EVALUATION OF ANTHOCYANIN-RICH STANDARDISED EXTRACT OF *Hibiscus sabdariffa* L. NIOSOMES FORMULATION AS ANTI-HYPERCHOLESTEROL AND CARDIOPROTECTIVE: A PILOT STUDY

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by

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Mohd Azmie Edwin

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LIST OF SYMBOLS

%	Percent
°C	Degree Celsius
>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
µA	Microampere
µL	Microlitre
µm	Micrometre
I.E.	International Units
g	Grams
mg	Milligrams
mg/kg	Milligram/kilogram
mm	Millimetre
mmHg	Millimetre mercury
mL	Millilitre
mmol/L	Millimoles per litre
mV	Millivolts

Cl-	Chloride ion
K+	Potassium ion
Na+	Sodium ion
kHz	Kilohertz
U	Unit
W	Watt

LIST OF ABBREVIATIONS

AEHS-Nio	Aqueous Extract *Hibiscus sabdariffa* L. – Niosome
AFR	African Region
AHA	American Heart Association
AHSE	Anthocyanin-Rich *Hibiscus sabdariffa* L. Ethanolic Extract
AHSW	Anthocyanin-Rich *Hibiscus sabdariffa* L. Water Extract
AMI	Acute Myocardial Infarction
ApoB	Apoliprotein B
ApoE	Apoliprotein E
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CAD	Coronary Artery Disease
CDC	Centre for Disease Control and Prevention
CETP	Cholesterol Ester Transfer Protein
CKD	Chronic Kidney Disease
CK-MB	Creatine Kinase Myocardial Band
CoA	Coenzyme A
CoQ	Ubiquinone
CVD	Cardiovascular Disease

COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DBP	Diastolic Blood Pressure
E3L	ApoE3Leiden
ECG	Electrocardiogram
EIM	Ether Injection Method
FDA	Food and Drug Administration
HC	Hypercholesterolaemic
HCA	Hibiscus Acid
HCD	High-Cholesterol Diet
HFD	High-Fat Diet
HC-MI	Hypercholesterolaemic-Myocardial Infarction
HDL	High-Density Lipoprotein
HIV	Human Immunodeficiency Virus
HMG-CoA	3-Hydroxy-3-Methylglutaryl Coenzyme A
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
GDP	Guanosine Diphosphate
GTP	Guanosine Triphosphate
ICD	In-Patient Cardioverter-Defibrillator
IDL	Intermediate-Density Lipoprotein

IP	Intraperitoneal
IPP	Isopentenyl Pyrophosphate
ISO	Isoprenaline
LAD	Ligation of the Left Anterior Descending Coronary Artery
LCA	Left Coronary Artery
LCx	Left Circumflex Coronary Artery
LDL	Low-Density Lipoprotein
LDLR	Low-Density Lipoprotein Receptor
LRP2	Receptor-Related Protein 2
IPH	Institute for Public Health
MetS	Metabolic Syndrome
MI	Myocardial Infarction
MT	Metallothionine
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
NAFLD	Non-Alcoholic Fatty Liver Disease
NHMS	National Health and Morbidity Survey
NSTEMI	Non-Short Term Myocardial Elevation Infarction
OHC	Obese-Hypercholesterolaemic
OS	Oxidative Stress

PAS	Periodic Acid-Schiff
PPSK	Pusat Pengajian Sains Kesihatan
PQ	Plastoquinone
PTCA	Percutaneous Transluminal Coronary Angioplasty
RCA	Right Coronary Artery
REV	Reverse Phase Evaporation Method
ROS	Reactive Oxygen Species
RU	Research University
SBP	Systolic Blood Pressure
$scCO_2$	Supercritical Carbon Dioxide Fluid
SC	Subcutaneous
SD	Sprague Dawley
SEAR	South-East Asia Region
SEM	Scanning Electron Microscope
STEMI	Short Term Myocardial Elevation Infarction
STZ	Streptozotocin
TC	Total Cholesterol
TFA	Trifluoroacetic Acid
TFH	Thin-Film Hydration Method
TNF	Tumour Necrosis Factor

UBD	Universiti Brunei Darussalam
UMK	Universiti Malaysia Kelantan
UMT	Universiti Malaysia Terengganu
UPM	Universiti Putra Malaysia
UQ	Ubiquinone
US	United States
USM	Universiti Sains Malaysia
VLDL	Very Low-Density Lipoprotein
WHO	World Health Organisation

PENILAIAN EKSTRAK STANDARD ANTOSIANIN *Hibiscus sabdariffa* L. YANG DIPERKAYA DENGAN FORMULASI NIOSOM SEBAGAI ANTI-HIPERKOLESTEROL DAN PERLINDUNGAN JANTUNG: KAJIAN RINTIS

ABSTRAK

Hiperkolesterolaemia meningkatkan risiko menghidap penyakit seperti kardiovaskular (CVD), penyakit ginjal kronik (CKD) dan hati. *Hibiscus sabdariffa* L. (Rosel) telah digunakan secara tradisional untuk mengurangkan berat badan dan tahap kolesterol. Rosel terbukti mempunyai kandungan antosianin yang lebih tinggi. Antosianin terbukti mempunyai kesan anti-hiperkolesterol dan perlindungan jantung. Walaubagaimanapun, aplikasi terapeutik Rosel mungkin terhalang disebabkan oleh kekurangannya seperti kadar kestabilan yang rendah, sifat farmakologi antosianin yang rendah dalam ekstrak yang secara tidak langsung menyebabkan pemberian dos terapeutik menjadi tidak realistik. Kajian ini bertujuan untuk menilai kesan ekstrak antosianin yang diperkaya dengan formulasi niosom Rosel (AEHS-Nio) pada tikus yang diaruh hiperkolesterolaemia (HC). Tikus diaruh menjadi HC selama 4 minggu. Terdapat tiga fasa yang telah dirancang dalam kajian ini. Fasa pertama (fasa induksi) bertujuan untuk menjadikan model tikus menjadi HC menggunakan HCD buatan sendiri diikuti dengan fasa kedua (fasa intervensi) akan dilakukan sejurus selepas fasa pertama selesai untuk menilai kesan AEHS-Nio terhadap indeks jisim tubuh (BMI), kadar jumlah kolesterol (TC), dan tekanan darah sistolik (SBP) pada tikus. Akhir sekali, fasa ketiga dimana tikus akan diaruh infaksi miokardium (HC-MI) pada hari ke-29 dan hari ke-30 menggunakan isoprenaline untuk menilai sama ada AEHS-Nio mampu melindungi jantung dengan meningkatkan aspek histologi dan penanda aras biokimia bagi kerosakkan jantung akibat daripada infarksi miokardium. Ekstrak Rosel

dijangka mampu mencegah perkembangan hiperkolesterolemia walaupun cuma terdapat sedikit peningkatan dalam morfologi aorta, ginjal dan hati. Tambahan pula, AEHS-Nio mampu mencegah kerosakkan jantung akibat daripada infarksi miokardium. Secara kolektif, hasil dapatan daripada kajian ini dijangka akan menunjukkan bahawa AEHS-Nio berpotensi untuk digunakan sebagai perawatan dan pencegahan hiperkolesterolemia serta rawatan tambahan sebagai perlindungan jantung.

