ANALYSIS OF THE ASSOCIATION BETWEEN APOA5 POLYMORPHISM AND CHOLESTEROL LOWERING EFFECTS AMONG STATIN USERS WITH HYPERCHOLESTEROLEMIA FROM HUSM, KELANTAN

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by

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

Abbreviation	Name
ABCA1	ATP Binding Cassette Transporters A1
ABCG5/G8	ATP Binding Cassette Transporter G5/G8
ACAT	Acyl cholesterol Acyl Transferase
Apo	Apolipoprotein
ASCVD	Atherosclerotic Cardiovascular Disease
BBB	Blood Brain Barrier
C	Cytosine
CCBs	Calcium Channel Blockers
CE	Cholesteryl Ester
CETP	Cholesteryl Ester Transfer Protein
Ch	Cholesterol
CHD	Coronary Heart Disease
CHF	Congestive Heart Failure
COQ2	Coenzyme Q2
CVDs	Cardiovascular Diseases
DM	Diabetes Mellitus
DMSO	Dimethyl Sulfoxide
dNTPs	Deoxynucleotides triphosphates
EDTA	Ethylenediamine Tetra acetic acid
ER	Endoplasmic Reticulum

FDA	Food and Drug Administration
FFAs	Free Fatty Acids
GWAS	Genome-Wide Association Studies
HBA1c	Hemoglobin A1c
HCH	Hypercholesterolemia
HDL	High Density Lipoproteins
HMGCR	3-Hydroxy-3- Methyl Glutaryl-CoA reductase
HPL	Hyperlipidaemia
HUSM	Hospital Universiti Sains Malaysia
IDL	Intermediate Density Lipoproteins
KIF6	Kinesin Family Member 6
KRK	Klinik Rawatan Keluarga
LAL	Lysosomal Acid Lipase
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low Density Lipoproteins
LDLR	Low Density Lipoprotein Receptor
LPA	Lipoprotein (a)
LPL	Lipoprotein Lipase
MAF	Minor Allele Frequency
$MgCl_2$	Magnesium Chloride
MW	Molecular Weight
NA	Nucleic Acids

NCBI	National Centre for Biotechnology Information
NCHS	National Center for Health Statistics
NPC1L1	Niemann Pick C1 Like 1 protein
OATP1B1	Organic Anion Transporting Polypeptide 1B1
PAI	Platelet Aggregation Inhibitors
PCI	Phenol-Chloroform Method
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
PCSK9	Proprotein Convertase Subtilisin Kexin type 9
PLTP	Phospholipid Transfer Protein
RCT	Reverse Cholesterol Transport
SAMS	Statin-Associated Muscle Symptoms
SNP	Single Nucleotide Polymorphism
SRB	Scavenger Receptor-B1
SREBPs	Sterol Regulatory Element-Binding Proteins
T	Thymine
TG	Triglyceride
VLDL	very Low Density Lipoproteins

Analisis Perkaitan di antara Polismorfisme APOA5 dan Kesan Penurunan Kolesterol Dalam Kalangan Pengguna Statin Dengan Hiperkolesterolemia Dari HUSM,Kelantan.

ABSTRAK

Statin, perencat enzim HMG-CoA reduktase, telah menunjukkan efikasinya dalam menurunkan salah satu faktor risiko major bagi penyakit kardiovaskular, iaitu aras kolesterol plasma. Tindakbalas pesakit terhadap rawatan dengan statin, walau bagaimanapun, didapati berbeza secara signifikan. Variasi inter-individu boleh disumbang oleh banyak faktor demografi dan klinikal termasuk faktor genetik. Polimorfik nukleotida tunggal (SNP) dalam gene Apolipoprotein A5 (APOA5) iaitu rs662799, telah dilaporkan menyebabkan kesan penurunan kolesterol oleh statin. Tiada kesimpulan yang jitu diperolehi sehingga kini dan tiada kajian hubungkait genetik dikendalikan dalam kalangan populasi rakyat Malaysia terhadap kesan SNP tersebut dalam menentukan kesan penuruan kolestrol disebabkan statin. Oleh itu, kajian ini bertujuan mengkaji perkaitan APOA5 rs6627999, termasuk faktor klinikal yang lain, terhadap statin yang dipreskripsikan dalam menurunkan aras kolesterol dalam kalangan pesakit hyperlipidemia di Kinik Rawatan Keluarga, Hospital Universiti Sains Malaysia, Kelantan. Ini merupakan kajian keratan rentas bukan intervensi melibatkan 84 orang pesakit. Data demografi dan klinikal telah diperolehi dari data berkomputer hospital dan fail perubatan pesakit di Unit Rekod. *Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)* merupakan teknik yang digunakan untuk penaipan DNA darah dalam kajian ini. Kecelaruan yang tidak dijangka ekoran pandemic Covid-19 menyebabkan perubahan polisi yang tidak dijangka termasuk urus selia kajian ini kerana kaedah penaipan tidak dapat dijalankan mengikut perancangan di awal kajian ini. Walau bagaimanapun, kami

menjangkakan bahawa kekerapan genotip SNP rs662799 adalah tidak berbeza dengan populasi Asia yang lain iaitu nilai Kekerapan Alel Minor (MAF) ialah 0.29 bagi populasi Asia Timur (https://asia.ensembl.org/index.html). Tambahan juga, perbezaan yang ketara juga dijangka terhadap kesan penurunan aras kolesterol oleh statin disebabkan oleh SNP rs662799, di mana pengurangan aras LDL-C yang lebih tinggi bagi genotip TT-1131 (alel major) berbanding pembawa C-1131 (alel minor). Tafsiran bagi jangkaan hasil kajian memerlukan kajian susulan bagi mengesahkan hubungkait SNP rs662799 terhadap keberkesanan statin.

