhibitor (PAI-1), all of which could increase the risk for thrombosis. Although the degree of subclinical atherosclerosis is related to clinical events, it appears that SAs have a higher propensity for clinical events compared with Europeans or Chinese, even after adjusting for all known risk factors and the degree of atherosclerosis. This probably suggests the potential for greater plaque rupture and thrombotic events among SAs (Yusuf S et al., 2001).

**Geographic Variations:** Marked increases in both CHD prevalence and risk factors is observed in urban India as compared with rural settings. A recent overview of prevalence surveys conducted over 2 decades in India reported a 9-fold increase of
CHD in urban centers, compared with a 2-fold increase in CHD rates among rural populations. This increase in CHD rates in urban areas is associated with an increase in the prevalence of lipid and glucose abnormalities as well as hypertension and obesity. By contrast, the rates of tobacco smoking are higher in rural compared with urban populations (Yusuf S et al., 2001).

Migration Patterns: SAs in the UK have higher risk factor levels compared with their siblings living in India (BMI, 27 versus 23 kg/m²; systolic BP, 144 versus 137 mm Hg; total cholesterol, 6.3 versus 5.0 mmol/L; lower HDL cholesterol, 1.14 versus 1.27 mmol/L; and higher fasting glucose, 5.4 versus 4.6 mmol/L). Therefore, adverse changes in CVD risk factors and disease rates are observed when South Asians adopt an urban lifestyle whether they live in India or abroad (Yusuf S et al., 2001).

2.5 LIPID RISK FACTORS

Serum LDL cholesterol as a major cause of CHD: The induction of hypercholesterolemia is a prerequisite for atherogenesis. LDL cholesterol as low as 25–60 mg/dL is physiologically sufficient (Brown MS and Goldstein JL, 1986). Moreover, persons who have extremely low levels of LDL throughout life due to familial hypobetalipoproteinemia have documented longevity (Glueck CJ et al., 1976). Epidemiological investigations of human populations incriminate high levels of LDL cholesterol as being atherogenic. The Framingham Heart Study (Wilson PWF et al., 1998), the Multiple Risk Factor Intervention Trial (MRFIT) (Stamler J et al., 1986), and the Lipid Research Clinics (LRC) trial (Lipid Research Clinics Program, 1984) found a direct relationship between levels of LDL cholesterol (or total cholesterol) and the rate of new-onset CHD in men and women who were initially free of CHD. Any LDL cholesterol above 100 mg/dL appears to be atherogenic. Only in populations that maintain very low levels of serum cholesterol, e.g., total cholesterol <150 mg/dL (or LDL cholesterol <100 mg/dL) throughout life do we find a near-absence of clinical CHD (National Cholesterol Education Program (NCEP) Guidelines, September 2002). Moreover, the cholesterol level in young adulthood predicts development of CHD later in life. Since LDL-cholesterol levels <100 mg/dL throughout life are associated with a very low risk for CHD in populations, they can be called optimal. Even when LDL-cholesterol concentrations are near optimal (100–
129 mg/dL), atherogenesis occurs; hence, such levels must also be called above optimal. At levels that are borderline high (130–159 mg/dL), atherogenesis proceeds at a significant rate, whereas at levels that are high (160–189 mg/dL) and very high (>190 mg/dL) it is markedly accelerated.

The first stage of atherogenesis is the fatty streak, which consists largely of cholesterol-filled macrophages; most of the cholesterol in fatty streaks is derived from LDL cholesterol. The second stage consists of fibrous plaques in which a layer of scar tissue overlies a lipid rich core. Other risk factors contribute to plaque growth at this phase. The third stage is represented by the development of unstable plaques that are prone to rupture and formation of luminal thrombosis. Plaque rupture (or erosion) is responsible for most acute coronary syndromes viz myocardial infarction, unstable angina and coronary death (Libby P, 1995; Libby P et al., 1998; Fuster V et al., 1999; and Theroux P and Fuster V, 1998). Elevated LDL cholesterol plays a role in the development of the mature coronary plaque & contributes to plaque instability as well. Conversely, LDL cholesterol lowering stabilizes plaques and reduces the likelihood of acute coronary syndromes (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

**Triglycerides:** Many prospective epidemiological studies have reported a positive relationship between serum triglyceride levels and incidence of CHD (Austin MA et al., 1998 and Assmann G et al., 1998). Following factors that contribute to elevated triglycerides in the general population are overweight and obesity, physical inactivity, cigarette smoking, excess alcohol intake, very high-carbohydrate diets (>60 percent of total energy), other diseases (type 2 diabetes, chronic renal failure, nephrotic syndrome), certain drugs (corticosteroids, protease inhibitors for HIV, beta-adrenergic blocking agents, estrogens) & genetic factors (Stone NJ, 1994; and Chait A and Brunzell JD, 1990). Although several factors can elevate triglycerides, most common are overweight/obesity and physical inactivity. Elevated triglycerides represent one factor within a set of risk-factor targets in persons who are overweight, obese, sedentary, or cigarette smokers. Life-habit changes—weight control, exercise, and smoking cessation—will favorably modify multiple risk factors including elevated triglycerides (Denke MA et al., 1993; Denke MA et al., 1994; Hardman AE et al., 1999 and Berg A et al., 1997). Thus, elevated serum triglycerides are a potential target for therapeutic lifestyle changes.
Non-HDL cholesterol: The sum of VLDL+LDL cholesterol is called non-HDL cholesterol. ATP III identifies non-HDL cholesterol as a secondary target of therapy in patients with high triglycerides (>200 mg/dL). Non-HDL cholesterol can be simply calculated by subtracting HDL cholesterol from total cholesterol. Based on the premise that VLDL cholesterol ≤30 mg/dL is normal, the non-HDL cholesterol goal is set at 30 mg/dL higher than the LDL cholesterol goal. Therefore, non-HDL cholesterol should be lowered to <130 mg/dL in patients with CHD or CHD risk equivalents, <160 mg/dL in those with ≥2 risk factors, and <190 mg/dL in those with no risk factors or 1 risk factor (Talbert RL, 2002).

High density lipoproteins: Strong epidemiological evidence links low levels of serum HDL cholesterol to increased CHD morbidity and mortality. It has been estimated that each 1 mg/dl (0.03 mmol/L) increase in HDL-C reduces the risk of cardiac events by 2% in men and by 3% in women (Tavintharan S et al., 2005; Wilson PWF et al., 1998; Gordon DJ et al., 1989). In addition to its role in reverse cholesterol transport, HDL-C has several other potentially significant proposed roles, including increasing fibrinolysis, antioxidant to LDL, anti-inflammatory, nitric oxide promoting, and also in decreasing platelet aggregability via prostacyclin, all contributing to decreasing the risk of atherothrombotic disease (Tavintharan S et al., 2005). Moreover, a low HDL level can be a sign of insulin resistance and its associated metabolic risk factors (Vega GL and Grundy SM, 1996).