EVALUATION OF ANTHOCYANIN-RICH STANDARDISED EXTRACT OF *Hibiscus sabdariffa* L. NIOSOMES FORMULATION AS ANTI-HYPERCHOLESTEROL AND CARDIOPROTECTIVE: PILOT STUDY

ABSTRACT

Hypercholesterolaemia increased the risk of developing cardiovascular diseases (CVDs), chronic kidney diseases (CKDs) and liver disorder. *Hibiscus sabdariffa* L. (Roselle) has been used traditionally to reduce weight and cholesterol level. Roselle has been shown to have a higher content of anthocyanin. Anthocyanin has been shown to have anti-hypercholesterol and cardioprotective effect. However, the therapeutic application of Roselle might be impeded by its shortcomings such as low stability, poor pharmacokinetics properties of anthocyanin in the extract causing administration at therapeutic dose unrealistic. The present study aims to evaluate effects of the anthocyanin-rich standardised aqueous extract of Roselle using niosomes formulation (AEHS-Nio) on hypercholesterolaemia (HC) rat. Rats were induced to be HC for 4 weeks. There are three phases planned for this study. The first phase (induction phase) was designed to develop a HC rat model using a self-made HCD diet followed by the second phase (intervention phase) after the first phase to assess the effects of AEHS-Nio on body mass index (BMI), total cholesterol (TC) levels and systolic blood pressure (SBP) in the rat. Lastly, the third phase where the rats will myocardial infarction induced (HC-MI) at days 29 and 30 using isoprenaline to evaluate whether AEHS-Nio able to protect the heart by improving the histological aspect and the biochemical markers of damaged heart secondary to MI. Roselle extracts were expected to improve the preventive impact of hypercholesterolaemia despite minor

improvement in the morphology of aorta, kidney and liver. In addition, AEHS-Nio will also able to prevent the damage to the heart secondary to MI. Collectively, these results were expected to be able to show that AEHS-Nio potentially can be utilised as treatment and prevention of hypercholesterolaemia as well as an adjuvant treatment as the cardioprotective.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Reports by World Health Organisation (WHO) has shown that both developed and developing countries are facing increased deaths (17.9 million) and cardiovascular disease-related disabilities (WHO, 2017). Cardiovascular disease (CVD), particularly myocardial infarction (MI), remains a major concern as it places a significant burden on public health and the economy by causing the highest number of deaths globally.

While considerable efforts have been made over the last decades to enhance the available therapeutic options, conventional therapy does not seem to be effective due to adverse effects, while recurrence tends to occur.

Roselle has been used as a traditional medicine for its health benefits, including atherosclerosis and hypertension (Wahabi et al., 2010; Hopkins et al., 2013). Earlier studies have shown that *Hibiscus sabdariffa* L. reduced cholesterol levels and the development of arteriosclerosis in rats (Rason et al., 2018). In addition, *H. sabdariffa* treatments have been able to restore cardiac function and attenuate decreased fibrosis cardiac hypertrophy as microscopically observed in the post-MI rat model (Si et al., 2017).

Despite its pharmacological properties, the therapeutic application of *H. sabdariffa* may be impeded by its inadequacies, such as low stability, poor aqueous solubility as well as absorption, and low bioavailability of anthocyanin in the extract, which caused unrealistic therapeutic dose administration (Rason et al., 2018). Anthocyanin-rich standardised extract of *Hibiscus sabdariffa* L. niosomes formulation (AEHS-Nio) is

believed to improve solubility and bioavailability before its therapeutic potential can be realised (Gharbavi et al., 2018).

This study will, therefore, use the isoprenaline-induced MI rat model to evaluate the potential of AEHS-Nio as cardioprotective and anti-hypercholesterol agents. Evidence from this study would provide information on the potential of *H. sabdariffa* as a possible adjunctive therapy to reduce post-MI complications in patients with heart disease and high cholesterol.

1.2 PROBLEM STATEMENT

The global prevalence of elevated TC in adults (e.g. 5.0 mmol/L) was 39% in 2008. Overall, the mean TC increased marginally between 1980 and 2008 and decreased by less than 0.1 mmol/L in both men and women per decade. The prevalence of pre-eminent TC was highest in the WHO region of Europe, which is 54% for both genders, followed by the WHO region of the Americas, which is 48% for both genders. The lowest percentages were 22.6% for African Region (AFR) and 29.0% for South-East Asia Region (SEAR) was reported by the WHO AFR and the WHO SEAR in 2015. The prevalence of elevated TC increased significantly based on the country's income status. Around a quarter of the adults had increased TC in low-income countries. This prevalence rose about a third of the adult population in lower middle-income countries. More than 50% of adults have increased overall cholesterol levels in high-income countries and more than double the low-income countries (WHO, 2015).

In the United States (US), more than 12% of adults aged 20 and older had TC higher than 13.32 mmol/L, and more than 18% had high-density lipoprotein (HDL) or "good "cholesterol lower than 2.22 mmol/L in 2015-2018 (Carroll and Fryar, 2020). Just over half of US adults (55% or 43 million) are taking cholesterol medication

(Mercado et al., 2015). Ninety-five million US people aged 20 or older have a total concentration of cholesterol in exceeding of 11.1 mmol/L. Almost 29 million Americans adults have TC levels of 13.32 mmol/L (Benjamin et al., 2017). Seven percent of U.S. children and teenagers between 6 and 19 years of age have elevated overall cholesterol levels (Nguyen, Kit and Carroll, 2015).

Malaysians have acquired heart disease such as MI-specific CVDs at 58 years of age compared to the population of 65 years of age in Thailand, 63 years in mainland China, 66 years in Western countries and 68 years in Canada (Ahmad et al., 2018). Malaysians experience heart disease at a younger age compared to their counterparts in other countries (Ahmad et al., 2018). Furthermore, the National Health Morbidity Survey (NHMS) (2019) has reported that CVDs, particularly strokes and coronary heart disease are the leading causes of death in Malaysia with 1.7 million people Malaysia currently live with three major risk factor such as diabetes (4.1%), high cholesterol (16.6%), and hypertension (9.3%). With that 38.1% from overall prevalence, almost 13.5% Malaysian have been diagnosed with hypercholesterolaemia and 24.6% with raised blood TC among unknown hypercholesterolaemia. Changes are seen from the prevalence in 2011 (35.0%) and 2015 (47.7%) (Institute for Public Health, 2019).

1.3 RATIONALE/JUSTIFICATION FOR STUDY

Hypercholesterolaemia is a global issue, especially in both developing and developed worlds, including Malaysia. The cardiac diseases among Malaysians such as CVD, particularly MI, are caused by hypercholesterolaemia at a younger age (Ahmad et al., 2018).

Although current lipid lowering agents such as Atorvastatin (Lipitor), Fluvastatin (Lescol XL), and Lovastatin (Altoprev) available, these medications are unable to treat the disease completely on its own without changing their lifestyles towards physically active by doing exercises and eating a balanced diet.

The highly accessible dietary trend in both developing and developed countries with a very fast-paced lifestyle has made people choose to consume fast food. This is because of their hectic lifestyle and the food is very much accessible and simply prepared rather than self-cooked food, which is time-consuming and does not work well in today's world. These circumstances lead to higher levels of cholesterol. Extra cholesterol may be concentrated in the arteries. They are blood vessels that carry blood from the heart to the rest of the body. Accumulation of cholesterol is known as an arterial plaque. Plaque can become tough over time, and narrow arteries. Large plaque deposits can completely block the artery. Cholesterol plaques may also break down, leading to the formation of a blood clot that prevents blood flow. When the blood supply is disrupted, it can also affect other vital organs in the human body, such as the aorta, kidneys, and liver (White, 1989). This will result in other serious health complications such as CVD, diabetes, high blood pressure (hypertension), peripheral vascular disease, and stroke.