ANALYSIS OF THE ASSOCIATION BETWEEN APOA5 POLYMORPHISM AND CHOLESTEROL LOWERING EFFECTS AMONG STATIN USERS WITH HYPERCHOLESTEROLEMIA FROM HUSM, KELANTAN

ABSTRACT

Statins, HMG-CoA reductase inhibitors, have shown their efficacy in reducing one of the major risk factors for the disease i.e., the plasma cholesterol level. Patients' responses to statin therapy have, however, been found to vary significantly. The interindividual variation can be attributed to many demographic and clinical factors including genetic factors. A single nucleotide polymorphism (SNP) in Apolipoprotein A5 (ApoA5) gene, i.e.,rs662799, was reported to result in the cholesterol-lowering effect of statins. No definitive conclusion has been drawn to date and no genetic association study among the Malaysian population has been explored on the SNP in determining statin's cholesterol-lowering effect. Therefore, this study aims to investigate the association of ApoA5 rs662799, along with other clinical factors, on the efficacy of prescribed statins in lowering the plasma cholesterol level among hyperlipidaemic patients at the Klinik Rawatan Keluarga, Hospital Universiti Sains Malaysia, Kelantan. This is a non-interventional cross-sectional study involving 84 patients. Demographic and clinical data for patients have been collected from the hospital's computerized database and medical folders at the Unit Record. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was the technique adopted for blood DNA genotyping in this study. Unprecedented damage

by the pandemic COVID-19 requires an unprecedented policy change including the arrangement of this study because the genotyping method was not accomplished as planned at the beginning of the study. Nevertheless, we consider the genotype frequencies of the rs662799 SNP to have been compatible with other Asian populations i.e., Minor Allele Frequency (MAF) value of 0.29 for East Asian populations (https://asia.ensembl.org/index.html). Also, a significant difference was expected in statin's cholesterol-lowering effects due to the rs662799 SNP, with a higher LDL-C reduction in TT-1131 genotype (the major allele) than the C-1131 allele (the minor allele) carriers. The interpretation of the expected results needs further investigation to validate the association of rs662799 SNP with respect to the effectiveness of the statin.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Classified as the number one cause of death worldwide, Cardiovascular diseases (CVDs) were defined as a group of abnormalities affecting heart and blood vessels, including a wide variety of conditions and diseases such as hypertension, hypercholesterolemia, coronary heart disease (CHD) and congestive heart failure (CHF). In 2016, around 17.9 million lives (31% of all deaths worldwide) were lost because of CVDs; approximately 85% of them were due to heart attack and strokes that resulted primarily from blocking or narrowing the coronary arteries by an accumulated lipidic plaque (World Health Organization, 2017). Therefore, the reduction of risk of CVDs has occupied a large space in recent medical concerns, leading to more prescribed for the cardiovascular drugs such as lipid-modifying drugs, platelet aggregation inhibitors (PAI), antihypertensive drugs and anticoagulants.

Hyperlipidemia, besides hypertension, diabetes mellitus (DM), smoking, and obesity, is one of the main five risk factors for CVDs (Rakel & Houston, 2018). It is a remarkable, well-recognized, and adjustable abnormality with widespread availability of treatment medication and lab diagnostic assays. Nevertheless, it is a prevalent disease, associated with a significant proportion of CVD deaths worldwide. People with

hyperlipidemia have almost double the chance to develop a CVD comparing with people with a normal level of total cholesterol (Karr, 2017). Moreover, hypercholesterolemia, which is a main type of dyslipidemia, has a high prevalence in Malaysia estimated by 44.9% in total, and distributed ethnically between Malays, Chinese, Indians and other races by 51.0%, 40.8%, 41.6% and 34.4% respectively (Lin *et al.*, 2018).

The classes of lipid modifying drugs are various in their indications and mechanisms. Among these drugs, the hydroxymethyl glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly known as the statins, are the first-line in hypercholesterolemia treatment (Grundy *et al.*, 2019). Numerous advantages have been investigated due to statin intake, classified as cholesterol-dependent and -independent effects. On one hand, statins hinder the synthesis of mevalonate in liver cells through inhibiting the catalyzing enzyme, HMG-CoA reductase, which is considered the rate-limiting factor in the intrinsic pathway of cholesterol synthesis. Responding to this action, the LDL-receptor expression on the hepatocytes` surface is increased, leading ultimately to lowering the LDL-C concentrations in plasma by about 20 to 55% (Natalie C. Ward, Watts & Eckel, 2019). On the other hand, statin can improve the endothelial function and reduce the oxidative stress in cells and perform many other pleiotropic effects (Allen & Mamotte, 2017).

In terms of the prevalence of use, statins are one of the most prescribed drugs, not only among cholesterol-lowering medication but also among all known drugs

(Aitken *et al.*, 2017). As the American National Center for Health Statistics (NCHS) has announced, the usage of statins raised from 18 to 26 percent, making them the most consumable anti-hypercholesteremic drugs. By 2011 to 2012, a statin has been used by 93 percent of people taking a cholesterol-lowering drug (Qiuping Gu *et al.*, 2014).

Despite statin efficacy, response to therapy and adverse effects have been found to vary significantly from one person to another. One study by (Gitt *et al.*, 2012) found that about half of the patients, who received statins, were unable to meet their lipid reduction goals, producing the term of "statins resistance". In addition, serious differences in the pharmacokinetics of each statin have been defined, with half-lives ranging from 1 to 19 hours and oral bioavailability from 5% to 60% (Schachter, 2005). These interindividual variations can be attributed to a number of factors such as gender, age, diet, concomitant diseases, drug-drug interactions, and many others (Peters *et al.*, 2009). Nonetheless, genetic variation can perfectly explain a large part of this diversity.

Single nucleotide polymorphisms (SNPs) have received special attention as key genetic factors causing considerable differences between individuals. Through genome-wide association studies (GWAS) and many other types of investigations, some of these SNPs have been proved for their association with the normal physical processes, such as lipid metabolism, and with the efficacy of drugs targeting these process, such as statins (Deshmukh *et al.*, 2012).

Statins, like other drugs, perform their therapeutic effects by interacting with several proteins that are normally produced from genes. From here, SNPs and other genetic factors can influence the drugs, whether pharmacodynamically or pharmacokinetically. Plasma binding proteins, transporter proteins, metabolizing enzymes and even the ultimate target of the drug are all susceptible to genetic variations and may get affected considerably by them.

This study is focusing on the cholesterol-lowering effect of statins and its alteration by a candidate SNP in the APOA5 gene, with studying the demographic and clinical characteristics of each genotype carriers.

1.2 The significance of study

The pharmacogenetic studies offer great advantages for the medical care service. This study investigates the contribution of APOA5 polymorphism, patient's demographic profiles, and clinical factors in determining the effectiveness of statins (as assessed by their cholesterol-lowering effect) among Kelantanese (statin users from outpatient statin users in HUSM) population. The data on this relationship in Malaysia is limited and the effect of this relationship on the regimens and treatments is still need to be examined.

Several reasons made this study important to be conducted. Firstly, this investigation may contribute to a more definite conclusion for APOA5 polymorphisms as the conclusion of some pharmacogenetic studies are indecisive. Secondly, APOA5 is a small protein discovered and described in 2001 (Su, Kong & Peng, 2018); therefore, further studies are required to clarify its effects on cholesterol blood levels and related drugs, despite what is known about its role on triglyceride (TG) metabolism. Thirdly, the studied variant (rs662799 -1131T>C) in the APOA5 gene has been found at a higher frequency in Asian populations compared with other races (Hubacek, 2016), suggesting more impact of this SNP on Malaysian patients. Fourthly, with regard to hypercholesterolemia (HCH), this disease is a major risk factor for CVDs, widely spread over the world, imposing high costs on individuals and the healthcare system. Thus, such a study may improve statin therapy and maximize its benefits. Fifthly, as pharmacogenetic research, this study could participate in developing more individualized targeted therapies to increase both the effectiveness and safety of statin treatment. Finally, statins, as one of the most prescribed drugs, have exhibited serious side effects which may have imposed dosage limitations and a recall. This study may help in reducing suffering from these side effects on the base of genetic diversity.

