3. REVIEW OF LITERATURE

3.1 Lipids

3.1.1 Introduction

As discussed by Botham KM\textsuperscript{13} “the lipids are a heterogeneous group of compounds, including fats, oils, steroids, waxes, and related compounds, that are related more by their physical than by their chemical properties. They have the common property of being (1) relatively insoluble in water and (2) soluble in nonpolar solvents such as ether and chloroform. Lipids have essential roles in nutrition and health and knowledge of lipid biochemistry is necessary for the understanding of many important biomedical conditions, including obesity, diabetes mellitus, and atherosclerosis.”

“Lipids of major physiologic significance include fatty acids and their esters, together with cholesterol and other steroids.”\textsuperscript{14}

3.1.2 Triacylglycerols (Triglycerides)

As per Spiegelman BM\textsuperscript{15} “triacylglycerols are esters of glycerol, a trihydric alcohol with three fatty acids, and are the main source of energy.” Porth CM\textsuperscript{16} stated that “the first step in using triglycerides involves their hydrolysis into fatty acids and glycerol, both of which are transported in the blood to the active tissues, where they are oxidized to give energy. Almost all the cells, with few exceptions, such as brain tissues and red blood cells, are able to use fatty acids as a source of energy.”

3.1.3 Cholesterol

Hu J\textsuperscript{17} stated that “Cholesterol is an amphipathic lipid which forms an essential structural component of membranes and of the outer layer of plasma lipoproteins. It is synthesized in many tissues from acetyl coenzyme A (acetyl CoA), and is the precursor of all other steroids in the body such as corticosteroids, sex hormones, bile acids and vit. D.” Champe PC\textsuperscript{18} stated that “it is synthesized by virtually all tissues in humans, although liver, intestine, adrenal cortex and reproductive tissues, including ovaries, testes and placenta; make the largest contributions to the body’s cholesterol pool.” Botham KM\textsuperscript{19} discussed that “it is present in plasma and tissues, either in the free form, or in combination with long-chain fatty acids as cholesteryl ester (storage form). In the
plasma, both forms are transported in lipoproteins. Tissue uptake of cholesterol and cholesteryl esters is accomplished with the help of plasma low-density lipoprotein (LDL) which acts as a vehicle. Free cholesterol is removed from tissues by plasma high-density lipoprotein (HDL) and transported to the liver, where it is eliminated from the body either unchanged or after conversion into bile acids in the process known as reverse cholesterol transport (RCT). Cholesterol is derived about equally from the diet and from biosynthesis. Major dietary sources are foods of animal origin such as egg yolk, meat, liver and brain. Endogenous synthesis of cholesterol occurs mainly in the liver and intestine. However, all tissues containing nucleated cells are capable of cholesterol synthesis, which occurs in the endoplasmic reticulum and the cytosol. It is a major constituent of gallstones. However, its chief role in the pathologic processes is as a factor in the genesis of atherosclerosis of vital arteries causing cerebrovascular, coronary and peripheral vascular disease.”

3.1.3.1 Cholesterol biosynthesis:

“The biosynthesis of cholesterol consists of following steps:

1. Synthesis of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) from acetyl CoA
2. Reduction of HMG-CoA to mevalonic acid (mevalonate)
3. Formation of isoprenoid units from mevalonate by loss of CO2
4. Condensation of six isoprenoid units to form squalene
5. Cyclization of squalene to form parent steroid, lanosterol
6. Finally, cholesterol is formed from lanosterol.”

3.1.3.2 Regulation of cholesterol synthesis:

Brown MS20 discussed that “cholesterol feeds back to inhibit its own synthesis by inhibiting HMG-CoA reductase, the enzyme that converts HMG-CoA to mevalonic acid. Thus, when dietary cholesterol intake is high, hepatic cholesterol synthesis is decreased and vice versa. However, the feedback compensation is incomplete, because, a diet that is low in cholesterol and saturated fats leads to only a modest decline in circulating plasma cholesterol.”
Figure-3.1.1: Pathway of Cholesterol Synthesis$^{17}$:
3.1.4 Lipoproteins:

Gallon GB\textsuperscript{21} stated that “in the post-absorptive state, after all the chylomicrons have been removed from the blood, more than 95\% of all the lipids in the plasma are in the form of lipoproteins. These are small particles, much smaller than the chylomicrons, but qualitatively similar in composition-containing triglycerides, cholesterol, phospholipids and proteins. The protein constituents of lipoproteins are called apoproteins (APO). The major APOs are APO-E, APO-C and APO-B. There are two forms of APO-B, a low molecular weight form APO-B48, which is characteristic of the exogenous system that transports exogenously ingested lipids, and a high molecular weight form called as APO-B100, which is the characteristic of the endogenous system.”

3.1.4.1 Types of Lipoproteins:

Lipoproteins are classified into five types based on their densities as measured by ultracentrifugation.

i) Very-low-density lipoproteins (VLDL): Lehninger AL\textsuperscript{22} discussed that “very-low-density lipoproteins contain high concentrations of triglycerides and moderate concentrations of both cholesterol and phospholipids. When the diet contains more fatty acids than are needed immediately as fuel, they are converted into triacylglycerols in the liver and packaged with specific apolipoproteins into VLDL. They contain some cholesterol and cholesteryl esters, as well as APO B-100, APO C-I, APO C-II, APO C-III and APO E. They are transported in the blood from liver to muscle and adipose tissue, where activation of lipoprotein lipase (LPL) by APO C-II causes the release of free fatty acids (FFA) from the triacylglycerols of VLDL. Most VLDL remnants are removed from circulation by hepatocytes, via receptor-mediated uptake and lysosomal degradation.”

ii) Intermediate-density lipoproteins (IDL): There is an elevation in the phospholipids and cholesterol concentration upon removal of a portion of triglycerides from the very low-density lipoproteins.

iii) Low-density lipoproteins (LDL): These are obtained from IDL when triglycerides are nearly completely removed from IDL. They have high concentrations of cholesteryl esters and cholesterol. APO-B-100 is their major apoprotein.
LDL carries out the transport of cholesterol to the tissues in the periphery, including the hepatic tissues. Recognition of APO B-100 by these peripheral tissues is possible because of the presence of specific receptors located on the surface. These receptors facilitate the uptake of cholesteryl esters and cholesterol.

iv) **High-density lipoproteins (HDL):** Sorci-Thomas MG²³ stated that “high density lipoproteins have a high concentration of proteins (about 50%) but much smaller concentrations of cholesterol, phospholipids and no cholesterol esters. They contain APO C-I and APO C-II among other lipoproteins, as well as the enzyme lecithin-cholesterol acyl transferase (LCAT) which catalyzes the formation of cholesterol esters from lecithin (phosphatidylcholine) and cholesterol. After release into the bloodstream, the nascent (newly synthesized) HDL collects cholesteryl esters from other circulating lipoproteins. Chylomicrons and VLDL, after the removal of their triacylglycerols by lipoprotein lipase (LPL), are rich in cholesterol and phosphatidylcholine. LCAT, on the surface of nascent HDL particles, converts the cholesterol and phosphatidylcholine of chylomicrons and VLDL remnants to cholesterol esters, which begin to form a core, transforming the disc-shaped nascent HDL to a mature, spherical HDL particle. This cholesterol-rich lipoprotein now returns to the liver, where the cholesterol is unloaded. Some of this cholesterol is converted to bile salts.”

