EFFECT OF PINEAPPLE ON LIPID METABOLISM IN HIGH CHOLESTEROL DIET-FED RATS

ATICHA NAMWONG

A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science in Physiology
2023

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By Aticha Namwong

has been approved by the Graduate School as partial fulfillment of the requirements for the Master of Science in Physiology of Naresuan University

Oral Defense Committee

..... Chair
(Assistant Professor Worawan Kitphati, Ph.D.)

..... Advisor
(Assistant Professor Wachirawadee Malakul, Ph.D.)

..... Co Advisor
(Assistant Professor Ittipon Phoungpetchara, Ph.D.)

..... Co Advisor
(Assistant Professor Nitirut Nernpermpisooth, Ph.D.)

..... Internal Examiner
(Assistant Professor Sakara Tunsophon, Ph.D.)

Approved

.....
(Associate Professor Krongkarn Chootip, Ph.D.)
Dean of the Graduate School

Title	EFFECT OF PINEAPPLE ON LIPID METABOLISM IN HIGH CHOLESTEROL DIET-FED RATS
Author	Aticha Namwong
Advisor	Assistant Professor Wachirawadee Malakul, Ph.D.
Co-Advisor	Assistant Professor Ittipon Phoungpetchara, Ph.D. Assistant Professor Nitirut Nernpermpisooth, Ph.D.
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ABSTRACT

Excessive consumption of a high cholesterol diet (HCD) leads to the development of hepatic steatosis, and dyslipidaemia. Previous studies have showed that many types of fruit are rich in fibre, vitamins, and phytochemicals, which are able to lower lipid levels in the blood and alleviate hepatic steatosis. Pineapples have been reported to exhibit anti-oxidants, anti-toxicity, anti-obesity, and anti-hepatic steatosis activities. The aim of this study was therefore to investigate the effects of powdered pineapple on hyperlipidaemia and hepatic lipid metabolism in the HCD-fed rats. Male Sprague-Dawley rats were divided into four groups: the control group (C) received normal diet, the high cholesterol diet group (HCD) received HCD, the pineapple group (HCD+P) received HCD plus 200 mg/kg body weight of powdered pineapple, and the simvastatin group (HCD+S) received HCD plus 40 mg/kg body weight of simvastatin. After 8 weeks of experiments, the lipid levels in the blood and livers of the rats were measured. The expression of the proteins involved in cholesterol metabolism was determined in the liver tissues by Western blot analysis. Our results showed that the powdered pineapple reduced body, and liver weights, and liver to body weight ratio in the HCD-fed rats. Pineapple-treated hypercholesterolemic rats exhibited significantly reduced serum TC, TG, LDL-c, AST, and ALT levels, as well as decreased hepatic TC and TG contents compared with the HCD group. Daily consumption of powdered pineapple also down-regulated the expression of HMG-CoA reductase (HMGCR) but upregulated the expression of LDL receptor (LDLR) in the livers of the HCD-fed rats. However, pineapple consumption had no effect on

hepatic CYP7A1 in HCD-fed rats. This study indicates that pineapple consumption ameliorates hyperlipidaemia in hypercholesterolemic rats. This effect is primarily mediated through the inhibition of lipid synthesis via suppressing the expression of HMGCR, and the activation of lipoprotein clearance via up-regulating the expression of LDLR.

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Aticha Namwong

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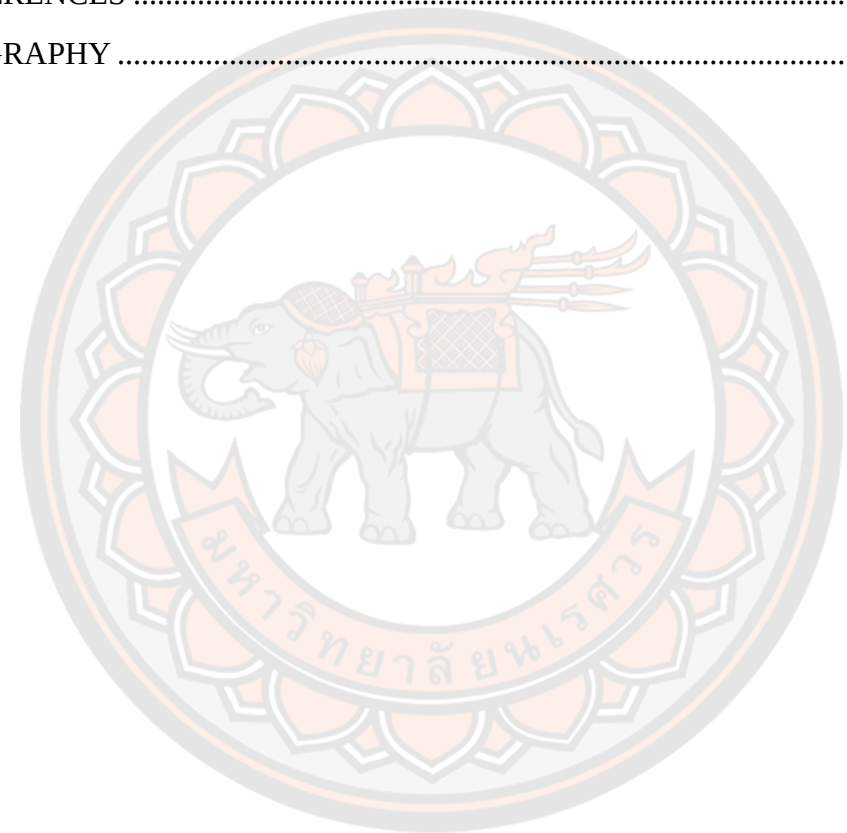

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ABBREVIATIONS

ABCA1 = ATP Binding Cassette Subfamily A Member 1

ABCG1 = ATP Binding Cassette Subfamily G Member 1

ABCG5 = ATP Binding Cassette Subfamily G Member 5

ABCG8 = ATP Binding Cassette Subfamily G Member 8

ACAT = Cholesterol acyltransferase

ACAT2 = Cholesterol acyltransferase 2

ADH = Autosomal dominant hypercholesterolemia

ADP = Adenosine diphosphate

AMPK = AMP-activated protein kinase

ALT = Alanine aminotransferase

Apo = Apoprotein

ARH = Autosomal recessive hypercholesterolemia

ASCVD = Atherosclerotic cardiovascular disease

AST = Aspartate aminotransferase

ATP = Adenosine Triphosphate

a = Hyperlipoproteinemia

CDCA = Chenodeoxychoyl-CoA

CE = Cholesteryl ester

CE'ase = Cholesterol esterase

CPT1 = Carnitine palmitoyltransferase 1

CVDs = Cardiovascular diseases

CYP27A1 = Cytochrome P450 family 27 subfamily A member 1 / Sterol-27-hydroxylase

CYP7A1 = Cytochrome P450 family 7 subfamily A member 1/ Cholesterol 7α-hydroxylase

CYP7B1 = Cytochrome P450 family 7 dubfamily B member 1/ Oxysterol 7α-hydroxylase

CYP8B1 = Cytochrome P450 family 8 subfamily B member 1 / Sterol-12α-hydroxylase

DCA = Deoxycholic acid

ER = Endoplasmic reticulum

FAS = Fatty acid synthase

FC = Free cholesterol

FH = Familial hypercholesterolemia

GCA= Glycocholic acid

HCD = High cholesterol diet

HFD = High fat diet

HDL = High-density lipoprotein

HMGCR = 3-hydroxy-3-methylglutanyl-CoA reductase / HMG-CoA reductase

HMGCS = HMG-CoA synthase

IDL = Intermediate density lipoprotein

LCA = Lithocholic acid

LCAT = Lecithin cholesterol acyl transferase

LDL = Low-density lipoproteins

LDLR = Low-density lipoprotein receptor

LPL = Lipoprotein lipase

LXRA = Liver X receptor α

MTP = Microsomal lipid transfer proteins

NAFLD = Non-alcoholic fatty liver disease

NADPH = Nicotinamide Adenine Dinucleotide Phosphate Hydrogen

NPC1L1 = Niemann–Pick type C1-like 1

PH = Polygenic hypercholesterolemia

PCSK9 = Proprotein convertase subtilisin/kexin type 9

PPAR α = Peroxisome proliferator activated-receptor α

SREBP1 = Sterol regulatory element-binding protein 1

SREBP2 = Sterol regulatory element-binding protein 2

TAG = Triacylglycerol

TC = Total cholesterol

TCA = Taurocholic acid

TF = Transcription factor

TG = Triglycerides

T/GCDCA = Tauro-and glycol-chenodeoxycholic acid

VLDL = Very-low-density lipoproteins

CHAPTER I

INTRODUCTION

Rationale for the study

Hypercholesterolemia is a major risk factor in the development of cardiovascular diseases (CVDs),(Ding et al., 2016; Islam, Hlushchenko, & Pfisterer, 2022) which is the most common cause of morbidity and mortality worldwide.(Bunnoy, Saenphet, Lumyong, Saenphet, & Chomdej, 2015) In 2019, approximately 32% of people worldwide died from CVDs. In addition, hypercholesterolemia has been considered as a major risk factor for strokes, diabetes, obesity, and non-alcoholic fatty liver disease (NAFLD).(Harlow et al., 2018; Shi et al., 2019)

Hypercholesterolaemia is characterised by an increase in low-density lipoproteins (LDL), and total cholesterol (TC) in the blood.(J. H. Wu et al., 2021; D. Yang et al., 2019) Cholesterol plays an important role in the structure of cellular and intracellular membranes of animals and is an important precursor of hormones and bile acid synthesis.(Benito-Vicente et al., 2018) The liver is a primary organ that plays a role in cholesterol homeostasis by regulating its absorption, cholesterol biosynthesis and excretion, and clearance of circulating LDL.(S. Wang, Sheng, Zou, Xiao, & Li, 2021; D. Yang et al., 2019; Yiu et al., 2011) The 3-hydroxy-3-methylglutanyl-CoA reductase (HMGCR) catalyses the conversion of HMG-CoA to mevalonic acid, an essential step for cholesterol biosynthesis. It is well known that HMGCR is a necessary enzyme for the control of cholesterol biosynthesis.(Ding et al., 2016; Hu et al., 2020; K. H. Lee et al., 2020) In cholesterol catabolism and bile acid synthesis, cholesterol 7α-hydroxylase (CYP7A1) is a rate-controlling enzyme for regulated bile acid synthesis. The CPY7A1 first catalyses the conversion of cholesterol to 7α-hydroxycholesterol, which is the primary substance for the conversion of primary bile acid.(Chen et al., 2019; X. Li et al., 2020; Zhu et al., 2017) In addition, the low-density lipoprotein receptor (LDLR) is an important receptor that removes cholesterol and LDL into the blood, thus important process for maintain cholesterol homeostasis

in the body.(Feingold, 2000; Kassaee, Goodarzi, & Kassaee, 2021) Previous studies reported that the excessive consumption of a high cholesterol diet (HCD) increased the protein expression levels of HMGCR (Islam et al., 2022; J. K. Lee, J. J. Lee, Y. K. Kim, Y. Lee, & J. H. Ha, 2020; Meneses et al., 2016) and CYP7A,(Shi et al., 2019; Zhu et al., 2017) and suppressed the LDLR expression in the liver of the HCD-fed rats,(Farahnak, Magri Tomaz, Bergeron, Chapados, & Lavoie, 2017; J. H. Wu et al., 2021; Yida, Al-Shuwayah, Ismail, & Imam, 2022) which led to impaired accumulation and metabolism of lipids and resulted in the development of hepatic steatosis and hypercholesterolaemia.(Chen et al., 2019; Meneses et al., 2016; Shi et al., 2019) Therefore, the modulation of hepatic cholesterol metabolism, may alleviate hepatic steatosis and hypercholesterolaemia in the HCD-fed rats.(Ibrahim et al., 2020; K. H. Lee et al., 2020; Shi et al., 2019) Statins are a common drug used to reduce cholesterol biosynthesis in patients with hypercholesterolaemia,(Sinaga et al., 2021) however, it has been reported that this drug is associated with several serious side effects.(Irvine, 2020; Won, Goh, & Hwang, 2020) Therefore, natural products exhibited cholesterol-lowering properties are of interest for this study. Fruits are valuable sources of dietary fibre and phytochemicals, which have many health effects as anti-oxidants and lipid-lowering effects.(Castro-Barquero, Lamuela-Raventós, Doménech, & Estruch, 2018; Dabeek & Marra, 2019; Mahmoudzadeh et al., 2022; Riaz et al., 2022)

Pineapples *(Ananas comosus (L) Merr.)* are widely grown in tropical and subtropical countries such as USA, Kenya, Malaysia, Philippines, South Africa, India, China, and Thailand. They contain a rich source of calcium, potassium, vitamin C, vitamin A, flavonoids, and phenolic compounds.(El-Shazly et al., 2018; Mohd Ali, Hashim, Abd Aziz, & Lasekan, 2020) Several studies showed that pineapples possess anti-obesity, anti-oxidants, and hepatoprotective properties.(El-Shazly et al., 2018; Mohd Ali et al., 2020; Yantih, Harahap, Sumaryono, Setiabudy, & Rahayu, 2017) Previous in vitro studies reported that pineapple is able to inhibit the activity of cholesterol absorption, pancreatic lipase, and the solubility of cholesterol in lipid micelles, which led to a reduction in lipid digestion in the small intestine, and serum lipid levels.(Duangjai, Ingkaninan, & Limpeanchob, 2011; Kanittaporn Trisat, 2016) A previous in vivo study reported that pineapple suppressed de novo lipogenesis in

the liver tissues via down-regulating the mRNA expression levels of sterol regulatory element-binding protein 1 (SREBP-1), and fatty acid synthase (FAS) in the liver tissues of the HFD-fed rats. In addition, the pineapple was reported to reduce the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and triglycerides (TG). (El-Shazly et al., 2018)

However, the effects of powdered pineapple on lowering cholesterol levels in the blood of the HCD-fed rats, and its underlying mechanisms are unclear. The aim of this study was to investigate the effects of powdered pineapple was able to be lowering cholesterol levels in the blood of HCD-fed rats, and to explain its underlying mechanisms of cholesterol biosynthesis and excretion, and LDL clearance in the liver of HCD-induced hypercholesterolaemia.

Purposes of the study

This study was conducted to assess the effects of orally administered powdered pineapple (8 weeks) on the metabolism of cholesterol in the rat model of hypercholesterolemia, which was induced by adding 1.5% cholesterol to their standard diet, with the following objectives.

1. To investigate the effect of powdered pineapple on the serum and hepatic lipid levels in the HCD-fed rats.
2. To investigate the effect of powered pineapple on the hepatic expression of proteins involved in the metabolism of cholesterol in the HCD-fed rats.

Expected outcome of the study

1. The results of this study were obtained by the efficacy of the effects of the powered pineapple on the serum and hepatic lipid levels and accumulations in the HCD-induced hypercholesterolaemic rats.
2. The information from this study regarding the beneficial effects of the powered pineapple, provided useful evidence that was invaluable for the development of the therapy to treat hypercholesterolemia.

Hypothesis

Administration of the powdered pineapple may attenuate the serum and hepatic lipid levels in the HCD induced hypercholesterolaemia, by reducing the protein expression level of the HMGCR, and increasing the protein expression levels of both LDLR, and CYP7A1 in the liver tissues.

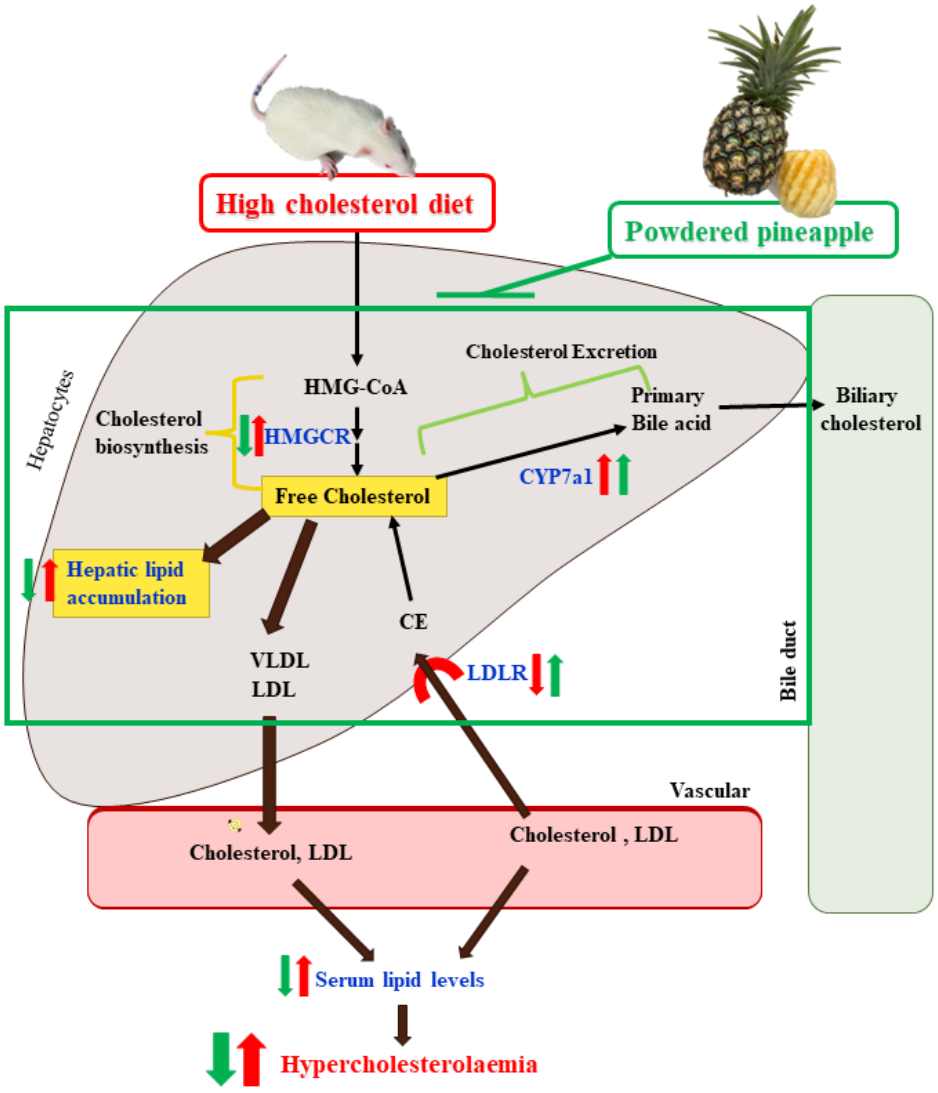


Figure 1 The hypothesis of this study.

CHAPTHER II

LITERATURE REVIEW

Liver

Anatomy of the liver

Gross anatomy of human liver

The liver is the second largest organ in the body, and weighs approximately 2-3% of the average body weight. The liver is located in the abdominal capacity, inferior to the diaphragm, right of the stomach, and superior to the intestine and the gallbladder. The liver has a general shape of a prism or wedge, and is a pinkish-brown colour. The liver is divided into two major lobes, which consists of the large right lobe and the small left lobe.

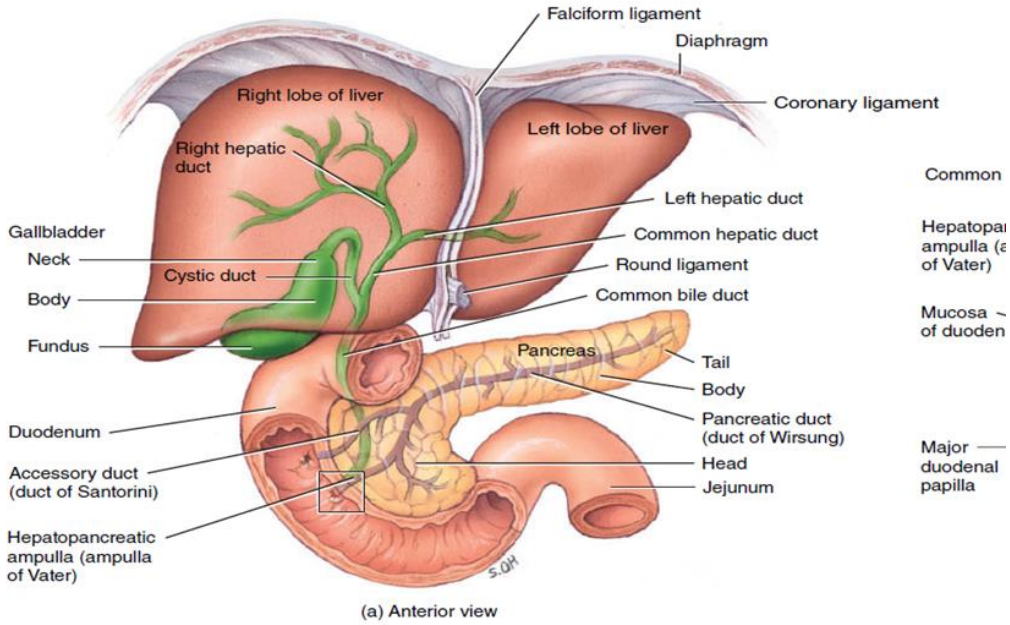


Figure 2 Gross anatomy of the human liver.

Source: (Gerard J. Tortora, 2016), p.920

Gross anatomy of a rat liver

A rat's liver has a similar function and structure as a human one, and is the second largest organ in its body, which weighs approximately 6 % of the average body weight, and its colour is dark red. The liver is located in the abdominal capacity,

inferior to the sub-diaphragm region, and superior to the intestine which is in the largest area of the abdominal cavity. The liver is divided into four major lobes that consist of the right (hemisected), the medial, the left, and the caudate lobes. The gallbladder is located below the central isthmus of the medial lobe when viewed from the ventral perspective. (Piper M. Treuting, 2017)

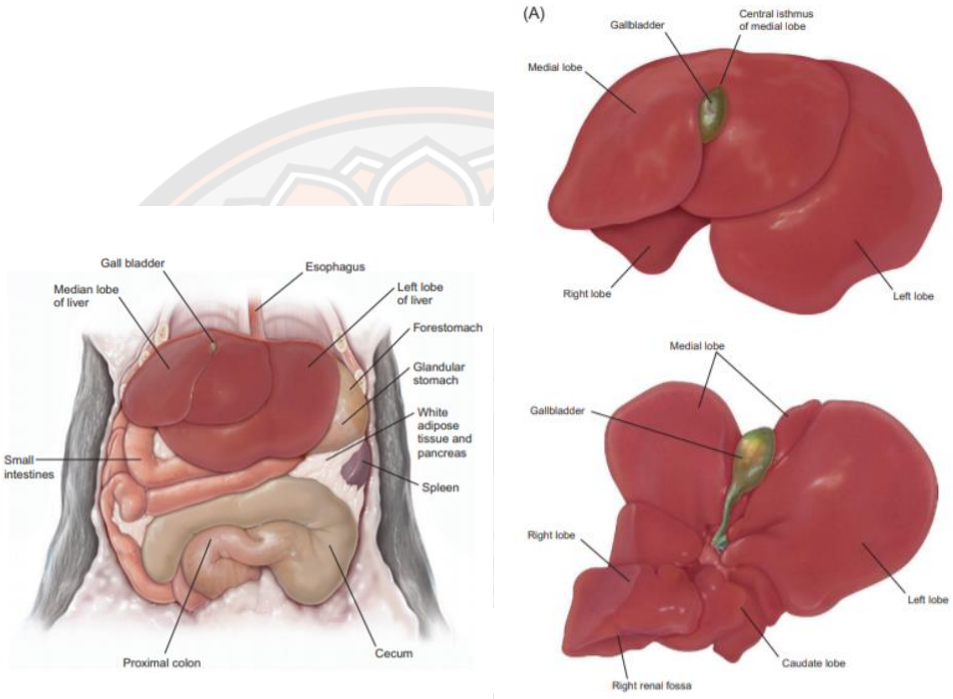


Figure 3 Gross anatomy of the rat liver.

Source: (Piper M. Treuting, 2017), p. 195-196

Microanatomy of the liver

In the liver, the hepatic lobule may be identified primarily by its two-dimensional hexagon appearance when observed under a light microscope. Hepatic lobule consists of the peripheral portal tracts in the periphery and the centrilobular vein in the centre. Each lobule consists of hepatocytes, and hepatic sinusoids. The portal tracts or portal triads contain branches of the hepatic artery, the hepatic portal vein, and the bile duct. A layer of hepatocytes surrounds the portal tracts (Florentina Radu-Ionita, 2020; Gary C. Kanel M.D., 2017; Mills, 2019) The cytotypes within the liver are composed of several components which include hepatocytes, endothelial cells, and Kupffer cells.

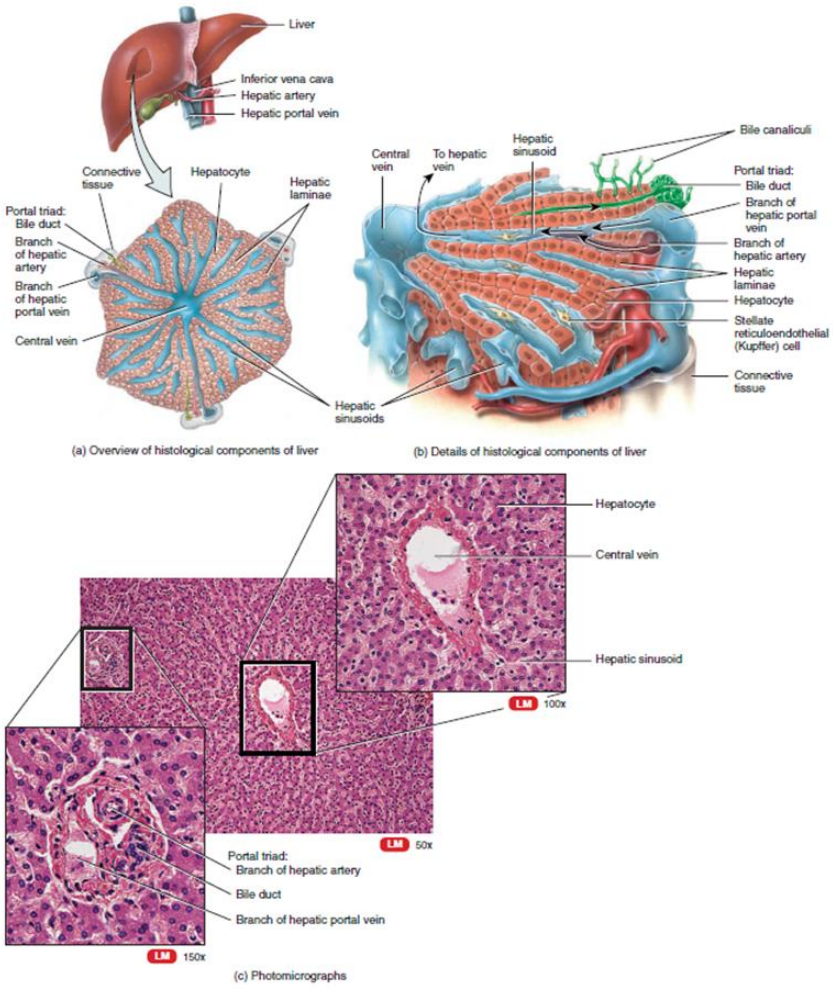


Figure 4 Micro anatomy of the liver.