1.3 Objectives of study

1.3.1 General Objective

To analyze the association between the selected genetic polymorphism and cholesterol-lowering effects among outpatient statin users with hypercholesterolemia from Klinik Rawatan Keluarga HUSM, Kelantan.

1.3.2 Specific Objectives

To perform SNP genotyping for APOA5 gene (rs662799) using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method.

To determine the frequencies of APOA5 rs662799 and its association with cholesterol-lowering effects among statin users with hypercholesterolemia.

To assess demographics profiles and clinical parameters of the statin users with hypercholesterolemia

CHAPTER 2

LITERATURE REVIEW

2.1 Cholesterol

Lipids are a large group of heterogenous hydrophobic compounds, soluble in organic solvents, insoluble in aqueous solution, and generated by condensing thioesters or isoprene units (Rifai, Horvath & Wittwer, 2018; Rodwell *et al.*, 2018). As a general classification, fats can be subdivided into simple, complex, and derived lipids, however, the fats that are important clinically can be classified under five major categories as shown in (Figure 2.1).

Functionally, fats perform vital roles in the human body, having the highest potential energy as a metabolic and storing fuel, contributing in the hormonal system as a precursor, participating physically and functionally in the cell membrane as a key compound, mediating neuronal conduction along myelinated nerves, and insulating the heat in the subcutaneous tissues (Rifai, Horvath & Wittwer, 2018). Cholesterol, in particular, occupies a significant place in physiology, despite the false impression probably people have. It constitutes a starting point for many metabolic processes and a precursor for a wide variety of important compounds including bile acids, adrenocortical hormones, sex hormones, vitamin D, and cardiac glycosides. Moreover, all living organisms contain cholesterol or its derivatives, due to its necessity in

preserving the required permeability and fluidity through the plasma membrane (Rifai, Horvath & Wittwer, 2018; Rodwell *et al.*, 2018).

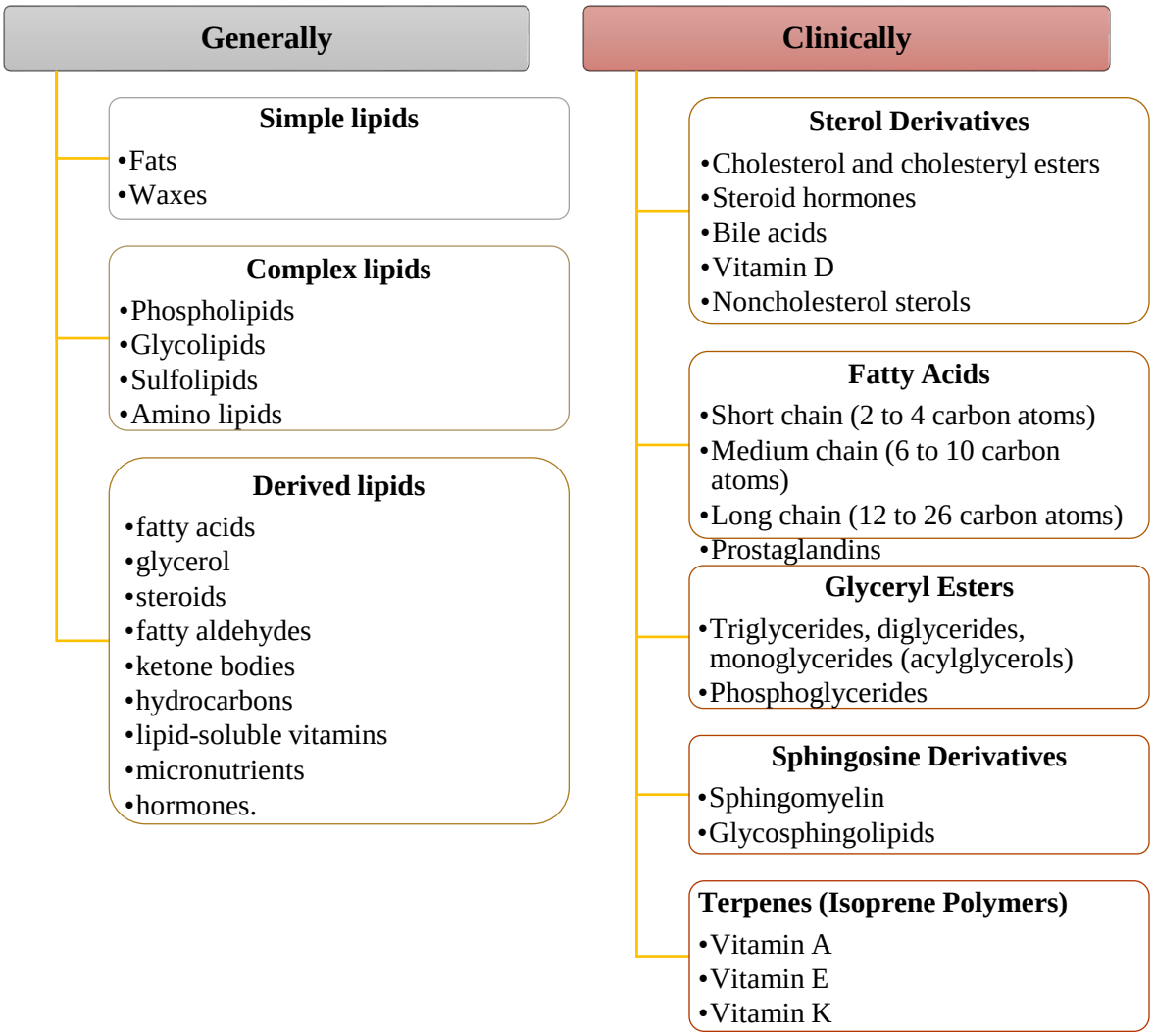

Figure 2.1 general and clinical classification of lipids.