There are several factors that contribute to low HDL cholesterol levels that need to be identified in clinical practice. These include: Elevated serum triglycerides, overweight and obesity, physical inactivity, cigarette smoking, very high carbohydrate intakes (>60 percent of total energy intake), type 2 diabetes, certain drugs (beta-blockers, anabolic steroids, progestational agents) & genetic factors. In the general population, about 50 percent of the variability of serum HDL-cholesterol levels derives from genetic factors; the other 50 percent presumably comes from the acquired factors listed above. Among these acquired factors, overweight and obesity appear to be most important (National Cholesterol Education Program (NCEP) Guidelines, September 2002).
Atherogenic dyslipidemia: A common form of dyslipidemia is characterized by three lipid abnormalities: elevated triglycerides, small LDL particles, and reduced HDL cholesterol. Often the lipoprotein concentrations in this lipid triad are not categorically abnormal, but are only marginally deranged. The lipid triad occurs commonly in persons with premature CHD, hence the designation atherogenic lipoprotein phenotype or atherogenic dyslipidemia. Typical characteristics of persons with atherogenic dyslipidemia are obesity, abdominal obesity, insulin resistance, and physical inactivity. Although there is evidence that each component of the lipid triad—low HDL, small LDL, and remnant lipoproteins—is individually atherogenic, the relative quantitative contribution of each cannot be determined. For this reason, it is reasonable to view the lipid triad as a whole as a “risk factor.” (Grundy SM et al., 1998; and National Cholesterol Education Program (NCEP) Guidelines, September 2002).

Therapy for atherogenic dyslipidemia includes dietary changes and drug therapy. Nicotinic acid reduces triglyceride and cholesterol levels while raising HDL concentrations. Fibric acids effectively lower triglyceride levels and are generally well tolerated but have little beneficial effect on the cholesterol profile. Statins offer marked reductions in total, LDL, and very low-density lipoprotein cholesterol levels and cause modest increases in HDL concentration. Combination therapy can enhance the efficacy of the individual drugs (Grundy SM, 1995).

2.6 NONLIPID RISK FACTORS

A number of nonlipid risk factors are associated with increased CHD risk and must be considered in preventive efforts. Some of these factors are modifiable. Several fixed risk factors cannot be modified; their presence signals the need for more intensive lowering of LDL cholesterol. Other risk factors, some of which are yet to be identified, undoubtedly influence risk independently of the major risk factors. Some of these other factors contributing to CHD risk include the life-habit risk factors (obesity, physical inactivity, and atherogenic diet), emerging risk factors, male sex, and genetic/racial/ethnic characteristics.
Modifiable risk factors

Control of the modifiable risk factors is especially important in preventing premature CHD events in men below 55 years or in women below 65 years. Observational studies suggest that modifiable risk factors account for 85% of excess risk (risk over and above that of individuals with optimal risk-factor profiles) for premature CHD (Stamler J et al., 1986; Wilson PWF et al., 1998).

Hypertension: Numerous observational studies have demonstrated unequivocally a powerful association of high blood pressure with risk for CHD (Stamler J et al., 1993; van den Hoogen PCW et al., 2000). This association holds for men and women and younger and older persons. Even below categorical hypertension, subjects with high-normal blood pressure (130–139 mmHg systolic and/or 85–89 mmHg diastolic) are at increased risk for CHD compared with those with optimal values. Clinical trials have established that blood pressure reduction in people with hypertension reduces risk for a variety of blood pressure-related endpoints including CHD (Rodgers A and MacMahon S, 1999 and Vasan RS et al., 1999).

Cigarette smoking: Cigarette smoking has been established as a powerful contributor to risk for CHD and other forms of CVD. The relationship of smoking to CVD risk is dose dependent and observed in men and women. Observational data suggest that smoking cessation reduces the risk for CVD events and that the decline in risk begins within months after quitting (Jonas MA et al., 1992; Wolf PA et al., 1988; and Doll R et al., 1980).

Diabetes: Risk for all forms of CVD, including CHD is increased substantially with type 1 and type 2 diabetes mellitus. Furthermore, the mortality rate in diabetic subjects who have experienced CHD is much higher than in non-diabetic subjects. The increase in risk attributed to hyperglycemia per se is independent of the overweight/obesity and dyslipidemia commonly observed in persons with diabetes (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

Overweight/obesity: Obesity is defined as a body mass index (BMI) (weight in kg divided by the square of height in meters) of ≥30 kg/m² and overweight as 25–29.9 kg/m². Overweight and obesity not only predispose to CHD, stroke, and numerous
other conditions, they also are associated with a greater all-cause mortality. Risk is accentuated when obesity has a predominant abdominal component. Obesity typically raises blood pressure and cholesterol levels and lowers HDL levels. It predisposes to type 2 diabetes. It also adversely affects other risk factors: triglycerides; small, dense LDL particles; insulin resistance; and prothrombotic factors. The Framingham Heart Study confirms that obesity is strongly predictive of CHD. Risk for CVD is particularly raised when abdominal obesity is present; abdominal obesity defined by a waist circumference greater than 102 cm in men or 88 cm (35 inches) in women. The association between excess body weight and CHD seems particularly strong in white Americans. For example, in one long-term prospective study, men aged 40 to 65 years with body mass index (BMI) 25 to 29 kg/m² were 72% more likely to develop fatal or nonfatal CHD than were men who were not overweight. In another study, women whose BMI was 23 to 25 kg/m² carried a 50% increase in risk for CHD compared with women with lower BMIs. Obesity is a strong risk factor for CHD and is a direct target for intervention. Prevention of obesity and weight reduction in overweight persons are integral parts of the strategy for long-term risk reduction (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

**Physical inactivity:** Physical inactivity is associated with increased risk for CHD. Physical inactivity reduces caloric expenditure and probably contributes to obesity and to its associated lipid and nonlipid risk factors, as well as to insulin resistance (Perseghin G et al., 1996). Conversely, physical activity favorably modifies several risk factors; it has been reported to lower LDL and triglyceride levels, raise HDL cholesterol, improve insulin sensitivity, and lower blood pressure (Helmrich SP et al., 1991; Forrest KY et al., 2001; and Grundy SM et al., 1999).