v) **Chylomicrons:** Karpe F²⁴ discussed that “chylomicrons contain a high concentration of triglycerides and a small amount of cholesterol and phospholipids. They are the largest of and the least dense of all the lipoprotein subtypes and contain a high concentration of triacylglycerols.” Lehninger AL²² stated that “they are synthesized in the endoplasmic reticulum of epithelial cells that line the small intestine, and then move through the lymphatic system, entering the bloodstream, through the left subclavian vein. The apolipoproteins of chylomicrons include APO B-48 (unique to this class of lipoproteins), APO E and APO C-II. APO C-II activates LPL in the capillaries of adipose tissue, heart, skeletal muscle and lactating mammary tissues; allowing the release of FFAs to these tissues.” The fatty acids that are obtained from food are transported to the tissues by chylomicrons and thereby used as fuel or stored. The Chylomicron remnants from which almost all triacylglycerols have been removed but still have APO E and APO B-48 and cholesterol, are carried to the liver along with blood, undergo lysosomal uptake and degradation and recycling of their constituents occurs.
3.1.5 **Free fatty acids (FFA):** Bernlohr DA\(^{25}\) discussed that “free fatty acids arise in the plasma from lipolysis of triacylglycerols in the adipose tissue or as a result of the action of LPL during the uptake of plasma triacylglycerols into the tissues. They are found in combination with albumin, in the concentrations varying between 0.1 and 2.0 μeq/mL of plasma. Levels are low in the fully fed condition and rise to 0.7 - 0.8 μeq/mL in the starved state. In uncontrolled diabetes mellitus, the levels may rise as high as 2 μeq/mL. They are removed from the blood extremely rapidly and oxidized (fulfilling 25 -50% of energy requirements in starvation) or esterified to form triacylglycerols in the tissues. The FFA uptake by the tissues is related directly to the plasma FFA concentration, which in turn is determined by the rate of lipolysis in the adipose tissue. After dissociation of the fatty acid-albumin complex at the plasma membrane, fatty acids bind to a membrane fatty acid transport protein that acts as a trans membrane co-transporter with Na+. On entering the cytosol, FFA are bound by intracellular fatty acid-binding proteins. Cardiac muscle, skeletal muscle, kidneys and other organs, where they are oxidized to CO₂ and H₂O in the mitochondria (β oxidation) and used as a source of energy. Fat cells, which either store the FFAs or use them to synthesize triglycerides. The liver is where the FFA are oxidized or used to synthesize triglycerides.”

**Figure-3.1.2: Structure of Lipoprotein** \(^{19}\)
Table-3.1.1: Composition of Lipoproteins of Human Plasma

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Source</th>
<th>Diameter (nm)</th>
<th>Density (g/mL)</th>
<th>Composition (%)</th>
<th>Main Lipid Components</th>
<th>Apolipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>Intestine</td>
<td>90-1000</td>
<td>&lt; 0.95</td>
<td>1-2</td>
<td>Triglycerol</td>
<td>A-I, A-II, A-IV, B-I, C-I, C-II, C-III, E</td>
</tr>
<tr>
<td>Chylomicron remnants</td>
<td>Chylomicrons</td>
<td>45-150</td>
<td>&lt; 1.006</td>
<td>6-8</td>
<td>Triglycerol, phospholipids, cholesterol</td>
<td>E, B, C, D</td>
</tr>
<tr>
<td>VLDL</td>
<td>Liver, intestine</td>
<td>30-90</td>
<td>0.95-1.006</td>
<td>7-10</td>
<td>Triglycerol</td>
<td>B-100, C-I, C-II, C-III</td>
</tr>
<tr>
<td>IDL</td>
<td>VLDL</td>
<td>25-35</td>
<td>1.006-1.019</td>
<td>11</td>
<td>Triglycerol, cholesterol</td>
<td>E, B-100, C-I, C-II, C-III</td>
</tr>
<tr>
<td>LDL</td>
<td>VLDL</td>
<td>20-25</td>
<td>1.019-1.063</td>
<td>21</td>
<td>Cholesterol</td>
<td>E-100</td>
</tr>
<tr>
<td>Pre-HDL$^4$</td>
<td></td>
<td>&lt; 5</td>
<td>&gt; 1.210</td>
<td>21</td>
<td>Free fatty acids</td>
<td>A-I</td>
</tr>
</tbody>
</table>

Abbreviations: HDL, high-density lipoproteins; IDL, intermediate-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins.
1. Secrete with chylomicrons but transfer to HDL.
2. Associated with HDL$_1$ and HDL$_2$ subfractions.
3. Part of a minor fraction known as very high density lipoproteins (VHDL).
Figure-3.1.3: Pathway of Lipoprotein Metabolism
3.2 Hyperlipidaemia

Bersot TP\textsuperscript{26} stated that “hyperlipidaemia is a major cause of atherosclerosis and atherosclerosis-related conditions such as coronary heart disease (CHD), ischaemic cerebrovascular disease and peripheral vascular disease. These conditions account for most morbidity and mortality among middle-aged and older adults. Dyslipidaemias, including hyperlipidaemia (hypercholesterolaemia) and low levels of HDL cholesterol, are the major causes of increased atherogenesis; genetic disorders and lifestyle (sedentary behavior and diet high in calories, saturated fat and cholesterol) contribute to the dyslipidaemias seen in the developed and developing countries.”

Hyperlipidaemia may manifest as hypercholesterolaemia or hypertriglyceridaemia or both. There is no ideal scheme to categorize these disorders, since the disorders are not exclusive to either endogenous or exogenous pathway of lipoprotein metabolism, and can run through family by inheriting trait or can arise from secondary disease in the body. Based on whether hyperlipoproteinaemia is inherent in nature or secondary to other disorders, it is broadly classified into two categories:

3.2.1 Primary category\textsuperscript{27} 

Inherited defects in lipoprotein metabolism lead to primary condition of either hypo- or hyperlipoproteinaemia.

Based on whether the primary elevation is in the serum triglycerides or cholesterol, it is divided into the following subtypes:

3.2.1.1 Primary hypertriglyceridaemias:

i) Primary chylomicronaemia (familial lipoprotein lipase or cofactor deficiency):

It is a rare autosomal recessive disorder characterized by hypertriacylglycerolaemia, that occurs as the result of abnormal lipoprotein lipase (LPL) or paucity of LPL or APO C–II resulting in inactive LPL, leading to failure in clearing chylomicrons. HDL and LDL are decreased.

ii) Familial hypertriglyceridaemia:

It is an autosomal dominant disorder characterized by the overproduction of VLDL and
the resultant increase of cholesterol. It is associated with glucose intolerance, hyperinsulinaemia and obesity.

iii) Familial combined hyperlipoproteinaemia:

It is an autosomal dominant disorder characterized by an elevated level of VLDL, LDL or both; leading to a moderate elevation in serum triglycerides, cholesterol or both.

iv) Familial dysbetalipoproteinaemia:

In this disorder, remnants of chylomicrons and VLDL accumulate and LDL levels are decreased due to an abnormality of APO E, leading to a deficiency in remnant clearance. Cholesteryl esters are present in abundance in these remnants, thereby cholesterol level may be as elevated as the level of triglycerides. Patients tend to have impaired glucose tolerance and be obese. They commonly suffer from characteristic plantar xanthomas of the palmer creases or there is occurrence of tuberous or tuberoeruptive xanthomas. Coronary and peripheral atherosclerosis occurs with increased frequency.

3.2.1.2 Primary hypercholesterolaemias:

i) Familial hypercholesterolaemia:

It is an autosomal dominant disorder characterized by the presence of LDL receptor defects. Cholesterol level tends to be very high with normal triglyceride level. Patient may suffer from xanthelasma and arcus corneae in the third decade of life. Occurrence of tendon xanthomas is fairly common., and arcus corneae and xanthelasma may appear in the third decade. There may be a tendency of premature occurrence of coronary disease.

ii) Familial ligand-defective apolipoprotein B:

Moderately severe hypercholesterolaemia occurs as the result of impaired LDL endocytosis, due to binding of defective APO B-100 domain to the receptor of LDL.

iii) Lp(a) hyperlipoproteinaemia:

Lp(a) comprises of APO A one mol bound to LDL one mol. Premature CHD due to atherosclerosis and thrombosis due to inhibition of fibrinolysis is seen.
iv) Familial LCAT (lecithin-cholesterol acyltransferase):

LCAT is responsible for esterification of HDL cholesterol; deficiency of which leads to a block in the RCT. It is associated with a low level of LDL. These patients tend to have premature atherosclerosis.

v) Familial hypoalphalipoproteinaemia:

It is associated with a low level of LDL. These patients tend to have premature atherosclerosis.

vi) Hepatic lipase deficiency:

There is an accumulation of VLDL remnants. Patients have xanthomas and cardiovascular disease (CVD).