From a chemical view, cholesterol follows the typical structure of steroids with a molecular weight of 386.65 g/mol. It comprises 27-carbon atoms that forming a

tetracyclic perhydrocyclopentanophenanthrene core and contains a single unsaturated double bond, single hydroxyl group, and a side chain at Carbon 17 (Figure 2.2). On this basis, cholesterol has a dual affinity for aqueous and organic solvents, makes it classified among amphipathic lipids. Despite its steroidal skeleton, cholesterol and many other lipids seem to be derived from fatty acids that contain long chains of hydrocarbons and combine with other molecules to give a different type of lipids as triglycerides (binding with glycerol) and phospholipid (binding with phosphate); the thing which might explain the physical and chemical similarity among these lipids (Hall, 2016).

Four dominant types of lipids have been defined in plasma, namely triacylglycerols (16%), phospholipids (30%), cholesterol (14%), and cholesteryl esters (36%). Meanwhile, the most active lipids metabolically found in a tiny fraction in plasma (only 4%) as unesterified long-chain fatty acid (free fatty acids (FFAs)) (Rodwell et al., 2018).

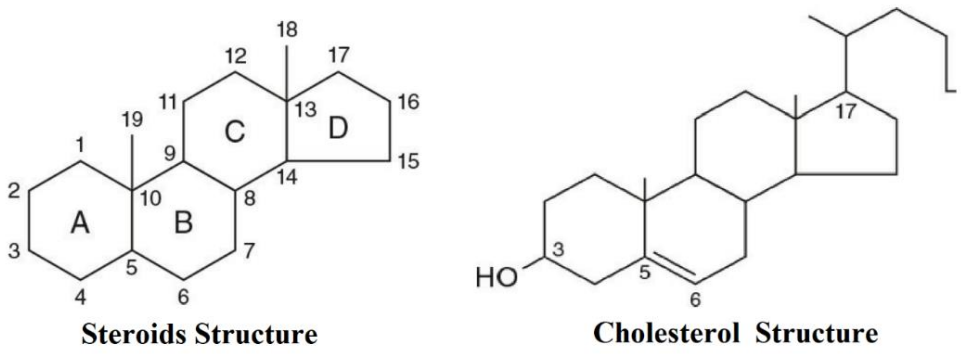

Figure 2.2 Common steroid skeleton and cholesterol structure. modified from (Rodwell et al., 2018)

2.1.1 Source

Most of the cholesterol in the human body comes from intrinsic synthesizing while the rest is supplied from the diet. At endoplasmic reticulum (ER) and cytosolic compartments, starting from Acetyl-CoA molecule, almost all nucleated cells are able to produce cholesterol, taking into account that liver and intestine cells are responsible for about 10% of total synthesized cholesterol (Rodwell et al., 2018).

2.1.2 Absorption

The majority of cholesterol molecules in the gastrointestinal tract have been found in an unesterified or free form, coming from intestinal synthesis, dietary source, or biliary secretion. Esterified cholesterol, which is approximately 15% to 20% of the dietary cholesterol, is rapidly hydrolyzed by the pancreatic-secreted cholesterol esterase in the intestine to free cholesterol and free fatty acids (Hall, 2016).

The absorption process starts with forming mixed micelles of lipids and bile acids which improves the solubility of cholesterol molecules and enhances their contact with the surface of luminal gut cells. After that, three main transporters are involved in adjusting the cholesterol amount entering the circulation, namely, Niemann Pick C1 Like 1 protein (NPC1L1), ATP binding cassette transporter G5/G8 (ABCG5/G8) and ATP binding cassette transporters A1 (ABCA1) (Rifai, Horvath & Wittwer, 2018). The NPC1L1 is a common target for Ezetimibe which inhibits cholesterol absorption

through inhibiting the function of this enterocyte influx sterol transporter when mediating the active transmission of cholesterol into cells. Parallelly, ABCG5/G8 has been found to balance NPC1L1 function by pumping the cholesterol molecules back into the intestine lumen, giving the intestine an important role as a secretory organ of cholesterol. Once the cholesterol is placed inside the enterocytes, ABCA1 transporters contribute in packaging some amount of cholesterol into high-density lipoprotein (HDL) particles, while the rest is conjugated with long-chain fatty acids and packed ultimately with other lipids in chylomicron (Rodwell *et al.*, 2018).

2.1.3 Transportation

While the free form is essential for the absorption, Cholesteryl ester (CE) was found the dominant form in the body, made by a combination of cholesterol molecules and free fatty acids. Lecithin-cholesterol acyltransferase (LCAT) and acylcholesterol acyltransferase (ACAT) are both typical enzymes responsible for esterification of cholesterol, exerts their effect in circulation and inside cells respectively with some differences (Figure 2.3). By this esterification, the capacity of transportation is improved and the toxicity of free cholesterol intracellularly is avoided (Rifai, Horvath & Wittwer, 2018).

After absorption in the small intestine, cholesterol molecules are incorporated into the circulation on lipoproteins and distributed throughout the body to the most, but not all,

tissues. In the brain, for example, no cholesterol can be delivered because of the Blood-Brain Barrier (BBB) and all existed there is from local synthesizing (Hall, 2016). Subsequent to distribution, cholesterol ester molecules are handled by cells and hydrolyzed via the intracellular lysosomal acid lipase (LAL) to be utilized, while the excess amount either remained for storing or transported back via circulated lipoproteins to the liver. In the liver, cholesterol could have many fates; either to be eliminated by biliary secretion unchanged, metabolized into bile acids, stored as cholesteryl ester (CE), utilized in hepatic bioprocesses, or integrated into new lipoproteins to be delivered to other tissues (Björkhem, 2013; Rifai, Horvath & Wittwer, 2018). Depend on that, the total cholesterol amount in the whole organism is based on the involvement of cholesterol production, absorption, elimination, and storage (Meaney, 2014).

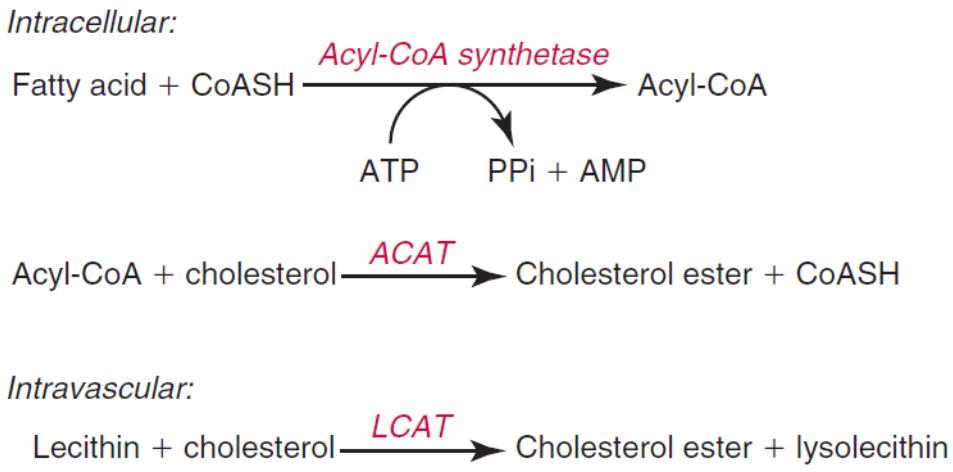

Figure 2.3 Intracellular and intravascular esterification of cholesterol via Lecithin-cholesterol acyltransferase (LCAT) and acylcholesterol acyltransferase (ACAT) respectively (Rifai, Horvath & Wittwer, 2018)