**Atherogenic diet:** Dietary patterns appear to influence baseline risk beyond the known risk factors. For example, populations that consume diets high in fruits, vegetables, whole grains, and unsaturated fatty acids appear to be at a lower baseline risk than can be explained by standard risk factors. The particular nutrients that impart this lower risk have not been adequately defined, but strong candidates include antioxidant nutrients, folic acid, other B-vitamins, omega-3 fatty acids, and other micronutrients (Krauss RM et al., 2000).
Non-modifiable Risk Factors

*Age:* Risk for coronary disease increases steeply with advancing age in men and women (Wilson PWF et al., 1998). The principal reason that risk rises with age is that age is a reflection of the progressive accumulation of coronary atherosclerosis, which in turn reflects the cumulative exposure to atherogenic risk factors, both known and unknown. Once atherosclerosis develops, the coronary plaque itself becomes a “risk factor” for development of clinical CHD. This is because plaque ruptures produce acute coronary events (unstable angina or myocardial infarction), or when plaques grow large, coronary obstructive symptoms (angina pectoris) occur (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

*Women:* CHD is a major cause of death in women as well as men and it ultimately kills as many women as men. In women, onset of CHD generally is delayed by some 10 to 15 years compared with that in men. It is only at age 75 and above that CHD rates of women approximate those of men (Denke MA and Grundy SM, 1990). The reasons for the disparity in ages of onset of CHD between women and men are not fully understood. Also, patterns of risk factors often differ between men and women. For example, blood pressure, LDL cholesterol, and triglycerides rise at an earlier age in men than in women. Moreover, HDL-cholesterol levels are on average some 10 mg/dL lower in adult men than in women. This latter difference is established at puberty when HDL-cholesterol levels decrease in males but not in females. Since a 10-mg/dL difference in HDL cholesterol is projected to account for a 20–30 percent difference in CHD event rates over the short term, (Gordon DJ et al., 1989) this difference over the adult lifespan could account for a significant portion of the gender disparity between men and women.

Although the magnitude of risk factors on average may vary between women and men, all of the major risk factors raise the risk for CHD in women (Wilson PWF et al., 1998). A commonly cited reason for the gender difference is a protective effect of estrogen in women. Observational studies have consistently suggested that postmenopausal estrogen users are at lower risk of CHD than non-users. However, these studies are confounded by a number of powerful biases that may account for a large overestimation of potential benefit (National Cholesterol Education Program (NCEP) Guidelines, September 2002).
Male sex: At any given age men are at greater risk for coronary disease than are women (Wilson PWF et al., 1998). The reasons for a gender difference in CHD risk are not fully understood. Part of the difference can be explained by the earlier onset of risk factors in men, e.g., elevations of LDL cholesterol and blood pressure, and lower HDL cholesterol (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

Family history of premature CHD: CHD tends to cluster in families, and a positive family history of premature CHD counts as a risk factor. Relative risk for CHD in first-degree relatives has been reported to range from two to as high as 12 times that of the general population (Slack J et al., 1969; Phillips RL et al., 1974 and Rissanen AM et al., 1979). Risk increases with the number of primary relatives affected and at younger ages of onset in the probands (Pohjola-Sintonen S et al., 1998).

2.7 EMERGING RISK FACTORS
Consequently there has been intensive research to identify new risk factors that will enhance predictive power in individuals. These newer factors can be called emerging risk factors. For present purposes, these can be conveniently divided into three categories: lipid risk factors, nonlipid risk factors, and subclinical atherosclerotic disease.

2.8 RISK ASSESSMENT: FIRST STEP IN RISK MANAGEMENT
A basic principle of prevention is that the intensity of risk-reduction therapy should be adjusted to a person's absolute risk. Hence, the first step in selection of LDL-lowering therapy is to assess a person's risk status. Risk assessment requires measurement of LDL cholesterol as part of lipoprotein analysis and identification of accompanying risk determinants. Risk determinants in addition to LDL cholesterol include the presence or absence of CHD, other clinical forms of atherosclerotic disease, and the major risk factors other than LDL (cigarette smoking, hypertension, low HDL-C, family history of premature CHD & age (men > 45 and women >55). Based on these other risk determinants, ATP III identifies 3 categories of risk that modify the goals and modalities of LDL-lowering therapy.
1. **CHD and CHD risk equivalents:** The category of highest risk consists of CHD and CHD risk equivalents. The latter carry a risk for major coronary events equal to that of established CHD, i.e., >20% per 10 years (i.e., more than 20 of 100 such individuals will develop CHD or have a recurrent CHD event within 10 years).

CHD risk equivalents comprise: Other clinical forms of atherosclerotic disease (peripheral arterial disease, abdominal aortic aneurysm, and symptomatic carotid artery disease), diabetes, multiple risk factors that confer a 10-year risk for CHD >20%.

Diabetes counts as a CHD risk equivalent because it confers a high risk of new CHD within 10 years, in part because of its frequent association with multiple risk factors. Furthermore, because persons with diabetes who experience a myocardial infarction have an unusually high death rate either immediately or in the long term, a more intensive prevention strategy is warranted. Persons with CHD or CHD risk equivalents have the lowest LDL cholesterol goal (<100 mg/dL).

2. **Persons with multiple (2+) risk factors in whom 10-year risk for CHD is ≤20%:** Risk is estimated from Framingham risk scores (see Appendix). The major risk factors, exclusive of elevated LDL cholesterol, are used to define the presence of multiple risk factors that modify the goals and cutpoints for LDL-lowering treatment. The LDL cholesterol goal for persons with multiple (2+) risk factors is <130 mg/dL.

3. **Persons having 0-1 risk factor:** with few exceptions, persons in this category have a 10-year risk <10%. Their LDL cholesterol goal is <160 mg/dL.

**Method of Risk Assessment: Counting Major Risk Factors and Estimating 10-Year CHD Risk**

Risk status in persons without clinically manifest CHD or other clinical forms of atherosclerotic disease is determined by a 2-step procedure. First, the number of risk factors is counted. Second, for persons with multiple (2+) risk factors, 10-year risk assessment is carried out with Framingham scoring to identify individuals whose short-term (10-year) risk warrants consideration of intensive treatment. Estimation of
the 10-year CHD risk adds a step to risk assessment beyond risk factor counting, but this step is warranted because it allows better targeting of intensive treatment to people who will benefit from it. When 0-1 risk factor is present, Framingham scoring is not necessary because 10-year risk rarely reaches levels for intensive intervention; a very high LDL level in such a person may nevertheless warrant consideration of drug therapy to reduce long-term risk. Risk factors used in Framingham scoring include age, total cholesterol, HDL cholesterol, blood pressure, and cigarette smoking. Total cholesterol is used for 10-year risk assessment because of a larger and more robust Framingham database for total than for LDL cholesterol, but LDL cholesterol is the primary target of therapy. Framingham scoring divides persons with multiple risk factors into those with 10-year risk for CHD of >20%, 10%-20%, and <10%. It should be noted that this 2-step sequence can be reversed with essentially the same results. Initial risk assessment in ATP III uses the major risk factors to define the core risk status. Only after the core risk status has been determined should any other risk modifiers be taken into consideration for adjusting the therapeutic approach.

