EFFECT OF BEE BREAD ON CARDIOVASCULAR CHANGES IN OBESE MALE RATS

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EFFECT OF BEE BREAD ON CARDIOVASCULAR CHANGES IN OBESE MALE RATS

by

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LIST OF ABBREVIATIONS

AC	abdominal circumference
ACh	acetylcholine
AI	atherogenic index
ANOVA	analysis of variance
AUC	area under curve
Bax	βcl-2 associated X protein
Bcl2	β-cell lymphoma 2
BH_4	tetrahydrobiopterin
BMI	body mass index
CAT	catalase
CDNB	2,4-Dinitrochlorobenzene
CE	cholesterol ester
cGMP	cyclic guanosine monophosphate
CKMB	creatinine kinase muscle and brain
CRI	Castelli's risk index
CVD	cardiovascular disease
DAB	3,3′-Diaminobenzidine
dH2O	distilled water
DNA	deoxyribonucleic acid
DNPH	2,4-Dinitrophenylhydrazine
DPPH	2,2-diphenyl-1-picrylhydrazyl.
DTNB	5,5′-dithiobis(2-nitrobenzoic acid)
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay

eNOS	endothelial nitric oxide synthase
FADD	Fas-associated protein with death domain
FAS	fatty acid synthase
FAT	fatty acid transporter
FDA	Food and Drug Administration
FFA	free fatty acids
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GPx	glutathione peroxidase
GR	glutathione reductase
GSH	reduced glutathione
GSSG	oxidised glutathione
GST	glutathione-S transferase
GTP	guanosine triphosphate
HDL	high-density lipoprotein
HFD	high-fat diet
HOMA-IR	homeostatic model assessment for insulin resistance
HPLC	high-performance liquid chromatography
HRP	horseradish peroxidase
H&E	haematoxylin and Eosin
ICAM-1	intracellular cell adhesion molecule-1
IDL	intermediate-density lipoprotein
IKβα	inhibitor of kappa β
IKK	inhibitor of kappa β kinase
IL	interleukin
iNOS	inducible nitric oxide synthase
KCI	potassium chloride
KH_2PO_4	potassium dihydrogen phosphate

KHB	Krebs-Henseleit buffer
LB	lithium borate
LCAT	lecithin–cholesterol acyltransferase
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
M	molar
MCP-1	macrophage chemoattractant protein-1
MDA	malondialdehyde
$MgSO_4.7H_2O$	magnesium sulphate heptahydrate
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate
NaCI	sodium chloride
NaOH	sodium hydroxide
$NaHCO_3$	sodium bicarbonate
NBT	nitro blue tetrazolium
NFκβ	nuclear factor kappa β
NO	nitric oxide
NQO1	NADPH quinone oxidoreductase 1
Nrf2	nuclear factor erythroid 2-related factor 2
OD	optical densitometry
OGTT	oral glucose tolerance test
PBS	phosphate buffer saline
PCO	protein carbonyl
PCR	polymerase chain reaction
PE	phenylephrine
PON1	paraoxonase-1

PUFA	polyunsaturated fatty acids
ROS	reactive oxygen species
RT qPCR	real-time qualitative polymerase chain reaction
sGc	soluble guanylyl cyclase
SD	standard deviation
SDS	Sodium dodecyl sulphate solution
SOD	superoxide dismutase
TAC	total antioxidant capacity
TBA	2-Thiobarbituuric acid
TBARS	thiobarbituric acid-reactive substance
TC	thoracic circumference
TC	total cholesterol
TCA	trichloroacetic acid
TEP	tetraethoxypropane
TG	triglyceride
TNB	5-thio-2-nitrobenzoic acid
TNF-α	tumour necrosis factor-α
VCAM-1	vascular cell adhesion molecule-1
VSMC	vascular smooth muscle cell
VVG	Verhoef Van Gieson

LIST OF APPENDICES

Appendix A Animal ethics approval

Appendix B Determination of 3,3'-diaminobenzidine (DAB)-stained area and staining intensity using ImageJ software

Appendix C Representative of gel electrophoresis of extracted cardiac RNA samples

Appendix D List of primers, sizes and concentration used for PCR amplification of oxidative stress, inflammation, apoptosis, and lipid metabolism genes