2.1.4 Synthesis

Regardless of the extrinsic source, almost all cells are capable to synthesize cholesterol in a sufficient amount to their needs, commencing from Acetyl-CoA. However, this process is extremely inhibited by high exogenous cholesterol income. Unlike ketogenesis which shares the same substrates but occurs inside the mitochondria, cholesterol synthesis is an extra-mitochondrial process involve 5 major steps. The first step is composed of many stages and includes the key regulatory reaction for the whole pathway. Initially, Two Acetyl CoA molecules combine and form Acetoacetyl-CoA, under the catalyzing of cytosolic thiolase. Subsequently, the previous Acetoacetyl-CoA is condensed with further Acetoacetyl-CoA by HMG-CoA synthase enzyme to end up with 3-hydroxy-3- methyl glutaryl-CoA (HMG-CoA). Next, the latter is reduced to Mevalonate by HMG-CoA reductase with NADPH, which considers the key regulatory reaction not only to this step but also to entire cholesterol synthesis pathway (Figure 2.4), therefore, it is an important target for the medication that reduce cholesterol plasma levels, such as statins.

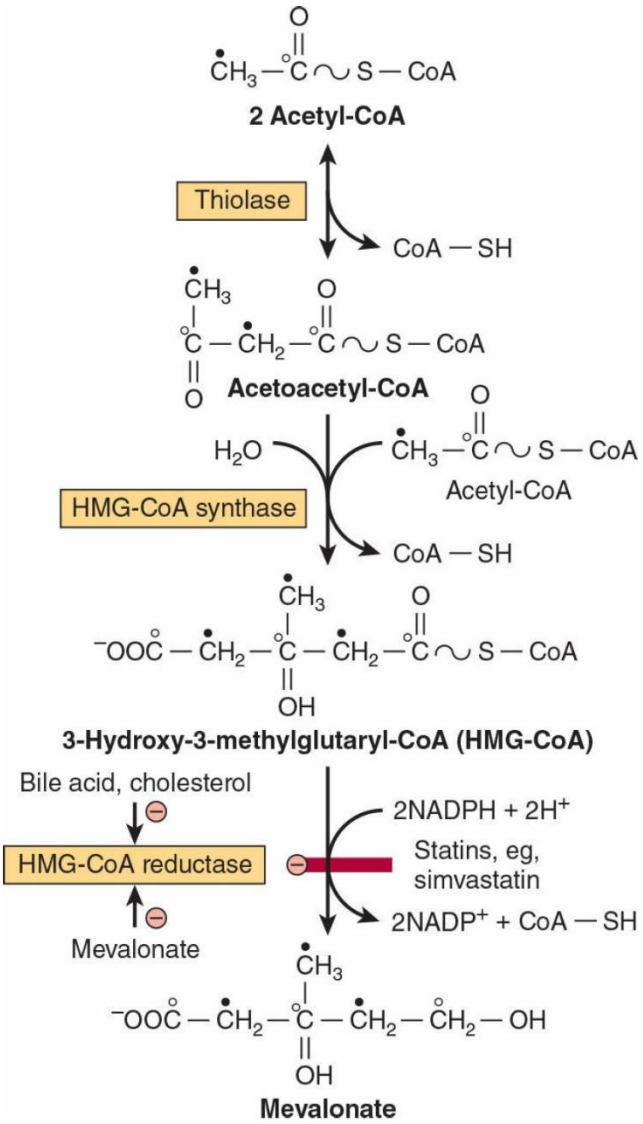

Figure 2.4 Biosynthesis of mevalonate
(Rodwell et al., 2018)

The next step comprises phosphorylation and decarboxylation reactions, applied sequentially on Mevalonate, and mediated by different enzymes to form ultimately one isoprenoid unit. In the third step, initial isomerization followed by two condensation reaction on the isoprenoid

units leads to form dimethylallyl diphosphate, geranyl diphosphate, and farnesyl diphosphate respectively. Then the squalene synthetase enzyme, along with NADPH, Mg^{+2}, and Mn^{+2}, mediates the condensation of two molecules of farnesyl diphosphate to produce squalene. Ultimately, the cyclization reaction of squalene produces lanosterol, the parent of steroid (Figure 2.5). Followed by the last step on the membranes of the endoplasmic reticulum, where several reactions occur, altering both the steroid nucleus and the side chain (Figure 2.5), to finish as Cholesterol (Hall, 2016; Rifai, Horvath & Wittwer, 2018; Rodwell *et al.*, 2018).