2.9 LDL-LOWERING THERAPY

The 2 major modalities of LDL-lowering therapy are therapeutic lifestyle changes (TLC) and drug therapy. The TLC Diet stresses reductions in saturated fat and cholesterol intakes. When the metabolic syndrome or its associated lipid risk factors (elevated triglyceride or low HDL cholesterol) are present, TLC also stresses weight reduction and increased physical activity. Table below defines LDL cholesterol goals and cutpoints for initiation of TLC and for drug consideration for persons with 3 categories of risk: CHD and CHD risk equivalents; multiple (2+) risk factors (10-year risk 10%-20% and <10%); and 0-1 risk factor.
Table 2.2: LDL Cholesterol Goals and Cutpoints for TLC and Drug Therapy in Different Risk Categories (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>LDL Goal (mg/dL)</th>
<th>LDL Level at Which to Initiate TLC (mg/dL)</th>
<th>LDL Level at Which to Consider Drug Therapy (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHD or CHD risk equivalents (10-year risk &gt;20%)</td>
<td>&lt;100</td>
<td>≥100</td>
<td>≥130 (100-129: drug optional)†</td>
</tr>
<tr>
<td>2+ Risk factors (10-year risk ≤20%)</td>
<td>&lt;130</td>
<td>≥130</td>
<td>10-year risk 10-20%: ≥130 10-year risk &lt;10%: ≥160</td>
</tr>
<tr>
<td>0-1 Risk factor‡</td>
<td>&lt;160</td>
<td>≥160</td>
<td>≥190 (160-189: LDL-lowering drug optional)</td>
</tr>
</tbody>
</table>

TLC = therapeutic lifestyle changes; *LDL indicates low-density lipoprotein; CHD, coronary heart disease.

† Some authorities recommend use of LDL-lowering drugs in this category if an LDL cholesterol level of <100 mg/dL cannot be achieved by therapeutic lifestyle changes. Others prefer use of drugs that primarily modify triglycerides and HDL, eg, nicotinic acid or fibrate. Clinical judgment also may call for deferring drug therapy in this subcategory.

‡ Almost all people with 0-1 risk factor have a 10-year risk <10%; thus, 10-year risk assessment in people with 0-1 risk factor is not necessary.

**CHD and CHD Risk Equivalents:** For persons with CHD and CHD risk equivalents, LDL-lowering therapy greatly reduces risk for major coronary events and stroke and yields highly favorable cost-effectiveness ratios. The cutpoints for initiating lifestyle and drug therapies are shown in Table above.

If baseline LDL cholesterol is 130 mg/dL, intensive lifestyle therapy and maximal control of other risk factors should be started. Moreover, for most patients, an LDL-lowering drug will be required to achieve an LDL cholesterol level of <100 mg/dL; thus an LDL-cholesterol lowering drug can be started simultaneously with TLC to attain the goal of therapy.

If LDL cholesterol levels are 100-129 mg/dL, either at baseline or on LDL-lowering therapy, several therapeutic approaches are available: initiate or intensify lifestyle and/or drug therapies specifically to lower LDL; emphasize weight reduction and increased physical activity in persons with the metabolic syndrome; delay use or
intensification of LDL-lowering therapies and institute treatment of other lipid or nonlipid risk factors; consider use of other lipid-modifying drugs (e.g., nicotinic acid or fibric acid) if the patient has elevated triglyceride or low HDL cholesterol.

If baseline LDL cholesterol is <100 mg/dL, further LDL-lowering therapy is not required. Patients should nonetheless be advised to follow the TLC Diet on their own to help keep the LDL level optimal. Several clinical trials are currently under way to assess benefit of lowering LDL cholesterol to well below 100 mg/dL. At present, emphasis should be placed on controlling other lipid and nonlipid risk factors and on treatment of the metabolic syndrome, if present.

**Multiple (2+) Risk Factors and 10-Year Risk of ≤20%:** For persons with multiple (2+) risk factors and 10-year risk ≤20%, intensity of therapy is adjusted according to 10-year risk and LDL cholesterol level.

**Multiple (2+) Risk Factors and a 10-Year Risk of 10%-20%:** In this category, the goal for LDL cholesterol is <130 mg/dL. The therapeutic aim is to reduce short-term risk as well as long-term risk for CMD. If baseline LDL cholesterol is ≥130 mg/dL, TLC is initiated and maintained for 3 months. If LDL remains ≥130 mg/dL after 3 months of TLC, consideration can be given to starting an LDL-lowering drug to achieve the LDL goal of <130 mg/dL. Use of LDL-lowering drugs at this risk level reduces CHD risk and is cost-effective. If the LDL falls to less than 130 mg/dL on TLC alone, TLC can be continued without adding drugs. In older persons (≥65 years), clinical judgment is required for how intensively to apply these guidelines; a variety of factors, including concomitant illnesses, general health status, and social issues, may influence treatment decisions and may suggest a more conservative approach.

**Multiple (2+) Risk Factors and a 10-Year Risk of <10%:** In this category, the goal for LDL cholesterol also is <130 mg/dL. The therapeutic aim, however, is primarily to reduce longer-term risk. If baseline LDL cholesterol is ≥130 mg/dL, the TLC Diet is initiated to reduce LDL cholesterol. If LDL is <160 mg/dL on TLC alone, it should be continued. LDL-lowering drugs generally are not recommended because the patient is not at high short-term risk. On the other hand, if LDL cholesterol is ≥160 mg/dL, drug therapy can be considered to achieve an LDL cholesterol level of <130 mg/dL; the
primary aim is to reduce long-term risk. Cost-effectiveness is marginal, but drug therapy can be justified to slow development of coronary atherosclerosis and to reduce long-term risk for CHD.

0-1 Risk Factor: Most persons with 0-1 risk factor have a 10-year risk <10%. They are managed according to Table 2.2. The goal for LDL cholesterol in this risk category is <160 mg/dL. The primary aim of therapy is to reduce long-term risk. First-line therapy is TLC. If after 3 months of TLC the LDL cholesterol is <160 mg/dL, TLC is continued. However, if LDL cholesterol is 160-189 mg/dL after an adequate trial of TLC, drug therapy is optional depending on clinical judgment. Factors favoring use of drugs include: a severe single risk factor (heavy cigarette smoking, poorly controlled hypertension, strong family history of premature CHD, or very low HDL cholesterol); multiple life-habit risk factors and emerging risk factors (if measured) & 10-year risk approaching 10%. If LDL cholesterol is ≥190 mg/dL despite TLC, drug therapy should be considered to achieve the LDL goal of <160 mg/dL.

