Faculty of Medicine and Health Sciences

A Study on Serum Leptin Levels and Lipid Profile in Patients with Stable Coronary Artery Disease (CAD)

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DECLARATION

I hereby declare that this particular thesis entitled “A Study on Serum Leptin Levels and Lipid Profile in Patients with Stable Coronary Artery Disease (CAD)” is solely the result of my research experiment interest, effort and hard work. This thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Cardiovascular diseases (CVD) are a group of disorders involving the heart and its related blood vessels. Cardiovascular diseases CVD is now a common cause of global mortality and largely contributed to the disease-related disabilities which poses a major impact on the costs of treatment and healthcare in most countries. Since the last decade, cardiovascular diseases have accounted for 31% of deaths worldwide and the single largest contributor to the global mortality. Leptin, has been shown to play important role in the development of cardiovascular diseases. However, the association of leptin and the long term outcomes of CVD patients is rarely being reported. This research aimed to determine the serum leptin levels and lipid profiles among patients with stable coronary artery disease. In this study, 100 participants were recruited from the pool of patients attending the Cardiac Clinic at the Sarawak Heart Centre (PJS) from the year 2015 till 2016. The serum leptin levels were determined by using ELISA method, whereas the lipid profile level were determined by using the blood biochemistry analyser Beckman Coulter AU680. Results showed a positive correlation between lipid profile parameter and serum leptin levels in patients with stable coronary artery disease (CAD). However only one parameter of the lipid profile, which is the triglyceride level shows positive correlation with correlation coefficient, r:0.318 and p value <0.005. The strength of linear relationship increased in the correlation of serum leptin level and triglyceride level among the non-diabetic stable CAD patients ($r = 0.439$, $p < 0.001$). Serum leptin levels correlate well with the body mass index of the stable CAD patients involved in the study (<0.001). Positive correlation between serum leptin levels and BMI also seen among non-diabetic groups (<0.001). Based on these findings, a combo test including drugs lower lipid levels and drugs lower leptin level might potentially useful

to treat and manage coronary artery disease patients. Therefore, a future study is required to test this intervention and hopefully this can bring a new insights regarding the role of these biomarkers in cardiovascular disease research, testing and its clinical management.

Keywords: Coronary artery disease, serum leptin, lipid profile

Kajian Terhadap Tahap Serum Leptin Dan Profil Lipid pada Pesakit Arteri Koronari yang Stabil

ABSTRAK

Penyakit kardiovaskular (CVD) merupakan kumpulan penyakit yang melibatkan jantung dan saluran darah. Penyakit kardiovaskular merupakan penyebab utama kematian global dan sebahagian besarnya menyumbang kepada implikasi besar terhadap kos rawatan dan penjagaan kesihatan di kebanyakkan negara. Sejak sedekad lalu, kematian yang dikaitkan dengan penyakit kardiovaskular adalah 31% kematian di seluruh dunia dan merupakan penyumbang terbesar kepada kematian global. Leptin adalah protein yang memainkan peranan penting dalam perkembangan penyakit kardiovaskular. Walaubagaimanapun, pertalian antara leptin dan hasil jangka panjang penyakit kardiovascular (CVD) sangat jarang dilaporkan. Kajian ini bertujuan menentukan hubungan antara serum leptin dan profil lipid di kalangan pesakit koroni arteri stabil. Dalam kajian ini, 100 orang calon adalah pesakit yang hadir di klinik jantung di Pusat Jantung Sarawak (PJS) dari 2015 hingga 2016. Serum leptin dikenalpasti menggunakan kaedah ELISA manakala profil lipid di analisis secara automatik menggunakan mesin biokimia Beckman Coulter AU680. Keputusan menunjukkan tidak ada perbezaan yang ketara antara paras serum leptin dan bangsa yang berbeza (nilai-p 0.007). Walaubagaimanapun, hanya satu parameter lipid iaitu trigliserida menunjukkan korelasi yang positif dengan serum leptin dengan pekali korelasi r = 0.318 dan nilai p <0.005. Kekuatan hubungan korelasi di antara tahap serum leptin dan level trigliserida telah meningkat pada kumpulan pesakit koronari arteri yang stabil dan bukan pesakit diabetes mellitus (r = 0.439, p<0.001). Level serum leptin berkolerasi baik dengan index jisim badan pada pesakit koronari arteri yang stabil dalam kajian ini (<0.001). Kolerasi yang positif juga berlaku antara level serum leptin dan index jisim badan

dalam kumpulan pesakit yang tiada diabetes mellitus (<0.001). Berdasarkan hasil kajian ini, ujian kombo yang merangkumi ubat yang merendahkan tahap lipid dan ubat yang merendahkan tahap leptin berkemungkinan berpotensi untuk merawat dan menguruskan pesakit koronari arteri. Oleh yang demikian, kajian di masa depan perlu dilakukan untuk menguji hasil kajian ini dan berharap ianya boleh membawa satu wawasan yang baru mengenai peranan biomarker ini dari segi ujian, penyelidikan dan pengurusan klinikal penyakit kardiovaskular.

Kata kunci: *Penyakit koronari arteri, serum leptin, profil lipid*

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LIST OF ABBREVIATIONS

↓	Decreased
↑	Increased
<	Less than
+	Increased
++	Greatly increased
≥	Greater than / Equal to
4-AA	4-aminoantipyrine
4-AAP	4-aminoantipyrine
Apo A-1	Apolipoprotein A-1
Apo B-48	Apolipoprotein B-48
Apo B-100	Apolipoprotein B-100
Apo C	Apolipoprotein C
Apo C I	Apolipoprotein C-I
Apo C-II	Apolipoprotein C-II
Apo C-III	Apolipoprotein C-III
Apo E	Apolipoprotein E
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BMI	Body mass index
CAD	Coronary Artery Disease
CE	Cholesterol esterase

CHD	Coronary Heart Disease
ChOx	Cholesterol oxidase
CO	Carbon monoxide
DAP	Dihydroxyacetone phosphate
DSBmT	*N*,*N*-bis-(4sulfobutyl)-ɱ-toluidinedisodium
DVT	Deep vein thrombosis
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase
H_2O_2	Hydrogen peroxide
HCL	Hydrochloric acid
HDL	High-density lipoprotein
IDL	Intermediate-density lipoprotein
IL-6	Interleukin -6
Kg/m^2	Kilogram per meter square
kU/L	Kilo unit per litre
LDL	Low-density lipoprotein
Lp	Lipoprotein
Lp(a)	Lipoprotein a
mg/dl	Milligrams per decilitre
N	Normal
Nm	Nanometer
O_2	Oxygen
PAI-1	Plasminogen activator inhibitor-1
TG	Triglyceride

TNF-α	Tumour factor α
μkat/L	Microkatal per litre
VLDL	Very-low density lipoprotein
WHR	Waist-hip ratio

CHAPTER 1

INTRODUCTION

1.1 Background

Cardiovascular diseases (CVD) are a group of disorders involving the heart and its related blood vessels. CVD is now a common cause of global mortality and largely contributed to the disease related disabilities which poses a major impact on the costs of treatment and healthcare in most countries. Since the last decade, death due to cardiovascular diseases accounted for 31% of deaths worldwide and CVD was the single largest contributor to global mortality and is expected to continue to be the major cause of mortality in the future. Among 16 million deaths among individuals younger than 70 years caused by non-communicable diseases (NCDs), 82% were in low- and middle-income countries, and 37% were caused by cardiovascular diseases (WHO, 2016).

In Malaysia, cardiovascular diseases are the dominant cause of morbidity and mortality in the last ten years. The National Health and Morbidity Surveys (NHMS) have shown that the prevalence of the cardiovascular (CV) risk factors which include hypertension, hypercholesterolemia, diabetes, overweight, obesity and smoking has been on an increasing trend. The National Cardiovascular Disease – Acute Coronary Syndrome (NCVD-ACS) Registry has also shown that Malaysians are developing heart disease at a younger age than that seen in the neighbouring countries.

Cardiovascular disease comprises of many types of conditions which include cerebrovascular disease, peripheral arterial disease and coronary artery disease. Coronary artery disease (CAD) involves impairment of blood flow through the coronary arteries, most commonly by atheromas. Coronary artery disease is the leading cause of death in both sexes, accounting for about one third of all deaths in developed countries. According to the World Health Organization, CAD accounted for 98.9 deaths per 100,000 populations in Malaysia in 2012 and it is the most common cause of deaths from non-communicable diseases in the country (WHO, 2015). The Malaysian burden of disease study conducted in 2000 found CAD to be the biggest cause of death with a total of 22,158 deaths or about one fifth of all deaths in the country (Yusoff et al., 2005). Information on the burden of disease which were obtained from death certifications and hospital admission records in Ministry of Health hospitals showed that circulatory disease accounted for 6.99% of total hospital admissions and 23.34% of all hospital deaths in 2014.

1.2 Leptin and cardiovascular diseases

Leptin, an adipokine, has been largely implicated in the increased cardiovascular risks. This hormone exerts several cardiovascular actions such as platelet aggregation, sympathetic activation, insulin resistance and proangiogenic effect, which suggest its important role in the development of cardiovascular diseases (Behowski, 2006). As the product of a 16- kDa obese gene found in early 1990s, leptin is primarily secreted by the white adipocytes and is one of the most prominent peptide that regulates appetite and energy expenditure (Ghalandari et al., 2015). High level of serum leptin has been implicated in inflammatory, metabolic and homeostatic factors involved in obesity, hypertension and cardiovascular disease (Chen et al., 2016). Previous studies have also revealed that leptin and its receptor's

isoforms are expressed in cardiomyocytes, vascular smooth muscle cells, and endothelia (Heida et al., 2010). Moreover, leptin resistance arose from hyperleptinemia was shown to enhance endothelial oxidative stress, stimulating the proliferation of vascular smooth muscle cells as well as inducing the thickening of injured arterial vascular walls, which in turn stimulates the formation of reactive oxygen species and activates the renin–angiotensin–aldosterone system, leading to the imbalance between vasoconstriction and vasodilatation with the consequent development of CVD.

1.3 Significance of study

Generally, CVD is the result of an interaction between various genetic, sociodemographic, economic, individual, environmental and health delivery system-related factors. Several recent studies have also reported that body mass index (BMI) is one of the strongest predictors of CVD (Chang & Chen, 2018). Essentially, efforts to prevent CVD should address all of these factors. However, because CVD involves interactions between multiple factors, using a single risk factor for predicting CVD is unreliable and may cause erroneous outcomes. One of the most efficient approaches to determining CVD risk is by considering the maximum number of all probable determinants to give a better picture and understanding, hence providing a more efficient steps in the management of patients with cardiovascular diseases.

Previous research has established various risk factors of CVD. Five modifiable risk factors, namely abnormal lipids, hypertension, current smoking, diabetes and abdominal obesity were shown to contribute to about 80% of myocardial infarcts (John et al., 2011). Diet and lifestyle factors such as smoking, physical inactivity and alcohol consumption, may

contribute by as much as 70% towards the development of other cardiovascular diseases risk factors such as abdominal obesity, hypertension, diabetes and hypercholesterolemia, in which collectively contributed to more than 955 of acute coronary events. Hypertension also called high blood pressure, is blood pressure that is higher than normal. Normal blood pressure is 120/80 mmHg. Blood pressure changes throughout the day based on activities. Having blood pressure measures consistently above normal may result in a diagnosis of high blood pressure or hypertension. Diabetes, also called *diabetes mellitus* (DM), is a condition when body doesn't use insulin to move glucose from blood into cells, which use it for energy like it should and too much glucose stays in bloodstream. While hypercholesterolemia is more commonly known as high cholesterol is a term for abnormally high levels of fats (lipids) in the blood. Although high cholesterol can be inherited, it's more often the result of unhealthy lifestyle choices. A decrease in these cardiovascular risk factors has been shown to reduce cardiovascular morbidity and mortality in both people without (primary prevention) and with established CVD (secondary prevention).

Adipokines, including leptin, has been implicated to play important role in the development of several risk factors of CVD such as obesity, hypercholesterolemia and diabetes. Although the link between adipokines and CAD has been well documented, the association between serum leptin and long-term cardiovascular outcomes in patients with CAD has been rarely reported. Moreover, conflicting data were seen concerning the association of leptin with future development of CVD. Serum leptin was a significant and independent predictor of recurrent CVD in men with earlier acute coronary syndrome and high plasma leptin levels was reported to predict the congestive heart failure or cardiac death and acute coronary syndrome or stroke in patients with CAD that were followed up for two years (Wallace et

al., 2001). As the knowledge on the role of leptin hormone in the prognosis of CAD is scarce, the current study aims to determine the relationship between serum leptin levels and lipid profiles among patients with stable CAD. The findings from this study will give deeper knowledge and insights of the role of these biomarkers in cardiovascular diseases which subsequently applied to identification of patients at risk and provide better modality towards the treatment and management of CAD patients.

1.4 Hypothesis

High serum leptin levels are associated with high lipid profiles level in patients with stable coronary artery disease.

1.5 Research Objectives

Broadly this research aims to determine the serum leptin levels and the parameters of the lipid profiles levels in patients with stable coronary artery diseases.

The specific objectives of this research are as follows:

a) To study the variations of serum leptin levels across race and specific risk factors of the coronary artery diseases in patients with stable CAD.
b) To study the relationship of serum leptin and lipid profiles in patients with stable CAD.
c) To study the correlation between serum leptin level and others clinical parameter in patients with stable coronary artery diseases (CAD).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction of Heart Disease

Heart diseases are truly global epidemics. Heart diseases are no longer diseases of the old men in developed countries. They are also diseases that plagued women, young adults and even children. Heart disease or also known as cardiovascular diseases, are the most common non-communicable diseases globally, responsible for more than 17 million deaths in 2017, of which more than three quarters were in low-income and middle-income countries (Roth et al., 2017). The prevalence and incidence of cardiovascular disease varies among different countries and most probably depending on income, culture, and the instituted healthcare systems (Lüscher, 2018). Previous research showed significant differences in the cardiovascular disease statistics between high income and low income populations with regard to cardiovascular disease risk factors, disease incidence, and mortality (Timmis et al., 2018). Diagnostically, cardiovascular diseases comprise of four major areas, which are the coronary heart diseases, manifested by myocardial infarction (MI), angina pectoris, heart failure, and coronary death; cerebrovascular disease, manifested by stroke and transient ischemic attack; peripheral artery disease, manifested by intermittent claudication and aortic atherosclerosis and thoracic or abdominal aortic aneurysm (Sesso et al., 2001).

Over the last 4 decades, major advances in cardiovascular epidemiology have improved the understanding of the cardiovascular pathogenesis, which include the identification and treatment of several major risk factors. The major risk factors for cardiovascular diseases

include high blood pressure, high blood cholesterol, tobacco used, physical inactivity unhealthy diet, obesity and family history of cardiovascular disease (Vasan & Benjamin, 2016). The goal of treating cardiovascular disease is to maximize the patient's quality of life and his life span. Prevention is the key to avoid cardiovascular disease and optimize treatment. For patients with heart disease, limitation of the disease progression can be achieved by maintaining a healthy lifestyle with routine exercise, healthy diet and controlled blood pressure. Risk prediction models can be a component of cardiovascular disease prevention and control efforts, as these models help to identify those at high risk of cardiovascular disease, who should benefit the most from preventive interventions (Lüscher, 2018).

The cardiovascular system consists of the heart and blood vessels and the problems that may arise within this system includes endocarditis, rheumatic heart disease and abnormalities in the conduction system (Benjamin et al., 2018). Figure 2.1 shows the worldwide prevalence of rheumatic heart disease in children over the world.

Since 1990, more people have died from coronary heart disease than from any other cause. Unlike stroke, coronary heart disease is a comparative newcomer on the world stage. Variation in death rates are marked: they are lower in populations with short life expectancy (Judith, 2004).

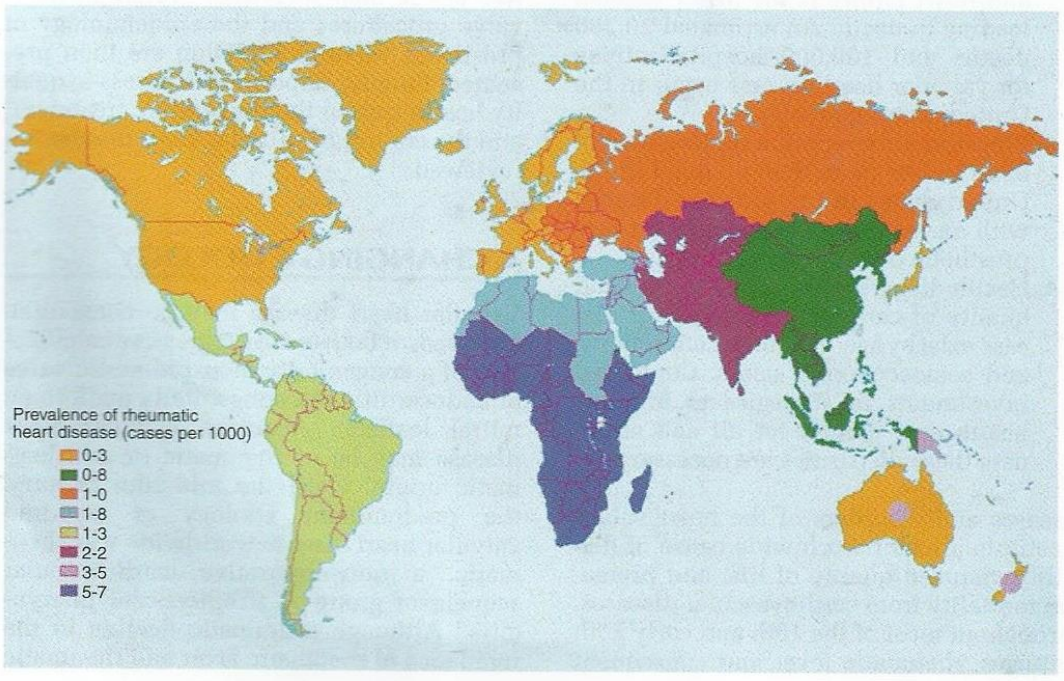


Figure 2.1: The worldwide prevalence (cases per 1000) of rheumatic heart disease in children age 5 – 14 years (Carapetis et al., 2005)

Heart disease mortality rates are also affected by differences between countries in the major risk factors especially blood pressure, blood cholesterol, smoking, physical activity and diet (Judith, 2004). While genetic factors play a part, 80% to 90% of people dying from coronary heart disease have one or more major risk factors that are influence by lifestyle (Judith, 2004). Of all coronary heart disease patients who die within 28 days after the onset of symptoms, about two-thirds die before reaching hospital. This highlights not only the need for early recognition of the warning signs of a heart attack, but also the need for prevention and keep the heart healthy (Judith, 2004).

The human heart is only the size of a fist, but it is the strongest muscle in the human body. The heart starts to beat in the uterus long before birth, usually by 21 to 28 days after conception. The average heart beats about 100 000 times daily or about two and a half billion

times over a 70 years of lifetime. With every heartbeat, the heat pumps blood around the body. It beats approximately 70 times a minute, although this rate can double during exercise or at times of extreme emotion (Sandra et al., 2019b).

Blood is pumped out from the right ventricle and left ventricle of the heart. It is transported through arteries of ever-decreasing size, finally reaching the capillaries in all the tissues, such as the skin and other body organs. Having delivered its oxygen and nutrients and having collected waste product, bloods is brought back to the right chambers of the heart through a system of ever-enlarging veins. During the circulation through the liver, waste products are removed (Sandra et al., 2019a).

Heart disease explain a range of conditions that affected heart. Heart diseases can be classified into several heart conditions include heart rhythm problems (heart arrhythmias), born with heart defect (congenital heart defect), disease of the heart muscle (cardiomyopathy), defect of heart valve, infection of the heart (endocarditis) and heart blood vessel diseases, such as coronary artery disease (Mendis et al., 2011). Many forms of heart disease can be prevented or treated with healthy lifestyle choices.

2.2 Types of cardiovascular disease

2.2.1 Arrhythmia

Arrhythmia is a heart problem when the electrical impulses that coordinate heartbeats not working properly and causing heart to beat too fast, too slow or irregularly (Gary, 2015). Normal heart is made up of four chambers with two chambers of atria and two chambers of ventricles. Heart rhythm is controlled by sinoatrial (SA) node located in the right atrium.

This natural peacemaker produces electrical impulse that act to start each heartbeat and causing blood can pump into the ventricles by contracting the atria muscle.

The electrical impulses then arrive at the atrioventricular (AV) node. Before sending it to the ventricles, the AV node will slow down the electrical signal. This slight delay allows the ventricles to fill with blood. Then blood pump to the lung and the rest of the body by contracting of the ventricles muscle, when electrical impulses reach the muscles of the ventricles. Normal resting heart rate is 60 to 100 beats a minute in a healthy heart (Paolo, 1999). When the resting heart rate going too slow and below than 60 beats a minute, this condition classify as bradycardia. While, if resting heart rate going too fast and more than 100 beats a minute, this condition is called tachycardia. Tachycardia can originate in the atria and the ventricles. Other than that, premature heart beat is actually an extra beat like a skipped heartbeat. Premature beat can trigger a longer lasting arrhythmia in people with heart disease.

Certain arrhythmias may increase risk of stroke and heart failure. Heart arrhythmias are well known to be associated with an increased risk of blood clots. When blood clot breaks loose it might block blood flow to brain and causing a stroke. Risk of stroke is increased with people have existing heart disease and with age 65 or older (Margaret et al., 2010).

Arrhythmias may not cause any signs or symptom, however noticeable may include fluttering in chest, tachycardia, bradycardia, chest pain and shortness of breath. Others symptoms may include anxiety, fatigue, dizziness, sweating, near fainting and syncope.

There are certain conditions that can lead an arrhythmia including scarring of heart tissue from a prior heart attack, cardiomyopathy, blocked arteries in heart, hyperthyroidism, hypothyroidism, uncontrolled high blood pressure, uncontrolled diabetes and obstructive sleep apnea (Tim, 2017). Other than that, arrhythmia can also cause by certain medication and supplements, drug abuse, genetics factor, smoking and drinking too much alcohol and caffeine.

It is important to live a heart-healthy lifestyle to reduce risk of heart disease by the preventing of heart arrhythmia. Few examples of heart-healthy lifestyles include eating more vegetables and fruits, whole grains with rich of fiber and other nutrients. Other activities that are known as heart-healthy lifestyle are keeping physically active with healthy weight by exercising for at least 150 minutes a week. Avoiding smoking, alcohol and limiting caffeine that can affect the electrical impulses in heart and causes the heart to beat faster are shown to prevent the development of more serious arrhythmias. As intense stress and anger can cause heart rhythm problems, controlling and reducing stress are important to prevent arrhythmia. Furthermore, using certain drugs and supplements may contribute to arrhythmia development and should always be taken with caution and upon advice by associated medical specialist.

2.2.2 Congenital heart disease (Congenital heart defect)

Congenital heart disease refers to children that were born with problem in the structure of the heart. The heart begins taking shape and start beating during the first six weeks of pregnancy. The major blood vessels that control blood circulation to and from heart also begin to form. Simple congenital heart defects usually do not require treatment while some

more complex cases may require several surgeries. Successful treatment during childhood may also lead to problems in adult later in life, such as scar tissue in heart that contributes to an arrhythmia where the electrical impulses do not function properly. Other congenital heart disease complications that might develop include heart infection (endocarditis), stroke, heart failure, heart valve problem and pulmonary hypertension where, high blood pressure causing more blood flow to the lung and making heart work harder and finally causes heart muscle to weaken and sometime to fail (Ioana & Mathue, 2018).

Sign and symptoms of serious congenital heart defects usually become evident soon after birth. However, some symptoms will be evident only during first few months of life including cyanosis where the child skin colour is pale gray or blue. Other than that, some child has swelling in the abdomen, legs or areas around the eyes. Rapid breathing, shortness of breath during feedings and poor weight gains also other symptoms of the serious congenital heart defects. Different with serious congenital heart defect, less serious congenital heart defects only diagnose until later in childhood. Symptoms of less serious congenital heart defect include easily tiring and easily becoming short of breath during exercise or activity. Some symptoms maybe swelling in the hands, ankles or feet and fainting during exercise (Ioana & Mathue, 2018).

There are several type of heart defect that cause congenital heart disease including holes in the heart, abnormal blood vessels, heart valve abnormalities, underdeveloped heart and combination of defects (Rong, 2015). Holes in the heart can form in the walls between heart chambers such as a hole in the wall between the right and left chambers of ventricles called ventricular septal defect. When the hole form between atria chambers occurs it is called atrial

septal defect. Presence of hole between major blood vessels exiting the heart may give rise to the condition such as complete atrioventricular canal defect. These holes allow oxygenated blood mix with deoxygenated blood, resulting in less oxygen being carried to the body. This lack of sufficient oxygen can cause the child skin or fingernails to appear blue depending on the size of the hole.

Abnormal blood vessels occur when the blood vessels are not formed and positioned correctly. When the pulmonary artery and the aorta are on the wrong sides of the heart, the defect is called transposition of the great arteries. When the main blood vessel supplying blood to the body is too narrow, the condition is called coarctation of the aorta defect while the total anomalous pulmonary venous connection defect occurs when the blood vessels from the lungs attach to the wrong area of the heart (Ioana & Mathue, 2018).

Heart valve abnormalities is the condition when the heart valves failed to open and close correctly, leading to the interruption of normal blood flow. Ebstein's anomaly is the defect of tricuspid valve that is malformed and often leaks. While, the pulmonary atresia is referring to the condition when the pulmonary valve is missing and causing abnormal blood flow to the lung.

In certain condition, a major portion of the heart are not developed properly such as in hypoplastic left heart syndrome where the heart has not properly developed and affect the amount of blood pumped to the body. Some infants are born with combination of heart defects known as Tetralogy of Fallot where the disease is a combination of four defects in

the heart including hole in the wall heart, narrow passage, shift in the great arteries connection and thickened right ventricle muscle.

The cause of most congenital heart defects is unknown, however genetic factors and certain environmental may play a role as a risk factors including having rubella during pregnancy, uncontrolled diabetes, certain medication taken during pregnancy such as thalidomide and statin, drinking alcohol and smoking during pregnancy (Rong, 2015). Even though it may be impossible to prevent, it was reported that some factors may reduce the congenital heart defects including obtaining rubella vaccine prior to conception and control of chronic medical conditions, such as diabetes where the mothers should maintain their blood sugar level in normal range. Other than that, avoidance of harmful substances such as painting or cleaning with strong-smelling products, drugs, smoking, alcohol consumption, taking herbs or dietary supplements without consulting medical specialist during pregnancy. Previous research showed that taking multivitamin with 400 mg folic acids daily may help reduce the risk of heart defect as well as reduce birth defects in brain and spinal cord (Bibbins et al., 2017).

2.2.3 Cardiomyopathy

Cardiomyopathy is a disease of the heart muscle involving the enlarged of left ventricle (hypertrophic cardiomyopathy), thickened ventricular septum (hypertrophic cardiomyopathy) and rigid and less elastic heart muscle (restrictive cardiomyopathy) resulting in the impaired pumping of blood to the rest of body. Cardiomyopathy may lead to other heart conditions including heart failure, blood clots, valve problems, cardiac arrest and sudden death. Cardiomyopathy is a treatable condition depending on the type and level of

severity of the disease with medications, surgically implanted devices and heart transplant (Ramaraj, 2009).

In early stages of cardiomyopathy, there might be no signs or symptoms. When cardiomyopathy occurs, signs and symptoms include dizziness, fainting, swelling of the legs, ankles and feet. Other symptoms are chest discomfort, such as fatigue, shortness of breath, heartbeats that feel rapid, pounding or fluttering. These sign and symptoms tend to worsen unless properly treated. The cause of the cardiomyopathy is unknown but have been shown to be acquired or inherited. Related factors for acquired cardiomyopathy including long-term high blood pressure, heart tissue damage from a heart attack, chronic rapid heart rate, heart valve problems, connective tissue disorders and metabolic disorders such as obesity, thyroid disease or diabetes (Ramaraj, 2009). Other factors are nutritional deficiency of essential vitamins or minerals, pregnancy complications, hemochromatosis, and unhealthy lifestyle including use of drugs such as cocaine and excessive alcohol consumption. In many cases cardiomyopathy cannot be prevented but can reduce by living in healthy lifestyle such as avoiding the use of alcohol or cocaine, controlling high blood pressure, controlling cholesterol level and controlling sugar level of diabetes people by eating a healthy diet and staying physically active with regular exercise and reducing stress by getting enough rest and sleep.

2.2.4 Endocarditis

Endocarditis is defined as an infection of the heart. The infections normally occur at the inner lining of heart chambers and heart valves called endocardium. Endocarditis can damage heart valves and lead to life-threating complications if it is not treated quickly by antibiotics or surgery in certain cases. Generally, endocarditis occurs when bacteria, fungi

or other germs from another part of body, spread through bloodstream and attach to damage areas in the heart to form clumps of bacteria and cell fragments (Yvette, 2018). When this clumps or vegetation break loose, it will travel to brain, lungs, abdominal organs, kidneys or limbs through blood stream. As a result, endocarditis can cause several complications including seizure and pulmonary embolism that can cause heart problem, such as heart murmur, heart valve damage and heart failure. Other complications may occur when the abscesses develop in the brain, lungs and other organs, paralysis due to stroke, kidney damage and enlarged of spleen (Yvette, 2018).

Usually, immune system will destroy harmful bacteria in the bloodstream. However, bacteria that live in mouth, throat, skin or gut can sometimes cause serious infections. Bacteria, fungi and germs that causes endocarditis might enter the bloodstream through everyday oral activities such as brushing teeth or dental procedure. Bacteria may enter the bloodstream through unhealthy teeth and gum or may spread from an infected area such as skin sore or by sexually transmitted infection. Other causes include prolonged use of catheter, unsterilized needles used for tattoos and body piercing as well as illegal intravenous drugs. Bacteria is able to attach easily to the endocardium if the linings surface is rough, faulty and diseased or due to damaged heart valves (Yvette, 2018).

Endocarditis can be prevented in several ways including by avoiding procedures that may lead to skin infections such as tattoos or body piercing and taking antibiotics before dental or medical procedures (Yvette, 2018). Dental hygiene through brushing and flossing and regular dental exams are important to maintaining good oral health and reduce the risk of germs entering bloodstream and indirectly reducing the risk of endocarditis.

2.2.5 Stable coronary artery disease (CAD)

Coronary artery disease occurs when the major blood vessels that supply blood to heart become damaged and diseased. This condition normally happens during cholesterol-containing deposits (plaque) build up, which narrows the coronary arteries in the heart, leading to arteriosclerosis (Joseph, 2017). However, Stable coronary artery disease (CAD), or stable ischemic heart disease (SIHD), refers to the syndrome of a reversible supply or demand mismatch related to ischemia, a history of myocardial infarction, or the presence of plaque documented by catheterization or computed tomography angiography (Ford et al., 2018; Fox et al., 2020). Patients are considered stable if they are asymptomatic or their symptoms are controlled by medications or revascularization. Treatment involves risk factor management, antiplatelet therapy, and antianginal medications. Tobacco cessation, exercise, and weight loss are the most important lifestyle modifications. Treatment of comorbidities such as diabetes mellitus, hyperlipidemia, and hypertension should be optimized to reduce cardiovascular risk (Michael et al., 2018).