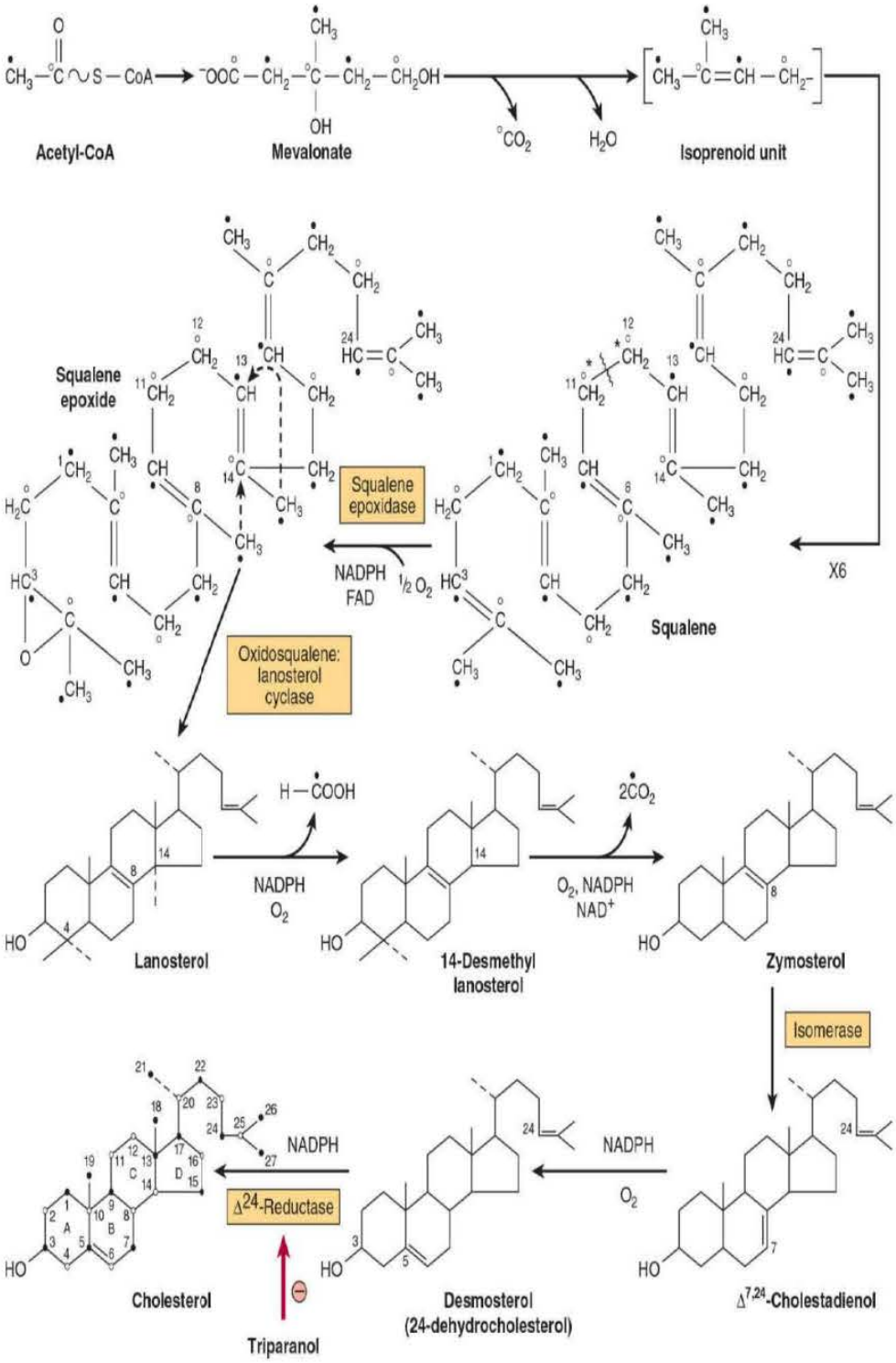

Figure 2.5 Biosynthesis of cholesterol
(Rodwell et al., 2018)

2.1.5 Regulation of Cholesterol synthesis

Since the overabundance of cholesterol has serious harmful effects on both cell and body, its synthesis rate should be precisely controlled and regulated. The high level of hepatic secreted cholesterol leads to saturation of bile acids and accumulation of cholesterol in the gall to end up with the common gallstone. In addition, the majority of cells, except hepatic and some endocrinal tissues, are limited in utilizing cholesterol. For that, the accumulated cellular cholesterol is associated with atherosclerosis incidence by its contributing in apoptosis or by forming extracellular crystals (Rifai, Horvath & Wittwer, 2018). The balance inside the cells can be shifted up by the overexpressing of the cholesterol-containing lipoproteins receptors (LDL receptor, CD36), maximizing the uptake of free cholesterol, the increasing of cholesterol synthesis, and the hydrolysis of esterified storage. In contrast, cellular cholesterol could be reduced when overabundance by suppressing the endogenous synthesis pathway controlled by HMG-CoA reductase, improving cholesterol esterification catalyzed by ACAT, reducing superficial LDL receptors through downregulate their gene, increase cholesterol utilization for cellular needs and increasing cholesterol effluxion from the membrane to HDL via ABCA1 and SR-B1 (Shimano, 2009; Rodwell *et al.*, 2018)

At the molecular level, the regulation of cholesterol synthesis adjusted through HMG-CoA reductase enzyme follows one out of two mechanisms, either by modifying the expression of the enzyme at a genetic level or by regulating the posttranslational modification of synthesized enzyme. On one hand, both Mevalonate (the immediate product of the regulatory step) and Cholesterol (the major product of the pathway)

practice an inhibitory role on HMG-CoA reductase. More deeply, the negative feedback control is precisely achieved when the level of cholesterol or its metabolites are enough to inhibit the activity of sterol regulatory element-binding proteins (SREBPs), a group of molecules that govern the expression of a range of genes. The impedance of these proteins will cause, in our case, a reduction in the transcription of HMG-CoA reductase gene and a subsequent inhibition to the overall productivity of the synthesis pathway. This pattern of regulation is highly observed in active organs, like the liver, and can be triggered by any mechanism elevating the plasma cholesterol level, intrinsically or nutritionally. On the other hand, hormones exert some of their divergent effects on HMG-CoA reductase by posttranslational modifications (such as phosphorylation and dephosphorylation), providing a rapid response to the short-term needs (Figure 2.6). For instance, insulin and thyroid hormones upregulate the activity of the HMG-CoA reductase while glucagon and glucocorticoids act as inhibitors (Rifai, Horvath & Wittwer, 2018; Rodwell *et al.*, 2018)