The purpose of using LDL-lowering drugs in persons with 0-1 risk factor and elevated LDL cholesterol (≥160 mg/dL) is to slow the development of coronary atherosclerosis, which will reduce long-term risk. For persons whose LDL cholesterol levels are already below goal levels upon first encounter, instructions for appropriate changes in life habits, periodic follow-up, and control of other risk factors are needed.

2.10 THERAPEUTIC LIFESTYLE CHANGES IN LDL-LOWERING THERAPY

ATP III recommends a multifaceted lifestyle approach to reduce risk for CHD. This approach is designated TLC. Its essential features are:

1. Reduced intakes of saturated fats (<7% of total calories) and cholesterol (<200 mg/d)
2. Therapeutic options for enhancing LDL lowering such as plant stanols/sterols (2 g/d) and increased viscous (soluble) fiber (10-25 g/d)
3. Weight reduction
4. Increased physical activity

After maximum reduction of LDL cholesterol with dietary therapy, emphasis shifts to management of the metabolic syndrome and associated lipid risk factors. The majority of persons with these latter abnormalities are overweight or obese and sedentary. Weight reduction therapy for overweight or obese patients will enhance LDL lowering and will provide other health benefits including modifying other lipid and nonlipid risk factors.

Table 2.3: Essential Components of TLC (National Cholesterol Education Program (NCEP) Guidelines, September 2002).

<table>
<thead>
<tr>
<th>Component</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-raising nutrients</td>
<td>Less than 7% of total calories</td>
</tr>
<tr>
<td>Saturated fats*</td>
<td>Less than 200 mg/day</td>
</tr>
<tr>
<td>Dietary cholesterol</td>
<td></td>
</tr>
<tr>
<td>Therapeutic options for LDL lowering</td>
<td>2 grams per day</td>
</tr>
<tr>
<td>Plant stanols/sterols</td>
<td></td>
</tr>
<tr>
<td>Increased viscous (soluble) fiber</td>
<td>10–25 grams per day</td>
</tr>
<tr>
<td>Total calories (energy)</td>
<td>Adjust total caloric intake to maintain desirable body weight/prevent weight gain</td>
</tr>
<tr>
<td>Physical activity</td>
<td>Include enough moderate exercise to expend at least 200 kcal per day</td>
</tr>
</tbody>
</table>

Macronutrient Recommendations for the TLC Diet

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyunsaturated fat</td>
<td>Up to 10% of total calories</td>
</tr>
<tr>
<td>Monounsaturated fat</td>
<td>Up to 20% of total calories</td>
</tr>
<tr>
<td>Total fat</td>
<td>25–35% of total calories*</td>
</tr>
<tr>
<td>Carbohydrate†</td>
<td>50–60% of total calories*</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>20–30 grams per day</td>
</tr>
<tr>
<td>Protein</td>
<td>Approximately 15% of total calories</td>
</tr>
</tbody>
</table>

* ATP III allows an increase of total fat to 35 percent of total calories and a reduction in carbohydrate to 50 percent for persons with the metabolic syndrome. Any increase in fat intake should be in the form of either polyunsaturated or monounsaturated fat.

† Carbohydrate should derive predominantly from foods rich in complex carbohydrates including grains—especially whole grains—fruits, and vegetables.

Increasing viscous fiber in the diet: Viscous (soluble) forms of dietary fiber can reduce LDL cholesterol levels. In contrast, insoluble fiber does not significantly affect
LDL cholesterol (Anderson JW and Hanna TJ, 1999). On average, an increase in viscous fiber of 5–10 grams per day is accompanied by an approximately 5 percent reduction in LDL cholesterol. Because of the favorable effect of viscous fiber on LDL cholesterol levels, the ATP III panel recommends that the therapeutic diet be enriched by foods that provide a total of at least 5–10 grams of viscous fiber daily. Even higher intakes of 10–25 grams per day can be beneficial.

2.11 DRUG THERAPY TO ACHIEVE LDL CHOLESTEROL GOALS

A portion of the population whose short-term or long-term risk for CHD is high will require LDL-lowering drugs in addition to TLC to reach the designated goal for LDL cholesterol. When drugs are prescribed, attention to TLC should always be maintained and reinforced.

Primary Prevention With LDL-Lowering Therapy

The clinical approach to primary prevention is founded on the public health approach that calls for lifestyle changes, including (1) reduced intakes of saturated fat and cholesterol, (2) increased physical activity, and (3) weight control, to lower population cholesterol levels and reduce CHD risk, but the clinical approach intensifies preventive strategies for higher-risk persons. One aim of primary prevention is to reduce long-term risk (>10 years) as well as short-term risk (10 years). LDL goals in primary prevention depend on a person's absolute risk for CHD (i.e., the probability of having a CHD event in the short term or the long term)—the higher the risk, the lower the goal. Therapeutic lifestyle changes are the foundation of clinical primary prevention. Nonetheless, some persons at higher risk because of high or very high LDL cholesterol levels or because of multiple risk factors are candidates for LDL-lowering drugs. Recent primary prevention trials show that LDL-lowering drugs reduce risk for major coronary events and coronary death even in the short term.

When drug therapy for primary prevention is a consideration, the third visit of dietary therapy will typically be the visit to initiate drug treatment. Even if drug treatment is started, TLC should be continued. As with TLC, the first priority of drug therapy is to
achieve the goal for LDL cholesterol. For this reason, an LDL-lowering drug should be started. The usual drug will be a statin, but alternatives are a bile acid sequestrant or nicotinic acid. In most cases, the statin should be started at a moderate dose. In many patients, the LDL cholesterol goal will be achieved, and higher doses will not be necessary. The patient's response should be evaluated about 6 weeks after starting drug therapy. If the goal of therapy has been achieved, the current dose can be maintained. However, if the goal has not been achieved, LDL-lowering therapy can be intensified, either by increasing the dose of statin or by combining a statin with a bile acid sequestrant or nicotinic acid.

After 12 weeks of drug therapy, the response to therapy should again be assessed. If the LDL cholesterol goal is still not achieved, consideration can be given to further intensification of drug therapy. If the LDL goal cannot be attained by standard lipid-lowering therapy, consideration should be given to seeking consultation from a lipid specialist. Once the goal for LDL cholesterol has been attained, attention can turn to other lipid risk factors and nonlipid factors. Thereafter, patients can be monitored for response to therapy every 4 to 6 months, or more often if considered necessary.

Secondary Prevention With LDL-Lowering Therapy
Recent clinical trials demonstrate that LDL-lowering therapy reduces total mortality, coronary mortality, major coronary events, coronary artery procedures, and stroke in persons with established CHD. An LDL cholesterol level of <100 mg/dL is optimal; therefore, ATP III specifies an LDL cholesterol level of <100 mg/dL as the goal of therapy in secondary prevention. This goal is supported by clinical trials with both clinical and angiographic end points and by prospective epidemiological studies. The same goal should apply for persons with CHD risk equivalents. When persons are hospitalized for acute coronary syndromes or coronary procedures, lipid measures should be taken on admission or within 24 hours. These values can guide the physician on initiation of LDL-lowering therapy before or at discharge. Adjustment of therapy may be needed after 12 weeks.

