EFFECTS OF BEE BREAD ON CARDIOVASCULAR PARAMETERS IN HIGH-FAT DIET-INDUCED OBESE RATS

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BBA	bee bread aqueous
BBE	bee bread ethanol 70%
BMI	body mass index
CAT	catalase
DPPH	1, 1-diphenyl-2-picryldrazyl
ELISA	enzyme-link immunosorbent assay
Eq	equivalent
$FeCI_3.6H_2O$	ferric chloride hexahydrate
$FeSO_4.7H_2O$	ferrous sulphate heptahydrate
FRAP	ferric reducing antioxidant power
GPx	glutathione peroxidase
HCL	hydrochloric acid
HDL	high-density lipoprotein
LCMS	liquid chromatography-mass spectrometry
LDL	low-density lipoprotein
MDA	malondialdehyde
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
ORO	Oil-Red O
OxLDL	Oxidised-low density lipoprotein
ROS	reactive oxygen species
SOD	superoxide dismutase
TG	triglyceride

KESAN ROTI LEBAH KE ATAS PARAMETER KARDIOVASKULAR DALAM TIKUS ARUHAN OBESITI SECARA DIET TINGGI LEMAK

ABSTRAK

Obesiti dikategorikan sebagai pengumpulan lemak yang berlebihan dalam badan yang membawa kepada risiko penyakit kardiovaskular seperti hiperlipidemia, hipertensi dan aterosklerosis. Roti lebah adalah produk semulajadi yang digunakan secara tradisi untuk mengekalkan dan meningkatkan taraf kesihatan. Walaubagaimanapun, peranannya sebagai pelindung terhadap parameter kardiovaskular dalam tikus aruhan obesiti secara diet tinggi lemak (HFD) masih belum diketahui. Objektif kajian ini adalah (1) untuk menilai dan menganalisa nutrien, fitokimia, dan aktiviti antioksidan roti lebah (*Heterotrigona itama*) dari tiga kawasan berlainan di Malaysia termasuk Kelantan, Selangor dan Perak (Kajian Fasa 1), (2) untuk menubuhkan model tikus aruhan obesiti dan menentukan dos roti lebah terbaik (Kajian Fasa 2), dan (3) untuk menentukan kesan roti lebah terhadap parameter kardiovaskular dalam tikus aruhan obesiti secara HFD (Kajian Fasa 3). Dalam kajian Fasa 2, dos roti lebah yang berlainan (0.5, 1.0, dan 1.5 g/kg/hari) telah diberikan kepada tikus aruhan obesiti secara HFD melalui oral gavaj selama 6 minggu. Dalam kajian Fasa 3, kesan perlindungan terhadap parameter kardiovaskular dari roti lebah (0.5 g/kg/hari) dan orlistat (10 mg/kg/hari) ditentukan dalam tikus aruhan obesiti secara HFD. Pada akhir eksperimen, tikus-tikus dikorbankan untuk menilai parameter kardiovaskular seperti indek obesiti Lee, pengambilan makanan dan kalori, profil lemak, indek adipositi dan aterogenik, status antioksidan, dan histologi arkus aorta, sel lemak dan miokardium. Semua sampel roti lebah

mempunyai nutrien, fitokimia dan aktiviti antioksidan yang tinggi. Kandungan fenolik dan flavonoid tertinggi didapati dari sampel roti lebah dari Perak dan aktiviti pengurangan radikal bebas tertinggi didapati dalam sampel roti lebah dari Selangor. Roti lebah pada dos 0.5 g/kg/hari dipilih sebagai dos terbaik kerana ia mengurangkan indek Lee obesiti, jumlah kolesterol, dan lipoprotein berkepadatan rendah (LDL) dalam tikus aruhan obesiti secara HFD. Roti lebah memberi kesan perlindungan terhadap risiko penyakit kardiovaskular kerana ia mengurangkan indek Lee obesiti, jumlah kolesterol, LDL, LDL teroksidasi, index aterogenik dan malondialdehid, serta meningkatkan aktiviti enzim antioksidan seperti superoksida dismutase dan glutation peroksidase secara signifikan dalam tisu aorta. Roti lebah juga mengurangkan aktiviti enzim asid lemak sintase, saiz sel-sel lemak dan kawasan nekrosis dalam tisu miokardium berbanding kumpulan obesiti. Tiada plak aterosklerosis didapati pada segmen arkus aorta dalam tikus yang diberi roti lebah. Kajian ini menunjukkan bahawa pemberian roti lebah pada dos 0.5 g/kg/hari selama 6 minggu mempunyai kesan perlindungan terhadap obesiti, hiperlipidemia, paras oksidatif dan pembentukan plak aterosklerosis mungkin dengan cara meningkatkan aktiviti enzim antioksidan dalam tikus aruhan obesiti secara HFD. Kesan-kesan ini boleh dikaitkan dengan kehadiran sebatian fitokimia seperti apigenin, asid kafein, asid ferulik, isorhamnetin and kaempferol. Penemuan ini mungkin menunjukkan potensi penggunaan roti lebah dalam mengurangkan risiko penyakit kardiovaskular yang memerlukan kajian lanjut untuk menentukan mekanisme molekulnya yang tepat.