3.2.2 Secondary category:

Hyperlipoproteinaemia secondary to underlying disorders:

Table 3.2.1: Secondary causes of Hyperlipoproteinaemia

<table>
<thead>
<tr>
<th>Hypertriglyceridaemia</th>
<th>Hypercholesterolaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Mellitus</td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Alcohol ingestion</td>
<td>Early nephrosis</td>
</tr>
<tr>
<td>Severe nephrosis</td>
<td>Resolving lipaemia</td>
</tr>
<tr>
<td>Oestrogens</td>
<td>Immunoglobulin- lipoprotein complex disorders</td>
</tr>
<tr>
<td>Uraemia</td>
<td>Anorexia nervosa</td>
</tr>
<tr>
<td>Corticosteroid excess</td>
<td>Cholestasis</td>
</tr>
<tr>
<td>Myxoedema</td>
<td>Hypopituitarism</td>
</tr>
<tr>
<td>Glycogen storage disease</td>
<td>Corticosteroid excess</td>
</tr>
<tr>
<td>Hypopituitarism</td>
<td></td>
</tr>
<tr>
<td>Acromegaly</td>
<td></td>
</tr>
<tr>
<td>Immunoglobulin- lipoprotein complex disorders</td>
<td></td>
</tr>
<tr>
<td>Lipodystrophy</td>
<td></td>
</tr>
<tr>
<td>Drugs: Isotretinoin, Protease inhibitors</td>
<td></td>
</tr>
</tbody>
</table>
3.3 Atherosclerosis

3.3.1 Introduction

Santiago GM\textsuperscript{28} stated that “atherosclerosis is characterized by accumulation of lipids and fibrous elements in the large arteries.” Mohammadi M\textsuperscript{29} discussed that “it is the leading cause of mortality and morbidity in the developed world and most of the developing countries. It is a complex process, and is possibly related to a high fat diet and sedentary lifestyle.” Sharma M\textsuperscript{30} stated that “the gravity of this situation is emphasized by a recent projection from the World Health Organization (WHO) and the Indian Council of Medical Research (ICMR), which predicts that India will be the myocardial infarct (MI) capital of the world by 2020.” Atherosclerosis mainly affects the circulatory system.

Angina pectoris and myocardial infarction occur commonly as the result of coronary artery atherosclerosis. Transient cerebral ischaemia and stroke are provoked usually because of atherosclerosis occurring in the arteries that supply the CNS. Viability of the limbs may be at risk due to gangrene and intermittent claudication as the consequence of atherosclerosis in the peripheral circulation. Mesenteric ischaemia may occur due to atherosclerosis in the splanchnic circulation. Renal artery stenosis can occur as the direct consequence of atherosclerosis in the renal circulation. It may also indirectly affect the kidneys by acting as the common site of occurrence of atheroembolic disorder.

3.3.2 Epidemiology and risk factors\textsuperscript{31}

Mukherjee AK\textsuperscript{32} argued that “virtually ubiquitous among most of the developed nations, atherosclerosis is much less prevalent in Central and South America, Africa, and Asia; though its incidence is steadily growing. The mortality rate for ischaemic heart disease (IHD) in India is among the highest in the world. CVD in India cause 3 million deaths/year, accounting for 25% of all mortality.” Castelli WP\textsuperscript{33} discussed that “the risk factors that predispose to atherosclerosis and resultant IHD have been identified by means of a number of prospective studies in well-defined population groups, most notably the Framingham Study (Massachusetts) and the Multiple Risk Factor Intervention Trial. The constitutional risk factors include age, sex and genetics.”
Table-3.3.1: Risk Factors for Atherosclerosis\textsuperscript{31}

<table>
<thead>
<tr>
<th>Major</th>
<th>Lesser, Uncertain or Non-quantitated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-modifiable</td>
<td></td>
</tr>
<tr>
<td>Increasing age</td>
<td>Obesity</td>
</tr>
<tr>
<td>Male gender</td>
<td>Physical inactivity</td>
</tr>
<tr>
<td>Family history</td>
<td>Stress (“Type A” personality)</td>
</tr>
<tr>
<td>Genetic abnormalities</td>
<td>Postmenopausal oestrogen deficiency</td>
</tr>
<tr>
<td>High carbohydrate intake</td>
<td></td>
</tr>
<tr>
<td>Potentially controllable</td>
<td></td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>Alcohol</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Lipoprotein Lp(a)</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>Hardened (\textit{trans}) unsaturated fat intake</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Chlamydial pneumonia</td>
</tr>
</tbody>
</table>

3.3.3 Role of Cholesterol and Lipoproteins in Atherosclerosis

Jaarin K\textsuperscript{34} stated that “hypercholesterolaemia is a known major risk factor in the development of atherosclerosis. It can result from endogenous causes or from exogenous dietary source. Dietary hypercholesterolaemia may result from high cholesterol intake or as a result of increased intake of saturated fatty acids. Hypercholesterolaemia, regardless of cause, influences the development of atherosclerosis.” Chen L\textsuperscript{35} argued that “in particular, elevated levels of total cholesterol and LDL in plasma are the major risk factors for the development of atherosclerosis. Much evidence provides support for the concept that the oxidized form of LDL causes oxidant stress and increases intracellular Ca\textsuperscript{2+} in the vessel wall, and represents the pathogenic element in hypercholesterolaemia.”

Grundy SM\textsuperscript{36} discussed that “in contrast, HDL is believed to mobilize cholesterol from developing and existing atheromas, and transport it to the liver for excretion in the bile, hence its designation as the ‘good cholesterol’. Therefore, the higher the level of HDL, the lower is the risk.” Understandably, there is thus a great interest in the dietary,
pharmacologic and behavioral methods of lowering the plasma LDL and raising the HDL. Exercise raises the level of HDL, whereas, smoking and obesity lower it.

High dietary intake of cholesterol and saturated fats, such as those present in egg yolk, animal fats, and butter; raises the plasma cholesterol level. Conversely, a diet low in cholesterol and low in the ratio of saturated-to-polyunsaturated fats lowers the plasma cholesterol levels. Moreover, omega-3 fatty acids, abundant in fish oils, are likely beneficial, whereas, hardened (trans) unsaturated fats produced by artificial hydrogenation of polyunsaturated vegetable fats and used in baked goods and margarine, may adversely affect the cholesterol profiles and contribute to atherosclerosis.

3.3.4 Pathogenesis of atherosclerosis: 31

Understandably, the overwhelming importance of atherosclerosis has stimulated enormous efforts to discover its cause. Historically, two hypotheses for atherogenesis were dominant: One emphasized the cellular proliferation in the intima, whereas, the other emphasized organization and repetitive growth of thrombi. The contemporary view of the pathogenesis of atherosclerosis incorporates the elements of both older theories, and accommodates the risk factors discussed previously. This concept, called the response to injury hypothesis, considers atherosclerosis to be a chronic inflammatory response of the arterial wall, initiated by an injury to the endothelium. Moreover, lesion progression is sustained by the interaction between modified lipoproteins, monocyte-derived macrophages, T-lymphocytes and the normal cellular constituents of the arterial wall.

Central to this hypothesis are the following: 31

- Chronic endothelial injury, usually subtle, with resultant endothelial dysfunction, yielding increased permeability, leucocyte adhesion and thrombotic potential.
- Accumulation of lipoproteins, mainly LDL, with its high cholesterol content, in the vessel wall.
- Modification of lesional lipoproteins by oxidation.
- Adhesion of blood monocytes (and other leucocytes) to the endothelium, followed by their migration into the intima and their transformation into macrophages and foam cells.
- Adhesion of platelets.
- Smooth muscle cells (SMC) from the middle coat of blood vessels into the inner coat migration owing to the factors that are released from vascular cells, macrophages and the activated platelets.
- Multiplication or reproduction of similar SMC in the inner coat of blood vessels and development of extracellular matrix thereby resulting in the buildup of proteoglycans and collagen.
- Increased buildup of lipids inside the SMC and macrophages as well as outside these cells.