Statins
HMG CoA reductase inhibitors are the most effective and practical class of drugs for reducing LDL-cholesterol concentrations. Results from four clinical trials with a
mean duration of 5.4 years have documented a decrease in CHD and total mortality, reductions in myocardial infarctions, revascularization procedures, stroke, and peripheral vascular disease [Downs JR et al. 1998, Shepherd J et al. 1995, Sacks FM et al. 1996 LaRosa JC et al 1999]. These trials documented benefits in men and women, in middle-aged and older persons, and in primary and secondary prevention. Approximately 30,000 individuals were randomized to either placebo or statin therapy in these five clinical outcome trials. Statin therapy proved remarkably safe, with no major or unexpected adverse effects observed. Several other types of clinical trials with statin therapy also showed favorable results. Beneficial outcomes in CHD parameters have been reported with almost all of the statins. Thus, statins are highly effective in lowering LDL-cholesterol levels (the primary target of therapy). Statin therapy reduces the risk of essentially every clinical manifestation of the atherosclerotic process; they are easy to administer with good patient acceptance. They have few drug-drug interactions, and they have a good record for safety (Knatterud GL et al, 2000).
2.12 ATORVASTATIN

DESCRIPTION
Atorvastatin is a synthetic lipid-lowering agent. Atorvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting event in cholesterol biosynthesis. Atorvastatin calcium is \( [R-(R^*, R^*)]-2'(4\text{-fluorophenyl})-\beta, \delta\text{-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate.} \) The empirical formula of atorvastatin calcium is \( (C_{33}H_{34}FN_2O_5)2\text{Ca\cdot3H}_2\text{O} \) and its molecular weight is 1209.42. Its structural formula is:

\[
\begin{align*}
\text{H} & \quad \text{CH} \quad \text{N} \\
\text{CH}_2 & \quad \text{CH}_2 & \quad \text{OH} \\
\text{CH}_2 & \quad \text{CH}_2 & \quad \text{OH} \\
\text{O} & \quad \text{F} \\
\text{N} & \quad \text{NH} \\
\end{align*}
\]

Atorvastatin calcium is a white to off-white crystalline powder that is insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer, and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

CLINICAL PHARMACOLOGY

Mechanism of Action
Atorvastatin is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate, a precursor of sterols, including cholesterol. Cholesterol and triglycerides circulate in the bloodstream as part of lipoprotein complexes. With ultracentrifugation, these complexes separate into HDL (high-density lipoprotein),
IDL (intermediate-density lipoprotein), LDL (low-density lipoprotein), and VLDL (very-low-density lipoprotein) fractions. Triglycerides (TG) and cholesterol in the liver are incorporated into VLDL and released into the plasma for delivery to peripheral tissues. LDL is formed from VLDL and is catabolized primarily through the high-affinity LDL receptor. Clinical and pathologic studies show that elevated plasma levels of total cholesterol (total-C), LDL-cholesterol (LDL-C), and apolipoprotein B (apo B) promote human atherosclerosis and are risk factors for developing cardiovascular disease, while increased levels of HDL-C are associated with a decreased cardiovascular risk.

In animal models, atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell-surface to enhance uptake and catabolism of LDL; atorvastatin also reduces LDL production and the number of LDL particles. Atorvastatin reduces LDL-C in some patients with homozygous familial hypercholesterolemia (FH), a population that rarely responds to other lipid-lowering medication(s).


**Pharmacodynamics**

Atorvastatin as well as some of its metabolites are pharmacologically active in humans. The liver is the primary site of action and the principal site of cholesterol synthesis and LDL clearance. Drug dosage rather than systemic drug concentration correlates better with LDL-C reduction. Individualization of drug dosage should be based on therapeutic response.
Pharmacokinetics and Drug Metabolism

Absorption: Atorvastatin is rapidly absorbed after oral administration; maximum plasma concentrations occur within 1 to 2 hours. Extent of absorption increases in proportion to atorvastatin dose. The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic availability is attributed to presystemic clearance in gastrointestinal mucosa and/or hepatic first-pass metabolism. Although food decreases the rate and extent of drug absorption by approximately 25% and 9%, respectively, as assessed by $C_{\text{max}}$ and AUC, LDL-C reduction is similar whether atorvastatin is given with or without food. Plasma atorvastatin concentrations are lower (approximately 30% for $C_{\text{max}}$ and AUC) following evening drug administration compared with morning. However, LDL-C reduction is the same regardless of the time of day of drug administration.

Distribution: Mean volume of distribution of atorvastatin is approximately 381 liters. Atorvastatin is $\geq 98\%$ bound to plasma proteins. A blood/plasma ratio of approximately 0.25 indicates poor drug penetration into red blood cells. Based on observations in rats, atorvastatin is likely to be secreted in human milk.

Metabolism: Atorvastatin is extensively metabolized to ortho- and para-hydroxylated derivatives and various beta-oxidation products. In vitro inhibition of HMG-CoA reductase by ortho- and para-hydroxylated metabolites is equivalent to that of atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites. In vitro studies suggest the importance of atorvastatin metabolism by cytochrome P450 3A4, consistent with increased plasma concentrations of atorvastatin in humans following co-administration with erythromycin, a known inhibitor of this isozyme. In animals, the ortho-hydroxy metabolite undergoes further glucuronidation.

Excretion: Atorvastatin and its metabolites are eliminated primarily in bile following hepatic and/or extra-hepatic metabolism; however, the drug does not appear to undergo enterohepatic recirculation. Mean plasma elimination half-life of atorvastatin in humans is approximately 14 hours, but the half-life of inhibitory activity for HMG-
CoA reductase is 20 to 30 hours due to the contribution of active metabolites. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration.

Special Populations

Geriatric: Plasma concentrations of atorvastatin are higher (approximately 40% for C_{\text{max}} and 30% for AUC) in healthy elderly subjects (age ≥65 years) than in young adults. Clinical data suggest a greater degree of LDL-lowering at any dose of drug in the elderly patient population compared to younger adults.

Gender: Plasma concentrations of atorvastatin in women differ from those in men (approximately 20% higher for C_{\text{max}} and 10% lower for AUC); however, there is no clinically significant difference in LDL-C reduction with atorvastatin between men and women.

Renal Insufficiency: Renal disease has no influence on the plasma concentrations or LDL-C reduction of atorvastatin; thus, dose adjustment in patients with renal dysfunction is not necessary.