The heart vascular system comprised of three major great vessels which directly carry blood into and out of the heart, which are the pulmonary vessel (pulmonary artery and pulmonary vein), vena cava (superior and inferior vena cava) and aorta (Onwuka et al., 2018). Pulmonary artery brings deoxygenated blood away from the right ventricle of the heart into the lungs for gas exchange or oxygenation. While, aorta the largest artery of the heart functions as to carry oxygenated blood into systemic circulation. The deoxygenated blood from systemic circulation flows to the heart via both vena cava. Coronary artery disease may include of one vessel disease (single vessel disease), two vessels disease (double vessel

disease) or three vessels disease (triple vessel disease) that represents the most severe form of coronary atherosclerosis.

Narrowing of the coronary arteries decreases the blood flow to the heart, and resulted in the blood rich with oxygen cannot be adequately supplied to the heart and other organs when it in greatest demand especially during exercise or strenuous activity. This condition may cause pressure or tightness on the middle or left chest and this pain is referred to as angina, which will subside with the cessation of the activity or with rest. Generally, angina can be triggered by physical or emotional stress. Other symptoms of CAD are shortness of breath or extreme fatigue which happens when insufficient blood is pumped to meet the body's needs. The severe symptom is heart attack which occurs when the coronary arteries are completely blocked. The classic signs come together with heart attack including pain in shoulder or arm, sweating, shortness breath and crushing pressure in the chest (Adam, 2019).

Coronary arteries damage or injury may also happen as early as during childhood. Over the time, the defect inner wall of arteries can lead to the development of atherosclerosis in the heart arteries and cause coronary artery disease. Increasing risk of coronary artery disease may be due to various risk factors including age, sex, family history, smoking, high blood pressure, high blood cholesterol level, uncontrolled diabetes, obesity, physical inactivity, high stress and unhealthy diet. The risk of damaged and narrowed arteries will increase with increasing of the age and people who are unmanaged stress. However, men are generally a higher risk compared to women, where the women mostly have CAD after menopause (Karen, 2001). People who have family members with heart disease has a higher risk to have a coronary artery disease especially if a close relative getting heart disease at an early age.

Unhealthy life style such as smoking and eating too much unhealthy food that rich with a fat or sugar also have significantly increased risk of coronary artery disease. Too high sugar level and blood cholesterol level can increase the risk of formation of plague and atherosclerosis that lead to cause CAD. While, narrowing of arteries cause by hardening and thickening arteries will happen when blood pressure is uncontrolled. Other than unhealthy diet, less of exercise also contribute to overweight and obesity an increased the risk factor of CAD. There are other possible factors that consider can develops CAD including sleep apnea, high sensitivity C-reactive protein, high triglycerides, homocysteine, preeclampsia, alcohol use and autoimmune disease. Risk factors often occur in grouped together and cause greater risk of coronary artery disease (Adam, 2019).

However, improve healthy lifestyle can help to prevent from developing of atherosclerosis in heart arteries that's cause coronary artery disease by avoiding or quit smoking, control blood pressure, control blood cholesterol and sugar level with healthy diet, maintain a healthy weight with active exercise, and keep to reduce and manage stress.

Compare to other heart diseases, coronary artery disease (CAD) is the leading cause of death, for both men and women in the Malaysia and worldwide. The number of death increased every year. Department of Statistics Malaysia (JSM) reported that the higher principal causes of death in Malaysia is ischemic heart disease or CAD from 2005 to 2014. In 2017, JSM reported the mortality cause by CAD is still in higher percentage, 13.2% (2016). This data supported by WHO data published in 2017 where, Malaysia are in ranking 63 in the world with coronary heart disease death with reached 30,598 or 22.13% of total deaths. Coronary artery disease is more than just health problem but causes have major financial implications

of governments, businesses and negative impact on the quality of individual life. Generally, more than 90% of heart disease in Malaysia have at least one of main risk factor of cardiovascular disease such as high cholesterol levels.

Therefore, National Heart Association Malaysia (NHAM) and the Academy of Medicine Malaysia with cooperation Ministry of Health Malaysia (KKM), published Clinical Practice Guidelines in Management of Heart Failure 4th edition 2019 as a guideline to manage in prevention, diagnosis, treatment, rehabilitation and palliative care of heart disease patients. Generally, heart disease especially coronary artery disease usually related to cholesterol level in the blood and blood cholesterol level can check by lipid profile test. A lipid profile test consists of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides are the recommended test in preventing of coronary artery disease become worsen. The desirable level of total cholesterol is <5.2 mmol/L, HDL-cholesterol is >1.0 mmol/L, LDL-cholesterol is <3.4 mmol/L and triglyceride is <2.3 mmol/L. As a step of prevention of CAD, Ministry of Health Malaysia recommended all healthy Malaysian to do lipid profile test per year as a health screening. While, patients under high cholesterol treatment usually more frequently check to make sure the dose and type of medicine are suitable.

2.3 Lipid profile

Lipid are category of fats and fat-like substances. It is source of energy and important part to build of cells. A lipid profile or lipid panel measure the level of specific lipids in the blood that's useful in diagnose people have a risk of cardiovascular heart disease. There are two important lipids in the blood, that's is cholesterol and triglyceride.

These two lipids transported in the blood by lipoprotein particles. Every one particles consist of cholesterol, triglyceride, protein and phospholipids molecules. The particle measured with a lipid profile are classified by their density. Three class density of lipid profile that's is high-density lipoprotein (HDL), low-density lipoproteins (LDL), and very low-density lipoprotein (VLDL) (Fisher, 2012).

Lipid profile assessment of total cholesterol, low density lipoprotein, high density lipoprotein and triglyceride levels that's is important in the diagnosis of cardiovascular disease.

2.4 Lipid

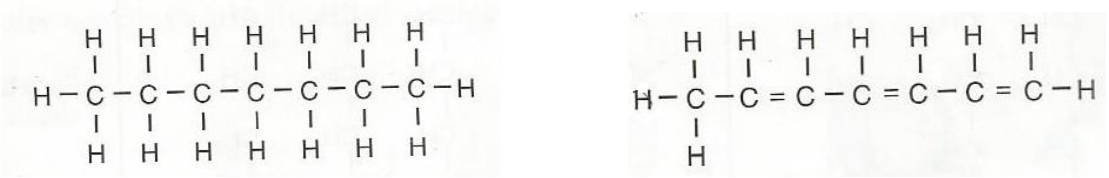
2.4.1 Introduction of lipids

Lipids are found throughout the body as structural components, hormones and nerve insulators. As essential as lipids are to our bodies, blood lipids are also play a big role in cardiovascular disease, and clinical practise guidelines suggest regular monitoring. The clinical significance of lipids is their link to coronary heart disease, cardiovascular disease, and lipoprotein disorders.

Biochemically, lipids are insoluble in water and soluble in organic solvents. The clinical significant classes of lipids include fatty acids, triglyceride, phospholipids, sterols, sphingolipids and cholesterol (Fisher, 2012).

2.4.2 Fatty acids

Fatty acids are carboxylic acids with long chains of carbon group. The chains can contain single bonds between the carbon groups (saturated fatty acids), or they can have both single and double bonds between the carbon atoms (unsaturated fatty acids). Fatty acids from plants sources are mostly unsaturated, and those from animal sources are saturated (Figure 2.2). The body uses and produces many fatty acids, but some fatty acids are classified as "essential," meaning that the body cannot synthesize them. Essential fatty acids include α-linoleic acids and linolenic acid.



A. Saturated hydrocarbon B. Polyunsaturated hydrocarbon

Figure 2.2: Saturated and unsaturated fats. A, Saturated fats with no double bonds within the carbon chain. B, a polyunsaturated fat with several double bonds with the carbon chain within the carbon chain. (From Karin,V.M., Robert, H., & William, V.M. *Microbiology for the Healthcare Professional.* St. Louis: Elsevier; 2010.)

In additional to using fatty acids for structural components, the body also uses long-chain fatty acids as energy sources. A fatty acid produces approximately twice as much energy as a comparable carbohydrate molecule. The body stores fatty acids efficiently, with stored molecules providing excellent insulating.

Excessive starvation or uncontrolled diabetes mellitus may cause the body to use fatty acids from adipose tissue for energy. These fatty acids undergo catabolism in the liver, resulting

in the production of three ketone bodies-acetoacetic acids, β-hydroxybutyric acids, and acetone (Fisher, 2012). Ketones are toxic acids, and increased production of these acids leads to ketosis or ketoacidosis or both. Ketosis is an increased level of ketones in blood. Ketoacidosis is an extreme case of ketosis in which the body does not regulate ketone production; this causes excessive amounts of ketones to accumulate in the blood, resulting in a lower blood pH.

2.4.3 Triglycerides

Triglycerides are large molecules that contain a glycerol molecule and three fatty acids (Figure 2.3). The body synthesizes triglycerides to effective transport fatty acids.

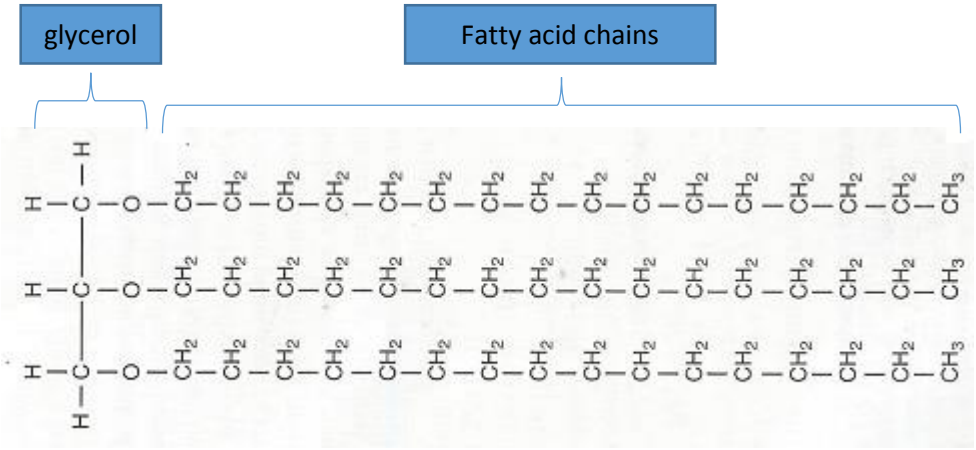


Figure 2.3: Triglyceride consist of fatty acid and glycerol

Cells require glucose inside the cell to synthesize triglycerides. If glucose is not present, the triglycerides from fat deposits are broken down to release the fatty acids. Intracellular glucose may not be present in conditions such as starvations, fasting, or uncontrolled diabetes mellitus. Free fatty acids are not soluble in water; once they are released from triglycerides they attach to albumin for transport (Donald & Robert, 1958).

Excess carbohydrates are stored as triglycerides. Insulin increases the synthesis of triglycerides from carbohydrates. Excess fatty acids in the blood are resynthesized into triglycerides for storage in adipose tissue.

Triglycerides are increased in diabetes mellitus, pancreatitis, alcoholism, glycogen storage disease, hypothyroidism, nephrosis, pregnancy, gout and use of oral contraceptive. Triglycerides are decreased in hyperthyroidism (Durrington, 2004).

2.4.4 Phospholipids

Phospholipids are cell membrane components. They are polar compounds with phosphates on one end and lipids on the other; as a result, they position themselves between water (phosphates end) and lipids (lipids end). Phospholipids are major component of the surfactant that allows the alveoli to distend during breathing. They are important for mitochondrial metabolism, blood coagulation, and lipids transport, in addition to serving as cellular membrane structural units (Bacha & Torres, 2016).

2.4.5 Sterols

Sterols consist of several ringed structural connected to a long aliphatic chain and at least one hydroxyl group (Figure 2.4). Plants sterols resemble cholesterol. However, plants sterols are not well absorbed and can even be used to reduce the absorption of cholesterol.

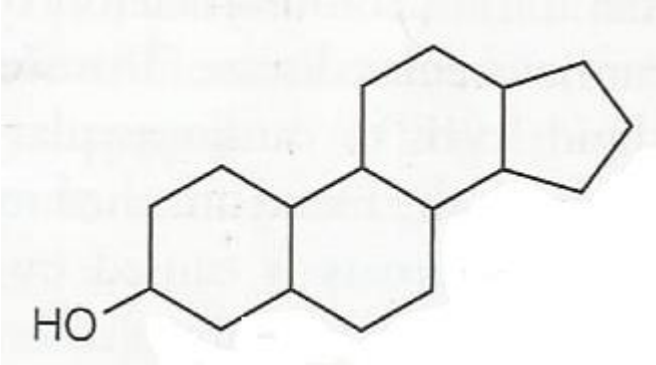


Figure 2.4: Basic chemical structure of sterols

2.4.6 Spingolipids

Spingolipids are cell membrane component in red blood cells and brain and nerve cells. They are involves in the recognitions of cells as self and in recognition of blood group antigens.

2.4.7 Cholesterol

Cholesterol is a sterol composed of several ring compounds, an aliphatic chain, and one hydroxyl group (Figure 2.5). All living organism contain sterols, and cholesterol is the primary sterol in human and animals.

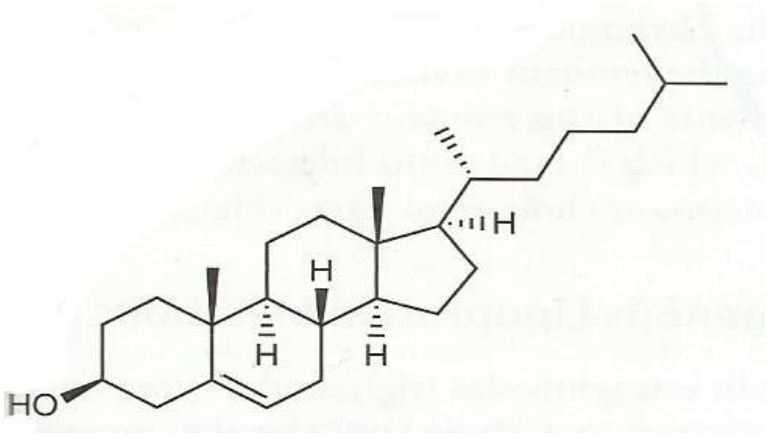


Figure 2.5: Structure of cholesterol

Cholesterol functions as a structural component in the body as well as precursor in the synthesis of steroid hormones. Some cholesterol is consumed in the diet, but most of the cholesterol in the body is synthesize by the liver and other body tissues. Cholesterol is required for the production of bile acids, steroids and cell membranes. The clinical conditions that produce elevated cholesterol levels include hyperlipidemia, atherosclerosis, and liver disease. Measurement of cholesterol and other lipids is routinely ordered to diagnose atherosclerosis (Cui et al., 2001).

2.5 Lipoprotein

2.5.1 Introduction of lipoprotein

Lipoprotein contain triglycerides, phospholipids, cholesterol, and apolipoprotein; they are used by the body to transport insoluble fats through blood (Figure 2.6). Lipoproteins are classified on the basis of their density as well as the apolipoproteins on their surface. The amount of lipid in a lipoprotein affects its density; the more lipids in a molecule, the lower its density. The five categories of lipoprotein include chylomicrons, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Fisher, 2012).

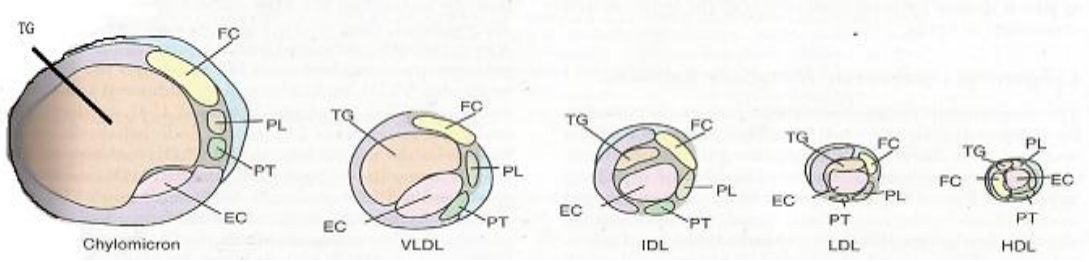


Figure 2.6: Lipoproteins (left to right); chylomicron, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). *EC*, Esterified cholesterol; *FC*, free cholesterol; *PL*, phospholipids; *PT*, protein; *TG*, triglyceride.

The five types of lipoproteins contain differing amounts of cholesterol, triglycerides, phospholipids, protein, and apolipoproteins. Chylomicrons are the largest and least dense of all the lipoproteins, and they are synthesized by cells in the small intestine. They transport triglycerides to muscle cells and adipose tissue. Chylomicrons contain 85% triglycerides, 3% cholesterol, 8% phospholipids and 4% proteins. Very-low-density lipoproteins (VLDL) are synthesized in the liver but a bit smaller and denser than chylomicrons. These lipoproteins carry triglycerides to muscle and adipose tissue. VLDL contain 25% cholesterol, 55% triglycerides, 14% phospholipids, and 6% proteins (Marcovinci & Morrisett, 1995).

IDL is formed when VLDL loses its triglycerides molecules. The remaining apoproteins C, free cholesterol, and phospholipids, apoprotein E and B-100, compose the IDL. Low-density lipoprotein (LDL) are cholesterol rich and are formed by the removal of triglycerides from VLDLs during catabolism. The major function of LDL particle is to carry cholesterol in the plasma. LDL carries cholesterol to the liver for bile formations, to the tissue for use in the cell membrane, to endocrine organs for steroid hormone productions, and to the cholesterol storage sites. LDL contains 50% 10% triglycerides. 29% phospholipids, and 11% protein.

High-density lipoprotein (HDL) are synthesized by the liver and the intestines. They are also formed through the catabolism of chylomicrons and VLDL particles. Their functions is to transport cholesterol from the cell to the liver. They are the smallest and densest of the lipoprotein particles. They also have higher concentrations of proteins and phospholipids than the other lipoprotein groups. HDL contain 25% cholesterol, 5% triglycerides, 26% phospholipids, and 44% protein (Table 2.1) (Fisher, 2012).

Table 2.1: Type of lipoproteins composition, characteristic and function (Fisher, 2012)

Name	Composition	Characteristic	Function
Chylomicrons	85% T 3% C 8% PH 4% P	Largest and least dense of all lipoproteins Synthesize in the intestine	Transport ingested triglycerides to muscle cells and adipose tissue
Very-low-density lipoprotein (VLDL)	25% T 55% C 14% PH 6% P	Smaller and more dense than chylomicrons Synthesized in the liver	Transport triglycerides synthesized by the liver to the muscle cells and adipose tissue
Low-density lipoprotein (LDL)	50% T 10% C 29% PH 11% P	Smaller and more dense than VLDL particles Synthesized by the liver or in the plasma	Carry cholesterol in the plasma to liver, tissues, endocrine organs, or storage
High-density lipoprotein (HDL)	25% T 5% C 26% PH 44%	Smallest and most dense of the lipoprotein molecules Synthesized in intestine and hepatic cells from simple molecules or from breakdown products of chylomicron and VLDL catabolism	Transport cholesterol from the cells to the liver

T, triglyceride; *C*, Cholesterol; *PH*, phospholipid; *P*, Protein

2.5.2 Apolipoproteins

Apolipoproteins are protein portion of lipoprotein molecules. The structure of these molecules is unique because they are soluble in plasma and bind to nonpolar lipids. Certain apolipoproteins are part of specific lipoprotein molecules: Apo A-I is found on HDL, apo B-48 on chylomicrons, and apo B-100 on LDL. Apo C-I, apo C-II, apo C-III, and apo E are found on all lipoproteins (Fisher, 2012). The main function of apolipoproteins is to allow lipids (neutral molecules) to be transported through the plasma. Other functions of apolipoproteins include activating or inhibiting enzymes that act on lipoproteins, acting as cofactors to allow lipoprotein to be removed from plasma, and maintaining the structure of a lipoprotein molecule.

Lipoprotein (a) (Lp(a)) is a distinct class of lipoprotein molecules. The Lp(a) structure is similar to that of LDL because both molecules contain a protein called apolipoprotein (a) (apo(a)) that is bound to the apo B-100 particle. LP(a) is considered an independent risk factor cardiovascular disease, cerebrovascular disease, atherosclerosis, thrombosis, and stroke. It is most strongly associated with cardiovascular disease and atherosclerosis. An increased Lp(a) predicts early atherosclerosis independent of other cardiac risk factor. In advanced cardiovascular disease, Lp(a) can predict a risk of plaque thrombosis because Lp(a) binds to blood vessels walls an increases the risk of clotting.

2.6 Normal lipoprotein metabolism

The body uses four main pathways for lipid and lipoprotein metabolism: the exogenous lipoprotein metabolic pathway, the endogenous lipoprotein metabolic pathway, the low-density lipoprotein receptor pathway, and the reverse cholesterol transport pathway (Khera et al., 2011). Although each pathway is discussed as a distinct entity, there are overlapping points at which dietary fat intake can influence the synthesis and catabolism of lipids.

2.6.1 Exogenous lipoprotein metabolic pathway

The exogenous lipoprotein metabolic pathway begins after fat is ingested. The intestinal enterocytes assemble chylomicrons from dietary fat and cholesterol. The chylomicron is encapsulated in secretory vesicles in the Golgi apparatus. The cell uses exocytosis to transport packaged chylomicrons to the extracellular space. The packages are absorbed by the intestine villi and introduced into the circulation. The packaged chylomicrons acquire additional apo C and apo E from circulating HDL to complete the chylomicron molecules.

With the apo C-II attached to the surface of the chylomicron, lipoprotein lipase (LPL) on the surface of endothelial cells is activated. Triglycerides inside the chylomicron molecule are quickly hydrolyzed into fatty acids. The fatty acids attach to albumin for uptake into muscle or adipose cells. At the same time, circulating HDL molecules absorb phospholipids and apo A from the chylomicron remnants. The apo B-48 and apo E on the chylomicron remnants act as liver cell receptors, allowing the remnants to be taken up by the liver cells. The components of the remnant are broken down into cholesterol, which is made into bile acids, newly synthesized lipoproteins, or cholesterol esters (Figure 2.7) (Khera et al., 2011).

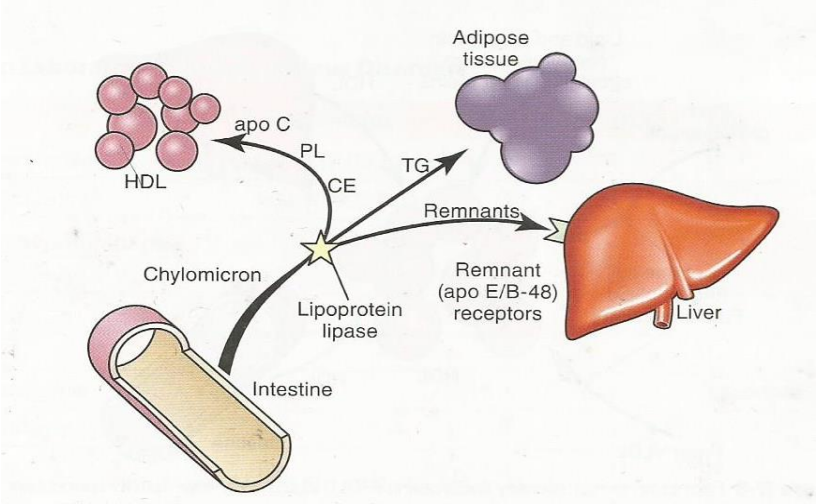


Figure 2.7: Metabolism of chylomicron. Chylomicron are absorbed by the intestine and enter the blood. In the blood, lipoprotein lipase acts on the chylomicron to produced chylomicron remnants, triglycerides, and cholesterol esters. The remnants go to the liver and combine with the apo E/B-48 receptors on the liver. The triglyceride is stored in the adipose tissue and the cholesterol ester is combined with phospholipid and apo C to create HDL molecules. (apo C, Apolipoprotein C; apo E, apolipoprotein E; B-48, apolipoprotein B-48; CE, cholesterol ester; HDL, high-density lipoprotein; PL, phospholipid; TG, triglyceride.)

2.6.2 Endogenous lipoprotein metabolic pathway

Liver cells can synthesised triglycerides (from fatty acids and carbohydrates) and cholesterol. Insulin promote triglyceride synthesis by signalling the liver to make

triglycerides from carbohydrates and fatty acids. Once this molecule is produced, they are packaged in secretory vesicles and transported (by exocytosis) into the extracellular space. The packages travel to the circulation and begin life as VLDL molecules. VLDL molecules acquire additional apo C from circulating HDL molecules. The apo C-II on the VLDL molecule activates the LPL on the endothelial cells, which hydrolyzes the triglycerides in the VLDL molecule to fatty acids. During the triglyceride hydrolysis, HDL absorbs the apo C on the VLDL molecule. Some remaining VLDL remnants go back to the liver and others form smaller, denser particles called intermediate-density lipoproteins (IDL). Eventually, the IDL is removed from circulation by the liver. The remnant molecules are recycle into newly synthesized lipoproteins (Figure 2.8) (Khera et al., 2011).

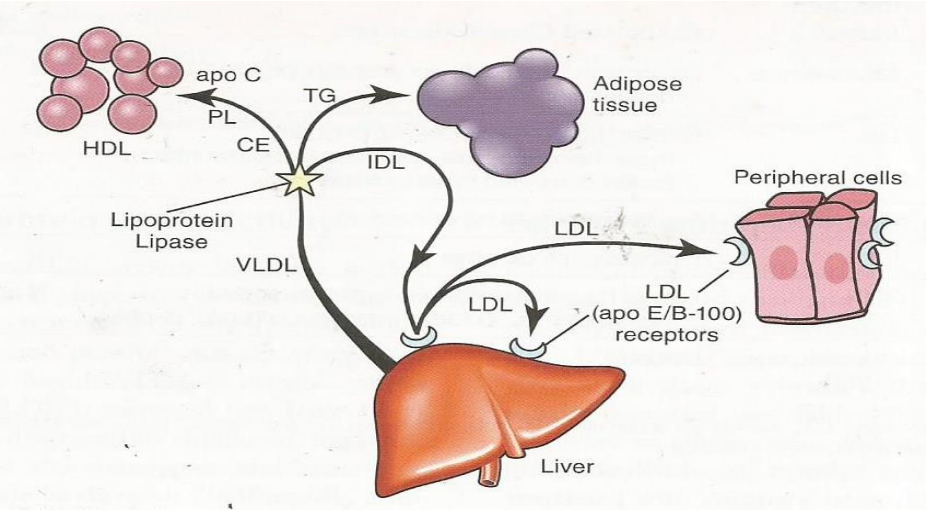


Figure 2.8: Metabolism of very-low-density lipoproteins (VLDL). VLDL is released into circulation where lipoprotein lipase breaks it into cholesterol ester, phospholipid, triglyceride and IDL molecules. The cholesterol ester and phospholipid along with apo C are combined together to make HDL. The triglyceride is store in adipose tissue. The IDL goes to the liver where it is converted to the LDL. Some LDL remains in the liver and other LDL goes to the peripheral cells. (apo C, Apolipoprotein C; apo E, apolipoprotein E; B-48, apolipoprotein B-48; CE, cholesterol ester; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; PL,phospholipid; TG, triglyceride).

2.6.3 Low-density lipoprotein (LDL) receptor pathway

Cell receptors recognize and bind to the apo B-100 of LDL molecules. After the cell binds to the apo B -100, the LDL molecule is taken into a vesicle that fuses with the LDL molecule to become an endosome. At this point, the LDL receptors separate from the LDL and travel back to the cell surface. The LDL migrates to a lysosome, where the apo B-100 is broken down into peptides, amino acids, and cholesterol (Khera et al., 2011).

The cholesterol is used by the cell to make cell membranes, steroid hormones (in cells that make them), and bile acids (in liver cells). Cells regulate the amount of free cholesterol by decreasing the amount of endogenous cholesterol esters, and inhibiting LDL synthesis when cholesterol levels are high. Excess cholesterol esters are taken up by macrophages. When macrophages become engorged with cholesterol esters, they are called foam cells. Foam cells are the earliest indications of atherogenic lesions (Yu et al., 2013).

2.6.4 Reverse Cholesterol Transport Pathway

High density lipoprotein (HDL) is secreted from the liver as crescent-shaped molecules containing mostly phospholipids and apo A-I. High density lipoprotein (HDL) molecule gain their spherical shape as they absorb more phospholipids, cholesterol esters, and certain apolipoproteins in the extracellular space. Hepatic cells can take up the cholesterol in the HDL remnants in three ways.

First, HDL receptors can attach to HDL cholesterol esters and transfer the cholesterol esters to the liver. Second, HDL remnants can provide the cholesterol esters to the lipoprotein containing apo B-100. Third, the apo E present on HDL remnats can be recognized by liver

cells. These processes represent a reverse cholesterol pathway that's allows cholesterol to be reused by the liver or disposed of (Figure 2.9) (Fisher, 2012).

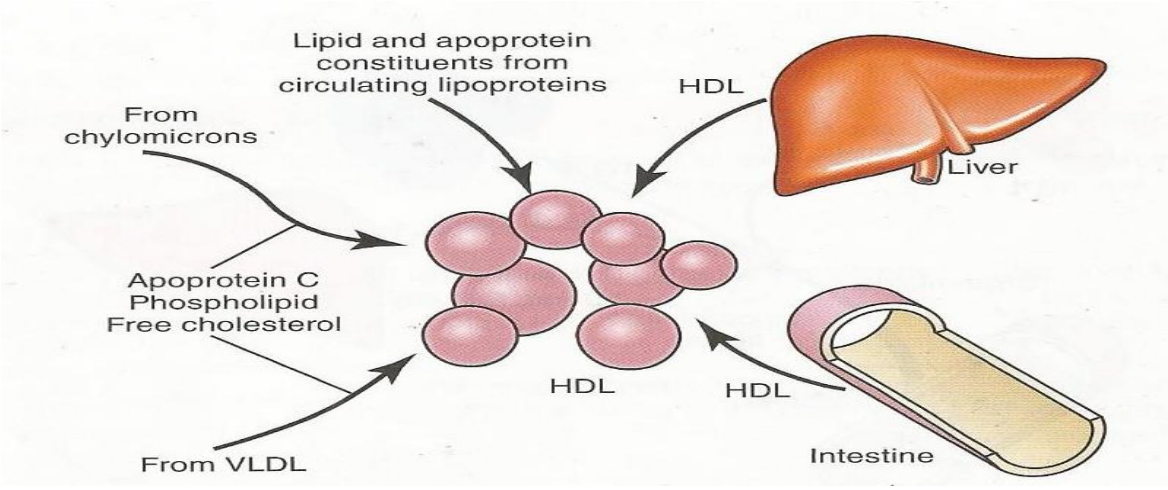


Figure 2.9: Formation of high-density lipoproteins (HDL). VLDL, very-low-density lipoprotein.

2.7 Abnormal lipoprotein metabolism

Abnormal lipoprotein metabolism is the result of genetic or acquired causes. Genetic defects in lipoprotein metabolism usually involve defects in surface apolipoproteins of the lipoprotein molecules, cell surface receptors of the liver and peripheral cells, or enzymes that regulate synthesis or catabolism (Khera et al., 2011). The following sections describe clinically significant conditions involving abnormal lipoprotein metabolism or production. Lipoprotein phenotypes were previously classified by the Fredrickson classification of lipid disorders system, but these condition are more appropriately classified by metabolic pathways. The Fredrickson classification table is provided for reference (Table 2.2) (Yeshura et al., 1995). The laboratory values seen with these conditions are summarized in Table 2.3 (Myers, 2000).