EFFECTS OF BEE BREADS ON CARDIOVASCULAR PARAMETERS IN HIGH-FAT DIET-INDUCED OBESE RATS

ABSTRACT

Obesity is characterised by excessive fat accumulation in the body thereby contributing to cardiovascular disease particularly hyperlipidaemia, hypertension and atherosclerosis. Bee bread is a natural product used traditionally to maintain and improve general health. However, its possible protective effect on cardiovascular parameters in high-fat diet (HFD) induced obese rats has not yet established. The objectives of this study were (1) to evaluate and analyse nutritional, phytochemical and antioxidant activity of bee bread of stingless bee (*Heterotrigona itama*) from three different regions in Malaysia namely Kelantan, Selangor and Perak (Phase 1 Study), (2) to establish animal model of obesity and to determine the best dose of bee bread (Phase 2 Study), and (3) to determine the effects of bee bread on cardiovascular parameters in HFD induced obese rats (Phase 3 Study). In Phase 2 study, different doses of bee bread (0.5, 1.0 and 1.5 g/kg/day) were administered to HFD-induced obese rats via oral gavage for 6 week. In Phase 3 study, cardioprotective and antioxidative effects of bee bread (0.5 g/kg/day) and orlistat (10 mg/kg/day) were determined in HFD-induced obese rats. At the end of the experiment, rats were sacrificed to assess cardiovascular parameters such as Lee obesity index, food and calorie intake, lipid profiles, adiposity and atherogenic index, antioxidative status, and histology of aortic arch, adipose tissue and myocardium. All bee bread samples had relatively high nutritional value, amino acids, phytochemical and *in-vitro* antioxidant activities. Highest total phenolic and flavonoid contents were found in bee bread sample from Perak whereby highest free radical scavenging

activity was found in sample from Selangor. Bee bread at the dose of 0.5 g/kg/day was chosen as the best dose as it reduced the Lee obesity index, total cholesterol, and low-density lipoprotein (LDL) in HFD-induced obese rats. Bee bread exerts protective effects against the risk of cardiovascular disease as it significantly reduced Lee obesity index and levels of total cholesterol, LDL, oxidised-LDL and malondialdehyde, as well as significantly increased the antioxidant activities such as superoxide dismutase and glutathione peroxidase in aortic tissues. Bee bread also significantly reduced fatty acid synthase activity, size of adipocytes and area of necrotic patch in myocardium compared to HFD group. There was an absence of atherosclerotic plaque in aortic arch segment in rats supplemented with bee bread. This study suggests that bee bread supplementation at 0.5 g/kg/day for 6 weeks significantly protects against obesity, hyperlipidaemia, oxidative stress and atherosclerotic plaque formation possibly by increasing antioxidant enzymes activities and decreasing fatty acid synthase activity in HFD-induced obese rats. These beneficial effects could be attributed to the presence of phytochemical compounds such as apigenin, caffeic acids, ferulic acids, isorhamnetin and kaempferol in bee bread. These findings may indicate the potential use of bee bread in reducing the risk of cardiovascular disease which needs further study to determine its exact molecular mechanism of action.

CHAPTER 1

INTRODUCTION

1.1 Background and justification of study

Obesity has been declared as global public health concern with approximately two-thirds of adults in the United States are classified as overweight, while one-third of the adults are identified as obese (Flegal et al., 2012). The global prevalence of obesity is projected to rise from 396 million in 2005 to 573 million in 2030 (Kelly et al., 2008). The complication of obesity has tremendously worrying as higher incidence of obesity-related complication involving cardiovascular system is reported especially coronary heart disease (Rossi et al., 2011). Besides, obesity has direct correlation with acute and chronic multisystem co-morbidities such as hypertension, dyslipidaemia, impaired glucose tolerance, type 2 diabetes mellitus, osteoarthritis, cerebrovascular disease and reproductive disease such as polycystic ovarian syndrome (Segula, 2014).

Mechanism of obesity can be divided into environmental and genetic factors. Environmental factors such as overconsumption of high-calorie diet and sedentary life activities have increased the positive energy balance thereby increasing weight gain in an individual. The discovery of monogenic genes of obesity has contributed to a new emerging research potential. Deficiency role of leptin and melanocortin-4 receptors have shown to impair regulation of energy homeostasis in which mutations of these genes were found in obese individual (Heymsfield and Wadden, 2017). Systemic oxidative stress is believed to contribute to deleterious effect of obesity mechanism and increase cardiovascular risk in obese individual (Keaney et al.,

2003). Excess of adipose tissue accumulation is found to have a metabolic role to adverse outcome of obesity. Adipose tissue is a precursor to pro-inflammatory cytokines production contributing to insulin resistance which is frequently present in obese individual (Balistreri et al., 2010).

Management of obesity is a kind of broad-spectrum area involving many organising healthcare setting. Lifestyle and dietary changes are the ultimate way of losing weight, however it needs high motivation and consistency in doing it (Adachi, 2005). Orlistat is a non-centrally anti-obesity drug of widely used and has shown to reduce weight gain, body mass index, lipid profiles, and inflammatory markers in obese individual after 6 months of treatment (Bougoulia et al., 2006). However, orlistat has shown to produce weight regain during second year of usage and some residual side effects such as gastrointestinal discomfort, faecal loss, and diarrhoea (Rossner et al., 2000; Teo et al., 2014). Rimonabant, an antiobesity drugs of centrally act is reported to decrease food-motivated behaviour and decrease weight gain in animal laboratory studies. However due to its potential effect of psychiatric disorders, Rimonabant has been withdrawn from the market in 2008 (Bellocchio et al., 2008). Another drug of commonly used is sibutramine, which is an appetite suppressant. It has been reported to exert tremendous side effects to cardiovascular system such as increases heart rate and blood pressure (Snow, 2005).