Source: (Gerard J. Tortora, 2016), p.923

Hepatocytes

The Hepatocyte is a polygonal epithelial cell which comprises about 80 % of the volume of the liver, and is also a major functional cell of it. The hepatocyte has three distinct cell boundaries which are (1) the sinusoidal (basolateral) surface area consists of microvilli that extend into the perisinusoidal spaces for secretory and absorptive functions, (2) the lateral (intercellular) membrane lies between two adjacent hepatocytes that occur in the gaps between the cells, and transports metabolites and (3) the canalicular membranes lie next to the microvilli and secretes bile acid. (Florentina Radu-Ionita, 2020; Gary C. Kanel M.D., 2017)

Endothelial cells

Flattened endothelial cells form the sinusoidal wall which contains numerous cytoplasmic projections and clustered fenestrae. The function of fenestrae is a filtration barrier. The major function of the sinusoidal wall is to filtrate macromolecules from the sinusoidal blood. (Florentina Radu-Ionita, 2020)

Kupffer cells

Kupffer cells are sinusoid lining cells that function as hepatocyte macrophages. The cells are star-shaped, which are stellate cells and contain lysosomes in their cytoplasm. The primary function is related to phagocytosis and clearance of particulate material. (Florentina Radu-Ionita, 2020)

Function of the liver

The liver is responsible for many functions, which are the secretion and synthesis of bile acid, metabolism of food, detoxification of drugs, hormones, and toxic substances, the storage of vitamins and minerals, the inactivation of hormones, vascular and hematologic functions, and the metabolism of bilirubin. (Gerard J. Tortora, 2016)

Table 1 The function of the liver

Secretion and synthesis of bile acid
Metabolism of nutrition o Fats - Fatty acid β-oxidation, synthesis of cholesterol, triglyceride and lipoprotein, production of ketoacids, and conversion of cholesterol into bile acid. o Carbohydrates – Conversion of galactose and fructose into glucose, conversion of glucose into glycogen, gluconeogenesis, and release the glucose into the blood circulation o Proteins – Synthesis of plasma proteins (alpha and beta globulins, albumin, prothrombin, and fibrinogen), removes the nitrogenous portion from amino acids, and breaks down nucleic acids.
Detoxification of drug, hormones, and toxic substances o Phase I (oxidation, reduction and hydrolysis) o Phase II (conjugation/ cytochrome P450 system)

Storage of vitamins and minerals o Fat-soluble vitamins: vitamin A, D, E, K o Water-soluble vitamins: vitamin B12. o Iron o Copper
Inactive of hormones o Insulin o Glucagon o Cortisol o Aldosterone o Thyroid o Sex hormones.
Vascular and hematologic functions o Important blood reservoir
Metabolism of bilirubin
Phagocytosis o Phagocytize aged red blood cells

Lipids

Lipids are divided into cholesterol, fatty acids, triglyceride, eicosanoid, phospholipids, sphingolipids, and fat-soluble vitamins (A, D, E and K), and their functions are summerized in Table 2.(Lindsay H Allen, 2013; Ronald Watson, 2016)

Table 2 The function of lipids.

Cholesterol o The component of membrane structure o Precursor of bile acid and steroid synthesis
Fatty acids o Source of energy
Triglycerides o Energy store
Eicosanoid o Blood coagulation

○ Bronchial and vascular contractility
Phospholipids ○ The component of membrane structure
Sphingolipids ○ The components of the neuronal membranes ○ The antigenic determinants on the surfaces of the erythrocytes
Fat-soluble vitamins ○ Vitamin A - Vision ○ Vitamin D - Calcium homoeostasis and maintenance of bone integrity ○ Vitamin E - Neural function and antioxidant ○ Vitamin K - Activation of clotting factors

Cholesterol

Cholesterol is a major sterol that is present in both animal and human tissues. The cholesterol structure is $C_{27}H_{46}O$, which consists of four fused carbon rings that form the steroid nucleus, and a total of twenty-seven carbon atoms. The hydroxyl (OH) group is attached to the first ring, the hydrocarbon side chain to the fourth ring,(Cerqueira et al., 2016) and the double bond between the carbon atoms fifth and sixth are an amphipathic lipid, as shown in Figure 1. Cholesterol is an important component of the cell membrane and the precursor for the synthesis of steroid hormones, bile acids and vitamin D.(Cerqueira et al., 2016) The crucial functions of cholesterol in diverse physiological contexts, can cause cardiovascular diseases and many others, when its metabolism is disrupted.

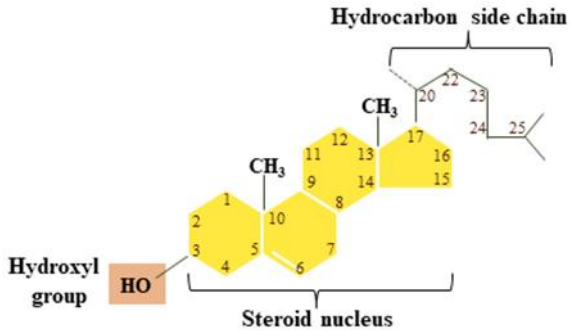
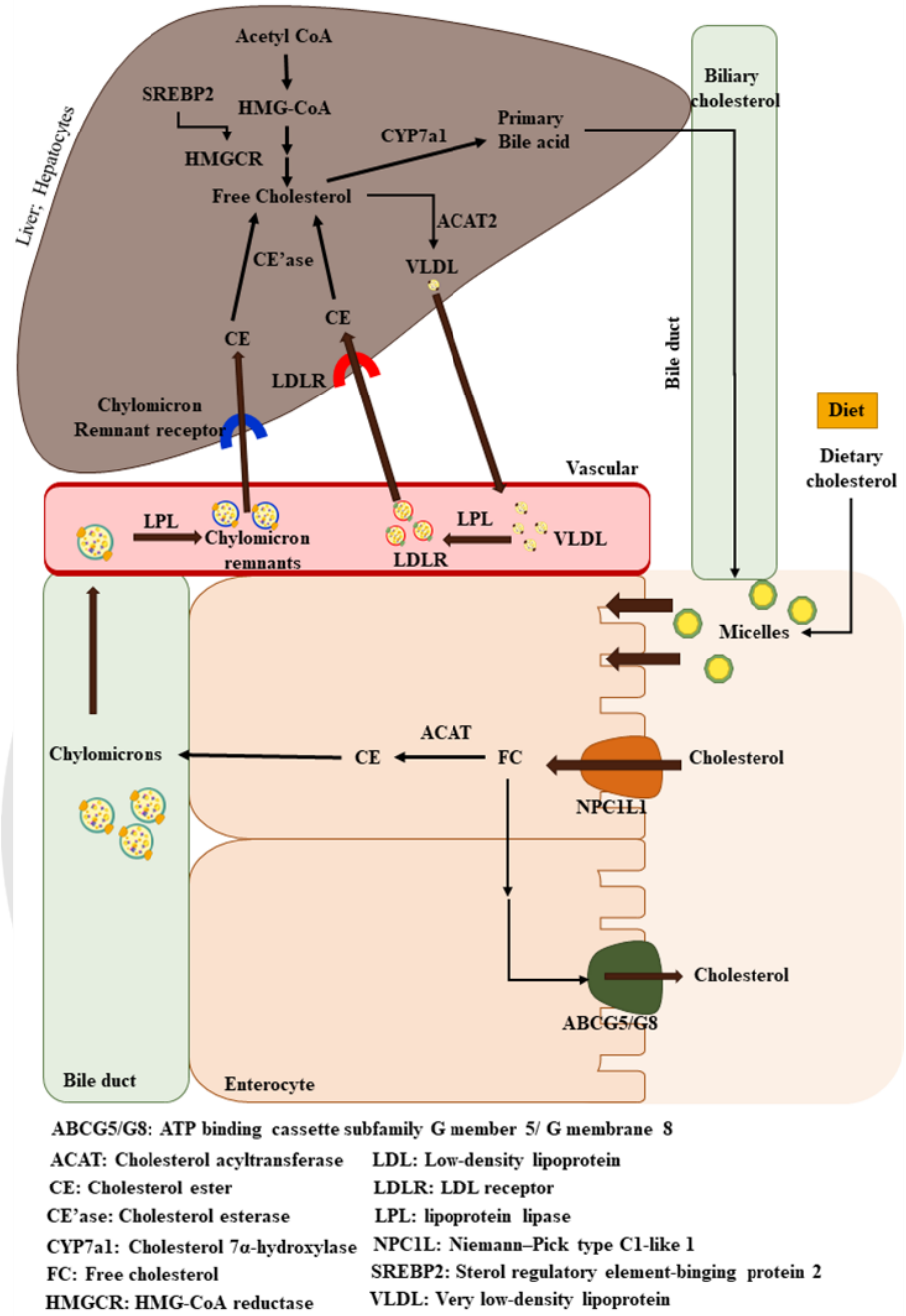


Figure 5 The cholesterol structure.

Source: (Rodwell, 2015)

Cholesterol absorption

The diet is presented in the form of cholesterol ester, which is hydrolysed to free cholesterol and fatty acids by the act of cholesterol esterase and phospholipase A2. The free cholesterol is incorporated into molecular aggregates that consist of glycerol, long-chain free fatty acids, phospholipids and lysophospholipids to form mixed micelle or bile salt micelle, which is then absorbed into the mucosal cells of the small intestine.(McLeod, 2016) The cholesterol is also absorbed into the enterocyte of the small intestine by the Niemann–Pick type C1-like 1 (NPC1L1) protein transporter, and located on the surface of the enterocyte. (Afonso et al., 2018; Rodwell, 2015) The cholesterol is re-esterified and metabolised into chylomicrons in the enterocyte of the small intestine, then they enter the lacteal lymphatic vessel, and are transported into the bloodstream. Lipoprotein lipases break down the chylomicrons which are converted to the chylomicron remnants, and then transported into the hepatocytes by the low-density lipoprotein receptor (LDLR).(Luo, Yang, & Song, 2020; McLeod, 2016) Cholesterol ester from chylomicron remnant converts to cholesterol by cholesterol esterase (CE'ase), which then generates free cholesterol that is used as a precursor for bile acid synthesis,(McLeod, 2016) or it could be converted back to cholesterol ester by cholesterol acyltransferase 2 (ACAT2) for the formation of very-low-density lipoproteins (VLDL). Cholesterol is secreted into the bloodstream by converting to VLDL,(Rudel & Shelness, 2000) as shown in Figure 6.



ABCG5/G8: ATP binding cassette subfamily G member 5/ G membrane 8
ACAT: Cholesterol acyltransferase
CE: Cholesterol ester
CE'ase: Cholesterol esterase
CYP7a1: Cholesterol 7α-hydroxylase
FC: Free cholesterol
HMGCR: HMG-CoA reductase
LDL: Low-density lipoprotein
LDLR: LDL receptor
LPL: lipoprotein lipase
NPC1L: Niemann–Pick type C1-like 1
SREBP2: Sterol regulatory element-binging protein 2
VLDL: Very low-density lipoprotein

Figure 6 The metabolism of cholesterol.

Source: Modified from (Carotti et al., 2020; Nurmohamed, Navar, & Kastelein, 2021; Rodwell, 2015; H. H. Wang, Garruti, Liu, Portincasa, & Wang, 2017)

Cholesterol biosynthesis

Approximately 50% of the time, cholesterol biosynthesis occurs in the hepatocytes of animals and humans.(Luo et al., 2020) The acetyl-CoA is a precursor of the cholesterol biosynthesis, which is a process that occurs in the endoplasmic

reticulum (ER) and the cytosol. The cholesterol biosynthesis is divided into five steps: 1) the mevalonate synthesis from the acetyl-CoA; 2) the mevalonate converts to the formation of the isoprenoid units; 3) the condensation of the isoprenoid units converts to squalene; 4) the cyclization of squalene converts to the lanosterol; and 5) the formation of cholesterol from the lanosterol,(Rodwell, 2015) as shown in Figure 7.

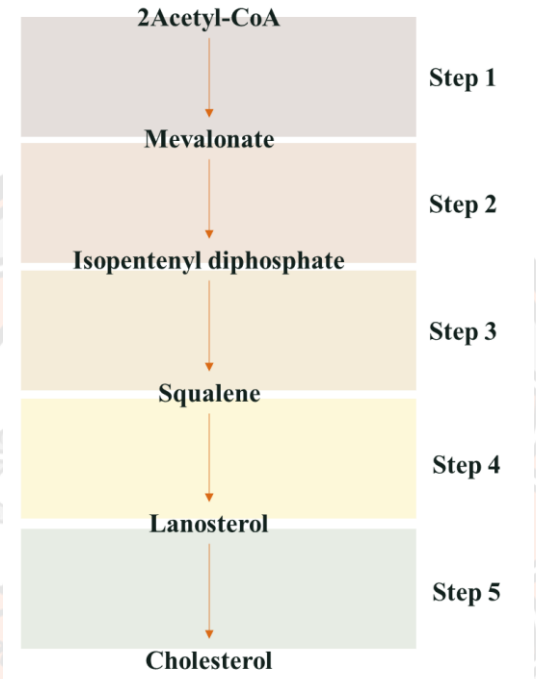


Figure 7 Total 5 steps of cholesterol biosynthesis.

Step 1: The mevalonate synthesis from the acetyl-CoA:

The formation of the acetoacetyl-CoA occurs from the condensation of two molecules of the acetyl-CoA catalysed by the cytosolic thiolase. Then, a molecule of the acetoacetyl-CoA condenses with a molecule of the acetyl-CoA, which is then converted to HMG-CoA using an HMG-CoA synthase catalyst. The HMG-CoA is covert to mevalonate by the HMG-CoA reductase catalyst and the NADPH reaction. The conversion of HMG-CoA to mevalonate is a crucial step in regulating the cholesterol biosynthesis pathway.(McLeod, 2016; Rodwell, 2015) This enzyme reaction is a rate determining step of the cholesterol biosynthesis pathway, as shown in Figure 8. This last step of the mevalonate synthesis from acetyl-CoA, is the most important for effectively lowering the cholesterol drug known as the HMG-CoA reductase inhibitor.(McLeod, 2016; Rodwell, 2015)

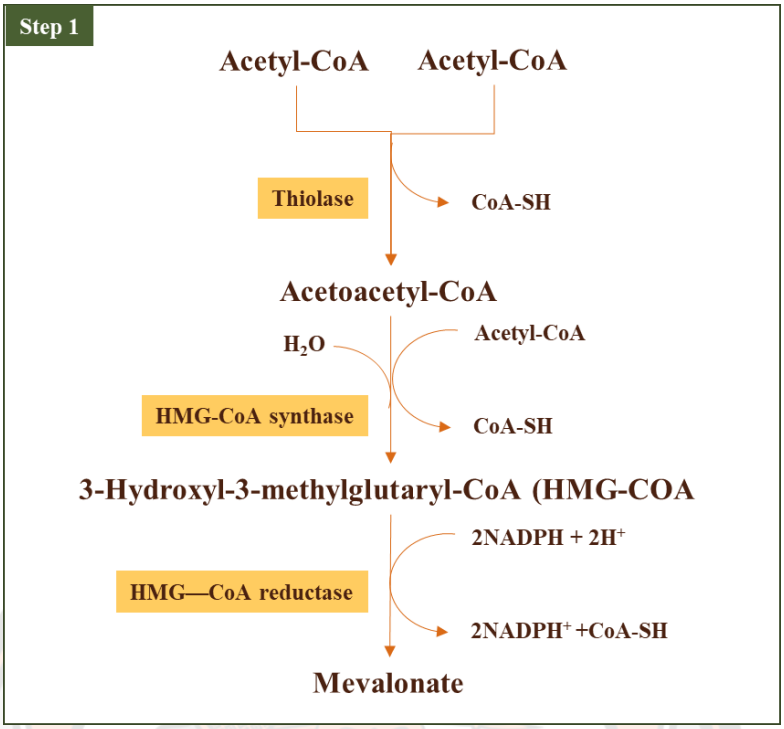


Figure 8 The mevalonate synthesis from acetyl-CoA.

Source: (Rodwell, 2015)

Step 2: the mevalonate converts to the formation of the isoprenoid units:

The mevalonate was phosphorylated using ATP by catalyzing mevalonate kinase and converting it to mevalonate 5-phosphate. This is then converted to mevalonate 5-diphosphate and mevalonate 3-phospho-5-diphosphate by phosphorylation, using ATP and the catalyst of phosphomevalonate kinase and di-phosphomevalonate kinase, respectively. The final step is the mevalonate 3-phoapho-5-diphophate is converted to isopentenyl diphosphate using the catalyst of diphospho-mevaloante decarboxylase,(Biochemical Pathways An Atlas of Biochemistry and Molecular Biology 2012) as shown in Figure 9.

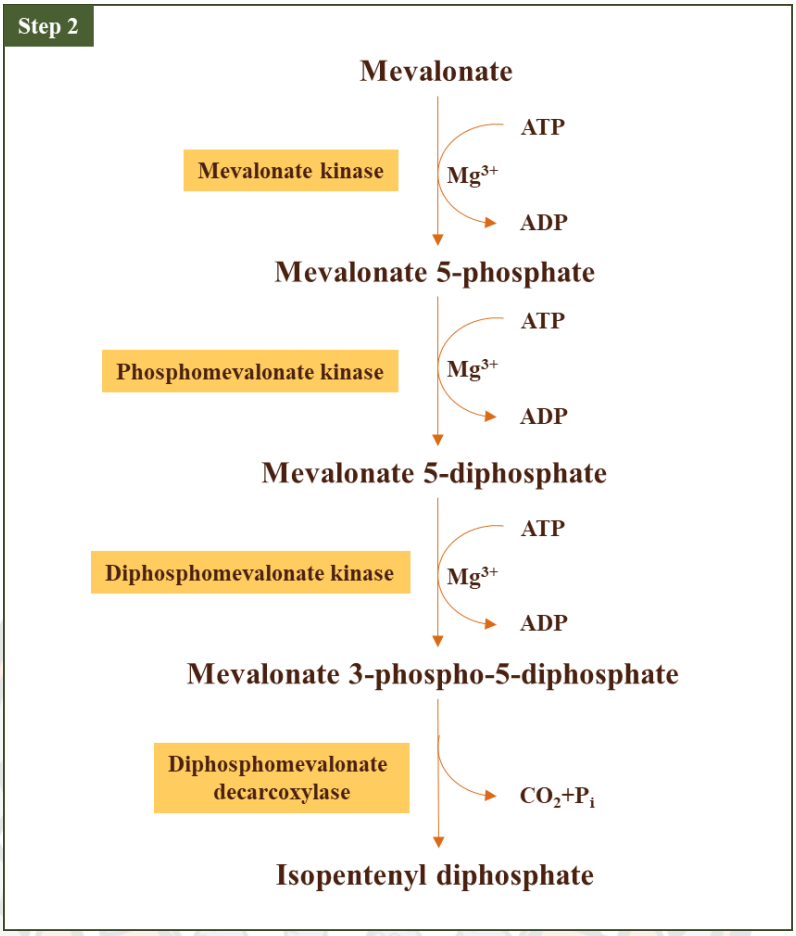


Figure 9 The mevalonate converts to the formation of the isoprenoid units.

Source: (Rodwell, 2015)

Step 3: the condensation of the isopentenyl unit converts to squalene:

The formation of the 3,3-dimethylallyl diphosphate occurs from the isomerization of the isopentenyl diphosphate by a shift of double bond between C1-C2 to C2- C3, using the isopentenyl diphosphate isomerase. The 3,3-dimethylally diphosphate is condensed using molecules of isopentenyl diphosphate, which are converted to the 10-carbon intermediate geranyl diphosphate form. This was condensed with another isopentenyl diphosphate to form farnesyl diphosphate, and then two molecules of farnesyl diphosphate were condensed to the squalene using NADPH, and finally catalysed by the squalene synthetase,(Biochemical Pathways An Atlas of Biochemistry and Molecular Biology 2012) as shown in Figure 10.

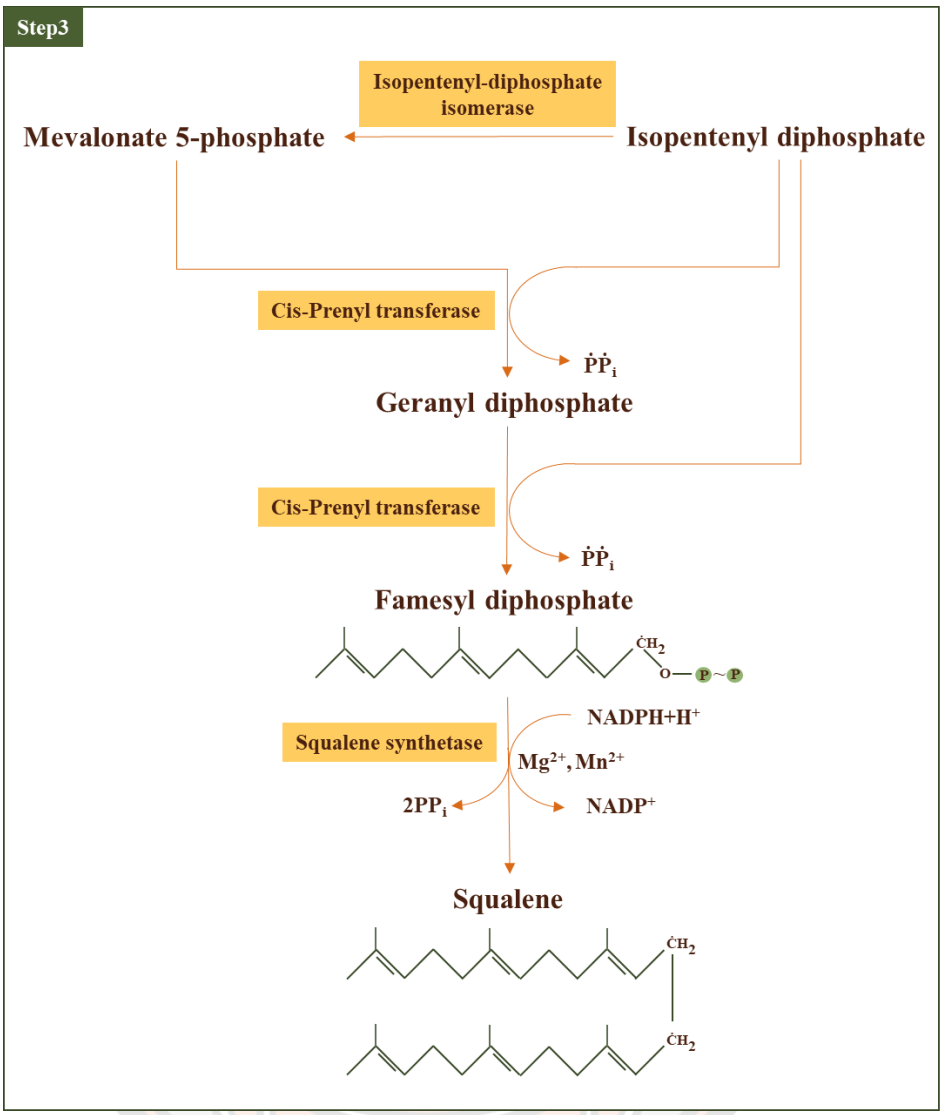


Figure 10 The condensation of the isopentenyl unit converted to squalene.

Source: (Rodwell, 2015)

Step 4: the cyclization of squalene converted to the lanosterol:

The folding structure of the squalene is very similar to that of the steroid nucleus. The initial step before the ring of squalene closes is to convert it to the squalene 2,3-epoxide, using the catalyst of the squalene epoxidase from the ER. The squalene 2,3-epoxide is transferred to both the methyl groups from C14 to C13 and C8 to C14, which are the cyclisation by the catalysation of oxidosqualene-lanosterol cyclase, which is lanosterol,(Rodwell, 2015) as shown in Figure 11.

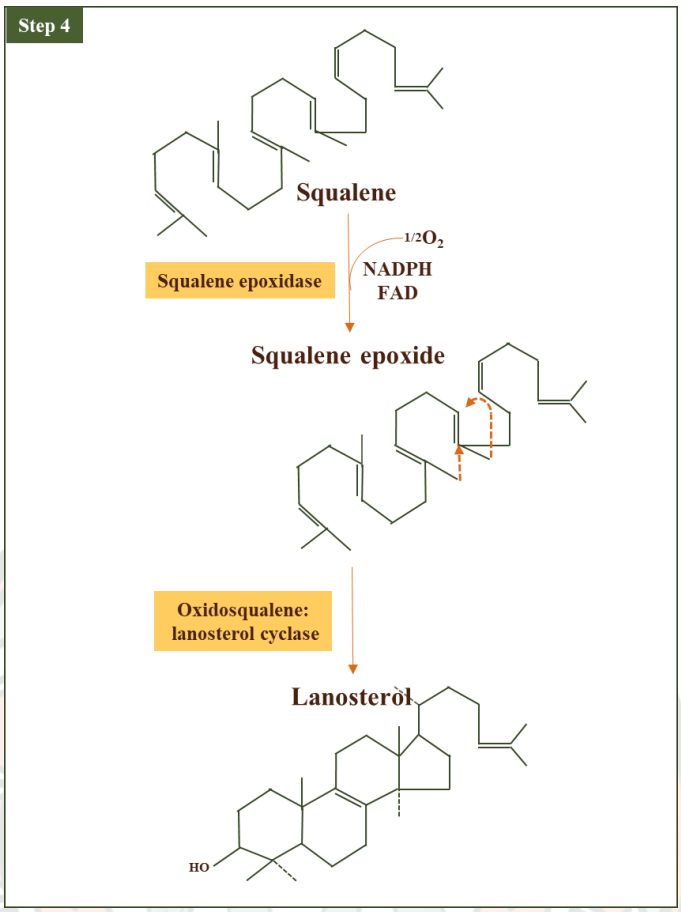


Figure 11 The cyclization of squalene converted to lanosterol.