Table 2.2: Fredrickson classification of lipid disorders (Yeshura et al., 1995)

Type	Description of serum	Elevated particles	Asspciated Clinical Disorders	Serum TC	Serum TG
I	Creamy top layer	Chylomicrons	Lipoprotein lipase deficiency, Apolipoprotein C-II deficiency	N	++
IIa	Clear	LDL	Familial hypercholesterolemia, Polygenic hypercholesterolemia, nephrosis, hypothyroidism, familial combined hyperlipidemia	++	N
IIb	Clear	LDL, VLDL	Familial combined Hyperlipidemia	++	+
III	Turbid	IDL	Dysbetalipoproteinemia	+	+
IV	Turbid	VLDL	Familial hypertriglyseridemia, Familial combined hyperlipidemia, sporadic hypertriglyceridemia, diabetes	N or +	++
V	Creamy top, Turbid bottom	Chylomicrons VLDL	Diabetes	+	++

IDL,intermediate-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG, triglyceride; VLDL, very-low-density lipoprotein; +, increased; ++, greatly increased; N, normal; N or +, normal or increased

Table 2.3: Lipoprotein laboratory values in various disorders (Myers, 2000)

Disorder	Triglycerides	VLDL	HDL	LDL	Total Cholesterol
Deficiency in lipoprotein lipase	Up to 10,000 mg/dL	N	↓	↑	N
Apolipoprotein C-II defiency	500-10,0000 mg/dL	↑	↓	↓	150-890 mg/dL
Familial combined hyperlipidemia Type IIa		↑	N	↑	↑
Type IIb	↑		↓	↑	↑
Type IV	↑		↓	↑	↑
Hyperapobetalipoproteinemia	N or ↑	↑	↓	↑ (Moderate)	↑
Familial hypertriglyceridemia	↑	↑	↓	N	N
Type V hypertriglyceridemia	↑	↑	N	N	↑
Dysbetalipoproteinamia (type III)	↑	↑	↓	↓	↑
Familial hypercholesterolemia	N or ↑	N	↓	↑	↑
Familial defective apolipoprotein B-100	N	N	N	↑	N
Familial hypoalphalipoproteinemia	N	N	↓	N	N or ↑
Defects in the synthesis of apolipoprotein A-I	N	N	↓	N	↓
Defects in the catabolism of apolipoprotein A-I (Tangier disease)	N	N	↓	N	N

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; ↑ increased; ↓ decreased

2.7.1 Deficiency in lipoprotein lipase activity

Characteristic of deficiency LPL activity include a marked hyperchylomicronemia and hypertriglyceridemia (up to 10,000 mg/dL). The absence of LPL leads to the inability to catabolize dietary fat, and massive amounts of chylomicrons accumulate in the blood. VLDL is usually normal, HDL decreased and LDL increased (see Table 2.3). The condition is usually diagnosed in childhood.

Signs and symptoms include severe abdominal pain and acute pancreatitis (Durrington, 2004). When triglyceride level reach 2000 mg/dL, eruptive xanthomas appear; when they reach 4000 mg/dL, lipemia retinalis appears. The severity of the symptoms is related to the triglyceride level. This condition is a rare (1 in 1,000,000 individuals), autosomal recessive disorder. Individuals with this condition are not predisposed to atherosclerosis (Khera et al., 2011).

2.7.2 Apolipoprotein C-II deficiency

When apolipoprotein C-II is altered or absent, chylomicrons do not break down, and the triglyceride level can increase from 500 to 10,000 mg/dL. The individual's total cholesterol level can vary from 150 to 890 mg/dL, with HDL and LDL values extremely decreased (see Table 2.3). The symptoms of this condition are milder than those seen with deficiency of LPL activity and occur at an older age. Symptoms for this condition do not include eruptive xanthomas or lipemia retinalis. Individual with this disease are not predisposed to atherosclerosis (Khera et al., 2011).

2.7.3 Familial combined hyperlipidemia

Characteristics of familial combined hyperlipidemia include increased levels of total and LDL cholesterol (Fredrickson type IIa), increased triglycerides (type IV), or both (type IIb). Low density lipoprotein (LDL) level are approximately 190 mg/dL, and triglyceride levels are between 200 and 400 mg/dL. High density lipoprotein (HDL) is usually decreased (see Table 2.3). This disease is usually expressed in adolescence, and it is relatively common (1 in 100 individuals). Familial combined hyperlipidemia is associated with coronary heart disease, and atherosclerosis is usually the only symptom (Khera et al., 2011).

2.7.4 Hyperopobetalipoproteinemia

Characteristics of hyperapobetalipoproteinemia include increased levels of apo B-100, total cholesterol, triglycerides, and LDL with decreased HDL levels. Upraised lipid levels are caused by the overproduction of VLDL by the liver (see Table 2.3). Atherosclerosis is usually the only symptom, and this condition is associated with coronary heart disease.

2.7.5 Familial hypertriglyceridemia

Characteristics of familial hypertriglyceridemia include normal LDL, decreased HDL and increased triglycerides (see Table 2.3). This condition has an autosomal dominant inheritance pattern with delayed expression. It is a fairly common disease with a frequency of 1 in 500 individuals. The VLDL in this condition is large with an abnormally high triglyceride content. Symptoms include acute pancreatitis if the triglyceride level reaches 4000 mg/dL.

2.7.6 Type V hyperlipoproteinemia

Characteristics of type V hyperlipoproteinemia include increased chylomicrons and VLDL. This disorder has an autosomal dominant inheritance pattern and is expressed in adulthood. Symptoms include eruptive xanthomas, lipemia retinalis, pancreatitis, and abnormal glucose tolerance with hyperinsulinemia. This condition is not linked to premature atherosclerosis (Durrington & Soran, 2004).

2.7.7 Dysbetalipoproteinemia (Type III)

Characteristics of dysbetalipoprotienemia (Fredrickson type III) include increased total cholesterol and triglycerides as well as decreased LDL and HDL (see Table 2.3). This disease is caused by a primary genetic defect in the removal of chylomicron and VLDL remnants. It is expressed in adulthood. Clinical symptoms include characteristic palmar xanthomas (yellow deposits of cholesterol in the creases of the palms). Eruptive xanthomas also appear on the tendons. Premature atherosclerosis develops in 30% to 50% of the patients affected by this condition, which occurs in 1 in 1000 individuals.

2.7.8 Familial hypercholesterolemia

Characteristic of familial hypercholesterolemia include increased LDL (two to three times normal), increased total cholesterol (500 to 1200 mg/dL), increased triglycerides, and decreased HDL (see Table 2.3). This condition affects 1 in 500 individuals. It is inherited in an autosomal dominant pattern and is caused by a genetic mutation in the LDL receptor gene. Cholesterol deposits form in the skin, tendons and arteries. The yellow-orange cutaneous xanthomas are characteristic of this disorder, and they appear before age 30. Homozygotes usually die before 40 years of age from a myocardial infraction (Chobanian, 2003).

2.7.9 Familial defective apolipoprotein B-100

Characteristics of familial defective apo B-100 include normal or very elevated levels of total cholesterol, increased LDL, normal triglyceride and normal HDL (see Table 2.3). This condition is caused by a mutation in the apo B-100 gene in individuals of European descent. It occurs in 1 of 500 individuals.

2.7.10 Familial hypoalphalipoproteinemia

Characteristics of familial hypoalphalipoproteinemia include normal triglyceride, normal LDL, normal VLDL and decreased HDL levels (see Table 2.3). This condition is inherited in an autosomal dominant pattern (Chobanian, 2003). Affected individuals have a very high incidence of coronary heart disease.

2.7.11 Defects in the synthesis of apolipoprotein A-I

Individuals with defects in the synthesis of apolipoprotein A-I characteristically have normal total cholesterol, normal triglyceride, normal LDL, normal VLDL and decreased HDL level; heterozygotes have half the normal HDL value and homozygotes have only a trace amounts (see Table 2.3). Symptoms include corneal clouding and a risk of developing premature coronary heart disease (Chobanian, 2003). Ten percent of the general population have this condition.

2.7.12 Defects in catabolism of apolipoprotein A-I (Tangier Disease)

Tangier disease was named for Tangier Island, on which it was first discovered. Characteristics include decreased HDL, decreased total cholesterol (heterozygotes, 170 mg/dL; homozygotes, 70 mg/dL), normal LDL, normal VLDL and normal triglycerides (see

Table 2.3). This condition is inherited in an autosomal dominant pattern. It resulted in the accumulation of cholesterol esters in body tissue. Symptoms include hyperplastic orange tonsils, splenomegaly, peripheral neuropathy, hepatomegaly and corneal clouding. Homozygotes show an increased incidence of coronary heart disease (Chobanian, 2003).

2.7.13 Lipoproteins and atherogenesis

Lipoproteins are implicated in the development of atherogenesis. Atherogenes is associated with elevated LDL levels, elevated VLDL levels and chylomicron remnants, and decreased levels of HDL (Figure 2.10) (Khera et al., 2011).

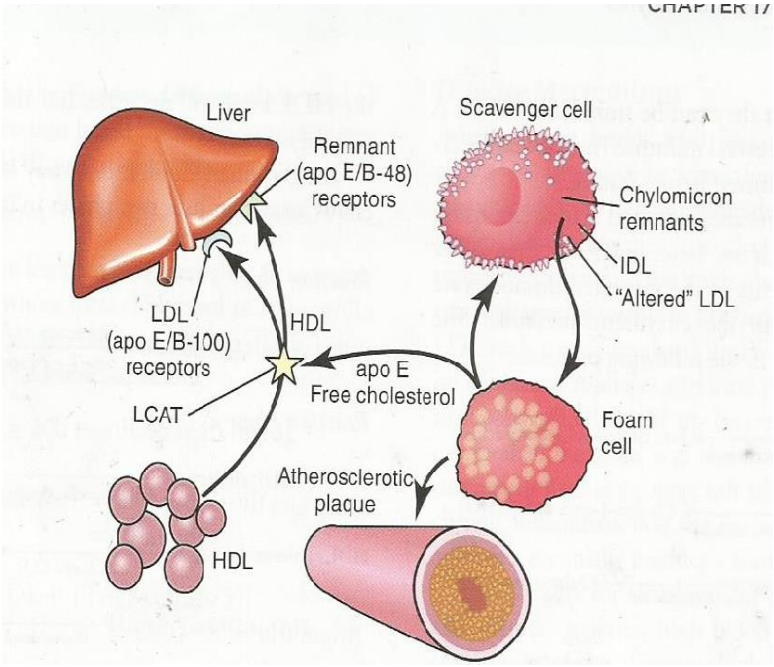


Figure 2.10: Lipoproteins and atherosclerosis. One hypothesis linking arthrogenesis with lipoproteins centers on foam cell formation. Foam cells adhere to the wall of blood vessels to form atherosclerotic plaques. Foam cell formation is promoted by chylomicron remnants, IDL and altered LDL molecules. HDL molecules promote free cholesterol excretion from foam cells, thus preventing the development of atherosclerotic plaque. apo E, Apolipoprotein E; B-48, apolipoprotein B-48;B-100,apolipoprotein B-100; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LCAT, lecithin cholesterol acetyl transferase; LDL, low-density lipoprotein.

2.8 Obesity

2.8.1 Introduction of obesity in cardiovascular morbidity and mortality

Obesity and overweight are independent risk factors for cardiovascular morbidity and mortality (Allison et al., 1999; Sjostrom, 1992). This figures are mainly based on the epidemiological studies in a white population. There may be gender and ethnic differences, as for example, coronary heart disease risk is proportionately increased at a lower body mass index (BMI) in the Asian population. Risk of morbidity and mortality begins to rise at BMI >25 kg/m^2 and the risk increases sharply at BMI >30 kg/m^2. Although BMI is used as a surrogate indicator of cardiovascular risks, central or abdominal obesity is considered to be a better predictor.

The mechanism by which obesity causes increased cardiovascular morbidity and mortality is attributed to associated co-morbidities and risk factor such as hypertension, dyslipidaemia, type 2 diabetes and insulin resistance. The co-occurrence of some or all of these risk factor along with obesity is termed the cardiometabolic syndrome.

Until recently the mechanism of atherosclerosis in obesity was not well understood. The recognition of adipose tissue as a metabolically active endocrine organ, capable of synthesizing and secreting mediators like tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), plasminogen activator inhibitor -1 (PAI-1) and angiotensin II (AII) may help explain the process of accelerated atherosclerosis. Endothelial dysfunction, which is a recognized complication of obesity and type 2 diabetes mellitus, play an important role in thrombus formation.

The secretion of adipocytokines like adiponectin may be implicated in the pathogenesis of type 2 diabetes mellitus. Thus, favourable modifying lipids, decreasing blood pressure, achieving near normoglycaemia, and reducing pro-inflammatory may prevent progression of atherosclerosis or occurrence of acute coronary syndrome events in the obese high risk populations with type 2 diabetes.

2.8.2 Obesity and mortality

The association between excess body weight and death is confirmed by the Nurse' Health Study, with mortality rising progressively in women with BMI >29 kg/m^2 (Manson et al., 1995). The increased mortality was also noted in the American Cancer Society's Cancer Prevention Study I and II. Cancer Prevention Study II involved 457 785 men and 588 369 women followed for 14 years. The lowest mortality for men was within BMI 23.5 – 24.9 kg/m^2 and for women 22.0 – 23.4 kg/m^2. For BMI >40 kg/m^2, the relative risk of death was 2.6 times higher for men and 2 times higher for women compared with BMI between 23.5 and 24.9. There was an ethnic difference with the relative risk of death – 1.4 for black men and 1.2 black women with BMI >40 kg/m^2 (Calle et al., 1999).

2.8.3 Obesity and cardiovascular disease

Obesity is a major contributor to the risk of cardiovascular disease. In the Framingham Heart Study, the 26 years incidence of coronary heart disease (CHD) was increased by a factor of 2.4 in obese women and 2 in obese men under age of 50 years (Hubert et al., 1983). Excess weight was an independent predictor of coronary artery disease, coronary death and congestive heart failure after adjusting for other know recognized risk factor.

The Nurse' Health Study from the United States, the risk of developing CHD increased 3.3 fold with BMI >29 kg/m^2 and 1.8-fold between 25 and 29 kg/m^2 compared to those women with BMI <21 kg/m^2 (Manson et al., 1995). Each kg of weight gained from age of 18 years was associated with 3.1% higher risk of cardiovascular disease (Willett et al., 1995).

This increased risk extends to overweight children and adolescents, who are at risk of premature cardiovascular morbidity and death. Excess weight in adolescence was a better predictor of these risks than excessive weight in adulthood (Gunnell et al., 1998).

Along with an increased risk of CHD, obese populations experience a higher recurrence of cardiac event rates after acute myocardial infarction. The relative risk of recurrent infarction or death was 1.5 with BMI 30 - 34.5 kg/m^2 and 1.8 with BMI >35 kg/m^2 compared to BMI 16 - 24 kg/m^2 as seen in a population based study of 2541 patients (Rea et al., 2001).

The increased CHD risk is better correlated with abdominal or central obesity than simple BMI (Rich-Edwards et al., 1995). In the Nurse' Health Study, a waist-hip ratio (WHR) of ≥0.88 versus WHR <0.72 was associated with an increased relative risk of CHD of 3.25 (Rexrode et al., 1998).

The increased CHD morbidity and mortality could be related to traditional risk factors like hypertension and dyslipidaemia or due to the effect of obesity on the cardiovasculature. Obesity is associated with disturbances in cardiac function and structural changes in the absence of hypertension and underlying organic heart disease. There is an increase in total blood volume in proportion to body weight resulting in higher cardiac output. Volume

overload of the left ventricle results in increased left ventricular stress which stimulates eccentric hypertrophy of the ventricle with resultant diastolic dysfunction, termed obesity cardiomyopathy. The presence of hypertension in obesity exacerbates left ventricular wall changes, which can increase progression towards heart failure (Albert & Hashini, 1993).

Left ventricular wall abnormality is implicated in the propensity for sudden death seen in obesity. The reason for sudden death from cardiomyopathy may be due to complex ventricular arrhythmias. Prolonged Q-T interval which predispose to cardiac arrhythmias, occurs in up to one third of obese subjects (Frank et al., 1986). Other ECG changes observed in a study of 100 obese subjects compared with 100 normal subjects, without any evidence of cardiac disease included more leftward shift of P, QRS and T axes, evidence of left ventricular hypertrophy and left atrial abnormality and T-wave flattening seen in the inferior and lateral leads (Alpert et al., 2000). Autonomic dysfunction due to alteration in parasympathetic and symphathetic cardiac innervation may also contribute towards arrhythmias.

The sturture and functional shanges are also seen in the right side of the heart in obesity. Right ventricular dysfunction could be secondary to left ventricular dysfunction or due to obstructive sleep apnoea and/or obesity hyperventilation syndrome which occurs in 5 percent of morbidly obese individuals (Alpert & Hashini, 1993).

2.9 Adipose-tissue derived factors

Adipose tissue product a large number of cytokines which include: leptin, the product of the ob gene (Zhang et al., 1994), TNF-α (Hotamisligil et al., 1993; Kern et al., 1995), resistin

(Holcom et al., 2000; Mc & Ternan, 2002; Steppan et al., 2001) adiponectin (Hotta et al., 2000; Maeda et al., 1996) and interleukin-6 (IL-6) (Mora & Pessin, 2002; Spranger et al., 2003), which may serve as important factors determining the pathogenesis of type 2 diabetes from obesity.

2.9.1 Leptin

Leptin is secreted and predominantly produced by adipose tissue, which circulates in the blood as a protein of 146 amino acids with a molecular mass of 16kDa (Madej et al., 1995; Zhang et al., 1994). Leptin is presently viewed as a hormone that adapts and responds to metabolic effects on peripheral tissues as well as a satiety signal. Data presently suggest that leptin regulates energy expenditure mainly by acting on the brain. Leptin is actively transported across the blood-brain barrier and reaches the hypothalamus where it binds to specific leptin receptors located on the surface of neuropeptide Y, a tyrosine-containing peptide with powerful stimulatory effects on appetite.

This leads to suppression of appetite, and ultimately activates the release of noradrenaline from the sympathetic nerve terminals that innervate adipose tissue and influence insulin actions in adipose tissue, liver, pancreas and potentially reproductive organs (Lonnqvist et al., 1999). The central action of leptin was first demonstrated by studies carried out on mice that were induce to have a homozygous defective(*ob/ob*) gene. The mice become obese and developed diabetes but, upon treatment with recombinant leptin, they lost weight and their diabetes improved (Pelleymounter et al., 1995). This generated intense interest and leptin was postulated to be the key mediator in the negative feedback loop from adipose tissue to

the brain, enabling the central nervous system to sense the size of the body fat depot (Considine et al., 1996; Van Gaal et al., 1999; Figure 2.11).

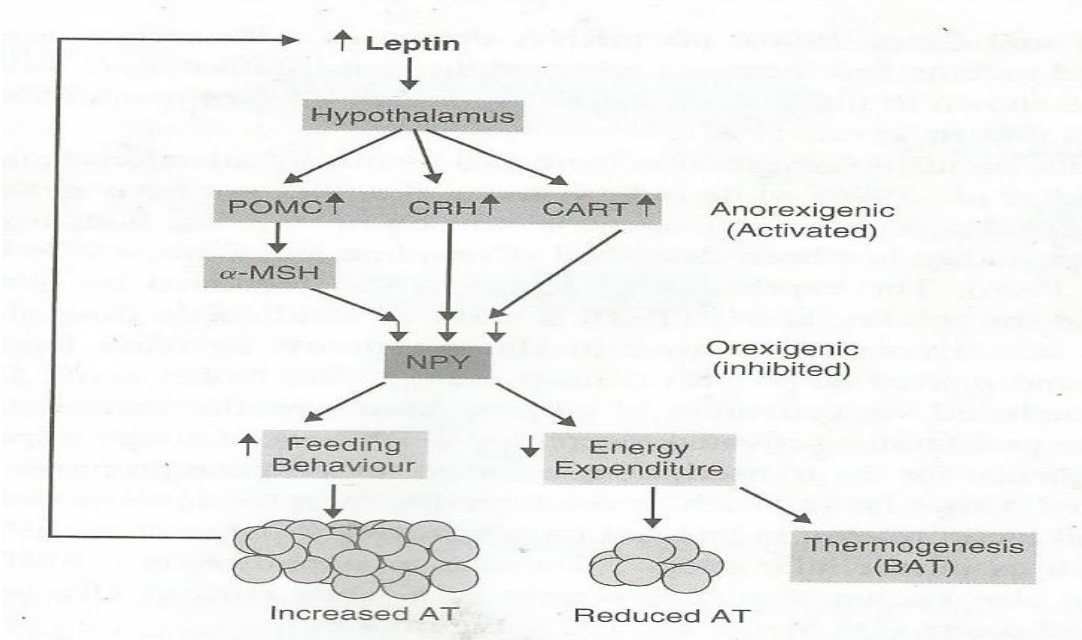


Figure 2.11: The schematic action of leptin in the human body

The role of leptin has turned out to be far more complex than first thought as leptin mutations inducing obesity are rare and, conversely, plasma leptin concentrations are elevated in obese humans and other rodent models depot leptin (Considine et al., 1996). Furthermore, these is a strong positive correlation between plasma leptin levels and fat mass (Zhang et al., 1994). Consequently, it is suggested that the hyperleptinaemia found in obese subjects may be a consequence of leptin resistance in these individuals.

The receptors for leptin (OB-R) have been identified in the hypothalamus and surrounding brain regions- a finding that is consistent with its centrally mediated effects on appetite, metabolism and other endocrine systems involved in the starvation response (Schwart et al., 1996; Tartaglia et al., 1995). However, leptin receptors have also been identified in many

other tissues such as possible role in the regulation of glucose uptake into skeletal muscle tissue and adipose tissue as well as being implicated in the development of insulin resistance (Muller et al., 1997; Shimomura et al., 1999; Yaspelkis et al., 1999).

Circulating leptin concentrations appear to be in direct proportion to the amount of *ob* mRNA in the adipose tissue, which is increased in obese subjects (Considine & Caro, 1996). *ob* mRNA decreases with weight loss in both humans and rodents and increases with weight gain (Maffei et al., 1995). Overfeeding has shown to increase *ob* mRNA in the absence of a significant weight gain in rats (Harris et al., 1996)

In rodents, on mRNA expression increased by insulin injection (Leroy et al., 1996; Saladin et al., 1995) while in human insulin injection does not have an acute effect and in vitro studies has been described (Considine & Caro, 1997); Kolaczynski et al., 1996). The expression of leptin is also regulated by other factors such as the thiazilidinediones (TZD) a class of antidiabetic drug also known as insulin sensitizers that improve insulin sensitivity, decrease leptin mRNA in human and murine adipocytes (Nolan et al., 1996; Zhang et al., 1996).

The effect is mediated via activation of adipose tissue-specific transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ), although a specific consensus sequence for the transcription factor on the ob gene promoter could not be identified. Other factors such as β-adrenergic receptor agonists reduce leptin mRNA and leptin release in isolated rat adipocytes (Slieker et al., 1996). Other agents such as intracellular cyclic

adenosine monophosphate (cAMP), isoproterenol are also known to reduce weight gain while cortisol effects on leptin increased adiposity (De Vos et al., 1995; Figure 2.12).

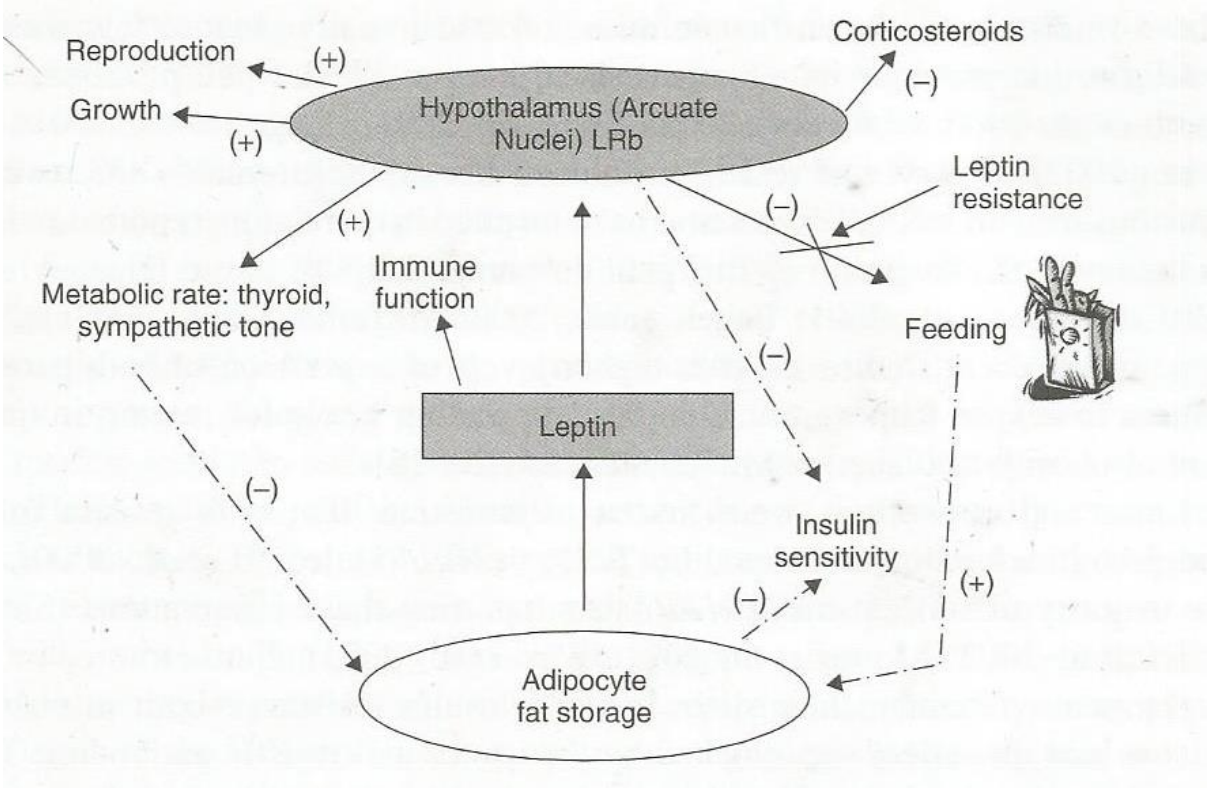


Figure 2.12: The Multi Roles of Leptin

2.9.2 Leptin and coronary artery disease (CAD)

Leptin has a variety of important central and peripheral actions to regulate energy balance and metabolism, fertility, and bone metabolism that are mediated by specific cell surface leptin receptors (Margetic et al., 2002; Seafert et al., 2004), Importantly, leptin may also exert actions related to cardiovascular homeostasis that are potentially atherogenic, thrombotic, and angiogenic (Beltowski, 2006; Correia & Haynes, 2004; Werner & Nickenig, 2004). Leptin has peripheral actions to stimulate vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy that may contribute to pathogenesis of type 2 *diabetes*

mellitus (T2DM), hypertension, atherosclerosis, and coronary heart disease (Beltowski, 2006; Seafert et al., 2004). Insulin resistance, systemic hypertension, and hypercholesterolemia.

All contribute independently to vascular endothelial dysfunction that promotes atherosclerosis and coronary heart disease (Koh, 2000; Muniyappa et al., 2007). Reciprocal relationships between endothelial dysfunction and insulin resistance are characterized by impaired insulin-stimulated nitric oxide (NO) production from endothelium that decreases blood flow to insulin target tissues (Han et al., 2007; Kim et al., 2006). Relationships among obesity, metabolic syndrome, diabetes mellitus, and their cardiovascular complications are well established. However, the mechanisms by which excess adiposity causes both insulin resistance and vascular dysfunction are not well understood. Direct vascular effects of adipokines such as leptin are attractive candidates that may help to explain underlying pathophysiological mechanisms. Several clinical studies demonstrate that hyperleptinemia predicts acute cardiovascular events, restenosis after coronary injury such as angioplasty, and cerebral stroke independent of traditional risk factors (Pjatti et al., 2003; Soderberg et al., 1999; Wallace et al., 2001). Leptin-deficient hyperlipidemic mice (ob/ob; apolipoprotein E [apoE]$^{-/-}$ mice) develop significantly less atherosclerosis than apoE$^{-/-}$ mice on an atherogenic diet. Exogenous leptin significantly increases atherosclerotic areas in apoE$^{-/-}$ mice. Taken together, these findings support the notion that leptin accelerates atherosclerosis (Chiba et al., 2008). By contrast, some data indicate that leptin may protect against atherosclerosis in specific animal models. For example, low-density lipoprotein–receptor (LDLR) knockout mice lacking leptin (LDLR$^{-/-}$ ob/ob) develop more atherosclerotic lesions than LDLR$^{-/-}$ control mice (Hasty et al., 2001). Moreover, in 207 women with normal

glucose tolerance, impaired glucose tolerance, or type 2 diabetes mellitus, low plasma leptin predicted cardiovascular mortality during a 7-year follow-up period (Piemonti et al., 2003).

Leptin stimulates lipoprotein lipase secretion in cultured human and murine macrophages (Maingrette & Renier, 2003). Leptin increases accumulation of cholesterol esters in foam cells, especially at high glucose concentrations (O'Rourke et al., 2001). However, under normoglycemic conditions leptin may protect macrophages from cholesterol overload (O'Rourke et al., 2002). Several studies demonstrate an inverse relationship between leptin and high-density lipoprotein (HDL) cholesterol and/or apolipoprotein A-I in humans (Rainwater et al., 1997). Leptin promotes hepatic HDL clearance by upregulating scavenger receptor type B1 and decreases plasma HDL level in mice (Lundasen et al., 2003). Thus, in the context of hyperglycemia, leptin may impair cholesterol removal from peripheral tissues by lowering HDL and unfavorably affect local cholesterol balance in diabetic patients.

Leptin stimulates migration and proliferation of vascular smooth muscle cells and expression of matrix metalloproteinase-2 in human aorta in vitro (Li et al., 2005). Interestingly, stretching the vascular wall induces expression of both leptin and its receptor in rabbit portal vein (Zeidan et al., 2005). Leptin stimulates synthesis and secretion of endothelin-1 in human umbilical vein endothelial cells and expression of preproendothelin-1 and endothelin ETA receptor genes, angiotensinogen, and angiotensin type 1 receptor expression in rabbit portal vein smooth muscle cells (Zeidan et al., 2005). Finally, leptin stimulates osteoblastic differentiation and hydroxyappatite production by calcifying vascular smooth muscle cells (Parhami et al., 2001).

Leptin increases expression of P-selectin on human platelets in vitro (Wallaschofski et al., 2004). Interestingly, enhancement of the effects of leptin on ADP-induced aggregation is attenuated in platelets obtained from overweight or obese individuals compared with normal-weight subjects (Corsonello et al., 2003). However, in other studies, no effect of leptin is observed at concentrations up to 500 ng/mL from normal-weight or obese subjects (Ozata et al., 2001). In a Swedish population-based study, leptin positively correlates with plasma fibrinogen and inversely correlates with tissue plasminogen activator concentration in plasma (Soderberg et al., 1999). In some contexts leptin may contribute to platelet hyperactivity and a pathological shift in the coagulation-fibrinolysis balance observed in the metabolic syndrome.

Leptin increases insulin sensitivity in rats and may improve vascular responses to insulin in states of insulin resistance (Sivitz et al., 1997). Leptin secretion by adipocytes is stimulated by insulin, and plasma leptin significantly correlates with plasma insulin (Courten et al., 1997). By contrast, under some conditions, leptin negatively regulates insulin signaling66 and glucose uptake Hennige et al., 2006). Leptin increases free fatty acid oxidation in isolated mouse soleus muscle by 42%, whereas insulin decreases this by 40%. When both hormones are administered, leptin attenuates both the antioxidative and lipogenic effects of insulin by 50% (Muoio et al., 1997).