Despite of well-established diagnostic and therapeutic guidelines for the standard management and treatment of obesity targeting on excess adiposity, this serious public health challenges has dramatically increased possibly due to psychological factor (D'Argenio et al., 2009). The abundance of weight management programme is able to attract obese individual nowadays. However, healthcare providers are still encounter the rise in obesity trend in some situation that they are unable to control. Besides alternative diet plan and healthy lifestyles changes which are considered as self-control to an individual, researchers must construct current strategies as an adjunct option to obese individual to prevent the most serious complication of obesity especially involving cardiovascular system. These include hypertension, dyslipidaemia, atherosclerosis, congestive heart failure, and arrhythmia which can further lead to sudden death (Poirier et al., 2006).

A variety of natural products has been traditionally used as a step to prevent or treat obesity (Nimmi and George, 2017; Bais et al., 2014). The existence of high antioxidant activity of natural products such as *Tamarindus indica* (Azman et al., 2012), *Citrus aurandium* (Angelica and Fong, 2008), *Rhinacanthus nasutus* (Wannasiri et al., 2016), and *Gymnema sylvestre* (Kumar et al., 2012) have been shown to improve metabolic abnormalities such as lipid profile, blood glucose, insulin, leptin, adiponentin and reduced oxidative stress status in obese animal model. Moreover, bioactive compounds found in natural products specifically tocotrienols and resveratrol have been shown to improve cardiovascular parameters and function in high-fat diet induced rats (Wong et al., 2012; Meng et al., 2014). Bioactive compounds present in bee bread from Poland are mainly from phenol group such as p-coumaric acid, kaempherol and isorhamnetin (Isidorov et al., 2009).

These medicinal properties of natural products have also shown a significant improvement of morphological changes of myocardium tissues in obese animal model (Noratto et al., 2015) and rats treated with high-cholesterol diet (Suanarunsawat et al., 2010). Thus, herbal remedies are one of the potential approaches to meet the needs of consumer as a complementary approach to encounter the rise in obesity.

Bee bread is a natural product manufactured by bees made up from mixture of bee pollen and its digestive enzymes which is stored in bee hive. Anaerobic lactic acid fermentation process contributed to a greater nutritive value of bee bread (Zuluaga et al., 2015). It is interesting to note that study of antioxidant activity of bee bread has been published world-wide including Lithuania (Ceksteryte et al., 2006), Araucania (Duran et al., 2014), Poland (Sobral et al., 2017), Ukraine (Ivanisova et al., 2015), Romania (Cocan et al., 2009) and Georgia (Tavdidishvili et al., 2014). Total phenolic and flavonoid contents of bee bread are high (Duran et al., 2014) and this high antioxidant property is believed to contribute to medicinal properties of bee bread such as anti-tumour (Sobral et al., 2017), anti-microbial (Eswaran and Bhargava, 2014) and hepatoprotective (Ceksteryte and Balzekas, 2012) properties. Bee bread also has been shown to exert hypolipidaemic properties when combined with honey by significantly reducing total cholesterol by 15.7 % and LDL cholesterol by 20.5 % in overweight and obese patients (Kas'ianenko et al., 2011).

However, to date, no study has been reported to determine whether bee bread supplementation is able to exert cardioprotective effects in high-fat diet (HFD) induced obese rats. Therefore, general objective of this study was to determine the effects of bee bread on cardiovascular parameters in HFD-induced obese rats.

1.2 Specific objectives

1. To evaluate and compare the nutritional, phytochemical, and antioxidant properties of bee bread from three different regions in Malaysia namely Kelantan (east coast), Selangor (central region), and Perak (northern region), Malaysia (Phase 1 Study).
2. To establish animal model of obesity and to determine the best dose of bee bread in improving some cardiovascular parameters (Phase 2 Study).
3. To determine the effects of bee bread on Lee obesity index, weight gain, food and calorie intake, total fat pad, adiposity and atherogenic index, lipid profiles [total cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL)], and fatty acid synthase (FAS) in HFD-induced obese rats (Phase 3 Study).
4. To determine the presence of atherosclerotic plaque, histology of adipose tissue and myocardium in HFD-induced obese rats (Phase 3 Study).
5. To determine the effects of bee bread on aortic oxidative stress status such as oxidised-LDL (oxLDL), malondialdehyde (MDA), and activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in HFD-induced obese rats (Phase 3 Study).

1.3 Hypothesis

The hypothesis of the present study are as follows:

1. Bee bread samples from Kelantan, Selangor and Perak have high nutrition, phytochemical composition and antioxidant properties.
2. Bee bread significantly improves Lee obesity index, weight gain, food and calorie intake, total fat pad, adiposity and atherogenic index, lipid profiles (Total cholesterol, TG, LDL, and HDL), and FAS in HFD-induced obese rats.
3. Bee bread significantly reduces formation of atherosclerotic plaque and improves the histology of adipose tissue and myocardium in HFD-induced obese rats.
4. Bee bread significantly improves aortic tissue oxidative stress status (MDA, oxLDL) as well as antioxidant enzymes (SOD, GPx, and CAT) activities in HFD-induced obese rats.

1.4 Significance of study

Herbal remedies are still an infancy topic in Malaysia, however it is growing in interest among researchers world-wide. There is a need to develop an optional approach to the community at the stage of obesity prevention as classical management of obesity really required long-term efficacy. Thus, herbal remedies provide another approach which is affordable and with minimal side effects. The goal of this study is to provide a new scientific finding on the protective or improving effects of bee bread supplementation against the changes of some cardiovascular parameters in HFD-induced obese male rats.

With the evidence of positive findings in the present study, further studies may be carried out specifically to investigate on the molecular mechanism of bee bread as well as clinical trial on obese patients. Besides, this study also aims to promote Malaysian bee bread either locally or internationally.