Source: (Rodwell, 2015)

Step 5: the formation of cholesterol from lanosterol:

The initial step is removing the methyl group from C4 and C 14 to be converted to 14-desmethyl lanosterol, and then to zymosterol. The double bond of the zymosterol structure migrates from the C8-C9 position to the C5-C6 position, which results in the densmosterol form. The final step is the double bond between C24-C25 position is reduced by NADPH to convert to the cholesterol form,(McLeod, 2016; Rodwell, 2015) as shown in Figure 12.

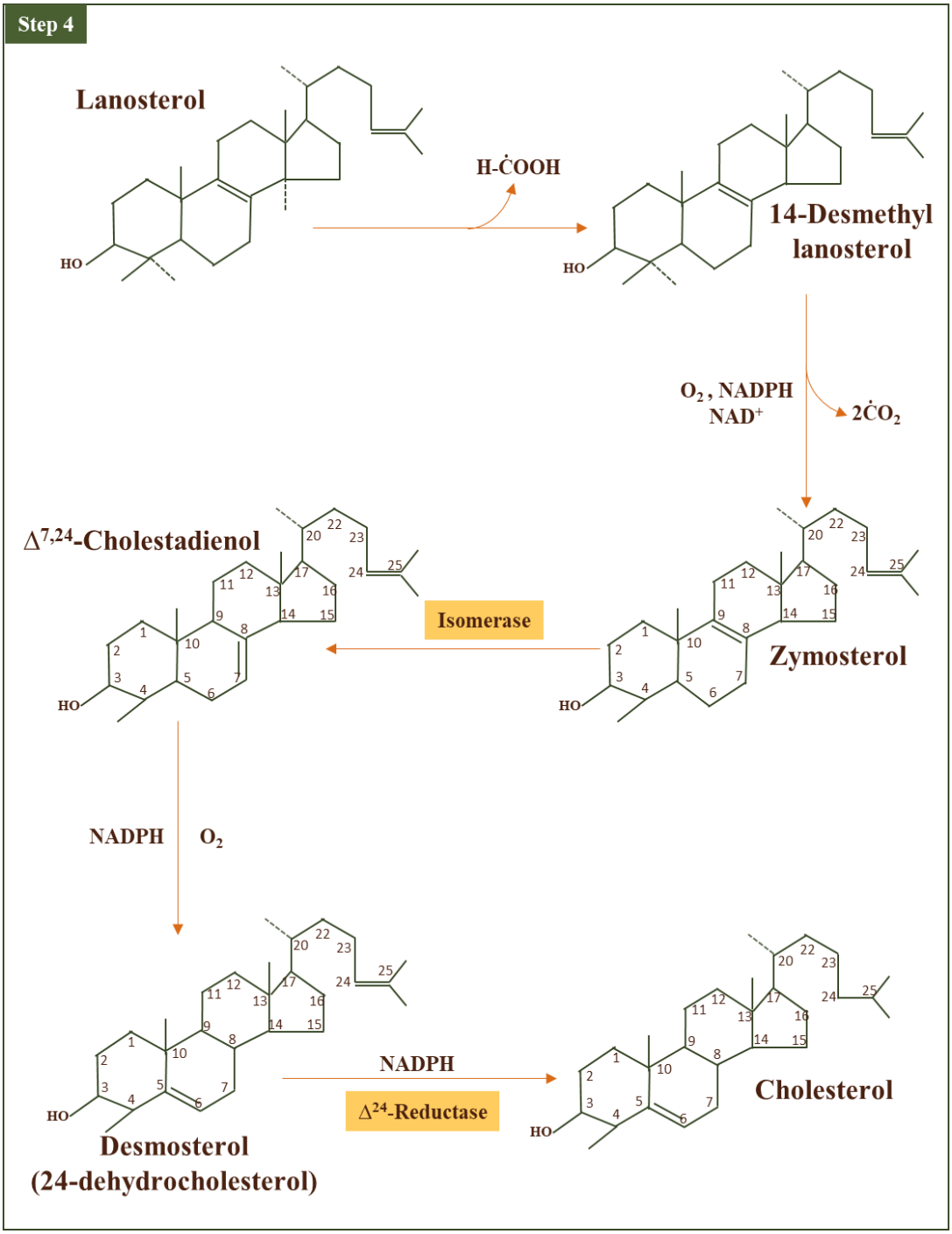


Figure 12 The formation of cholesterol from the lanosterol.

Source: (Rodwell, 2015)

The regulation of cholesterol biosynthesis

The regulation of the HMG-CoA reductase activity is effective in controlling the cholesterol biosynthesis in the liver. Several factors are regulated by the HMG-CoA reductase expression or activity in the liver, such as cholesterol dietary uptake; the activation of transcription factors; and the activation of protein kinase, etc.(McLeod, 2016) Cholesterol dietary uptake is an exogenous supply in the body when its uptake supply is adequate, and its biosynthesis is decreased to maintain the cholesterol balance in the body.(McLeod, 2016) Additionally, the activation of the sterol regulatory element-binging protein 2 (SREBP2) regulates cholesterol uptake and biosynthesis, which is a transcription factor and appears in the endoplasmic reticulum. The activation of SREBP2 is involved in the transcription of genes such as LDLR and HMGCR, while its increased expression or activity induces the gene and/or protein expression levels of them both.(McLeod, 2016) The increased LDLR expression and/or activity increases cholesterol uptake in the liver, and in the same way, the cholesterol biosynthesis is decreased.(Gabcova-Balaziova et al., 2015) The HMGCR expression is stimulated by the SREBP2 expression, which increases the cholesterol biosynthesis.(Afonso et al., 2018) While the alteration of the cholesterol in the body can be controlled via the activity of SREBP2 in the liver, as shown in Figure 13. Additionally, the other way to regulate the cholesterol mechanism is by AMP-activated protein kinase (AMPK) activity, which is an enzyme. The inactive AMPK form is phosphorylated for the AMPK active form, using AMPK kinase (AMPKK) and AMP for allosteric modification. HMGCR is inactivated via phosphorylation by phosphor-AMPK and using ATP, as shown in Figure 14, while the inactivated form of HMGCR reduced the formation of mevalonate, which resulted in reduced cholesterol biosynthesis. (Bathaie, Ashrafi, Azizian, & Tamanoi, 2017; McLeod, 2016)

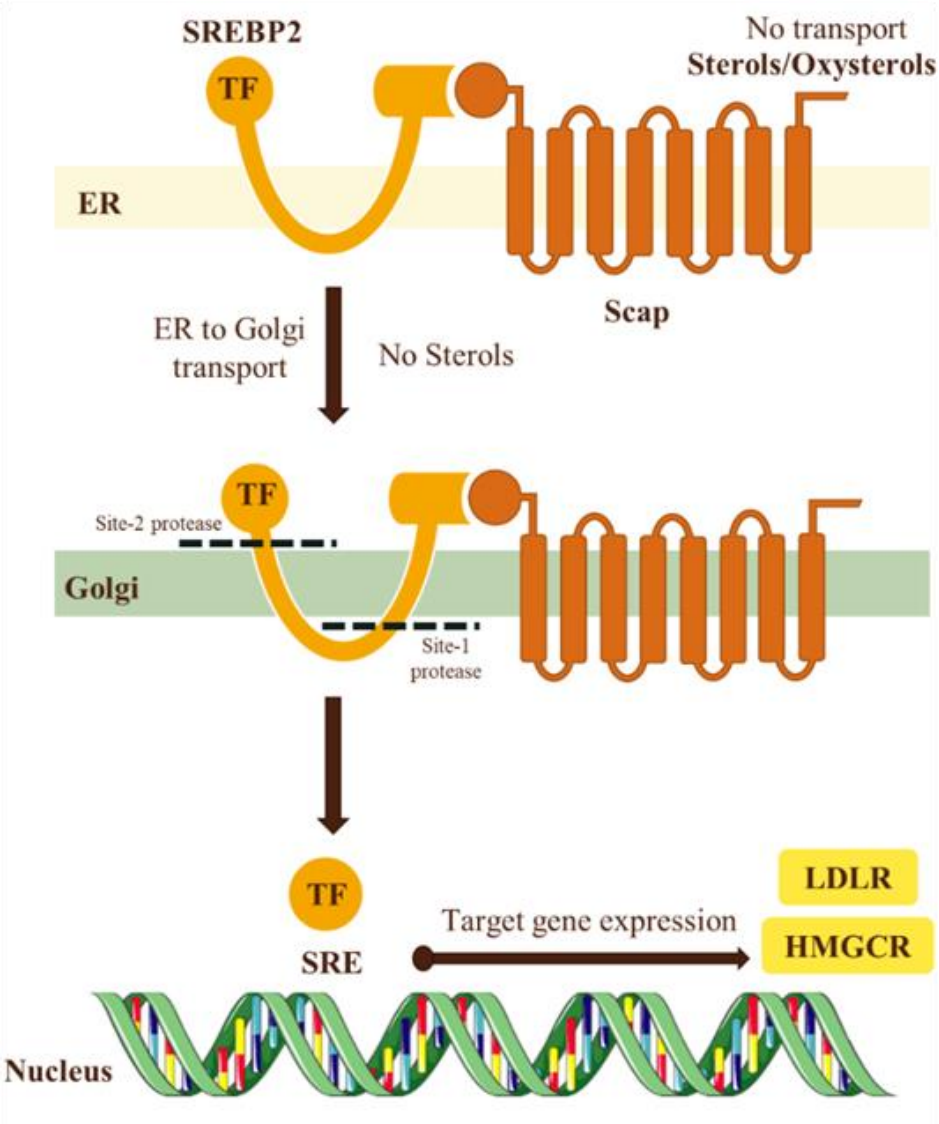


Figure 13 The process of SREBP2 pathway.

Source: (McLeod, 2016)

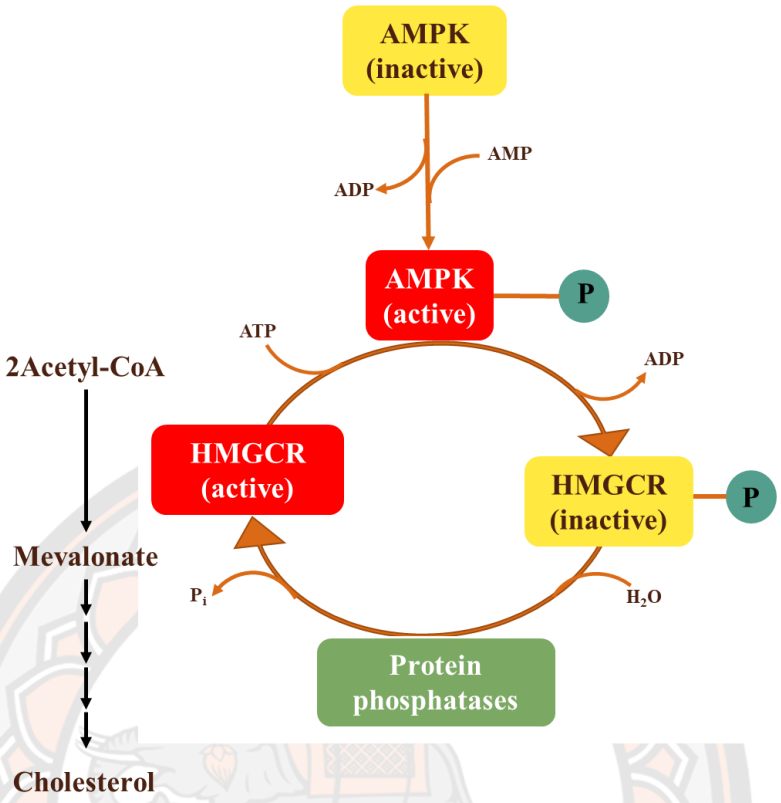


Figure 14 The regulation of HMGCR by AMP-activated protein kinase (AMPK).

Source: Modified from (Bathaie et al., 2017)

Cholesterol transport.

The cholesterol is transported between tissues in the bloodstream using the lipoprotein form, which is the formation of cholesteryl ester and apoprotein. The transport of cholesterol in the lipoprotein form starts in the small intestine, which is then transported to either the liver or other organs in the body. Ninety-five percent of the cholesterol transport in the chylomicron remnant form is delivered to the liver, after the degradation of chylomicron by lipoprotein lipase. (Rodwell, 2015) Most of the cholesterol secreted from the liver is in the very low-density lipoprotein (VLDL) form, which is then degraded by lipoprotein lipase and converted to intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL), respectively. The LDL was up-taken into the liver and the extrahepatic tissues by the LDLR.(Rodwell, 2015; William J. Marshall, 2014)

Lipoprotein.

lipoproteins are macromolecular complexes of lipids (cholesterol, triglycerides, phospholipids) and proteins (apolipoproteins, enzymes). Their basic structures consist of a hydrophobic core of triglycerides and/or cholesterol esters, surrounded by a layer of amphipathic phospholipids, unesterified cholesterol and proteins,(William J. Marshall, 2014) as shown in Figure 15.

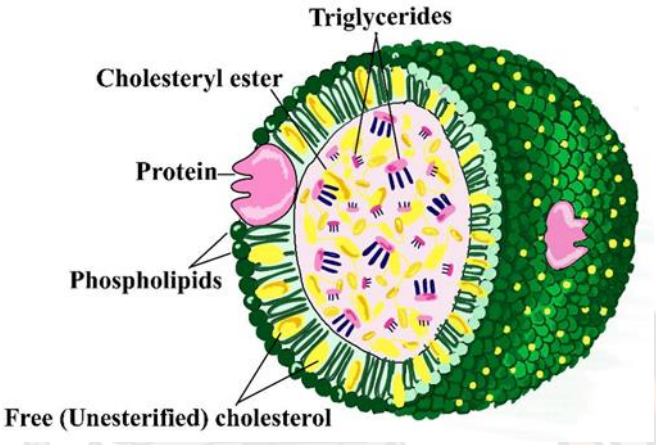


Figure 15 The generic lipoprotein structure.

Source: Modified from (Rodwell, 2015)

The lipoproteins play an important role in the absorption and transport of lipids. The classification of lipoproteins is divided into 6 classes according to their size or density, lipid composition, and apolipoproteins. The classes are chylomicrons, chylomicron remnants, very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) as shown in Table 3 and Figure 16.

Chylomicrons

Chylomicrons occur in the mucosal cells of the intestine, which is the largest lipoprotein structure. They are derived from the recombination of monoacylglycerols and fatty acids, which form triacylglycerols (TAG) with enzymes that are then combined with cholesterol, to form lipoproteins. Chylomicrons contain apoprotein 48 (Apo 48), along with apoprotein C-II, apoprotein E, and apoprotein A, which attach to their surfaces and assist in the metabolic process. They are absorbed through the lacteal lymphatic vessel by the facilitation of the microsomal lipid transfer proteins (MTP), which are then transferred to the thoracic duct. The chylomicrons

were first hydrolysed so they could be converted to chylomicron remnants by the enzyme lipoprotein lipase (LPL), and then absorbed by the liver via a receptor.

Very low-density lipoprotein

Very low-density lipoproteins (VLDL) are a rich TAG structure that are mostly secreted from the liver. The surface of them contains unesterified cholesterol, phspholipids, and apolipoprotein. VLDL consists mostly of apoprotein B-100 (apo-100) and the other apoproteins are apo-Es, and the apo C-II, which stimulates LPL active. They were hydrolysed by LPL to release TAG to other organs and transformed to VLDL remnants, which were up-taken into the liver via LDLR or glycoproteins, and in addition, they were converted to LDL by LPL hydrolysis.

Low-density lipoproteins

Approximately two-thirds of low-density lipoproteins (LDL) consist of cholesterol, while the other third consists of phospholipids and TAG. The surface of the LDL is coated only with apo B-100. The LDL is removed from the bloodstream by LDLR on hepatic tissue surfaces, which are formed when the apo B-100 binds to hepatic LDLR. This is due to it being an important risk factor for lipid accumulation in the bloodstream, which causes the development of atherosclerosis.

High-density lipoproteins

High-density lipoproteins (HDL) mainly contain proteins and cholesterol ester, and a small amount of TAG, which carry the cholesterol from the peripheral tissues to the liver. The surface of HDL is mainly coated by apo AI, but sometimes it is coated by apo AII, apo C and apo E. The apo AI of HDL stimulates lecithin cholesterol acyl transferase (LCAT), for the esterification of free cholesterol to be converted to cholesterol ester. Additionally, the surface of HDL consists of phospholipids and unesterified cholesterol.

Table 3 The classification of lipoproteins.

Lipoprotein	Density (g/ml)	Size (nm)	Major Lipids	Major Apoproteins
Chylomicrons	<0.930	75-1200	Triglycerides	Apo B-48, Apo C, Apo E, Apo A-I, A-II, A-IV

Chylomicron Remnants	0.930-1.006	30-80	Triglycerides Cholesterol	Apo B-48, Apo E
VLDL	0.930-1.006	30-80	Triglycerides	Apo B-100, Apo E, Apo C
IDL	1.006-1.019	25-35	Triglycerides Cholesterol	Apo B-100, Apo E, Apo C
LDL	1.019-1.063	18- 25	Cholesterol	Apo B-100
HDL	1.063-1.210	5- 12	Cholesterol Phospholipids	Apo A-I, Apo A-II, Apo C, Apo E

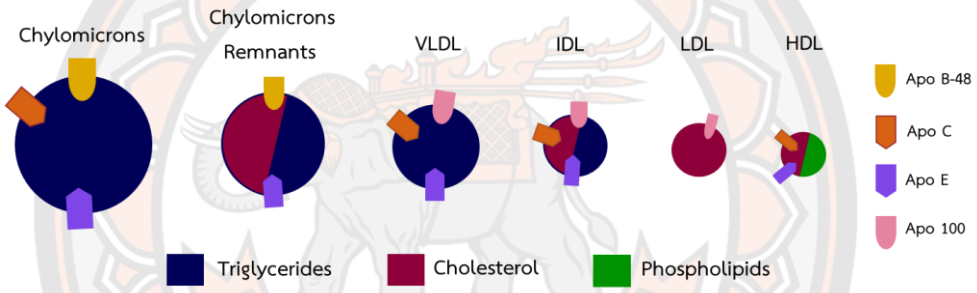


Figure 16 The classification of lipoproteins.

The regulation of cholesterol balance in tissue

The cholesterol balance is regulated by its influx and efflux into the cells.(McLeod, 2016; Rodwell, 2015) The cholesterol in the cells was increased by the influx of cholesterol-containing lipoproteins by the receptors. The LDL receptor (LDLR) and the scavenger receptor uptake the cholesteryl ester (CE), which was broken down by the lysosome and then hydrolysed by the CE'ase to convert to free cholesterol.(Luo et al., 2020) The cholesterol efflux into the cells by the formation of HDL via the ATP Binding Cassette Subfamily A Member 1(ABCA1), and the G Member 1 (ABCG1), which are protein transporters.(McLeod, 2016; Vourakis, Mayer, & Rousseau, 2021) HDL occurs from the esterification of the free cholesterol by ACAT2, while another form of cholesterol efflux into the cells is the VLDL form. In addition, the free cholesterols were utilized as hormones and bile acid synthesis in the liver.(Ortsäter, Sjöholm, & Rafacho, 2012)

The important factor for regulating the cholesterol balance in the tissues is the LDLR, which is a receptor on the cell surface that consists of apo B-100 and, E.(Enjoji, Kohjima, & Nakamuta, 2016) The LDLR endocytosis is the process that allows the CE to enter the cells, after they were hydrolysed in the lysosome to degenerate to free cholesterol. The influx of cholesterol by LDLR inhibits the enzymes involved in the cholesterol biosynthesis, such as HMG-CoA synthase (HMGCS), and HMGCR etc.(Gabcova-Balaziova et al., 2015) Furthermore, the cholesterol influx inhibits the LDLR expression via the regulation of the SREBP2 pathway. The increased cholesterol influx suppresses the cholesterol biosynthesis and uptake in the tissues. (McLeod, 2016) The LDLR activity is controlled by the supply and demand of cholesterol, steroid hormones, and bile acid biosynthesis.(Ortsäter et al., 2012; Rodwell, 2015) The enhanced LDLR expression or activity in the tissues increased cholesterol accumulation and decreased it in the blood stream. The lowering of cholesterol in the bloodstream leads to a decrease of the important factors that develop cardiovascular diseases (CVDs),(Malakul, Pengnet, Kumchoom, & Tunsophon, 2018; Pengnet, Prommaouan, Sumarithum, & Malakul, 2019) which is hypercholesterolemia. It is known that the proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme for the degradation of LDLR.(Gabcova-Balaziova et al., 2015) This enzyme regulates the LDL level that leads to the accumulation of cholesterol in the bloodstream, which results in hypercholesterolemia and the development of atherosclerosis.(Pengnet et al., 2019) The PCSK 9 inhibitor is one type of drug used for cholesterol-lowering in the blood stream. The LDLR plays an important role in decreasing cholesterol in the blood stream by up-taking it into the tissues.(Gabcova-Balaziova et al., 2015)

Cholesterol excretion

Excess cholesterol is excreted from the body in the unesterified form via bile acid, and/or the conversion of cholesterol to bile acid in the liver.(McLeod, 2016; Rodwell, 2015) The initial step of bile acid synthesis occurs in the liver, and are called the primary bile acids, which are then converted to metabolic products, called secondary bile acids that are excreted from the gut by microbiota. The cholesterol is converted to the 7α-hydroxycholesterol by the 7α-hydroxylation of the cholesterol, and catalysed by the cholesterol 7α-hydroxylase (CYP7A1). This step is crucial for

the regulation of bile acid biosynthesis, because the cholesterol 7α-hydroxylase is a rate limiting enzyme for converting cholesterol into bile acid, which is a microsomal cytochrome P450 enzyme.(Chambers, Day, Aboufarrag, & Kroon, 2019) The bile acid biosynthesis was divided into two neutral pathways after the conversion of cholesterol to 7α-hydroxycholesterol. The first one contained the 7α-hydroxycholesterol, which was hydroxylated on the C12 position by catalyst of the Cytochrome P450 family 8 subfamily B member 1 (CYP8B1) and the mitochondrial Cytochrome P450 family 27 subfamily A member 1 (CYP27A1), respectively, resulting in cholyl-CoA form.(Chambers et al., 2019; McLeod, 2016) This was then converted to primary bile acid that consisted of taurocholic acid (TCA) and glycocholic acid (GCA) using the taurine and glycine conjugates, respectively, which occurred in the liver. The deconjugation and the 7α-dehydroxylated process of the TCA and GCA were for the conversion to deoxycholic acid (DCA), which is the secondary bile acid that is represented in the gut, as shown in Figure 17.

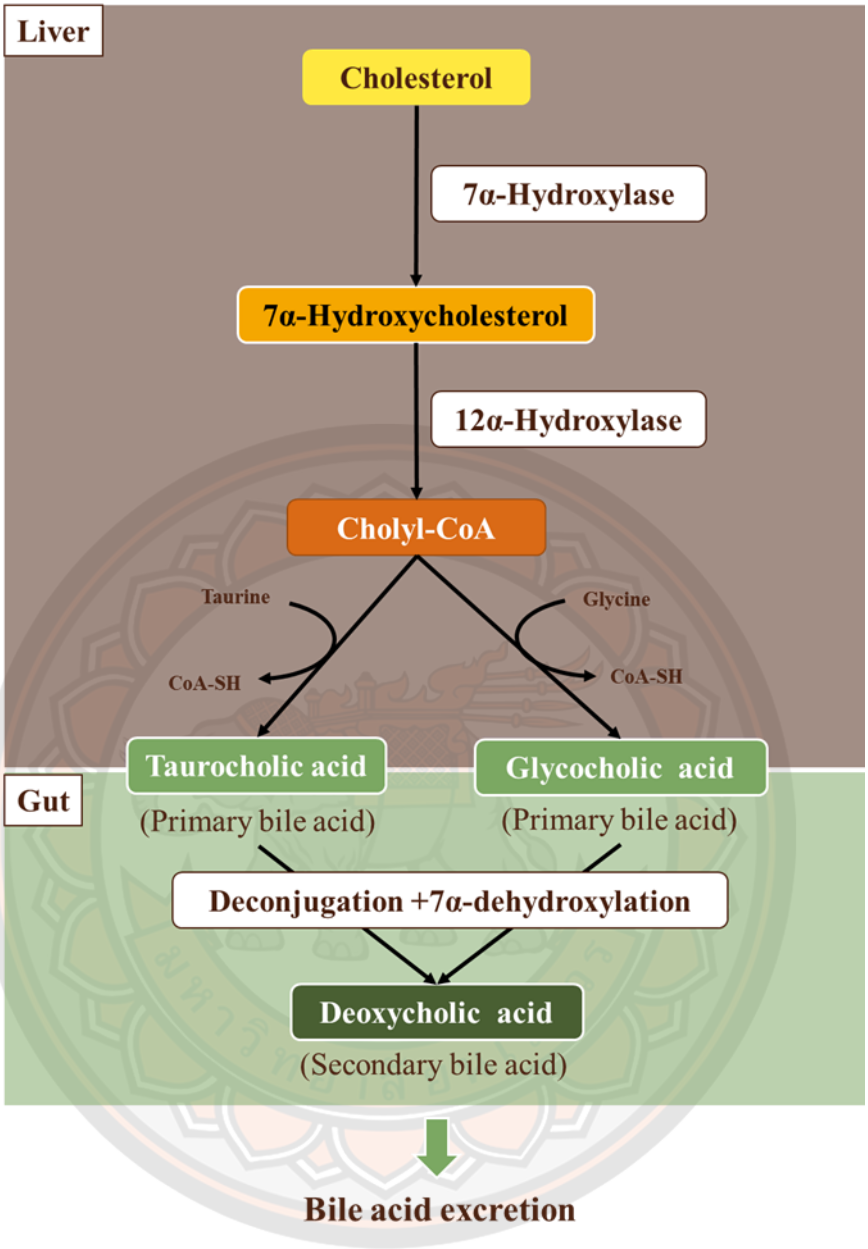


Figure 17 The first neutral pathway of bile acid biosynthesis.