Leptin may potentiate pressor effects of hyperinsulinemia in insulin-resistant states. Therefore, interactions between Angiotensin II (Ang II) and insulin with leptin may have deleterious cardiovascular effects in the setting of obesity. Leptin administered short term has no net effect on blood pressure under healthy conditions. Although a cause-and-effect

relationship between leptin and high blood pressure in humans has not been demonstrated directly, many clinical studies have shown elevated plasma leptin in patients with essential hypertension. In addition, a significant positive correlation exists between leptin and blood pressure independent of body adiposity in both normotensive and hypertensive individuals (Agata et al., 1997). Correlations between plasma leptin levels and blood pressure before efonidipine therapy was shown no significant different (Singhal et al., 2002). Correlations between leptin and blood pressure also influenced by gender. Despite higher serum leptin levels in women, leptin and blood pressure associations have been reported more frequently in men than in women, regardless of hypertension and adiposity (Mallamaci et al., 2000). Ethnic and racial background may also influence the relationship between leptin and blood pressure.

In pathological conditions such as obesity, the balance of leptin actions may shift to stimulate vascular inflammation, oxidative stress, and vascular smooth muscle hypertrophy. These actions may contribute to the pathogenesis of hypertension, atherosclerosis, left ventricular hypertrophy, and type 2 *diabetes mellitus* (T2DM). Several clinical studies demonstrate that hyperleptinemia predicts acute cardiovascular events, restenosis after coronary injury, and cerebral stroke independent of traditional risk factors (Piatti et al., 2003; Soderberg et al., 1999; Wallace et al., 2001). By contrast, some data indicate that leptin may protect against atherosclerosis in specific animal models (Chiba et al., 2008). Indeed, low plasma leptin predicts cardiovascular mortality (Piemonti et al., 2003). However, the effects of leptin on cardiovascular pathophysiology are complex and not completely understood.

2.9.3 Leptin and lipid metabolism

Lipid metabolism is regulated by numerous hormones and leptin is the most recently discovered among them (Zhang et al., 1994). Previous study shown that the leptin modulates appetite and lipid storage through additional signaling pathways that are independent from neuropeptide Y (NPY) as mice lacking neuropeptide Y (NPY) have normal food intake and body weight, yet still show a decrease in food intake, body mass and fat mass when treated with leptin (Erickson et al., 1996).

Although, the direct effects of leptin on substrate cycling rates have not yet been investigated, it has been shown that the triacylglyceride and free fatty acid substrate cycling rate of human adipocytes is negatively correlated with obesity (Bottcher & Furst, 1997). Leptin treatment of in vitro adipocytes also increases the ratio of glycerol:fatty acids that are released from the cells (Wang et al., 1999). These results suggest that the triacylglyceride and free fatty acid cycling rate may be increased by leptin. Leptin appears to mediate fatty acid metabolism by changing enzyme mRNA levels and concentration. For example, the presence of leptin inhibits the expression of acetyl CoA carboxylase in adipocytes (Bai et al., 1996).

Using a different experimental approach, long-term treatment of wild-type mice with high concentrations of leptin increased mRNA expression of hormone-sensitive lipase, the key lipolytic enzyme, while causing a decrease in mRNA expression of the lipogenic enzyme, fatty acid synthase (Sarmiento et al., 1997). Leptin also regulates lipolysis by controlling the activity of hormone-sensitive lipase. This is an enzyme which is controlled by cellular levels of cyclic adenosine monophosphate (cAMP) and although the regulation of lipolysis by

leptin at the molecular level has not yet been fully described, preliminary evidence suggests that leptin, like glucagon and catecholamines, stimulates lipolysis by increasing cyclic adenosine monophosphate (cAMP) concentrations (Takekoshi et al., 1999).

Leptin is able to mediate lipid metabolism by way of many different routes. Its effects are exerted both indirectly through the central nervous system and directly on the peripheral tissues. Both pathways end up with similar outcomes, a decrease in triacylglycerol synthesis and increases in lipolytic rates and lipid oxidation. This is achieved by a reduction in caloric intake, causing an increased reliance on internal energy stores, mainly triacylglycerol. Modulation of energy expenditure by leptin through its inhibition of normal drops in metabolic rate, essentially acts to increase triacylglycerol use further. This shift in metabolism towards a catabolic state coupled with the direct lipolytic and oxidative effects of leptin on peripheral tissues sum together to cause more rapid utilization of triacylglycerol stores. The ability of leptin to dampen stimulation of lipogenesis by insulin and possibly stimulate adipocyte apoptosis, further enhances the capacity of leptin to regulate fat mass. The limited number of studies that have specifically looked at the direct effects of leptin on lipid metabolic rates have given us some important, yet still preliminary information about leptin. The effect that leptin has on lipolytic pathways in in vitro systems is becoming more clear, yet the effect that leptin treatment has on in vivo rates of lipid metabolism is still unknown.

2.9.4 Effect of treatment on serum leptin level in stable coronary artery disease (CAD)

The American Heart Association/American College of Cardiology (AHA/ACC) 2013 guidelines for the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk

in adults recommends life style modifications prior to, and in concert with, use of moderate-to-high intensity statins to achieve lipid-lowering goals (Stone et al., 2014). Interestingly, statin users in the general US population (as represented in the National Health and Nutrition Examination Survey, NHANES) report higher total calorie intake as well higher fat intake compared to non-statin users (Sugiyama et al., 2014). In the previous study by Maeda and Horiuchi (2009) simvastatin-mediated decreases in leptin mRNA were shown to be dependent on cellular increases in cyclic adenosine monophosphate (Camp) and activation of the protein kinase A (PKA) pathway. Besides that, approximate 20% reduction in leptin transcription in response to atorvastatin and simvastatin treatment (Prachi et al., 2018). Considering simvastatin and atorvastatin reduce leptin expression and secretion. However, the role of statins in regulation of leptin is conflicting. While several clinical studies suggest that statin therapy is associated with decreased systemic leptin (Krysiak et al., 2014), some studies have shown that statin therapy does not contribute to any change in leptin levels (Al-Azzam et al., 2013; Szotowska et al., 2012). These discrepancies may be related to differences in study populations, presence of comorbidities, dosage of statins, length of statin treatment, as well as use of different statins.

Other than that, metformin treatment for diabetic patients of coronary artery disease (CAD) effectively reduces fasting insulin and leptin in 31 morbidity obese, non-diabetic subject with BMI >30 kg/m^2 (Glueck et al., 2001). Metformin treatment also not associated with decrease in blood leptin level in patients with type 2 *diabetes mellitus* (T2DM) compared with levels in patients in the control group (Satoshi et al., 2017).

Previous studies have elucidated that some antihypertensives might be more relevant than others in terms of reducing leptin levels in obesity and coronary artery disease. When the angiotensin receptor blocker (ARB) candesartan was employed prior to angiotensin II administration in human fat cells it effects angiotensin II to stimulate leptin production (Skurk et al., 2005). Similarly, valsartan, another ARB, aside from lowering blood pressure, decreased leptin levels and BMI in obese individuals, when compared with the calcium channel blocker (CCB) felodipine (Fogari et al., 2005). Likewise, the angiotensinconverting enzyme (ACE) inhibitor, enalapril, in combination with a weight reduction program, evidenced the greatest benefits in terms of weight loss and diminution of plasma norepinephrine, insulin, and leptin levels in comparison with control groups treated with weight reduction program alone or combined with the CCB amlodipine (Masuo et al., 2001). In another study, the beta-blocker (BB) pindolol showed a marked suppressive effect on serum leptin levels, not seen in hypertensive individuals on perindopril, or felodipine (Ficek et al., 2002). In contrast to these findings, ARB Losartan has no effect on leptin levels in young hypertensive individuals despite its hypotensive action (Sonmez et al., 2001). Likewise, both enalapril and clonidine reduced heart sympathetic activity and blood pressure in another clinical trial, but failed to decrease serum leptin levels in normotensive obese and non-obese subjects after 7 days of treatment (Amador et al., 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Materials

3.1.1 Chemicals and reagents

All reagents purchased and used in this research were of analytical grade. Most of the reagents were pre-prepared and ready for use, while certain solutions and dilutions were prepared using ultra filtered water, produced using Millipure ultrapure water system from Thermo Scientific, USA. For sample collection and separation, sterile, non-toxic and non-pyrogenic 10mL syringe and 10mL vacutainer plain tube used were purchased from Becton, Dickinson and Company (BD), America.

For the determination of lipid profile, the levels of plasma triglyceride and LDL-cholesterol were measured using the pre-prepared reagents which were purchased from Beckman Coulter, USA. Final concentration of the reaction solution in the triglyceride reagent comprised of piperazine-N,N'-bis (PIPES) buffer (pH 7.5), 50 mmol/L; lipase (*Pseudomonas*) ≥1.5 kU/L (25 µkat/L); glycerol kinase (*Bacillus stearothemophilus*): ≥0.5 kU/L (8.3 µkat/L); glycerol phosphate oxidase (*Pseudomonas*): ≥1.5 kU/L (25 µkat/L); ascorbate oxidase (*Curcubita species*): ≥1.5 kU/L (25 µkat/L); peroxidase (horseradish): ≥0.98 kU/L (16.3 µkat/L); adenosine triphosphate (ATP): 1.4 mmol/L; 4-aminoantipyrine: 0.50 mmol/L; magnesium acetate: 4.6 mmol/L; N,N-bis (4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB): 0.25 mmol/L and sodium azide as preservative.

Final concentration of the reaction solution in LDL-cholesterol reagent including compound of 2-ethanesulfonic acid (MES) buffer at pH 6.3, cholesterol esterase (*Pseudomonas*), 1875 U/L; cholesterol oxidase (nocardia), 1125 U/L; peroxidase (horseradish), 975 U/L; detergent 1, 0.75%; detergent 2, 0.25%; N.N-bis-(4-sulfobutyl)-m-toluidine disodium (DSBmT), 0.25 mmol/L; 4-aminoantipyrine, 0.375 mmol/L; ascorbate oxidase, 2250 U/L and sodium azide as preservative.

HDL-cholesterol reagent and total cholesterol reagent were purchased from Roche, USA and Diasys, Germany, respectively. HDL-cholesterol reagent consists of two working solutions, reagent 1 (R1) and reagent 2 (R2). The working solution of reagent 1 comprised of 4-(2-hydroxyethil)-1-piperazine ethanesulfonic acid (HEPES) buffer, 10.07 mmol/L; 96.95 mmol/L of 2-(N-cyclohexylamino)-ethanesulfonic acid (CHES) at pH 7.4; dextran sulfate, 1.5 g/L; ≥11.7 mmol/L of magnesium nitrate hexahydrate; 0.96 mmol/L of N-(2-hydroxyl-3-sulfopropyl)-3,5-dimethoxyaniline(HSDA); ≥50 µkat/L (3.0 kU/L) of ascorbate oxidase (*Eupenicillium species.*, recombinant) and ≥16.7 µkat/L (1.0 kU/L) of peroxidase (horseradish). Meanwhile, the working solution of reagent 2 contained HEPES buffer, 10.07 mmol/L, pH 7.0; polyethylene glycol (PEG) – cholesteroloxidase (Pseudomonas spec.) ≥3.33 µkat/L (0.2 kU/L); PEG-cholesterol oxidase (*Streptomyces species.*, recombinant) ≥127 µkat/L (7.6 kU/L); peroxidase (horseradish) ≥333 µkat/L (20 kU/L) and 4-amino-antipyrine: 2.46 mmol/L. Both working solutions are ready for use and contained preservative to maintain the reagent quality.

Reagent components and concentrations for total cholesterol test comprised of 50 mmol/L of ammonium phosphate buffer at pH 6.7; 0.3 mmol/L 4-aminoantipyrine; 5 mmol/L phenol;

cholesterol esterase (CE), ≥200 U/L; ≥50 U/L of cholesterol oxidase (ChOx); peroxidase (POD), ≥3 kU/L and 0.95 g/L of sodium azide as a preservative.

For the determination of leptin hormone concentration level, Human Leptin ELISA kits were purchased from Merck, USA. Human leptin ELISA kits contains one human leptin ELISA plate coated with Rabbit anti-Human leptin antibody, two sheets of adhesive plate sealer, two bottles of 10x HRP wash buffer (50 Mm Tris buffered saline containing Tween 20), eight different concentrations of human leptin ELISA standards (0.78 ng/ml, 1.56 ng/ml, 3.125 ng/ml, 6.25 ng/ml, 12.5 ng/ml, 25 ng/ml, 50 ng/ml and 100 ng/ml in 1 mL), quality controls 1 (QC1) with range (3.6 – 7.5 ng/ml) and quality control 2 (QC2) with range (13.0 -26.9 ng/ml), 0.05M PBS assay buffer at pH 7.4, containing 0.025M EDTA, 0.08% Sodium Azide, 1% BSA and 0.05% Triton X-100. For the leptin concentration determination, human leptin detection antibody (biotinylated mouse anti-human leptin antibody), enzyme solution (streptavidin-horseradish peroxidase conjugated in buffer), substrate (3,3',5,5'-tetramethylbenzidine) and stop solution (0.3M HCL) were used in the assay.

3.2 Methods

3.2.1 Study design

This research is a cross-sectional study, involving patients attending the Cardiac Clinic of Sarawak General Hospital Heart Centre. The centre, which is situated in Kota Samarahan, is the only public cardiology referral centre in Sarawak that serves more than 2.5 million populations in the state. Study is an exploratory study. Sample size calculation was based on prevalence of ischemic heart disease (IHD)hospital admission and not powered to show differences between diabetes mellitus and non-diabetes mellitus in terms of leptin levels.

3.2.2 Sample collection and selection

In this study, 100 participants were recruited from the pool of patients attending the Cardiac Clinic at the Heart Centre of Sarawak General Hospital from the year 2015 till 2016. Sample size of the participants was calculated using the formula (Equation 3.1) below:

Sample size formula; $\mathbf{n = t^2 \times p(1-p) / m^2}$ Equation 3.1

n = required sample size

t = confidence level@95% (1.96)

p = estimated prevalence

m = margin of error@5% (0.05)

Prevalence of patients with Acute Coronary Syndrome in Sarawak General Hospital (2008) – 10% in Annual Report of National Cardiovascular Disease-ACS, National Registry Malaysia (Azman & Sim, 2008).

$n = (1.96)^2 \times 0.1(1-0.1) / (0.05)^2$ Equation 3.2

$= 138.29 \approx 100$

3.2.3 Sample criteria

Patients with more than fifty per cents stenosis in any segment after coronary angiography were identified as having CAD. There are three main criteria that must be fulfilled by the participants prior to the recruitment into this study. Firstly, all participants were categorised as stable CAD patients, which was defined by non-event for the past three months. Secondly, the patients were those who has no plan to undergo any coronary procedures and lastly, the

serum triglyceride level of these patients were in the category of moderate high with the range between 0.7 – 2.8 mmol/L.

This study had been approved by UNIMAS Ethical Committee. All participants were thoroughly briefed by the attending cardiologist and subsequently gave written informed consent prior to study participation.

All participants were monitored for three months subsequent to the enrolment into the study. The primary endpoint was the incidence of major adverse cardiovascular events (MACEs), including death from cardiovascular causes, cardiac arrest, myocardial infarction, stroke, non-fatal stroke or other arterial thrombotic events, and hospitalization due to cardiovascular conditions such as unstable or progressive angina and heart failure.

3.2.4 Sample collection and handling

The laboratory where the study was developed is accredited by Kementerian Kesihatan Malaysia (KKM) in Handbook of Pathology Service 4th Edition 2013. Selection of the participants in this study was specified by moderate triglyceride level for the past three months. After reviewing the patients' medical record, each identified participant was called for an appointment. The participants were required to fast by consuming no food or liquids other than plain water, for 9 to 12 hours. Blood taking was done via venepuncture during the appointment session. The morning blood pressure of all participants who had rested for at least 10 minutes were measured on the right arm. Hypertension was defined as systolic blood pressure of equal or more than 140 mmHg and/or diastolic blood pressure of equal or more than 90 mmHg or having received any anti-hypertensive drugs in the past two weeks.

Patients having fasting plasma glucose levels of more than 7.0 mmol/ L or those using oral hypoglycemic medications or insulin were diagnosed with *diabetes mellitus* (DM).

Body weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively, with participants in light clothing without shoes. Body mass index was calculated using Quetelet's formula: weight in kilograms divided by the height in meters squared.

After an overnight fasting, 10 ml of whole blood was collected from all participating patients. All the samples were collected by using traditional methods of antecubital venepuncture and under aseptic conditions, at room temperature (21 – 25 ^{0}C). After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature for 30 minutes, but not more than 1 hour. The clot was removed by centrifuging at 700 x g for 10 minutes using the benchtop Beckman Coulter centrifuge. The resulting supernatant was the serum used for subsequent biochemical analysis.

Serum was made into aliquots using 6 Eppendorf tubes with the average volume 1.0 ml in each tube. 2 tubes were used for the analysis of serum lipid profile (total cholesterol, triglyceride, LDL and HDL level) which was carried out at the Biochemistry Lab Units, Sarawak General Hospital. The remaining 4 tubes were used for the leptin assay using the ELISA technique (Conlon & Sonnevand, 2011). Full Lipid profile test was performed on the same day. Only those patients with the triglyceride levels between 0.7 to 2.8 mmol/L were selected for the subsequent phase of this study.

3.2.5 Preparation of quality control (QC) solutions and buffers

All solutions were prepared as indicated in the manufacturer's instructions booklet. Lyphochek Assayed Chemistry Control for lipid profile level analysis is human serum based and it is assayed for more than 100 methodologies and over 30 instruments. These two levels of quality controls were prepared by adding 5.0 mL distilled water each. QC solution mixed by pipet or gently invert for 3 to 5 times and leave at room temperature for at least 5 minutes before use. While, wash buffer for human leptin ELISA assay procedure was prepared by diluting 1:10 of the concentrated buffer with ultrapure water. Both bottles buffer containing 50 mL each were transferred into a conical flask. Then, 900 mL of ultrapure water was added and the buffer solution was mixed gently prior to use.

3.2.6 Determination of full lipid profile

Full lipid profile analysis was performed at Biochemistry Lab units, Department of Pathology Sarawak General Hospital. Full lipid profile (FLP) test parameters consist of total cholesterol level, triglyceride level, HDL-cholesterol level and LDL-cholesterol level. Prior to analysis of the sample for full lipid profile, internal quality controls were performed to ensure the accuracy of the analytical testing. There are two levels of internal control that is control 1 as a normal control and control 2 as a high control. The internal control results were plotted as a Levey Jennings chart to give a visual indication whether a full lipid profile test is working well.

All serum samples were analysed by Beckman Coulter AU680 analyser (Beckman Coulter, USA). Before the analysis of the samples, each sample and its respective request form were

verified for the patient's full name and identity card number. This procedure is very important to avoid sample mixed up and wrong results.

Before FLP test was performed, serum sample was transferred into a 3 ml plain tube. Then, the request form and the tube were labelled with same number of barcode for identification. Sample labelled with barcode arranged in the sample rack. Then, identity patient sample was registered in lab information system (LIS) version Omega 2000 by Roche. Every sample registered with different barcode. Patient name, IC number and full lipid profile test were recorded and registered in LIS. Lab information system(LIS) will send test information to Beckman Coulter analyser. After finishing sample registered, sample rack assembled in analyser for analysis.

Beckman Coulter AU 680 is a chemistry analyser which optically measures the concentration or activity of sample serum to be assayed. The system is a discrete, single-line simultaneous multi test analyser with automatic rerun function. Generally, the main processes such as serum dispensing, reagent dispensing, mixing and photometry are fully automated. First, reagent from reagent chamber was dispensed into a cuvette, and then a sample was added. After mixing the sample with the reagent, the system measure the absorbance of the sample produced, when the reaction was completed. Finally, the optical density of the sample was calculated according to the absorbance measured.

3.2.6.1 Determination of total cholesterol

Total cholesterol measured using enzymatic methods, which are faster n safer than older methods. Total cholesterol level determined using cholesterol FS reagent by Diasys,

German. 200 microliter reagent dispensed into cuvette and mixed with 2.0 microliter sample. When reaction between sample and reagent was completed, the absorbance was measured with wave length 520 nm and 700 nm. Determination of cholesterol after enzymatic hydrolysis and oxidation (Artiss et al., 1997; Deeg et al., 1983), shows in the Figure 3.1:

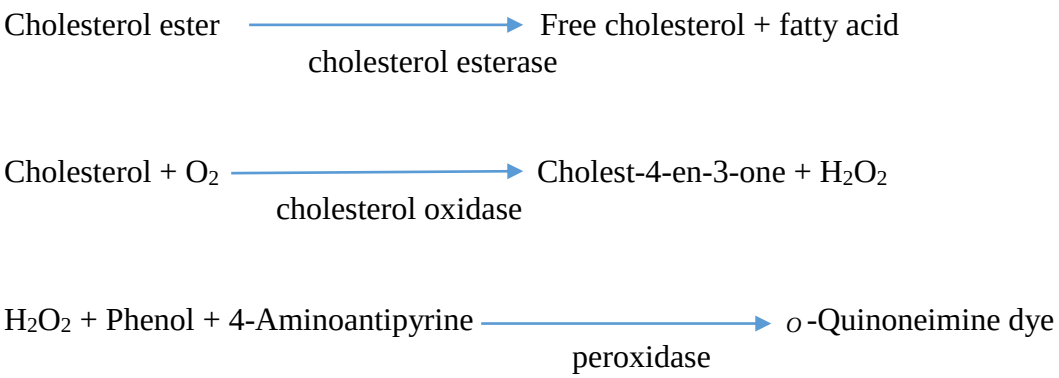


Figure 3.1: The enzymatic method reactions in determining of cholesterol

Cholesterol esters were cleaved through the action of cholesterol esterase producing free cholesterol and fatty acids. Cholesterol oxidase catalysed the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of oxidase, the hydrogen peroxide formed affects the oxidative coupling of phenol and 4-aminoantipyrine, forming a quinone-imine red dye.

The intensity coloured product (o-quinoneimine dye) is measured at 520nm and 700nm, and the intensity of the colour is directly proportional to the amount of cholesterol in the specimen. Enzymatic methods are usually linear to 500 mg/dl. If a sample result indicates more than 500 mg/dl, the samples were diluted with saline and retested for an accurate result.

3.2.6.2 Determination of triglyceride level

Determination of triglyceride is based on a series of coupled enzymatic reactions (Bucolo, 1973 & Trinder, 1969). A volume of 66 microliter reagent 1(R1) and 17 microliter reagent 2(R2) dispensed into cuvette and mixed with 1.6 microliter sample. When reaction between sample and reagent was completed, the absorbance was measured with wave length 660nm and 800 nm. The enzymatic method for determining triglycerides in serum uses the following steps shows in Figure 3.2:

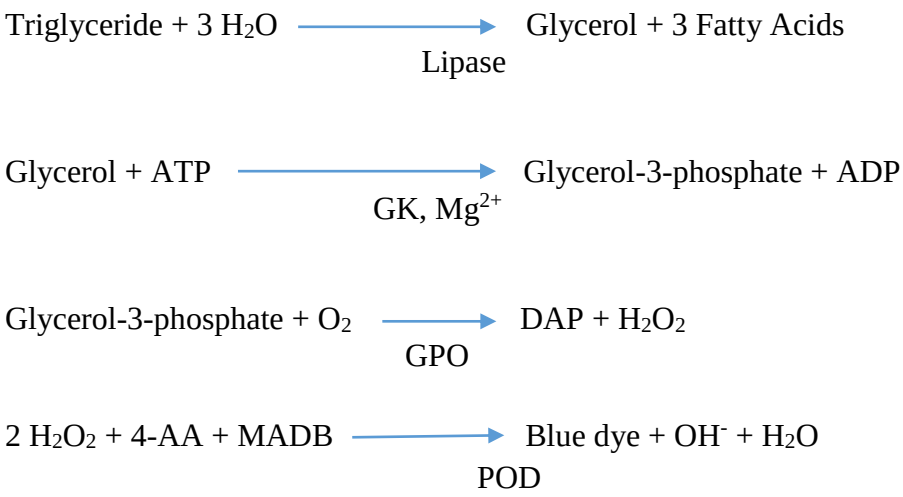


Figure 3.2: The enzymatic method reactions in determining of triglyceride

Where ATP is adenosine triphosphate, ADP is adenosine diphosphate, GK is glycerol kinase, DAP is dihydroxyacetone phosphate, GPO is glycerol phosphate oxidase, 4-AA is 4-aminoantipyrine, MADB is N,N-bis (4-sulfobutyl)-3,5-dimethylaniline,disodium salt and POD is peroxidase.

Triglyceride rapidly and completely hydrolysed by a combination of microbial lipase lipoprotein lipase into glycerol and fatty acids followed by phosphorylated of glycerol by

adenosine triphosphate (ATP) in presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate was oxidized by molecular oxygen to produced hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate in the presence of glycerol phosphate oxidase (GPO). In presence of peroxidase catalytic action, the hydrogen peroxide (H_2O_2) then reacts with 4-aminophenazone and N,N-bis (4-sulfobutyl)-3,5-dimethylaniline,disodium salt (MADB) to produce a chromophore (blue dye). The absorbance of the dye is read at 660 nm and 800 nm in proportional to the triglyceride concentration of the sample. Enzymatic methods are linear to 1000 mg/dl. Specimens with values greater than 1000 mg/dl was diluted with saline and re-tested.

3.2.6.3 Determination of high-density lipoprotein (HDL)

Determination of high density lipoprotein is based on a homogeneous enzymatic reactions. A volume of 210 microliter reagent 1 (R1) and 70 microliter reagent 2 (R2) were dispensed into cuvette and mixed with 3.0 microliter sample. When reaction between sample and reagent was completed, the absorbance was measured with wave length 600 nm and 700 nm. The enzymatic method for determining high density lipoprotein in serum uses the following steps shows in Figure 3.3 (Donna, 2017):

When serum sample mixed with reagent 1 (R1), free cholesterol from lipoproteins other than HDL is consumed and forms a colourless end product in presence of magnesium ions and dextran sulphate. Dextran sulphate selectively forms water soluble compounds with LDL-C, VLDL-C and chylomicrons, which are resistant to polyethylene glycol-modified enzymes. Under the influence of the cholesterol esterase enzyme, the cholesterol ester was quantitatively decomposed into free cholesterol and fatty acids.

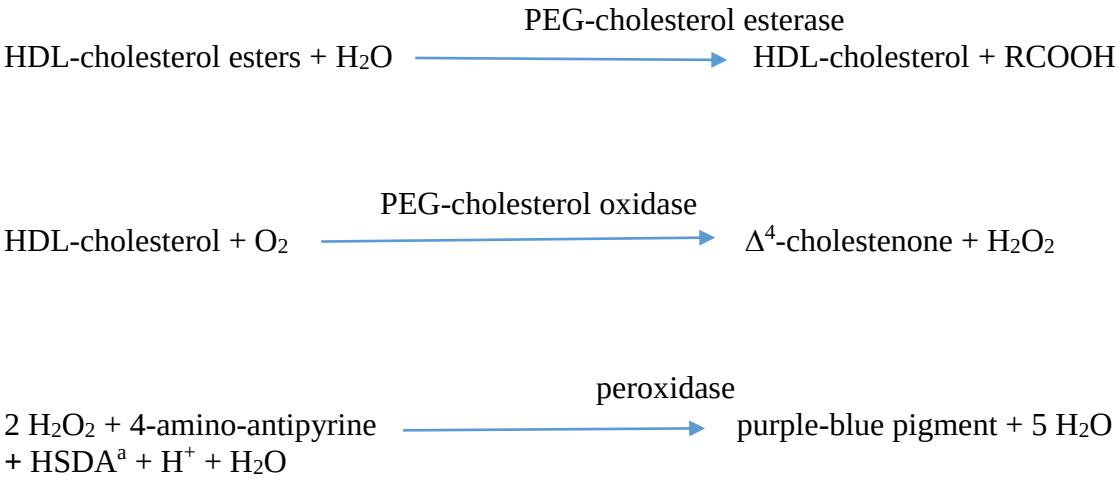


Figure 3.3: The enzymatic method reactions in determining of HDL

With presence of oxygen, cholesterol oxidase enzyme oxidized cholesterol to Δ^4-cholestenone and hydrogen peroxide. The hydrogen peroxide generated reacts with 4-aminoantipyrine and N-(2-hydroxyl-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) in the presence of peroxidation to forming a blue product. The blue colour complex is measured bichromatically at 600 nm and 700 nm. The resulting increased in absorbance is directly proportional to the amount of the HDL in the sample.

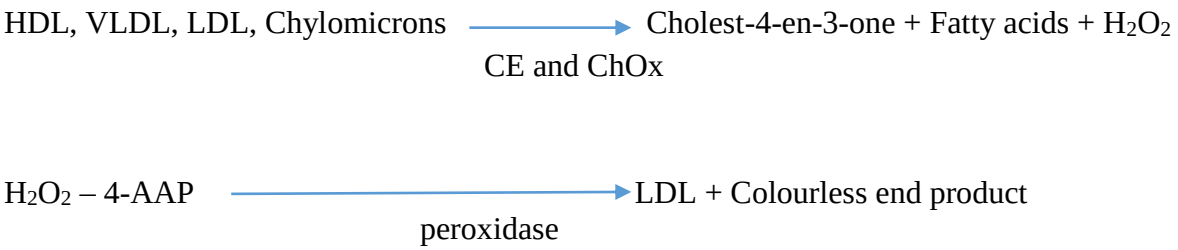
3.2.6.4 Determination of low-density lipoprotein (LDL)

Determination of low density lipoprotein is based on a homogeneous enzymatic reactions system with two distinct phase (Figure 3.4) (Donna, 2017). A volume of 210 microliter reagent 1 (R1) and 70 microliter reagent 2 (R2) were dispensed into cuvette and mixed with 3.0 microliter sample. When reaction between sample and reagent was completed, the absorbance was measured with wave length 540 nm and 660 nm.

Phase 1 is consist of mixing the serum sample with a detergent in reagent 1 (R1) to dissolve the cholesterol from non –LDL particles. Then, Cholesterol oxidase (ChOx), cholesterol

esterase (CE), peroxidase and 4-aminoantipyrine (4-AAP) combine to form a colourless end product.

Reaction Phase 1



Reaction Phase 2

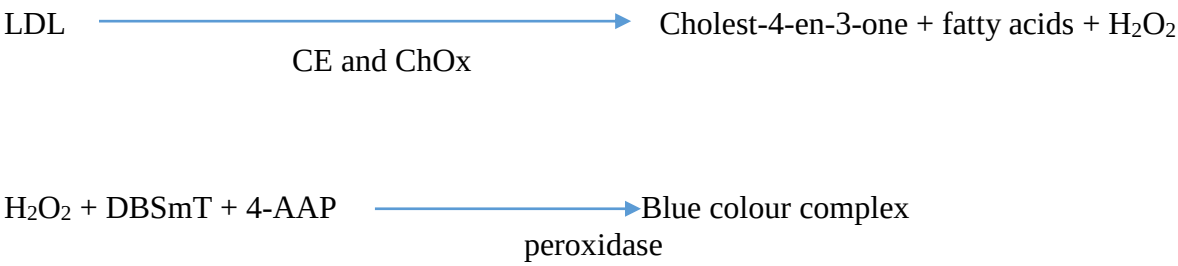


Figure 3.4: The enzymatic method reactions in determining of LDL

In phase 2 is by adding reagent 2 (R2), another detergent dissolves the cholesterol in the LDL particles. This cholesterol then reacts with CE, ChOx and a chromogen to yield a blue-coloured product that is measured bichromatically at 540 nm and 660 nm. The LDL concentration in the sample is directly proportional to the resulting increase in absorbance of the blue compound.