KESAN ROTI LEBAH KE ATAS PERUBAHAN KARDIOVASKULAR DALAM TIKUS OBES JANTAN

ABSTRAK

Obesiti dan hiperlipidemia merupakan faktor utama yang menyebabkan penyakit kardiovaskular seperti kardiomiopati dan aterosklerosis. Roti lebah dilaporkan memiliki komposisi bioaktif dan menunjukkan aktiviti biologikal terhadap tekanan oksidatif, inflamasi, dan juga apoptosis dalam tisu buah pinggang dan testis. Walaubagaimanapun, kesan rawatan roti lebah terhadap perubahan kardiovaskular pada tikus obes masih belum dilaporkan. Oleh itu, objektif kajian ini adalah untuk menentukan (1) kandungan mineral, vitamin, dan asid lemak di dalam roti lebah. (2) kesan roti lebah ke atas parameter obesiti dan kardiometabolik juga progressi obesiti-kardiomiopati, (3) kesan roti lebah ke atas disfungsi endotelial dan progressi aterosklerosis menggunakan tikus obes *Sprague Dawley*. Empat puluh tikus obes yang mempunyai berat 180-230 g telah dibahagikan kepada dua kumpulan iaitu diet normal (kumpulan Normal, n = 10/kumpulan) dan diet tinggi lemak (kumpulan HFD, n = 30/kumpulan). Selepas enam minggu, tikus obes daripada kumpulan HFD diberi samaada air suling (kumpulan OB), roti lebah 0.5 g/kg/hari (kumpulan OB/BB), dan orlistat 10 mg/kg/hari (kumpulan OB/OR) untuk enam minggu seterusnya (n = 10/kumpulan). Roti lebah dan orlistat di larutkan dalam 1 mL air suling sebelum diberikan kepada tikus obes melalui oral gavaj. Pada akhir minggu ke-12, tikus telah dikorbankan untuk memperoleh serum, jantung, aorta dan tisu adipos. Roti lebah mempunyai pelbagai komponen mineral, vitamin, dan asid lemak. Roti lebah memperbaiki parameter obesiti dan kardiometabolik dalam tikus obes secara signifikan seperti indek Lee obesiti, jisim badan dan adipositi, ratio risiko kardiovaskular, profile lemak, dan tahap asid lemak bebas, gula, laktat

dehydrogenase, kreatinina kinase untuk otot dan otak, adiponektin, dan leptin. Secara serupa, roti lebah juga memperbaiki status oksidan-antioksidan, inflamasi, dan penanda apoptosis secara signifikan di dalam jantung dan aorta pada tikus obes. Roti lebah memperbaiki vasokenduran pada endotelial-dependen aruhan asetilkolina secara signifikan berkemungkinan melalui pengaktifan signal eNOS/NO/cGMP. Tambahan pula, terdapat pembaikian secara signifikan pada tahap asid lemak sintase di dalam jantung dan aorta tikus obes. Penemuan ini telah disokong oleh pembaikian pada perubahan histologi berkaitan obesiti pada jantung, aorta, dan tisu adipos pada tikus obes yang diberi intervensi roti lebah. Kajian ini menunjukkan kesan bermanfaat roti lebah dalam rawatan terhadap penyakit kardiovaskular berkaitan obesiti berkemungkinan melalui aktivitinya terhadap respon antioksidan, perencatan kaskad inflamasi dan juga sel apoptosis dalam jantung dan aorta dalam model tikus obes. Walaubagaimanapun, kajian lanjut diperlukan untuk menjelaskan mekanisma tindakbalas molekul roti lebah dan keselamatannya untuk digunakan sebagai rawatan adjuvant atau alternatif yang berpotensi kepada pesakit obes.