The development and mass production of chemically synthesised drugs has revolutionised healthcare in most parts of the world over the last 100 years. However, large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for primary treatment (Wachtel-Galor and Benzie, 2011). Natural products and traditional medicines are vital and essential. In some parts of the world, such types of medicine as traditional Chinese medicine, Ayurveda, Kampo, traditional Korean medicine, and Unani have been practiced and evolved into

regulated systems of medicine (Yuan et al., 2016). With a small burst of published research studies, in particular, in the field of dyslipidaemia and hypertension, scientific interest in *H. sabdariffa* in the last few years, has grown. Water extracts of hibiscus flowers have been reported to have a calming effect on the uterus and lower blood pressure over 20 years ago (Hudson, 2011). Preclinical animal studies (Ali et al., 1991; Adegunloye et al., 1996; Onyenekwe et al., 1999; Odigie, Ettarh, and Adigun, 2003) and human models (clinical) have shown that extracts or infusions influence the mechanisms of atherosclerosis, blood sugar, lipids and blood pressure (Chen et al., 2004; Herrera-Arellano et al., 2004). However, despite their pharmacological properties, the research study shows that *H. sabdariffa* used therapeutically may be hindered by their active compounds (anthocyanin) pharmacokinetics weakness, resulting in unrealistic therapeutic dose administration (Rason et al., 2018).

Thus, a new formulation of niosomal with *H. sabdariffa* aqueous extract (AEHS-Nio) in the form of nanoencapsulation will be formulated as it is important to enhance solubility and bioavailability before its therapeutic potential can be realised. Niosomes considered novel drug delivery mechanisms that could improve the solubility and stability of natural pharmaceutical molecules (Gharbavi et al., 2018).

1.4 SIGNIFICANCE OF THE RESEARCH

Approximately 1.5 million cases of MI occur annually in the US, with an annual incidence rate of around 600 cases per 100,000 people. Despite a significant decrease in age-adjusted death rates due to AMI since the mid-1970s, the overall number of MI-related deaths in the US has not decreased (Rogers et al., 2008). In the European Union, the death rate for coronary artery disease (CAD) increased in the early 1990s, followed by a subsequent fall. Cardiovascular mortality remained the

same across the Russian Federation (Levi et al., 2002). The prevalence of CAD and associated mortality is expected to increase significantly in several other developing countries, including India, Latin America, the Middle East, and sub-Saharan Africa, with an estimated 80% increase from about 9 million in 1990 to 20 million by 2020 (Reddy, 2004). According to the World Health Organisation, in 2012 CAD accounted for 98.9 deaths per 100,000 Malaysian people, or 29,400 deaths (20.1% of all deaths), it is the country's most common cause of death. The Malaysian disease burden study conducted in 2000 found that CAD was the largest cause of death with a total of 22,158 deaths or about one-fifth of all deaths. Much information on the disease burden was also obtained from death certificates and hospital admission records in the Ministry of Health (KKM) hospitals where circulatory disease accounted for 6.99% of total hospital admissions and 23.34% of all hospital deaths in 2014 (Seong and John, 2016).

Although current treatment methods, such as statins, are available to overcome elevated cholesterol leading to MI, the choice of drugs or combinations of drugs cannot provide 100% efficacy, as the treatment itself depends on various factors, including personal risk factors, age, health, and possible adverse drug reactions.

Furthermore, the nutraceuticals have been sought out as the alternative for synthetic drugs. A new niosomal formulation of *H. sabdariffa* aqueous extract (AEHS-Nio) nanoencapsulation is formulated as it is vital to regenerate solubility and bioavailability before its therapeutic potential can be realised. Niosomes were considered to be new drug delivery mechanisms that could improve the solubility and stability of natural pharmaceutical molecules (Gharbavi et al., 2018).

1.5 RESEARCH QUESTION

Is the AEHS-Nio capable of protecting the heart and lowering the cholesterol level in HC-MI SD-induced rats?

1.6 OBJECTIVES OF STUDY

1.6.1 General Objective

To evaluate the cardioprotective and anti-hypercholesterol effects of AEHS-Nio in isoprenaline (ISO)-induced MI rats' model.

1.6.2 Specific Objectives

The specific objectives of this study as below:

1. To induce the hypercholesterolaemia-state in rats.
2. To determine the effects of AEHS-Nio on cardiac enzymes parameter (to be achieved).
3. To determine the effects of AEHS-Nio on lipid profiles (to be achieved).
4. To determine the effects on AEHS-Nio on cardiac histology (to be achieved).

1.7 RESEARCH HYPOTHESES

The hypotheses of the study are as follows:

1. AEHS-Nio significantly improves the cardiac enzyme parameter.
2. AEHS-Nio significantly improves the lipid profile.
3. AEHS-Nio significantly reduces the cardiac histology damage.

CHAPTER 2

LITERATURE REVIEW

2.1 CHOLESTEROL

2.1.1 Overview of Cholesterol

Cholesterol is the most common and vital steroid. Steroids are an essential group of terpenes. Steroids perform a number of functions in the cell. There are five classes of hormones in steroids that are androgens, e.g. testosterone (male sex characteristics), oestrogens e.g. oestradiol (female sex characteristics), progastrin includes progesterone (period and pregnancy), glucocorticoid, e.g. cortisol in which lipid, carbohydrate, and protein metabolism are regulated, mineralocorticoid in which potassium ion (K+), sodium ion (Na+) dan chloride ion (Cl-) balance in tissues, and bile acids, for instance, cholic acid that helps in the absorption of fats in the intestines (McEwen et al., 1982).

Steroids belong to one of seven lipid classifications. Lipids are molecules containing hydrocarbons. It is known as the main component of the cell structure and function of living cells. Lipids act as the primary energy storage molecule (triglyceride/triacylglycerol). Lipids are not polar. Polar lipids are essential components of the biological membrane of the lipid bilayer. Lipids also act as fat-soluble vitamins such as A, D, E, and K. Lipids responsible for transporting electrons in chloroplasts and mitochondria (ubiquinone (UQ or CoQ) and plastoquinone (PQ)) (Liu and Lu, 2016). Lipids also act as signal molecules like prostaglandins.

2.1.2 Characteristics of Cholesterol

Cholesterol is a waxy, white-yellow fat that is available in many of the foods we consume and is a key building block in cell membranes. Since blood is water-based, because of the nature of the cholesterol itself, which is an oil-based substance, cholesterol does not mix with it. Cholesterol has three-six rings, called phenanthrene, which are linked to one-five rings, called pentane. It consists of a nucleus of sterane (cyclopentanoperhydrophenanthrene) compounds (Gokula, 2015).