3.2.7 Determination of serum leptin level

Serum leptin level were determined by using human leptin ELISA kit purchased from Merck, USA. Before setting up the assay, serum sample and all reagents were thawed or pre-warm at room temperature. The concentrated wash buffer was diluted 1:10, by adding the entire contents of both bottles of buffer to 900 mL distilled water. Strips assemble in an empty plate holder and 300 uL of diluted wash buffer added to each well. Then, the plate was incubated at room temperature for 5 minutes. After 5 minutes, the residual amount wash buffer decanted and removed by inverting the plate and tapping it smartly onto absorbent towels several times.

Before wells plate dry, 75 uL assay buffer was added into every wells. Then, 25 uL assay buffer was added in duplicate to blank wells. 25 uL Human leptin standard was added in duplicate in order of ascending concentration to the appropriate wells. 25 uL QC1 and 25 uL QC2 also were added in duplicate to the appropriate wells then sequentially followed by added 25 uL of serum samples in triplet to the remaining wells.

After all additions were completed, the plate was covered with a plate sealer and was incubated at room temperature for 2 hours on an orbital microtiter plate shaker that set to rotate at moderate speed, about 400 to 500 rpm. After 2 hours, the plate sealer was removed and the solution was decanted from the plate. Residual solutions in the wells were removed by tapping several times as before. Then, every wells were washed 3 times with 300 uL with diluted wash buffer. Residual buffer was removed by decanted and tapping after each wash.

After that, 100 uL detection antibody was added to each well. The Plate was covered with sealer and was incubated at room temperature for 30 minutes on the microtiter plate shaker. After 30 minutes, sealer was removed and solution was decanted from plate by tapping several times onto absorbent towel.

Then, 100 uL enzyme solution was added to each well. The plate was covered again with sealer and was incubated at room temperature for 30 minutes on the microtiter plate shaker. After 30 minutes, sealer was removed and solution was decanted from the plate. The residual fluid was removed from plate by tapping several times onto absorbent towel. The wells were washed 5 times with diluted wash buffer, 300 uL per well per wash. Then the solution was decanted by tapping firmly to remove residual buffer for every wash. 100 uL of substrate solution was added to each well, then plate was covered with sealer and shake on the plate shaker for 5 to 20 minutes. Blue colour was formed in wells of leptin standards with intensity proportional to increasing concentration of leptin. Then, sealer was removed and 100uL stop solution was added after 15 minutes. The plate was shaked by hand to ensure complete mixing of solution in all wells. The blue colour was turned to yellow after acidification. Finally, within 5 minutes, serum leptin concentration was measured by the turbidity of solution colour as the optical density (OD) at 450 nm and 590 nm using Zynth ELISA plate reader. The serum leptin concentration in the sample is directly proportional to the resulting increase in absorbance of the yellow compound.

3.3 Statistical analysis

All analysis were carryout IBM SPSS version 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp). Normally distributed

variables were expressed as mean ± standard deviation and with differences between patients being compared using the Student's independent *t*-test (two-tailed). Data not normally distributed were expressed as medians and interquartile ranges with differences between patients being compared using the Mann–Whitney *U* test or Kruskal Wallis test. Spearman'rho correlation was used to determine any significant correlation between 2 variable parameters. Data was significantly different when $p < 0.005$.

CHAPTER 4

RESULTS

4.1 Participants' demographic profile

A total of one hundred patients with stable CAD were recruited into this study. The majority of the patients were males, which comprised 84% of the total number of participants while female made up the remaining 16%. Among the participants, Malay patients made up the highest proportion with 32%, followed by Chinese with 31%. Other races were represented by the indigenous groups, which were the Ibans and Bidayuh, with 17% and 10%, respectively. A small percentage of Melanau patients, with 6%, and others with 4%, made up the total of 100 stable CAD patients sample population. The percentages of patients participated in this research according to their respective race and indigenous groups is shown in Figure 4.1.

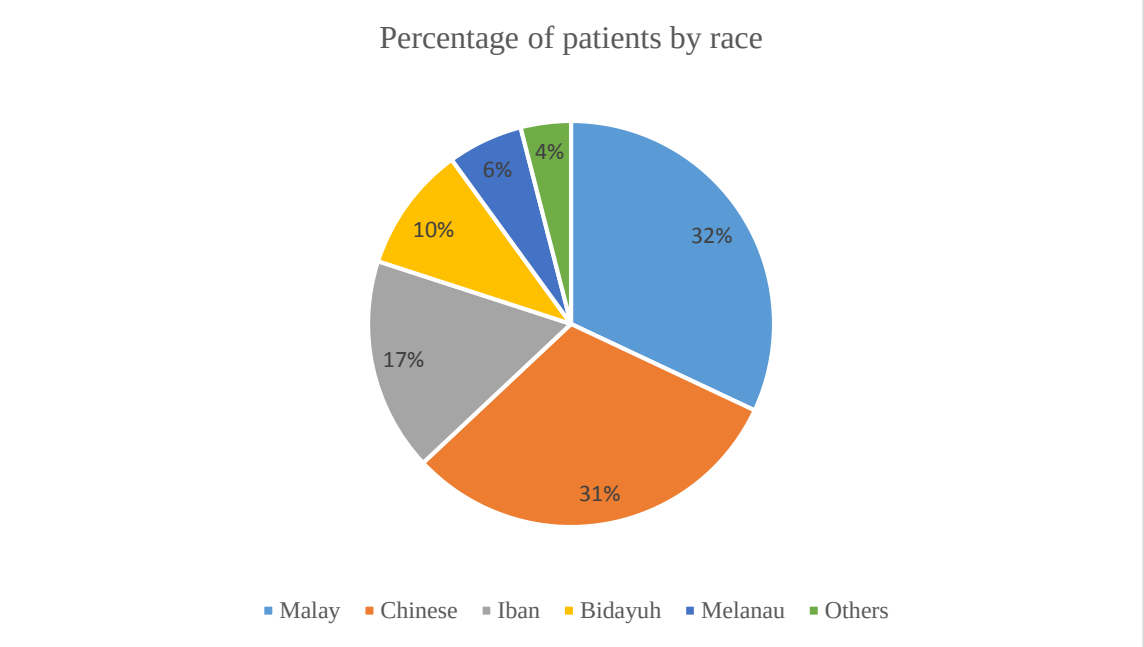


Figure 4.1: The percentage of stable CAD patients according to race

Almost half of the participants (41%) involved in this study aged between 60 - 69 years old and twenty-nine per cent of the patients were in the range of 50 - 59 years old. Twenty per cents of the participants falls in the age group of 70 - 79 years old, while eight per cents of the patients were in the age group between 40 - 49 years old. A small portion of the participants (2%), that make up the remaining of the total sample population, were in the age group of 80 - 89 years old.

Most of the patients were married (98%) and only two per cents of the participants were not married. Almost half of the participated patients were working, while fifty-one per cents were not working. The demographic profiles of the participating patients with stable CAD, indicated by the percentage distribution of the patients by age range, gender, race, marital status and work status are summarised in Table 4.1.

Table 4.1: Demographic profile of one hundred patients with stable CAD

Demographic profile	Numbers (n)	Percentage (%)
Age range		
40-49	8	8
50-59	29	29
60-69	41	41
70-79	20	20
80-89	2	2
Total	100	100
Gender		
Male	84	84
Female	16	16
Total	100	100

Table 4.1 continued

Race		
Malay	32	32
Chinese	31	31
Iban	17	17
Bidayuh	10	10
Melanau	6	6
Others	4	4
Total	100	100
Marital status		
Single	2	2
Married	98	98
Total	100	100
Work status		
Working	49	49
Not working	51	51
Total	100	100

4.2 Stable CAD patients and the related risk factors

The risk factors and the percentage of the participants with respective CAD risk factor are tabulated in Table 4.2.

Table 4.2: Number and percentage of participants with CAD risk factors

Risk factors	Number (n)	Percentage (%)
Hypertension status		
Non-hypertensive	78	78
Hypertensive	22	22
Total cholesterol level status		
Normal cholesterol	0	0
High cholesterol	100	100
Diabetes mellitus status		
Non-diabetic	63	63
Diabetes mellitus	37	37
Smoking status		
Non Smoking	86	86
Smoking	14	14
Family history of CAD (parents and/ sibling only)		
No	74	74
Yes	26	26

Table 4.2 continued

Exercise at least once per week		
No	56	56
Yes	44	44
Body mass index status (kg/m^2)		
Underweight	2	2
Ideal	40	40
Overweight	35	35
Obese	23	23

All of the patients enrolled in this study were known to have high cholesterol level, while thirty per cents are with diabetes mellitus. On the other hand, only twenty-two per cents of the patients were reported to be hypertensive and twenty- six percent of the patients were recorded to have family history of CAD.

In term of patients' attitude to smoking and exercise, majority of the patients (86%) were non-smokers, where only 14% were active smokers. The smaller difference was seen in the percentage of patients who perform exercises, where 44% were reported to exercise at least once per week, while the 56% of the participants did not do any exercises.

Body mass index (BMI) is grouped into specific classes according to the values. The categories of BMI are underweight (BMI < 18.5 kg/m^2), ideal (BMI 18.5-24.99 kg/m^2), overweight (BMI 25-29.99 kg/m^2) and obese (BMI ≥30 kg/m^2). The value derived from the person's mass (weight) in kilograms, divided by the square of heights in meters. Based on Table 4.2, even though the BMI of most of the patients were ideal (40%), more than half of the total patients were reported to be overweight (35%) and obese (23%). Only two percent were recorded as underweight.

4.3 Smoking and CAD risk factors

Smoking increased the risk of developing cardiovascular disease which includes coronary artery disease. In this study, the percentage of stable CAD patients who were smokers were analysed based on the presence of the respective CAD risk factors. The results are shown in Table 4.3.

Table 4.3: Number and percentage of stable CAD patients who were smokers with respective risk factors based on genders

Gender	Number (n)	Percentage (%)
Male smokers	13	13
Female smokers	1	1
Total	14	14
Hypertensive		
Male	10	10
Female	0	0
Total	10	10
High cholesterol		
Male	13	13
Female	1	1
Total	14	14
Diabetic		
Male	5	5
Female	0	0
Total	5	5

From the total of 14 patients who were smokers in this study, one was a female (1%) while the majority were male (13%). Most of the male smokers were reported to have hypertension, high cholesterol levels and diabetes mellitus. From the data tabulated, ten per cents of the male smokers were recorded to have hypertension and thirteen per cents with high cholesterol levels, while five per cents of the total male smokers were recorded to be

diabetic. On the other hand, the female smoker was recorded to have hypertension and high cholesterol level but was not diabetic.

4.4 Types and percentage of specific medication taken by CAD patients

The numbers and percentage of the specific drugs taken by the participants are summarised in Table 4.4.

Table 4.4: Number and percentage of medicine of hundred stable CAD patients

Medicine	Number (n)	Percentage (%)
Metformin HCL 500mg	37	37
Perindopril Tert-butylamine 8mg	22	22
Amlodipine 5mg	22	22
Simvastatin 10mg	100	100

Other than high cholesterol level which contributes largely as the main cause of CAD, certain patients that were involved this study also have other risk factors such as hypertension (HPT) and *diabetes mellitus* (DM). Taking appropriate drugs is important to avoid CAD from getting worse. Among one hundreds of the patients presented with stable CAD in this study, thirty-seven per cents were taking metformin HCL as measure to control the diabetes. While, twenty-two per cents of the patients were prescribed with both perindopril and amlodipine for their hypertension and all of the patients in this study (100%) took simvastatin as cholesterol lowering drug. Metformin is a hypoglycaemic agent which is used to control blood sugar level of *diabetes mellitus* (DM) patients. Perindopril and amlodipine is the oral anti-hypertension medicine that are used to treat high blood pressure in hypertensive patients. While simvastatin is an oral high cholesterol medicine that's used to reduce the

amount of cholesterol made by liver including total cholesterol, LDL-cholesterol and triglyceride, at the same time help in raising of HDL-cholesterol.

4.5 Numbers of diseased arteries in stable CAD patients

The numbers of arteries diseased collected from the following coronary angiography result showed the presence of single vessel disease (SVD), 2-vessel disease (2VD) or 3-vessel disease (3VD). Table 4.5 shows the percentage and the number of diseased artery among the stable CAD patients in this study.

Table 4.5: Numbers of diseased arteries among patients with stable CAD

Numbers of arteries	Number (n)	Percentage (%)
1	45	45
2	29	29
3	26	26

Among the total 100 patients presented with stable CAD, 45% had a single vessel disease (SVD). Twenty-nine per cents of the patients were shown to have 2-vessel disease (2VD) while the patients with 3-vessel disease (3VD) were only 26%. Since there are outlier in a sample data, interquartile range (iqr) and median used to summerize atypical value and the variable in the sample data respectively. The summary of serum leptin level of one hundred stable CAD based on number of vessel disease involved in this study recorded in the Table 4.6.

Table 4.6: Serum leptin level of one hundred stable CAD based on number of vessel disease

Number vessel disease	Median	Interquartile range	Minimum	Maximum	p-value
1-vessel disease	1.92	3.22	0.20	21.67	
2-vessel disease	2.97	1.71	0.94	14.31	0.184
3-vessel disease	1.80	3.38	0.20	19.67	

From Table 4.6, the minimum and maximum value of serum leptin of one vessel disease were 0.20 ng/mL and 21.67 ng/mL, two vessel disease were 0.94 ng/mL and 14.31 ng/mL while three vessel disease were 0.20 ng/mL and 19.67 ng/mL. Median value of serum leptin for one vessel disease is 1.92 ng/mL, two vessel disease is 2.97 ng/mL and three vessel disease is 1.80 ng/mL. Interquartile range for 2 vessel disease is the lowest compare to one vessel disease and three vessel disease it is 1.72 ng/mL. While, interquartile range value for one vessel disease and three vessel disease were 3.22 ng/mL and 3.38 ng/mL each. The p-value is 0.184, thus no significant difference exists between the serum leptin level and the number of vessel diseased. Serum leptin level and its relation to the number of vessel diseased were subsequently analysed among the *non-diabetes mellitus* (non-DM) patients, as shown in Table 4.7 and among the *diabetes mellitus* (DM) patients shows in Table 4.8.

Table 4.7: Serum leptin level among non-DM patients based on number of vessel disease

Number vessel disease	Median	Interquartile range	Minimum	Maximum	p-value
1-vessel disease	1.51	3.08	0.20	12.37	
2-vessel disease	2.76	1.71	0.94	12.83	0.076
3-vessel disease	1.60	2.87	0.29	19.67	

The p-value (0.076) shows no significant difference exists between serum leptin level and the different number of diseased vessel among non-diabetic patients. The minimum and maximum value level of serum leptin in one vessel diseased patients is 0.20 ng/mL and 12.37 ng/mL each. Patients with two vessels disease shows a slightly higher leptin level as compared to one vessel disease with 0.90 ng/mL (minimum) and 12.83 ng/mL (maximum). While the non-DM patients with three vessel disease shows minimum level lower than two vessel disease with 0.29 ng/mL and the highest maximum value level of 19.67 ng/mL. Median value of one vessel disease is 1.51 ng/mL, followed by three vessel disease (1.60

ng/mL) and two vessel disease (2.76 ng/mL). While the interquartile range for two vessel disease is 1.71 ng/mL followed by three vessel disease (2.87 ng/mL) and one vessel disease it is 3.08 ng/mL.

Table 4.8: Serum leptin level among DM patients based on number of vessel disease

Number vessel disease	Median	Interquartile range	Minimum	Maximum	p-value
1-vessel disease	3.30	3.29	0.35	21.67	
2-vessel disease	3.24	3.16	1.49	14.31	0.950
3-vessel disease	3.14	6.21	0.20	11.90	

The minimum and maximum value of one vessel disease patients is 0.35 ng/ml and 21.67 ng/mL each. While the median and interquartile range of one vessel disease is 3.30 ng/mL and 3.29 ng/mL each. Group of two vessel disease has median value 3.24ng/mL, interquartile range is 3.16 ng/mL, minimum value is 1.49 ng/mL and maximum value is 14.31ng/mL. While, the median value of three vessel disease is 3.14 ng/mL, interquartile range is 6.21 ng/mL, minimum value is 0.20 ng/mL and the maximum value is 11.90 ng/mL. Since p-value is 0.950, it shows no significant difference exists of serum leptin level in different number of vessel disease among *diabetes mellitus* (DM) patients involved this study.

4.6 Clinical parameters of stable CAD patient participants

Among 100 of patients stable CAD, BMI, heart rate and blood were recorded during the survey interview. While the clinical parameters involving serum including total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and serum leptin recorded after the sample done for determination and analysis. The summary of

clinical parameters of one hundred of stable CAD patients involving in this study recorded in the Table 4.9.

Table 4.9: Summary of clinical parameters of one hundred stable CAD patients participated in the study

Parameter	Median	Interquartile range	Minimum	Maximum
BMI	26.01	5.9	16.5	36.7
Heart rate	66.5	15	40	135
BP systolic	140	29	90	207
BP diastolic	82	14	52	112
Total cholesterol	3.87	1.27	2.31	7.41
Triglyceride	1.28	0.74	0.7	2.80
LDL	2.03	0.97	0.61	5.31
HDL	1.20	0.36	0.68	2.53
Serum leptin	2.35	2.93	0.20	21.67

The clinical parameters outcome in this study reported in median level, interquartile range, minimum level and maximum level. As the data set has outliers and extreme values, we summarize a typical value using the median as opposed to the mean. When a data set has outliers, variability is often summarized by the interquartile range, which is the difference between the first and third quartiles. The minimum and maximum level were important in calculation of the range and the calculation of the range is very straightforward.

From the data in Table 4.9, the median of the Body Mass Index (BMI) of 100 patients in this study is 26.01 kg/m^2 and the interquartile range is 5.9 kg/m^2. Respectively the minimum and maximum level is 16.5 kg/m^2 and 36.7 kg/m^2 each. Within of the heart rate level, the median of the heart rate from the total patients of this study is 66.5 bpm and the interquartile range

is 15 bpm. The minimum level is 40 bpm and the maximum level is 135 bpm. While from the statistic finding, the BP systolic median is 140 mm Hg with interquartile range is 29 mm Hg. The minimum and the maximum level are 90 mm Hg and 207 mm Hg respectively. Other than that, the median of BP Diastolic is 82 mm Hg with minimum level is 52 mm Hg and maximum level of BP diastolic is 112 mm Hg. Then, the interquartile range is 14 mm Hg.

In terms of the group of lipid, total cholesterol and triglyceride were analysed between this coronary artery disease patients. The median and the interquartile range of total cholesterol level is 3.87 mmol/l and 1,27 mmol/l respectively. Then the minimum level is 2.31 mmol/l and the maximum level is 7.41 mmol/l. While the triglyceride median level is 1.28 mmol/l and the interquartile range is 0.74 mmol/l. 0.7 mmol/l is the minimum level of the triglyceride in this study. Then the maximum level of triglyceride is 2.80 mmol/l.

Group of lipoprotein includes low-density lipoprotein (LDL) and high-density lipoprotein (HDL) was analysed and the data showed in Table 4.9. The median level of LDL is 2.03 mmol/l and the interquartile range level is 0.97 mmol/l. Then, the minimum level of LDL is 0.61 mmol/l and the maximum level of LDL is 5.31 mmol/l. High-density lipoprotein is also known as good cholesterol showed the median level is 1.20 mmol/l with 0.36 mmol/l of interquartile range level. The minimum level of HDL is 0.68 mmol/l and the maximum level of HDL is 2.53 mmol/l. The last and the main parameter analysed in this study is serum leptin level. From the data in the Table 4.9, the median level of leptin is 2.35 ng/ml. The interquartile range level is 2.93 ng/ml. Then, 0.20 ng/ml and 21.67ng/ml for each minimum and maximum level.

4.7 Levels of serum leptin and lipid profile of stable CAD based on race

In an analysis of this study involving 100 patients with stable CAD, the clinical parameters of serum leptin, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride were arranged in Table 4.10 where each parameter analysed was based on race.

Table 4.10: Levels of serum leptin and lipid profile parameters of stable CAD patients based on race

Parameters	Race	Median	Interquartile range	Minimum	Maximum	p-value
Serum leptin	Malay	3.25	2.46	0.30	21.67	0.07
	Chinese	1.66	1.84	0.20	12.83	
	Iban	3.81	8.43	0.20	14.31	
	Bidayuh	3.03	3.30	0.24	12.37	
	Melanau	1.44	1.32	0.86	2.97	
	Others	3.49	8.28	1.09	10.80	
Low density lipoprotein	Malay	2.06	0.93	0.7	3.85	0.58
	Chinese	1.94	1.02	0.61	5.31	
	Iban	1.84	1.14	1.20	3.91	
	Bidayuh	1.89	1.46	1.21	4.52	
	Melanau	2.52	0.92	1.70	2.86	
	Others	2.44	1.47	1.66	3.60	
High Density Lipoprotein	Malay	1.18	0.28	0.83	2.01	0.283
	Chinese	1.39	0.47	0.68	1.75	
	Iban	1.13	0.40	0.92	2.53	
	Bidayuh	1.15	0.17	0.86	1.30	
	Melanau	1.22	0.41	0.97	1.93	
	Others	1.23	0.25	1.10	1.41	
Triglyceride	Malay	1.21	0.66	0.70	2.82	0.26
	Chinese	1.19	0.69	0.70	2.42	
	Iban	1.26	0.60	0.77	2.43	
	Bidayuh	1.50	0.76	1.02	2.51	
	Melanau	1.87	0.92	1.20	2.30	
	Others	1.66	1.35	0.70	2.29	

Among the serum leptin level, most of the race includes Malay (3.25 ng/ml),Iban (3.81 ng/ml) Bidayuh (3.03 ng/ml) and Others show the median are slightly in the same level. Compare to Chinese and Melanau the median level were 1.66 ng/ml and 1.44 ng/ml respectively. Interquartile range for Iban (8.43 ng/ml) and Others (8.28 ng/ml) show the higher level compare to Malay (2.46 ng/ml), Bidayuh (3.30 ng/ml) and Chinese (1.84 ng/ml). While, Melanau (1.32 ng/ml) shows the lowest interquartile range.

Both of the minimum level of serum leptin for Chinese and Iban was 0.20 ng/ml. This is followed by Bidayuh (0.24 ng/ml), Malay (0.30 ng/ml) ,Melanau (0.86 ng/ml) and Others (1.09 ng/ml).The maximum level of serum leptin shows Malay (21.67 ng/ml) was the highest level and the Melanau (2.97 ng/ml) was the lowest level. Chinese (12,83 ng/ml) and Bidayuh (12.37 ng/ml) were closely at the same level. Iban (14.31 ng/ml) was a bit higher compare to others (10.80 ng/ml). As a whole, the p value of the serum leptin level versus ethnic group was ($p= 0.07$).

Within Low-density lipoprotein (LDL), the median level of Chinese (1.94 mmol/l), Iban (1.84 mmol/l) and Bidayuh (1.89 mmol/l) were a bit lower than Malay (2.06 mmol/l), Melanau (2.54 mmol/l) and Others (2.44 mmol/l). While the interquartile range show four ethnics are closely at the same level with Malay (0.93 mmol/l) and Melanau (0.92 mmol/l); Bidayuh (1.46 mmol/l) and Others (1.47 mmol/l).

The interquartile range for Chinese and Iban respectively was 1.02 mmol/l and 1.14 mmol/l each. For the minimum level of LDL, Malay (0.7 mmol/l) and Chinese (0.61 mmol/l) ; Iban (1.14 mmol/l) and Bidayuh (1.21 mmol/l) ; Melanau (1.70 mmol/l) and Others (1.66 mmol/l)

show nearly LDL level for each other. Compare to maximum level of LDL, it did not show any closely result level. Malay (3.85 mmol/l),Chinese (5.31 mmol/l), Iban (3.91 mmol/l), Bidayuh (4.52 mmol/l), Melanau (2.86 mmol/l) and others (3.60 mmol/l). Therefore, the p value between LDL versus ethnic group was 0.58 (p = 0.58).

Another parameter to compare with ethnic group was High-density lipoprotein (HDL). Among the ethnic groups, the Chinese (1.39 mmol/l) shows a slightly more of median level compare to Malay (1.18 mmol/l), Iban (1.13 mmol/l) and Bidayuh (1.15 mmol/l). While Melanau (1.22 mmol/l) was closely at the same level with Others (1.23 mmol/l). Bidayuh (0.17 mmol/l) shows the lowest level of interquartile range and the Iban (0.40 mmol/l), Melanau (0.41 mmol/l) and Chinese (0.47 mmol/l) were a bit higher. However, Malay (0.28 mmol/l) and Others (0.25 mmol/l) were closely same result level of interquartile range. Compare to others ethnic groups, Iban shows a wider range with minimum level was 0.92 mmol/l and the maximum level was 2.53 mmol/l.

The minimum level of Chinese (0.68 mmol/l), Malay (0.83 mmol/l), Bidayuh (0.86 mmol/l), Melanau (0.97 mmol/l) and Others (1.10 mmol/l) as show in the Table 4.3. Other than Iban, (2.53 mmol/l) the maximum level of HDL in Malay (2.01 mmol/l) shows a bit higher compare to Chinese (1.75mmol/l,), Bidayuh (1.30 mmol/l), Melanau (1.93 mmol/l) and Others (1.41 mmol/l). Lastly, the p value of the HDL versus ethnic group was 0.283 (p = 0.283).

The last parameter to compare with ethnic group was triglyceride. Malay, Chinese and Iban show a closely same result level in median, interquartile range and minimum level of

triglyceride. Malay (1.21 mmol/l), Chinese (1.19 mmol/l) and Iban (1.26 mmol/l) in level of median. Then, the interquartile range was Malay (0.66 mmol/l), Chinese (0.69 mmol/l) and Iban (0.60 mmol/l). The minimum level of triglyceride in Malay was 0.70 mmol/l, Chinese was 0.70 mmol/l and the Iban was 0.77 mmol/l.

The median level of Bidayuh (1.50 mmol/l), Melanau (1.87 mmol/l) and Others (1.66 mmol/l) respectively shows in the Table 4.3. Compare to Bidayuh (0.76 mmol/l) and Melanau (0.92 mmol/l) the interquartile range of others (1.35 mmol/l) are slightly higher. The minimum level of triglyceride of Others (0.70mmol/l) shows the same level with Malay and Chinese. While Melanau and Bidayuh respectively were 1.02 mmol/l and 1.20 mmol/l each.

The higher level of maximum shown in Malay (2.82 mmol/l) and the lower was shown in Others (2.29 mmol/l). Chinese (2.42 mmol/l) and Iban (2.43 mmol/l) show closely the same result. While the maximum level of triglyceride of Bidayuh and Melanau was 2.30 mmol/l and 2.29mmol/l each. The statistic shows the p value of triglyceride versus ethnic group was 0.26 (p-value 0.26).

4.8 Descriptive of clinical parameters based on exercise activity

Data of exercise activity acquired during the survey interview. The exercise activity including brisk walking or jogging recorded for at least once per week. Median data and the level of minimum and maximum of each parameter based on exercise activity reported in Table 4.11.

Table 4.11: Summary of clinical parameters of one hundred stable CAD patients participated in the study based on exercise activity

	Median	Interquartile range	Minimum	Maximum
No exercise even once per week				
Total cholesterol	3.91	1.11	2.31	6.89
Triglyceride	1.30	0.79	0.70	2.51
High-density lipoprotein	1.23	0.30	0.83	2.01
Low-density lipoprotein	2.09	1.04	0.61	4.52
Serum leptin	2.40	2.43	0.20	21.67
Exercise at least once per week				
Total cholesterol	3.82	1.39	2.69	7.41
Triglyceride	1.27	0.79	0.72	2.80
High-density lipoprotein	1.14	0.40	0.68	2.53
Low-density lipoprotein	1.93	0.94	0.97	5.31
Serum leptin	2.17	3.58	0.20	14.31

4.8.1 Descriptive of clinical parameters based on no exercise even once per week

In terms of the group of lipid, total cholesterol and triglyceride were analysed between this coronary artery disease patients. The median of total cholesterol level was 3.91 mmol/. Then, the minimum level was 2.31 mmol/l and the maximum level was 6.89 mmol/l. While the triglyceride median level was 1.30 mmol/l 0.70 mmol/l was the minimum level of the triglyceride in this study. Then, the maximum level of triglyceride was 2.51 mmol/l.

Group of lipoprotein include low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were analysed and data showed in the Table 4.11. The median level of LDL was 1.93 mmol/l. Then, the minimum level of LDL was 0.97 mmol/l and the maximum level of LDL was 4.52 mmol/l. High-density lipoprotein as known as good cholesterol showed the median level was 1.23 mmol/l. The minimum level of HDL was 0.83 mmol/l and the maximum level of HDL was 2.01 mmol/l. The last and the main parameter analysed in this study is serum

leptin level. From the data in the Table 4.8, the median level of leptin was 2.40 ng/ml. Then, 0.20 ng/ml and 21.67 ng/ml for each minimum and maximum level.

4.8.2 Descriptive of clinical parameters based on exercise at least once per week

From the Table 4.11, overall median level data of all parameters by the group of exercise showed decreasing compared to median level data by the group of non-exercise patients. The median of total cholesterol level was 3.82 mmol/l. Then, the median level of triglyceride was 1.27, HDL and LDL was 1.14 mmol/l and 1.93mmol/l each. Follow by median level of serum leptin was 2.17 ng/ml.

While the minimum level of each parameters showed approximately same between groups exercise and non-exercise patients. The minimum level of total cholesterol was 2.69 mmol/l. Then the minimum level of triglyceride was 0.72 mmol/l and the minimum level of HDL was 1.14 mmol/l. follow by minimum level of LDL was 0.97 mmol/l. Serum leptin level group of exercise and non-exercise patients showed the same level it was 0.20 ng/ml.

The maximum level of clinical parameters in group of exercise patients showed the cholesterol maximum level was 7.41 mmol/l compare to non-exercise patients only 6.89 mmol/l. The maximum level of triglyceride and LDL also showed higher in exercise patients which was 2.80 mmol/l and 0.97 mmol/l each. Then, maximum level of HDL was 2.53 mmol/l. While the maximum level serum leptin in exercise group of patients stable CAD was 14.31 ng/ml

4.9 Serum leptin level based on body mass index (BMI) status

Among the 100 participants in this study analysis, body mass index (BMI) for each patient calculated during patients came for interview. BMI calculate by weight (kg) and height (m). Then, the patients grouped based on BMI result. The level of median, minimum and maximum serum leptin arranged on the table based on BMI status (Table 4.12).

Table 4.12: Levels of serum leptin of one hundred stable CAD patients based on BMI status

BMI Status	Median	Interquartile range	Minimum	Maximum
Underweight	0.25	-	0.20	0.30
Ideal	1.53	1.98	0.20	12.54
Overweight	2.76	2.46	0.56	12.83
Obesity	4.32	6.52	1.13	21.67

From the Table 4.12, the median of leptin showed the increased trend from underweight group to obesity group. Underweight (0.25 ng/ml), idea (1.53 ng/ml), overweight (2.76 ng/ml) and the obesity group (4.32 ng/ml). While the minimum level of leptin for underweight and ideal group are at the same level (0.20 ng/ml). Compare to overweight group (0.56 ng/ml) the minimum level of group obesity was the higher (1.13 ng/ml). Same goes to the median level of leptin in different group of BMI, the maximum level of leptin by group of BMI also show the increasing between the groups. But, the maximum level of ideal group (12.54 ng/ml) and overweight group (12.83 ng/ml) were just slightly different. The obesity group was 21.67 ng/ml while underweight group was only 0.30 ng/ml.