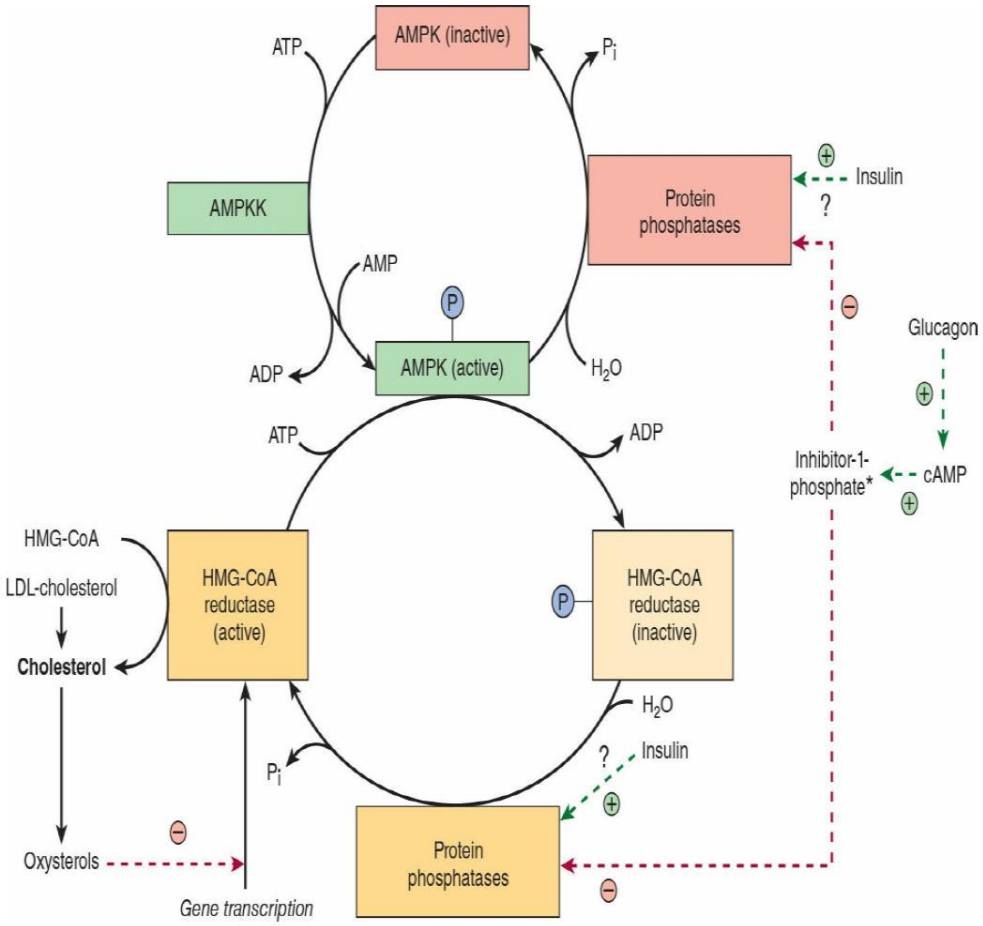

Figure 2.6 Possible posttranslational mechanisms in the regulation of cholesterol synthesis by HMG-CoA reductase Insulin has a dominant role compared with glucagon. (AMPK, AMP-activated protein kinase; AMPKK, AMP-activated protein kinase kinase) (Rodwell et al., 2018)

2.1.6 Elimination

Two major organs are responsible for cholesterol excretion; small intestine and liver. intestinal cells secret the excessive amount via ABCG5/G8 transporter, while hepatic cells integrate cholesterol as a main compound in the bile either as a free form or as a

form of bile acids. Saying that, the synthesis of bile acids and its regulation closely associates with cholesterol homeostasis.

Two pathways of bile acids synthesis have been deciphered; the classical and the alternative pathway. Classical pathway occurs in the microsomes of endoplasmic reticulum with multi hydroxylation steps mediated by several monooxygenase enzymes, mainly CYP7A1, the central regulatory enzyme in this pathway also called cholesterol 7α-hydroxylase. The alternative pathway, in turn, is also a hydroxylation reaction but occurs in mitochondria and is controlled by CYP27A1 (sterol 27- hydroxylase).

Both pathways end up with producing the primary bile acids: cholic acid and chenodeoxycholic acid, which conjugated partially with glycine and taurine before the excretion from the liver to make up a pool of salts and acids with a pH range of 7.6-8.4. In the intestine, a further metabolism including deconjugation and 7α- dehydroxylation by the intestinal microbes mediates the formation of the secondary bile acids: deoxycholic acid, and lithocholic acid (Figure 2.7) (Rodwell et al., 2018).

After performing their function in facilitating the digestion and absorption of lipid, the majority of primary and secondary bile acids (98-99%) are absorbed in ileum and returned to the liver via portal circulation by a process known as enterohepatic circulation. Otherwise, the few rests insoluble of bile acids are taken away with feces, which lead to stimulating a feedback system to compensate what has lost and consume an additional amount of cholesterol. By repeating this circulation 6 to 10 times every

day, bile acids are able to perform a vital role in lipid metabolism, in term of both absorption and elimination (Rodwell *et al.*, 2018).

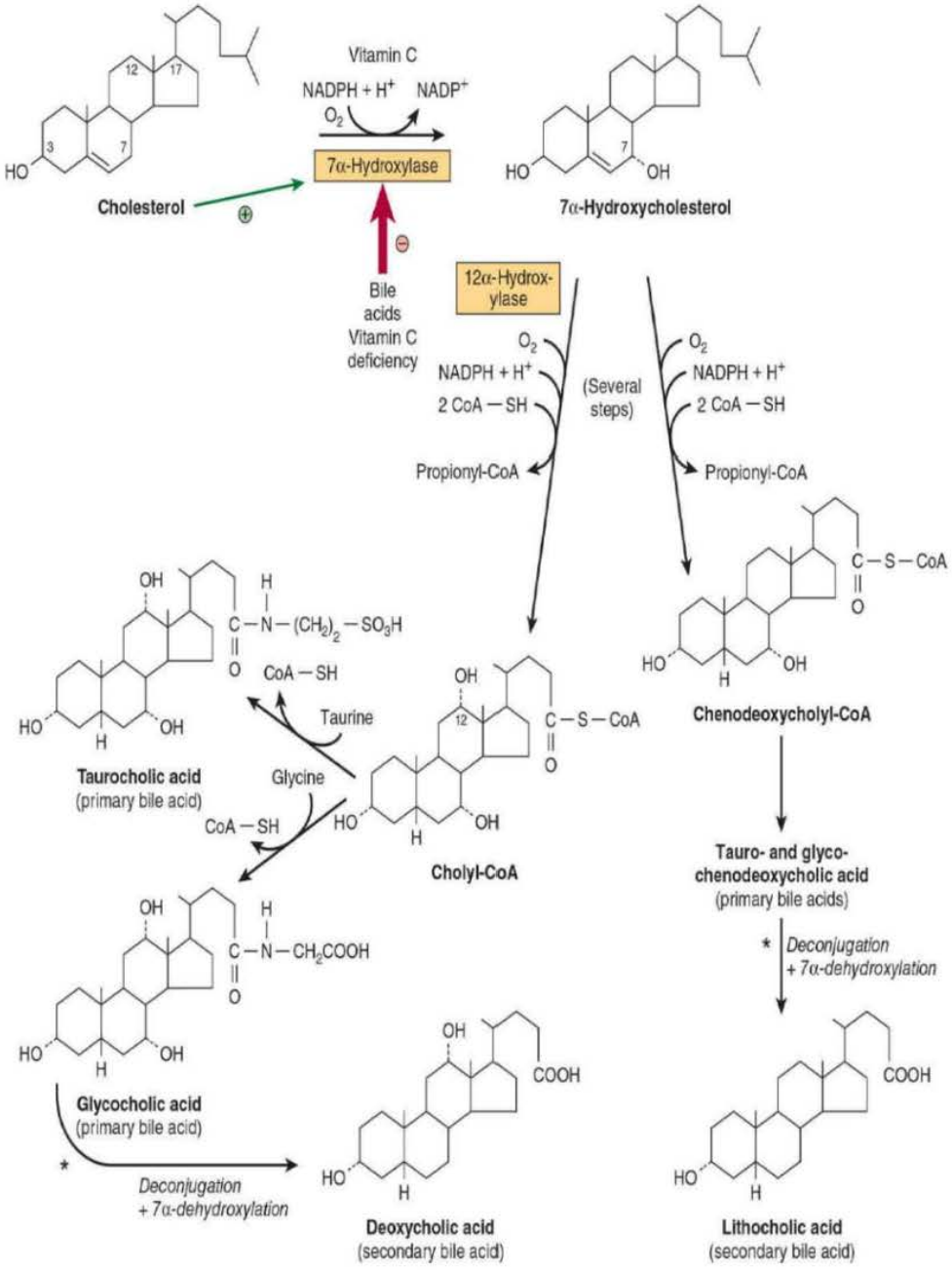

Figure 2.7 Biosynthesis and degradation of bile acids**.** *Catalyzed by microbial enzymes. (Rodwell et al., 2018)

