CHAPTER 3

ANIMAL INTRODUCTION

1.1 CHOLESTEROL

Cholesterol is an essential biological molecule in eukaryotes that is involved in structural and metabolic roles, vital to the biological systems (Podar *et al.,* 2006) and is a key regulator for membrane fluidity. Cholesterol is the most important sterol in human body and liver is the major site for its homeostasis. Its molecular formula is $C_{27}H_{45}OH$andits structural formula. It possess "cyclopentanoperhydrophenanthrene nucleus", a –OH group at C3,a double bond between C5 andC6 and two –CH3 groups at C10 and C13.

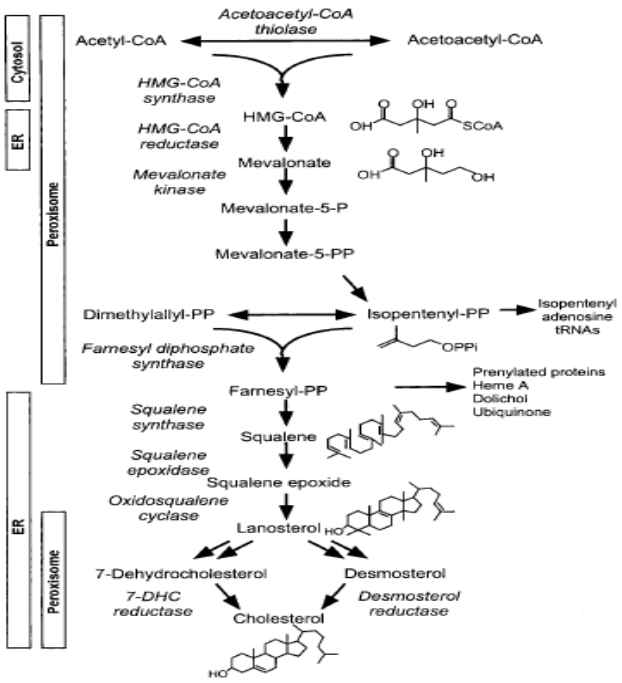


Fig. 1 The cholesterol biosynthetic pathway [D.E. Vance and J.E. Vance (Eds.) Biochemist of Lipids, Lipoproteins and Membranes 4th edition 2002 Elsevier Science B.V

1.2 CHOLESTEROL METABOLISM AND HOMEOSTATSIS

Cholesterol is an important precursor in metabolic pathways, including steroid hormone and bile acid synthesis. Cholesterol is exchanged within/ between organelle membranes, and in the whole body. Cells synthesize cholesterol and are also taken up with food. Cholesterol homeostasis is under tight regulation, and reflects on the net effect of *de novo* synthesis, intestinal absorption (of dietary and biliary cholesterol), circulatory clearance and excretion. The perturbations in lipid metabolism are associated with disease states, including atherosclerosis, metabolic syndrome, and type 2 diabetes (Glass *et al.,* 2001). The cholesterol biosynthesis pathway is represented in Fig: 1.2.and liver is the principal site for cholesterol homeostasis (Dietschy J.M*et al.,* 1993).The homeostasis is maintained through many mechanisms, such as biosynthesis via 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), uptake through LDL receptors, lipoprotein release in the blood, storage by esterification, degradation and conversion into bile acids (Weber L W*et al.,* 2004).

1.2.1 Cholesterol regulation

In humans, cholesterol is derived from two sources – diet and *de novo* synthesis. All nucleated cells synthesize cholesterol in *de novo* from acetyl CoA through the mevalonate pathway, which occurs in the ER. The rate limiting step is the conversion of HMG-CoA to mevalonate by HMG-CoA reductase (HMGR), a negative feedback system modulated by the SREBP pathway (Brown *et al.,* 1997). In mammalians, there are two SREBP genes, *SREBP1* and *SREBP2* that express three major SREBP proteins. The SREBP2 is the main regulator of cholesterol metabolism (e.g., HMGR, LDLR, and PCSK9) (Horton *et al.,* 2003: Xiao *et al.,* 2013). When sterol levels are restored, cholesterol synthesis and uptake are co-ordinately suppressed. Cholesterol synthesis has a diurnal rhythm with a nadir during the day and peaks during the night from midnight to 4:00, both in humans and rats (Edwards *et al.,* 1972; Miettinen 1982; Kopito*et al.,*

1982). Insulin and thyroid hormone increase HMGR activity whereas glucagon and glucocorticoids decrease it. HMGR inhibitor, statin, competitively inhibits the enzyme. The total cholesterol content of a 70 kg human body is about 140g and slightly less than 1% (~1200 mg) gets degraded daily (Turley and Dietschy, J.M 2003).