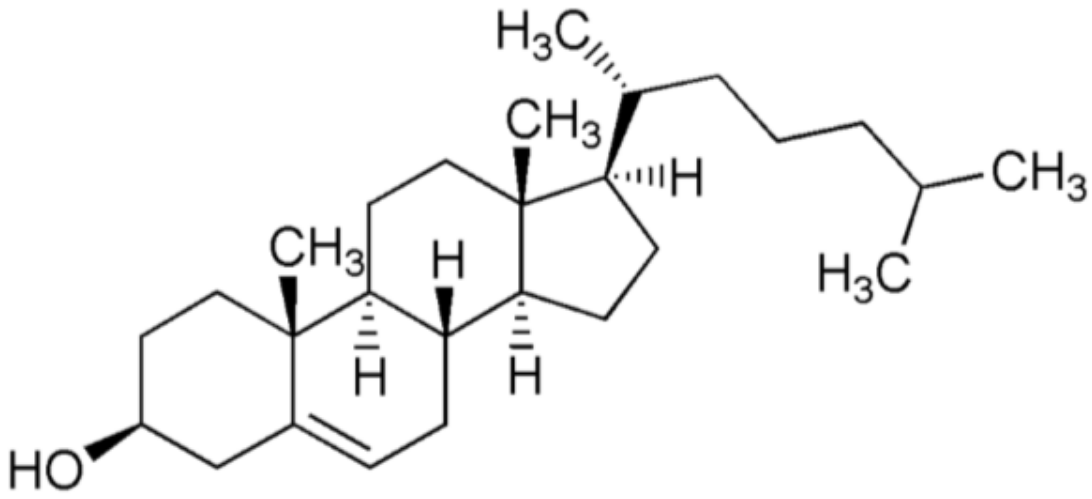

Figure 2.1: Cholesterol 3-6 membered rings (phenanthrene) linked to 1-5 membered rings (pentane).

Adopted from Gokula, (2015).

2.1.3 The Importance of Cholesterol

Cholesterol is not necessarily "bad". The body needs cholesterol to build cells. In order to function normally, the body needs some cholesterol and can make all the cholesterol it needs. Hormones (including testosterone and oestrogen) and vitamin D are made of cholesterol. It has a vital part to play indigestion. Cholesterol development is so essential that the liver and intestines make up 80% of the cholesterol needed to

remain healthy. Only about 20% of cholesterol comes from the food consumed (Corliss, 2019).

2.1.4 Types of Cholesterol

A number of specific types of cholesterol and other lipids circulate and accumulate in the bloodstream. Of these, low-density lipoprotein (LDL) or "bad" cholesterol is the one that has the most coverage. However, lipoproteins come in a variety of types and sizes, and each has its tasks. They also change from one form to another. Four other major types of cholesterol other than LDL include chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and HDL. Chylomicrons are very large particles, which mainly contain triglycerides (fatty acids from food). Chylomicrons produced in the digestive system are also affected by what food is consumed.

Very low-density lipoprotein (VLDL) particles also have triglycerides in their tissues. Yet they are produced by the liver. When the body's cells remove fatty acids from VLDL, the particles turn into IDL and LDL particles with further extraction. Intermediate-density lipoprotein (IDL) particles form VLDL and lose their fatty acids. Some are rapidly absorbed by the liver and others are converted to LDL. Low-density lipoprotein (LDL) particulate matter is also more abundant in pure cholesterol. A lot of the triglycerides they bear are gone. Low-density lipoprotein (LDL) is considered to be "bad" cholesterol because it delivers cholesterol to tissues and is closely correlated with artery-blocking plaque accumulation. High-density lipoprotein (HDL) particles are referred to as "good" cholesterol as some of them extract cholesterol from circulation and artery walls and return it to the liver for excretion (Corliss, 2019; Centers for Disease Control and Prevention (CDC), 2020).

2.1.5 Sources of Cholesterol

Cholesterol comes from two sources. The liver contains all the cholesterol that the body needs. Most of the body's cholesterol comes from animal-derived foods. For example, all foods contain cholesterol, called cholesterol dietary, in meat, poultry, and whole-grain dairy foods. Both animal products contain some cholesterol (American Heart Association (AHA), 2017).

2.1.6 Biosynthesis of Cholesterol

Approximately 80% of the total daily cholesterol production is in the liver and intestines (Corliss, 2019). Including adrenal glands and reproductive organs that are known to have higher synthesis rates at sites. Synthesis within the body begins with the mevalonate pathway in which two molecules of acetyl coenzyme A (CoA) are condensed to form acetoacetyl-CoA. This is followed by a second 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) condensation between acetyl CoA and acetoacetyl-CoA. The enzyme HMG-CoA reductase reduces the molecule to mevalonate. Mevalonate development is a rate-limiting and irreversible step in cholesterol synthesis and a site of action for statins (a class of drugs that lower cholesterol). Through two phases of phosphorylation and one phase of decarboxylation involving adenosine triphosphate (ATP), mevalonate is eventually converted to isopentenyl pyrophosphate (IPP).

Three IPP molecules are condensed by geranyl transferase to form farnesyl pyrophosphate. Two molecules of farnesyl pyrophosphate are then condensed in the endoplasmic reticulum to form squalene by the activity of squalene synthase. The oxidosqualene cyclase then cyclicates squalene to form lanosterol. Finally, through a 19-step process, lanosterol is converted to cholesterol. The final 19 steps to cholesterol

include nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) and oxygen to help oxidise methyl groups to remove carbon. This is followed by alkene group movement mutations and ketone reduction by nicotinamide adenine dinucleotide hydrogen (NADH) (Berg, Tymoczko and Stryer, 2002).