EFFECT OF BEE BREAD ON CARDIOVASCULAR CHANGES IN OBESE MALE RATS

ABSTRACT

Obesity and hyperlipidaemia are major risk factors for developing cardiovascular disease such as cardiomyopathy and atherosclerosis. Bee bread has been reported to contains bioactive compositions and exhibit some biological actions against oxidative stress, inflammation, as well as apoptosis in renal and testicular tissues. However, to date, the therapeutic effect of bee bread on cardiovascular changes in obese rats has not been reported. The objectives of this study therefore were to determine (1) the mineral, vitamin, and fatty acid contents of bee bread, (2) the effects of bee bread on obesity and cardiometabolic parameters as well as obesity-cardiomyopathy progression, (3) the effects of bee bread on endothelial dysfunction and atherosclerosis progression using obese *Sprague Dawley* rats. Forty male rats weighing 180-230 g were divided into two groups i.e., normal diet (Normal group, n = 10/group) and high-fat diet (HFD group, n = 30). After six weeks, obese rats in the HFD group were administered either with distilled water (OB group), 0.5 g/kg/day bee bread (OB/BB group), and 10 mg/kg/day orlistat (OB/OR group) for another six weeks (n = 10/group). Bee bread and orlistat were suspended in 1 mL distilled water before being administered to the obese rats via oral gavage. At the end of 12th weeks, the rats were sacrificed to obtain serum, heart, aorta, and adipose tissues. Bee bread possesses multi-components of minerals, vitamins, and fatty acids. Bee bread significantly improved obesity and cardiometabolic parameters in obese rats such as Lee obesity, body mass and adiposity indexes, cardiovascular risk ratios, lipid profile, and levels of free fatty acid, glucose, lactate dehydrogenase, creatinine kinase for muscle and brain, adiponectin, and leptin. Similarly, bee bread also

significantly improved oxidant-antioxidant status, inflammation and apoptosis markers in the heart and aorta of the obese rats. Bee bread significantly improved acetylcholine-induced endothelial-dependent vasorelaxation response possibly through activation of eNOS/NO/cGMP-signaling pathway. Moreover, there was a significant improvement in the level of fatty acid synthase in the heart and aorta of obese rats. The findings were further supported by the improvement of obesity-related histopathological changes in the heart, aorta and adipose tissues of obese rats treated with bee bread. The present study indicates that the beneficial effect of bee bread in treating obesity-related cardiovascular diseases could be through its action by mediating the antioxidant response, inhibiting the inflammatory cascades as well as cellular apoptosis in the heart and aorta of obese animal model. However, further studies are warranted to elucidate the exact molecular mechanisms of action of bee bread and its safety to be included as a potential adjuvant or alternative treatment in obese patients.

CHAPTER 1

INTRODUCTION

1.1 Background of study

In this 21st century, obesity has threatened the health system and quality of life among millions of people in all walks of life. Generally, obesity is described as an excess accumulation of stored fat in the body. Theoretically, overweight and obesity are described by the values of body mass index (BMI) of equal or more than 25kg/m^2 and 30 kg/m^2, respectively (Chew et al., 2014). Substantial evidence indicate that total consumption of dietary fat is the largest food-related contributing factor for developing excessive weight gain (Blundell and Cooling, 1999). The prevalence of obesity among Malaysian has exhibited an upward trend with 4.4% of the population were categorised as obese in 1996 and this continued to increase up to 12.2% in 2003, 14% in 2006, 15.1% in 2011, and 18.5% in 2014. Latest population-based survey in 2015 according to The National Health and Morbidity Surveys (NHMS) has come out with 19.1% of Malaysian had obesity that is equal to one fifth of the population (Zaki et al., 2018).

Obesity has implicated numerous diseases which are associated with the complications that arise due to prolong accumulation of fat in the body. These includes cardiovascular diseases (CVD)s such as atherosclerosis, myocardial infarction and hypertension, diabetes mellitus, stroke, metabolic syndrome, and certain forms of cancer which include oesophageal and colon cancers (Kinlen et al., 2018). The obesity progression caused great impact to the structural and functional status of the heart and aorta and often related to cardiomyopathy, endothelial

dysfunction, and atherosclerosis (Van Gaal et al., 2006; Hsieh et al., 2016). In another observational study, obesity is associated with greater risk of developing cardiovascular complications. For instance, 11% increase in cardiac failure cases in men while 14% increase in women attributed to cardiac lipotoxicity and dysfunction (Ebong et al., 2014). Obesity is also associated with the increases in the metabolic risks such as glucose imbalance, increased in the levels of insulin and lipids which further increased the prevalence of obesity-related complications (Bobbioni-Harsch et al., 2012).