Source: (Biochemical Pathways An Atlas of Biochemistry and Molecular Biology 2012)

The secondary pathway contained the 27α-hydroxylation of the 7α-hydroxycholesterol that was first converted to chenodeoxychoyl-CoA (CDCA), and then to tauro-and glycol-chenodeoxycholic acid (T/GCDCA), which is a primary bile acid. The deconjugation and the 7α-dehydroxylated process of the T/GCDCA were for the conversion to lithocholic acid (LCA), which is a secondary bile acid that is represented in the gut, as shown in Figure 18.

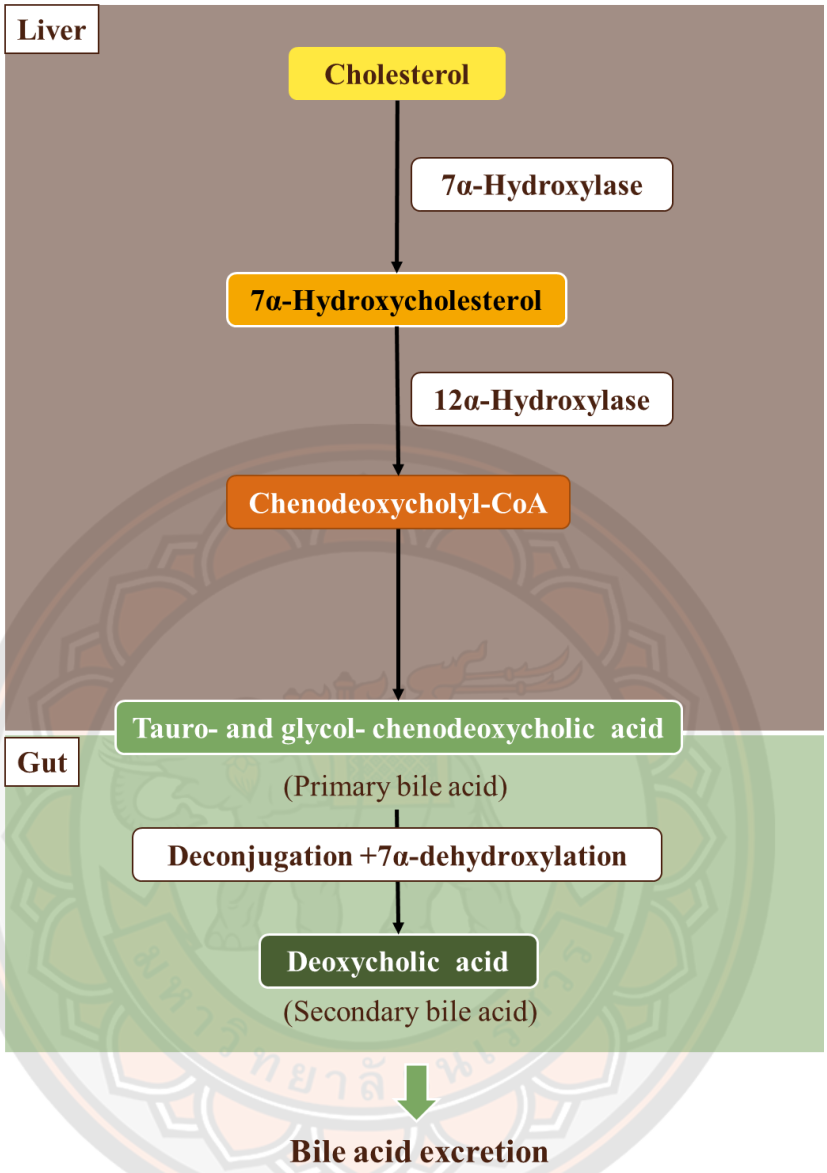


Figure 18 The secondary neutral pathway of bile acid biosynthesis.

Source: (Biochemical Pathways An Atlas of Biochemistry and Molecular Biology 2012)

Additionally, an alternative pathway for the bile acid biosynthesis from the cholesterol, is represented in the mitochondrial. The first step involved the 27-hydroxylation of the cholesterol that is a catalyst of the CYP27A1, which is a cytochrome P450 enzyme. The cholesterol was oxidized by the CYP27A1 then converted to the 27-hydroxycholesterol, and to the CDCA by the oxysterol 7α-hydroxylase (CYP7B1). The CDCA is oxidized to a primary bile acid, a T/GCDCA

product, which is then deconjugated and 7α-dehydroxylated for the conversion to LDA in the gut, as shown in Figure 19.

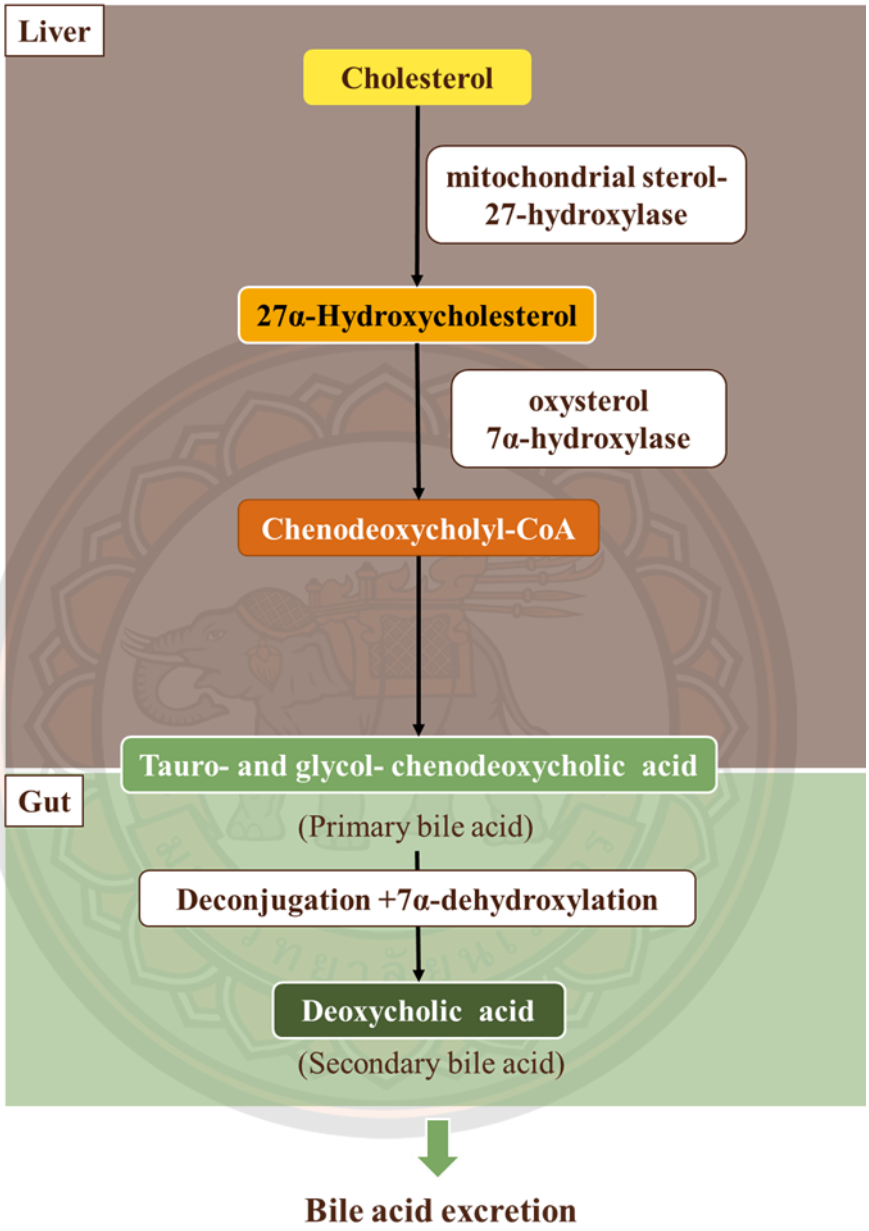


Figure 19 The alternative pathway of bile acid biosynthesis.

Source: (Biochemical Pathways An Atlas of Biochemistry and Molecular Biology 2012)

The cholesterol homeostasis

The cholesterol homeostasis in the body is regulated by cholesterol absorption, synthesis, and its excretion is via the bile acid.(Chambers et al., 2019;

McLeod, 2016; Rodwell, 2015) Excess cholesterol in the body downregulates cholesterol biosynthesis and up-regulates cholesterol excretion.(Bunnoy et al., 2015; J. K. Lee, J.-J. Lee, Y.-K. Kim, Y. Lee, & J.-H. Ha, 2020; Zhao et al., 2020) The cholesterol biosynthesis is controlled by the expression and activity levels of HMGCR, which is a rate-limiting enzyme. The HMGCR expression level was regulated by the SREBP2 expression, and the cholesterol uptake into the cells.(Afonso et al., 2018; Luo et al., 2020) In addition, the influx of cholesterol into the cells was regulated by the LDLR. Excess cholesterol reduces LDLR activity, which causes high cholesterol in the bloodstream, and results in hypercholesterolemia.(Al-Allaf et al., 2010; Jennifer K. Lee et al., 2020) Cholesterol is excreted in the form of unesterified cholesterol and bile acid, which controls the levels of cholesterol in the body. The CYP7A1 is an important rate-limiting enzyme that is used to regulate bile acid.(Chambers et al., 2019) Normal cholesterol levels in the body are determined by the balance between input and output. The input is regulated by the uptake and synthesis of the cholesterol, while the output is controlled by its excretion via the biliary system and the bile acids.

Dietary cholesterol

The Dietary Guidelines for Americans recommend that cholesterol consumption be limited to no more than 300 mg/day across calorie levels ≤3200 kcal/day. In 2015, the National Lipid Association Recommendations for Patient-Centered Management of Dyslipidaemia, focused on individuals with established hypercholesterolemia, recommended limiting dietary cholesterol to <200 mg/day to lower LDL and non–HDL cholesterol concentrations.(Carson et al., 2020) All cholesterol diets are created from animal products. These are divided into two groups which are dietary cholesterol with high saturated fats and low saturated fats, as shown in Table 4.

Table 4 Foods contain cholesterol with high and low saturated fats.

Cholesterol diet with high saturated fats	Cholesterol diet with low saturated fats
Full fat dairy foods such as milk, cheese, yogurt and cream.	Lean meat, especially offal, such as liver, kidney, sweetbreads, heart and tripe
Animal fats, such as butter, ghee, margarines and spreads made from animal fats, lard, suet and dripping	Prawns, crab, lobster, squid, octopus and cuttlefish.
Fatty meat and processed meat products such as sausages.	Eggs (the cholesterol is in the yolk).

Dietary cholesterol absorption

Cholesterol absorption starts in duodenum and proximal jejunum of the small intestine and is dependent on the presence of bile acids. On average, 50-60 % of cholesterol is absorbed in the small intestine, but there is a large variance in absorption between 20%-80%. The intestinal transit time of cholesterol absorption is slow transit times, which results in the high cholesterol fractional absorption rate. Dietary factors that affect the cholesterol absorption rate include the total mass of dietary cholesterol, the concentration of plant sterols in the diet, and the type and amount of dietary fibre. Biliary cholesterol and dietary cholesterol are absorbed in the small intestine in the form of micelle but cholesterol ester in the diet is hydrolysed by pancreatic cholesterol esterase (CE'ase) before cholesterol absorption. The micelle cholesterol absorbed in the apical membrane of the small intestine by the Niemann–Pick type C1-like 1 (NPC1L1), protein transporter. In contrast, the ATP Binding Cassette Subfamily G Member 5 (ABCG5) and ATP Binding Cassette Subfamily G Member 8 (ABCG8) on the apical membrane of the small intestine is excreted cholesterol. (Afonso et al., 2018; Jia, Betters, & Yu, 2011; Rodwell, 2015) Then cholesterol is esterified in the intestinal mucosa cells by cholesterol acyltransferase 2 (ACAT2) to form cholesterol ester, which is exocytosized from the basement membrane of the small intestine in the form of chylomicrons and entered into the lymph system, as shown in Figure 20. (Lindsay H Allen, 2013)

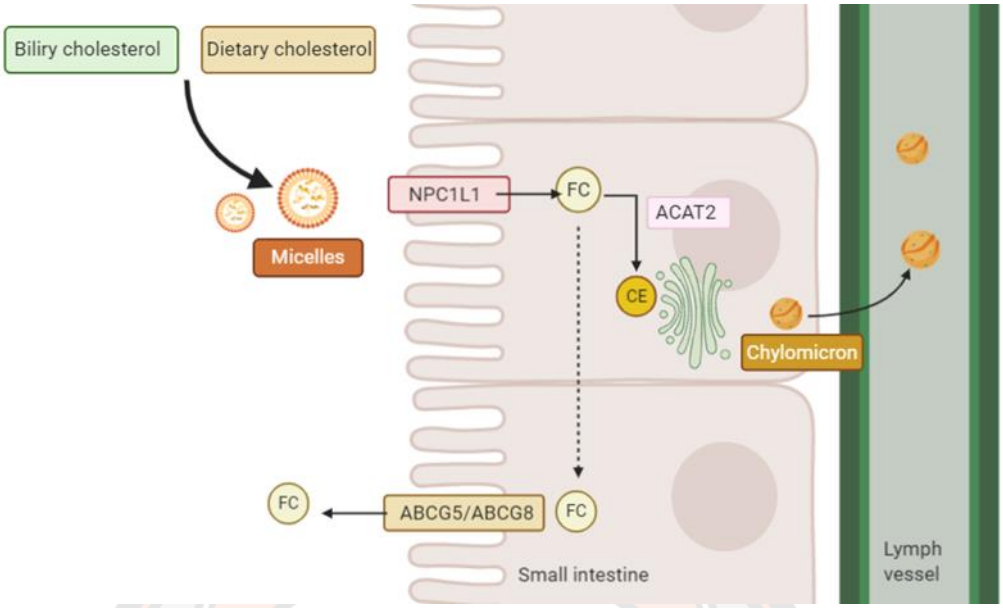


Figure 20 Dietary cholesterol absorption.

FC: Free cholesterol, CE: Cholesterol ester

Source: Modified from (H. H. Wang et al., 2017)

Dietary cholesterol and hypercholesterolemia

Previous studies showed that overconsumption of high-cholesterol diet increased serum cholesterol level (Cha & Park, 2019; Pengnet et al., 2019) and oxidative stress. This condition could contribute to endothelial dysfunction, which is a major cause of CVDs.(Al-Muzafar & Amin, 2017) Over-loaded cholesterol increases the hepatic metabolic burden, stimulating the expression of sterol regulatory element-binding protein-2 (SREBP2), a nuclear transcription factor, which increases HMGCR activity. (Afonso et al., 2018; Chavez-Santoscoy et al., 2014; Shi et al., 2019) In addition, they can increase ACAT2 activity.(Ding et al., 2016) In contrast, high-cholesterol diet down-regulate CYP7A1, liver X receptor α (LXRA), peroxisome proliferator activated-receptor α (PPAR α), and LDLR. (Hu et al., 2020; Kiran, Prasada Rao, Salimath, & Chilkunda, 2017; D. Yang et al., 2019) This leads to high LDL levels in the bloodstream and hepatic lipid accumulation.

Hypercholesterolemia

Hypercholesterolemia is characterized as high cholesterol levels in plasma with normal triglyceride levels,(Encyclopedia of Endocrine Diseases, 2018) and as a consequence of the rise of LDLs, the serum total cholesterol is 190 mg/dl or more.

The classifications are divided into two major classes, primary and secondary hypercholesterolemia as shown below:

Primary hypercholesterolemia

Hypercholesterolemia is characterized as high cholesterol levels in plasma with normal triglyceride levels, (Martinez-Hervas & Ascaso, 2019) and as a consequence of the rise of LDLs, the serum total cholesterol is 190 mg/dl or more. The classifications are divided into two major classes, primary and secondary hypercholesterolemia as shown below:

Familial hypercholesterolemia (FH), which includes:

- Autosomal dominant hypercholesterolemia (ADH)
- FH is caused by the mutations of low-lipoprotein receptor (LDLR) results in the loss of the receptor for ligand binding.
- FH is caused by familial defective apolipoprotein B 100, which results in the loss of ligand binding to the LDL receptor.
- FH is caused by the mutations of proprotein convertase subtilisin/kexin type 9 (PCSK9), which results in low LDLR and high LDL in plasma.
- Autosomal recessive hypercholesterolemia (ARH)

Polygenic hypercholesterolemia (PH)

The different characteristics of these entities are shown in Table 5. (Encyclopedia of Endocrine Diseases, 2018)

- Hyperlipoproteinemia (a)

Table 5 The characteristics of different types of primary hypercholesterolemia

	ADH	ARH	PH
Beginning	From birth	From birth	From 20 years old
Frequency	0.5%	Rare	More than 10%
Xanthomas	Yes	Yes	NO
CVDs	+++	+++	++
Phenotype	IIa	IIa	IIa
Heredity	Autosomal dominant	Autosomal recessive	Polygenic

Etiology	Mutations of LDLR Defection of apoB 100 Mutation of PCSK9	ARH protein	Various Polymorphisms Exogenous factors
Gen	LDLR:Chromosome 19 ApoB:Chromosome 2 CSK9: Chromosome 1	Chromosome 1	Various polymorphisms

Secondary hypercholesterolemia

Is basically caused by diets, diseases, metabolic disorders, and drugs, as shown in Table 6.(Rhee et al., 2019)

Table 6 Potential causes of secondary hypercholesterolemia.

Diets	Saturated fat intake Trans fat intake Excessive energy intake
Diseases	Obstructive liver disease Nephrotic syndrome Anorexia nervosa
Metabolism disorders	Obesity Pregnancy Hypothyroidism
Drugs	Diuretics Glucocorticoids Amiodarone Cyclosporin

Management of hypercholesterolemia

Hypercholesterolemia is an important factor in the development of CVDs as atherosclerosis and coronary artery disease, the prevention and treatment of hypercholesterolemia is essential in the management of cholesterol-lowering. It includes:

Non-pharmacological cholesterol-lowering approaches

Non-pharmacological is the adaptation of behaviour and lifestyle change that can improve and protect hypercholesterolemia. Previous studies have reported that changes in lifestyle such as the reduction of high cholesterol diet, enhances physical activity or physical fitness, cessation or reduction of smoking and the drinking alcohol, the consumption of a healthy diet (almond, nuts, avocado, fish, green vegetables, and fruits) reduced cholesterol levels in the blood steam, leading to a reduction in this risk factor for hypercholesterolemia. (Kelly, 2010; Mahmood, 2015; Mannu, Zaman, Gupta, Rehman, & Myint, 2013)

Pharmacological cholesterol-lowering approaches

Ezetimibe

Ezetimibe ($C_{24}H_{21}F_2NO_3$) is an azetidinone derivative and a drug that inhibits intestinal absorption by blocking the internalization of NPC1L1 protein with cholesterol-lowering activity.(T. Y. Chang & Chang, 2008) Ezetimibe, which is also known as zetia or ezetrol, belongs to the class of organic compounds known as monobactams which causes a decrease in the cholesterol levels in the blood stream. Overall, the following effects are observed: a reduction of hepatic lipid accumulation. Total cholesterol, LDL, and other triglycerides in the blood are also reduced.(Agarwala, Kajani, Miedema, & Virani, 2016; Bin Abdulhak & Robinson, 2018; Strilchuk, Tocci, Fogacci, & Cicero, 2020)

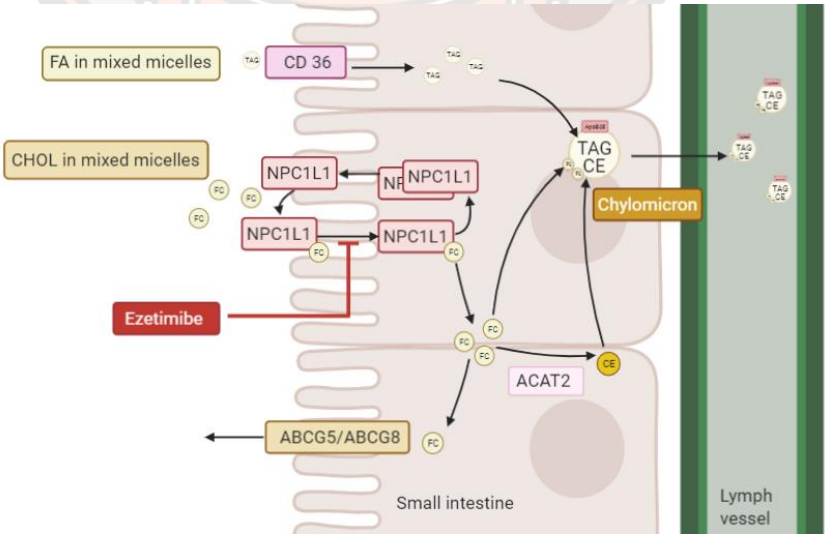


Figure 21 The function of ezetimibe

Source: Modified from (Huff, Pollex, & Hegele, 2006)

Colesevelam

Colesevelam ($C_{31}H_{67}Cl_3N_4O$) is a hydrophilic, water-insoluble polymer not hydrolyzed by digestive enzymes, and non-absorbable, which is the bile acid sequestrant. The trade name for colesevelam is Cholestagel®, and WelChol®. They bind with high affinity to bile acids within the gastrointestinal tract, thereby inhibiting the reabsorption of bile acids. For this reason, colesevelam decreases the level of LDL in bloodstream.(Davidson, Dicklin, Maki, & Kleinpell, 2000; Melian & Plosker, 2001)

Lomitapide

Lomitapide ($C_{39}H_{37}F_6N_3O_2$) is a cholesterol-lowering agent that performs by the inhibition of microsomal triglyceride transfer protein (MTP), an enzyme located in the endoplasmic reticulum. Lumen is responsible for absorbing dietary fats and transferring TG onto apo-B in the assembly of metabolic precursors of VLDL. Lomitapide blocks the assembly of Apo-B-containing lipoprotein, thus reducing the LDL levels in the bloodstream.(Berberich & Hegele, 2017; Brahm & Hegele, 2016)

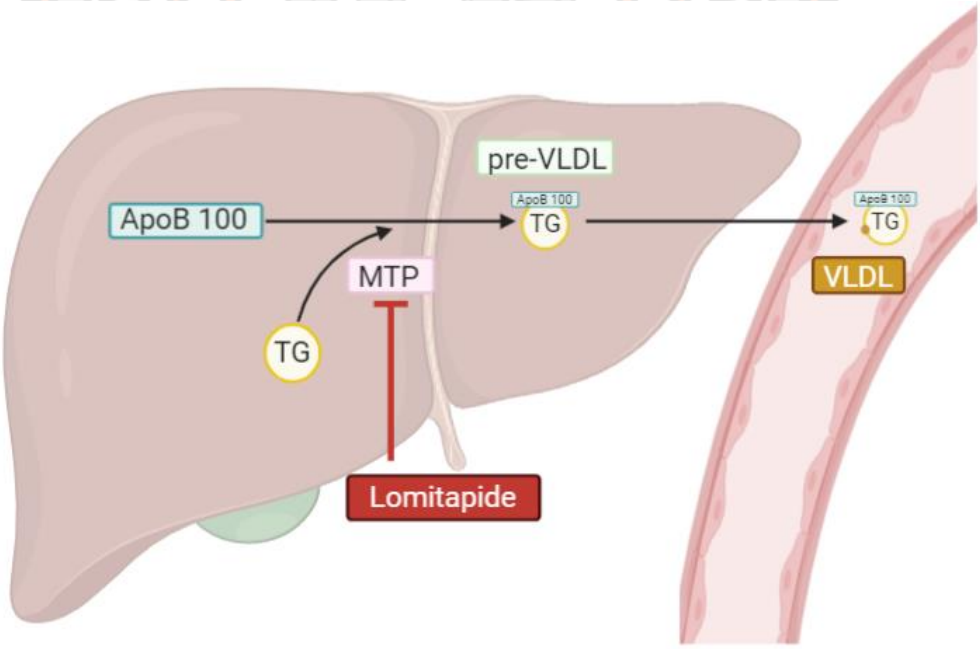


Figure 22 The function of Lomitapide.

Source: Modified from (Goulooze, Cohen, & Rissmann, 2015)

Mipomersen

Mipomersen ($C_{230}H_{324}N_{67}O_{122}P_{19}S_{19}$) is an anti-sense oligonucleotide that prevents the formation of ApoB-100 leading to a decrease in the levels of VLDL and LDL in patients with homozygous and heterozygous familial hypercholesterolemia.(Hashemi et al., 2014; Parham & Goldberg, 2019)

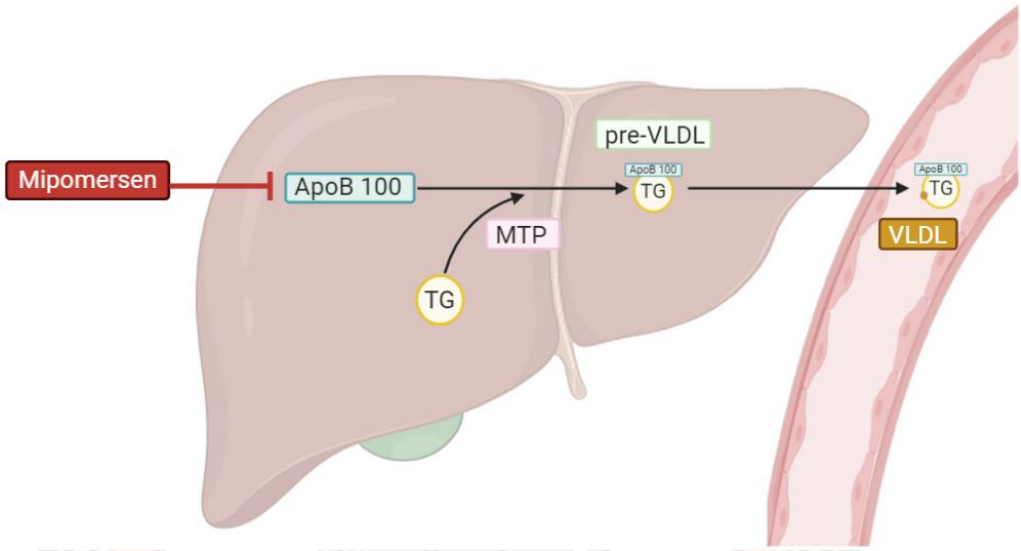


Figure 23 Function of Mipomersen.