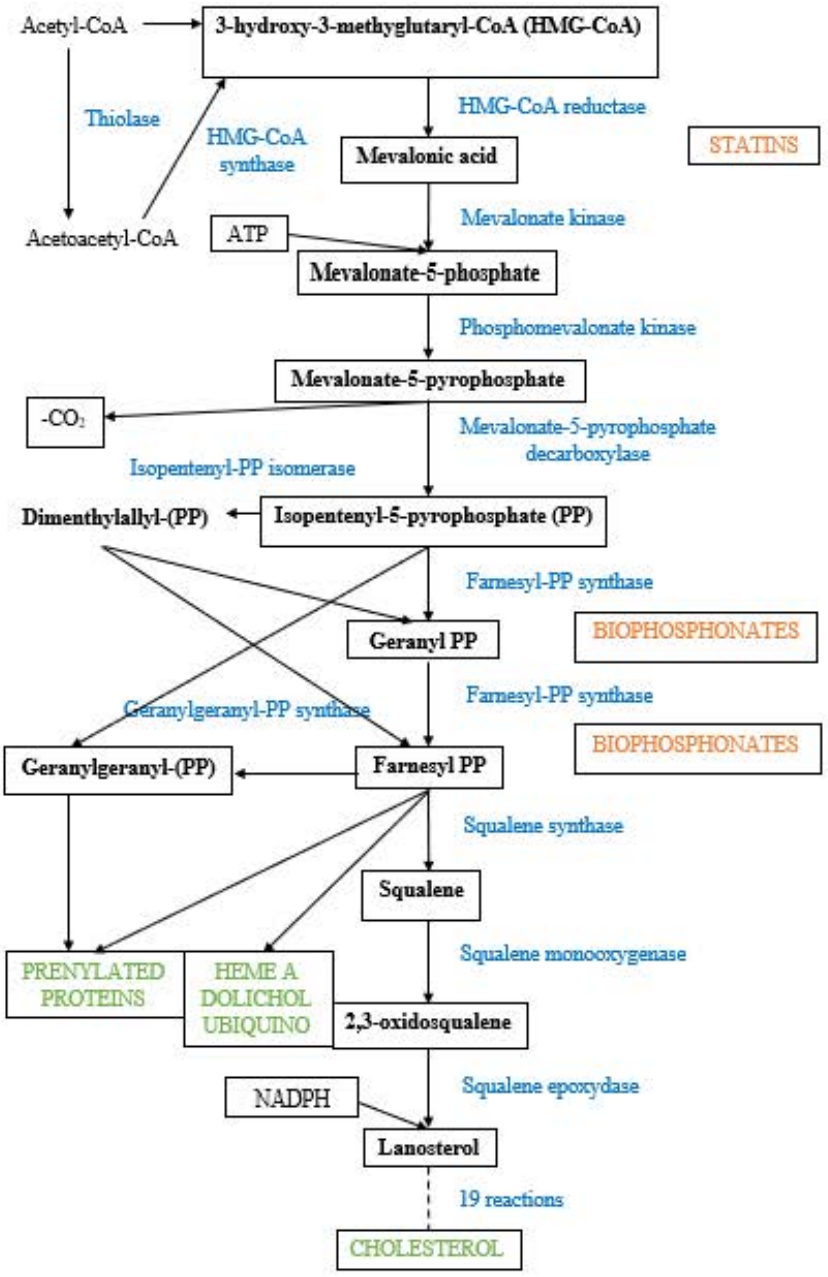

Figure 2.2: Cholesterol biosynthesis pathway.

Adapted from Fakheri and Javitt, (2011).

2.2 HYPERCHOLESTEROLAEMIA

2.2.1 Overview of Hypercholesterolaemia

Hypercholesterolaemia is the production of elevated blood cholesterol levels, also called excessive or high cholesterol. It is a combination of hyperlipidaemia, which is high blood lipids and hyperlipoproteinemia or high blood lipoprotein levels. Hyperlipidaemia is a medical disorder characterised by elevated levels of one or more plasma lipids, including triglycerides, cholesterol, cholesterol esters, phospholipids, and plasma lipoproteins, particularly VLDL and LDL, together with reduced HDL. This increase in plasma lipids is one of the major risk factors associated with CVD (Shattat, 2014). Inherited (genetic) diseases such as LDL hypercholesterolaemia and conditions such as type 2 diabetes as well as underactive thyroid disease, and obesity are result caused by elevated levels of non-HDL and LDL cholesterol (Durrington, 2003).

Cholesterol is one of the three major lipid groups used by all animal cells to create their membranes. Plant cells contain cholesterol in minimal quantities. Steroid receptors and bile acids are precursors as well. Since cholesterol is insoluble in water, it is distributed to the blood plasma inside protein particles called lipoproteins. Lipoproteins are categorised according to their mass as VLDL, LDL, IDL, and HDL (Biggerstaff and Wooten, 2004). All lipoproteins carry cholesterol but high levels of lipoprotein other than HDL, such as non-HDL cholesterol, particularly LDL cholesterol, are associated with an increased risk of atherosclerosis and coronary heart disease (Carmena, Duriez and Fruchart, 2004). Conversely, higher levels of HDL cholesterol are safe (Kontush and Chapman, 2006).

2.2.2 Signs and Symptoms of Hypercholesterolaemia

Despite the fact that hypercholesterolaemia is asymptomatic itself, the long-term elevation of serum cholesterol can lead to the hardening of the arteries, also known as atherosclerosis (Bhatnagar, Soran and Durrington, 2008). Elevated serum cholesterol has been responsible for the development of atheromatous plaques in the arteries for decades (Figure 2.3). It may lead to a gradual narrowing of the arteries involved. Alternatively, smaller plaques may rupture and allow blood flow to form a clot and block it (Finn et al., 2010). Sudden blockage of the coronary artery can cause a heart attack. A blockage of the artery that supplies the brain may result in a stroke. If the development of stenosis or occlusion is incremental, the blood supply to the tissues and organs will gradually diminish until the function of the organ is compromised.

At this point, restrictions on the blood supply of tissue, also known as ischemia, may manifest as particular symptoms. For example, temporary ischaemia of the brain, commonly referred to as a transient ischaemic attack, may manifest as temporary loss of vision, dizziness and impaired balance, difficulty speaking, weakness or numbness, or tingling, typically on one side of the body. Insufficient blood flow to the legs may manifest as calf pain while walking and may present as abdominal pain in the intestines after eating a meal (Grundy et al., 1998; Durrington, 2003).

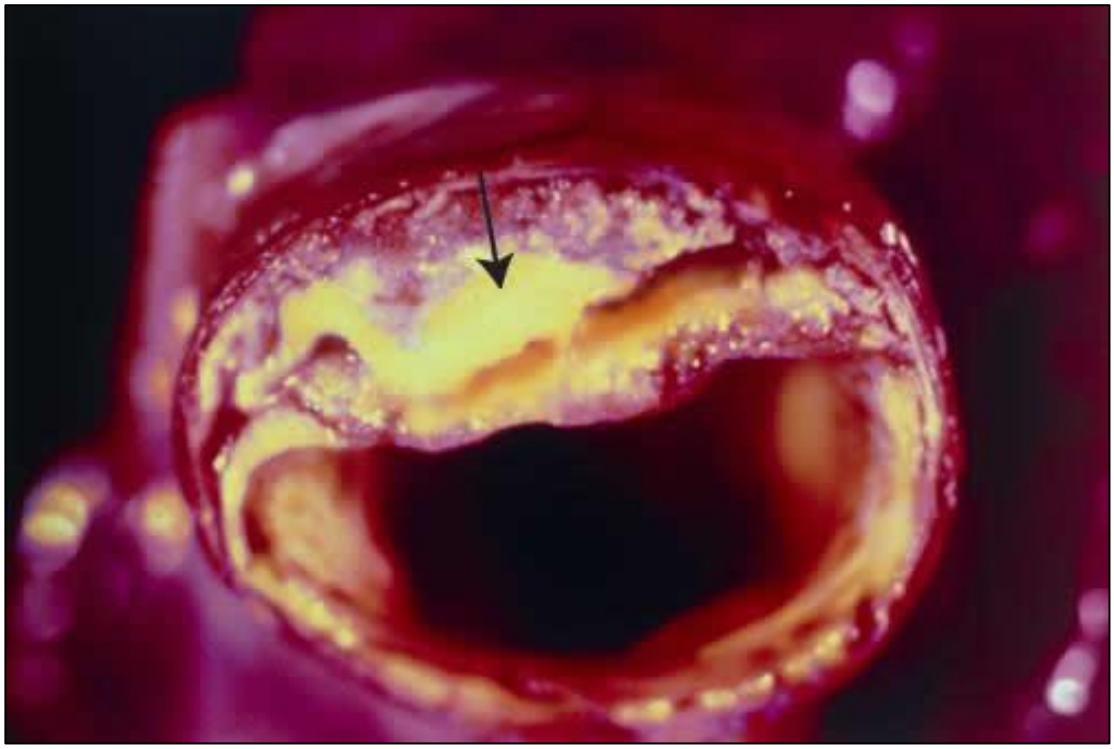

Figure 2.3: This photograph reveals a noticeable atherosclerotic plaque (atheroma; see arrow) in an artery (Thanassoulis and Afshar, 2019).