### 3.3.5 Role of Oxidized-Low-Density Lipoproteins in Atherogenesis:

Sezer ED\(^{37}\) discussed that “atherogenesis is a chronic inflammatory process that involves a complex interplay between the circulating cellular and blood elements, and the cells of the arterial wall. While many factors are involved, the ‘oxidative hypothesis’ has been a central focus in the investigation of pathogenesis of atherosclerosis, suggesting that the oxidative modification of LDL is central, if not obligatory, for the atherogenic process. The oxidative modification of LDL has been implicated, both in the atherosclerosis and the development of abnormal endothelium dependent control of vascular tone.”

Keaney JF\(^{38}\) argued that “abnormalities in the endothelium-dependent arterial relaxation develop early in the course of atherosclerosis, and may result in part from the effects of oxidized LDL on agonist-mediated endothelium-derived relaxing factor (EDRF) release and EDRF degradation. In vitro, oxidized-low-density lipoprotein (ox-LDL) inhibits the receptor-mediated endothelium-dependent arterial relaxation and the endothelial-cell signal transduction. Moreover, ox-LDL is cytotoxic to the endothelial cells and chemotactic for the monocytes; leading to the accumulation of vascular endothelial cells, local production of oxygen-derived free radicals, and the degradation of EDRF.”
Sezer ED\textsuperscript{37} discussed that “oxidation of LDL is a lipid peroxidation process, resulting in the formation of a wide range of biologically active products, including peroxide and malondialdehyde. Oxidation of LDL results in the generation of aldehydes, that substitute lysine residues in the APO B-100 moiety of LDL and causes their fragmentation. As a result, the particle loses its affinity for the LDL receptor and binds avidly to the scavenger receptors on macrophages, resulting in foam cell formation.”

Witztum JL\textsuperscript{39} stated that “interest in the studies on ox-LDL stemmed originally from the observations that this modification of LDL led to its enhanced uptake in the macrophages. However, the ability of ox-LDL to cause cholesteryl ester accumulation, may in part, also be related to an inability of macrophages to degrade ox-LDL as readily as native LDL, resulting in the accumulation of undegraded ox-LDL. The recent demonstration that arterial wall macrophages, isolated from cholesterol-fed rabbits, can contain as much as 600 μg of cholesterol per mg cell protein, and that they also stain for epitopes of ox-LDL even after being in culture for several days, is consistent with this suggestion. In addition, various oxidized products, such as oxidized sterols, may interfere with a variety of intracellular enzymes, for example, inhibiting the normal cellular processing of ingested lipids and proteins, and possibly, even leading to the accumulation within the cells of toxic intermediates.”
3.3.6 Natural history of atherosclerosis

The American Heart Association classification divides atherosclerotic lesions into six types, beginning with isolated foam cells (‘fatty dots’) through the stages of fatty streaks, atheromas, and fibroatheromas, to the complicated lesion as following:

**Type 1:** Isolated macrophage foam cells.

**Type 2:** According to the results obtained from clinical and animal studies regarding atherosclerosis, the “fatty streak” represents the earliest lesion of atherosclerosis. They are composed of lipid-filled foam cells. They are not significantly raised and thus do not cause any disturbance to the blood flow. Fatty streaks begin as multiple yellow flat spots less than 1 mm in diameter that coalesce into elongated streaks, 1 cm long or more. They contain T lymphocytes and extracellular lipid in smaller amounts than in plaques.

**Type 3:** (Intermediate lesion): There is a small extracellular lipid accumulation.
Type 4: (Atheroma): Formation of an extracellular lipid core.

Type 5: (Fibroatheroma lesion): Single or multiple lipid core and fibrotic layer, mainly calcific or fibrotic.

Type 6: (Complicated lesion): Surface defect, haemorrhage, thrombus formation.

3.3.7 Diagnosis

Erkelens DW\textsuperscript{40} stated that “as hypercholesterolaemia is an essential risk factor for atherosclerosis, a strategy for diagnosis and treatment of hyperlipidaemia is indispensable.” In routine practice, determination of triglycerides, total cholesterol, LDL and HDL cholesterol allows us to (a) diagnose hyperlipidaemia and (b) to type the hyperlipoproteinaemia, based on the known triglyceride and cholesterol content of the different classes of lipoproteins. The commonest hyperlipoproteinaemias are secondary to dietary excess, uncontrolled diabetes mellitus, hyperthyroidism, alcoholism and chronic renal failure. Therefore, every patient with hyperlipidaemia should be investigated for these factors.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Desirable level (Low Risk)</th>
<th>Borderline level (Moderate Risk)</th>
<th>Abnormal Level (High Risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>&lt; 200</td>
<td>200 – 240</td>
<td>&gt; 240</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>&lt; 130</td>
<td>130 - 160</td>
<td>&gt; 160</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>&gt; 60</td>
<td>40 - 60</td>
<td>&lt; 40</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt; 200</td>
<td>200 – 240</td>
<td>&gt; 400</td>
</tr>
</tbody>
</table>

*The risk increases further with other risk factors such as smoking, diabetes and Hypertension.*

3.3.8 Management:

The current NCEP (National Cholesterol Education Programme 1990) guidelines for the management of patients with lipid disorders are of two types. One is the population-based approach, which is intended to lower the blood cholesterol by dietary recommendations: Reduce total calories from fat to less than 305 kcal and from saturated
fat to less than 10%, consume less than 300 mg of cholesterol per day, and maintain desirable body weight.

The second is the patient-based approach described in the 2001 report of the NCEP, Adult treatment Panel-III (ATP-III), which continues to focus on lowering the LDL levels as the primary goals of therapy. The NCEP–ATP III guidelines regarding the hypolipidaemic therapy for decreasing the risk of coronary heart disease comprise of many new features that include the modification in lipid/ lipoprotein levels, increased focus on the primary prevention through the use of Framingham risk scoring, to define the risk in a person with multiple lipid/ non-lipid risk factors, and increased focus on the association of the metabolic syndrome with CHD risk. Use of new secondary therapeutic target of non-high density lipoprotein cholesterol, should improve the management of lipid risk factors in the patients who have elevated triglyceride levels after LDL goals have been met.

Various drugs are used to decrease LDL cholesterol levels in the plasma. As stated by Rang HP, “drug therapy to lower the plasma lipids is used in addition to dietary management and correction of other modifiable cardiovascular risk factors.” Chaudhari SK stated that “according to the European Atherosclerotic Society guidelines, serum cholesterol values should be less than 200 mg/dl. Where the values are between 200 – 250 mg/dl, treatment to lower cholesterol value should be started, but should be by non-drug regimen. Where the values are still higher, drugs should be included. HDL values below 35 mg/dl demand lipid lowering therapy strongly.”

As per the report from ATP-III, there are four different types of drugs available for the management of lipid disorders. They comprise of statins that act by causing competitive inhibition of the enzyme HMG-CoA, niacin, binding resins (bile acid sequestrants), and derivatives of fibric acid.

**i) HMG-CoA Reductase Inhibitors (Statins):**

For the management of dyslipidaemia, statins are currently the most effective, most commonly used, as well as the best tolerated drugs. They act by causing competitive inhibition of the enzyme HMG-CoA reductase. This enzyme acts as the catalyst for an early, rate-limiting step in the synthesis of cholesterol in the body. Some of the statins
that have greater potency such as simvastatin, rosuvastatin and atorvastatin etc, when given in larger doses, cause decrease in triglyceride levels also that occur due to increased VLDL levels. Increase in the levels of HDL may also be an indication for the use of some statins, however its clinical importance has not yet been proved unequivocally.

Isolation of statins was done by Endo and his colleagues in 1976 from *Penicillium citrinum* mould. Later on, Brown and Goldstein discovered that statins cause decrease in the synthesis of cholesterol in the body by competitive inhibition of enzyme HMG-CoA reductase.

Mode of Action: Statins cause decrease in the LDL levels owing to a mevalonic acid-type moiety which results in competitive inhibition of the enzyme HMG-CoA reductase. Statins cause inhibition of synthesis of cholesterol in the liver, thereby affecting its levels in the blood, with resultant rise in LDL gene expression. Decrease in LDL receptors’ degradation causes an increase in the number of receptors of LDL on liver cells’ surface with resultant rise in the rate of LDL removal from blood, culminating in lowered levels of LDL in the blood.\(^{26}\)

Different statins differ in their potency and maximal efficacy in reducing LDL.