CHAPTER 2

LITERATURE REVIEW

2.1 Obesity definition and classification

Obesity is a consequence of imbalance between an excess calorie intake and reduced physical activity. Generally obesity is defined as excess body fat, or operationally is determined as calculated body mass index (BMI) of equal or more than 30 kg/m^2. Body mass index is calculated by dividing an individual's weight in kilograms (kg) by the square of height in metres (m). Obesity can be classified into class I obesity (BMI 30.0 to 34.9 kg/m^2), class II obesity (BMI 35.0 to 39.9 kg/m^2), and class III or extreme obesity (BMI $\geq$ 40 kg/m^2) (WHO, 1995). As the anthropometric data are different in western and Asian countries, WHO has come out with redefining criteria of obesity among Asian population into class I obesity (BMI 25 to 29.9 kg/m^2) and class II obesity (BMI, $\geq$ 30 kg/m^2) (WHO, 2000). The BMI is the standard anthropometric most often used in adults and children. However, it does have its own limitation as it does not account for fat distribution, degree of muscularity and bone density (Prentice and Jebb, 2001).

Lee obesity index is widely used for determination of obesity in animal model as it represents the body fat accumulation in an individual rat (Angeloco et al., 2012). The excess of body fat in human has been defined as more than 25% in men and 35% in women. For human subjects, the waist-hip ratio has been a standard variable use to determine abdominal fat accumulation (WHO, 1995). The recommended cut-off points for waist-hip ratio in male and female are $\leq$0.90 cm and $\leq$0.85 cm, respectively (WHO, 2008).

2.1.1 Animal model of obesity

Animal models have been used on development of obesity by various methods. Massive obesity has been genetically developed by mutation of gene product such as leptin and neuropeptide Y. Despite of expensive cost, generation of obesity by spontaneous genetic mutation also shows a slow progress in understanding its energy regulation (Speakman et al., 2007). Other researchers use a different method which is costly effective such as by using high sucrose diet to develop model of obesity in rats as it increases total energy intake due to its high sugar content. The excess carbohydrate intake is converted for fat storage therefore increases the fat tissue accumulation. Administration of sucrose 300 g/L of water for 91 days has shown significant increases in BMI, gonadal fat and retroperitoneal fat weight compared to normal group (Malafaia et al., 2013). Induction of obesity by administration of high-fat diet is a classical method used widely by researchers. Administration of high fat diet for 4 weeks (Valcheva-Kuzmanova et al., 2007), 45 days (Dhandapani, 2007) and 12 weeks (Bahijri et al., 2017) by using different diet regimes have shown to establish successful models of obesity in animal.

2.1.2 Lee obesity index as determinants of obesity

Generally, there has been a variety of anthropometrical parameters to identify the obesity in rat including Lee obesity index, BMI, and rate of body weight gain (g). Lee index has been proven to estimate the body fat in rat fed with high-fat diet and it has been used world-wide in study related to animal model of obesity (Angeloco et al., 2012).

2.1.3 Prevalence

The prevalence of obesity has increased tragically recently. Obesity accelerates the prevalence of cardiovascular disease partly through the factors such as hypertension, dyslipidaemia, insulin resistance and glucose intolerance (Poirier et al., 2006). It has been a pandemic issue recently with over 115 million people suffering from obesity-related problem (WHO, 2000). Generally, the prevalence of obesity among adults has increased from 4.0 % in 1996 to 10.0 % in 2006, with male predominant (Nor et al., 2008). Obesity prevalence is higher in developing countries with cardiovascular disease as the leading cause of death by 2030 (Wild et al., 2004).

In 2008, it was calculated that 19.5% from a total of 4428 Malaysian adults were categorised as obese (Wan Mohamud et al., 2011). The prevalence of obesity among Malaysian was relatively higher in 1990 (38.3%) to 2013 (46.3%) with calculated changes of 20.9%. Apart from that, most of population in Southeast Asia countries had extremely increased in the prevalence of obesity based on the data reported by Asia Development Bank Institute (ADBI) from 1990 to 2013 including Brunei (17.7%), Indonesia (75.7%), Philippines (37.6%), Singapore (24.4%), and Thailand (73.1%) (Helble and Francisco, 2017). National Health and Nutrition Examination Survey had reported that the occurrence of obesity in 2011-2012 at United States was 34.9% which was equal to about one third of the nation (Ogden et al., 2014). Similar trend was also seen in China whereby the calculated obesity prevalence in adult male was 2.8 % in 2001 (Reynolds et al., 2007), rising up to 11.8 % in 2011 (Mi et al., 2015). It can be concluded that the trend of obesity prevalence is increasing world-wide. Such growing numbers have been a paradigm to the major health concern among researchers.

2.1.4 Predisposing factors

In some population, due to hectic lifestyle, fast food and take-away meals have always been an alternative pathway nowadays. Snack time and inappropriate time of having meal are also part of predisposing factors to obesity (McCullough et al., 2017). Current trends of food service are focusing on the larger portion of size per individual without considering its calculated calorie intake. Besides, the advance of advertisement has attracted the customer well to get into westernised diet (Wardle, 2007). Subsequently, this eating behaviour can contribute to excess calorie input in human body.