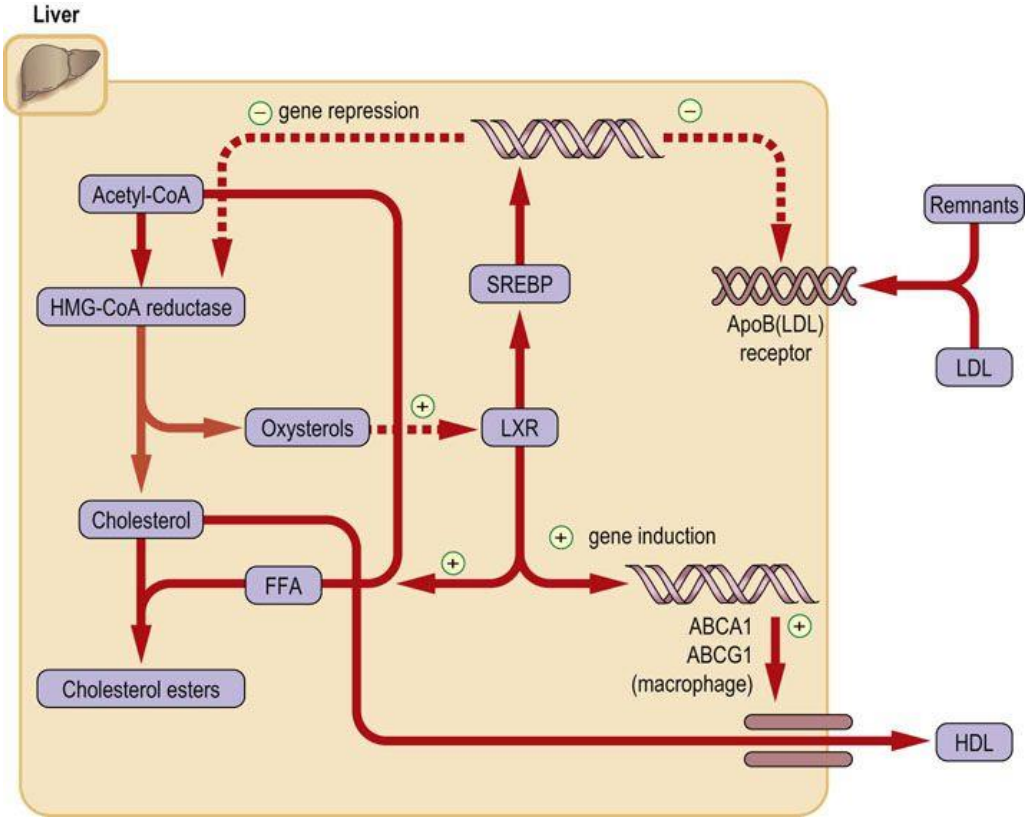


Fig.2 Regulation of intracellular cholesterol level. Free membrane cholesterol (and oxysterols) regulates intracellular cholesterol level by inducing or suppressing gene expression. The increase in intracellular cholesterol concentration will suppress the synthesis of HMGR and ApoB/E receptor and at the same time increase cholesterol esterification and its transport from cells. FFA: free fatty acid, acetyl-CoA: acetyl coenzyme A, LXR: liver X receptor, SREBP: sterol regulatory element-binding protein.
(Source : http://rgd.mcw.edu/rgdweb/pathway/pathwayRecord.html?acc_id=PW:0000454.)

1.2.2 Cholesterol excretion

Many cells cannot catabolize cholesterol, and small amounts are lost via. The excretion of steroid hormones in the urine, and through the sequestration of dead cells from the skin. The role of cholesterol elimination through the excretion of dead

intestinal cells is less clear and the possible potential active secretion is thro' plasma lipoprotein-derived cholesterol by enterocytes, a process referred as cholesterol excretion (TICE) (Stanley *et al.,* 1959; Le May *et al.,* 2013). The major net excretion from the body is through hepatic elimination in which is actively regulated by dietary and pharmacological treatments. This occurs through direct secretion of free cholesterol into the bile, or conversion of cholesterol to bile acids (figure 3).

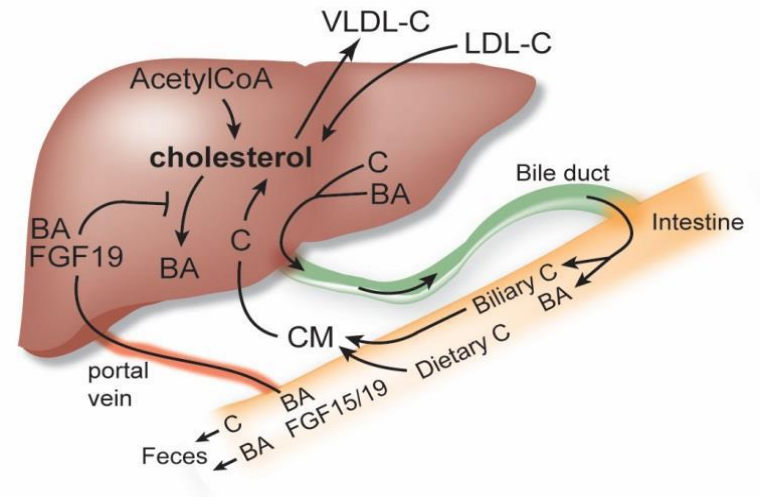


Fig. 3 A Simplified overview of cholesterol metabolism and entero-hepatic circulation of bile acids. (Source : http://themedicalbiochemistrypage.org/steroid-hormones.php)

1.2.3 Reverse cholesterol transport and HDL metabolism

Most of the cells in the body are capable of synthesizing cholesterol but are unable degrade. The reverse cholesterol transport removes cholesterol from peripheral tissue including lipid laden macrophages in the artery wall– to the liver for excretion as free cholesterol or is converted to bile acids (Rosenson *et al.,* 2012; Hellerstein *et al.,* 2014; Temel *et al.,* 2015; Zannis, *et al.,* 2015). HDL has a key role in cholesterol efflux from cells, and as a transporter of cholesterol from the plasma to the liver.HDL formation begins when lipid poor in apoA-I is secreted by the liver or intestine through interaction with the transporter ABCA1 in the hepatocyte or enterocyte. Lipid-free/lipid poor apoA- I is continuously generated during the remodeling of mature HDLs

(Rosenson *et al.*, 2012: Zannis, *et al.*, 2015). ApoA-I is the main apoprotein in HDL and participates in the formation of HDL and also interacts with the scavenger receptor B-1 (SR-B1), the principal HDL receptor. SR-B1 is an integral membrane protein primarily expressed in the liver, steroidogenic tissues, and endothelial cells. HDL heterogeneity is a consequence of constant remodeling; and two circulating proteins are responsible for intravascular maturation of HDL; lecithin cholesterol acyl transferase (LCAT) and phospholipid transfer protein (PLTP). Further, hepatic lipase and endothelial lipase are important for HDL remodeling (Rosenson *et al.*, 2012; Zannis *et al.*, 2015; Rye *et al*., 2014). HDL-cholesterol esters are transferred by cholesterol ester transferase (CETP) to VLDL/IDL/LDL in exchange for triglycerides (Rye *et al.*, 2014). The CETP decreased HDL-cholesterol and enriched LDL-cholesterol. SR-B1 mediates selective removal of cholesterol esters, TAGs, phospholipids and vitamin E from the HDL core into the cell without endocytotic uptake and degradation of the whole HDL particle (Favari *et al.*, 2015). As mentioned above, ABCA1 mediates lipid efflux to lipid poor apoA1. A second transporter, ABCG1, mediates further cholesterol efflux to lapidated HDL.