Tannous M\(^{45}\) discussed that “atorvastatin has also been shown to elevate nitrous oxide production. It was found to reduce the size of atherosclerotic lesion and decrease vascular smooth muscle cell proliferation in in-vitro model. These effects raise a hope that atorvastatin may promote platelet de aggregation and vasodilatation in patients of dyslipidaemia.”

**ii) (Binding Resins) Sequestrants of Bile Acids:**

Resins such as colesvelam, colestipol, cholestyramine etc are the oldest in the category of lipid lowering drugs. Compared to other lipid lowering drugs, they have greater safety profile, as their direct intestinal absorption does not occur. They are usually used as second line drugs in those cases where statins can’t cause adequate decrease in the levels of LDL in the blood. They are also recommended for patients 11 – 20 years of age. When used with a statin, they are usually given at sub-maximal doses since maximal
doses are associated with unacceptable gastric side effects (bloating and constipation) that limit compliance.26

**Mechanism of Action:**

As the binding resins bear positive charge, they bind with high affinity to the bile acids that bear negative charge. Binding resins have large molecular size, and thus do not undergo intestinal absorption, with resultant excretion of the bile acids along with the faeces. Increased excretion of bile acids breaks the continuity of their intestinal reabsorption process, that normally occurs in a very significant proportion. This leads to a major decrease in the quantity of bile acids in the body, ultimately causing an increase in their production in the liver that requires cholesterol. Thus, there occurs a lowering of cholesterol content in the liver, augmenting the synthesis of receptors of LDL, thereby causing a rise in clearance of LDL culminating in depletion of levels of LDL in body.

**iii) Nicotinic Acid (Niacin)**

Amongst the drugs used in the treatment of dyslipidaemia, it is one of the earliest drugs available. It belongs to the B-complex group of vitamins, is water soluble, can perform the role of a vitamin after it gets converted to nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). It exists as an amide in it. Oral administration is permissible for niacin as well as its amide, however, the levels of lipids are affected by niacin only. It has a favourable impact on almost all parameters of lipids. Higher doses are needed for its hypolipidaemic effect, as compared to the doses needed in order to get its effects as a vitamin. Its use may be preferred for causing a rise in the levels of HDL (30-40%); it also lowers triglycerides by 35-45% and reduces LDL by 20-30%.26

**Mode of Action:**

Niacin, in the adipose tissue, causes inhibition of decomposition of triglycerides mediated by hormone-sensitive lipase, thereby causing a decrease in the free fatty acid carriage towards the liver. It causes a decrease in hepatic lipase, which decreases the carriage of free fatty acids towards the liver and results in lowering of synthesis of triglycerides in the liver. Niacin also causes inhibition of enzyme diacyl glycerol acyltransferase-2, that is a rate limiting enzyme involved in the synthesis of triglycerides.
iv) Fibric Acid Derivatives (PPAR Activators):

They act by binding to peroxisome proliferator-activated receptor-α (PPAR-α). These receptors are densely located in liver and brown fat tissue, and comparatively less densely in the cardiac and renal tissues and skeletal muscles. They cause an increase in the oxidation of fatty acids by causing the stimulation of PPAR-α receptors, thereby resulting in decrease in triglycerides. They also cause decrease in triglycerides by virtue of causing activation of LPL and less formation of APO C-III. Fibrates also mediate rise of HDL levels because of rise in production of APO A-I and APO A-II by liver, which causes lowering of LDL.

v) Other Drugs:

a) Sterol Absorption inhibitors:

Ezetimibe, a comparatively newer drug, acts by causing inhibition of absorption from the intestine of phytosterols and cholesterol, that are present in the diet as well as bile. Its main action is to cause a decrease in the levels of LDL.

b) Probucol:

A hypocholesterolaemic drug with an antioxidant property that appears to exert antiatherogenic effect by inhibiting oxidation of LDL, rather than by lowering cholesterol. It causes a modest lowering of total plasma cholesterol, which is more due to reduction of LDL cholesterol, it has little effect on VLDL and triglyceride levels.

c) Gugulipid:

It is a mixture of sterones obtained from ‘gum guggul’ which has been used in Ayurveda. Modest lowering of plasma cholesterol and triglycerides occurs after continued use of gugulipid.

d) Microsomal triglyceride transfer protein (MTP) Inhibitors:

MTP inhibitors targeted to the liver would decrease VLDL production, thereby decreasing plasma triglyceride levels and ultimately reducing LDL production from VLDL.
3.4 Carotenoids

Rao AV\textsuperscript{46} discussed that “there is convincing scientific evidence in support of the association between diet and the chronic diseases. Based on such evidence, dietary guidelines have been formulated around the world for the prevention of chronic diseases such as cancer, cardiovascular disease, diabetes and osteoporosis. One of the main recommendations of these dietary guidelines is to increase the consumption of plant-based foods, including fruits and vegetables, that are good sources of carotenoids and other biologically active phytochemicals. In recent years, oxidative stress induced by Reactive Oxygen Species (ROS) that are generated by normal metabolic activity, as well as lifestyle factors such as smoking, exercise and diet, have been implicated in the causation and progression of several chronic diseases. Antioxidants that can mitigate the damaging effects of ROS have been the focus of research.”

Mangels AR\textsuperscript{47} stated that “carotenoids are a family of pigmented compounds that are synthesized by the plants and microorganisms, but not by animals. Fruits and vegetables constitute the major source of carotenoids in the human diet.” Paiva S\textsuperscript{48} stated that “they are present as micro components in fruits and vegetables and are responsible for their yellow, orange and red colours. They are thought to be responsible for the beneficial properties of fruits and vegetables in preventing human diseases including CVD, cancer and other chronic diseases.” Gerster H\textsuperscript{49} discussed that “more than 600 carotenoids have so far been identified in nature. However, only about 40 are present in the typical human diet, of which, about 20 have been identified in the human blood and tissues. Close to 90% of these carotenoids in the diet and human body are represented by \( \beta \)-carotene, \( \alpha \)-carotene, lycopene, lutein and cryptoxanthin.”

Parker RS\textsuperscript{50} stated that “several factors influence the absorption of carotenoids. Food processing and cooking that causes mechanical breakdown of the tissue releasing the carotenoids, improves their absorption.” Erdman Jr JW\textsuperscript{51} discussed that “in the intestine, carotenoids are absorbed by passive diffusion after being incorporated into the micelles that are formed by the dietary fat and bile acids. The micellar carotenoids are then incorporated into the chylomicrons and released into the lymphatic system. They are then incorporated into the lipoproteins in the liver and released into the blood.”
3.5 Lycopene

Stahl W$^{52}$ stated that “lycopene is an unsaturated acyclic carotenoid, with 11 linear conjugated and two non-conjugated double bonds. It is not a precursor for vitamin A, since it lacks the terminal β-ionic ring found in the basic structure of vitamin A. It is the pigment that gives red colour to the fruits and vegetables such as tomatoes, watermelons, grapefruits and red grapes. It is reported as the most efficient singlet oxygen quencher in the carotenoids group, whose quenching ability is mainly dependent on the number of conjugated double bonds, and to a lesser extent, to the presence of cyclic or acyclic end groups.”

As per Stahl W$^{53}$, “this red colored pigment was first discovered in the tomato by Millardet in 1876. It was later named lycopene by Schunck. Lycopene from the natural plant sources exists predominantly in the trans configuration, the most thermodynamically stable form. In human plasma, lycopene is an isomeric mixture containing 50 % of the total lycopene as cis isomers. Lycopene, ingested in its natural trans form found in tomatoes, is poorly absorbed. Recent studies have shown that heat processing of tomatoes and tomato products induces isomerisation of lycopene to the cis form, which in turn increases its bioavailability.”