Physical inactivity may be a crucial factor than excess calorie intake in explaining high trends of obesity. Time spend on sitting and web surfing are examples of sedentary lifestyles which reduce the number of calories burned. Besides, advance research has shown that some individuals have tendency to store more fat thus gain weight through genetic predisposition factor (Jaaskelainen et al., 2013). The exact genetic distribution has been a famous factor with the discovery of obese gene and its gene product which contributes to an explosion in obesity research study area (Aouadi et al., 2013). Deficiency of the leptin receptors and heterozygous mutations in melanocortin-4 receptors recently have shown their role in regulating energy homeostasis, which are expressed mainly in the hypothalamus (Olza et al., 2017; Song et al., 2017).

2.1.5 Implication

Obesity is associated with negative outcomes in various aspects of life. Literature has documented negative outcome of obesity in social aspects related to education, employment, socio-economic status, healthcare, interpersonal relationships, and psychology health (Wee et al., 2014). However, the most difficult situation for obese individual is when it impacts body system which leads to higher morbidity and mortality rates. The impact of obesity on cardiovascular system is widely reported, which is associated with dyslipidemia, hypertension, atherosclerosis, abnormal coagulation profile, obstructive sleep apnoea and sudden death (Poirier et al., 2006).

2.1.6 Pathophysiological changes in obesity

2.1.6.1 Obesity and dyslipidaemia

The increased BMI is associated with increases in total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL), and decrease in high-density lipoprotein (HDL) (Marsh, 2003). In obese condition, the abdominal fat leads to overproduction of very low-density lipoprotein (VLDL), thus leads to an increase in TG in liver. Consequently, the lipolytic activities of adipocytes stimulate free fatty acid flux to the liver hence increase VLDL secretion. Together with several studies, such findings have led to the suggestion that insulin resistance play an important effect to the activity of lipoprotein lipase (Kim et al., 2001). This situation has resulted in reduced HDL production, and increased dense LDL particles due to alteration in VLDL metabolism which is also known as dyslipidaemia. Dyslipidaemia has been well established as cardiovascular risk factor referring to an increase in any of the following TC, TG, and LDL or decrease in HDL (Bays et al., 2007).

2.1.6.2 Obesity and atherosclerosis

Obese individuals are at high risk to develop cardiovascular disease directly or by its association with other known risk factors such as hypertension and diabetes (Jean et al., 2014). Several studies have reported that abnormal abdominal fat distribution may be a relevant determinant of cardiac risk (Arcaro et al., 1999). Atherosclerosis is one of the conditions occurs in obese adults as the effects of excessive fat distribution in obesity (De Michele et al., 2002). Atherosclerosis is a disease involving large and medium size arteries as a result of accumulation of fatty streaks with subsequent cascade of inflammatory process within wall of arteries. The pathophysiology of atherosclerosis among obese individual remains as a critical question to be explained. The inflammation process is classically reported as a factor in pathophysiology of this acute vascular syndrome (Tabas, 2010). The inflammatory plaque triggers the formation of thrombosis thus impairs blood flow and perfusion to distal arteries. Atherosclerosis gives major implications to circulatory system such as coronary artery disease and stroke. Obesity has been proven to influence the severity and extent of early atherosclerosis in adult men.

2.2 Adipose tissue: Morphology and Metabolism

2.2.1 Morphology

2.2.1(a) White adipose tissue

White adipose tissue plays an important role in fuel storage and for thermal and mechanical insulation. In obese individual, the sizes of adipocyte are ranged from 150 to 200 μm in diameter. The unique feature of adipocyte cell is related to its centrally located unilocular lipid droplet, surrounded by a thin layered cytoplasm and

a nucleus. It has less extensive vascular supply. The sympathetic innervation is linked to the plasma membrane of adipocyte (Cinti, 2001).

It is interesting to note that visceral fats in human are strongly linked to the disease associated with obesity. Generally, function of white adipose tissue is crucial when there is a need of energy flux, for example, during fasting, starvation, exercise, or as response to cold environment. It acts by releasing the fatty acid to the area needed for fuel regeneration. The anatomical arrangement of adipocytes which are close to one another and surrounded by extensive capillary network have providing a short distance for transport molecules or substrates in between adipocyte cell (Poirier et al., 2006). The finding of a hormone known as leptin, a key protein hormone released from adipocytes, has further classified the adipose tissue as an endocrine organ. The secretory and signalling role of white adipose tissue is further established when other researchers have found out the presence of lipoprotein lipase and adipokine which has contributed a big impact in research area involving the discovery of obesity (Trayhurn and Beattie, 2001).

Adipokines have variety of molecular structure and confers a dynamic physiological function with other organs which is responsible for lipid metabolism, blood pressure regulation, insulin sensitivity, inflammation and immunity, as well as energy balance (Rajala and Scherer, 2003). Therefore, adipose tissue is metabolically active organ that communicates with endocrine, paracrine, and autocrine systems as it is producing a large number of proteins including hormones, enzymes, and cytokines via signalling mechanism. It has a capability to stimulate, modulate, and inhibit the targeting molecules by different mechanism of action. Hence, it is

interesting to note that the anatomical and biochemical changes of adipose tissue are thought to play an important role in the morphological and functional changes of heart and circulatory system of body (Jung and Choi, 2014).

2.2.1(b) Brown adipose tissue

Brown adipose tissue is initially known as hibernating glands. The brown adipose tissue plays an important role in maintenance of body temperature in mammals by generating heat. It is interesting to note that the brown adipose tissue is more prominent in small mammalian individual. In neonate of human being, the brown adipose tissue is more concentrated at area of the neck and upper back. It is only present during the early postnatal life. The brown adipose tissue can be transformed into white adipose tissue over first few days and weeks of life (Lean et al., 1986). Histologically, the brown adipose tissue can be easily differentiated from white adipose tissue by the arrangement of lipid droplet in adipocyte. The brown adipose tissue has multiple lipid droplets (multilocular structure) surrounded by a thin cytoplasm and a nucleus. Besides, the brown adipose tissue is thermogenically active tissue consisting of numerous mitochondria which gives its cristae structure. It is innervated with sympathetic nervous system and more vascularised than white adipose tissue, and responsible for heat-generating mechanism (Cinti, 2001).