1.3 ATP BINDING CASSETTE (ABC) TRANSPORTERS

In the reverse cholesterol transport the excess cholesterol in peripheral tissues is transported to liver for elimination (Glomset, *et al.*, 1968). The liver produces lipid-poor apolipoprotein apoA-I that circulates to peripheral cells and picks up cholesterol and phospholipids. A fraction of this apoA-I may also interact with hepatocytes and acquire lipids before exiting into the portal circulation. By a complex series of steps involving acquisition of more lipids and proteins and esterification of cholesterol, this partially lipidated apoA-I matures into spherical particles that represent the bulk of HDL. These particles are processed and remodeled by the combined actions of cholesterol ester transfer protein, phospholipid transfer protein, scavenger receptor B1,

and hepatic lipase, which transfer HDL cholesterol esters to other lipoproteins and cells and regenerate lipid-poor apoA-I. The gatekeeper of this reverse cholesterol transport pathway is by the ATP-binding cassette (ABC) transporter ABCA1, an integral membrane protein (Brooks-Wilson 1999; Bodzioch *et al.,* 1999; Rust S., 1999; Lawn *et al.,*1999; Oram, JF., Santamarina-Fojo *et al.,* 2001; Hayden *et al.,*2001).ABCA1proteincouplesthe hydrolysis of ATP to the transport of various substrates across cellular membranes. The ABCA1 protein is critical for the efflux of excess cellular cholesterol to Apo acceptors such as ApoA-I, the first step in reverse cholesterol transport. The importance of ABCA1 in systemic cholesterol metabolism has become clear from the study of patients with Tangier disease, which is caused by mutations in this gene (Bodzioch M *et al.,* 1999).

The interaction of apolipoproteins with cholesterol-loaded cells stimulates the translocation of free cholesterol away from intracellular esterifying enzymes to sites accessible to apolipoproteins (Rogler *et al.,* 1995) that presumably contain ABCA1. The interaction of apolipoproteins with ABCA1 or a partner protein stimulates the translocation of intracellular cholesterol and phospholipids from the Golgi to plasma-membrane ABCA1 by a signal-responsive vesicular transport pathway. As another possibility, ABCA1-containing vesicles travel to intracellular lipid deposits, and pumps lipids into the vesicle lumen, and the vesicles transport their lipid cargo back to the plasma membrane (Santamarina-Fojo *et al.*, 2001).

1.4 BILE ACID METABOLISM

Bile acid transporters and bile acid synthesis is under strict and coordinated regulation via nuclear receptors. The liver is crucial for metabolic homeostasis, and the liver hepatocytes are responsible for most of the synthetic and metabolic functions. Hepatocytes are polar cells with a canalicular and a sinousoidal surface (Gissen *et al.,* 2015), and the sinousid endothelial cells lack basal membrane and are perforated with

pores (fenestrae) (Braet, *et al*., 2012; DeLeve 2015; McLean *et al.*, 2003). The classical major pathway of bile acid synthesis accounts for more than 90% of bile acid synthesis in humans. Two bile acids are synthesized from cholesterol in the liver: CDCA (3α, 7α-dihydroxy) and CA (3α, 7α, and 12α-trihydroxy).The rate limiting step is the hydroxylation at the carbon C-7 mediated by cholesterol 7α-hydroxylase (CYP7A1) located in the ER and largely determines the bile acid pool size (Li, and Chiang 2014). Primary bile acids synthesized from cholesterol in the liver are further metabolized into secondary and tertiary bile acids by the gut microbiota.

In the hepatocyte, bile acids are conjugated to glycine or taurine preceding secretion into the bile canicula (Hofmann *et al.*, 1992). Conjugation increases the amphipathicity and enhances the solubility of the molecules, which makes them impermeable to cell membranes. Several bile acid transporter proteins ensure proper excretion and up take and have different transport affinities for various bile acids species, and also for other endogenous and exogenous compounds such as toxins and drugs (Halilbasic *et al*., 2013). Efficient hepatic excretion, intestinal reabsorption, and hepatic uptake of bile acids restrict bile acids to hepatobiliary and intestinal compartments.

1.4.1 Regulation of bile acid synthesis

Bile acid synthesis is regulated by negative feedback mechanisms. Bile acids mediate inhibition of the rate limiting enzyme *in* bile acid synthesis, CYP7A1, through activation of the nuclear receptor FXR in the liver and in the intestine.

1.5 LIPOPROTEINS

Hyperlipidemia results in excess cholesterol in the blood. Hyperlipidemia usually mean high cholesterol and high triglyceride levels the blood. Excess cholesterol becomes a problem when too much is ingested through regular eating of unhealthy

foods. Cholesterol is carried through the blood to cells by lipoproteins that are either low density (LDL) or high density (HDL). The lipoproteins are the vehicles and cholesterol the passenger. HDL is the good lipoprotein because it carries extra cholesterol back to the liver where it can be eliminated. LDL is bad, since it builds up excess cholesterol in the blood. Triacylglycerol is another type of fat in the blood, and because of its strong association with heart disease, triacylglycerol is measured as well. Both the LDL and triacylglcerols are elevated in hyperlipidemia. In the fasting state, plasma triacylglycerols are present mostly as VLDL and in the non-fasting state as chylomicrons and appear transiently and contribute significantly to total plasma triacylglycerols level. LDL contains about 70% of total plasma cholesterol and HDL contains about 20% to 30% of plasma cholesterol.