Source: Modified from (Pang, Chan, & Watts, 2015)

Statins

Statins are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, used in the treatment of lipid disorders that are characterized by elevations in LDL. Statins have revolutionized the primary and secondary prevention of atherosclerotic cardiovascular disease (ASCVD), due to their cholesterol-lowering properties.(Abd & Jacobson, 2011; Sirtori, 2014)

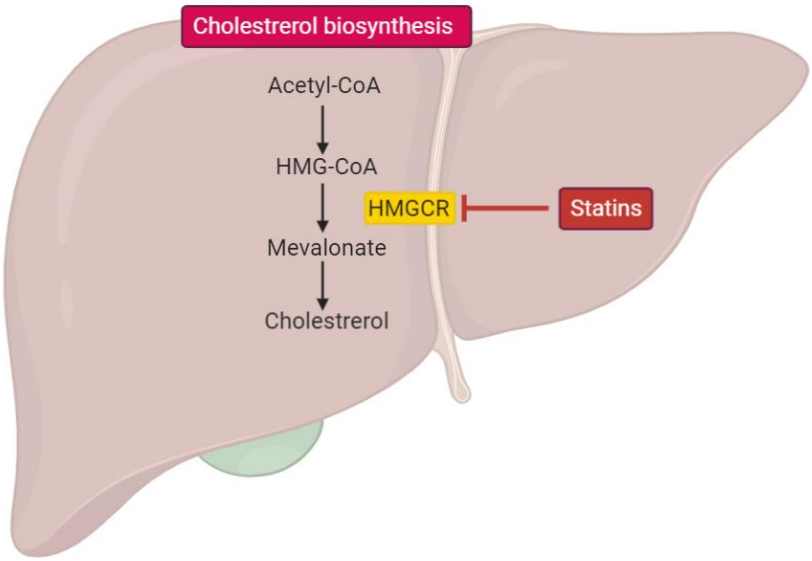


Figure 24 The function of statin.

Source: Modified from (Pang et al., 2015)

Simvastatin

Simvastatin ($C_{25}H_{38}O_5$) is an HMG-CoA reductase (HMGCR) drug that belongs to the group of statins and is used to lower lipid levels and reduce the risk of cardiovascular disease. It is a lipid-lowering agent synthetically derived from a fermentation product of Aspergillus terreus. Simvastatin is available as a generic drug and under the trade name Zocor in tablets of 5, 10, 20, 40 and 80 mg. The recommended dose is 5 to 80 mg daily, depending on tolerance and lipid levels. It can lower cholesterol levels by inhibiting the endogenous production of cholesterol in the liver.

Simvastatin competitively inhibits the enzyme HMGCR, a hepatic microsomal enzyme that catalyses the conversion of HMG-CoA to mevalonic acid. This is an important step in the control of metabolic reactions involved in the production of various compounds involved in lipid metabolism and transport, including cholesterol, LDL and VLDL. The major active metabolites of simvastatin are β-hydroxyacid metabolite and its 6'-hydroxy, 6'-hydroxymethyl, and 6'-exomethylene derivatives. In addition, it acts primarily in the liver, where reduced hepatic cholesterol concentrations stimulate the upregulation of hepatic LDLR, which increases hepatic endocytosis of LDL. Simvastatin is also administered as an inactive

lactone derivative, which is then converted to its β-hydroxy acid form by a combination of spontaneous chemical transformation and enzyme-mediated hydrolysis by non-specific carboxyesterases in the intestinal wall, liver, and plasma. A drug transporter, the organic anion- transporting polypeptide 1B1 (encoded by SLCO1B1), facilitates the hepatic uptake of some statins and other compounds.(Drug Metabolism in Diseases, 2017) All of these actions are capable of lowering plasma LDL and VLDL levels. Oxidative metabolism in the liver is mediated mainly by CYP3A4 and CYP3A5, with the remaining metabolism by CYP2C8 and CYP2C9. (Sampson, 2018)

Absorption of the drug.

Peak plasma concentrations of both active and total inhibitors were reached within 1.3 to 2.4 hours post-dose. Although the recommended therapeutic dose range is 10 to 40 mg/day, there was no substantial deviation from linearity of the area under the concentration-time curve (AUC) when the dose was increased up to 120 mg. The half-life of simvastatin is 4.85 hours. Compared to the fasting state, the plasma profile of the inhibitors was not affected when simvastatin was administered immediately before a test meal. Simvastatin undergoes extensive first-pass extraction in the liver, the target organ for the inhibition of HMG-CoA reductase and the primary site of action.(2012)

Alirocumab

Alirocumab (Praluent®; Sanofi) is a monoclonal antibody that binds to PCSK9, which is a second inhibitor of proprotein convertase PCSK9. The PCSK9- mediated of LDLR is inhibited, resulting in increased LDLR and leading to increased LDL uptake in hepatocyte and decreased LDL levels in the bloodstream. Therefore, alirocumab can decrease LDL levels in patients with familial hypercholesterolemia.("Alirocumab for hypercholesterolaemia," 2019; Della Pepa, Bozzetto, Annuzzi, & Rivellese, 2017)

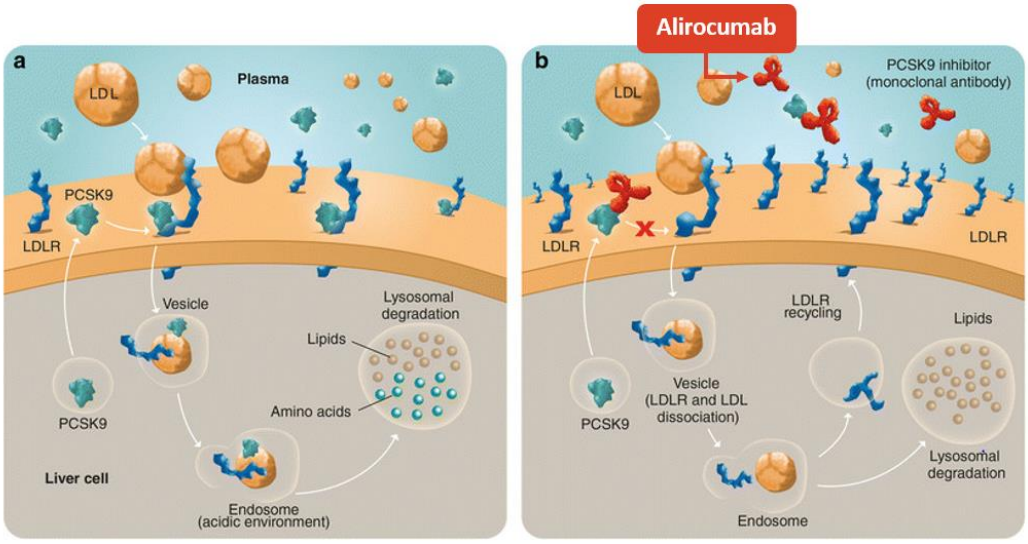


Figure 25 The function of alirocumab.

Source: https://www.biovendor.com/pcsk9-and-its-inhibitors-a-new-approach-in-lipid-lowering

Evolocumab

Evolocumab (Repatha®) is a monoclonal antibody targeting proprotein convertase PCSK9, which is a PCSK9 inhibitor. It results in the removal of LDL in the blood circulation, leading to cholesterol-lowering in patients with familial hypercholesterolaemia. ("Evolocumab," 2016; Sabatine et al., 2017)

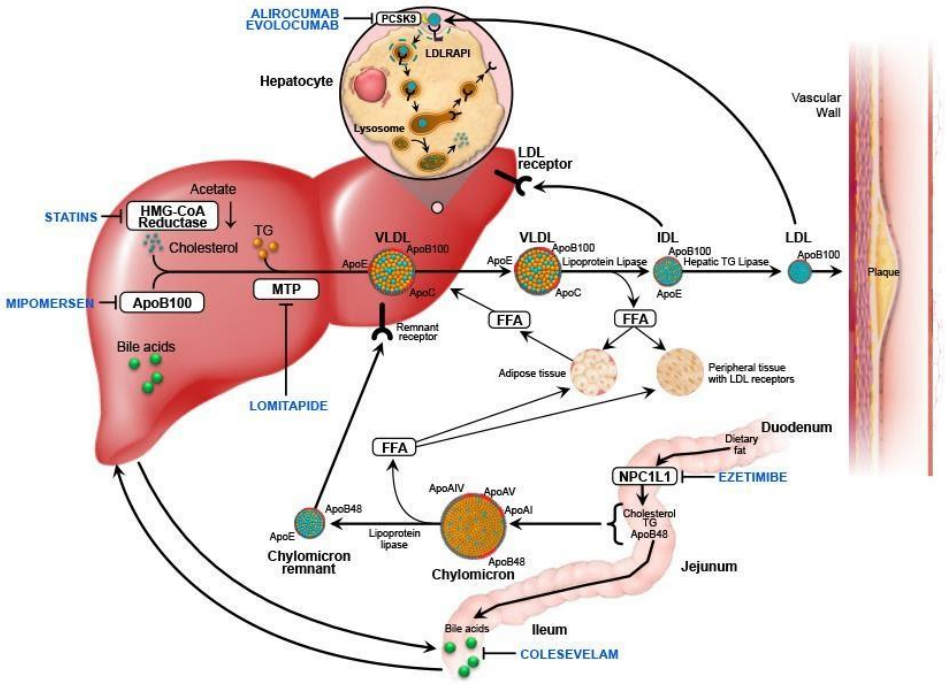


Figure 26 An overview of pharmacological cholesterol-lowering approaches.

Source: (Safarova & Kullo, 2016)

Dietary Fibre

Dietary fibre is a complex group of carbohydrates and lignin's, which include nondigestible polysaccharides, oligosaccharides, and associated plant substances that are not hydrolysed by human enzymes, and are not absorbed in the human body.(La Torre, Verbeke, & Dalile, 2021) Dietary fibre comes from a wide range of sources that include vegetables, fruits, grains, as well as others, which are divided into 2 categories: insoluble and soluble fibres.(He et al., 2022)

Insoluble fibre

Insoluble fibre consists of cellulose, some hemicellulose, and lignin. Cellulose is a long linear polymer made from β (1-4)-linked glucose units, and the hydrogen bond between the glucose units forms the 3-dimensional structure. Hemicellulose is formed of both hexose, and pentose of sucrose with the backbone linked by β (1-4) bonds, while the side chain includes galactose, arabinose, and glucuronic acid linked via both β (1-2), and β (1-3) bonds. Lignin is formed of phenol polymers that are highly branched with strong intramolecular bonds, and have the functions that decrease transit time, and increase faecal bulk, which helps to relieve

constipation. This fibre is found in whole grain, wheat, bran, nuts, seeds, some fruits, and some vegetables.(He et al., 2022; Soliman, 2019)

Soluble fibre

Soluble fibre includes pectin, gums, mucilage extracted from psyllium husk, glucan, fructans, and some hemicellulose. Pectin is a heterogeneous polysaccharide that is formed by unbranched chains of α (1-4)-linked D-galacturonic acid backbone, which is attached to both of the pentose and hexoses chains. Gums are formed from galactose backbone linked by both β (1-3), and β (1-6) bonds with side chains of arabinoses, glucuronic acid, methyl-glucuronic acid or galactose, which are secreted from the site of the plant injury.(He et al., 2022; Soliman, 2019) Mucilage is found in the plant psyllium and its structure is similar to gums, which is a viscous, gel-forming water-soluble fibre that contains up to 80% of soluble polysaccharide. While, β-glucans are formed from homopolymers of glucose subunits, and the fructans have the structure of the polymers of fructose, including oligofructoses, and inulin. It has the ability to resist the hydrolysis of small intestinal enzymes, but is fermented by bacteria to short-chain fatty acids in the human colon. The production of short-chain fatty acids leads to alterations in the intestinal microbiota, which contributes to the hypercholesterolaemic effect.(Soliman, 2019) In addition, soluble fibre is able to delay gastric emptying, decreases nutrient absorption, and slows digestion of the soluble fibre found in legumes, oats, barley, vegetables such as carrots, broccoli, onion and artichokes, and fruits such as bananas, berries, apples, pears, etc.(La Torre et al., 2021; Soliman, 2019)

Dietary fibre with different structures have different physicochemical properties, which play a vital role in controlling the health mechanism of the human body.(Carson et al., 2020; La Torre et al., 2021) In addition, dietary fibre can work in the human metabolism to help prevent and treat cardiovascular diseases, obesity, diabetes, liver diseases, and other non-communicable diseases that are increasing year by year, which are causing an increase in morbidity, and mortality worldwide. The physicochemical properties of dietary fibre, includes the ability to bind water-holding, water-swelling, oil-holding, glucose adsorption, and cholesterol adsorption and visibility, are closely related to physiological functions, and functional foods.(He et al., 2022)

Physicochemical properties

Water holding capacity

The water-holding capacity is somewhat related to the structure of the dietary fibre. When the contact area and the hydrophilic group of the dietary fibres increase, the water is able to be retained in the hydrophilic part or in the interstices of the network fibre structure, which improves the water holding capacity (WHC). The WHC property is mostly found in vegetable dietary fibres, which are irregular shaped, loosely bound together, and have greater porous surface areas, which facilitate water retention.(He et al., 2022) Dietary fibres have a good WHC value, which is one of the main reasons for their role in improving human health. The function of dietary fibre's WHC properties includes a lubricating effect after water absorption, which is able to promote intestinal peristalsis and motility. The function of WHC in the fibre increases the food volume after the intake of water, which reduces the amount of food intake.(He et al., 2022) Dietary fibre is fermented in the intestine to produce short-chain fatty acids (SCFAs), which are able to promote the release of satiety hormones that reduce the risk factors of obesity. After the dietary fibre absorbs water, it is able to increase the stool volume and the number of carcinogens removed during bowel movements.(He et al., 2022)

Water swelling capacity

Dietary fibres have water swelling capacity (WSC) for the following reasons: 1) Water has surface tension strength, which can be maintained in the capillary structures of dietary fibres. 2) Hydrogen bonds and dipolar hydrophilic groups bind to water. 3) This is directly related to the contact surface of dietary fibres. In general, the WSC of dietary fibres is similarly beneficial to the WHC for their functional properties.(He et al., 2022)

Oil holding capacity

The oil holding capacity (OHC) is related to the total charge density, hydrophobicity, surface properties and treatment conditions. Generally, the OHC of vegetable dietary fibres is higher than that of fruit, and cereal dietary fibres. The OHC of dietary fibre is also the basis for important functional properties, which can absorb fat and reduce the intake of excess calories in the body, and can prevent the occurrence of obesity.(He et al., 2022)

Glucose adsorption capacity

The glucose adsorption capacity (GAC) of dietary fibres is related to their physical properties, which allows glucose to adhere to the network structure of them, and reduces contact within the human intestinal tract. The GAC of dietary fibre in foods and studies on GAC mainly focus on vegetables. It has been reported that GAC shows a significantly improved effect at high hydrostatic pressure. In addition, the physical properties are associated with the function of glucose metabolism, as dietary fibre is able to absorb sugars and produce SCFAs via fermentation in the body, which is able to inhibit diabetes.(He et al., 2022)

Cholesterol absorption capacity

Cholesterol absorption capacity (CAC) is expressed as the mass of cholesterol absorbed by a 1 g sample of fruit. The CAC of dietary fibre is divided into two types: physical and chemical adsorption. Physical adsorption is related to the particle size, porosity, specific surface area and reaction temperature of dietary fibres. Whereas the chemical adsorption is related to the charge and hydrophobic group of dietary fibres. In a previous study, dietary fibre from bamboo shoots was found to be able to absorb bile acids and cholesterol, which led to a reduction in cholesterol levels.(He et al., 2022) The soluble dietary fibre in soybean hulls have good viscosity, which leads to a high CAC content and thus lowers blood cholesterol levels, and can protect against and/or treat obesity. Due to the dietary fibres being able to absorb cholesterol and the SCFAs produced by fermentation, they are able to participate in the body's metabolism and improve dyslipidaemia, which is the reason why they can protect against obesity. (Dreher, 2018)

Viscosity

Viscosity is related to the flow of a fluid and the force directed on it. Factors that affect viscosity include chemical composition, structure, particle size, surface area and processing conditions. A previous study found that the viscosity property in oatmeal was able to promote an increase of the water film thickness on the intestinal mucosa, which increased the viscosity of the supernatant intestinal contents. The viscosity of dietary fibre may prevent metabolic disorders such as obesity and diabetes, by delaying fat formation and gastric emptying while increasing insulin sensitivity.(He et al., 2022; La Torre et al., 2021)

Dietary fibre and lipid levels.

Dietary fibre is able to inhibit lipid absorption and reduce energy intake by controlling food intake, digestion, absorption, and metabolism. Body weight loss caused by dietary fibre is as follows: 1) The viscosity of soluble dietary fibre such as gum and gum arabic, which can slow down the migration of nutrients. Dietary fibres have good WHC, OHC, and CAC, which are able to increase the absorption of lipids, and the network structure of insoluble dietary fibre also has a good CAC. A previous study found that dietary fibre in broccoli contained sticky pectin, that decreases the intake of dietary fat, and increases the removal of cholesterol and bile acid from enterohepatic circulation, which are the reasons for the reduction of cholesterol levels in the blood.(Soliman, 2019) Another study reported that bamboo shoot shells contained a large amount of insoluble dietary fibre, which is able to absorb bile acids and cholesterol, which results in lower cholesterol levels.(Liu et al., 2016) 2) dietary fibre has good WSC, which is able to enhance satiety and decrease food intake. A previous study found that the consumption of dietary fibre was able to absorb water and increase satiety, which was able to slow down the absorption of nutrients and effectively reduce energy intake.(Lambert et al., 2017) (ref) 3) Dietary fibre produces SCFAs through intestinal microbial fermentation, which exhibits the secretion of glucagon-like peptide 1 (GLP-1) and YY peptide (PYY).(Isken, Klaus, Osterhoff, Pfeiffer, & Weickert, 2010) The GLP-1 is secreted from intestinal L-cells that promotes the secretion of insulin and the proliferation of pancreatic cells, which are able to control glycogen synthesis in the muscle cells and at the same time enhance satiety.(He et al., 2022; Isken et al., 2010) Whereas PYY is an intestinal secretion hormone with anti-obesity effects, which can suppress the appetite and reduce the amount of food intake.(S. Chang et al., 2017) An existing study found that the insoluble dietary fibre of pomace pears was able to dilute energy, increase the consumption of energy, and secretes GLP-1 and PYY after intestinal fermentation, which reduces fat cells and effectively ameliorates obesity.(S. Chang et al., 2017; Isken et al., 2010) In addition, a few previous rat studies, showed that the administration of dietary fibre was associated with decreased fat absorption. In addition, some former studies found that dietary fibre increased LDL-ApoB 100 that led to the upregulation of the LDL receptors of the liver, which led to faster

catabolism and clearance. The hypocholesterolaemia effect of dietary fibre was due to the reduced number of secreted VLDL particles, and reduced activity of cholesteryl ester transfer protein (CEPT), which led to decreased CE in the VLDL particles that are converted to LDL, and at the same time increased the VLDL and LDL apo B 100 are reabsorbed by the liver.(Soliman, 2019)

Studies conducted in several animal models showed that the beneficial effects of dietary fibres were able to reduce the risk factors of cardiovascular diseases. A previous study found that dietary fibre from soybeans was able to prevent atherosclerosis in animal models, as well as another study that found that pectin in grapefruit was also able to reduce atherosclerosis. Additionally, another study found that the consumption of dietary fibre was able to decrease the total cholesterol in the blood by reducing the LDL cholesterol synthesis. It has been reported that insoluble dietary fibre was able to ameliorate hypercholesterolaemia in animal models, while several other studies, found that the dietary fibre was also beneficial in lowering the cholesterol levels in the blood of mice.(Soliman, 2019)

The beneficial effects of hypocholesterolaemia, and anti-obesity by dietary fibre mainly comes from the physicochemical properties such as WHC, WSC, OHC, CAC, and viscosity, which slow down the absorption of dietary fat. Both soluble and insoluble dietary fibres, have the effects of ameliorating hypercholesterolaemia and obesity by lowering lipid levels.(He et al., 2022)

Phenolic compounds

Plants are a natural source of bioactive compounds, such as secondary metabolism of plants and anti-oxidants. They absorb sun light, which produces high levels of oxygen, and secondary metabolism by photosynthesis. The secondary metabolites of a plant are mostly involved in the defence against ultraviolet radiation and/or aggression by pathogens.(Vasantha Rupasinghe, Nair, & Robinson, 2014) Flavonoids and phenolic acids are important groups of secondary metabolisms, and the bioactive compounds that are found in fruits and vegetables are phenolic compounds. They are natural products that possess anti-oxidants, anti-dyslipidaemia, anti- aging, anti-obesity, and anti-cancer properties.(Asghar et al., 2018; Toma et al., 2020) It was found that flavonoids decreased serum levels of lipids and glucose in humans. Flavonoids contain a wide range of substances that play a crucial role in

protecting biological systems from harmful effects of oxidative processes of macronutrients, such as carbohydrates, proteins, and lipids.(Scalbert & Williamson, 2000) However, polyphenols or phenolic compounds are a widespread group of phytochemicals, which possess different physiological activities associated with their chemical structures. The fruits are rich in phenolic compounds, which are highly variable, ranging from simple molecules to highly polymerised compounds. One or more aromatic rings with one or more hydroxyl groups are the structure of phenolic compounds, the molecules that are biosynthesised via the pathways of shikimic acid (shikimate), malonic acid (acetate), or shikimate- acetate combined. Phenolic compounds are classified according to the number of rings they contain and the structural elements connecting these rings, and can also be classified according to its biosynthesis pathway. These are common components of fruits and are partly responsible for the overall organoleptic properties of them. The bitterness and astringency of fruits results from the interaction between phenolic compounds and saliva.(Dabeek & Marra, 2019)

Classification of phenolic compounds

The general classes of phenolic compounds are mostly found in fruits, which include phenolic acids, flavonoids, stilbenes, lignans, and tannins (proanthocyanins). These compounds are part of our daily dietary anti-oxidants, and are consumed throughout the world. Phenolic acids and flavonoids account for 60 and 30 per cent, respectively, of the total phenolic compounds found mainly in foods.(Vasantha Rupasinghe et al., 2014)

Phenolic acids

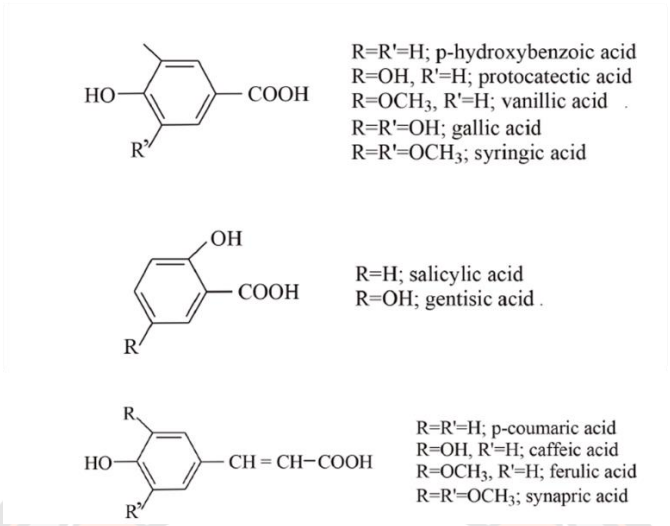


Figure 27 Chemical structure of phenolic acids

Source: (Vasantha Rupasinghe et al., 2014)

Phenolic acids are found primarily in the majority of all fruits and are divided into three classifications, which are derivatives of benzoic acid (C6-C1), cinnamic acid (C6-C3), and acetophenones and phenylacetic (C6-C2), as shown in Figure 27. The most common hydroxybenzoic acids in fruits are *p*-hydroxybenzoic, protocatechuic, syringic and vanillic, which are components of complex structures such as hydrolysable tannins in red fruits, e.g., strawberries, blackberries, and raspberries.(Padmanabhan, Correa-Betanzo, & Paliyath, 2016) The hydroxycinnamic acids, on the other hand, consist mainly of p-coumaric, ferulic, caffeic and sinapic acids, which occur in bound form as glycosylated derivatives or esters of quinic, shikimic and tartaric acids. For example, chlorogenic or 5-caffeoulquinic acids result from combining both caffeic and quinic acids, and is found in many types of fruits such as blueberries and apples,(Padmanabhan et al., 2016) which both possess anti-atherogenic, and anti-cancer properties.(Luna-Guevara, Luna-Guevara, Hernández-Carranza, Ruíz-Espinosa, & Ochoa-Velasco, 2018)

Potential health effects

Even though the protective effects of phenolic acids are not understood yet, it is known that they may be linked to free radical scavenging (ROS) via the donation of hydrogen or electrons, or through singlet-oxygen extinction actions, which result in a stable compound formation. It was also found that phenolic

acids exhibited other biological activities, such as anti-inflammatory, anti-cancer, and anti-obesity properties. Previous studies found that the consumption of phenolic acids, related to decreased risk factors of cardiovascular diseases and diabetes.(Luna-Guevara et al., 2018) It is believed that phenolic acids can protect the cell membranes due to their ROS ability to interact with various biomacromolecules, and inhibit the activity of enzymes associated with CVDs and diabetes.(Rana, Samtiya, Dhewa, Mishra, & Aluko, 2022) A previous study reported that the hydroxycinnamic and hydroxybenzoic acids were able to inhibit pancreatic lipase.(Buchholz & Melzig, 2015) In addition, phenolic acids reduced serum levels of triglycerides and LDL-cholesterols, which are risk factors for the development of CVDs and dyslipidaemia.(Borochov-Neori et al., 2015; Chambers, Day, Aboufarrag, & Kroon, 2019) They were also found to be able to induce apoptosis of cancer cells, such as leukaemia, melanoma, colon cancer, etc.(Anantharaju, Gowda, Vimalambike, & Madhunapantula, 2016; Yang, Zhang, Qin, Luo, & Ren, 2022)

Flavonoids

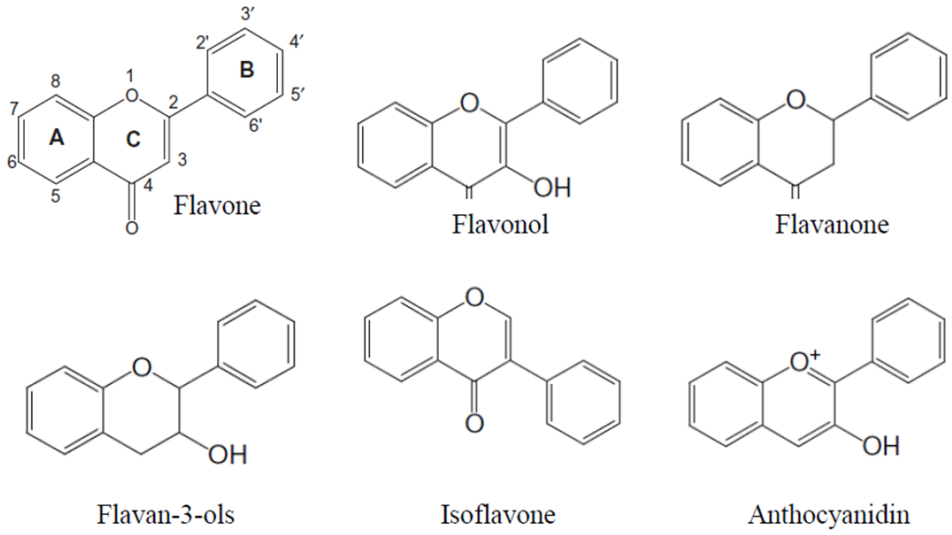


Figure 28 Structure of flavonoids

Source: (G. Ali, 2011)

Flavonoids are the largest group of phenolic compounds from plants, of which more than 8000 species were known by 2006. Flavonoids are similar to phenolic acids that originate from secondary metabolism of plants. They are synthesised via the phenylpropanoid pathway, which has phenylalanine as a substrate.