**Table-3.5.1: Physical Properties of Lycopene$^{54}$**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>C_{40}H_{56}</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>556.83 Da</td>
</tr>
<tr>
<td>Melting point</td>
<td>172-175 °C</td>
</tr>
<tr>
<td>Crystal form</td>
<td>Long red needles separate from a mixture of carbon disulfide and ethanol</td>
</tr>
<tr>
<td>Powder form</td>
<td>Dark reddish-brown</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in chloroform, hexane, benzene, carbon disulfide, acetone, petroleum ether and oil. Insoluble in water, ethanol and methanol</td>
</tr>
<tr>
<td>Stability</td>
<td>Sensitive to light oxygen, high temperature, acids, catalyst and metal ions</td>
</tr>
</tbody>
</table>
Figure-3.5.1: Molecular structures of lycopene isomers$^{54}$
3.5.1 Absorption, Transportation and Distribution of Lycopene in Humans:

3.5.1.1 Absorption:

Agarwal S\textsuperscript{56} stated that “in the stomach and duodenum, lycopene separates from the food matrix and subsequently dissolves in the lipid phase. Prior to absorption, the lipid phase forms droplets, resulting from the reaction with bile salts and pancreatic lipases. Then, it enters the duodenum and appears as the multi-lamellar lipid vesicles. Finally, the lipid vesicles are absorbed into small intestine via passive diffusion process.” The bioaccessibility of lycopene in the intestine has also been discussed by Goni I\textsuperscript{57} who stated that “the release of lycopene was higher in the large intestine (57%) than in the small intestine (40%), but the potential for lycopene to be absorbed in the large intestine is negligible.” As per Failla ML\textsuperscript{58} “furthermore, an in vitro study using Caco-2 cells showed that the uptake of \textit{cis} lycopene was significantly greater than for all \textit{trans} isomer.” Thus, \textit{cis} isomers have a higher bioavailability than all \textit{trans} isomers.

Table-3.5.2: Lycopene content of various foods\textsuperscript{55}

<table>
<thead>
<tr>
<th>Source</th>
<th>Lycopene content (mg/100 g wet basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatoes, fresh\textsuperscript{a}</td>
<td>0.72–20</td>
</tr>
<tr>
<td>Tomato juice\textsuperscript{b}</td>
<td>5.00–11.60</td>
</tr>
<tr>
<td>Tomato sauce\textsuperscript{b}</td>
<td>6.20</td>
</tr>
<tr>
<td>Tomato paste\textsuperscript{b}</td>
<td>5.40–150.00</td>
</tr>
<tr>
<td>Tomato soup, condensed\textsuperscript{b}</td>
<td>7.99</td>
</tr>
<tr>
<td>Ketchup\textsuperscript{b}</td>
<td>9.90–13.44</td>
</tr>
<tr>
<td>Pizza sauce, canned\textsuperscript{b}</td>
<td>12.71</td>
</tr>
<tr>
<td>Spaghetti sauce\textsuperscript{c}</td>
<td>9.3–18.2</td>
</tr>
<tr>
<td>Barbecue sauce\textsuperscript{c}</td>
<td>7.6</td>
</tr>
<tr>
<td>Watermelon\textsuperscript{d}</td>
<td>2.3–7.2</td>
</tr>
<tr>
<td>Pink guava\textsuperscript{a}</td>
<td>5.23–5.50</td>
</tr>
<tr>
<td>Pink grapefruit\textsuperscript{e}</td>
<td>0.35–3.36</td>
</tr>
<tr>
<td>Papaya\textsuperscript{e}</td>
<td>0.11–5.3</td>
</tr>
<tr>
<td>Carrot\textsuperscript{a}</td>
<td>0.65–0.78</td>
</tr>
<tr>
<td>Pumpkin\textsuperscript{a}</td>
<td>0.38–0.46</td>
</tr>
<tr>
<td>Sweet potato\textsuperscript{a}</td>
<td>0.02–0.11</td>
</tr>
<tr>
<td>Apricot\textsuperscript{a}</td>
<td>0.01–0.05</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Adapted from Shi (2000).
\textsuperscript{b} Adapted from Clinton (1998).
\textsuperscript{c} Adapted from Lugasi, Hovari, Biro, Brandt, and Helyes (2004).
Richelle M\textsuperscript{59} stated that “the nature of the human body is believed to cause the isomerisation of lycopene along the digestive tract. A study reported that 60\% of cis lycopene isomers occurred in human plasma, even though the early consumption of the lycopene rich food that mostly consisted of all trans lycopene (>90\%).” Re R\textsuperscript{60} discussed that “an in vivo study also explained that the acidic condition in gastric milieu enhances the isomerisation of all trans lycopene to cis isomers.” This mechanism will further improve the absorption of lycopene once it reaches the small intestine.

Unlu NZ\textsuperscript{61} stated that “food processing is one of the factors which can affect the bioavailability of lycopene, and thus increase its absorption. The heating of tomato sauce purposely to induce the isomerisation of all trans lycopene to cis isomers, could increase the bioavailability of lycopene.” Besides that, an in vitro study by Karakaya S\textsuperscript{62} showed that “sun dried tomatoes give the highest bioavailability of lycopene as compared to fresh and canned tomatoes.” Ahuja KDK\textsuperscript{63} stated that “besides, ingestion of lycopene together with oil also helps in increasing its bioavailability.” Brown MJ\textsuperscript{64} discussed that “a human study has shown that a combination of salad dressing and canola oil increased the lycopene content in plasma chylomicrons, as compared to fat free salad dressing.” This is in agreement with the results of Fielding et al\textsuperscript{65} showing that “the tomatoes cooked with olive oil greatly increase the lycopene level in human plasma, as compared to the tomatoes cooked without olive oil.” Cardinault N\textsuperscript{66} stated that “moreover, bioavailability of lycopene was found to be impaired in elderly people.”

\textbf{3.5.1.2 Transportation:}

Roldan-Gutierrez JM\textsuperscript{67} discussed that “after the uptake by intestinal mucosa, lycopene is parcelled into the triacylglycerol-rich chylomicrons and is secreted into the lymph transport system, and lastly transferred to the liver.” Clinton SK\textsuperscript{68} stated that “it is prone to accumulate in the lipophilic compartments of membrane or lipoprotein. As a hydrophobic compound, lycopene is found at the lipophilic part of lipoproteins which is the core of the lipoprotein, while other polar carotenoids are found at the surface of the lipoproteins. Therefore, lycopene is mostly transported by LDL, while other oxygenated carotenoids are transported by both LDL and HDL.” Boileau TW\textsuperscript{69} discussed that “in addition, cis isomers of lycopene were reported to have a higher ability to be incorporated in the lipoprotein and other protein compared to all trans isomers, due to its shorter chain length.”
3.5.1.3 Distribution:

The distribution of lycopene in the human organs and plasma has been reported by Erdman JW Jr\textsuperscript{70} where “higher concentrations of lycopene are found in the liver, adrenals and reproductive organs (ten times higher than other tissues). The concentrations were within the range of 0.2-21.4 nmol/g tissue.” Goraleczyk R\textsuperscript{71} described that “lycopene concentration was highest in human testes, followed by adrenal gland > liver > prostate > breast > pancreas > skin > colon > ovary > lung > stomach > kidney > fat tissue > cervix.” A review by Rao and Agarwal\textsuperscript{72} quoted that “lycopene concentrations in the human tissues are around 0.15 – 21.36 nmol/g tissue, but are not detectable in the brainstem tissue.” On the other hand, a study on rats carried out by Zaripheh et al\textsuperscript{73} showed that lycopene was highly distributed in the liver. Besides, high lycopene content was found in the adipose tissue, spleen and adrenal tissue. The excretion of lycopene through faeces and urine was also reported.

In humans, total serum carotenoids is about 1 – 2 μM, with lycopene being one of the major carotenoids present in the human serum. As per Porrini et al\textsuperscript{74} “the eating behaviour of different individuals makes the lycopene level vary among people.”

The lycopene metabolite products were recently studied by Lindshield et al\textsuperscript{75} and lycopene metabolites were formed by reacting with carotenoid monooxygenase (CMO) II. A Study using post-mitochondrial fraction of rat mucosa with soy lipoxygenase reviewed that cleavage products and oxidation products were formed from lycopene metabolism.