4.10 Correlation analysis between serum leptin level and lipid profile parameters in total of 100 patients stable CAD

The relationship between two quantitative outcomes in this study was analysed with SPSS version 21.0. The correlation coefficient (r) was used to describe the degree of linear relationships between variable. Spearman's correlation coefficient presented because of the both variable are non-normal distribution. Summary of correlation analysis shows in Table 4.13.

Table 4.13: Correlation analysis between serum leptin level and others clinical parameter of one hundred stable CAD patients

Parameter	r_s-value	p-value
Age	0.140	0.166
BP systolic	0.099	0.326
BP diastolic	0.086	0.397
BMI	0.548	<0.001
Total cholesterol	0.084	0.406
Triglyceride	0.318	<0.005
LDL-cholesterol	0.065	0.522
HDL-cholesterol	0.134	0.183

Table 4.13 shows the correlation analysis between serum leptin level with age, blood pressure (BP), body mass index (BMI) and serum lipid profile. Most of the parameter showed no correlation with serum leptin level since p-value >0.05 except body mass index (BMI) and triglyceride. Both BMI and triglyceride were positive correlation with serum leptin level with p-value is <0.001 and <0.005 each, then the coefficient of spearman's rho correlation is 0.548 for BMI and p-value and the coefficient of Spearman's rho correlation of triglyceride is <0.005 and 0.318 each. The correlation analysis between serum leptin level and serum lipid profile parameter level which is shown graphically by a scatter plot shows in Figure 4.2, Figure 4.3, Figure 4.4 and Figure 4.5.

4.10.1 Correlation analysis between serum leptin and total cholesterol level

Figure 4.2 shows the analysis of relationship between two quantitative outcomes, serum leptin level and total cholesterol level which are shown graphically by a scatter plot. From the plot, it is obvious that there is no linear relationship between the serum leptin level and total cholesterol level.

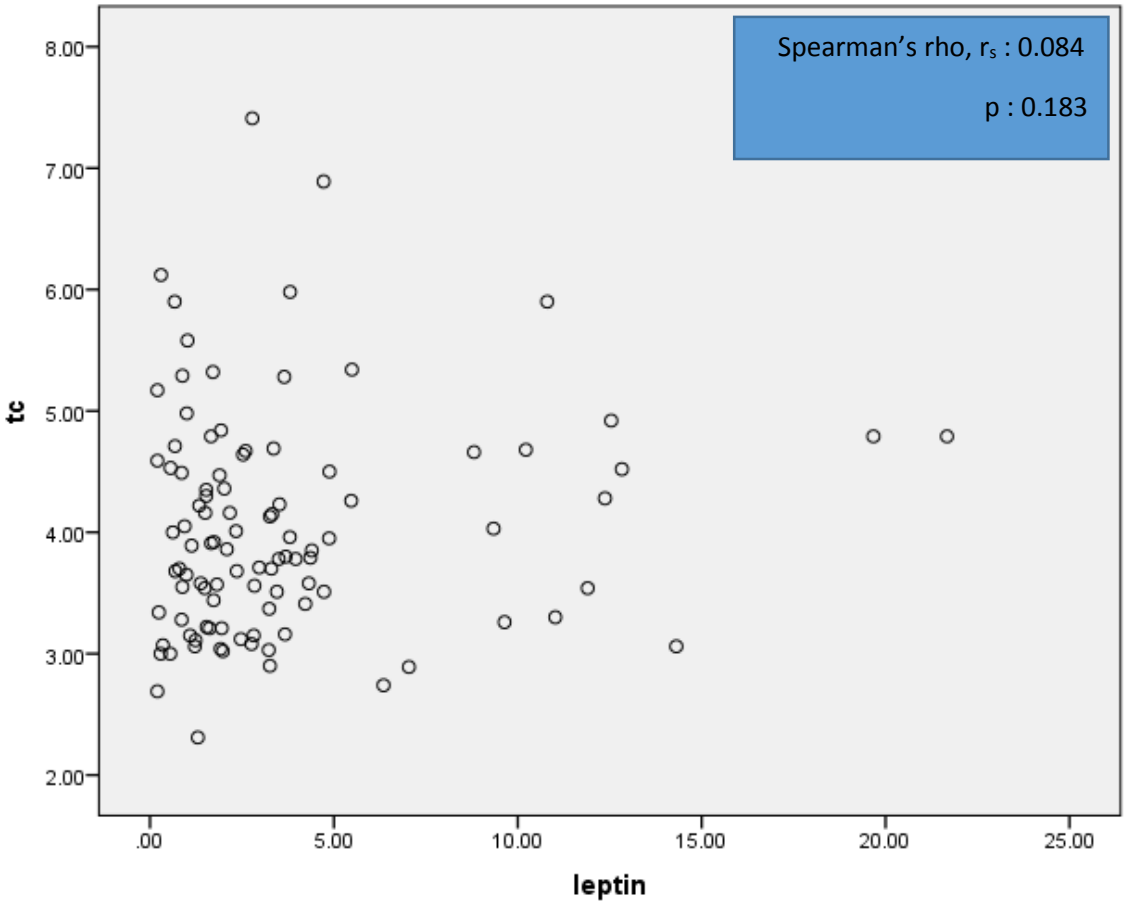


Figure 4.2: Serum leptin versus total cholesterol level

Since both of the parameters are non-normally distribution, Spearman's correlation coefficient were presented. Figure 4.2 shows the correlation coefficient between serum leptin level and cholesterol level (both variable are non-normally distributed): Spearman's rho, r_s

= 0.084 (p = 0.183). There is no correlation between serum leptin level and total cholesterol (p = 0.183).

4.10.2 Correlation analysis between serum leptin and triglyceride level

Figure 4.3 show the analysis of relationship between two quantitative outcomes, serum leptin level and triglyceride which is shown graphically by a scatter plot. From the plot, it is obvious that there is no linear relationship between the serum leptin level and triglyceride level.

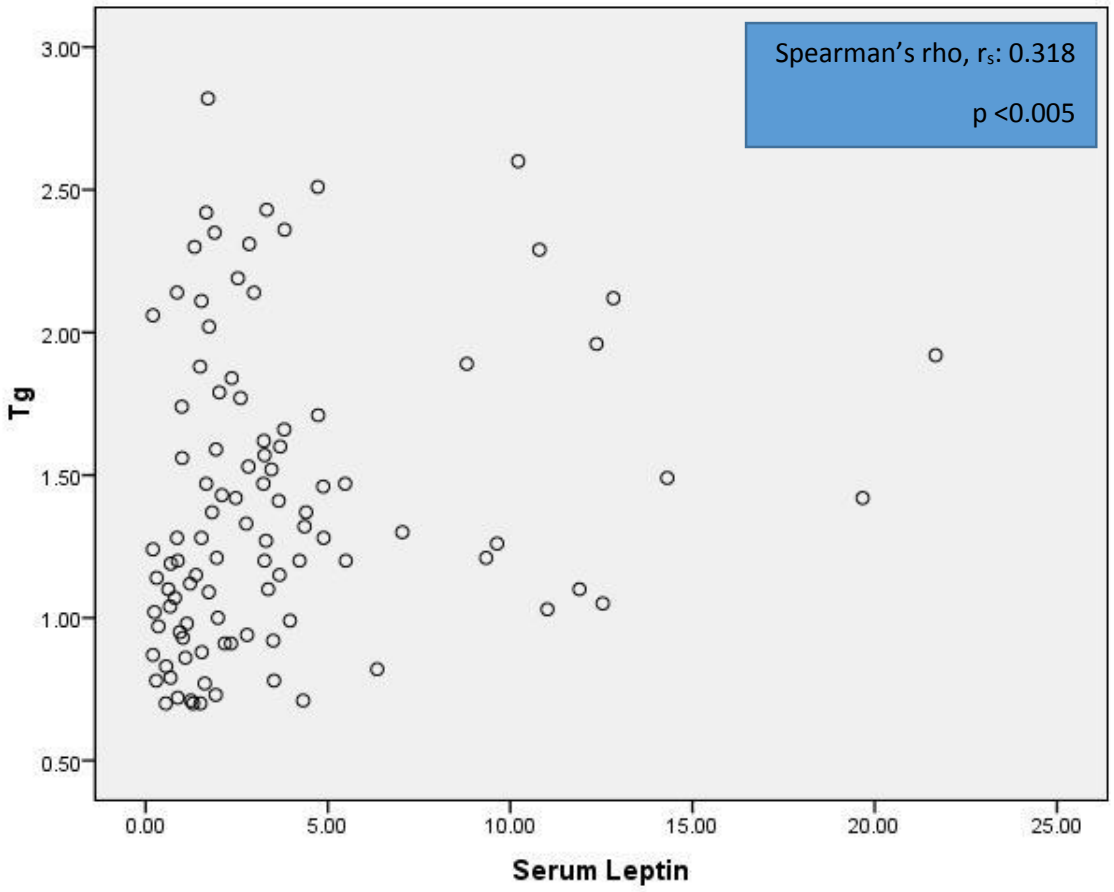


Figure 4.3: Serum leptin versus triglyceride level

Since both of the parameters are non-normally distribution, Spearman's correlation coefficient was presented. Figure 4.3 shows the correlation coefficient between serum leptin

level and triglyceride (both variable are non-normally distributed): Spearman's rho, r_s = 0.318 (p <0.005). There is a positive correlation between serum leptin and triglyceride, since p <0.005 and the coefficient of Spearman's rho correlation is 0.318.

4.10.3 Correlation analysis between serum leptin and low-density lipoprotein (LDL)

Figure 4.4 shows the analysis of relationship between two quantitative outcomes, serum leptin level and low-density lipoprotein which is shown graphically by a scatter plot. From the plot, it is obvious that there is no linear relationship between the serum leptin level and low-density lipoprotein.

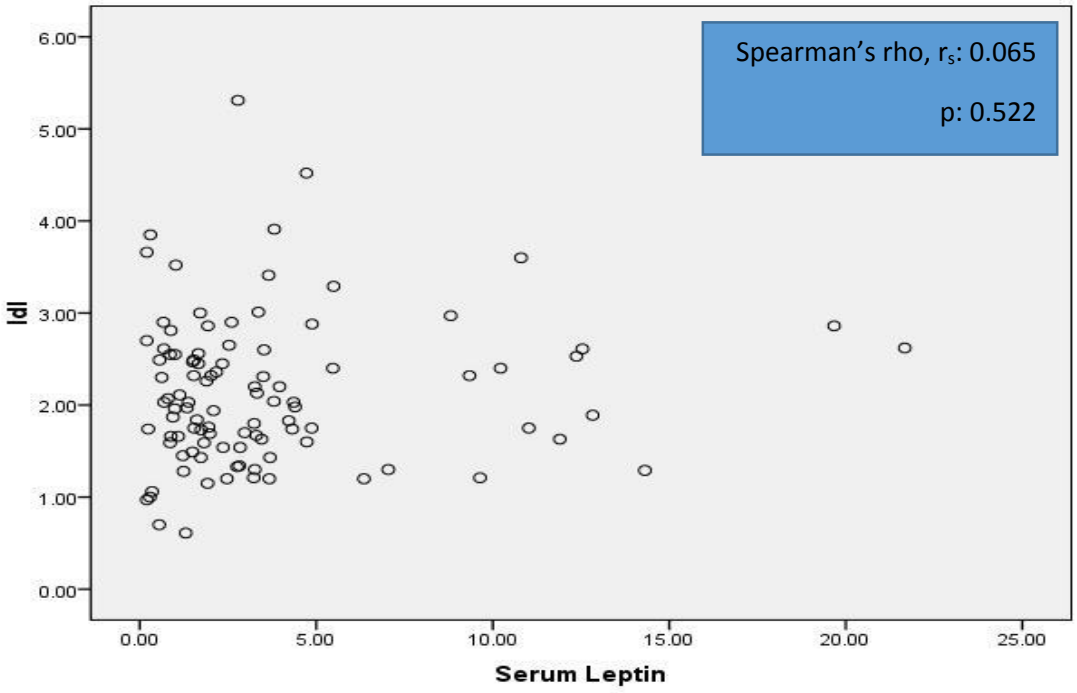


Figure 4.4: Serum leptin versus low density lipoprotein level

Since both of the parameters are non-normally distribution, Spearman's correlation coefficient was presented. Figure 4.4 shows the correlation coefficient between serum leptin level and LDL (both variable are non-normally distributed): Spearman's rho, r_s = 0.065 (p–

value 0.522). There is no correlation between serum leptin level and low-density lipoprotein (p-value 0.522).

4.10.4 Correlation analysis between serum leptin and high-density lipoprotein (HDL)

Figure 4.5 shows the analysis of relationship between two quantitative outcomes, serum leptin level and high-density lipoprotein which is shown graphically by a scatter plot. From the plot, it is obvious that there is no linear relationship between the serum leptin level and high-density lipoprotein.

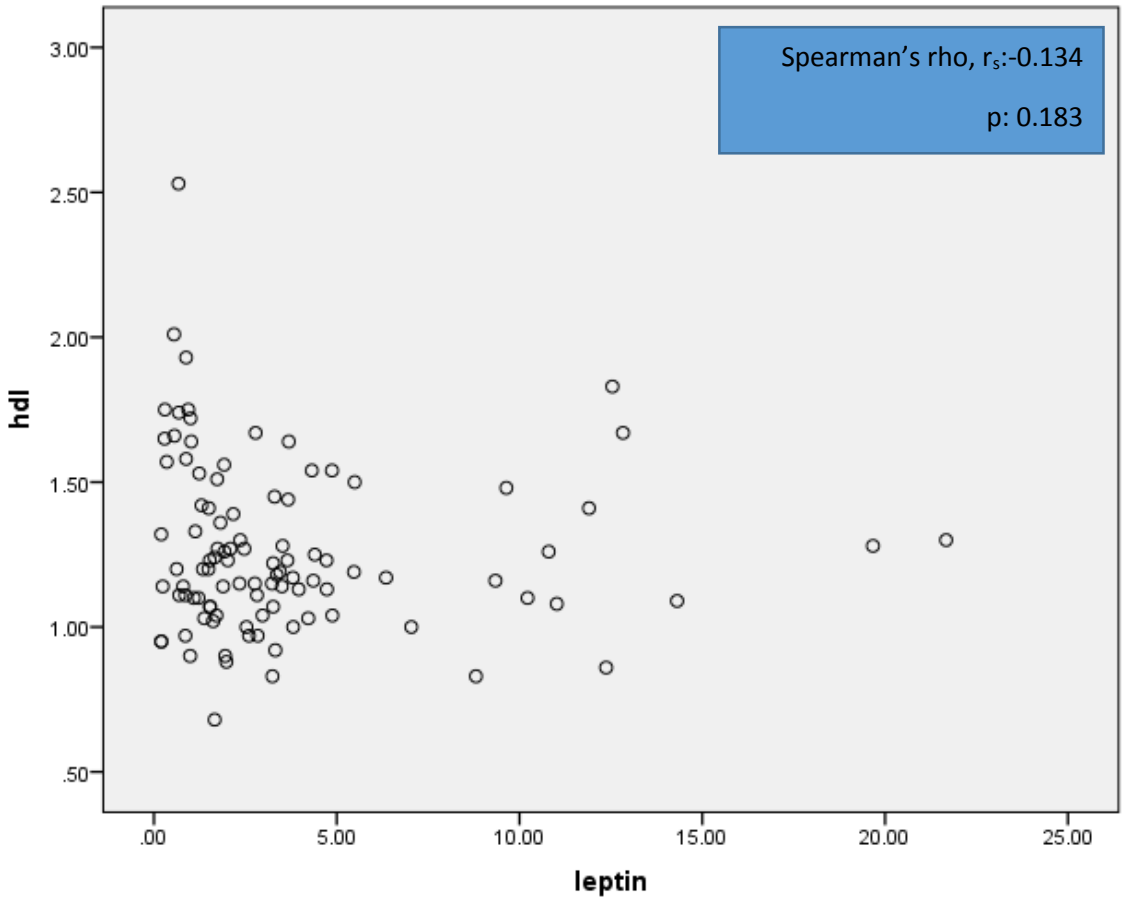


Figure 4.5: Serum leptin versus high-density lipoprotein level

Since both of the parameters are non-normally distribution, Spearman's correlation coefficient was presented. From this Figure 4.5 shows the correlation coefficient between

serum leptin level and LDL (both variable are non-normally distributed): Spearman's rho, r_s = -0.134 (p = 0.183). There is no correlation between serum leptin level and low-density lipoprotein (p = 0.183).

4.11 Correlation analysis between serum leptin level and others clinical parameter among DM patients

Table 4.14 show the analysis of relationship between serum leptin level and age, blood pressure (BP), body mass index (BMI) and serum lipid profile level among *diabetes mellitus* (DM) patients involved this study.

Table 4.14: Correlation analysis data between serum leptin level and others clinical parameter among DM patients

Parameter	r_s-value	p-value
Age	0.046	0.787
BP systolic	0.148	0.383
BP diastolic	0.113	0.505
BMI	0.419	0.010
Total cholesterol	0.128	0.451
Triglyceride	0.071	0.677
LDL-cholesterol	0.161	0.342
HDL-cholesterol	0.064	0.706

Most of the r_s-value is less than 0.3, means that the poor relationship between serum leptin level with others clinical parameter. From the table data, there is no correlation between serum leptin level with others clinical parameter among DM patients. However, the strength of linear relationship between leptin level and BMI is fair with correlation coefficient value 0.419 (p-value 0.010). The correlation analysis between serum leptin level and triglyceride level which is shown graphically by a scatter plot shows in Figure 4.6.

4.11.1 Correlation analysis between serum leptin and triglyceride among *diabetes mellitus* patients

Figure 4.6 shows the analysis of relationship between two quantitative outcomes, serum leptin level and triglyceride among *diabetes mellitus* patients which is shown graphically by a scatter plot. From the plot, it is obvious that there is no linear relationship between the serum leptin level and triglyceride level.

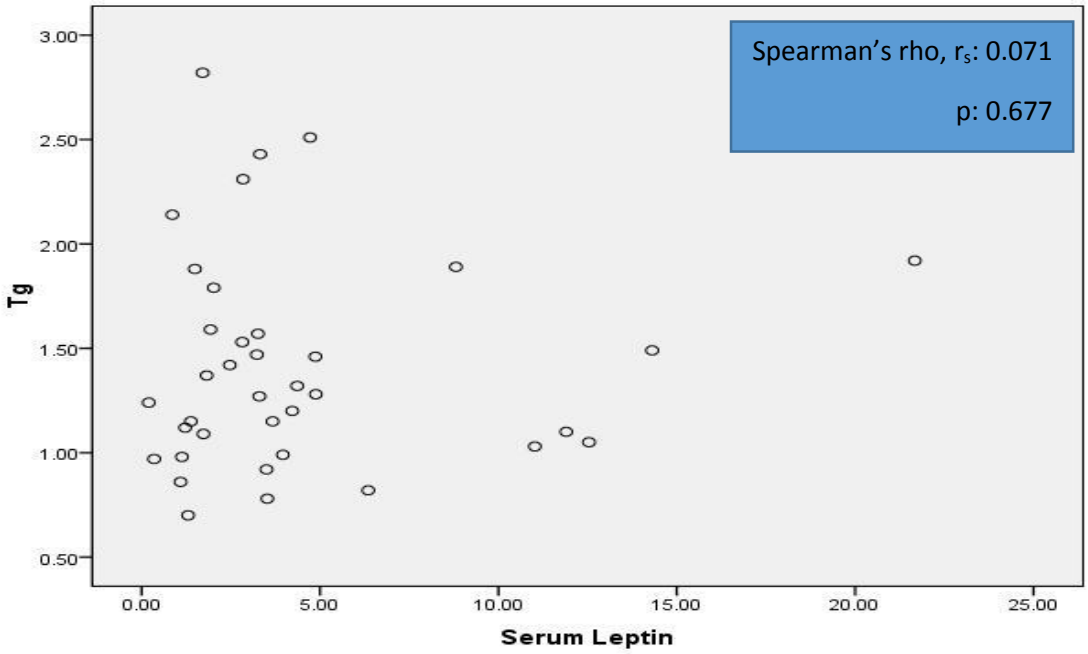


Figure 4.6: Serum leptin versus triglyceride in patients with *diabetes mellitus*

Since both of the parameters are non-normally distribution, Spearman's correlation coefficient were presented. Figure 4.6, shows the correlation coefficient between serum leptin level and triglyceride (both variable are non normally distributed): Spearman's rho, $r_s = 0.071$ ($p < 0.677$). Among patients with *diabetes mellitus*, there is no correlation between serum leptin level and triglyceride level ($p = 0.677$).

4.12 Correlation analysis between serum leptin level and others clinical parameter among non-DM patients

The summary of correlation analysis between serum leptin level and age, blood pressure (BP) and serum lipid profile among *non-diabetes mellitus* (DM) patients involved this study showed in Table 4.15.

Table 4.15: Correlation analysis between serum leptin level and others clinical parameter among non-DM patients

Parameter	r_s-value	p-value
Age	0.308	0.014
BP systolic	0.030	0.818
BP diastolic	0.163	0.202
BMI	0.615	<0.001
Total cholesterol	0.110	0.389
Triglyceride	0.439	<0.001
LDL-cholesterol	0.072	0.577
HDL-cholesterol	0.235	0.064

Since p-value is >0.05, age and blood pressure shows no correlation with serum leptin level. As well as no correlation serum leptin level with total cholesterol, LDL-cholesterol and HDL-cholesterol. While, there are strong positive correlation between serum leptin level with BMI and triglyceride. Both the p-value of body mass index (BMI) and triglyceride is <0.001. While different in the correlation value(r_s-value) where serum leptin level and BMI has moderate strong relationship (r_s, 0.615) and serum leptin level has fair relationship with triglyceride (r_s, 0.439). The correlation analysis between serum leptin level and triglyceride level which is shown graphically by a scatter plot shows in Figure 4.7.

4.12.1 Correlation analysis between serum leptin and triglyceride among *non-diabetes mellitus* patients

Figure 4.7 shows the analysis of relationship between two quantitative outcomes, serum leptin level and triglyceride among *non-diabetes mellitus* patients which is shown graphically by a scatter plot. From the plot, it is obvious that there is no linear relationship between the serum leptin level and triglyceride level.

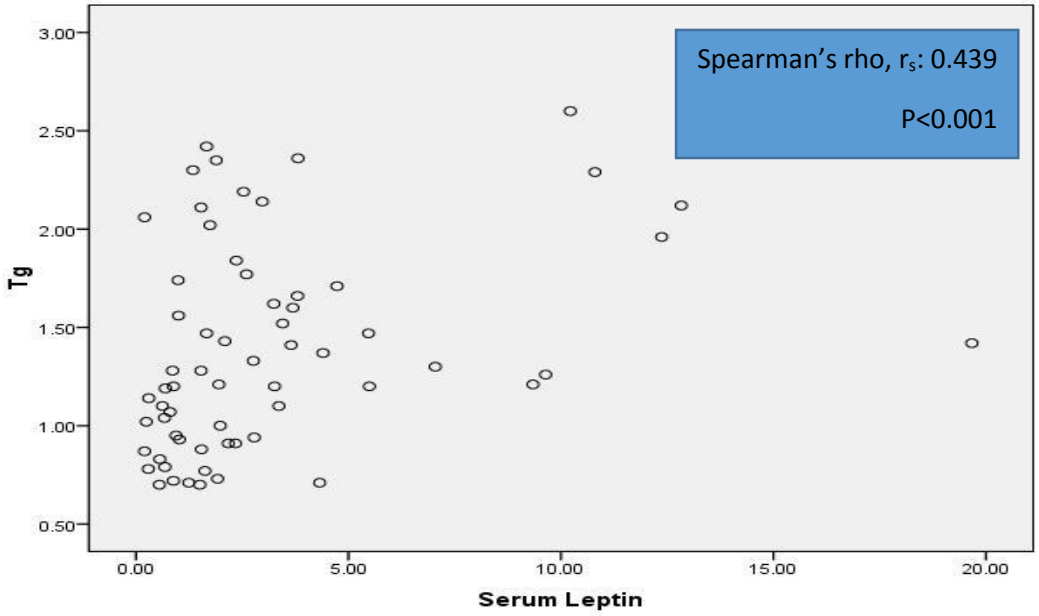


Figure 4.7: Serum leptin versus triglyceride in patients with *non-diabetes mellitus*

Since both of the parameters are non-normally distribution, Spearman's correlation coefficient was presented. Figure 4.7 shows the correlation coefficient between serum leptin level and triglyceride (both variable are non-normally distributed): Spearman's rho, r_s = 0.439 ($p < 0.001$). There is a positive correlation between serum leptin and triglyceride among the *non-diabetes mellitus* patients . Since $p < 0.001$ and the coefficient of Spearman's rho correlation is 0.439.

4.13 Determination of serum leptin level and serum lipid profile parameters between obese and non-obese of stable CAD patients

The summary of serum leptin level and lipid profile parameters between obese and non-obese of stable CAD patients involved this study shows Table 4.16.

Table 4.16: Levels of serum leptin and serum lipid profile parameters between obese and non-obese of stable CAD patients

Parameter	Obesity	Median	Interquartile range	Minimum	Maximum	p-value
Serum Leptin	Non-obese	1.53	2.02	0.20	12.54	<0.001
	Obese	4.36	6.67	1.13	21.67	
Total Cholesterol	Non-obese	3.95	1.44	2.31	7.41	0.995
	Obese	3.92	1.47	2.89	6.89	
Triglyceride	Non-obese	1.19	0.66	0.70	2.43	0.101
	Obese	1.44	0.62	0.71	2.82	
HDL-Cholesterol	Non-obese	1.23	0.47	0.68	2.53	0.245
	Obese	1.18	0.27	0.88	1.54	
LDL-Cholesterol	Non-obese	2.16	1.04	0.61	5.31	0.801
	Obese	2.01	1.07	1.21	4.52	
LDL:HDL Ratio	Non-obese	1.80	1.02	0.35	3.85	0.568
	Obese	1.77	1.11	0.82	3.91	

From this data table, obese is classify when body mass index (BMI) is >30 kg/m^2. While non-obese is classify when BMI <25 kg/m^2. Compares to serum lipid profile parameter, serum leptin level show significance difference in median value, interquartile range, minimum value and maximum value between obese and non-obese patients. The correlation serum leptin level between obese and non-obese patients also show positive correlation with p-value <0.001. Overall, the lipid profile parameters level shows no significance difference

between obese and non-obese in median, interquartile range, minimum value and maximum value. Others than that, all the p-value of lipid profile parameters is negative correlation between obese and non-obese patients.

4.13.1 Correlation analysis between serum lipid profile level and others clinical parameter among non-obese patients

Table 4.17 show summary of correlation analysis between serum lipid profile parameter including total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and LDL:HDL ratio level with age, blood pressure (BP) and serum leptin among non-obese patients involve this study.

Table 4.17: Correlation analysis between serum lipid profile level and others clinical parameter among non-obese patients

Parameter	Serum lipid profile	r_s-value	p-value
Age	Total cholesterol	0.175	0.255
	Triglyceride	0.136	0.378
	LDL-cholesterol	0.188	0.221
	HDL-cholesterol	0.142	0.359
	LDL:HDL ratio	0.011	0.943
BP systolic	Total cholesterol	0.241	0.115
	Triglyceride	0.072	0.640
	LDL-cholesterol	0.274	0.875
	HDL-cholesterol	0.082	0.596
	LDL:HDL ratio	0.229	0.135
BP diastolic	Total cholesterol	0.006	0.971
	Triglyceride	0.086	0.581
	LDL-cholesterol	0.024	0.875
	HDL-cholesterol	0.080	0.605
	LDL:HDL ratio	0.102	0.510
Serum leptin	Total cholesterol	0.123	0.426
	Triglyceride	0.263	0.085
	LDL-cholesterol	0.138	0.373
	HDL-cholesterol	0.135	0.381
	LDL:HDL ratio	0.180	0.243

Generally, all of the R-value from the table is less than 0.3 and all p-value from the table are >0.05. Since the p-value is large (>0.05) and R-value is less than 0.3, there is no any correlation and poor relationship between serum lipid profile parameter with others clinical parameter among non-obese patients.

4.13.2 Correlation analysis between serum lipid profile level and others clinical parameter among obese patients

Table 4.18 show summary of correlation analysis between serum lipid profile parameter including total cholesterol, triglyceride, LDL-cholesterol, HDL-cholesterol and LDL:HDL ratio level with age, blood pressure (BP) and serum leptin among obese patients involve this study.

Table 4.18: Correlation analysis between serum lipid profile level and others clinical parameter among obese patients

Parameter	Serum lipid profile	r_s-value	p-value
Age	Total cholesterol	0.111	0.624
	Triglyceride	0.366	0.094
	LDL-cholesterol	0.066	0.772
	HDL-cholesterol	0.004	0.987
	LDL:HDL ratio	0.062	0.785
BP systolic	Total cholesterol	0.097	0.669
	Triglyceride	0.207	0.356
	LDL-cholesterol	0.015	0.947
	HDL-cholesterol	0.067	0.767
	LDL:HDL ratio	0.085	0.708
BP diastolic	Total cholesterol	0.154	0.494
	Triglyceride	0.250	0.261
	LDL-cholesterol	0.221	0.322
	HDL-cholesterol	0.236	0.290
	LDL:HDL ratio	0.227	0.309
	LDL:HDL ratio	0.234	0.294

Table 4.18 continued

Serum leptin	Total cholesterol	0.060	0.791
	Triglyceride	0.076	0.736
	LDL-cholesterol	0.164	0.466
	HDL-cholesterol	0.407	0.060
	LDL:HDL ratio	0.234	0.294

Generally, all of the r_s-value from the table is less than 0.3 except correlation between HDL-cholesterol with serum leptin level and all p-value from the table are >0.05. Since the p-value is large (>0.05) and r_s-value is less than 0.3, there is no any correlation and poor relationship between serum lipid profile parameter with others clinical parameter among obese patients. Even though HDL-cholesterol has fair relationship with serum leptin level with r_s-value 0.407, but the p-value is still large (0.060).

CHAPTER 5

DISCUSSION

5.1 Participants' demographic profile

A total of 100 stable CAD patients who were attending the Cardiac Clinic at the Heart Centre of Sarawak General Hospital have been recruited into this study. Majority of the participants were males (84%) as compared to the percentage of female patients which was only 16%. This proportion between male and female participants is similar to the actual statistical pattern reported of coronary heart disease in Malaysia in 2018 whereby 80% of the patients were male. Since 2006 until 2015, there was an increasing number of coronary disease involving male reported. The percentage of increase was from 75% in 2006 to 79.3% in 2015. The factor that may contribute to this is increase smoking during the early age in males while oestrogen hormone in female can lower the risk for coronary artery disease.

The Malay patients made up the highest number of the participants with 32 per cents, followed by Chinese with 31 per cents. While Iban, Bidayuh and Melanau were at 17% ,10% and 6%, respectively. In 2018, Health Information Centre, Ministry of Health Malaysia reported that Malays were the highest race suffered from coronary disease as compared to two others main races in Malaysia, Chinese and Indians, since 2006. The increasing of percentage of coronary disease based on three main races in Malaysia were the Malays with 52% - 56%, Chinese (24% -23%) and Indians (24% - 21%).