2.2.2 Adipose tissue metabolism

Adipocytes contribute greatly in generation of adenosine triphosphate by producing and breaking down lipids component for metabolism purposes. Adipose tissue especially white adipose tissue has the capability to store lipid in the form of triacylglycerol during postprandial period and meet the energy need to other organs.

After fat ingestion, fatty acids are bound to albumin as its protein transporter in the circulation, which generally known as non-esterified fatty acids. Fatty acids in adipose tissue coupled with coenzyme A in serial manner to form thioester and subsequently re-esterified to produce triacylglycerols. Triacylglycerols are then hydrolysed by hormone-sensitive lipase to form glycerol and free fatty acids (Yu and Ginsberg, 2005).

2.3 Lipid and lipoprotein metabolism

The overall mechanism of lipid and lipoprotein metabolism can be divided into exogenous and endogenous lipid pathways, followed by reverse cholesterol transport. Cholesterol and TG, both are hydrophobic in nature which are transported in the blood stream by a core full of fat called lipoprotein. Four major lipoproteins are classified as chylomicrons, VLDL, LDL, and HDL.

2.3.1 Exogenous lipid pathway

The major components of dietary lipids are derived from triglycerides and the remaining are cholesterol, phospholipids and free fatty acids. Subsequently after food ingestion, the dietary fats are reassembled into chylomicrons and further transported into the blood stream via capillaries. Chylomicron is a large structural protein with large lipid core at the centre and contains various types of apolipoprotein such as apolipoprotein B48, CII, CIII, AI, AII, AIV and E (Redgrave, 1966). The major role of chylomicron is to transport the dietary fats from intestinal lymphatic system into the blood stream to reach targeting organ. In muscle and adipose tissue for example, the chylomicron is further hydrolysed into fatty acids and glycerol by the action of lipoprotein lipase for the purpose of energy storage. The remaining cholesterol in

chylomicron remnants is transported to the liver through chylomicron receptors (Brahm and Hegele, 2015).

2.3.2 Endogenous lipid pathway

Endogenous metabolism refers to the formation of various lipoproteins in the liver by the presence of cholesterol remnants. The triglycerides and cholesterol ester are transformed into VLDL which mediated by apolipoprotein B-100. High-density lipoprotein donates parts of its apolipoprotein CII and E to the VLDL molecule to subsequently produce intermediate-density lipoprotein (IDL) which has higher concentration of cholesterol. The IDL is either circulated in the blood stream or being reuptake by the liver through the presence of apolipoprotein E. The circulating IDL is further hydrolysed by hepatic lipase to produce LDL through the release of more glycerol and fatty acids. The LDL molecule possesses large concentration of cholesterol and can be reuptaken by the liver and peripheral cells through the involvement of LDL receptor by the action of apolipoprotein B 100 (Sacks, 2015). In the context of cardiovascular disease, LDL acts as pro-atherogenic components through the process of LDL oxidation. It facilitates the inflammatory cascade, subsequently leading to cell damage and death (Fernandez-Sanchez et al., 2011).

2.3.3 Reverse cholesterol transport

Reverse cholesterol transport refers to the mechanism of removing the dense cholesterol to lesser particles from the circulation, thus reducing the total cholesterol content. HDL has anti-atherogenic properties by the act of special apolipoprotein presence on its surface predominantly apolipoprotein AI followed by apolipoprotein AII, and some other proteins. Formation of HDL involves a series of maturation

processes generated from nascent HDL molecule by involvement of lecithin-cholesterol acyltransferase. Matured HDL has the ability to bind free cholesterol into the lipid core and packages them to be reuptaken to the liver which is subsequently released from the circulation (Rothblat and Phillips, 2010).

2.3.4 Fatty acid synthase (FAS)

In normal conditions, FAS converts excess carbohydrate into fatty acids through serial of reaction sequences involving substrate loading, chain extension, β-oxidation, and chain termination. *De novo* fatty acid synthesis is highly active during embryogenesis and in fetal lungs, where fatty acids are used for the production of lung surfactant. In adults, FAS is highly sensitive and is regulated by both oestradiol and progesterone. It is used for *de novo* lipogenesis in which it catalyses the synthesis of palmitate acid, a long polyunsaturated chain (C16:0) from acetyl-CoA and malonyl-CoA (Rangan and Smith, 2002). FAS comprises of multi-enzyme system which is responsible to catalyse fatty acid synthesis. It consists of two identical 272 kDa polypeptides. The subunit consists of three N-terminal domains known as b-ketoacyl synthase, malonyl/acetyl transferase and dehydrase which are separated by a structural core from four C-terminal domains (enoyl reductase, b-ketoacyl reductase, acyl carrier protein and thios- terase). The biosynthesis of palmitic acid involves serial decarboxylative condensation processes in the presence of nicotinamide adenine dinucleotide phosphate (Smith et al., 2003). The pathophysiology of lipid and lipoprotein metabolism are summarised in Figure 2.1.