Many forms of hypercholesterolaemia give rise to unique physical discoveries. For example, xanthelasma palpebrarum (Figure 2.4), which shows yellowish patches under the skin around the eyelids, may be associated with family hypercholesterolaemia (Type 2a hyperlipoproteinaemia) (Shields and Shields, 2015), white or brown, peripheral corneal discolouration known as arcus senilis (Zech and Hoeg, 2008) and yellowish cholesterol-rich tendon deposition, especially of fingers known as arcus senilis. Type 3 hyperlipidaemia, on the other hand, can be correlated with hand, knee, and elbow xanthomata (James, Elston and Treat, 2020).

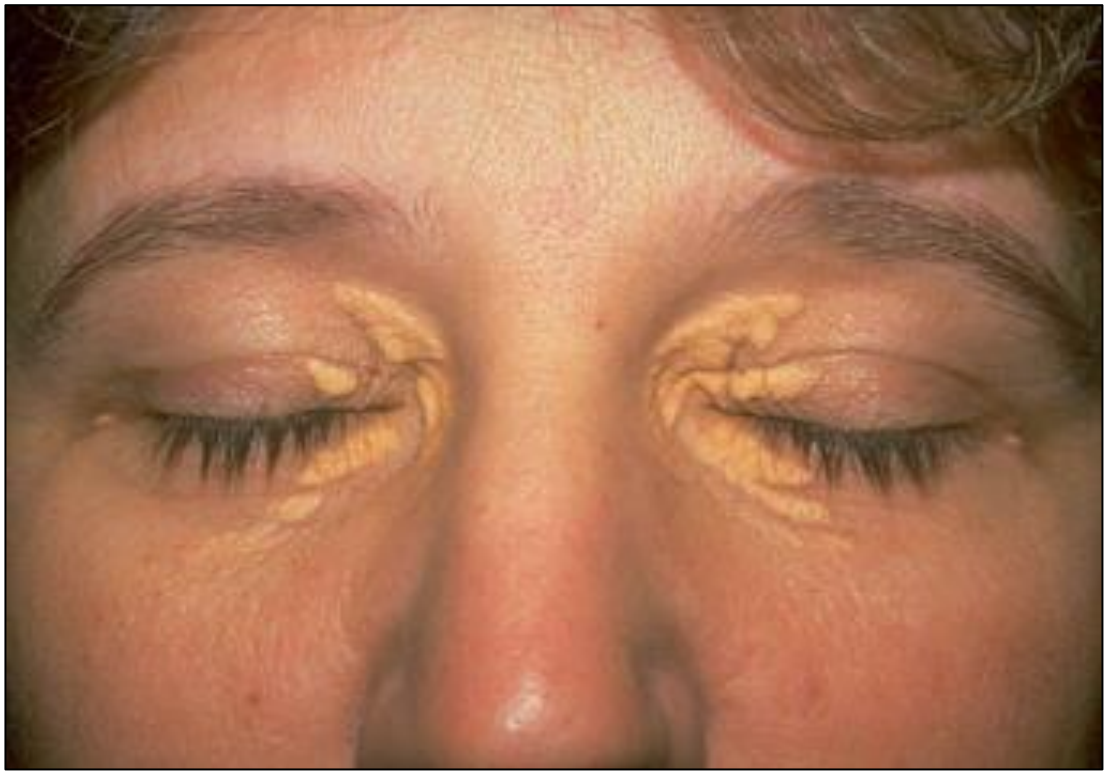

Figure 2.4: Xanthelasma of four eyelids in a patient with hypercholesterolaemia (Nair, 2018).

2.2.3 Causes of Hypercholesterolaemia

Hypercholesterolaemia is typically caused by a combination of environmental and genetic factors. Weight, diet, and stress are environmental factors (Calderon et al., 1999). Some conditions may also increase cholesterol levels, including type 2 diabetes mellitus, alcohol use, anorexia nervosa, Cushing's syndrome, dialysis, hypothyroidism, monoclonal gammopathy, nephrotic syndrome, and obesity. Various drugs or drug groups may interfere with lipid metabolism, such as thiazide diuretics, cyclosporine, glucocorticoids, beta-blockers, retinoic acid, antipsychotics (Bhatnagar, Soran and Durrington, 2008). It is also suggested that some drugs may interfere with lipid metabolism, such as anticonvulsants and human immunodeficiency virus (HIV) drugs, as well as interferons (Herink and Ito, 2000).

2.3 MYOCARDIAL INFARCTION (MI)

2.3.1 Overview of Myocardial Infarction (MI)

Colloquially referred to as a heart attack. Most of MI is due to underlying coronary artery disease (Thygesen, Alpert and White, 2007). With coronary artery occlusion, heart muscle myocardium is deprived of oxygen. Long-term depletion of myocardial oxygen supply may escalate to myocardial cell death and necrosis (Ojha and Dhamoon, 2019). Myocardial ischemia may be associated with changes in the electrocardiogram (ECG) and elevated biochemical markers such as cardiac troponins (Goodman et al., 2006; Apple et al., 2017). If there is evidence of MI, it may be categorised as a short-term (ST) myocardial elevation infarction (STEMI) or a non-ST myocardial elevation infarction (NSTEMI) based on ECG tests (Thygesen et al., 2012). The term "heart attack" is also used to refer to MI in an unspecific manner. The MI is different but may result in cardiac arrest, where the heart does not contract at all or so severely that all vital organs do not function and can, therefore, lead to death (Blumenthal and Margolis, 2007). Approximately 1.5 million MI cases occur annually in the US (Sabatine, 2017).

2.3.2 Signs and Symptoms of Myocardial Infarction (MI)

The most common and important symptom of MI is chest pain, which may or may not radiate to other parts of the body. Other signs, including sweating, may also be followed (Morrow, 2016). Chest pain has been defined most often as tightness, pressure, or pressing of the acute myocardial infarction (AMI). Pain most often radiates to the left arm but may also radiate to the lower jaw, spine, right arm, back, and upper abdomen (Jameson et al., 2018). In some cases, chest pain may be atypical. It's increasing in strength over a few minutes. The pain may begin with exercise or

psychological stress, but most commonly, AMI occurs without apparent precipitation (Malik et al., 2012). The Levine sign, in which a person finds chest pain by tightening one of the two fists over their sternum, has traditionally been seen as a predictor of heart chest pain. The prospective retrospective analysis found, however, that it had a poor predictive value (Marcus et al., 2007).

2.3.3 Mechanism of Myocardial Infarction (MI)

The most important cause of MI is the rupture of an atherosclerotic plaque on an artery that supplies myocardium. Plaques may become brittle, rupture, and thus promote the development of a pulmonary embolism that blocks the artery. This can happen in a matter of minutes. Blocking the artery will result in the delivery of tissue necrosis to the artery in the tissue. Atherosclerotic plaques are often present for decades before symptoms develop (Reed, Rossi and Cannon, 2017). If the reduced blood flow to the heart persists long enough, it causes a cycle called an ischemic cascade, the heart cells in the blocked coronary artery area die (infarction), mainly through necrosis, and do not develop again. A collagen scar is created in their position. Cells lose oxygen when the artery is blocked and ATP needs to be generated in mitochondria. Adenosine triphosphate (ATP) is necessary for the maintenance of the electrolyte balance, in particular through the Na/K ATPase. This leads to an ischemic cascade of infected cells with intracellular modification, necrosis, and apoptosis (Buja, 2005). The most vulnerable to injuries are cells in the area with a weaker blood supply under the inner heart surface (endocardium) (Bolooki and Askari, 2010).