1.6 EPIDEMIOLOGY OF DYSLIPIDEMIA IN INDIA

Cardiovascular disease (CVD) is the leading cause of death worldwide, and mortality due to CVD is higher in low- and middle-income countries (Mathers *et al.,* 2008; Fuster 2010). In India, there has been an alarming increase in the prevalence of CVD over the past two decades and accounts for 24% of all deaths among adults aged between 25–69 years. Asian Indians have been found to develop CVD at a younger age than other populations (Enas *et al.,* 1992). The likely causes for the increase in the CVD rates include lifestyle changes associated with urbanization and the epidemiologic and nutritional transitions that have accompanied the economic development (Omran AR 1972). Dyslipidemia has been closely linked to the pathophysiology of CVD and is a key independent risk factor for cardiovascular disease (Groundy, S.M.1997; Haffnar, M. 1999). While Asian Indians are known to have a unique pattern of dyslipidemia with lower HDL cholesterol, increased triacylglycerol levels and higher proportion of small dense LDL cholesterol, there have been no large scale representative studies on dyslipidemia to assess the magnitude of the problem in India. The estimation of the

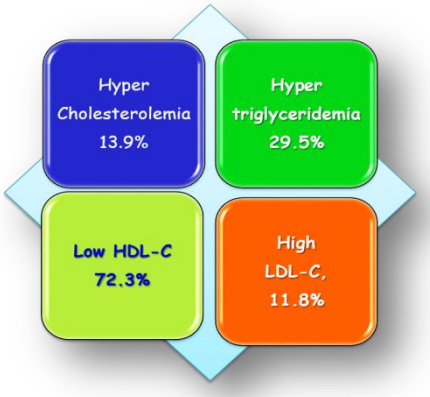
prevalence of dyslipidemia will ensure proper planning of health care resources for both primary and secondary prevention of CVDs. The report on the lipid patterns and prevalence of lipid abnormalities of the Indian population in Phase I of the Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study, involved three states and one union territory, representing the north, south, east and west of the country.

1.6.1 Lipid trends in India

India is undergoing a rapid epidemiological transition with increasing population, economic prosperity, urbanization and aging with associated risk factor transition (Gupta*et al.,* 2009). Increase in cardiovascular risk and hypercholesterolemia is also associated with increase in adverse lifestyles such as greater smoking and tobacco use, change in nutritional habits with greater intake of unhealthy diets and increasing sedentary lifestyle (Defo 2014; Patel *et al*., 2011). All have contributed to the rising burden of non-communicable diseases, especially CHD. Even in rural areas of India non-communicable and chronic diseases have become the leading causes for death (Gupta *et al.,* 2012).The INTERHEART study has reported that apolipoproteins such as high ApoB and low ApoA1 as well as high total and LDL cholesterol are the most important risk factor for CHD globally as well as in South Asian countries (Karthikeyan *et al.,* 2009). Trends in these metabolic risk factors have not been well studied.

1.6.2 Epidemiology reports in India

Prevalence	All four regions		
	Urban	Rural	Overall
n	590	1452	2042
Hypercholesterolemia	19.2	11.7**	13.9
Hypertriglyceridemia	36.6	26.6**	29.5
Low HDL cholesterol	73.2	71.9	72.3
High LDL cholesterol	15.9	10.1**	11.8
High total cholesterol: HDL ratio	44.2	33.5**	36.6
Isolated hypercholesterolemia	4.6	4.5	4.6
Isolated hypertriglyceridemia	22.0	19.4	20.2
Isolated low HDL-C	39.0	47.2*	44.9



1.6.3 Epidemiology reports in Tamilnadu

Tamilnadu		
Urban	Rural	Overall
194	463	657
23.7	16.0*	18.3
33.0	29.6	30.6
72.2	67.6	68.9
22.2	13.2*	15.8
48.5	39.7*	42.3
5.7	5.8	5.8
14.9	19.4	18.1
39.2	41.0	40.5

Table 2 presents the prevalence of lipid abnormalities in all the four regions studied. Overall, in the four regions studied, prevalence of at least one lipid abnormality was 79% with highest rates found in Chandigarh (82.9%), followed by Jharkhand (80%), Tamilnadu (76.9%) and Maharashtra (77%) with no urban rural differences observed in any of the four regions. Hypercholesterolemia was highest in Tamilnadu

(18.3%) and least in Jharkhand (4.9%) and was significantly higher in urban compared to rural areas in all regions except Maharashtra. Hypertriglyceridemia was highest in Chandigarh (38.6%) and least in Maharashtra (22.8%), and the rates were significantly higher in urban compared to rural areas in Jharkhand and Chandigarh. Low HDL-C was highest in Jharkhand (76.8%) and least in Tamilnadu (68.9%), and no urban rural differences were observed in any of the four regions. High LDL-C was highest in Tamilnadu (15.8%), followed by Maharashtra (13.3%), Chandigarh (12%) and Jharkhand (3.4%), with high rates in urban, areas compared to rural areas, except in Maharashtra. High cholesterol to HDL-C ratio was highest in Tamilnadu (42.3%), followed by Chandigarh (40.4%), Maharashtra (34.5%) and Jharkhand (25.4%) and the rates were significantly higher in urban compared to rural areas in all regions except in Maharashtra.

1.7 CAUSES AND RISK FACTORS OF HYPERLIPIDEMIA

1.7.1 Dietary Causes

Dietary fatty acids are divided into three major classes (saturated, monounsaturated and polyunsaturated fatty acids). The foods that contribute to saturated fatty acids (e.g. myristic acid, palmitic acid, stearic acid, etc) (1)meats (e.g. beef, pork, processed meat products, poultry), (2) milk and other dairy products (e.g., butter, cheese, ice cream, yoghurt), (3) tropical fats (e.g. coconut, palm oils) and (4) egg (contains less saturated fat compared to other animal food sources). Monounsaturated fatty acids are present as oleic acid in olive oil, avocado, animal fats, etc. Polyunsaturated fatty acids are the omega-3 fatty acids (e.g., linoleic acid) and omega-6 fatty acids (e.g. linolenic acid) (Fauci *et al.,* 2008). Food choices made by individuals can influence intake of the different saturated fatty acids. The leaner meats are high in palmitic acid and limiting the lean meat would help in lowering saturated fat intake (Sereday *et al.,* 2004). Milk and other dairy products are high in myristic acid

content. Substituting skim milk and non-fat dairy products for whole milk products will result in a reduction of saturated fat such as myristic acid intake.