In term of the age of the participants, nearly half of them (41%) falls into the 60-69 years of age group. Twenty-nine per cent of the patients were in the 50-59 years old, followed closely by the 70-79 years old group with 20%. Eight per cent of the patients were in the younger age group of 40-49 years old. The smallest group were also the oldest among the participants (2%), that consisted of 80-89 years old. Based on record, the average age of coronary disease in Malaysia are much younger as compared to developed countries which is reported to be 59 years old. Referring to the report by Ministry of Health Malaysia in 2017, Malaysian citizens suffered coronary disease as early as 59 years old as compared to Thailand (65 years old), China (63 years old) and Canada (68 years old). This trend is reflected in the current study, whereby the average age between 60 years old to 89 years old shows the higher number of stable CAD patients as compared to age less than 60 years old (37%).

Most of the patients are married (98%) with a small percentage of non-married (2%) and almost half of the patients were working. Being unmarried is associated with decreased survival in the general population. Whether married, divorced separated, widowed or never-married status affects outcomes in patients with cardiovascular disease has not been well characterized. However, marriage indirectly will increase the population with family history of CAD. In other words, the potential number of CAD in Malaysia will increased. Working or not working status in patients with CAD also has not been well characterized. Not working status can be already pension or never working. Referring to age group of the participants in this study, the majority of patients aged more than 60 years old, which is the age of pension in Malaysia.

5.2 Demographic of participants with CAD risk factors

A low percentage of patients involved in the study were reported to have the CAD risk factors such as high cholesterol, hypertension, *diabetes mellitus* (DM) and family history of CAD. Only 26% of the patients were reported to have family history of CAD. It is showed that the coronary artery disease is not only inherited but life style factor also plays an important role contributing to coronary artery disease (CAD). However, 56% of the patients recruited into this study admitted that they do not perform any exercise for at least once per week. It is possible that this may contributed to the high number of patients in this study with high body mass index (BMI). From the total, 58 per cents of participants has the BMI of more than 25 kg/m^2, 35 per cents were overweight and 23 per cents were obese. Physical inactivity and obesity were among the earliest factors that have been identified as an independent predictor for the development of coronary artery disease (CAD) (Morris et al., 1973). Fifty years ago in Framingham, the association of obesity and coronary heart disease was first noted by Kannel et al., 1970. Obesity is also an independent risk factor for all-cause mortality and it is a metabolic disorder associated with comorbidities such as coronary heart disease, type 2 *diabetes mellitus* (T2DM) and hypertension.

In this study, the comparison of the stable CAD patients who were smoking with CAD risk factors according to gender cannot be done as the total numbers of the smokers were too small and not equal for each gender. There were only 14 patients who were smoking with one female smoker and thirteen male smokers. Patients who smoked with more than one risk factors has higher potential in the risk of myocardial infarction (MI) or sudden death and the risk was associated to the number of cigarettes smoked each day (Doyle et al., 1962). The deleterious effect of smoking on health has been proven in many studies (Lakier et al.,

1992). All of the smokers involved in this study has high cholesterol level and 10 patients has hypertension while, 5 of them has diabetic mellitus (DM). Acrolein in cigarettes is easily absorb into the blood stream through the lungs, and scientists believe it contributes to heart disease by affecting the way the body metabolizes cholesterol (Henning, 2017). From the previous study on the association between smoking and type 2 diabetes reported that heavy smoking (>20 cigarettes per day) carried an increased relative risk of type 2 diabetes (Carlsson et al., 2004). According to Tuomilehto et al. (1982), smoking caused an acute increased in blood pressure (BP) and heart rate and has been found to be associated with malignant hypertension. Thus, smoking is directly associated with increased of high cholesterol, diabetes and hypertension. When risk factors often occur in clusters certain risk factors will cause greater risk of coronary artery disease. Every risk factor given the different level of risk in the CAD patients. However, the risk of mortality and morbidity are associated in the number of risk factor involved.

5.3 Descriptive of clinical parameters based on exercise activity

Exercise is part of healthy lifestyle and has shown to have health benefits through optimization of the physiological processes in the body. It is recommended that regular exercise training ≥3 three or more times a week and at 30-45 min per session (Roffi, 2016) In this study, data on exercise activity were acquired during the survey interview. Median data and the level of minimum and maximum of each parameter based on the exercise activity was categorised into group of no exercise even once per week and group of exercise at least once per week.

The patients that did not exercise even once per week displayed a median of total cholesterol of 3.91 mmol/L (IQR=1.11), median level of triglyceride of 1.30 mmol/L (IQR=0.79), median level of LDL-cholesterol of 2.09 mmol/L (IQR=1.04), median level of HDL-cholesterol of 1.23 mmol/L (IQR=0.30) and median level of serum leptin of 2.40 ng/mL (IQR=2.43). While, the patients that has exercise at least once per week displayed a median of total cholesterol of 3.82 mmol/L (IQR=1.39), median level of triglyceride of 1.27 mmol/L (IQR=0.79), median level of LDL-cholesterol of 1.93 mmol/L (IQR=0.94), median level of HDL-cholesterol of 1.14 mmol/L (IQR=0.40) and median level of serum leptin of 2.17 ng/mL (IQR=3.58). Overall, there are no significance differences in the levels of various clinical parameters between exercise and non-exercise patients. From this study findings, physical activity has not affect the levels of serum leptin and serum lipid profile of the participating stable CAD patients. In previous study, it has been reported that in some patients with mild to moderate dyslipidemia, few months of aerobic exercise did not significantly reduce the LDL-cholesterol, but the concentration of atherogenic small LDL particle was found to be decreased, and the average size of LDL particles increased (Varady et al., 2005). However, according to Elosua et al., 2003, aerobic exercise had no effect on LDL particle diameter. Many studies have shown that sedentary individuals have no change in triglyceride levels after a single exercise session (Kantor, 1984). Although the mechanism of exercise-induced lipid changes is unclear, exercise itself may increase blood lipid consumption hence to decrease lipids level (Earnest, 2013).

5.4 Correlation analysis between serum leptin level and the number of vessel disease

Majority of the stable CAD patients in this study were reported to have single vessel disease (SVD) at 45 per cents. 29 per cents of the patients were with 2-vessel disease (2VD). While the patients with 3-vessel disease (3VD) is at 26%.

Serum leptin level by number of vessel disease has been statistically analysed to study the significance of the serum leptin levels in different number of vessel disease group. The one vessel disease displayed a median of 1.92ng/mL serum leptin level (IQR=3.22), two vessel disease displayed a median 2.97ng/mL serum leptin level (IQR=1.71) and three vessel disease displayed a median 1.80ng/mL serum leptin level (IQR=3.38). The median of serum leptin level of two vessel disease is in normal range. However, the median of serum leptin level in one vessel disease and three vessel disease is less than normal range. Normal range leptin level for men is 3.8 ng/mL ±1.8 ng/ml and female 7.4 ng/mL ± 3.7 ng/ml (EMD, 2015). Analysis statistic found p-value is 0.184 and it shows no significant difference exist between serum leptin level with different number of vessel disease. Previous study about association between serum leptin and the number of vessel disease are not reported. However, Tsai et al. in 2016 reported that increasing serum concentration of leptin correlated positively with the total number of stenotic coronary arteries and serum leptin level may predict the development of arterial stiffness in CAD patients. Besides, in 2018, Chen et al. also reported that high serum leptin level is a risk marker for peripheral artery disease in geriatric individuals. Different findings analysis in my study maybe cause of total participant for every number of vessel disease and the gender of participants are not equal. The participants also consist of various disease such as hypertension and diabetic, so that different medicine taken may be affected serum leptin level. However, from this study

finding, serum leptin level not a contributing factor for determination number of arterial damage or disease.

5.5 Correlation between serum leptin level and lipid profile of stable CAD based on race

In an analysis of this study involving 100 patients with stable CAD, the clinical parameters of serum leptin, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglyceride were arranged, where each parameter analysed based on the race.

Serum leptin level by race has been statistically analysed to obtain the significant different in between race group. The malay displayed a median of 3.25 ng/mL serum leptin level (IQR=2.46), Chinese displayed a median of 1.66 ng/mL serum leptin level (IQR=1.84), Iban displayed a median of 3.81ng/mL serum leptin (IQR=8.34), Bidayuh displayed a median of 3.03 ng/mL(IQR=3.30), Melanau displayed a median of 1.14 ng/mL serum leptin (IQR=1.32) and others race displayed a median of 3.49 ng/mL serum leptin level (IQR=3.38). The median of serum leptin level of Malay, Iban, Bidayuh and Others is in normal range. However, the median of serum leptin level Chinese and Melanau is less than normal range. Normal range leptin level for men is 3.8 ng/mL ±1.8 ng/ml and female 7.4 ng/mL ± 3.7 ng/ml (EMD, 2015). Since, correlation analysis found p-value is 0.07 and it shows no association and no significant difference exist between serum leptin level within different race.

Then, LDL-cholesterol by race has been statistically analysed to obtain the significant different in between race group. The malay displayed a median of 2.06 mmol/L LDL_cholesterol level (IQR=0.93), Chinese displayed a median of 1.94 mmol/L LDL-

cholesterol level (IQR=1.02), Iban displayed a median of 1.84 mmol/L LDL-cholesterol level (IQR=1.14), Bidayuh displayed a median of 1.89 mmol/L LDL-cholesterol level (IQR=1.46), Melanau displayed a median of 2.52 mmol/L LDL-cholesterol level (IQR=0.92) and others race displayed a median of 2.44 mmol/L LDL-cholesterol level (IQR=1.47). All of the median of LDL-cholesterol within race group shows in normal range. Normal range of LDL-cholesterol is <3.4 mmol/L. Since, correlation analysis found p-value is 0.58 and it shows no association and no significant difference exist between LDL-cholesterol level within different race.

Then, HDL-cholesterol by race has been statistically analysed to obtain the significant different in between race group. The malay displayed a median of 1.18 mmol/L HDL_cholesterol level (IQR=0.28), Chinese displayed a median of 1.39 mmol/L HDL-cholesterol level (IQR=0.47), Iban displayed a median of 1.13 mmol/L HDL-cholesterol level (IQR=0.40), Bidayuh displayed a median of 1.15 mmol/L HDL-cholesterol level (IQR=1.46), Melanau displayed a median of 1.22 mmol/L HDL-cholesterol level (IQR=0.41) and others race displayed a median of 1.23 mmol/L HDL-cholesterol level (IQR=0.25). All of the median of HDL-cholesterol within race group shows in normal range. Normal range of HDL-cholesterol is >1.0 mmol/L. Since, correlation analysis found p-value is 0.283 and it shows no association and no significant difference exist between HDL-cholesterol level within different race.

Then, triglyceride by race has been statistically analysed to obtain the significant different in between race group. The malay displayed a median of 1.21 mmol/L triglyceride level (IQR=0.66), Chinese displayed a median of 1.19 mmol/L triglyceride level (IQR=0.69), Iban

displayed a median of 1.26 mmol/L triglyceride level (IQR=0.60), Bidayuh displayed a median of 1.50 mmol/L triglyceride level (IQR=0.76), Melanau displayed a median of 1.87 mmol/L triglyceride level (IQR=0.92) and others race displayed a median of 1.66 mmol/L triglyceride level (IQR=1.35). All of the median of triglyceride within race group shows in normal range. Normal range of triglyceride is <2.3 mmol/L. Since, correlation analysis found p-value is 0.26 and it shows no association and no significant difference exist between triglyceride level within different race.

Overall, the correlation analysis of serum leptin level and serum lipid profile parameter within race shows no association and no significant difference exist. From this study finding, there is no different of value level of serum leptin and serum lipid profile of stable CAD patients with racial differences. Similarly in 1998, Perez et al. also reported the similar leptin levels were observed among the males Caucasian and Mapuche. However, in 2010 American Association Journal was reported serum leptin was significantly higher in South Asians and Aboriginal people than in Europeans and Chinese. The different analysis findings maybe cause of economy factors, human activity and geography factors.

5.6 Correlation analysis between serum leptin level and others clinical parameter

The correlation between serum leptin level with age, blood pressure (BP), body mass index (BMI) and serum lipid profile parameter including total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol has been statistically analysed. Only body mass index (BMI) and triglyceride has showed correlation with serum leptin level since p-value is <0.005. Both BMI and triglyceride were positive correlation with serum leptin level with p-value is <0.001 and the coefficient of spearman's rho correlation is 0.548 for BMI. While,

p-value of triglyceride is 0.001 and the coefficient of spearman's rho correlation of triglyceride is 0.318. This data analysis supported by Erkin et al. in year 2014 where their research found out that leptin is associated with general and abdominal obesity, dyslipidemia and insulin resistance in Kyrgyz patients. However, Keiko et al. (2006) reported that leptin was associated with diastolic blood pressure (DBP) but not with systolic blood pressure (SBP). Since all the blood pressure of participants in this study are well control and within normal range the serum leptin level were not correlated with both diastolic blood pressure (DBP) and systolic blood pressure (SBP).

5.7 Correlation analysis between serum leptin level and others clinical parameter among *diabetes mellitus* (DM) patients and *non-diabetes mellitus* (non-DM) patients

In type 2 *diabetes mellitus* (T2DM), it has been reported there is a link between high leptin concentrations and increased cardiovascular disease risk, as well as the presence of microvascular complications and cardiac autonomic dysfunction (Rodriguez et al., 2016). That is why in this study we decided analysis *diabetes mellitus* (DM) patients separately. The correlation between serum leptin level with others clinical parameter were analysed among patients has *diabetes mellitus* (DM) and *non-diabetes mellitus* (non-DM). The correlation analysis has found two different result.

Analysis among *non-diabetes mellitus* (non-DM) patients found that is strong positive correlation between serum leptin level with body mass index (BMI) and triglyceride level. The p-value for both BMI and triglyceride are <0.001. Different with correlation between serum leptin level and others parameter among *diabetes mellitus* (DM) patients. Statistics analysis found that is only body mass index (BMI) has correlation and fair relationship with

serum leptin level with p-value 0.010 and correlation coefficient value 0.419. Two correlation analysis found that serum leptin level has positive correlation and has different strength of relationship with body mass index (BMI) and triglyceride except among *diabetes mellitus* (DM) patients. This study supported by Mona at el., 2017 that has reported no significant relationship between body mass index (BMI) and leptin in diabetes mellitus (DM) male and positive relationship between body mass index (BMI) and leptin in *non-diabetes mellitus* (non-DM). Besides that, statin can decrease leptin concentration in cardiovascular disease (CVD) patients (Takashi et al., 2012) that well known taken by all participants. Whether this statin-induced effect is involved in the atheroprotective properties of statin should be elucidated in future studies. Apart from statins, several drugs including metformin also decreased leptin concentrations in type 2 *diabetes mellitus* (T2DM) patients (Farooq et al., 2017). Beside small size of sample *diabetes mellitus* (DM) patients this metformin and statin maybe cause no correlation any parameter of lipid profile risk factor including body mass index (BMI) among *diabetes mellitus* (DM) patients group. By not taking of metformin drug possibly cause the strength of correlation analysis of body mass index (BMI) and triglyceride among *non-diabetes mellitus* (non-DM) were increased and the significant difference also increased to maximum value compare to general correlation.

5.8 Correlation analysis of serum lipid profile parameters with others clinical parameter between obese and non-obese of stable CAD patients

The summary of serum leptin level and lipid profile parameters between obese and non-obese of stable CAD patients involved this study has been analysed. Statistical analysis has been found that is serum leptin level clearly show significance difference in median value, interquartile range, minimum value and maximum value between obese and non-obese patients. The correlation serum leptin level between obese and non-obese patients also show

positive correlation with p-value <0.001. Different with the lipid profile parameters level shows no significance difference between obese and non-obese in median, interquartile range, minimum value and maximum value. Others than that, all the serum lipid profile parameters are negative correlation between obese and non-obese patients. Then, correlation between serum lipid profile parameter level with others clinical parameter among obese and non-obese patients has analysed. The correlation analysis found that is no correlation and poor relationship between serum lipid profile parameter with age, blood pressure (BP) and serum leptin level among obese and non-obese patients. However, HDL-cholesterol has fair relationship but no correlation with serum leptin level among obese patients. This correlation analysis found that, serum leptin level that has positive correlation between obese and non-obese patients also has fair relationship with HDL-cholesterol among obese patients. Previous study showed conflict result. These study showed the serum lipid profile parameter shows no correlation with obese and non-obese patients. Similar results were obtained in the study LIPIDOGRAM 2004, where higher serum levels of total cholesterol, low density lipoprotein and triglyceride were found in overweight people compared with the obese (Mastej et al., 2006). Other than that, Anna et al. (2014) also reported the group of patients with Class III obesity (BMI >40 kg/m^2) had lower mean serum levels of TC and LDL-C compared with patients from the other 2 groups, and this difference was statistically significant when compared to the patients with Class I (BMI 30.0 – 34.9 kg/m^2) obesity. In another study by Mohammad & Mohamad (2016) found out the obese group, significant higher cholesterol and triglycerides were observed compared to non-obese group. Similar reported by Bhati et al. (2001) where all the lipids parameters except serum HDL level showed significant increase in obese persons while HDL level was significantly decreased. Therefore, Howard et al. (2003) concluded, based on results of clinical studies, that the

relationship between BMI and low density lipoprotein serum levels is complex and depends on numerous factors such as age or sex. This has showed that serum lipid profile parameter level is not a major factor that cause obesity.

CHAPTER 6

CONCLUSION

6.1 Conclusion

One hundred fasting serum patients with stable CAD was successfully assayed for their serum leptin levels using the ELISA kit manufactured by MERCK, USA, along with other biochemical parameters analysed in this study. From this research, it is observed that there is no significant difference in the overall circulating serum leptin levels among different races and ethnic groups involved in this study. Small sample size and uneven numbers of sample group is likely caused correlation analysis cant do well especially correlation among race and indigenous groups.

There is a positive correlation between lipid profile parameter and serum leptin levels in patients with stable coronary artery disease (CAD). However only one parameter of the lipid profile, which is the triglyceride level shows positive correlation with correlation coefficient, r:0.318 and p value <0.005. The strength of linear relationship increased in the correlation of serum leptin level and triglyceride level among the non-diabetic stable CAD patients ($r = 0.439$, $p < 0.001$). Serum leptin levels correlate well with the body mass index of the stable CAD patients involved in the study. Positive correlation between serum leptin levels and BMI were seen in both diabetic and non-diabetic groups.

However, statin can decrease leptin concentration in CVD patients that well known taken by all participant. Whether this statin-induced effect is involved in the atheroprotective

properties of statin shoud be elucidated in future studies. Apart from statins, several drugs including metformin also decreased leptin concentrations in T2DM patients. Beside very small size of sample *diabetes mellitus* (DM) patients in this study, the metformin maybe caused no correlation any parameter of lipid profile and risk factor including BMI and triglyceride among *diabetes mellitus* (DM) group. However, the strenght of correlation analysis of BMI and triglyceride among *non-diabetes mellitus* (non-DM) were increased and the significance difference also increased to maximum value.

6.2 Limitations of research

Initially, this study had faced some technical issues where the numbers of subjects recruited in this study were not comparatively equal for each ethnic. This limitation issue caused the investigation in variation of serum leptin level for ethnic cannot be fully studied. Secondly, there was no control sample from healthy individuals and small numbers of participants. Higher number of patients may yield better results and thus may increase the validity of the findings. For future research, the increasing of total sample and setting the sample group of gender and ethnics may solve this issue.

6.3 Recommendations

The findings from this study are hoped to bring new insights regarding the role of biomarkers in cardiovascular disease research, testing and its clinical management. Determination of association and correlation of specific biomarkers with the progression and outcomes of cardiovascular diseases and with the medium high of risk level lipid profile analysis, potentially can assist clinicians to identify more at-risk patients and target treatment to each patient's individual risk. Similar study with increased number of sampling size and variables/

parameters will offer better understanding and improve the management of CAD patients. As dietary factor has been shown to influence serum leptin level, establishing the association trend of this hormone in patients with stable CAD in Sarawak would also provide additional information which can be used towards the betterment of patient management. Thus further study can be done using a higher number of sample populations, targeting various race and ethnic groups, in relation to their specific and traditional dietary pattern.

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APPENDICES

Appendix A: Participant Information sheet

PARTICIPANT INFORMATION SHEET

Relationship between Atherogenic Lipid Profile and Serum Leptin Levels in Patients with Stable Coronary Artery Disease (CAD)

Principal Investigator:

Dr Mohamad Lizan bin Mohamad Buang[1]

Co-Investigator:

Dr Muna binti Sabri[1]

Dr Asri bin Said[1]

Dr Alan Fong Yean Yip[2]

1 Faculty of Medicine and Health Sciences, UNIMAS, Kuching 93250 Sarawak

2 Cardiology Department, Sarawak General Hospital, Heart Centre, Kota Samarahan 94300 Sarawak

PARTICIPANT INFORMATION SHEET

1. Study Information

Protocol Title:

Relationship between Artherogenic Lipid Profile and Serum Leptin Levels in Patients with Stable Coronary Artery Disease (CAD)

Principal investigator & contact details:

Dr Mohamad Lizan bin Mohamad Buang, Medical Lecturer, Faculty of Medicine and Health Sciences, UNIMAS, Lot 77, Jalan Tun Ahmad Zaidi Adruce 93250 Kuching, Sarawak, Malaysia, Tel: 082-416550, Fax: 082-416550

Study Sponsor:

Ministry of Higher Education (MOHE)

2. Purpose of the research study

You are invited to participate in a research study because you have had a history of stable angina or heart attack which occurred more than 3 months ago and investigators at the Faculty of Medicine and Health Sciences (FMHS) UNIMAS are interested in discovering the relationship between the serum leptin levels and the artherogenic lipid profile (especially lowdensity lipoprotein) in the blood. It is important to us that you first take time to read through and understand the information provided in this sheet. Nevertheless, before you take part in this research study, the study will be explained to you and you will be given the chance to ask questions. After you are properly satisfied that you understand this study, and that you wish to take part in the study, you must sign this informed consent form. You will be given a copy of this consent form to take home with you.

The purpose of the study is to collect blood samples and medical information in order to establish the pattern of serum leptin levels and its variations in the multi-ethnic population of patients with stable coronary artery diseases (CAD). Besides that, we will also try to establish the relationship between the plasma LDL profile and serum leptin levels in these patients. We will then perform laboratory analysis on your blood to find specific particle sizes of LDL that are responsible for the development of CAD. This is part of an international multicentre study of patients with history of angina or heart attacks, of which we will be

enrolling up to 100 patients from Sarawak General Hospital Heart Center (SGHHC) over a period of 12 months from 1st November 2012 to 1st November 2013.

Leptin, a hormone secreted by adipose tissue, has been largely implicated in the increased cardiovascular risks. Basically, it is known as the satiety factor that plays a central role in the control of food intake and energy homeostasis. This hormone exerts several cardiovascular actions such as platelet aggregation, sympathetic activation, insulin resistance and proangiogenic effect, which suggest its important role in the development of cardiovascular diseases. This hormone can be found abundantly in the plasma of obese patient due to leptin resistance. Many previous epidemiologic Coronary Artery Disease (CAD) studies have demonstrated a relationship between obesity and increased risk for cardiovascular disease, however the human studies on leptin and CAD have reported conflicting results. Besides its plasma concentration level in the blood, low-density lipoprotein (LDL) particle size has been indicated to be an important predictor of cardiovascular events and progression of coronary artery disease, whereas a predominance of small, dense LDL has been accepted as an emerging cardiovascular risk factor. As both leptin and LDL size are genetically and environmentally influenced, we thus seek to investigate the serum leptin level pattern and its relationship with atherogenic lipoprotein phenotype in patients with stable coronary artery diseases in the multi-ethnic populations of Sarawak.

3. What procedures will be followed in this study

If you take part in this study, you will have to give a sufficient amount of blood. This blood may be taken during your follow up in the Cardiac Clinic at the SGHHC. You will be asked to be fasted 10 hours before the blood taking and you need to provide one blood specimen equivalent to 2 teaspoons of blood (10mls) if you decide to participate in the full study which includes for the detection of serum leptin and plasma lipid profile. Your participation in the study will last 12 months. For the purpose of this study, you will not be required to return for any further visits beyond the 1st visit however, you will be follow up during your regular check-ups at the Cardiac Clinic. Participating in this study does not in any way exempt you from seeing your regular doctor during your scheduled clinical visits.

4. Your responsibilities in this study

If you agree to participate in this study, you should follow the advice given to you by the study team. You should be prepared to answer several questions about your medical condition especially at 3 months before this procedure.

5. What is not standard care or experimental in this study

We will not be administering any experimental medications to you in this study. However, please note that additional blood collected and the administration of questions via telephone in this study are not part of standard care.

6. Possible risks and side effects

This research study only involves taking blood on one occasion thus the possible risks are very low. Anyway, obtaining blood can cause pain, bleeding, bruising, or swelling at the site of the needle stick. Fainting sometimes occurs and infection rarely occurs. We will maintain strict confidentiality of your entire plasma lipid profile, serum leptin and other data pertaining to this study. We will not release your blood information to any 3rd party who is not an investigator on this study. However, we will release any blood information to you at your request with prior approval from the UNIMAS or the Ministry of Higher Education, Malaysia (MOHE). Please note that this blood information, if released to you, may have significant social and psychological risk.

There is a risk for discrimination against individuals who are at risk for a medical disorder or have a medical disorder/condition in their family. Discrimination may include barriers to obtaining health, life or long-term care insurance, or obtaining employment. Extensive efforts are made to protect all research subjects from prejudice, discrimination, or uses of this information that will negatively affect them. When results from this study are reported in medical journals or at meetings, the identification of research subjects is withheld. Study records that identify you will be kept confidential as required by law.

7. Possible benefits from participating in the study

There is no known benefit from participation in this study. However, your participation in this study may add to medical knowledge that may benefit future patients.

8. Use of specimens and ownership of samples

Blood removed from you during the course of this study may be valuable for scientific, research, or teaching purposes or for the development of a new medical product. By agreeing to participate in this research, you authorize the UNIMAS and members of its staff to use your blood specimens for these purposes. The PI and his team will maintain these samples indefinitely or until the samples are exhausted. These samples are unavailable for clinical (diagnostic) purposes. Therefore, any future diagnostic testing must be performed using a new sample. In the event this research project results in a product which could be sold commercially, the UNIMAS and its collaborators will assert all rights to any revenue from the sale of such a product. Scientists who have access to your information and biological

materials may form collaboration(s) with private companies in order to study samples and information collected in the study. You should understand that such scientists and companies have an economic interest in using the information found from testing your blood, along with serum leptin from other participants, for the eventual development of commercial products. These companies may patent products or sell discoveries based on this research. Some of the investigators involved in the analysis of your blood and information that you provide may get some financial benefit from this work. There are no plans to provide any compensation to you or to your heirs should this occur.

9. Incidental findings

If you are found to have an inherited risk for a disease known at the time of testing to be likely to cause premature death if untreated, we will attempt to notify you by way of certified mail to contact Dr Mohamad Lizan bin Mohamad Buang at FMHS UNIMAS. UNIMAS staff will not release these specific research findings over the telephone or by mail. Please provide us with any change in your address, since such notification will be sent to the last address you provided to the study personnel. Your blood samples and medical and family history data may be shared with other investigators or other research institutions including pharmaceutical companies for research purposes. Your identity will remain confidential and will not be available under any circumstances to these collaborators. The researchers directly involved with this study will review the potential scientific value of research proposed by other investigators before agreeing to the use of the resources of the project. Any proposed research with your samples and information must also be approved by the UNIMAS Medical Research Ethics Committee (MREC). Researchers who ask to study samples, medical and family information will not be given your name or other information that might reveal your identity, such as your initials or date of birth. Samples and data shared with other investigators will be given a unique code number. The key to the code will be kept in a locked file available only to Dr. Mohamad Lizan and his research staff. The UNIMAS or MOHE Investigators need to maintain a link between the coded identification number by which samples, photographs and data are identified to other investigators and the confidential information kept by the Investigators at the UNIMAS. This will allow the Investigators to study test results provided by the collaborators using your samples and data to better understand heart disease and other related conditions.

10. Alternatives to participation

Whether you choose to or choose not to take part in this study, you will receive standard care for your condition.

11. Costs & payments if participating in the study

You will be granted with a token of RM30 if you join the study. Besides, you will still receive the same treatment and you will pay the same hospital bill, whether or not you decide to participate in this study.

12. Voluntary Participation

Your participation in this study is voluntary. You may stop participating in this study at any time. Your decision not to take part in this study or to stop your participation will not affect your medical care or any benefits to which you are entitled. Your doctor, the Investigator of this study may stop your participation in the study at any time if they decide that it is in your best interests. They may also do this if you do not follow instructions required to complete the study adequately. If you have other medical problems or side effects, the doctor and/or nurse will decide if you may continue in the research study. In the event of any new information becoming available that may be relevant to your willingness to continue in this study, you will be informed in a timely manner by the Principal Investigator or his/her representative.

13. Consent for future use of biological samples

For each patient, we will bank unused blood, for serum leptin and lipid profile analysis, at the Clinical Research Laboratory, FMHS UNIMAS. All blood specimens stored for future use will be kept in the UNIMAS laboratory, which is a secured facility under 24 hour surveillance. All blood specimens will be de-identified with identifiers kept in a secured separate location.

You should understand that the investigators involved in this study may have an economic interest in using the information found from testing your blood for the eventual development of commercial products. The investigators may patent products or sell discoveries based on this research. Some of the investigators involved in the analysis of your blood and information that you provide may get some financial benefit from this work. There are no plans to provide any compensation to you or to your heirs should this occur.

You may withdraw consent for the use of these samples at any time you see fit. Any information about future research will not be made known to your clinician, but you may obtain such information from the principal investigator if you so wish.

14. Confidentiality of study and medical records

Information collected for this study will be kept confidential. Your records, to the extent of the applicable laws and regulations, will not be made publicly available. However, the UNIMAS or UNIMAS MREC will be granted direct access to your original medical records to check study procedures and data, without making any of your information public. By signing the Informed Consent Form attached, you are authorizing such access to your study and medical records.

Data collected and entered into the Case Report Forms and blood specimens are the property of the UNIMAS. In the event of any publication regarding this study, your identity will remain confidential.

Only the Investigators and specifically designated members of their research staff will have access to information which would allow a link to be made between your medical information and the results of your blood testing. All information collected during this research study will not be released to anyone in an identified way, including you or other family members.

Research records that identify you by name (for example, this signed consent form) will be kept in a separate research file that is not part of your usual medical record. Your records will be assigned a unique code number. Research records or reports sent to other researchers, including the study Sponsors, will identify you by the unique code number, not by your name.

Your records may be reviewed in order to meet federal or state regulations.

This study has a long-range goal to establish the relationship between serum leptin and plasma lipid profile in the stable CAD patients. No end date has been set for analyzing results from the study. As new information about heart disease becomes available and new methods of analyzing research results are developed, we hope to use the samples and data over time to continue to advance knowledge about the disease. At such time as the study is considered completed, either the research information not already in your medical record will be destroyed or information identifying you will be removed from study results at the UNIMAS.

15. Who to contact if you have questions

If you have questions about this research study, you may contact the Principal Investigator:

Dr Mohamad Lizan bin Mohamad Buang

Faculty of Medicine and Health Sciences UNIMAS Tel: 082-416550 Fax: 082-416550 Sarawak General Hospital, Heart Centre Tel: 082-281296 Fax: 082-281296

The study has been reviewed by the UNIMAS MREC for ethics approval. If you have any complaints about this research study, you may contact the Principal Investigator or the MREC Secretariat at: NIH Secretariat, Ministry of Health Malaysia, c/o Institute of Health Management, Jalan Rumah Sakit, Bangsar, 50900 Kuala Lumpur, Tel: 03-22874032, Fax: 03-22874030

Appendix B: Consent form

CONSENT FORM

Protocol Title:

Relationship between Lipid Profile and Serum Leptin Levels in Patients with Stable Coronary Artery Disease (CAD)

Principal Investigator & Contact Details: Dr Mohamad Lizan bin Mohamad Buang Faculty of Medicine and Health Sciences UNIMAS Tel: 082-416550 Fax: 082-416550
Sarawak General Hospital, Heart Centre Tel: 082-281296 Fax: 082-281296

I voluntarily consent to take part in this research study. I have fully discussed and understood the purpose and procedures of this study. This study has been explained to me in a language that I understand. I have been given enough time to ask any questions that I have about the study, and all my questions have been answered to my satisfaction.