Hemodialysis: While studies have not been conducted in patients with end-stage renal disease, hemodialysis is not expected to significantly enhance clearance of atorvastatin since the drug is extensively bound to plasma proteins.

Hepatic Insufficiency: In patients with chronic alcoholic liver disease, plasma concentrations of atorvastatin are markedly increased. C_{\text{max}} and AUC are each 4-fold greater in patients with Childs-Pugh A disease. C_{\text{max}} and AUC are approximately 16-fold and 11-fold increased, respectively, in patients with Childs-Pugh B disease.

INDICATIONS AND USAGE

Prevention of Cardiovascular Disease: In adult patients without clinically evident coronary heart disease, but with multiple risk factors for coronary heart disease such as age ≥ 55 years, smoking, hypertension, low HDL-C, or a family history of early coronary heart disease, atorvastatin is indicated to:

- Reduce the risk of myocardial infarction.
- Reduce the risk for revascularization procedures and angina.
Hypercholesterolemia

Atorvastatin is indicated:
1. As an adjunct to diet to reduce elevated total-C, LDL-C, apo B, and TG levels and to increase HDL-C in patients with primary hypercholesterolemia (heterozygous familial and nonfamilial) and mixed dyslipidemia (Fredrickson Types IIa and IIb).
2. As an adjunct to diet for the treatment of patients with elevated serum TG.
3. For the treatment of patients with primary dysbetalipoproteinemia (Fredrickson Type III) who do not respond adequately to diet.
4. To reduce total-C and LDL-C in patients with homozygous familial hypercholesterolemia as an adjunct to other lipid-lowering treatments (eg, LDL apheresis) or if such treatments are unavailable.
5. As an adjunct to diet to reduce total-C, LDL-C, and apo B levels in boys and postmenarchal girls, 10 to 17 years of age, with heterozygous familial hypercholesterolemia if after an adequate trial of diet therapy the following findings are present:
   a. LDL-C remains ≥ 190 mg/dL or
   b. LDL-C remains ≥ 160 mg/dL and:
      • there is a positive family history of premature cardiovascular disease or
      • two or more other CVD risk factors are present in the pediatric patient.

Therapy with lipid-altering agents should be a component of multiple-risk-factor intervention in individuals at increased risk for atherosclerotic vascular disease due to hypercholesterolemia. Lipid-altering agents should be used in addition to a diet restricted in saturated fat and cholesterol only when the response to diet and other nonpharmacological measures has been inadequate.

After the LDL-C goal has been achieved, if the TG is still ≥200 mg/dL, non HDL-C (total-C minus HDL-C) becomes a secondary target of therapy. Non-HDL-C goals are set 30 mg/dL higher than LDL-C goals for each risk category.

Prior to initiating therapy with atorvastatin, secondary causes for hypercholesterolemia (eg, poorly controlled diabetes mellitus, hypothyroidism,
nephrotic syndrome, dysproteinemias, obstructive liver disease, other drug therapy, and alcoholism) should be excluded, and a lipid profile performed to measure total-C, LDL-C, HDL-C, and TG.

**DOSAGE AND ADMINISTRATION**

The usual starting dose is 10 mg once a day. The maximum dose is 80 mg once a day. Doses may be given at any time of day with or without food.

**CONTRAINDICATIONS**

Active liver disease or unexplained persistent elevations of serum transaminases. Hypersensitivity to any component of this medication.

*Pregnancy and Lactation:* Atherosclerosis is a chronic process and discontinuation of lipid-lowering drugs during pregnancy should have little impact on the outcome of long-term therapy of primary hypercholesterolemia. Cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other biologically active substances derived from cholesterol, they may cause fetal harm when administered to pregnant women. Therefore, HMG-CoA reductase inhibitors are contraindicated during pregnancy and in nursing mothers.

If the patient becomes pregnant while taking this drug, therapy should be discontinued and the patient apprised of the potential hazard to the fetus.

**WARNINGS**

*Liver Dysfunction:* HMG-CoA reductase inhibitors, like some other lipid-lowering therapies, have been associated with biochemical abnormalities of liver function. Persistent elevations (>3 times the upper limit of normal [ULN] occurring on 2 or more occasions) in serum transaminases occurred in 0.7% of patients who received atorvastatin in clinical trials. The incidence of these abnormalities was 0.2%, 0.2%, 0.6%, and 2.3% for 10, 20, 40, and 80 mg, respectively. One patient in clinical trials developed jaundice. Increases in liver function tests (LFT) in other patients were not associated with jaundice or other clinical signs or symptoms. Upon dose reduction,
drug interruption, or discontinuation, transaminase levels returned to or near pretreatment levels without sequelae. Eighteen of 30 patients with persistent LFT elevations continued treatment with a reduced dose of atorvastatin.

It is recommended that liver function tests be performed prior to and at 12 weeks following both the initiation of therapy and any elevation of dose, and periodically (e.g., semiannually) thereafter. Liver enzyme changes generally occur in the first 3 months of treatment with atorvastatin. Patients who develop increased transaminase levels should be monitored until the abnormalities resolve. Should an increase in ALT or AST of >3 times ULN persist, reduction of dose or withdrawal of atorvastatin is recommended.

Atorvastatin should be used with caution in patients who consume substantial quantities of alcohol and/or have a history of liver disease. Active liver disease or unexplained persistent transaminase elevations are contraindications to the use of atorvastatin.

Skeletal Muscle: Rare cases of rhabdomyolysis with acute renal failure secondary to myoglobinuria have been reported with atorvastatin and with other drugs in this class. Uncomplicated myalgia has been reported in atorvastatin-treated patients. Myopathy, defined as muscle aches or muscle weakness in conjunction with increases in creatine phosphokinase (CPK) values >10 times ULN, should be considered in any patient with diffuse myalgias, muscle tenderness or weakness, and/or marked elevation of CPK. Patients should be advised to report promptly unexplained muscle pain, tenderness or weakness, particularly if accompanied by malaise or fever.

Atorvastatin therapy should be discontinued if markedly elevated CPK levels occur or myopathy is diagnosed or suspected. The risk of myopathy during treatment with drugs in this class is increased with concurrent administration of cyclosporine, fibric acid derivatives, erythromycin, niacin, orazole antifungals.
PRECAUTIONS

General: Before instituting therapy with atorvastatin, an attempt should be made to control hypercholesterolemia with appropriate diet, exercise, and weight reduction in obese patients, and to treat other underlying medical problems.

Information for Patients: Patients should be advised to report promptly unexplained muscle pain, tenderness, or weakness, particularly if accompanied by malaise or fever.

Drug Interactions: The risk of myopathy during treatment with drugs of this class is increased with concurrent administration of cyclosporine, fibrin acid derivatives, niacin (nicotinic acid), erythromycin, azole antifungals.