2.2 Lipoprotein

As principal metabolic compounds, lipids must be transported to cells via blood to be utilized or stored. Through different types of water-soluble macromolecule complexes, called lipoproteins, the lipids are transported in circulation despite their insolubility in the aqueous mediums. In other words, lipoproteins package the lipids regardless their source, transfer them as cargo between peripheral tissues and the metabolic organs, along with shipping the hydrophobic and amphipathic substances such as drugs, and vitamins (Feingold & Grunfeld, 2018).

2.2.1 General Structure of lipoprotein

The general structure of lipoprotein can be split into two compartments; nonpolar core and hydrophilic surface**.** The lipidic nature of the core made it suitable for high hydrophobic triacylglycerol and cholesteryl ester molecules to locate and nestle away from the polarity of the blood. At the same time, the amphipathic nature of phospholipids, free cholesterol, and proteins allows them to confront the external aqueous medium by their hydrophilic groups while facing the internal lipid core by their hydrophobic moieties**,** forming a surrounding single layer, structurally similar to the cell membrane **(**Rodwell et al., 2018**)**. Knowing that, the components of lipoproteins, illustrated in Figure 2.8, are : (1) structural apolipoprotein, (2) peripheral apolipoprotein, (3) structural lipids at the surface (phospholipids and free cholesterol) and (4) cargo lipids in the core (triacylglycerols, sterol esters, and other high hydrophobic lipid types) (Meaney, 2014). While the bonds between these compounds are tight enough to provide stable complexes able to exchange free cholesterol molecules smoothly and

spontaneously with cells membranes, the other highly lipophilic elements need special transporters for smooth exchanging, namely, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) (Rifai, Horvath & Wittwer, 2018).

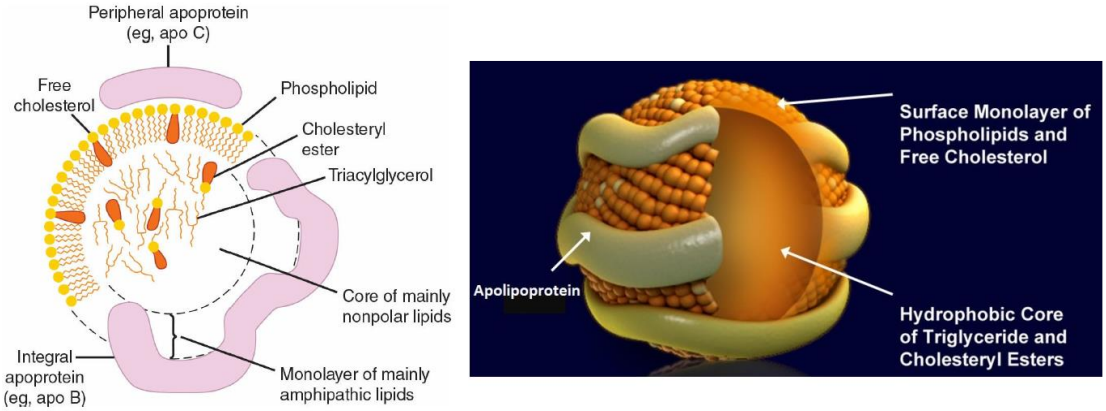

Figure 2.8 Generalized structure of a plasma lipoprotein.
Modified from (Feingold & Grunfeld, 2018; Rodwell *et al.*, 2018)

As long the protein portion determines the function and the fate of lipoproteins, a suggestion has been made to classify lipoproteins on the base of their structural apolipoprotein, into two major types; APO B-contained particles and APO A-contained particles (Vance & Vance, 2008). In addition, another classification was used according to the electrophoretic properties (weight and charge), represented in three types; α- (HDL), β- (LDL), and pre-β (VLDL)-lipoproteins (Rifai, Horvath & Wittwer, 2018). In any case, four primary types of lipoprotein have been described widely based on their size, lipid composition, and apolipoproteins, along with their importance in physiology and diagnosis, namely: chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high-density lipoproteins (HDL) (Table 2.1) (Rodwell *et al.*, 2018), respecting that other lipoproteins types may be mentioned elsewhere depending on the technology of segregation.

Table 2.1 Lipoprotein classes and characteristics.

Modified from (Rifai, Horvath & Wittwer, 2018; Rodwell *et al.*, 2018)

Variable		Chylomicron	VLDL	LDL	HDL
Source		**Intestine**	**Liver (intestine)**	**VLDL**	**Liver, intestine, VLDL, Chylomicrons**
Density, g/mL		<0.95	0.95–1.006	1.019–1.063	1.063–1.210
Electrophoretic mobility		Origin	Pre-β	β	α
Approximate Molecular weight, Da		$0.4–30 \times 10^9$	$5–10 \times 10^6$	2.75×10^6	$1.8–3.6 \times 105$
Diameter, nm		>70	27–70	19–23	4–10
Protein %		1-2%	7-10%	21%	32-57%
Lipid %		98-99%	90-93%	79%	43-68%
Major lipids		Exogenous triglycerides	Endogenous triglycerides	Cholesteryl esters	Phospholipids
Major Apolipoproteins		B-48 A-I A-II A-IV C-I C-II C-III	B-100 C-I C-II C-III E	B-100	A-I A-II A-IV C-I C-II C-III D E
Surface components*	**Ch**	2	7	8	04-May
	Phoso	7	18	22	25-33
	Apo	2	19	22	40-55
Core lipids*	**TG**	86	55	6	03-May
	Ch-ester	3	12	42	13-17

* Surface components and core lipids are given as a percentage of dry mass.

* HDL, High-density lipoprotein; LDL, low density lipoprotein; VLDL, very low-density lipoprotein; Ch, Cholesterol; Phoso, Phospholipids; Apo, Apolipoproteins; TG, Triglycerides; Ch-ester, Cholesterol ester.