…….. ……. ……

Name of Participant Signature Date

……....

Identification Card No (I/C No)

Witness Statement I, the undersigned, certify to the best of my knowledge that the participant signing this informed consent form had the study fully explained in a language understood by him / her and clearly understands the nature, risks and benefits of his / her participation in the study.

…….. ……. ……

Name of Witness Signature Date

…….

Identification Card No (I/C No)

Investigator Statement

I, the undersigned, certify that I explained the study to the participant and to the best of my knowledge the participant signing this informed consent form clearly understands the nature, risks and benefits of her participation in the study.

……. ……. ……

Name of Investigator Signature Date
person administering consent

Please indicate your preference on future use of your stored blood:

- o Yes, I agree to have my unused blood kept for future research and there are no restrictions on the kind of research that may be done with my blood specimen.

- o Yes, I agree to have my unused blood kept for future research and the investigator may use my blood specimen for future research as long as the research does not involve genetic analysis.

- o Yes, I agree to have my unused blood kept for future research and the investigator may use my blood specimen for future research as long as the research is related to cardiovascular disease.

- o No, I did not agree my unused blood keep for future use.

Appendix C: Acknowledgement Receipt

UNIVERSITI MALAYSIA SARAWAK

FACULTY OF MEDICINE AND HEALTH SCIENCES

Acknowledgement of Receipt / Cash Payment Form

Name: _____
No IC: _____
Address: _____

I am as above hereby had received cash of
_____
(RM
_____)

For_____

_____

On (date) _____ from the following officer: -

Name: _____
No IC: _____
Address: _____

For the purpose of reimbursement through the vot/account
_____

Signature of Payee: Signature of Payer:

Date: Date:

Appendix D: Human leptin ELISA Kits used in human leptin level analysis

EZHL-80SK Sigma-AldrichHuman Leptin "Dual Range" ELISA

- 

This Human Leptin "Dual Range" ELISA is used to measure & quantify Leptin levels in Metabolism & Endocrine & Neuroscience research.

Human Leptin "Dual Range" ELISA MSDS (material safety data sheet) or SDS, CoA and CoQ, dossiers, brochures and other available documents.

Analytes Available	Species Reactivity	Key Applications	Detection Methods
Leptin	H	ELISA	Fluorescent, Colorimetric
Description			
Catalogue Number	EZHL-80SK		
Description	Human Leptin "Dual Range" ELISA		

Description	
Background Information	Leptin is synthesized in adipocytes as a 16 kDa molecule and the circulating level is directly proportional to the total amount of fat in the body.
Product Information	
Detection method	Fluorescent Colorimetric
Precision, %	• Inter-assay: 2.6–6.2 Sensitive Assay 1.3–8.6 • Intra-assay: 2.6–4.6 Sensitive Assay 1.4–4.9
Applications	
Application	This Human Leptin "Dual Range" ELISA is used to measure & quantify Leptin levels in Metabolism & Endocrine & Neuroscience research.
Key Applications	• ELISA
Application Notes	Room temperature, 3.5 hour assay 25 µL sample size (Sensitive Assay: 50µL)
Biological Information	
Species Reactivity	• Human
Analytes Available	• Leptin

Biological Information	
Entrez Gene Number	• NM_000230.1
Entrez Gene Summary	This gene encodes a protein that is secreted by white adipocytes, and which plays a major role in the regulation of body weight. This protein, which acts through the leptin receptor, functions as part of a signaling pathway that can inhibit food intake and/or regulate energy expenditure to maintain constancy of the adipose mass. This protein also has several endocrine functions, and is involved in the regulation of immune and inflammatory responses, hematopoiesis, angiogenesis and wound healing. Mutations in this gene and/or its regulatory regions cause severe obesity, and morbid obesity with hypogonadism. This gene has also been linked to type 2 diabetes mellitus development.
Gene Symbol	• LEP • leptin • OB • obesity • OBS
UniProt Number	• P41159
UniProt Summary	**FUNCTION:** SwissProt: P41159 # May function as part of a signaling pathway that acts to regulate the size of the body fat depot. An increase in the level of LEP may act directly or indirectly on the CNS to inhibit food intake and/or regulate energy expenditure as part of a homeostatic mechanism to maintain constancy of the adipose mass. **SIZE:** 167 amino acids; 18641 Da **SUBUNIT:** Interacts with SIGLEC6. **SUBCELLULAR LOCATION:** Secreted. **DISEASE:**SwissProt: P41159 # Defects in LEP may be a cause of autosomal recessive obesity [MIM:601665]. **SIMILARITY:** SwissProt: P41159 ## Belongs to the leptin family.
Accuracy	• 94–114% • Sensitive Assay 86–102%

Physicochemical Information	
Sensitivity	• 0.5 ng/mL • Sensitive Assay 0.125 ng/mL
Linearity of Dilution	80–105% Sensitive Assay 96–125%
Standard Curve Range	• 0.5–100 ng/mL • Sensitive Assay 0.125–20 ng/mL
Product Usage Statements	
Usage Statement	• For research use only. Not for use in diagnostic procedures.
Packaging Information	
Material Size	96-well strip plate

Appendix E: Beckman Coulter AU680 Analyser using in full lipid profile analysis

Beckman Coulter AU680 analyser

Sample rack use in assembling specimen

Appendix F: Number and percentage of participants by category:

Gender

Gender					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	84	84.0	84.0	84.0
	Female	16	16.0	16.0	100.0
	Total	100	100.0	100.0	

Race

Race					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Malay	32	32.0	32.0	32.0
	Chinese	31	31.0	31.0	63.0
	Iban	17	17.0	17.0	80.0
	Bidayuh	10	10.0	10.0	90.0
	Melanau	6	6.0	6.0	96.0
	Others	4	4.0	4.0	100.0
	Total	100	100.0	100.0	

Marital status

Marital status					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Single	2	2.0	2.0	2.0
	Married	98	98.0	98.0	100.0
	Total	100	100.0	100.0	

Hypertension status

Hypertension status					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Non HPT	22	22.0	22.0	22.0
	HPT	78	78.0	78.0	100.0
	Total	100	100.0	100.0	

Diabetes mellitus status

Diabetes mellitus status					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Non DM	63	63.0	63.0	63.0
	DM	37	37.0	37.0	100.0
	Total	100	100.0	100.0	

Numbers of artery disease

No of artery involved					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	45	45.0	45.0	45.0
	2	29	29.0	29.0	74.0
	3	26	26.0	26.0	100.0
	Total	100	100.0	100.0	

High cholesterol patient

Cholesterol status					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1	100	100.0	100.0	100.0

Status of active smoker and the number of cigarettes per day

No of cigarettes per day					
			Smoker		
			Non Smoker	Active smoker	Total
No of cigarettes per day	0	Count	86	0	86
		% within rokok	100.0%	.0%	86.0%
	1	Count	0	2	2
		% within rokok	.0%	14.3%	2.0%
	2	Count	0	2	2
		% within rokok	.0%	14.3%	2.0%
	5	Count	0	1	1
		% within rokok	.0%	7.1%	1.0%
	10	Count	0	5	5
		% within rokok	.0%	35.7%	5.0%
	20	Count	0	4	4
		% within rokok	.0%	28.6%	4.0%
Total		Count	86	14	100
		% within rokok	100.0%	100.0%	100.0%

Smoker compare to gender

Crosstab					
			Smoker		
			Non Smoker	Active Smoker	Total
Gender	Male	Count	71	13	84
		% within rokok	82.6%	92.9%	84.0%
	Female	Count	15	1	16
		% within rokok	17.4%	7.1%	16.0%
Total		Count	86	14	100
		% within rokok	100.0%	100.0%	100.0%

Appendix G: Descriptive statistic data of clinical parameter by category Body mass index (BMI)

Descriptives				
			Statistic	Std. Error
Body Mass Index	Mean		26.640	.4297
	95% Confidence Interval for Mean	Lower Bound	25.787	
		Upper Bound	27.492	
	5% Trimmed Mean		26.577	
	Median		26.010	
	Variance		18.465	
	Std. Deviation		4.2970	
	Minimum		16.5	
	Maximum		36.7	
	Range		20.2	
	Interquartile Range		5.9	
	Skewness		.293	.241
	Kurtosis		-.010	.478

Heart rate

Descriptives				
			Statistic	Std. Error
Heart Rate	Mean		68.94	1.583
	95% Confidence Interval for Mean	Lower Bound	65.80	
		Upper Bound	72.08	
	5% Trimmed Mean		67.50	
	Median		66.50	
	Variance		250.542	
	Std. Deviation		15.829	
	Minimum		40	
	Maximum		135	
	Range		95	
	Interquartile Range		15	
	Skewness		1.746	.241
	Kurtosis		4.782	.478

Blood pressure (BP) (Systolic)

Descriptives				
			Statistic	Std. Error
BP Systolic	Mean		141.88	2.081
	95% Confidence Interval for Mean	Lower Bound	137.75	
		Upper Bound	146.01	
	5% Trimmed Mean		140.94	
	Median		140.00	
	Variance		432.874	
	Std. Deviation		20.806	
	Minimum		90	
	Maximum		207	
	Range		117	
	Interquartile Range		29	
	Skewness		.690	.241
	Kurtosis		1.038	.478

Blood pressure (BP) (Diastolic)

Descriptives				
BP Diastolic	Mean		82.79	1.155
	95% Confidence Interval for Mean	Lower Bound	80.50	
		Upper Bound	85.08	
	5% Trimmed Mean		82.67	
	Median		82.00	
	Variance		133.299	
	Std. Deviation		11.546	
	Minimum		52	
	Maximum		112	
	Range		60	
	Interquartile Range		14	
	Skewness		.212	.241
	Kurtosis		.154	.478

Total cholesterol level

Descriptives				
			Statistic	Std. Error
Total Cholesterol	Mean		4.0330	.09202
	95% Confidence Interval for Mean	Lower Bound	3.8504	
		Upper Bound	4.2156	
	5% Trimmed Mean		3.9719	
	Median		3.8750	
	Variance		.847	
	Std. Deviation		.92024	
	Minimum		2.31	
	Maximum		7.41	
	Range		5.10	
	Interquartile Range		1.27	
	Skewness		1.060	.241
	Kurtosis		1.552	.478

Triglyceride level

Descriptives				
			Statistic	Std. Error
Triglyceride	Mean		1.4008	.05199
	95% Confidence Interval for Mean	Lower Bound	1.2976	
		Upper Bound	1.5040	
	5% Trimmed Mean		1.3753	
	Median		1.2800	
	Variance		.270	
	Std. Deviation		.51986	
	Minimum		.70	
	Maximum		2.82	
	Range		2.12	
	Interquartile Range		.74	
	Skewness		.727	.241
	Kurtosis		-.313	.478

High-density lipoprotein (HDL)

Descriptives				
			Statistic	Std. Error
High-Density Lipoprotein	Mean		1.2656	.02934
	95% Confidence Interval for Mean	Lower Bound	1.2074	
		Upper Bound	1.3238	
	5% Trimmed Mean		1.2492	
	Median		1.2000	
	Variance		.086	
	Std. Deviation		.29341	
	Minimum		.68	
	Maximum		2.53	
	Range		1.85	
	Interquartile Range		.36	
	Skewness		1.188	.241
	Kurtosis		2.603	.478

Low-density lipoprotein (LDL)

Descriptives				
			Statistic	Std. Error
Low-Density Lipoprotein	Mean		2.1337	.08044
	95% Confidence Interval for Mean	Lower Bound	1.9741	
		Upper Bound	2.2933	
	5% Trimmed Mean		2.0864	
	Median		2.0300	
	Variance		.647	
	Std. Deviation		.80436	
	Minimum		.61	
	Maximum		5.31	
	Range		4.70	
	Interquartile Range		.97	
	Skewness		1.036	.241
	Kurtosis		2.006	.478

Serum leptin level

Descriptives				
			Statistic	Std. Error
Serum Leptin	Mean		3.6642	.40264
	95% Confidence Interval for Mean	Lower Bound	2.8653	
		Upper Bound	4.4631	
	5% Trimmed Mean		3.1586	
	Median		2.3500	
	Variance		16.212	
	Std. Deviation		4.02639	
	Minimum		.20	
	Maximum		21.67	
	Range		21.47	
	Interquartile Range		2.93	
	Skewness		2.305	.241
	Kurtosis		5.902	.478

Appendix H: Descriptive statistic data of clinical parameter among obesity status

Serum leptin level

Descriptives					
	status_obese			Statistic	Std. Error
Serum Leptin	non-obese	Mean		2.0843	.33124
		95% Confidence Interval for Mean	Lower Bound	1.4163	
			Upper Bound	2.7523	
		5% Trimmed Mean		1.8001	
		Median		1.5300	
		Variance		4.828	
		Std. Deviation		2.19722	
		Minimum		.20	
		Maximum		12.54	
		Range		12.34	
		Interquartile Range		2.02	
		Skewness		2.858	.357
		Kurtosis		11.298	.702
	obese	Mean		6.5255	1.21943
		95% Confidence Interval for Mean	Lower Bound	3.9895	
			Upper Bound	9.0614	
		5% Trimmed Mean		5.9915	
		Median		4.3600	
		Variance		32.714	
		Std. Deviation		5.71965	
		Minimum		1.13	
		Maximum		21.67	
		Range		20.54	
		Interquartile Range		6.67	
		Skewness		1.595	.491
		Kurtosis		1.870	.953

Total cholesterol level

Descriptives					
	status_obese			Statistic	Std. Error
Total cholesterol	non-obese	Mean		4.0607	.15295
		95% Confidence Interval for Mean	Lower Bound	3.7522	
			Upper Bound	4.3691	
		5% Trimmed Mean		4.0003	
		Median		3.9550	
		Variance		1.029	
		Std. Deviation		1.01454	
		Minimum		2.31	
		Maximum		7.41	
		Range		5.10	
		Interquartile Range		1.44	
		Skewness		.959	.357
		Kurtosis		1.547	.702
	obese	Mean		4.1027	.21634
		95% Confidence Interval for Mean	Lower Bound	3.6528	
			Upper Bound	4.5526	
		5% Trimmed Mean		4.0192	
		Median		3.9200	
		Variance		1.030	
		Std. Deviation		1.01471	
		Minimum		2.89	
		Maximum		6.89	
		Range		4.00	
		Interquartile Range		1.47	
		Skewness		1.211	.491
		Kurtosis		1.459	.953

Triglyceride level

Descriptives					
	status_obese			Statistic	Std. Error
Triglyveride	non-obese	Mean		1.2995	.07252
		95% Confidence Interval for Mean	Lower Bound	1.1533	
			Upper Bound	1.4458	
		5% Trimmed Mean		1.2706	
		Median		1.1950	
		Variance		.231	
		Std. Deviation		.48103	
		Minimum		.70	
		Maximum		2.43	
		Range		1.73	
		Interquartile Range		.66	
		Skewness		.875	.357
		Kurtosis		-.034	.702
	obese	Mean		1.5068	.11756
		95% Confidence Interval for Mean	Lower Bound	1.2623	
			Upper Bound	1.7513	
		5% Trimmed Mean		1.4794	
		Median		1.4400	
		Variance		.304	
		Std. Deviation		.55143	
		Minimum		.71	
		Maximum		2.82	
		Range		2.11	
		Interquartile Range		.62	
		Skewness		.912	.491
		Kurtosis		.409	.953

High density lipoprotein (HDL)

Descriptives					
	status_obese			Statistic	Std. Error
HDL	non-obese	Mean		1.3170	.05249
		95% Confidence Interval for Mean	Lower Bound	1.2112	
			Upper Bound	1.4229	
		5% Trimmed Mean		1.2951	
		Median		1.2300	
		Variance		.121	
		Std. Deviation		.34818	
		Minimum		.68	
		Maximum		2.53	
		Range		1.85	
		Interquartile Range		.47	
		Skewness		1.151	.357
		Kurtosis		2.149	.702
	obese	Mean		1.1995	.03876
		95% Confidence Interval for Mean	Lower Bound	1.1189	
			Upper Bound	1.2802	
		5% Trimmed Mean		1.1978	
		Median		1.1850	
		Variance		.033	
		Std. Deviation		.18180	
		Minimum		.88	
		Maximum		1.54	
		Range		.66	
		Interquartile Range		.27	
		Skewness		.399	.491
		Kurtosis		-.432	.953

Low density lipoprotein (LDL)

Descriptives					
	status_obese			Statistic	Std. Error
LDL	non-obese	Mean		2.1575	.13607
		95% Confidence Interval for Mean	Lower Bound	1.8831	
			Upper Bound	2.4319	
		5% Trimmed Mean		2.1094	
		Median		2.1650	
		Variance		.815	
		Std. Deviation		.90257	
		Minimum		.61	
		Maximum		5.31	
		Range		4.70	
		Interquartile Range		1.04	
		Skewness		.954	.357
		Kurtosis		2.283	.702
	obese	Mean		2.2200	.18231
		95% Confidence Interval for Mean	Lower Bound	1.8409	
			Upper Bound	2.5991	
		5% Trimmed Mean		2.1510	
		Median		2.0100	
		Variance		.731	
		Std. Deviation		.85513	
		Minimum		1.21	
		Maximum		4.52	
		Range		3.31	
		Interquartile Range		1.07	
		Skewness		1.192	.491
		Kurtosis		1.343	.953

LDL:HDL ratio level

Descriptives					
	status_obese			Statistic	Std. Error
LDL:HDL Ratio	non-obese	Mean		1.7422	.12077
		95% Confidence Interval for Mean	Lower Bound	1.4987	
			Upper Bound	1.9858	
		5% Trimmed Mean		1.7047	
		Median		1.8095	
		Variance		.642	
		Std. Deviation		.80112	
		Minimum		.35	
		Maximum		3.85	
		Range		3.50	
		Interquartile Range		1.02	
		Skewness		.614	.357
		Kurtosis		.555	.702
	obese	Mean		1.9034	.17446
		95% Confidence Interval for Mean	Lower Bound	1.5406	
			Upper Bound	2.2663	
		5% Trimmed Mean		1.8519	
		Median		1.7738	
		Variance		.670	
		Std. Deviation		.81831	
		Minimum		.82	
		Maximum		3.91	
		Range		3.09	
		Interquartile Range		1.11	
		Skewness		1.104	.491
		Kurtosis		.849	.953

Blood pressure (BP) systolic level

Descriptives					
	status_obese			Statistic	Std. Error
BPsys	non-obese	Mean		144.73	3.824
		95% Confidence Interval for Mean	Lower Bound	137.02	
			Upper Bound	152.44	
		5% Trimmed Mean		144.12	
		Median		140.50	
		Variance		643.273	
		Std. Deviation		25.363	
		Minimum		90	
		Maximum		207	
		Range		117	
		Interquartile Range		33	
		Skewness		.532	.357
		Kurtosis		.205	.702
	Obese	Mean		139.64	3.308
		95% Confidence Interval for Mean	Lower Bound	132.76	
			Upper Bound	146.52	
		5% Trimmed Mean		139.94	
		Median		140.50	
		Variance		240.719	
		Std. Deviation		15.515	
		Minimum		109	
		Maximum		164	
		Range		55	
		Interquartile Range		26	
		Skewness		-.077	.491
		Kurtosis		-.928	.953

Blood pressure (BP) diastolic level

Descriptives					
	status_obese			Statistic	Std. Error
BPdias	non-obese	Mean		81.52	1.788
		95% Confidence Interval for Mean	Lower Bound	77.92	
			Upper Bound	85.13	
		5% Trimmed Mean		81.58	
		Median		82.50	
		Variance		140.627	
		Std. Deviation		11.859	
		Minimum		52	
		Maximum		110	
		Range		58	
		Interquartile Range		14	
		Skewness		-.041	.357
		Kurtosis		.288	.702
	Obese	Mean		85.23	2.173
		95% Confidence Interval for Mean	Lower Bound	80.71	
			Upper Bound	89.75	
		5% Trimmed Mean		85.21	
		Median		84.00	
		Variance		103.898	
		Std. Deviation		10.193	
		Minimum		66	
		Maximum		105	
		Range		39	
		Interquartile Range		18	
		Skewness		.046	.491
		Kurtosis		-.645	.953

Appendix I: Correlation statistic of between clinical parameter and obesity status

Test Statistics[a]								
	Serum Leptin	Total choles-terol	Trigly -ceride	HDL	LDL	LDL: HDL ratio	BPsys	BPdias
Mann-Whitney U	152.0	483.5	363.5	398.5	465.5	442.0	446.0	392.0
Wilcoxon W	1142.0	1473.5	1353.5	651.5	1455.5	1432.0	699.0	1382.0
Z	-4.516	-.007	-1.639	-1.163	-.252	-.571	-.517	-1.253
Asymp. Sig. (2-tailed)	.000	.995	.101	.245	.801	.568	.605	.210
a. Grouping Variable: status_obese								

Appendix J: Statistic data of correlation between serum lipid profile parameter and others clinical parameter among non-obese patients

			Leptin	BMI	BPSys	BPDias	Age
Spearman's rho	LDL	Correlation Coefficient	.138	.062	.274	.024	.188
		Sig. (2-tailed)	.373	.690	.072	.875	.221
		N	44	44	44	44	44
	HDL	Correlation Coefficient	-.135	-.314*	.082	-.080	.142
		Sig. (2-tailed)	.381	.038	.596	.605	.359
		N	44	44	44	44	44
	Triglyceride	Correlation Coefficient	.263	.229	.072	.086	-.136
		Sig. (2-tailed)	.085	.135	.640	.581	.378
		N	44	44	44	44	44
	Total cholesterol	Correlation Coefficient	.123	.008	.241	-.006	.175
		Sig. (2-tailed)	.426	.961	.115	.971	.255
		N	44	44	44	44	44
	LDL:HDL ratio	Correlation Coefficient	.180	.228	.229	.102	.011
		Sig. (2-tailed)	.243	.137	.135	.510	.943
		N	44	44	44	44	44

Appendix K: Statistic data of correlation between serum lipid profile parameter and others clinical parameter among obese patients

			Leptin	BMI	BPsys	BPdias	Age
Spearman's Rho	LDL	Correlation Coefficient	-.164	-.443*	.015	-.221	.066
		Sig. (2-tailed)	.466	.039	.947	.322	.772
		N	22	22	22	22	22
	HDL	Correlation Coefficient	.407	-.175	-.067	.236	-.004
		Sig. (2-tailed)	.060	.435	.767	.290	.987
		N	22	22	22	22	22
	Triglyceride	Correlation Coefficient	.076	.162	.207	-.250	.366
		Sig. (2-tailed)	.736	.471	.356	.261	.094
		N	22	22	22	22	22
	Total cholesterol	Correlation Coefficient	.060	-.370	.097	-.154	.111
		Sig. (2-tailed)	.791	.090	.669	.494	.624
		N	22	22	22	22	22
	LDL:HDL ratio	Correlation Coefficient	-.234	-.252	.085	-.227	.062
		Sig. (2-tailed)	.294	.258	.708	.309	.785
		N	22	22	22	22	22

Appendix L: Descriptive data of serum leptin level among the number of artery disease

Descriptives					
	BIL ARTERI TERLIBAT			Statistic	Std. Error
Serum Leptin	1	Mean		3.4976	.63437
		95% Confidence Interval for Mean	Lower Bound	2.2191	
			Upper Bound	4.7760	
		5% Trimmed Mean		2.9538	
		Median		1.9200	
		Variance		18.109	
		Std. Deviation		4.25547	
		Minimum		.20	
		Maximum		21.67	
		Range		21.47	
		Interquartile Range		3.22	
		Skewness		2.452	.354
		Kurtosis		7.070	.695
	2	Mean		3.9310	.62229
		95% Confidence Interval for Mean	Lower Bound	2.6563	
			Upper Bound	5.2057	
		5% Trimmed Mean		3.5447	
		Median		2.9700	
		Variance		11.230	
		Std. Deviation		3.35113	
		Minimum		.94	
		Maximum		14.31	
		Range		13.37	
		Interquartile Range		1.71	
		Skewness		2.026	.434
		Kurtosis		3.572	.845

Appendix L continued

Descriptives					
	BIL ARTERI TERLIBAT			Statistic	Std. Error
Serum Leptin	3	Mean		3.6550	.86860
		95% Confidence Interval for Mean	Lower Bound	1.8661	
			Upper Bound	5.4439	
		5% Trimmed Mean		3.0557	
		Median		1.8000	
		Variance		19.616	
		Std. Deviation		4.42903	
		Minimum		.20	
		Maximum		19.67	
		Range		19.47	
		Interquartile Range		3.38	
		Skewness		2.407	.456
		Kurtosis		6.408	.887

Appendix M: Descriptive data between serum leptin level and the number of artery disease among non-diabetes mellitus (DM) patients

Descriptives					
	BIL ARTERI TERLIBAT			Statistic	Std. Error
Serum Leptin	1	Mean		2.9496	.66689
		95% Confidence Interval for Mean	Lower Bound	1.5813	
			Upper Bound	4.3180	
		5% Trimmed Mean		2.6040	
		Median		1.5150	
		Variance		12.453	
		Std. Deviation		3.52887	
		Minimum		.20	
		Maximum		12.37	
		Range		12.17	
		Interquartile Range		3.08	
		Skewness		1.675	.441
		Kurtosis		1.689	.858
	2	Mean		3.6679	.69042
		95% Confidence Interval for Mean	Lower Bound	2.2174	
			Upper Bound	5.1184	
		5% Trimmed Mean		3.3104	
		Median		2.7600	
		Variance		9.057	
		Std. Deviation		3.00949	
		Minimum		.94	
		Maximum		12.83	
		Range		11.89	
		Interquartile Range		1.71	
		Skewness		2.097	.524
		Kurtosis		4.345	1.014

Appendix M continued

Descriptives					
	BIL ARTERI TERLIBAT			Statistic	Std. Error
Serum leptin	3	Mean		3.1481	1.16320
		95% Confidence Interval for Mean	Lower Bound	.6688	
			Upper Bound	5.6274	
		5% Trimmed Mean		2.3890	
		Median		1.6000	
		Variance		21.649	
		Std. Deviation		4.65281	
		Minimum		.29	
		Maximum		19.67	
		Range		19.38	
		Interquartile Range		2.87	
		Skewness		3.346	.564
		Kurtosis		12.190	1.091

Appendix N: Descriptive data between serum leptin level and the number of artery disease among diabetes mellitus (DM) patients

Descriptives					
	BIL ARTERI TERLIBAT			Statistic	Std. Error
Serum Leptin	1	Mean		4.4000	1.26944
		95% Confidence Interval for Mean	Lower Bound	1.7089	
			Upper Bound	7.0911	
		5% Trimmed Mean		3.6656	
		Median		3.3000	
		Variance		27.395	
		Std. Deviation		5.23402	
		Minimum		.35	
		Maximum		21.67	
		Range		21.32	
		Interquartile Range		3.29	
		Skewness		2.696	.550
		Kurtosis		7.765	1.063
	2	Mean		4.4310	1.28063
		95% Confidence Interval for Mean	Lower Bound	1.5340	
			Upper Bound	7.3280	
		5% Trimmed Mean		4.0456	
		Median		3.2450	
		Variance		16.400	
		Std. Deviation		4.04970	
		Minimum		1.49	
		Maximum		14.31	
		Range		12.82	
		Interquartile Range		3.16	
		Skewness		2.035	.687
		Kurtosis		3.846	1.334

Appendix N continued

Descriptives					
	BIL ARTERI TERLIBAT			Statistic	Std. Error
Serum leptin	3	Mean		4.4660	1.31230
		95% Confidence Interval for Mean	Lower Bound	1.4974	
			Upper Bound	7.4346	
		5% Trimmed Mean		4.2900	
		Median		3.1450	
		Variance		17.221	
		Std. Deviation		4.14984	
		Minimum		.20	
		Maximum		11.90	
		Range		11.70	
		Interquartile Range		6.21	
		Skewness		1.003	.687
		Kurtosis		-.246	1.334

Appendix O: Statistic data of correlation between serum leptin level and others clinical parameter

			Serum Leptin
Spearman's rho	Serum Leptin	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	100
	Ldl	Correlation Coefficient	.065
		Sig. (2-tailed)	.522
		N	100
	Hdl	Correlation Coefficient	-.134
		Sig. (2-tailed)	.183
		N	100
	Tg	Correlation Coefficient	.318
		Sig. (2-tailed)	.001
		N	100
	Tc	Correlation Coefficient	.084
		Sig. (2-tailed)	.406
		N	100
	Ratio	Correlation Coefficient	.132
		Sig. (2-tailed)	.191
		N	100
	Bmi	Correlation Coefficient	.548
		Sig. (2-tailed)	.000
		N	100
	bpsys	Correlation Coefficient	.099
		Sig. (2-tailed)	.326
		N	100
	bpdias	Correlation Coefficient	.086
		Sig. (2-tailed)	.397
		N	100
	Umur	Correlation Coefficient	-.140
		Sig. (2-tailed)	.166
		N	100

Appendix P: Statistic data of correlation between serum leptin level and others clinical parameter among non-diabetes mellitus (DM) patients

			Serum Leptin
Spearman's rho	Serum Leptin	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	63
	Ldl	Correlation Coefficient	.072
		Sig. (2-tailed)	.577
		N	63
	Hdl	Correlation Coefficient	-.235
		Sig. (2-tailed)	.064
		N	63
	Tg	Correlation Coefficient	.439
		Sig. (2-tailed)	.000
		N	63
	tc	Correlation Coefficient	.110
		Sig. (2-tailed)	.389
		N	63
	ratio	Correlation Coefficient	.237
		Sig. (2-tailed)	.062
		N	63
	bmi	Correlation Coefficient	.615
		Sig. (2-tailed)	.000
		N	63
	bpsys	Correlation Coefficient	.030
		Sig. (2-tailed)	.818
		N	63
	bpdias	Correlation Coefficient	.163
		Sig. (2-tailed)	.202
		N	63
	Umur	Correlation Coefficient	-.308
		Sig. (2-tailed)	.014
		N	63

Appendix Q: Statistic data of correlation between serum leptin level and others clinical parameter among diabetes mellitus (DM) patients

			Serum Leptin
Spearman's rho	Serum Leptin	Correlation Coefficient	1.000
		Sig. (2-tailed)	.
		N	37
	Ldl	Correlation Coefficient	.161
		Sig. (2-tailed)	.342
		N	37
	Hdl	Correlation Coefficient	.064
		Sig. (2-tailed)	.706
		N	37
	Tg	Correlation Coefficient	.071
		Sig. (2-tailed)	.677
		N	37
	Tc	Correlation Coefficient	.128
		Sig. (2-tailed)	.451
		N	37
	Ratio	Correlation Coefficient	.060
		Sig. (2-tailed)	.724
		N	37
	Bmi	Correlation Coefficient	.419
		Sig. (2-tailed)	.010
		N	37
	Bpsys	Correlation Coefficient	.148
		Sig. (2-tailed)	.383
		N	37
	Bpdias	Correlation Coefficient	.113
		Sig. (2-tailed)	.505
		N	37
	Umur	Correlation Coefficient	.046
		Sig. (2-tailed)	.787
		N	37