1.7.2 Dietary Cholesterol

Cholesterol is a steroid and is a type of lipid found in foods such as eggs and dairy products and is also manufactured in the body, especially the liver. Cholesterol also stabilizes a cell against temperature changes. It is a major part of the membranes of the nervous system, the brain, the spinal cord and the peripheral nerves. In particular, it is incorporated into the myelin sheath that insulates the nerves from the surrounding tissue. Cholesterol is also the forerunner of important hormones such as the female sex hormone, oestradiol and the male sex hormone, testosterone and of vitamin D. Cholesterol is also used to produce the bile which is required to digest the fats in food. Nearly most of the body tissues are capable of making cholesterol, but the liver and intestines make the most. The dietary cholesterol is responsible for both the development of hypercholesterolemia and atherosclerosis has been the focus of many investigators. Many studies in rabbits (and other animal models), in human and epidemiologic investigations, indicated the importance of dietary cholesterol on serum cholesterol levels and its associated effects (Sereday *et al.*, 2004). However, other investigations have come to opposite conclusions after reviewing numerous human feeding studies (although many continue to support the view that dietary cholesterol is the major hypercholesterolemic and atherogenic nutrient in the diet) (Ruixing*et al.*, 2006)

1.7.3 Other Dietary Factors

Carbohydrates: Recommendations to lower the total fat intake and increase the dietary carbohydrate intake because favorable plasma lipid and lipoprotein levels have been reported for populations and individuals whose habitual diet is rich in carbohydrates. High carbohydrate consumption being associated with a decrease in

HDL cholesterol levels. Plasma triacylglycerol levels are not elevated in these individuals, possibly because obesity is rare (Charney, P. 1999).

Fiber: Studies have shown that only water-soluble fiber plays a role in lipoprotein metabolism in humans. A meta-analysis of 20 studies found that intake of oat products reduces serum cholesterol levels (Charney, P. 1999). The mechanism by which dietary fiber affects plasma lipid levels is unknown. Insoluble fibers in wheat and vegetables do not to reduce cholesterol, but they do have other beneficial effects.

Protein: Soy protein also lowers serum cholesterol levels in animals and in hypercholesterolemic individuals when compared with casein (a dairy protein) and beef proteins. The mechanism underlying these changes is unknown, but it has been stated that soy protein affects cholesterol absorption, bile acid absorption, the insulin-glucagon ratio, serum thyroxine levels and hepatic LDL-receptor activity.

Obesity: For a given level of body mass index (BMI), obesity is associated with hyperlipidemia, insulin resistance and hypertension and independent predictor of coronary artery disease (CAD). A meta-analysis of 70 studies indicated that weight reduction was related to increasing in HDL cholesterol levels and significant decrease in total, LDL and VLDL cholesterol and triglyceride level (Woollett *et al.,* 1992). Although not always, obesity is often accompanied with hyperlipidemia. Both obesity and hyperlipidemia are independently associated with atherosclerosis, non-alcoholic fatty liver disease and insulin resistance (Cortse *et al.,* 1983).

Diabetes and Insulin Resistance: Insulin resistance (type II diabetes) is associated with a number of lipid and lipoprotein abnormalities. The lipid abnormality associated with insulin resistance is hyperinsulinemia and hypertriglyceridemia. VLDL and total triglycerides are elevated in individuals with type II diabetes although the exact roles of insulin resistance and hypertriglyceridemia are disputed.

Physical Exercise/Activity: Sedentary lifestyles contribute to the development and maintenance of obesity (Keys *et al.,* 1985). Diet changes the plasma lipoprotein concentrations that occur with exercise.

Alcohol Intake: Low dose of ethanol consumption in healthy volunteers modestly activates hepatic *de novo* lipogenesis and that the major quantitative fate of ethanol is acetate produced in the liver. The acetate released into the plasma inhibits lipolysis in peripheral tissues by 53% and whole body lipid oxidation is decreased by 73%. Alcohol intake is second only to diabetes mellitus as a cause of hyperlipidemia in the population, about 25% of hospitalized alcoholics have fasting blood triacylglycerol concentrations above normal limits, and 17% have concentrations >3 mmol/L Hypertriglyceridemia is seen mostly in patients with fatty liver and rarely in cirrhosis patients. Patients with cirrhosis have a lower capacity to produce blood lipids than do subjects without liver injury when challenged with diet and alcohol experimentally.

Lipid-lowering drugs: Lipids are important biomolecules. Cholesterol, is an essential component of the human cell membrane and a precursor for steroid hormones and bile acids. Triglycerides have a important role in transferring energy from food to body cells. Elevation of different lipids in the bloodstream, a condition generally termed hyperlipidemia, causes a constant health problem. Lipids are transported in the bloodstream, and so hyperlipidemia is always a threat to coronary arteries and an important risk factor for coronary heart disease. One group of drugs (statins) lowers cholesterol by interfering with the cholesterol biosynthetic pathway (Krukemyer 1987; Hebert *et al.,* 1997). Statins are the popular anti-hyperlipidemic drugs. The most common side effect is statin-associated muscle symptoms (SAMS), and is reported as 10% (1) to 25% (Cohen *et al.,* 2012). In patients receiving statin therapy. In an internet survey of former statin users, 60% reported SAMS (2) and 62% reported stopping statin therapy because of side effects (Cohen *et al.,* 2012). Cessation of statin treatment is

associated with worse cardiovascular outcomes. A meta-analysis of 15 statin studies observed a 45% increase in all-cause mortality and a 15% increase in CVD events in patients taking <80% of their prescribed statin therapy versus patients who were more adherent (Chowdhury *et al.,* 2013).