The structure of flavonoids consists of 15 carbon atoms arranged in three rings (C6-C3-C6) divided into two aromatic rings (A and B) and one heterocyclic pyrone ring (C), with C linked to B by a three-carbon bridge, as shown in the Figure 28. Flavonoids are divided into twelve subgroups, which are flavones, flavonols, dihydroflavonols, catechins, flavans, anthocyanidins, proanthocyanidins, isoflavonoids, 4-phenyl-coumarins, neoflavonoids, chalcones and dihydrochalcones, with Flavonols (3-hydroxyflavones) and flavones being the most common. Many types of food are rich in flavonoids, such as green leafy vegetables like spinach, lettuce, celery, broccoli, etc, which contain flavonols, flavones and flavone glycosides. Various spices such as rosemary, oregano, thyme, ginger, basil, etc., as well as seeds and certain beverages like green tea and red wine are also important sources.(A. M. A. Ali, El-Nour, & Yagi, 2018; Bernatoniene & Kopustinskiene, 2018) While flavanones, are found almost exclusively in grapes and citrus fruits. Flavanols are found in apricots, apples, pears, peaches, cocoa, chocolate products and broad beans.(S. Park, Kim, & Park, 2022; Yeh et al., 2021) Anthocyanidins are a type of flavonoid that are found in various red and purple fruits and vegetables, such as berries, cherries, red grapes, nectarines, red cabbage, and aubergines, etc., while isoflavonoids are also found in selected vegetables such as onions and peppers, and especially in legumes such as soybeans and chickpeas.(Luna-Guevara et al., 2018; Padmanabhan et al., 2016)

Potential health effects

Several in vivo and in vitro studies have suggested that the consumption of a diet rich in flavonoids, may be associated with a number of human health benefits. These compounds have anti-oxidant, anti-obesity, anti-atherogenic and other functional properties.(Padmanabhan et al., 2016) Flavonoids have been reported to be able to protect DNA-induced damage from environmental genotoxic agents, and also inhibits ROS- which produces enzymes, such as xanthine oxidase, nitric oxide synthase, and myeloperoxidase. Previous studies reported that flavonoids are able to inhibit cancer and tumour activity, particularly in the colon, liver, esophagus and breast cancer. The anti-cancer effects of flavonoids are associated with flavonoid-induced oxidative stress, which leads to the dysfunction of the mitochondria of cancer cells, and causes apoptosis of tumours and/or cancer

cells.(Reyes-Farias & Carrasco-Pozo, 2019) In addition, Flavonoids also ameliorate CVDs by its activity of anti-aggregatory, anti-hypertension, vasodilation, anti-fibrotic, and anti-hypercholesterolemia.(Cao et al., 2021; Ciumărnean et al., 2020; Córdoba et al., 2015) Other studies found that dietary flavonoids were able to control metabolic disorders in humans, thus showing anti-diabetic, neuroprotective, and anti-dyslipidaemia properties.(Calis, Mogulkoc, & Baltaci, 2020; Eid & Haddad, 2017) It was also found that flavonoids could help maintain a healthy digestive system.(Szoka, Nazaruk, Stocki, & Isidorov, 2021)

Tannins

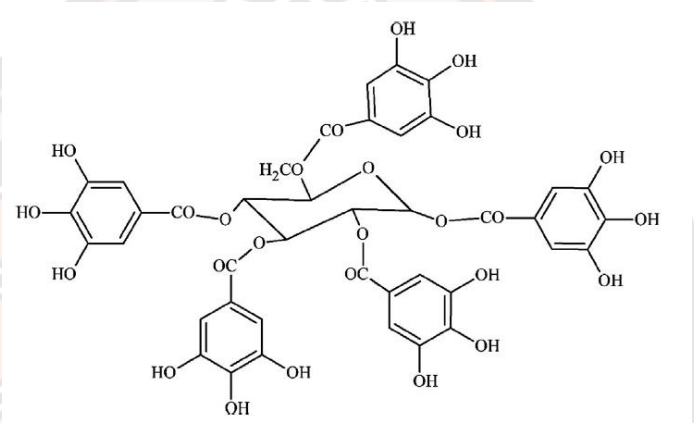


Figure 29 Structure of tannins

Source: (Luna-Guevara et al., 2018)

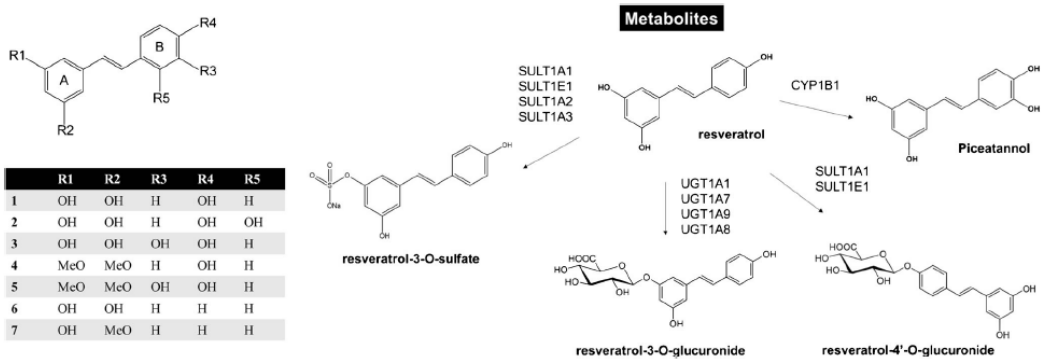
The flavonoids in some foods could polymerize into larger molecules, either by plant synthesis or food processes, which produce tannins. These compounds are found in more than 500 different plant species, where they accumulate in the roots, peel, fruits, and seeds. Leguminosae, Rosaceae, Polygonaceae, Rhizophoraceae, and Myrtaceae are all sources of tannins, which have large molecules and are a heterogeneous group of compounds, and have a molecular weight of up to 20,000 Da. They are a complex structure that consists of 12-16 hydroxyl groups in 5-7 aromatic rings as shown in Figure 29. However, some tannins are small and have molecular weights of 500 to over 3,000 Da. Tannins can bind to certain macromolecules such as carbohydrates and proteins through both covalent and non-covalent bonds, and they cause astringency to a number of foods. There are three main classes of tannins that are based on their monomer structures, which are gallic acid, phloroglucinol, and flavan-3-ol. Esterification reactions of gallic acids are bound to a central polyol core,

which are generally glucose or another carbohydrate form hydrolysable tannins, that are further classified as gallotannins and ellagitannins. Hydrolysable tannins are abundant in natural products and foods such as grapes, pomegranates, berries, red apples and wines. Phlorotannins are formed from the arrangement of phloroglucinol monomers, which have a molecular weight of 126 Da to 650 kDa and are further subdivided into eckols, phloroeckols, fucofuroeckols, fucols, fuhalols, and isofuhalols, and are also found in brown algae such as kelps and Ascophyllum nodosum or Sargassaceae species. Flavan-3-ol is the main monomer structure of condensed tannins, which are found in Schinopsis lorentzii, Acacia mollissima, grape seeds, pine and spruce bark. While phlobatannins are found in kola nut, chocolate liquor, and the red skins of peanut. Plants can accumulate tannins in various parts such as seeds, leaves, fruits, and roots. The concentration of tannins in vegetables and fruits can be influenced by edaphoclimatic conditions, plant genetics, plant maturity, or the ripening process, among other things.(Luna-Guevara et al., 2018)

Potential health effects

Epidemiological research has shown that the consumption of fruit and vegetables can help to prevent CVDs, dyslipidemia and cancer. Previous studies found that tannins can treat skin inflammation and injury, and help to delay and/or prevent CVDs. (J. B. Chang, Lane, Yang, & Heinrich, 2016; Macho-González et al., 2018) The potential health effects of tannins depend on their size and absorption ability. The large complex compounds cannot be absorbed, while the small molecules of tannins are able to be absorbed into the intestines. Previous studies found that the un-absorbable complex structures with binding properties may produce local effects in the gastrointestinal tract, which impacts the induction of the intracellular signalling pathways, and the modulation of genes.(Desrues, Mueller-Harvey, Pellikaan, Enemark, & Thamsborg, 2017; Tedeschi et al., 2021) The small tannin molecules that can be absorbed into the small intestine have also been reported to have other physiological effects, such as increasing blood clotting, lowering blood pressure and serum lipid levels, and modulating immune responses.(Luna-Guevara et al., 2018)

Stilbenes



	R1	R2	R3	R4	R5
1	OH	OH	H	OH	H
2	OH	OH	H	OH	OH
3	OH	OH	OH	OH	H
4	MeO	MeO	H	OH	H
5	MeO	MeO	OH	OH	H
6	OH	OH	H	H	H
7	OH	MeO	H	H	H

Figure 30 Structure of stilbenes compounds and metabolization of resveratrol

Source: (Chou, Ho, & Pan, 2018)

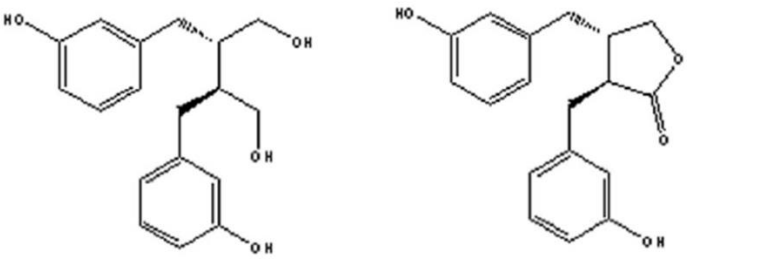
The structure of stilbenes contains two benzene rings that bind an ethanol or ethylene molecule, which has a skeleton that consists of 14-carbons and forms two stereoisomers, cis and trans, as shown in Figure 30. The formation of the stilbenes trans-isometric structures are usually found in natural products and foods. Currently, more than 400 different types of stilbenes are known. They are biosynthesised via the phenylpropanoid pathway, which is also responsible for the biosynthesis of primary and secondary metabolites such as flavonoids, coumarins, hydrolysable tannins, lignans, lignins, etc. They also possess anti-pathogens, such as anti-bacterial, anti-fungal, nematocidal and insecticidal properties in plants. Stilbenes are found in the plant families such as, Vitaceae, Leguminaceae, Gnetaceae, and Dipterocarpaceae. In foods such as grapes and berries,(Padmanabhan et al., 2016) stilbenes are present as *cis*- and *trans*-isomeric forms of resveratrol, which are mostly glycosylated. Resveratrol, pterostilbene and piceatannol are subcategories of stilbenes that are normally found in foods.

Potential health effects

Previous studies have shown that the resveratrol has potent biological properties such as anti-oxidants, anti-inflammatory, anti-ageing, anti-allergenic, anti-carcinogenic, and anti-mutagenic activities.(Padmanabhan et al., 2016) Another study reported that resveratrol was able to increase apoptosis of cancer cells, which increased tumour necrosis factor alpha (TNF-α) activity, and reduced NF-κB activity. In vivo studies have shown that the stilbenes are able to reduce lipid

accumulation, control glucose homeostasis, alleviate inflammation, and modulate the gut microbiota in the small intestine. Stilbenes are able to prevent obesity by regulating lipid metabolism, such as de novo lipogenesis, adipogenesis, and lipolysis.(Toma et al., 2020)

Lignans



Enterodiol Enterolactone

Figure 31 Chemicals structure of mammalians lignans

Source: (J. B. Park, 2014)

Lignans are small molecules of phenolic compounds that are water-soluble, which cannot be digested in the small intestine. The lignans in plants are derived from phenylalanine (pinoresinol, lericiresinol, secoisolariciresinol, syringaresinol, or sesamin), which interacts between the primary and secondary metabolism of plants. The metabolism of phenylalanine plays an important role in channelling carbon from photosynthesis to the biosynthesis of phenylpropanoids. Lignans accumulate in the roots, rhizomes, stems, leaves, seeds, and fruits of more than 70 different families of vascular plants, from bryophytes and ferns, to perennial plants. They can be converted by the gut microbiota in the colon into enerodiol and enterolactone, which are known as mammalian lignans, as shown in Figure 31. According to the pathway through which the oxygen is incorporated into the carbon structure and the cyclisation pattern, lignans are divided into eight classifications, which are furofuran, furan, dibenzylbutane, dibenzylbutyrolactone, aryltetralin, arylnaphthalene, dibenzocyclooctadiene, and dibenzylbutyrolactol. In contrast, they can be divided into 5 classifications depending on their structure: Lignans, Neolignans, Norlignans, Hybrid Lignans and Oligomeric Lignans. Structurally, lignans consist of two phenylpropane dimers linked by β-β'-bonds between the central

atoms of the corresponding side chains, usually located in position 8, and they may be linked to other C-C carbons, which are called neolignans. Lignans are compounds found in several foods, such as grains, nuts, seeds, vegetables, and beverages such as tea, coffee, or wine.(Luna-Guevara et al., 2018) While flaxseed is probably the richest source of lignans, containing 100 times the concentration compared to other foods, such as pumpkin seeds, sesame, cranberries, and tea.(J. B. Park, 2014) Among the various fruits, lignans are mainly found in kiwi, while they are found to a lesser extent in apricots, strawberries, peaches, pears, nectarines, raisins, grapefruit, cherries, oranges, and grapes.

Potential health effects

Enerodiol and enterolactone are formed in the intestine, and are absorbed into the liver from the blood stream, where they are conjugated with glucuronic acid or sulphate. Both enerdiol and enterolactone are known to be conjugated by various human and animal body fluids, mostly plasma and urine, where they are usually combined with glucuronide. Both compounds possess anti-oxidants properties due to their catechol-like structure, which can be reversibly oxidised to an O-quinone structure upon oxidation. A catechol-like structure is usually found in anti-oxidants compounds involved in the production of ROS in cells.(Dai, Wang, Fan, Du, & Zhou, 2018; Pinnataip & Lee, 2021) A previous study found that lignans in wheat may have a protective effect against cancer.(Majdalawieh & Mansour, 2019; Parikh et al., 2019) It was also found that lignans can increase cell apoptosis in rats given the carcinogen azoxymethane, which also has an anti-cancer effect.(Bommareddy, Arasada, Mathees, & Dwivedi, 2006) Enterolactone is used as a predictive biomarker in clinical trials of breast, prostate, and colon cancer and is also used to reduce the proliferation of colon cancer. Another study found that the consumption of a diet, rich in lignans may help to reduce oestrogen-dependent diseases, such as breast adenocarcinoma. Other studies found that a flaxseed-supplemented diet, rich in secoisolariciresinol diglucoside, a lignan compound, increased apoptosis in tumour cells, decreased CYP3A4 activity, and reduced tumour aggressiveness and invasiveness through decreased levels of 16-hydroxyestradiol. Consumption of flaxseed lowers serum levels of glucose and insulin, i.e., it improved insulin resistance

in mice.(Shayan, Kamalian, Sahebkar, & Tayarani-Najaran, 2020; Villarreal-Renteria et al., 2022)

Pineapple

Pineapple or Ananas comosus (L.) Merr. is the third tropical fruit after banana, and mango in terms of worldwide production.(Léchaudel, Darnaudery, Joët, Fournier, & Joas, 2018) It is widely grown in USA, Kenya, Malaysia, Philippines, South Africa, India, China, and Thailand (Mohamad et al., 2015). According to the FAO online database, the total amount of pineapples exported worldwide in 2020 was approximately 2.9 million tonnes. Currently, there are more than 130 genotypes of pineapples worldwide, and Thailand has more than 14 of them, including Nang Lae, Pattawia, Phuket, MD2, Phetchaburi 1, Phetchaburi 2, Singapore Spanish (Indrachit Daeng), Selangor green or Green Spanish (Indrachit Khao), Sriracha, Phu-Lea, Huay Mun, and Phuchawa. Pineapple is a sweet friut and rich in nutrients including calcium, potassium, vitamin C, vitamin A, and flavonoid.(El-Shazly et al., 2018; Lu, Sun, Wu, Liu, & Sun, 2014) Pineapple flesh is a rich source in bromelain which acts as an anti-oxidants,(El-Shazly et al., 2018) anti-inflamatory, anti-microbial, and anti-cancer.(Rathnavelu, Alitheen, Sohila, Kanagesan, & Ramesh, 2016) Interestingly, pineapple contains eight phenolic compounds, including gallic acid, gentisic acid, syringic acid, vanillin, ferulic acid, sinapic acid, isoferulic acid and o-coumaric acid, which exhibits an anti-oxidants activity and an anti-inflammatory function.(El-Shazly et al., 2018) Previous studies have reported that pineapple possesses anti-obesity, anti-oxidants, and hepatoprotective properties. (El-Shazly et al., 2018; González-Peña et al., 2017; Mohamad et al., 2015)

Morphology of Pattavia pineapple

Pattavia is a cultivar of pineapple in the Smooth Cayenne pineapple family which has a mixed sweet and sour flavour, it is very popular to plant, consume, and is used for canning. Pattavia weighing approximately 1.8 to 4.5 kilograms with green skin, and turns into orange-yellow skin when it matures. The pattavia has shallow eyes, and a cylindrical shape.(Dittakan, 2018) Pattavia is a rich source in scorbic acid, total phenolic compound, and flavonoid which is anti-oxidants property.

In vitro previous study

Pineapple inhibited cholesterol absorption and cholesterol biosynthesis by inhibition of HMG-CoA reductase is a rate-limiting enzyme for cholesterol biosynthesis.(Duangjai, Ingkaninan, & Limpeanchob, 2011) Pineapple inhibited pancreatic lipase activity and the solubility of cholesterol in lipid micelles which potentially inhibits lipid digestion in the small intestine and is consequently helpful in controlling body weight and serum lipid levels.(Kanittaporn Trisat, 2016)

In vivo previous study

Pineapple inhibited de novo lipogenesis in the liver and activated fatty acid β-oxidation in the muscle. It is effects exhibited significant down-regulation of sterol regulatory element-binding protein 1 (SREBP-1), a transcription factor that regulates fatty acid synthesis. In addition, pineapple down-regulated fatty acid synthase (FAS) expression is an enzyme protein that catalyses fatty acid synthesis, and the up-regulation of Carnitine palmitoyltransferase 1 (CPT1). CPT1 is an enzyme that catalyses acyl-CoA through the outer membrane of the mitochondria,(El-Shazly et al., 2018) causing a reduction in the hepatic and serum lipid levels. Furthermore, Pineapple reduced serum levels of AST, ALT, and TG. (Mohamad et al., 2015; Yantih, Harahap, Sumaryono, Setiabudy, & Rahayu, 2017)

CHAPTER III

RESEARCH METHODOLOGY

Materials

Chemicals and antibodies

All the chemicals and antibodies that were used in this study are listed below in Table 7 and Table 8, respectively.

Table 7 The chemicals used in this study.

30% acrylamide/Bis solution 29:1	Bio-Rad, USA
Ammonium persulfate (APS)	Merck KGaA, Germany
Bovine serum albumin (BSA)	GinbH, Austria
Chloroform	RCI Labscan limited, Thailand
Glycine	Bio-Rad, USA
2X Laemmli sample buffer	Bio-Rad, USA
Magnesium chloride ($MgCl_2$)	Ajex Finechem, Australia
Methanol	RCI Labscan limited, Thailand
Potassium dihydrogen phosphate (KH_2PO_4)	Ajex Finechem, Australia
Potassium chloride (KCl)	Ajex Finechem, Australia
Sodium chloride (NaCl)	Ajex Finechem, Australia
Sodium phosphate dibasic (Na_2HPO_4)	Ajex Finechem, Australia
Sodium Dodecyl Sulphate (SDS)	Merck KGaA, Germany
Tris (hydroxymethyl) aminomethane	Bio-Rad, USA
10X Triton	Bio Basic inc, USA
N,N,N',N'-tetramethylethylenediamine(TEMED)	Bio-Rad, USA
TWEEN	Sigma-Aldrich Chemic GmbH, UK
30% acrylamide/Bis solution 29:1	Bio-Rad, USA

Table 8 Antibodies

Agent	Company
Anti-beta actin (β-actin)	Cell signaling technology, USA
Anti-cholesterol 7 alpha-hydroxylase (CYP7A1)	Merck KGaA, Germany
Anti-HMG-CoA reductase (HMGCR)	Merck KGaA, Germany
Anti-LDLR polyclonal	Thermo fisher Scientific, USA
Goat anti-mouse IgG, (H+L) HRP conjugate	Merck KGaA, Germany
Goat anti-rabbit IgG, (H+L) HRP conjugate	Cell signaling technology, USA

Animals

Twenty-four male Sprague–Dawley rats weighing between 180–200 g were purchased from the Nomura Siam International Co.,Ltd., Lumpini, Pathumwan, Bangkok, Thailand. All the rats of this study were housed in the animal room of the Centre for Animal Research, Naresuan University at a constant temperature of 22±1°C, with a 12 hrs. light-dark cycle and fed on a standard diet and water ad libitum. After acclimation for one week, All the rats were randomly divided into four experimental groups for use in the experiment.

The protocol of the study was approved by the institution animal care and committee of Naresuan University (NU-AE610409).

Method

1. **Induction of an animal model of hypercholesterolemia and drug treatment**

Hypercholesterolemic (HC) rats were induced by feeding a high-cholesterol diet (HCD) that consist of 1.5% cholesterol and 0.37% cholic acid (as previously described (Seenak, Kumphune, Malakul, Chotima, & Nernpermpisooth, 2021)) for 8 weeks.

2. **Pineapple preparation**

Pineapple materials were sliced, peels and the eyes were cut out of the pineapple, chopped into small pieces, weighed approximal 4500 g of fresh pineapple,

and then dried at 60 °C in hot air oven for 4 days that gave product of dried pineapple for 600 g, which is gave a yield of 13.33% (w/w). After that, dried pineapple was mashed to a crude extract and weighed before being stored in the desiccator. Before the crude pineapple extract is analysed, it was dried under a vacuum.

3. PA and simvastatin treatment

Powdered Pineapple (PP) at a dose of 200 mg/kg BW and simvastatin (Sim) at the dose of 40 mg/kg BW were freshly-prepared in distilled water for oral administration daily in HC rats, in which the amount received per day were calculated from body weight each week.

4. Experimental animal designs

After an acclimatization period of 1 week, the rats were randomly divided into 4 groups including:

1. Control rats (Control) were received a standard diet for 8 weeks.

2. High cholesterol diet-fed rats (HCD) were received a high-cholesterol diet for 8 weeks.

3. HCD rats plus powdered pineapple (HCD+P) were received a high-cholesterol diet and 200 mg/kg/BW of dried pineapple for 8 weeks.

4. HCD rats plus simvastatin (HCD+S) were received a high-cholesterol diet and simvastatin at a dose of 40 mg/kg BW for 8 weeks.

The rats were orally administered test substances once daily at the same time, and received HCD every day for 8 weeks. Food intake in each rat group were recorded daily and the body weight were measured once a week through the experimental period. After 8 weeks feeding period, rats were anesthetized with an intraperitoneal injection with sodium thiopental (50 mg/kg BW) and blood samples were collected from the thoracic cavity after removal of the rat's heart. The collected blood sample were centrifuged at 1000 xg for 20 min at 4°C to obtain serum, which were stored at −20°C for analysis of the lipid profile. Serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) levels were measured by using colorimetric assay kits and low-density lipoprotein (LDL) level was calculated using the formula of Friedewald et al. (1972). The liver tissues were removed immediately and half of each of both organs were placed in 10% neutral formalin and fixed for histopathological changes by using haematoxylin and eosin (H&E) staining and

Hepatic lipid accumulation by using oil red o staining. In addition, the liver tissues were frozen stored in liquid nitrogen at –80 ˚C for analysis of hepatic lipid levels and western blot analysis.