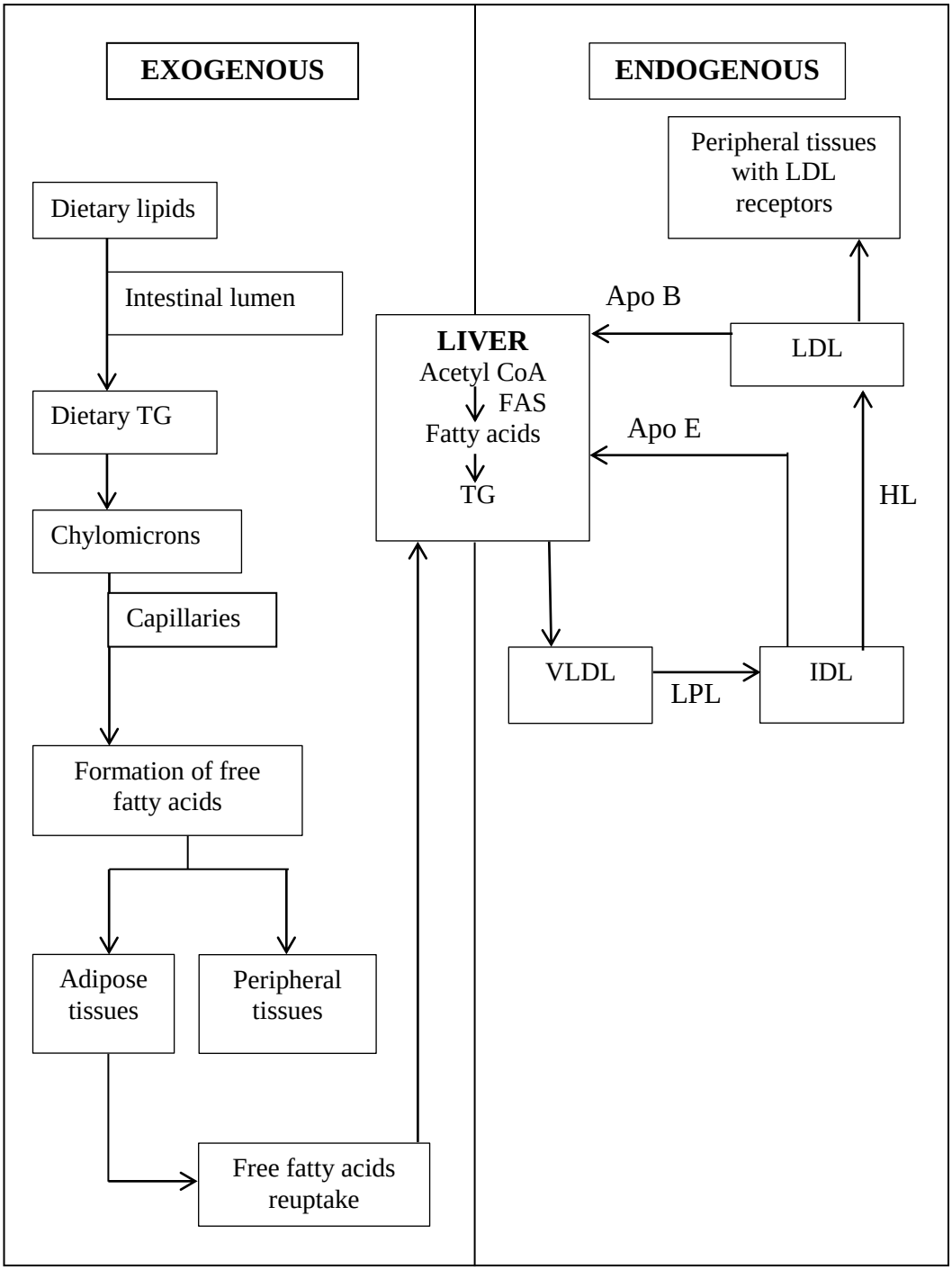

Figure 2.1 Summary of lipid and lipoprotein metabolism. TG, triglycerides;VLDL, very-low density lipoprotein; IDL, intermediate lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; FAS, fatty acid synthase; HL, hepatic lipase; Apo B, Apolipoprotein B; Apo E, Apolipoprotein E.

2.4 Free radicals and scavenging system

2.4.1 Free radicals and oxidative stress

Free radicals are atoms or groups of atoms which comprise of unpaired electron. They have a tendency to react with other free radicals and with a stable, doubled paired molecule. They have very short half-lives either in milli-, micro-, or nanoseconds. Biologically, free radical itself has its favourable role inclusive generation of adenosine triphosphate from adenosine diphosphate, detoxification process, apoptosis of defect cells, killing of organism and cancer cells, as well as generation of oxygenase to produce prostaglandin and leukotrienes. This regulated manner of free radicals is maintained by homeostasis system at cellular level via signalling molecule (Devasagayam et al., 2004).

Reactive oxygen species (ROS) include free radicals produced by oxidative enzymes such as superoxide anion radical and hydroxyl radical and non-radicals such as hydrogen peroxide, peroxyl radical, singlet oxygen, and ozone (Lakshmi et al., 2009). However, increase of ROS can insult the normal cell structure due to environmental stress. They can react with macromolecules including lipids, carbohydrates, proteins, DNAs and RNAs thus lead to intracellular damage and apoptosis (Kehrer and Klotz, 2015). The mechanisms of molecular damage of special target provoked by oxidative stress are as follows:

2.4.1(a) Lipid oxidation

Membrane lipids are more susceptible to peroxidation as polyunsaturated fatty acids which consist of double bonds are susceptible to free radicals reaction. Low homolytic dissociation energies are found to abstract the hydrogen atoms presence in lipid double bonds by affecting the ion channels and membrane transport protein. The action leads to destruction of lipid bilayer and cell rigidity (Szebeni et al., 1984). Previous study has reported the signalling effects of lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxy-2nonenal (HNE) which are primarily found in endothelial and smooth muscle cell destruction (Chapple et al., 2013; Yang et al., 2008).