However, to fight these problems, humans have acquired several drugs, commonly known as lipid-lowering drugs. The fibrates decrease fatty acid and triglyceride levels by stimulating the peroxisomal β -oxidation pathway (Watts G.F 1999; Ozasa *et al.,* 1985). Apart from these drugs, ezetimibe, which selectively inhibits intestinal cholesterol absorption (Vasudevan *et al.,* 2005), cholestyramine, colestipol, and colesevelam, which sequester bile acids (Steinmetz 2002), torcetrapib, which inhibits cholesterol ester transfer protein(Gauthier *et al.,* 2005), avasimibe, which inhibits acyl-CoA: cholesterol acyltransferase (Kharbanda *et al.,* 2005) implitapide, which inhibits microsomal triglyceride transfer protein (Ueshima *et al.,* 2005), and niacin, which modifies lipoproteins(Vasudevan *et al.,* 2005), are providing clinicians with several therapeutic options for lipid lowering. However, based on medical use, importance, and popularity, statins and fibrates are way ahead of the others. Recent experimental data have revealed that both statins and fibrates display a broad spectrum of activities in addition to their lipid-lowering properties. As a result, statins and fibrates are now being considered as possible medicines in a variety of human disorders.

1.8 LIPID- LOWERING THERAPIES

For patients who are intolerant to statins, other lipid-lowering therapies exist and demonstrate beneficial effects. Of note, minimal randomized control trials of alternative lipid-lowering therapies have been performed in statin intolerant patients (Stone *et al.,* 2013).

Fibrates

The well-studied alternative therapies are fibrates. Commonly used worldwide fibrates include gemfibrozil, fenofibrate, bezafibrate, and ciprofibrate. They appear to have beneficial effects on lipid profiles, and their mechanism of action involves the activation of peroxisome proliferators activated receptors (Fruchart *et al.,* 1998). Larger randomized control trials demonstrating efficacy of fibrates include the use of gemfibrozil for secondary prevention (Rubins *et al.,* 1999), and the use of fenofibrate in diabetics (Keech *et al.,* 2005). A recent meta-analysis reaffirms previous work that the greatest benefit for patients, with respect to cardiovascular risk reduction, is found in those patients with low HDL-c and elevated triglycerides (Lee *et al.,* 2011). Yet, clinical cardiovascular outcomes are less favorable than statins. A key factor which should be considered both when initiating and continuing fibrate therapy is renal function. Fenofibrate should not be prescribed if estimated glomerular filtration rate (GFR) is <30 and should be dose reduced if GFR <60. A population based cohort study demonstrated increase in serum creatinine in fibrate users 90 days after initiation (Zhao *et al.,* 2012), although the mechanism is unclear and longer term analysis has actually demonstrated reduced albuminuria and slowed GFR decline (Davis *et al.,* 2011). The most recent ACC/AHA Blood Cholesterol Guidelines (Stone *et al.,* 2013), suggest renal function measurement at 3 and 6 months after initiation, and every 6 months. The fibrates have also been associated with significant muscle toxicity and more pronounced effects on those already taking statins. Gemfibrozil should not be started on patients already on a statin given the risk of rhabdomyolysis (Pierce *et al.,* 1990). The interaction of fibrates with concurrent medications must be closely monitored, most notably warfarin and oral hypoglycemic when initiating fenofibrate.

Bile acid sequestrants

Mechanistically, bile acid sequestrates (BAS) interrupt reabsorption of bile acids in the gut, thus lowering intrahepatic cholesterol and causing an up-regulation of LDL receptors and reduction of blood cholesterol. The most commonly used BAS include cholestyramine, colestipol, and colesevelam. Trials of cholestyramine were among the earliest to demonstrate a strong relationship between LDL-c reduction and CHD primary prevention. The major limitation to their use is gastrointestinal side-effects, including nausea, abdominal cramping, transaminase elevation, and impaired absorption of other medications.

Niacin

Nicotinic acid (niacin) has complex effects on lipoprotein metabolism including decreasing LDL-c, increasing HDL-c, and the suggestion of anti-atherogenic effect is independent of its effect on lipids (Lukasova*et al.*, 2011). Classical studies before the statin era demonstrated dose dependent changes in lipid concentrations. The more recent studies have tested niacin in combination with statins (Julius and Fischer2013). The most recent randomized control trial of extended-release niacin and laropiprant (added to simvastatin treatment) was stopped early due to lack of efficacy and increased non-fatal side-effects, myopathy in particular. These results highlighted the potential poor tolerability of niacin, limited its widespread use. Flushing is the most common side-effect. Different preparations have been studied, and niacin has been combined with laropiprant (a highly selective prostaglandin receptor antagonist) to reduce the side effect profile, although currently, the best way to minimize prostaglandin mediated side-effects is pretreatment with aspirin or ibuprofen. Another detrimental side effect is an increase in fasting plasma glucose (Garg*et al.*, 1990), limiting its use in a population with a high-coincidence of diabetes.

Ezetimibe

Newer agents such as ezetimibe are also an option in the statin-intolerant patient. Ezetimibe is a cholesterol absorption inhibitor that targets uptake at the enterocyte brush border and exerts its main effect on the cholesterol transport protein, Niemann-Pick C1 like 1 protein. A recent meta analysis (Pandor *et al.*, 2009), looked at eight RCTs of ezetimibe monotherapy in the treatment of primary hypercholesterolemia. Although the meta-analysis was restricted to short term trials (12 weeks) a 19% decrease in LDL-c with ezetimibe, compared to placebo, was seen and was equally well tolerated. A combination of simvastatin and ezetimibe reduced major atherosclerotic events in patients with impaired renal function (Baigent *et al.*, 2011). However, controversy regarding ezetimibe for improving outcomes remains and is being examined in the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) which involves approximately 18,000 acute coronary syndrome patients and compares simvastatin 40 mg (with potential to increase to 80 mg during the study, depending on degree of LDL-c reduction) versus a combination of simvastatin plus ezetimibe on major adverse cardiovascular events (Cannon *et al.*, 2008).