Ischaemia first affects the subendocardial region and tissue begins to die within 15–30 minutes of blood loss. The area of potentially reversible ischaemia is covered by dead tissue, which is a complete thick transmural infarction (Aaronson, 2013). The

initial "wave" infarction may take 3–4 hours (Buja, 2005). The location, size, and severity of the infarction depend on the artery affected, the magnitude of the blockage, the duration of the blockage, the presence of collateral blood vessels, the demand for oxygen, and the effectiveness of interventional procedures (Reed, Rossi and Cannon, 2017). Tissue death and myocardial scarring alter the regular conduction pathways of the heart and weaken the affected areas. Size and position put a person at risk of irregular heart rhythms (arrhythmias) or heart damage, heart ventricle aneurysm, heart wall inflammation following infarction, and heart wall rupture that may have devastating consequences (Kutty, Jones, and Moorjani, 2013). Myocardium damage also occurs during the time of reperfusion (Figure 2.5). The infarct, in this case, is diffusely hemorrhagic. This posterior left ventricular transmural infarction has a rupture track through the middle. The death mechanism had been hemopericardium. This can be manifested as ventricular arrhythmia. Reperfusion damage is the result of the absorption of calcium and sodium from cardiac cells and the release of oxygen radicals during reperfusion. The no-reflow phenomenon also leads to the myocardial death-no-reflow phenomenon, which refers to the fact that the blood is still unable to deliver to the affected myocardium after the artery occlusion has been removed (Kloner, Dai and Hale, 2018).

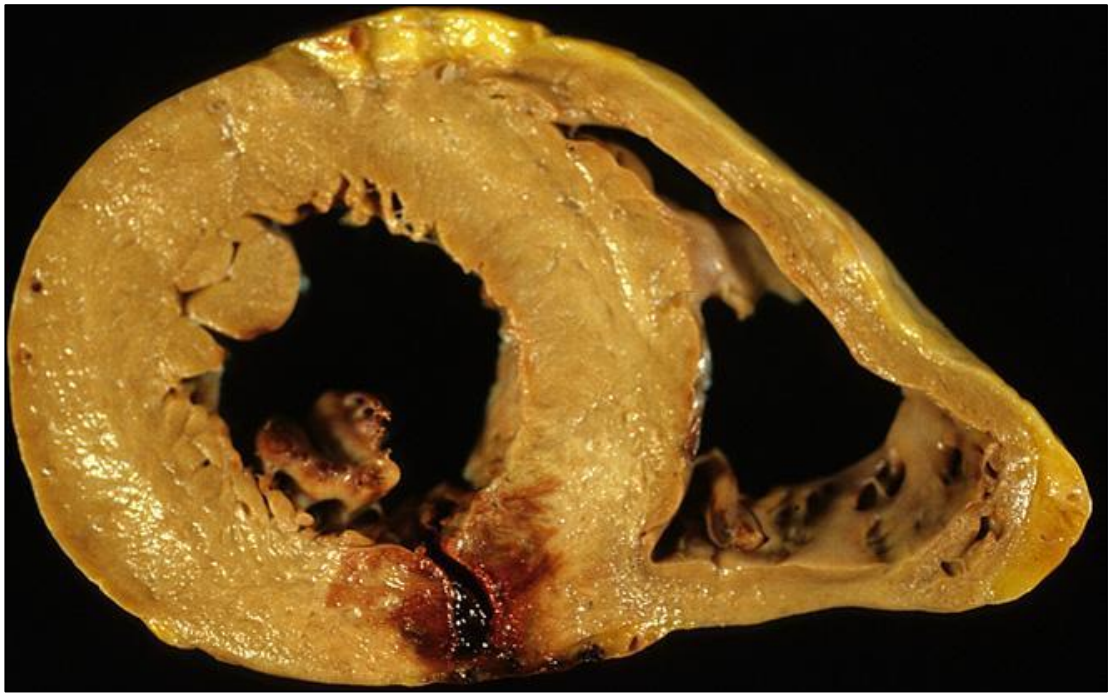

Figure 2.5: Acute infarction of the myocardial, reperfusion type (Sabatine, 2017).

2.3.4 Diagnosis of Myocardial Infarction (MI)

Several conventional biomarkers are used to assess the extent of cardiac muscle damage. Troponins, measured by blood tests, are considered the best (Anderson, 2013) and are preferred because they have a greater sensitivity to measure myocardial injury compared to other tests. Troponin increase occurs within 2-3 hours of injury in the heart muscle and peaks within 1-2 days. Troponin levels, as well as shifts over time, are useful for measuring and diagnosing or eliminating MI, and the diagnostic accuracy of troponin testing is increasing over time (Figure 2.6) (Reed, Rossi and Cannon, 2017). Any high-sensitivity cardiac troponin will rule out a heart attack as long as the ECG is normal (Chapman et al., 2017; Pickering et al., 2017). Other measures, such as creatine kinase myocardial band (CK-MB) or myoglobin, should be discouraged (Amsterdam et al., 2014). Creatine kinase myocardial band (CK-MB) is not as reliable as troponins for acute myocardial damage and may be elevated with previous cardiac surgery, inflammation or electrical cardioversion increases within 4–

8 hours (Table 2.1), and returns to normal within 2–3 days. Copeptin may be useful when used along with troponin to quickly rule out MI (Lipinski et al., 2014).

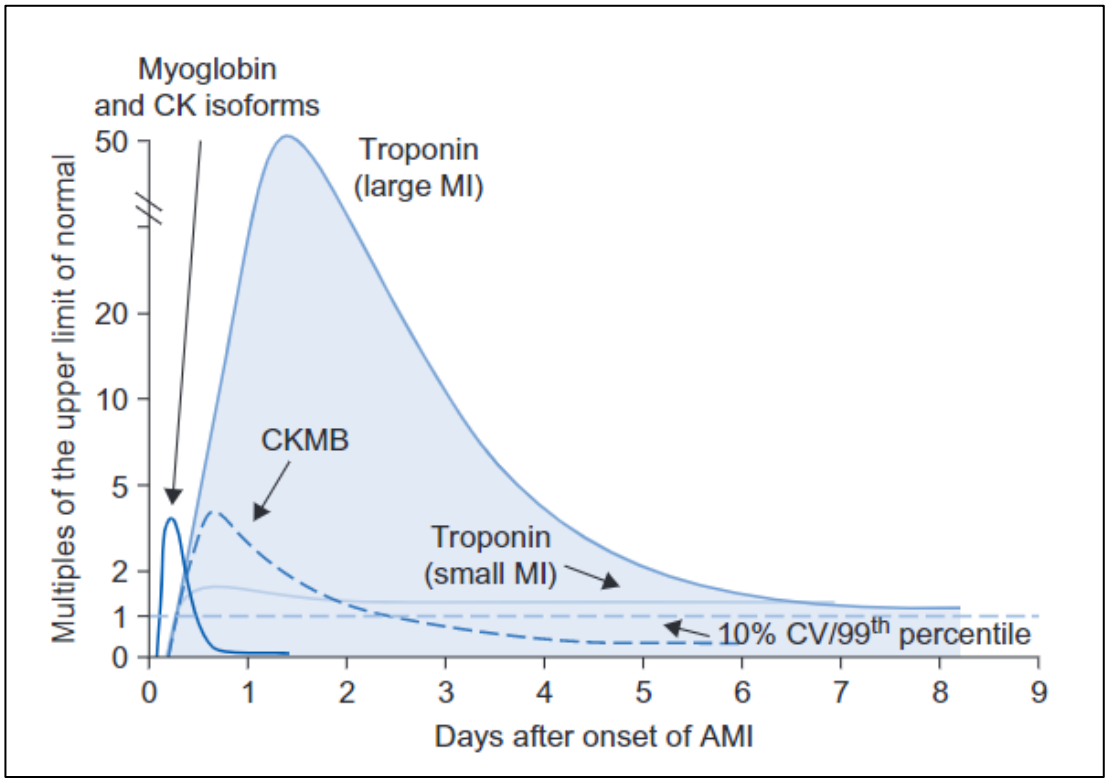

Figure 2.6: Timing of release of various cardiac biomarkers after myocardial injury (Anderson et al., 2007).