3.5.2 Mechanism of action:

Britton G\textsuperscript{76} stated that “the reactivity of carotenoids, especially lycopene, in the biological systems depends on their molecular and physical structure, location or site of action within the cells, ability to interact with other antioxidants, concentration and the partial pressure of oxygen.” As per Stahl W\textsuperscript{77} “biologically, lycopene tends to act as singlet oxygen (1O2) and peroxy radical scavenger (LOO•).” Young AJ\textsuperscript{78} discussed that “the highly conjugated double bonds of lycopene play the most important role in energy transfer reactions. Lycopene has quenching ability towards singlet oxygen (1O2), based on the excited energy state, and is greatly related to the length of the conjugated double bond system.” Di Mascio\textsuperscript{79} stated that “among the carotenoids, lycopene is the most
efficient singlet oxygen quencher. The physical quenching rate of lycopene was two times higher than β-carotene and 10 times higher than α-tocopherol.” As per El-Agamey A "basically, chain lipid auto-oxidation reactions can be interrupted by antioxidants such as phenols, vitamin E and flavonoids, which eliminate the lipid peroxyl radicals by donating the hydrogen atom to form lipid peroxide and a resonance-stabilized antioxidant radical.” However, as a carotenoids compound, lycopene may scavenge the radicals by other ways. The mechanism of action for lycopene towards the reactive species can be predicted through three possible mechanisms: (i) adduct formation, (ii) electron transfer to the radical and (iii) allylic hydrogen abstraction.

i. Adduct formation: Lycopene + R• R-Lycopene•

ii. Electron transfer: Lycopene + R• Lycopene•+ + R–

iii. Allylic H abstraction: Lycopene + R• Lycopene• + RH

3.5.3 Preventive effects of lycopene on various diseases:

The effects of lycopene on various diseases have been previously reviewed by many researchers. The protective effects of lycopene have been on oxidative stress, cardiovascular disease, hypertension, atherosclerosis, cancers, diabetes and others. However, there are still no conclusive results reported due to the fact that studies on the role of lycopene against these diseases are still ongoing.

3.5.3.1 Oxidative Stress:

It is one of the major risk factors for chronic diseases. Free radicals or oxidants are potential contributors leading to oxidative stress. In vitro, ex vivo and in vivo studies have been carried out to demonstrate the effects of lycopene against oxidative stress. In this context, lipid, protein and deoxyribonucleic acid (DNA) oxidation are closely related to oxidative stress.

As per Liu F “previous studies have reported that, lycopene-rich diet and lycopene supplementation provided protective effects against DNA damage, in both normal and cancerous human cells.” Matos HR stated that “in animals, reduction of lipid peroxidation products- Thiobarbituric acid reactive substances (TBARS) and DNA damage markers were found in monkey kidney fibroblast and rat hepatocytes
supplemented with lycopene (20 pmol/106 cells and 1.86 – 18.62 M, respectively). Rats injected with lycopene (10 mg/kg/day) for five days, also showed protective effect from iron-induced oxidative damage in the prostate tissue and reduction of lipid peroxidation.”

Zhao X\textsuperscript{83} discussed that “human plasma lycopene levels have shown an inverse relationship with oxidative DNA damage. Consumption of lycopene rich foods, juices or supplements has demonstrated protective effects against DNA damage in the lymphocytes. Besides, high protection of lymphocytes from the oxidative damage due to singlet oxygen, was found in human subjects with intake of lycopene-rich tomato juice.”

Jacob K\textsuperscript{84} stated that “a decrease of lipid and protein oxidation was also obtained in the humans, who consumed lycopene in the form of ketchup or oleoresin capsules. Besides, the LDL oxidation and urinary 8-iso-prostaglandin F2α was found to be lower after the consumption of tomato products (8 mg lycopene/day for three weeks). Lycopene capsule supplementation (4 mg/day for six months) could substitute the hormone replacement therapy in postmenopausal women to prevent the oxidative stress and atherosclerosis. Nevertheless, synergistic effect of lycopene with other antioxidants can be found. Tomato juice fortified with vit. C gave a higher antioxidant capacity in urine and lower TBARS in plasma and urine.”

3.5.3.2 Cardiovascular diseases:

The normal physiological working of cardiovascular system is adversely affected by the CVD. Plasma LDL is the major risk factor of CVD. Increase in LDL oxidation is hypothesized to be causally associated with increasing risk of atherosclerosis and coronary heart disease.

Agarwal S\textsuperscript{56} discussed that “a study has shown that dietary lycopene supplementation (once a day, 1 week each) provided through tomato juice, spaghetti sauce and tomato oleoresin significantly increased serum lycopene.” Sesso HD\textsuperscript{85} stated that “it also showed that serum lipid peroxidation and LDL oxidation significantly decreased after consuming lycopene rich foods, even though no difference was found in serum cholesterol levels. Besides, a high plasma level of lycopene was associated with a decreased risk of CVD in women.”
The circulating plasma lycopene has been thought to prevent the development of atherosclerosis, especially in smokers. Moreover, Rissanen TH\textsuperscript{86} exhibited an inverse relationship between lycopene and intima-media thickness of the carotid artery as the risk factor for CVD.

3.5.3.3 Cancers:

Cancer has emerged as a major public health problem around the world. It has raised the awareness of people for natural products and their therapeutic or preventive value. The beneficial effect of lycopene is associated with a decrease in cancer incidence worldwide, especially in prostate cancer.

Kong KW\textsuperscript{54} discussed that “the study reported that higher plasma lycopene was inversely associated with prostate cancer risk. Lycopene was able to delay the high-grade prostate intraepithelial neoplasia (HGPIN) from developing into prostate cancer, and also inversely related to the prostate specific antigen. Besides, lycopene (20 – 60 μM) was able to inhibit the proliferation of prostate cancer cells. The antioxidative properties of lycopene had significantly diminished the DNA damage in prostate tissues.” Protective effect was also achieved with increased consumption of lycopene-rich diet. According to Giovannuci et al\textsuperscript{87} “frequent intake of tomato or lycopene was associated with lower risk of prostate cancer.”

Hwang E-S\textsuperscript{88} argued that “on the other hand, lycopene (1 – 10 μM) was able to inhibit the proliferation of human liver cancerous cells and preventing them from metastatic process.” Burgess LC\textsuperscript{89} reported that “Lycopene has significantly inversed the proliferation of human colon carcinoma, chronic lymphocytic leukaemia, erythroleukaemia and Burkitt lymphoma cell lines. However, no anti-proliferation effect was found in lycopene treated skin carcinoma, prostate carcinoma, lung carcinoma and breast carcinoma.”

A study by Kong KW\textsuperscript{54} has shown that “there was no association between some carotenoids and breast cancer among Chinese women, but increased intake of lycopene is associated with a reduced risk of breast cancer. A cohort study concluded that neither high dietary nor plasma lycopene levels were associated with a reduced risk of breast
cancer in middle-aged and older women. Therefore, the beneficial effect of lycopene may be specific for certain organs.”

3.5.3.4 Diabetes mellitus:

Lycopene is closely related to various metabolic complications, especially diabetes mellitus (DM). Serum lycopene is inversely associated with type-2 DM and impaired glucose metabolism. The fact was proved by Coyne et al.\textsuperscript{90} that “the plasma glucose and fasting insulin concentrations decreased significantly, with increase in the serum lycopene.” Besides, Polidori et al.\textsuperscript{91} found that “plasma lycopene concentrations were significantly lower in very old diabetic patients, as compared to controls, while significant inverse correlations were found between the age and lycopene.”

In humans, dietary lycopene was directly related to baseline serum concentrations of non-esterified fatty acids. Besides that, there is also a concern about dietary lycopene and modulation of insulin-like growth factor (IGF). Riso\textsuperscript{P} has evaluated the effect of tomato drink intervention providing small amounts of lycopene, and other carotenoids, on serum levels of IGF – 1. The results indicated that, lycopene supplementation before and after each experimental period, were inversely and significantly correlated with those of IGF-1. However, Wang\textsuperscript{L} has found low evidence for an association between baseline plasma lycopene and the risk of type-2 DM in middle-aged and older women, after adjustment for multiple risk factors.