Red yeast rice

Chinese red yeast rice extracts (RYR) are popular lipid-lowering dietary supplements containing monacolins that have hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor activity. A meta-analysis published in 2004 (Liu *et al.*, 2006), examined the effectiveness of several different RYR formulations on lipid levels in patients with primary hyperlipidemia and found favorable results. Two recent randomized control trials examined RYR in populations with statin-associatedmyalgias. The earlier trial of 62 patients showed a significant LDL-c lowering effect of RYR compared to placebo(Becker*et al.*, 2009), and a more recent trial of 43 patients

demonstrated equal efficacy of LDL-c lowering when comparing RYR to 20 mg of pravastatin daily (Halbert*et al.,* 2010). The most promising role for red yeast rice is as an alternative lipid lowering therapy for patients who refuse to take statins because of philosophical reasons or patients who are unable to tolerate statin therapy due to statin-associated myalgias (Childress *et al.,* 2013). However, standards and oversight for RYR formulations are lacking, and many of these products may contain significant amounts of monacolin K and toxins such as citrinin, which are known for renal toxicity.

Coenzyme Q10

CoQ10, also known as ubiquinone, is a lipid-soluble antioxidant that is synthesized *de novo* by animal cells. Found in cell membranes, it is particularly well known for its role as a cofactor in the electron transport chain, playing a key role in mitochondrial energy production. Statins block the production of an intermediary needed to produce CoQ10. CoQ10 depletion results in mitochondrial dysfunction and theoretically results in myopathy (Larsen *et al.,* 2013). Strong evidence exists demonstrating that statin therapy lowers serum CoQ10 levels (Marcoff *et al.,* 2007), Smaller randomized control trials of between 28 and 76 patients (Caso*et al.,* 2007; Bookstaver *et al.,* 2012; Fedacko *et al.,* 2013; Zlatohlavek *et al.,* 2012; Young *et al.,* 2007; Bogsrud *et al.,* 2013), have yielded conflicting results regarding CoQ10 supplementation and its clinical value in decreasing statin intolerance. These trials used a variety of visual analog scales to quantify changes in myalgia, each studied different CoQ10 preparations, and examined distinct patient populations. As such, inadequate evidence exists to definitely recommend CoQ10 supplementation. A highly anticipated, more definitive trial is ongoing (Parker *et al.,* 2013). It will address the lack of conclusive scientific evidence for the utility of CoQ10 supplementation by examining its effects on the severity of muscle pain during statin treatment in patients with confirmed statin myalgia. This trial is the first to confirm the presence of statin-related

myalgia via a crossover run-in trial, during which the presence of symptoms will be documented when treating with a statin or placebo. Dosing of CoQ10 supplementation is also an issue. Supplements may be poorly absorbed and it is unclear how much dietary CoQ10 intake is necessary to offset statin-associated reductions in muscle tissue levels of ubiquinone. However, the safety profile of CoQ10 (Hathcock *et al.*, 2006), makes it an attractive medication at higher doses.

Vitamin D

A complex, and not fully understood, relationship exists between vitamin D and statins. Both affect skeletal muscle metabolism and function. Vitamin D deficiency alone has been linked to myalgias (Plotnikoff *et al.,* 2003). Serum levels of vitamin D may affect statin effectiveness and metabolism (Bhattacharyya *et al.,* 2012) Certain trials demonstrate that statins increase serum vitamin D levels, while others show no significant change (Gupta *et al.,* 2011). There is a suggestion that vitamin D deficiency is associated with increased statin intolerance due to myopathy (Ahmed *et al.*, 2009). Data are observational and no randomized control trials exist addressing this issue. The most recent data include 150 hypercholesterolemic patients, unable to tolerate statin because of myalgia, selected by low serum vitamin D. These patients were placed on vitamin D supplementation and restarted on a statin for a median time of 8 months. Eighty-seven percent were free of myalgia and appeared to tolerate statin re-initiation (Glueck *et al.*, 2011). Ultimately, myalgia in patients taking statins, with underlying vitamin D deficiency, may reflect a reversible interaction between vitamin D deficiency and statins on skeletal muscle. Vitamin D deficiency may potentiate statin myopathy or lead to drug-unrelated myalgias in a subset of statin-treated patients. Insufficient evidence supports testing for vitamin D deficiency in patients with statin-induced myalgia. However, in the patient known to be vitamin D deficient with a history of

statin intolerance, re-challenging with a statin once vitamin D levels are replete is a reasonable strategy.

Nowadays, cardiovascular complications are considered as the main factors of morbidity and mortality. Globally, the number of deaths from cardiovascular diseases has increased from 14.4 million in 1990 to 17.5 million in 2005, and it is estimated to be about 20 million in 2015(WHO 2004). Morbidity and mortality increase when combined with other prevalent diseases such as diabetes mellitus and hypertension (Eisenberg *et al.,* 1998). The formation of atherosclerotic plaque involves accumulation of LDL in intima, LDL oxidation and uptake of oxidized LDL by macrophage scavenger receptors, influence of macrophages on foam cells, and stabilization of plaque. In all steps of atherosclerosis, inflammatory cytokines are involved and make this process a chronic inflammatory disease (Rafieian-Kopaei *et al.,* 2014).