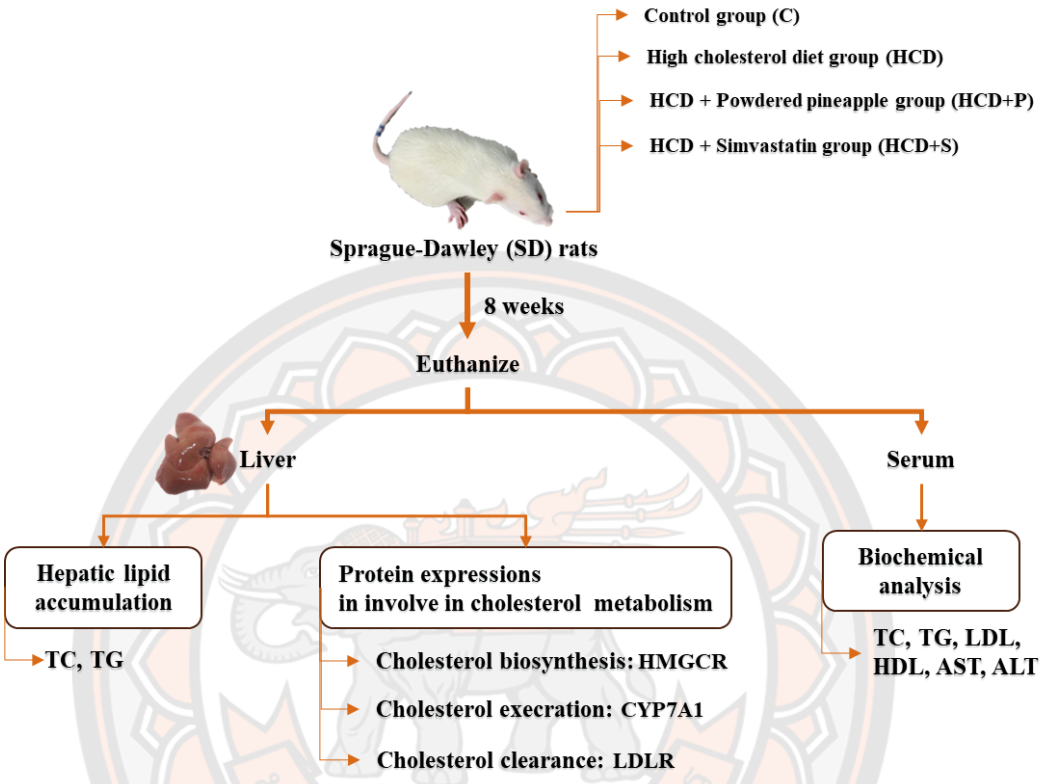


Figure 32 The experimental designs of this study.

5. Detection of serum and hepatic lipid levels

TC and TG levels were be determined by using colorimetric assay kits in hepatic lipid homogenate for the indication of hepatic lipid contents. Serum was measured TC, TG, and HDL levels by using colorimetric assay kits, which LDL level was calculated using the formula of (Friedewald, Levy, & Fredrickson, 1972) for determine lipid accumulation in the circulation system.

LDL = TC-HDL-(TG/5)

(Iqbal et al., 2022)

5.1 The preparation of lipid extraction in liver

Total liver lipids were extracted according to a modified method by Folch et al. (Löfgren, Forsberg, & Ståhlman, 2016) Liver tissues were cut into small pieces on ice by using a clean scalpel blade. 100 mg each of liver tissue were

homogenized in 200 µl of PBS solution (Phosphate-buffered saline solution) and then were added 2 ml of solvent mixture (chloroform /methanol 2:1; v/v) with a tube homogenizer until fully lysed. The homogenates were incubated for 2 hrs. on ice, and then centrifuged at 1650 xg for 20 minutes at 4 °C. The mixed sample was separated into 3 phase layers: the upper methanol layer, the middle protein disc, and the bottom chloroform and lipid. The bottom chloroform and lipid were transferred to a new glass tube on ice. The supernatant in the first tube was mixed with 1500 µl of chloroform and 600 µl of MgCl2, vortex for 1 minute, incubated for 30 minutes on ice, and then centrifuged at 1650 xg for 20 minutes at 4 °C. The mixed sample of the first tube were separated into 3 phase layers. The bottom phase of the first tube was mixed with the bottom phase of the second glass tube. The sample lipid homogenates were dried using nitrogen gas and resolubilized in 1 ml of chloroform with 1% (v/v) triton X-100, dried again and then were resuspended in 1 ml of distilled water.

5.2 Measurement of TC level

The serum and hepatic total cholesterol (TC) levels were determined enzymatically using a commercial kit (Human, Germany) according to the manufacturer's instructions. In this assay cholesterolester are converted to cholesterol and free fatty acids by cholesterolesterase. The cholesterol is further oxidized to cholestane-3-one and hydrogen peroxide by cholesteroloxidase. Peroxidase catalysed the reaction of dihydrogen peroxidase, 4-aminophenazone and phenol to produce a quinonimine dye with a pink colour that an absorbance maximum at 500 nm. The reaction sequence is as follows:

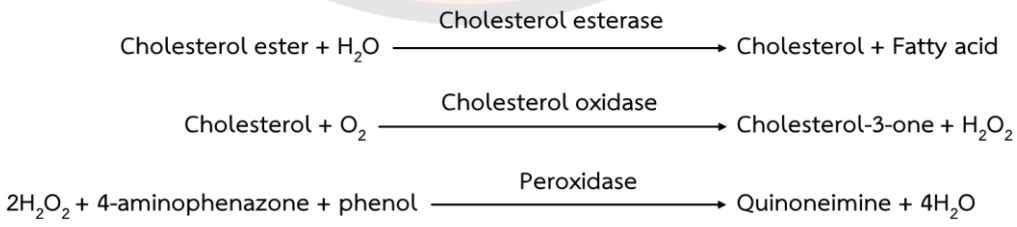


Figure 33 The reaction sequence of TC measurement.

Reagent:

Enzymatic reagent (Phosphate buffer (pH 6.5), 4 – aminoantipyrine, Phenol, Peroxidase, Cholesterolesterase, Cholesteroloxidase, Sodium Azide)

Cholesterol Standard (200 mg/dl of cholesterol and sodium azide)

Procedure:

3 µl of serum or hepatic lipid homogenates (1:10 dilution in ddH2O) were mixed with 150 µl of enzymatic reagent The reaction mixture was incubated at 37 °C for 5 minutes and absorbance of the pink colour formed was read at 500 nm in spectrophotometer (Biotex, USA) against a reagent blank.

Standard absorbance of triglyceride (200, 100, 50, and 25 mg/dl) were used to calculate the amount of TG levels in the samples and results were expressed as mg/dL serum or mg/mg liver.

5.3 Measurement of TG level

The serum and hepatic triglyceride (TG) levels were determined enzymatically using a commercial kit (Human, Germany) according to the manufacturer's instructions. In this assay TG are converted to glycerol and free fatty acids by lipase. The glycerol-3-phosphate is further oxidized to dihydroxyacetone phosphate and hydrogen peroxide by glycerol-3-phosphate oxidase. Peroxidase catalysed the reaction of hydrogen peroxidase and 4-aminophenazone to produce a quinonimine dye with a pink colour that an absorbance maximum at 500 nm. The reaction sequence is as follows:

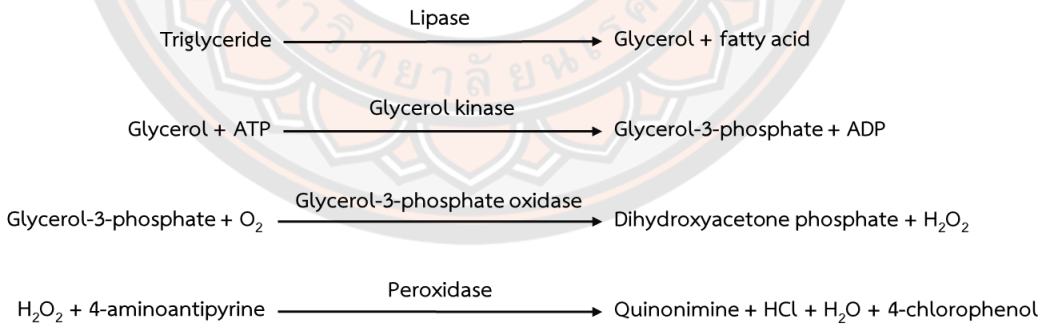


Figure 34 The reaction sequence of TG measurement.

Reagent:

Monoreagent (PIPES (pH7.5), 4-chloropnenol, 4-aminophenazone, magnesium ions, ATP, lipase, peroxidase, glycerol kinase, glycerol-3-phosphate oxidase, sodium azide)

Triglyceride standard (200 mg/dl)

Procedure:

Hepatic lipid homogenates (1:10 dilution in ddH_2O) or 3 µl of serum were mixed with 150 µl of monoreagent. The reaction mixture was incubated at 37 °C for 5 minutes and absorbance of the pink colour formed, was read at 500 nm in spectrophotometer (Biotex, USA) against a reagent blank.

Standard absorbance of triglyceride (200, 100, 50, and 25 mg/dl) were used to calculate the amount of TG levels in the samples and results were expressed as mg/dL serum or mg/mg liver.

5.4 Measurement of serum HDL level

The serum high-density lipoprotein (HDL) levels were determined enzymatically using a commercial kit (Human, Germany) according to the manufacturer's instructions. This assay combines 2 steps including: (1) eliminating chylomicrons, very low-density lipoprotein (VLDL) and LDL by specifically enzymatic reaction (2) determines the remaining HDL by the well-known enzymatic reaction cascade cholesterol esterase, cholesterol oxidase and peroxidase. In the final reaction, hydrogen peroxide oxidases a chromogen (N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline) under the catalytic action of peroxidase. The resulting colour change indicates an absorbance maximum at 593 nm. The reaction sequence is as follows:

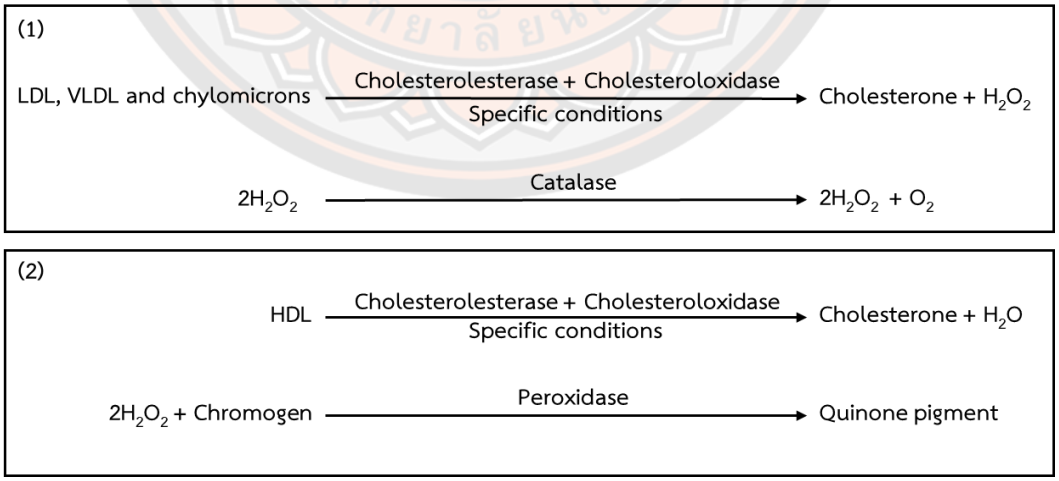


Figure 35 The reaction sequence of HDL measurement.

Procedure:

Mix 5 µl of serum with 225 µl of enzyme reagent in well. The reaction mixture was incubated at 37 °C for 5 minutes. After that, the serum and enzyme

reagent added in well was mixed with 75 µl of substate. The reaction mixture was incubated at 37 °C for 5 minutes and absorbance of the changed colour formed was read at 593 nm in spectrophotometer (Biotex, USA) against a reagent blank.

Standard absorbance of calibrator (56.4, 28.2, 14.1, and 7.05 mg/dL) was used to calculate the amount of HDL levels in the samples and the results were expressed as mg/dL serum.

6. Determination of the expression of proteins involved in lipid metabolism in liver tissues

To study the effect of PA on hepatic lipid accumulation was indicated by the protein expression levels involved in lipid metabolism including: HMGCR, CYP7A1 and LDLR. The expressions of protein levels were determined by western blotting.

6.1 Preparation of liver homogenates

Liver tissues were cut into small pieces on ice by using a clean scalpel blade. Tissue pieces (100 mg each) were homogenized in 300 µl of cold RIPA lysis buffer with a 1% protease inhibitor cocktail with a glass homogenizer until fully lysed. The homogenates were incubated for 30 minutes on ice, and then centrifuged for 30 minutes at 20,000 xg at 4°C. The resulting supernatants were transferred to a new tube for protein analysis.

6.2 Measurement of protein concentrations

The total protein level of liver tissues homogenate was determined by a bicinchinonic acid (BCA) assay kit (Merck KGaA, Germany) according to the manufacturer's instructions. This assay is based on a biuret reaction for the colorimetric detection, and this reaction combines the reduction of cupric ion (Cu2+) to cuprous ion (Cu+) by protein in an alkaline medium. The chelation of BCA with the Cu+ leads to the production of purple-coloured reaction complex which exhibits a strong absorbance at 562 nm. The reaction sequence is as follows

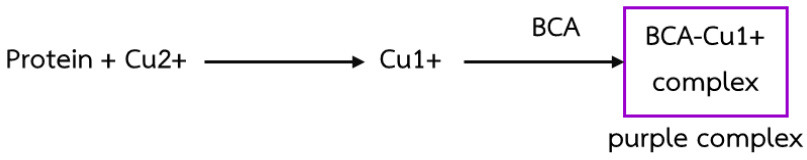


Figure 36 The reaction sequence of the levels of porteins measurement.

Reagent:

BCA solution (bicinchoninic acid, sodium carbonate, sodium tartrate, and sodium bicarbonate in 0.1 M NaOH pH11.25)

4% Cupric sulphate

BSA Standard (2 mg/ml)

Procedure:

25 µl of liver tissues homogenate (1:200 dilution in PBS) were mixed with 200 µl of BSA working reagent (BCA solution: 4% cupric sulphate; 49:1). The reaction mixture was incubated at 37°C for 30 minutes and then absorbance of the purple colour formed were read at 562 nm in spectrophotometer (Biotex, USA).

Standard absorbance of bovine serum albumin (1, 0.5, 0.25, 0.125, and 0.0625 mg/ml) were used to calculate the amount of total protein in the samples and results were expressed as mg/ml.

6.3 Western blot analysis

Protein expression levels of HMGCR, CYP7A1, and LDLR in the liver tissues were determined. 80 ug proteins of each sample of liver homogenate (80 µg proteins) was heat denatured in a loading buffer, sample: loading buffer (1:1) 20 µl. Samples were separated by 12.5% (w/v) sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in running buffer into two steps: first step is stacking, which was 80 volts for 20 minutes and the second step is separating, which was use 100 volts for 1 hr. After electrophoresis, proteins were transferred to polyvinylidene fluoride (PVDF) membrane using a Trans-Blot® wet electrophoretic transfer cell (Bio-Rad laboratories, USA) at 100 volts for 2 hrs. The membrane was incubated for 1 hr. with blocking buffer at room temperature. After washing 4 times for 7 minutes with washing buffer, the membranes were subsequently incubated with the appropriate primary antibodies (Table 9) diluted in diluent primary buffer at 4°C for 15 hrs. The membrane was washed 3 times for 5 minutes and then incubated with appropriate secondary antibody (Table 10) conjugated to horse-radish peroxidase (HRP) in diluent secondary buffer at room temperature for 1 hr. After that, the membrane was washing 3 times for 5 minutes and detected using enhanced chemiluminescence (ECL) (Bio-rad, USA) detection system and assayed by image lab™ software (Bio-Rad Laboratories, Hercules, CA, USA).

The levels of protein expression in each lane were determined by normalizing protein band intensity to actin band intensity. Data were expressed as a percentage of the control.

Table 9 Primary antibodies in this study

Agent	Dilution	Species	Company
Anti-beta actin	1:5000	Rabbit	Cell signaling technology, USA
Anti-cholesterol 7 alpha-hydroxylase (CYP7A1)	1:2500	Mouse	Merck KGaA, Germany
Anti-HMG-CoA reductase (HMGCR)	1:2500	Mouse	Merck KGaA, Germany
Anti-LDLR polyclonal antibody	1:2500	Rabbit	Thermo fisher Scientific, USA

Table 10 Secondary antibodies in this study

Agent	Dilution	Species	Company
Goat anti-mouse IgG, (H+L) HRP conjugate	1:5000	Mouse	Merck KGaA, Germany
Goat anti-rabbit IgG, (H+L) HRP conjugate	1:5000	Rabbit	Cell signaling technology, USA

7. Statistical analysis

Data were expressed by mean ± means ± SEM. The data were analysed by one-way analysis of variance coupled with the Tukey's test where appropriate with the use GraphPad Prism version 8. Statistical significance was accepted for $p < 0.05$.

CHAPTER IV

RESULTS

Effects of powdered pineapple on the body weight and organ weight of the HCD-induced hypercholesterolaemic rats.

As shown in Table 11 and Figure 37, the HCD group had a significantly higher body weight than the control group from week 5 to 8. Daily oral administration of both powdered pineapple and simvastatin for 8 weeks resulted in a significant reduction in the body weight from week 7 to 8. At week 8, the liver weight, and the ratio of liver to body weight of the HCD group were significantly higher than those of the control group by 149.49% and 122.27%, respectively, (Table 12 and Figure 38). In pineapple and simvastatin-treated groups, these levels were decreased compared to the HCD group (33.20% and 30.25% for liver weight, and 28.07% and 21.05% for the ratio of liver to body weight in pineapple and simvastatin-treated groups, respectively, vs HCD group). (Table 12 and Figure 38). However, there was no significant differences in daily food intake among the groups (Table 12). These results suggest that powdered pineapple decrease the body weight, liver weight, and the ratio of liver to body weight in HCD-induced hypercholesterolaemic rats.

Table 11 The body weight of all groups of rats for 8 weeks of the experimental period.

	Body weight (g) Mean ± SEM								
Weeks	0	1	2	3	4	5	6	7	8
C	271.29± 25.3	324.29± 17.3	375.57± 14.7	418.57± 8.7	451.43± 5.7	472.86± 6.1	496.86± 6.1	525.43± 8.0	552.57± 9.5
HCD	277.17± 26.8	345.50± 21.4	403.50± 15.3	454.00± 8.9	496.33± 3.1	533.67± 3.2	556.17± 11.1*	593.00± 6.3**	626.67± 9.7**
HCD+ P	298.20± 31.8	331.20± 27.5	389.40± 15.6	424.00± 9.8	454.80± 8.5	480.40± 9.3	510.00± 16.1	529.00± 15.2$^{\#}$	561.00± 16.8$^{\#}$
HCD+ S	271.80± 30.3	321.20± 22.3	373.80± 19.4	410.60± 8.7	450.40± 4.3	479.60± 6.7	507.60± 9.2	533.40± 11.1$^{\#}$	562.60± 9.9$^{\#}$

Results are shown as the mean ± SEM (n=5-6 rats). **p<0.01 vs. C group; $^{\#\#}$p<0.01 vs. HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin Treatment group.

Table 12 Effects of daily consumption of powdered pineapple for 8 weeks on food intake, liver weight, and the ratio of liver weight to body weight in the HCD-fed rats.

	C	HCD	HCD+P	HCD+S
Food intake (g/week)	27.05±0.35	29.55±0.65	28.18±0.87	27.86±0.78
Liver weight (g)	13.98±0.8	34.88±2.0**	23.30±1.7**,##	24.33±2.0**,##
Liver weight to body weight ratio (g/g)	0.026±0.001	0.057±0.005**	0.041±0.004*,#	0.045±0.004**

Results are shown as the mean ± SEM (n=5-6 rats). **p<0.01 vs. C group; ##p<0.01 vs. HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

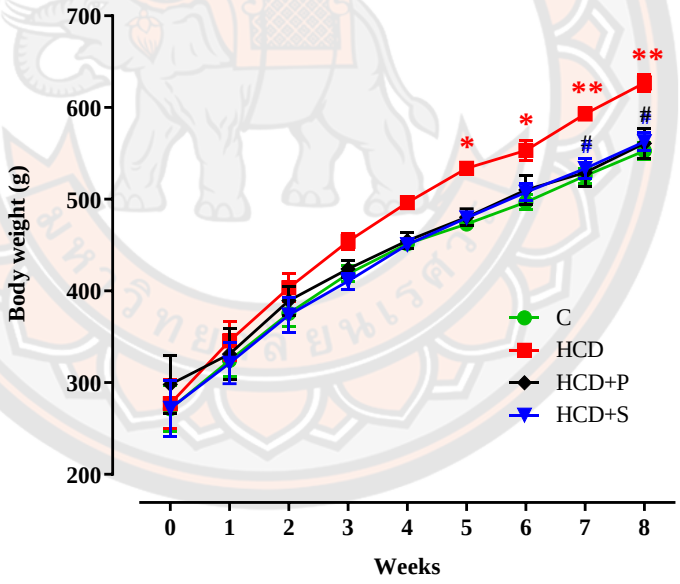


Figure 37 Effects of powdered pineapple consumption on body weight during different weeks in rats fed with normal and high cholesterol diets (HCD). Data are presented as mean ± SEM of 5-6 rats in each group. **p < 0.01, *p < 0.05 vs. the C group and ##p < 0.01, #p < 0.05 vs. the HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

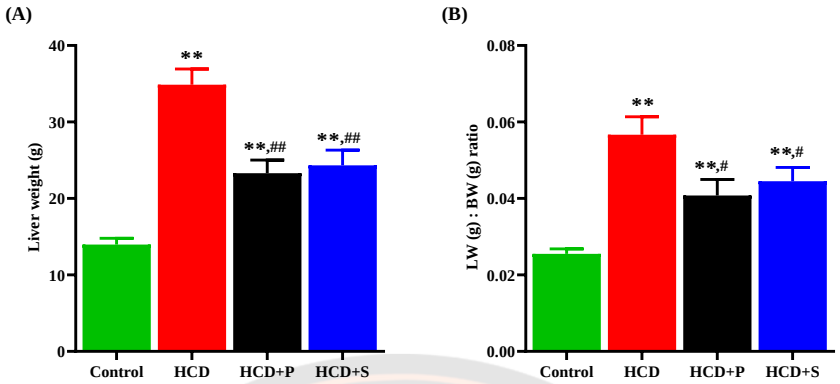


Figure 38 Effects of powdered pineapple consumption on liver weight and liver to body weight ratio in normal and high cholesterol diets (HCD) rats. Liver weight (A) and liver to body weights ratio (B) in all groups of rats. Data are presented as mean ± SEM of 5-6 rats in each group. **p < 0.01, *p < 0.05 vs. the C group and $^{\#\#}$p < 0.01, $^{\#}$p < 0.05 vs. the HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

Effects of powdered pineapple on serum lipid levels and liver function tests in HCD-fed rats.

As shown in Table 13 and Figure 39A-D, compared to the control group, the HCD significantly increased the serum levels of TG, TC, and LDL-C, with a concomitant reduction in serum HDL-C level.Pineapple or simvastatin treatment in the HCD-fed rats resulted in a significant reduction in the TC, TG, LDL-C levels, but caused no significant change in serum HDL-C level compared with the HCD group . In addition, the serum levels of AST and ALT were significantly increased in the HCD group compared with the control group (Figure 39 and Table 13). In pineapple and simvastatin-treated groups, these levels were decreased compared to the HCD group (60.29 % and 38.74 % for AST, and 43.36 % and 48.34 % for ALT in pineapple and simvastatin-treated groups, respectively, vs HCD group). These results suggest that pineapple reduce the release of transaminase due to hepatocyte injury in hypercholesterolemic rats.

Table 13 Effects of powdered pineapple consumption on serum biochemical parameters in HCD-fed rats.

Parameters	Mean ± SEM			
	C	HCD	HCD+P	HCD+S
TG (mg/dL)	23.00±1.78	36.42±4.01**	25.53±2.40$^{\#}$	19.75±1.03$^{\#\#}$
TC (mg/dL)	54.92±0.87	195.40±19.45**	123.50±11.72**,$^{\#\#}$	123.00±9.21**,$^{\#\#}$
LDL (mg/dL)	9.00±1.34	117.80±10.51**	81.50±4.92**,$^{\#\#}$	69.50±8.51**,$^{\#\#}$
HDL (mg/dL)	40.00±.00	26.75±2.87**	33.00±0.71	36.00±3.34
AST (U/L)	166.80±4.96	372.20±21.33**	147.80±26.10$^{\#\#,+}$	228.00±8.42*,$^{\#\#}$
ALT (U/L)	42.00±0.41	120.50±4.41**	68.25±5.95**,$^{\#\#}$	62.25±8.42**,$^{\#\#}$

Results are shown as the mean ± SEM (n=5-6 rats). **p<0.01 vs. C group; $^{\#\#}$p<0.01 vs. HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

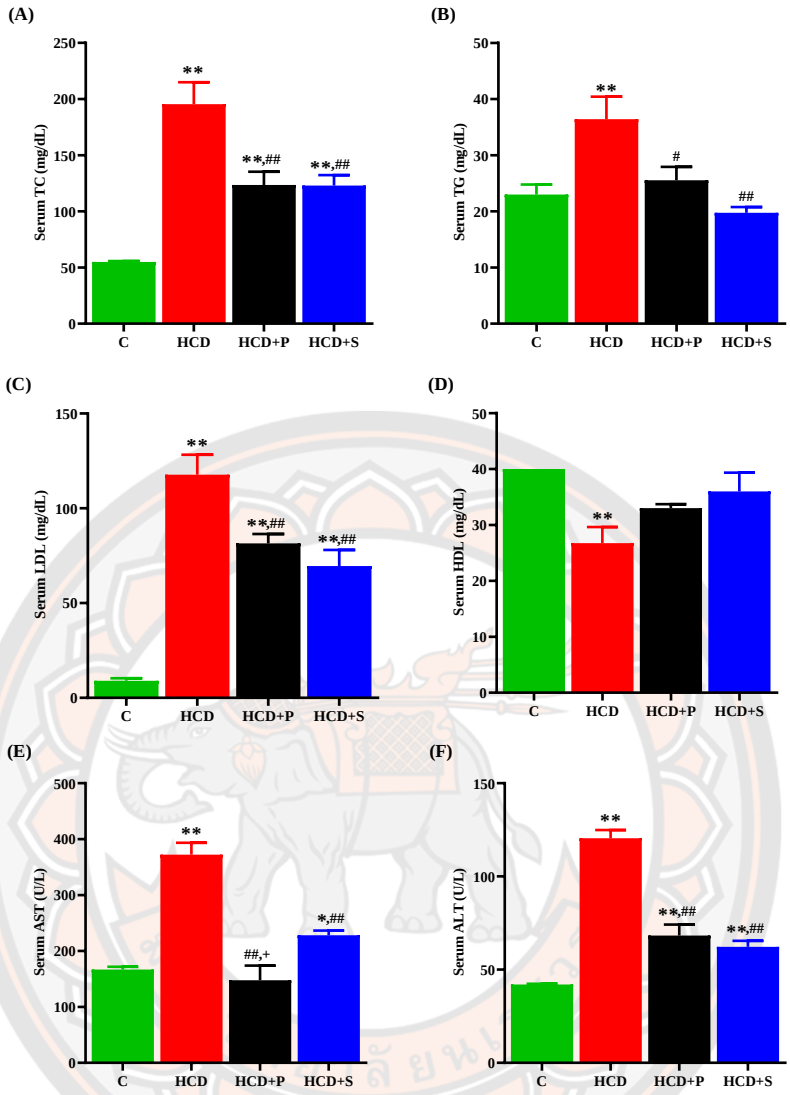


Figure 39 Effect of powdered pineapple consumption on serum biochemical parameters in HCD-fed rats. The serum levels of (A) total cholesterol (TC); (B) triglycerides (TG); (C) low-density lipoproteins (LDL); (D) high-density lipoproteins (HDL); (E) aspartate aminotransferase (AST); and (F) alanine aminotransferase (ALT) in all groups of rats. Data are presented as mean ± S SEM of 5-6 rats in each group. **p < 0.01, *p < 0.05 vs control group and ##p < 0.01, #p < 0.05 vs HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

Effects of powdered pineapple on hepatic triglycerides and cholesterol contents in HCD-fed rats.