2.4.1(b) Protein oxidation

Oxidation of amino acids has led to modifiable changes to its molecular structure including loss of sulfhydryl groups, formation of mixed disulfides or through oxidation of methionine residues to sulfoxides. Some of these structural alterations have attributed to production of aldehydes, hydroperoxides and caused ring cleavage in histidine residues thereby impair signalling properties of proteins (Spickett and Pitt, 2012).

2.4.1(c) Carbohydrate oxidation

Glucose undergoes several modification processes as a consequence of free radical attack such as by oxidation reaction and production of advanced glycation end products. Advanced glycation end products are reported to be involved in diabetes mellitus related complications (Ott et al., 2014).

2.4.1(d) DNA oxidation

Modification of DNA involves several mechanisms either oxidation process (by generation of 8-oxo-deoxyguanosine), adduction process (adduction of bases to lipid peroxidation product) or due to changes in transcription factor responsible for gene expression. Following DNA damage, the cell will lose its ability to produce adenosine triphosphate and lead to deleterious effect of cell death (Winczura et al., 2012).

2.4.1(e) RNA oxidation

RNA has important role in regulating genes and cell function particularly microRNAs. Modification of RNA usually involves base modification. Evidence of RNA oxidation is reported in several diseases including Alzheimer disease, diabetes mellitus and cardiovascular disease (Nunomura et al., 2009; Debard et al., 2004; Garnier et al., 2003). Following RNA modification, production of abnormal protein is increased as a result of misfolding thereby triggers apoptosis.

Oxidative stress has attributed to pathogenesis of many vascular related diseases including hyperlipidaemia, hypertension, atherosclerosis, diabetes mellitus and heart failure (Cai and Harrison, 2000). Several studies have proposed that oxidative stress is the causative mechanism which induces the inflammatory reaction as the result of formation of excessive fat tissue. Oxidative stress burden has provoked the lipid metabolism changes in heart and liver in rats fed with high-fat diet by significantly increases MDA and protein carbonyl level (Charradi et al., 2013). Some theories of mechanism of ROS production in obesity have been described previously. First factor is due to mitochondrial and peroxisomal oxidation process of fatty acids in which the ROS is directly produced by oxidation reaction. Secondly, it

is due to overconsumption of oxygen in which free radicals are generated in mitochondrial respiratory chain by coupling reaction with oxidative phosphorylation (Fernandez-Sanchez et al., 2011). Generally, sources of free radicals can be classified into reactive oxygen species and reactive nitrogen species.

2.4.2 Reactive oxygen species (ROS)

Oxygen-centered free radicals are the most common type found. ROS classically acts as second messenger of cellular process in tolerance to environmental stress such as lipid peroxidation, xenobiotics, cytokines, and microbial invasion (Shahidi and Zhong, 2010; Masella et al., 2005). Production of ROS is by stepwise reduction of molecular oxygen due to electron transfer reaction. Scavenging or eradication of excess ROS is achieved by effective role of antioxidant system either enzymatically or non-enzymatically system (Birben et al., 2012). Singlet ion is generated during lipid peroxidation process involving decomposition of lipid peroxides. It has a very short life-time (Weldor et al., 1999). Previous report has shown that singlet ion has a significant role in formation of nanoparticles in human keratinocytes and mediates the adverse effect of ultra-violet radiation (Fenoglio et al., 2013).

Superoxide, hydrogen peroxide, and hydroxyl radical are produced by biological system and have shown to actively participate with aerobic organism. Superoxide has been found to react with transition metals including sulphur, iron and other type of radical such as nitric oxide (Bielski and Cabelli, 1991). Hydrogen peroxide has a capability to penetrate cell membrane by diffusion method augmented by aquaporin, a water channel (Vieceli et al., 2014). It can receive an electron from transition metals like copper and ferrous resulting in generation of hydroxyl radical, a

highly reactive radical which has one nanosecond of half-life (Bielski and Cabelli, 1991). Other types of ROS includes peroxyl radical which is formed by oxidative damage of lipid and ozone that is usually present as atmospheric pollutant (Devasagayam et al., 2004).

2.4.3 Reactive nitrogen species (RNS)

Nitric oxide has a significant role as neurotransmitter and vasodilator by signalling the immune response (Yamawaki et al., 2017). It has potential to react with other radicals and results in damage of cellular function. For example, nitric oxide can react with superoxide and formed peroxynitrite which is a very strong type of RNS. Peroxynitrate causes oxidation of numerous biomolecules and alters signalling process which consequently leads to cell death (Pacher et al., 2007). Other types of RNS include peroxynitrous acid which is a protonated form of peroxynitrate and nitrogen dioxide which is formed during atmospheric pollutant (Coddington et al., 1999).

2.4.4 Oxidative stress and atherosclerosis

There are multiple sources of biochemical, physiological, and pharmacological studies regarding ROS which link toward cardiovascular diseases, either indirectly or directly affected by vascular risk factors such as hypertension, dyslipidaemia, diabetes, and smoking activity (Devasagayam et al., 2004). ROS mediate numerous signalling mechanisms for vascular inflammation which further lead to formation of atherosclerosis. ROS are produced in endothelium, smooth muscle and adventitia of the vessels (Lassegue and Clempus, 2003). Abundance of ROS affecting cardiovascular system specifically vascular wall are identified as hydrogen peroxide