3.5.3.5 Other health benefits:

Kuhad A\textsuperscript{94} discussed that “lycopene has the ability to scavenge free radicals. Thus, lycopene may have health benefit effects and improvement of other disease conditions. Treatment of lycopene (1, 2 and 4 mg/kg; p.o.) in streptozotocin-induced diabetes rats significantly attenuated the cognitive deficit, increased acetyl cholinesterase activity, oxidative-nitrosative stress and inflammation. Lycopene treatment has significantly improved the memory and restored the functioning of glutathione system in the 3-nitropropionic acid-induced rats.” Akbaraly NT\textsuperscript{95} also suggested that “low plasma lycopene levels could contribute to cognitive impairment.”

Kong KW\textsuperscript{54} discussed that “serum lycopene concentration was significantly lower in the asthmatics and the subjects having rheumatoid arthritis, than the control group. Dietary
supplementation or adequate dietary intake of lycopene and vit. A-rich foods, may therefore be beneficial in asthma and rheumatoid arthritis.”

### 3.5.4 Thermal Process on Lycopene Content:

Thermal processing is used in the food industry to preserve food products and maintain the nutritional quality. However, lycopene is a heat sensitive compound and is degraded when exposed to heat. The temperature is an important factor for thermal processing in order to remove the moisture, with minimum destruction of lycopene and other nutrients.

Chang CH\(^{96}\) reported that “thermal processing enhanced the lycopene isomerisation and increased the lycopene extracting ability, by breaking down the cell walls and weakening the interaction between lycopene and the tissue matrix of samples. Hot air drying at 80\(^{0}\) C for the first 2 hours, plus shifting the drying temperature to 60\(^{0}\) C for another 6 hours, were reported to yield higher lycopene content, as compared to fresh and freeze-dried samples. Besides, treatment of tomatoes with forced air drying at 42\(^{0}\) C for 48 hours, has shown a significant increase in the lycopene contents. In contrast, semi-drying method for drying of tomatoes, using a forced air drying at 42\(^{0}\) C for 8 hours, showed a significant decrease in the lycopene content.”

A study done by Shi J\(^{97}\) showed that “higher levels of lycopene \(cis\) isomers, and lower levels of total lycopene and \(trans\) isomers, were obtained from tomato using air drying method at 950 C for 6 – 10 h as compared to the vacuum drying and osmotic treatment methods. However, lycopene and other lipophilic antioxidant compounds in tomato pulps have high stability after air drying.”

Moisture content is closely related to lycopene degradation. When moisture is retained, the water-soluble compounds will react as catalyst during lycopene degradation. Goula AM\(^{98}\) reported that “degradation of lycopene in tomato pulp was reduced when the moisture content decreased from 95 % to 5 %, with a minimum degradation rate in between 50 to 55 % of moisture content. Thus, the catalytic effect of lycopene degradation will be eliminated when the moisture is removed.”
3.6 Animal models of hyperlipidaemia and atherosclerosis

Fernandez ML discussed that “the use of appropriate animal models to determine the effects of dietary interventions on metabolic process and gene expression regulating cholesterol and lipoprotein metabolism, is essential to understand the mechanisms underlying the reported effects on plasma lipids.”

**Figure-3.6.1: Comparisons between lipoprotein cholesterol distributions between several animal models and humans**

3.6.1 Introduction:

Nicolosi RJ discussed that “although, in general, the animal data supports the observations in humans, they should be interpreted cautiously because :i) Certain animal species have lipoprotein profiles, during their normal and hypercholesterolaemic states, that are dissimilar to those of humans; ii) Many studies used either no dietary cholesterol or pharmacologic doses, which can significantly influence the size of the fatty acid effect; iii) Some diet treatments used variable energy densities; iv) More than one dietary component was varied in a study; and v) The saturated vegetable oil or fat was substituted for an unsaturated vegetable oil instead of a neutral control.”
3.6.2 Mice:

Mice, by nature, are not affected adversely by development of atherosclerosis, as they exhibit resistance to it. However, a strain that is susceptible to atherosclerosis is available and as transgenic lines can be generated, they are the sought-after models. Advanced genetic modifications have been performed in this model that have made it possible to get detailed knowhow regarding the stages of atherosclerosis such as initiation, progression, formation of plaque and rupture. The mice that have been genetically manipulated and modified, and that do not have the genes responsible for lipid transportation and metabolism, help to know their role in atherosclerosis and disorders pertaining to the cardiovascular system.

Singh V\textsuperscript{101} stated that “the major disadvantages with mice are that they are highly resistant to atherosclerosis, need genetic manipulations and have high HDL. The RCT is absent and there are difficulties in blood sampling and dissection of miniature vessels. Correlation with human population is as yet unknown in some transgenic models. The model is not valuable for studies assessing dietary effects on lipoprotein metabolism.”

3.6.3 Rats:

Singh V\textsuperscript{101} discussed that “generally, rats are highly resistant to the development of atherosclerosis. HDL is dominating lipoprotein in these animals. Sprague-Dawley rats develop hyperlipidaemia by triton administration. Lymphatic cholesterol transport system and the rate of hepatic secretion of VLDL in triton-induced hyperlipidaemic rats, has been explored. Corpulent rat strain developed by cross breeding between Sprague-Dawley rats and spontaneously hypertensive rats (SHR) are hypertensive, obese, hyperlipidaemic and hyperinsulinaemic. However, rats lack physiological resemblance on many aspects with humans that are pathophysiologically important. Rat platelets are generally resistant in hyperlipidaemic condition.”

3.6.4 Hamsters:

As per Singh V\textsuperscript{101} “golden Syrian hamster, preferentially F1B strain, is used as a model for studying hyperlipidaemia and atherosclerosis. Hamsters have quite a few similarities with humans, which makes them a valuable model of atherosclerosis. These features include - LDL as a major circulating lipoprotein, similar cholesterol and bile acid
metabolism, and exclusive hepatic production of APO B100. They do show the influence of other metabolic disorders on lipoprotein metabolism, and can develop extensive hypertriglyceridaemia.

However, the major limitations include - lack of spontaneity in lesion formation and absence of advanced atherosclerotic lesions and plaque rupture. Furthermore, platelets are much less sensitive towards hyperlipidaemia in this species.”

3.6.5 Guinea Pigs:

Singh V\textsuperscript{101} reported that “the most striking similarity between guinea pigs and humans is that, the majority of circulating cholesterol is transported in LDL. Other key points include - higher concentrations of free compared to esterified cholesterol in the liver, possess plasma cholesteryl ester transfer protein (CETP) activity, a critical component for RCT, possess LCAT and LPL activities that contribute to remodelling of plasma lipoproteins. Comparable to humans, they exhibit moderate rates of hepatic cholesterol synthesis and catabolism. Females have higher HDL levels than males, and ovariectomized guinea pigs have a plasma lipid profile similar to post-menopausal women. In response to exercise, they lower plasma triglycerides and increase plasma HDL cholesterol. However, the major limitation of this model is the requirement of vit. C as a dietary supplement, which has antioxidant activity and may interfere in the development of atherosclerosis.”

3.6.6 Rabbits:

Singh V\textsuperscript{101} discussed that “rabbits are widely used in the biomedical research, and especially as animal models in the atherosclerosis studies. New Zealand White (NZW) rabbits are the commonly used strain. Although they have low plasma total cholesterol concentration and HDL as the dominant lipoprotein, VLDL becomes the major class of lipoproteins when exposed to cholesterol rich diet. In conjunction with chylomicron remnants, VLDL becomes highly atherogenic. Besides, it has pronounced CETP activity and human like APO–B. Due to their large size, imaging technology such as ultrasound, computer tomography (CT) and magnetic resonance imaging (MRI); can be effectively applied to determine the plaque composition, distribution pattern and vulnerability. This model is also suited to study the effects of atherosclerosis associated complications, such as hypertension and diabetes, on disease progression. Since this model does not produce
spontaneous plaque rupture, various manipulations (balloon injury) have been applied to study the various aspects of plaque rupture.”