When the blockage of the coronary arteries reach more than 75%, usually the symptoms of angina will gradually appear. Blood clot usually develops on the irregular surfaces of arteries, which then may become detached, thus blocking the downstream blood flow. Heart attacks and strokes are usually caused by such blood clots. Moreover, the atherosclerotic blood vessels are generally weak and can burst. The best treatment in diseases such as atherosclerosis is prevention. Therefore, conventional medical approaches generally focus on lifestyle changes, such as reduction in the consumption of saturated fats, quitting smoking, and increase in aerobic exercise. Drugs are also used to lower cholesterol levels or blood pressure.

Available Lipid-Lowering Medicinal Plants

Different remedies are used to treat hyperlipidemia in traditional medicine in which the role of medicinal plants is significant. Recent researches performed on medicinal plants and food supplements used in traditional medicine indicate that

compounds present in them including food fibers, vitamins, flavonoids, sterols, and other antioxidant compounds can lower lipids, inhibit LDL oxidation, eliminate oxygen free radicals, and possibly improve this disease by having an effect on the immune system and improving metabolic disorders of the body. (Asgary *et al.,* 2013; Madihi *et al.*, 2013; Asgary *et al.,* 2014; Madihi *et al.*, 2013).

***Cynara cardunculus* (Artichoke)**

In the early 20th century, French scientists stated this plant as liver and bile stimulator. Leaves of artichoke are used as diuretic to stimulate kidneys and as bile stimulator to release the flow of bile from the liver. Italian scientists used to prescribe the cynarin compound (effective substance of artichoke) to stimulate the liver and gallbladder and to treat elevated cholesterol (Brand and Cynara scolamus 1990). Use of artichoke leaves stimulates bile production and result in dyspeptic problems. There are no valid evidences indicating that leaves of this plant result in dyspeptic problems directly (Kupke *et al.,* 1999). A number of animal studies suggest that artichoke leaves inhibit cholesterol synthesis in liver cells and also protect the liver from damage caused by chemical toxins (Heidarian *et al.*, 2013). According to a study performed on 143 patients with high cholesterol, leaves of artichoke improved the level of cholesterol significantly (Englisch *et al.,* 2000). Compounds present in the leaf of artichoke like cynarin and luteolin may play a significant role in reducing the synthesis of cholesterol as well as its total level (Englisch *et al.,* 2000).

***Medicago sativa* (Alfalfa)**

In traditional medicine, *Medicago sativa* is used as a dietary supplement, antidiabetic, antihyperlipidemic, and anti-allergen. It is also used to treat menstrual disorders, gastrointestinal tract disorders, kidney and urinary tract problems, burns, etc. This plant is used as a dietary supplement, since it contains high amount of b-carotene and vitamins, including B, C, E, and K. Researchers have suggested that seeds of

Medicago sativa have the ability to decrease the blood cholesterol level in laboratory animals. The seeds of this plant in monkeys for 1 year had no side effects but also reduced the blood cholesterol level. (Radhakrishnamurthy *et al.,* 1982). In this study in which the effects of alfalfa on aortas from cynomolgus monkeys with diet-induced atherosclerosis were evaluated, alfalfa resulted in varied degrees of regression of lesions. Laboratory studies have reported the presence of plant estrogens in this plant that may be useful to treat menstrual disorders.

***Allium sativum* L (garlic)**

Allium sativum L (garlic) is used in the treatment of an extensive range of diseases. The aromatic compound alliin is one of the most important compounds that exist in Allium sativum L. When garlic is cut or pressed, an enzyme named Alinase affects alliin, transforming it to allicin, which is the main factor of the strong odor of the garlic (Schulz *et al.,* 1998). Today, garlic is used to treat gastrointestinal tract disorders, asthma, diabetes; cardiovascular diseases, hypercholesterolemia, common cold, and high blood pressure (Shirzad *et al.,* 2011).There are contradictive scientific evidences in the decrease in cholesterol and blood pressure. Although some studies performed over the 1980 to 1990 decades suggested that garlic can decrease the blood cholesterol level, some more recent studies have shown contrary results. (Shirzad *et al.,* 2011; Nasri *et al.,* 2013).

***Glycine max* (Soybean)**

Soya as a food product rich in protein is used in Asia and as free-cholesterol meat in traditional food of American people. The American Food and Drug Administration has allowed producers of food products containing soybean to use the healthy heart label on their products. In traditional medicine, soybean has been used to decrease blood cholesterol and also as an anti-cancer and anti-osteoporosis drug (Schulz *et al.,* 1998). Several studies have suggested that soybean can decrease LDL in

the blood. In a study performed on 38 individuals with high blood cholesterol, soybean decreased blood cholesterol and improved the LDL/HDL ratio. In this meta-analysis study the effect of soy protein intake was evaluated on serum lipid profile of hyperlipidemic patients. In most of the evaluated trials, the intake of cholesterol, fat, saturated fat, and energy was nearly equal in the control and soy-containing diet groups. Thus, soy protein rather than animal protein was able to significantly decrease the serum concentrations of triglycerides, total cholesterol, low-density lipoprotein cholesterol (Anderson *et al.,* 1995). Some observations indicate that isoflavones available in soybean have an important role in decreasing blood cholesterol but some studies say the proteins available may have more an important role in decreasing blood cholesterol compared with these flavones (Rafieian-Kopaei *et al.*, 2014).

***Commiphora mukul* (Gugulipid)**

Commiphora mukul is an adhesive gum that is obtained from the Mukul myrrh tree. In the traditional medicine of India, this material is combined with other plants and is applied to treat skin problems, nervous system pains, obesity, diabetes, digestive problems, rheumatoid pains, mouth infection, and menstrual problems (Satyavati GV 1988). To study the effect of this material on blood cholesterol, a study on 61 individuals was performed for 24 weeks. After 12 weeks of diet control, half of the individuals received placebo and other half received Guggul at 100 mg daily dose. Clofibrate had no effect on HDL; however, HDL was increased in 60% of cases who consumed gugulipid. A significant reduction in LDL cholesterol was observed in the responders to both drugs (Nityanand *et al.*, 1989).

In the present study to investigate the anti-hyperlipidemic activity of medicinal plant *Cassia auriculata.*