Table 2.1: Various established cardiac markers (Anderson et al., 2007).

Cardiac Marker	Increases	Peak	Return to Baseline
Myoglobin	1-4 h	4-12 h	24-36 h
CK-MB	4-9 h	24 h	48-72 h
Troponin I/T	4-9 h	12-24 h	7-14 h

Getting an ECG is a key part of the practice of AMI, and ECGs can be repeated several times over minutes to hours or in response to sign or symptom changes (Thygesen et al., 2012). The ECG reads a waveform element with different labeling features. In addition to an increase in biomarkers, changes in the ST portion, changes in the shape or flipping of the T waves, new Q waves, or a new left bundle branch block may also be used to diagnose the AMI. However, ST elevation may be used to diagnose the ST section of MI (STEMI). ST-elevation is associated with infarction, and changes that suggest ischemia, such as ST depression or T - wave inversion, may precede it. Abnormalities can help to distinguish the position of the infarct based on the leads affected by the changes (Colledge et al. , 2014). T waves can be peaked prior to early STEMIs (Anderson, 2013). Some ECG disorders, such as atrial or ventricular fibrillation, can also be seen with symptoms of AMI (Jameson et al. , 2018).

2.4 STATIN

2.4.1 Overview of Statin

In 1976, the Japanese microbiologist Akira Endo discovered the first statin as a result of the Penicillium citrinum fungus inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A reductase called 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR) activity of this first molecule called compactin (Endo, 2010). Initially, Merck Research Laboratory researchers discovered another HMGCR-derived inhibitor of Aspergillus terrus, originally called mevinolin, and later renamed lovastatin (Alberts et al., 1980). The molecule was the first statin approved by the food and drug administration (FDA). Since then, many drugs from the same family have been synthesised, revolutionising cardiovascular disease control. Statins block the rate-limiting step of cholesterol synthesis in which the HMGCR enzyme converts 3-hydroxy-3-methylglutaryl coenzyme A (HMGC) to mevalonate. However, the biosynthetic pathway of cholesterol also involves the synthesis of dolichols, the development of ubiquinone (coenzyme Q10), and important post-transcriptional modification of proteins in the prenylation process. Note that glycoprotein synthesis includes dolichols (Lennarz, 1975).

Ubiquinone plays a key role in the extraction of energy from muscle cells (Marcoff and Thompson, 2007). In addition, prenylation is needed for the activation of various proteins, including members of the molecular switch family GTPase (a large family of hydrolase enzymes that bind to and hydrolyse the nucleotide guanosine triphosphate (GTP) to guanosine diphosphate (GDP)) such as CDC42, RAC, or RHO (three Ras-related GTP-binding proteins, which regulate actin cytoskeleton assembly and disassembly in response to extracellular signals), which play a crucial role in regulating multiple signaling pathways (Greenwood et al., 2006). Although the key

therapeutic outcome desired with statin administration is lower cholesterol levels, the development of other downstream mevalonate products, which explain the other beneficial and harmful effects of these drugs, has decreased.

2.4.2 Action Mechanism of Statins

The mechanism of action of statins (Figure 2.7) began when statins inhibited HMG-CoA reductase, leading to a decrease in the concentration of cholesterol within the cell. Low intracellular cholesterol induces the synthesis of the LDL receptor. Increased LDL receptor numbers facilitate the absorption of LDLs by the blood. Low intracellular cholesterol decreases the secretion of VLDL (Ward et al., 2019).

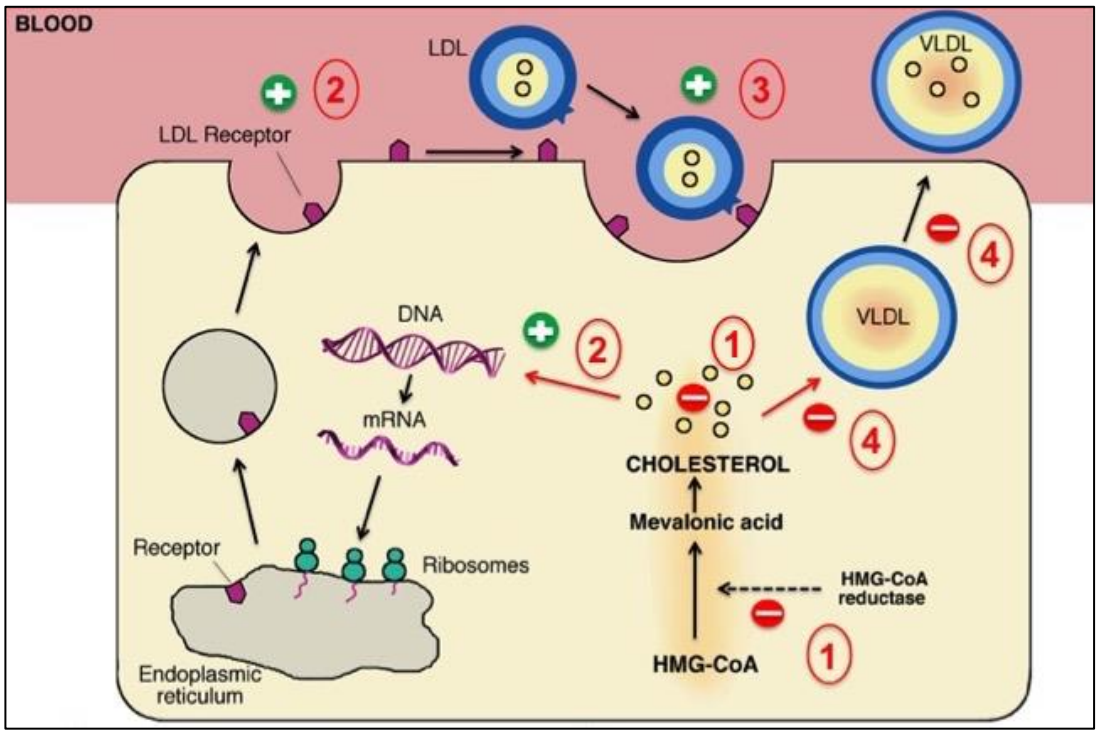

Figure 2.7: Mechanism action of statins (Ward et al., 2019).

2.4.3 Statins as Lipid-Lowering

Statins were significantly more effective in lowering LDL than previous approaches, and statins were found to have beneficial effects from lower cholesterol