As shown in Table 14 and Figure 40, the HCD group showed a significant increase in hepatic cholesterol ($P < 0.01$) and triglycerides ($P < 0.01$) contents compared with the control group. In HCD+P and HCD+S groups, these levels were decreased compared to the HCD group (39.38 % and 24.41 % for triglycerides content, and 47.47 % and 63.16 % for cholesterol level in pineapple and simvastatin-treated groups, respectively, vs HCD group).

Table 14 Effects of powdered pineapple consumption on hepatic triglycerides and cholesterol contents in HCD-fed rats.

Parameters	Mean ± SEM			
	C	HCD	HCD+P	HCD+S
Hepatic triglycerides content (mg/g liver)	11.62±1.6	24.58±1.1**	14.9±1.1##	18.58±1.9*,#
Hepatic cholesterol content (mg/g liver)	2.71±0.1	30.02±6.3**	15.77±2.5*,#	11.06±1.4##

Results are shown as the mean ± SEM (n=5-6 rats). **p<0.01 vs. C group; #p<0.05, ##p<0.01 vs. HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

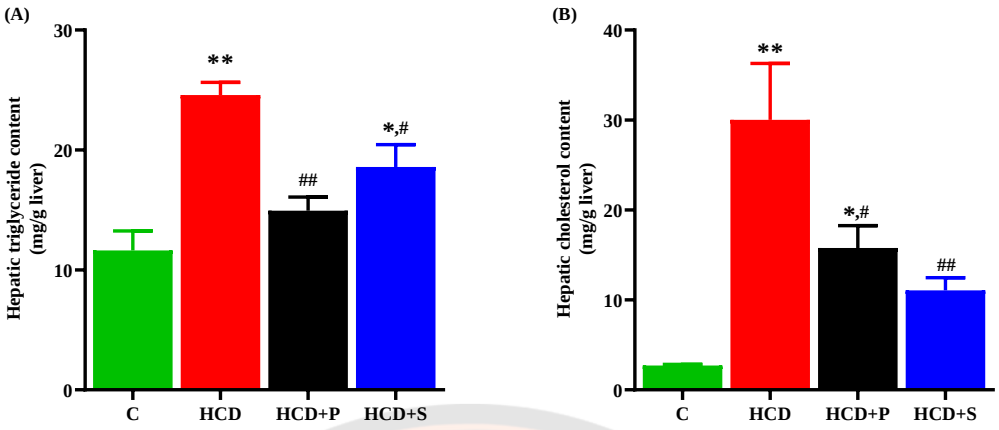


Figure 40 Effects of powdered pineapple consumption on hepatic triglycerides and cholesterol contents in HCD-fed rats. The hepatic cholesterol content (A) and the hepatic cholesteryl ester content (B). Data are presented as mean ± SEM of 5-6 rats in each group. **p < 0.01, *p < 0.05 vs control group and ##p < 0.01, #p < 0.05 vs HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

Effect of powdered pineapple on cholesterol metabolism in the liver of HCD-fed rats.

As shown in Figure 41 and Table 16, the elevated expressions of HMGCR, and CYP7A1 with a decreased LDLR expression in the liver were observed in the rats fed with the HCD. Compared to the HCD group, the pineapple- or simvastatin-treated group had significantly reduced hepatic HMGCR expression and increased hepatic LDLR expression. However, hepatic CYP7A1 expression were unaffected by pineapple or simvastatin.

These results suggest that daily consumption of powdered pineapple able to ameliorate HCD-induced hypercholesterolemia by the reduction of HMGCR expression, and the elevation of LDL expression.

Table 15 Effects of powdered pineapple consumption on protein expressions involved in cholesterol metabolism in the liver of HCD-fed rats.

	Mean ± SEM			
	C	HCD	HCD+P	HCD+S
LDLR	1.00±0.0	0.37±0.1**	0.79±0.1$^{\#\#}$	0.77±0.1$^{\#\#}$
HMGCR	1.00±0.0	1.42±0.1**	0.69±0.2$^{\#\#}$	0.79±0.0$^{\#\#}$
CYP7A1	1.00±0.0	1.24±0.1*	1.22±0.1*	1.26±0.1*

Results are shown as the mean ± SEM (n=5-6 rats). **p<0.01 vs. C group; $^{\#}$p<0.05, $^{\#\#}$p<0.01 vs. HCD group. C, control group; HCD, high-cholesterol diet group; HCD+P, HCD with powdered pineapple treatment group; HCD+S, HCD with simvastatin treatment group.

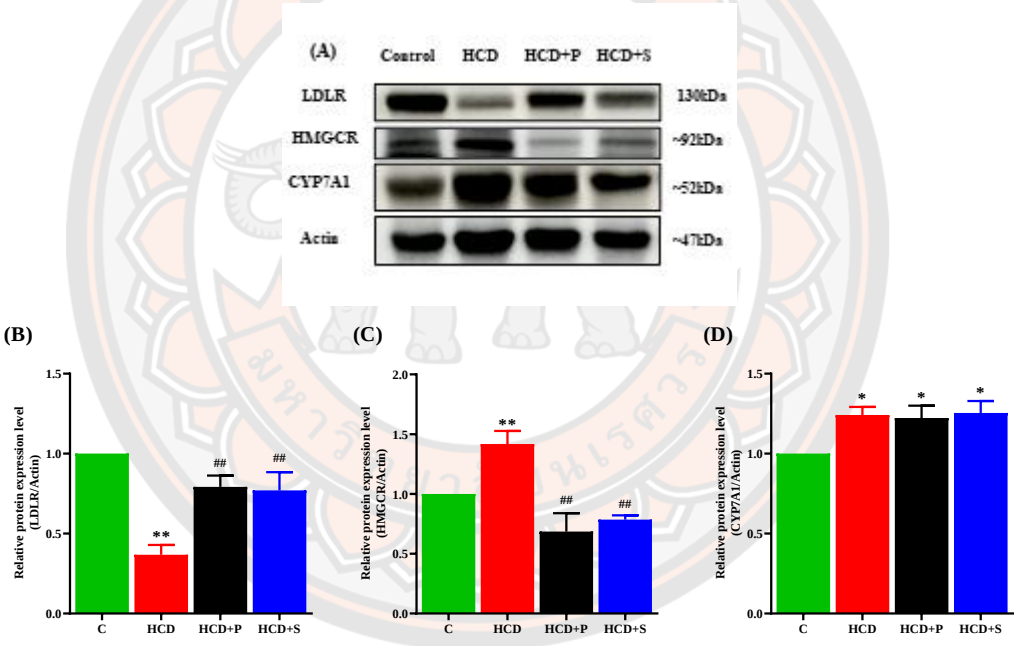


Figure 41 Effects of powdered pineapple consumption on protein expressions involved in cholesterol metabolism in the liver of HCD-fed rats. (A) Representative western blot analysis of HMGCR, CYP7A1, and LDLR. β-actin was used as a loading control. (B-E) Quantified levels of HMGCR, CYP7A1, and LDLR expressions in the livers of the control group, the high cholesterol diet (HCD) group, the HCD + powdered pineapple (HCD+P) group and the HCD+ simvastatin (HCD+S) group. The results are shown as mean ± SEM. n = 5 rats. **p<0.01, *p<0.05 vs control group, ##p<0.01, #p<0.05 vs HCD group.

CHAPTER V

DISCUSSION & CONCLUTION

Discussion

This study demonstrated that the consumption of pineapple powder has a protective effect against hypercholesterolaemia by reducing lipid levels in hepatic and serum of the HCD-fed rats, and it also reduced the AST and ALT in the serum. Additionally, powdered pineapple also regulated the protein expressions involved in cholesterol metabolism, by decreasing the cholesterol biosynthesis, and increasing the cholesterol removal from the livers of HCD-fed rats.

A high cholesterol diet (HCD) promotes high levels of plasma cholesterol, LDL, and triglycerides, known as hyperlipidaemia.(Encyclopedia of Endocrine Diseases, 2018; Stewart, McCallin, Martinez, Chacko, & Yusuf, 2020) It also promotes an imbalance in hepatic lipid metabolism and leads to excessive accumulation of lipid droplets within the hepatocytes, that largely consists of triglycerides, which causes fatty liver disease and contributes to severe liver disease.(Alves-Bezerra & Cohen, 2017; H. Li, Yu, Ou, Ouyang, & Tang, 2021) The elevated hepatic lipid accumulation caused by an HCD can trigger mitochondrial stress in hepatocytes that is led to increase in reactive oxygen species (ROS), which are the main product in various oxidative metabolism pathway, inducing liver disorder and damage.(Ahn et al., 2020; Shen et al., 2020) The increase of both AST and ALT in the bloodstream is a biomarker for liver disorder.(Iloon Kashkooli et al., 2015; Khan et al., 2022) Our study found that the HCD increased the serum levels of the TC, TG, LDL-C, and decreased the serum HDL-C level, as well as increasing the content of both hepatic TC, and TG in the rats. Previous studies also found that the HCD increased the serum levels of both AST and ALT in the rats that are indicated that liver dysfunction and damage, which is similar to our findings.(Prommaouan et al., 2022; Sakurai et al., 2019) In our study also found that the consumption of the HCD resulted in an increase in both body and liver weights, and liver to body weight ratio in the rats.These results suggest that an HCD causes obesity, dyslipidaemia, and hepatic steatosis, as well as impaired liver functions in rats.

It was discovered that the consumption of some fruits showed beneficial effects against oxidative stress, obesity, hyperlipidaemia, and hepatic steatosis. (Alissa & Ferns, 2017; Anhê et al., 2019; Prommaouan et al., 2022) This is due to them being a rich source of phytochemicals, such as phenolic compounds, flavonoids, and dietary fibre.(Ballard et al., 2020; Kumar et al., 2021) The majority of fruits generally contain more soluble fibre than insoluble fibre.(Soliman, 2019) Previous studies have shown that the consumption of fruit that are high in fibre such as apples, oranges, bananas, pears, etc. can lower elevated cholesterol in the blood, which leads to protection against liver damage and cardiovascular disease.(Dreher, 2018; Soliman, 2019) Dietary fibre also reduces the risk of becoming overweight, which causes obesity via its physicochemical properties such as water-holding, water-swelling, oil-holding, glucose-adsorption, and cholesterol-adsorption capacity's, and gut microbiota fermentation.(He et al., 2022) Pineapples are grown in tropical and sub-tropical regions, and have a pleasant taste and aroma, and are very juicy and sweet. According to the FAO online database, the total amount of pineapples exported worldwide in 2020 was approximately 2.9 million tonnes.(Nations, 2020) Currently, there are more than 130 genotypes of pineapples worldwide, and Thailand has more than 14 of them, which are include Pattavia, Tratseethong, MD2, Phetchaburi, Nanglae, etc.(X. H. Lu et al., 2014) This study used the "Pattavia" species of pineapple, which belongs to the smooth Cayenna pineapple family. The Pattavia is a popular pineapple grown specifically for the canning industry in Thailand.(Dittakan, 2018) A previous study found that Pattavia reduced ROS capacity more than the Tratseethong species, and superoxide dismutase (SOD) activity was also higher in Pattavia than in Tratseethong.(Korakot Chanjirakul, 2009) Previous studies have shown that pineapple contains an abundance of dietary fibre, phenolic compounds, and flavonoids.(Arampath & Dekker, 2020; X. H. Lu et al., 2014) In vitro studies have shown that pineapple inhibited the absorption and solubility of cholesterol in the small intestine.(Duangjai et al., 2011; Kanittaporn Trisat, 2016) While an in vivo study found that pineapples ameliorated high-fat diet (HFD)-induced hepatic steatosis, obesity, and hyperlipidaemia.(El-Shazly et al., 2018) However, the mechanisms by which pineapple lowers cholesterol levels in vivo, are still unclear.

Our study found that the consumption of powdered pineapple reduced TG, TC, and LDL levels in the serum of rats, as well as hepatic TG and TC. Daily consumption of pineapple also decreased body and liver weights, and liver to body weight ratios, consistent with previous study found that the consumption of pineapple juice can lower serum and hepatic lipid levels, and ameliorate liver and body overweight in HFD-fed rats.(El-Shazly et al., 2018) However, in this study, it was found that there were no significant differences in the food intake of the rats in all groups, which are indicated that daily consumption of pineapple had no effect on food intake. Additionally, our study found that the consumption of pineapple powder was also able to decrease the serum levels of both AST and ALT in the HCD-fed rats. Due to the consumption of powdered pineapple reduced lipid accumulation in the livers of HCD-fed rats, resulting in a reduction in markers of liver dysfunction. These results indicated that the pineapple had no toxic effects and was able to reduce damage to the livers. The results also suggested that daily consumption of pineapple powder attenuated dyslipidaemia, hepatic steatosis, and impaired liver function in the HCD-induced hypercholesterolaemic rats.

The liver is an organ that is primarily responsible for maintaining cholesterol homeostasis in the body. The balance of cholesterol in the body can be disturbed by the regulation of cholesterol biosynthesis, the conversion of cholesterol to bile acid, and the removal of LDL.(Jia et al., 2011; Vourakis et al., 2021) Most of the cholesterol in the body occur from cholesterol biosynthesis in the liver.(Luo et al., 2020; Rodwell, 2015) HMGCR is a rate-controlling enzyme in the mevalonate pathway that produces cholesterol, which then converts HMG-CoA to mevalonic acid in the cytoplasm of hepatocytes, which is a necessary step in the biosynthesis of cholesterol.(Baskaran et al., 2015; De Giorgi et al., 2020; Rodwell, 2015) Previous studies have shown that excessive consumption of cholesterol stimulates both the mRNA and protein HMGCR expressions in the liver, which causes the level of cholesterol in the liver to increase.(Jennifer K. Lee et al., 2020; K. H. Lee et al., 2020; Meneses et al., 2016) Nevertheless, other studies found that the HCD reduced the HMGCR expression in the liver of the rats.(Hwang et al., 2016; Min et al., 2012) This was due to excess cholesterol in the body that inhibits the transcription factor genes involved in stimulating the HMGCR gene expression, which is a negative feedback

loop for maintaining cholesterol levels in the body of normal liver function.(Kim, Yoon, & Jung, 2021) However, in our study, consumption of an HCD was found to increase HMGCR expression in the livers of the rats.

Excessive consumption of HCD leads to excessive accumulation of cholesterol, which contributes to oxidative stress in liver of the rat, leading to impaired hepatocyte function.(J. H. Lu et al., 2019) Previous study found that the liver cannot maintain cholesterol levels due to liver dysfunction a crucial role in the reduction of the inactive form of HMGCR to inhibit cholesterol biosynthesis.(Ma, Sun, Gao, & Liu, 2019; Min et al., 2012; N. Wu et al., 2013) Previous studies have reported that the daily consumption of fruit which contain high dietary fibre, phenolic compounds, and flavonoids, are able to decrease the HMGCR expression in the liver, and reduce cholesterol biosynthesis.(D. Li, Cui, Wang, Liu, & Li, 2021; D. Li, Liu, Wang, & Li, 2019) In contract, other studies have shown that dietary fibre consumption can inhibit cholesterol absorption into the small intestine, which leads to a decrease in blood cholesterol levels, that in turn can lead to an increase in the HMGCR expression in the liver.(D. Li et al., 2019) A previous study found that dietary fibre consumption does not effects on the alteration to triglycerides level and/or the protein expression involve in lipogenic genes in the liver, which include fatty acid synthetase (FAS), acetyl-CoA carboxylase (ACC) and HMGCR, as well as the key fatty acid receptor PPAR-γ.(Cronin, Joyce, O'Toole, & O'Connor, 2021) Many other studies have shown that phenolic compounds and flavonoids, are able to reduce the risk of lipid accumulation in the liver by reducing lipid synthesis and/or increasing lipid excretion by inhibit HMGCR and increase CYP7A1 activity via regulating transcription factor involved in gene expressions of them. Our study found that daily consumption of powdered pineapple decreased HMGCR expression in the liver of HCD-fed rats. These results suggest that daily consumption of powdered pineapple is able to lower lipid levels in the liver by reducing the expression of proteins involved in cholesterol biosynthesis.

It is known that the excretion of cholesterol accounts for 5% of cholesterol homeostasis in the body.(Chambers et al., 2019; Rodwell, 2015) This process is divided into two pathways: 1) cholesterol is converted to primary bile acid in the liver, and 2) cholesterol combines with bile acid, which is called biliary excretion of

cholesterol.(McLeod, 2016) It is known that bile acid, in combination with cholesterol and free fatty acid, has the function of forming micelles that enable the absorption of cholesterol and triglycerides in the small intestine.(Chambers et al., 2019) CYP7A1 is a microsomal cytochrome P450 enzyme that is located in the endoplasmic reticulum (ER), which plays an important role in controlling cholesterol levels in the body by being able to convert it to primary bile acid in the liver, which is the first step in bile acid synthesis.(Chambers et al., 2019) Previous studies have shown that an HCD increases the expression of CYP7A1 in the liver, which is due to excessive cholesterol uptake that leads to an increase in total cholesterol in the body. (Bunnoy et al., 2015; Meneses et al., 2016) Therefore, the liver stimulates genes and/or proteins involved in cholesterol excretion, which maintains the cholesterol levels in the body.(Meneses et al., 2016) Our study found that daily consumption of powdered pineapple and simvastatin did not affect the expression of CYP7A1 in the livers of the HCD-fed rats. This result indicated that the consumption of powdered pineapple is able to lower cholesterol levels in blood did not relate with cholesterol excretion in the liver of HCD-fed rats.

One of the major pathways through which cholesterol is balanced in the body, is by the clearance of LDL and cholesterol.(Gabcova-Balaziova et al., 2015) The LDLR is the main receptor for the uptake of LDL into the liver and other organs. (Gabcova-Balaziova et al., 2015) Its receptor-mediated endocytosis of specific ligands on the cell surface, which binds LDL and/or cholesterol, then enters the cells by endocytosis and is released in the lysosomes. (Gabcova-Balaziova et al., 2015; Ma et al., 2019; Trinh et al., 2020) Many factors can regulate the activity and expression of LDLR, including blood and liver cholesterol levels, etc.(Ma et al., 2019) Previous studies reported that the HMGCR expression can inhibit the LDLR expression in the liver, and showed that the protein and gene expressions of LDLR were decreased in the HCD-fed rats.(Bunnoy et al., 2015; Feng et al., 2022) Normally, the increase in LDLR expression is due to an excess of cholesterol in the blood caused by the consumption of HCD, leading to the uptake of cholesterol into the liver and the maintenance of cholesterol levels in the blood.(N. Wu et al., 2013) However, our study found that an HCD decreased the LDLR expression in the liver of rats, which is consistent with previous studies.(Feng et al., 2022; J. H. Wu et al., 2021) Excessive

HCD consumption contributed to high intracellular cholesterol levels in the liver, which suppresses the uptake of exogenous cholesterol, resulting in decreased LDL receptor-mediated uptake of cholesterol into the liver, which is in agreement with the results observed in animal model of previous studies.(Bunnoy et al., 2015; Heo et al., 2018; J. H. Wu et al., 2021) Furthermore, a previous study, HMGCR expression was found to be able to inhibit LDLR expression in the liver of the rats, which are similar to our founding.(Feng et al., 2022) Daily consumption of powdered pineapple and simvastatin increased the LDLR expressions in the livers of the HCD-fed rats. These results suggested that the effects of powdered pineapple were able to lower cholesterol levels, by upregulating the LDLR in the livers of the rats with HCD-induced hypercholesterolaemia.

All the results of this study demonstrated that daily intake of 200 mg/kg body weight of powdered pineapple, had a beneficial effect on hypercholesterolaemia. This was achieved by lowering the high cholesterol levels in the blood and livers, by downregulating the HMGCR expression and upregulating the LDLR expression. Our study also found that the daily consumption of pineapple or treatment with simvastatin for 8 weeks, was able to reduce the HCD-induced toxicity in the livers. All of these results show why pineapple should be included in the daily diet. Daily consumption of 200 mg/kg of powdered pineapple in animal models is equivalent to 2.188 g of powdered pineapple or 75.44 g of fresh pineapple for adults weighing 50 kg.

Conclusion

The present study provides evidence that the daily consumption of of pineapple reduces lipid levels in the blood and liver in HCD-induced hypercholesterolaemia in the rats. The potential cholesterol-lowering mechanisms of pineapple are mediated by the inhibition of cholesterol synthesis via suppressing hepatic HMGCR expression, and promoting the clearance of plasma LDL-c via up-regulating hepatic LDLR expression.

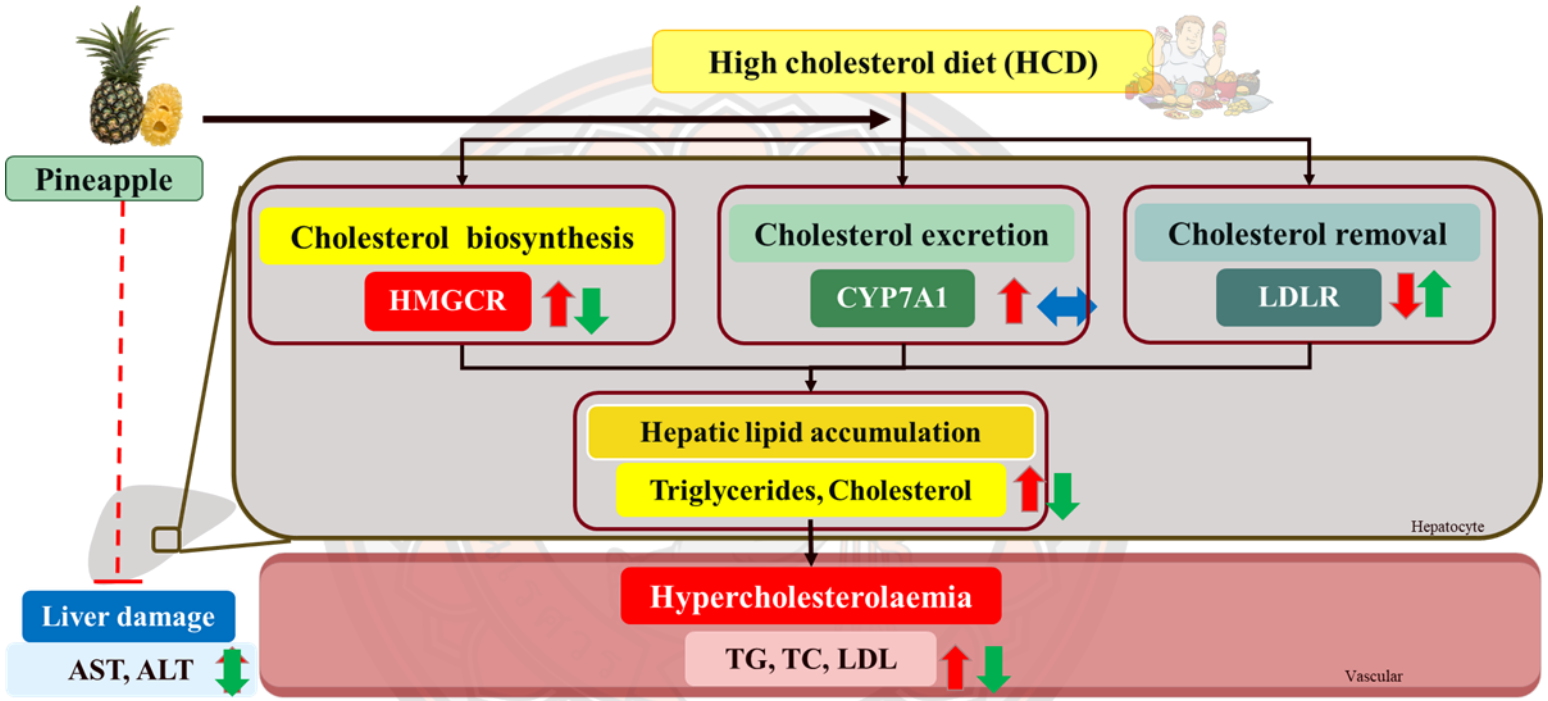


Figure 42 Conclusion of this study.

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