Antacid: When atorvastatin and Maalox® TC suspension were coadministered, plasma concentrations of atorvastatin decreased approximately 35%. However, LDL-C reduction was not altered.

Antipyrine: Because atorvastatin does not affect the pharmacokinetics of antipyrine, interactions with other drugs metabolized via the same cytochrome isozymes are not expected.

Colestipol: Plasma concentrations of atorvastatin decreased approximately 25% when colestipol and atorvastatin were co-administered. However, LDL-C reduction was greater when atorvastatin and colestipol were co-administered than when either drug was given alone.

Cimetidine: Atorvastatin plasma concentrations and LDL-C reduction were not altered by coadministration of cimetidine.

Digoxin: When multiple doses of atorvastatin and digoxin were co-administered, steady state plasma digoxin concentrations increased by approximately 20%. Patients taking digoxin should be monitored appropriately.

Erythromycin: In healthy individuals, plasma concentrations of atorvastatin increased approximately 40% with co-administration of atorvastatin and erythromycin, a known
inhibitor of cytochrome P450 3A4.

**Oral Contraceptives:** Co-administration of atorvastatin and an oral contraceptive increased AUC values for norethindrone and ethinyl estradiol by approximately 30% and 20%. These increases should be considered when selecting an oral contraceptive for a woman taking atorvastatin.

**Warfarin:** Atorvastatin had no clinically significant effect on prothrombin time when administered to patients receiving chronic warfarin treatment.

**Endocrine Function:** HMG-CoA reductase inhibitors interfere with cholesterol synthesis and theoretically might blunt adrenal and/or gonadal steroid production. Clinical studies have shown that atorvastatin does not reduce basal plasma cortisol concentration or impair adrenal reserve. The effects of HMG-CoA reductase inhibitors on male fertility have not been studied in adequate numbers of patients. The effects, if any, on the pituitary-gonadal axis in premenopausal women are unknown. Caution should be exercised if an HMG-CoA reductase inhibitor is administered concomitantly with drugs that may decrease the levels or activity of endogenous steroid hormones, such as ketoconazole, spironolactone, and cimetidine.

**Adverse Reactions**

Atorvastatin is generally well-tolerated. Adverse reactions have usually been mild and transient.

**Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT):** In ASCOT involving 10,305 participants treated with Lipitor 10 mg daily (n=5,168) or placebo (n=5,137), the safety and tolerability profile of the group treated with Lipitor was comparable to that of the group treated with placebo during a median of 3.3 years of follow-up.

The following adverse events were reported, regardless of causality assessment in patients treated with atorvastatin in clinical trials. The events in italics occurred in ≥2% of patients and the events in plain type occurred in <2% of patients.
CHAPTER II: LITERATURE REVIEW

Body as a Whole: Chest pain, face edema, fever, neck rigidity, malaise, photosensitivity reaction, generalized edema.

Digestive System: Nausea, gastroenteritis, liver function tests abnormal, colitis, vomiting, gastritis, dry mouth, rectal hemorrhage, esophagitis, eructation, glossitis, mouth ulceration, anorexia, increased appetite, stomatitis, biliary pain, cheilitis, duodenal ulcer, dysphagia, enteritis, melena, gum hemorrhage, stomach ulcer, tenesmus, ulcerative stomatitis, hepatitis, pancreatitis, cholestatic jaundice.

Respiratory System: Bronchitis, rhinitis, pneumonia, dyspnea, asthma, epistaxis.

Nervous System: Insomnia, dizziness, paresthesia, somnolence, amnesia, abnormal dreams, libido decreased, emotional lability, incoordination, peripheral neuropathy, torticollis, facial paralysis, hyperkinesia, depression, hypesthesia, hypertonia.

Musculoskeletal System: Arthritis, leg cramps, bursitis, tenosynovitis, myasthenia, tendinous contracture, myositis.

Skin and Appendages: Pruritus, contact dermatitis, alopecia, dry skin, sweating, acne, urticaria, eczema, seborrhea, skin ulcer.

Urogenital System: Urinary tract infection, urinary frequency, cystitis, hematuria, impotence, dysuria, kidney calculus, nocturia, epididymitis, fibrocystic breast, vaginal hemorrhage, albuminuria, breast enlargement, metrorrhagia, nephritis, urinary incontinence, urinary retention, urinary urgency, abnormal ejaculation, uterine hemorrhage.

Special Senses: Amblyopia, tinnitus, dry eyes, refraction disorder, eye hemorrhage, deafness, glaucoma, parosmia, taste loss, taste perversion.

Cardiovascular System: Palpitation, vasodilatation, syncope, migraine, postural hypotension, phlebitis, arrhythmia, angina pectoris, hypertension.

Metabolic and Nutritional Disorders: Peripheral edema, hyperglycemia, creatine phosphokinase increased, gout, weight gain, hypoglycemia.
Hemic and Lymphatic System: Ecchymosis, anemia, lymphadenopathy, thrombocytopenia, petechia.

In controlled clinical studies of 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to atorvastatin. The most frequent adverse events thought to be related to atorvastatin were constipation, flatulence, dyspepsia, and abdominal pain.

Adverse experiences reported in ≥2% of patients in placebo-controlled clinical studies of atorvastatin, regardless of causality assessment, are shown in Table:

<table>
<thead>
<tr>
<th>Body as whole</th>
<th>Placebo</th>
<th>Atorvastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 mg</td>
<td>20 mg</td>
</tr>
<tr>
<td>Infection</td>
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</tr>
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<td>0.9</td>
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<td>Flatulence</td>
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<td>Musculoskeletal System</td>
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<td>Myalgia</td>
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<td>3.2</td>
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Post-introduction Reports: Adverse events associated with Lipitor therapy reported since market introduction, that are not listed above, regardless of causality.
assessment, include the following: anaphylaxis, angioneurotic edema, bullous rashes (including erythema multiforme, Stevens-Johnson syndrome, and toxic epidermal necrolysis), and rhabdomyolysis.

Table 2.4: Summary of randomised clinical trials comparing the efficacy of atorvastatin (ATO) with other HMG-CoA reductase inhibitors

<table>
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<th>Reference</th>
<th>Design</th>
<th>N</th>
<th>Dose</th>
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<td>db, pg, mc 36 wk</td>
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</table>

A = Abstract; B = Extension of Crouse III JR et al (1999) Data are for weeks 18 to 36 of the study; co = crossover; db = double-blind; HDL = high-density lipoprotein; LDL = low-density lipoprotein; mc = multicentre; nb = nonblind; pc = placebo-controlled; pg = parallel group; P = placebo; PR = pravastatin; R = rosuvastatin; S = simvastatin; TG = triglycerides; tt = treat-to-target; * p < 0.05, ** p < 0.01, *** p < 0.001, † p ≤ 0.0001, all vs ATO.

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