The increased in the positive energy balance is believed to be a major contributing factor for development of body fat mass. The increment in the dietary fat intake has led to increase in the circulating fatty acids that further stored in the form of triacylglycerol in the tissues and fat cells (Díaz-Rúa et al., 2017). Obesity is also associated with the accumulation of higher fatty acids in the circulation which further increases the generation of isolated lipid fractions such as total cholesterol (TC), triglyceride (TG), and low-density lipoprotein (LDL) (Xu et al., 2016a). The increase in circulating lipids can also deposit in between the cell tissues and cause damage to the cell homeostasis system (Chtourou et al., 2015). The complication of obesity-related CVDs is also suggested to be due to oxidative stress in view of high production of free radicals which further dampen the antioxidant defence mechanism. It is reported that the imbalance of oxidant-antioxidant status in obese male rats has implicated in CVD risks such as endothelial dysfunction, atherosclerosis, cardiomyopathy, and heart failure (Neves et al., 2014; Martínez-martínez et al., 2016; Si et al., 2017). There is substantial evidence of association between the increased oxidative stress markers such as malondialdehyde (MDA) with the increased inflammatory markers of cytokines, chemokines and adhesion

molecules which can trigger macrophage migration and activation through stimulation of tumour necrosis factor-α (TNF-α), intercellular cell adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in the obese induce rats (Tang et al., 2021). How a cell can overcome oxidative stress is primarily dependent on the capacity in activating antioxidant response elements which is dominantly under the control of nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor (Han et al., 2019).

Excessive fatty acids in the adipose tissue and non-adipose tissues also play a major role in the activation of inflammatory cascade through the release of leptin, resistin, adiponectin and cytokines such as TNF-α, interleukin (IL)-1 and 6, and macrophage chemoattractant protein-1 (MCP-1) which can act on the immune cells leading to local and systemic inflammation (Balistreri et al., 2010). Few pathways are closely related to the cascade of inflammatory process including phosphatidylinositol-3'-kiase (PI3K)/ protein kinase B(Akt) and nuclear factor-kappa β (NF-κβ) signaling pathways (Sun et al., 2014; Ajala-Lawal et al., 2020). The increased in peroxidation products could also increase the risk of apoptotic cell death. This is supported by few studies which demonstrated significantly higher apoptotic cell death and damage in obese induce rats and mice models with involvement of Fas-dependent and mitochondrial dependent apoptotic pathways (Lee et al., 2007; Hsieh et al., 2016). Hence, macrophage activation which mediate the cascade of inflammatory response and apoptotic cell death could be the predominates in the pathogenesis of obesity-related cardiovascular tissue inflammation.

Bee bread has been used traditionally for maintaining general health. It possesses good antioxidant activity and contains bioactive ingredients comprising of polyphenols compounds that contribute to its antioxidant action (Ulusoy and Kolayli,

2014; Othman et al., 2020). Bee bread is a well-balanced food supplement as it contains carbohydrate, protein, fat, with considerable amount of minerals and vitamins in addition to significant quantities of active polyphenolic compounds. The presence of essential and non-essential amino acids is also demonstrated in the bee bread which is important for maintaining biological functions (Othman et al., 2019a). Bee bread has demonstrated good range of biological properties such as antimicrobial (Ivanišová et al., 2015), antifungal (Janashia et al., 2018), and antihyperlipidaemia (Kas'ianenko et al., 2011), antitumour (Sobral et al., 2017), as well as hepatoprotective properties (Čeksterytė et al., 2012). Previous study showed that administration of bee bread at 0.5 g/kg/day for 6 weeks significantly improved Lee obesity index, serum levels of TC and LDL, aortic oxidative stress markers such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) as well of histology of myocardium suggestive of some cardioprotective effects of bee bread against the risk of CVDs in HFD-induced obese rats (Othman et al., 2020). However, to date, it is not known whether bee bread may also have therapeutic effect on cardiovascular changes in obese male rats.

1.2 Justification and significant of study

The prevalence of obesity is in increasing globally and CVD is estimated to be the first leading cause of disease burden in 2020 (Khan et al., 2020). In the past decades, a number of obesity-induced animal models have been developed. Previous human study indicated that HFD contents of more than 30% energy from fat can easily induced obesity (Hill et al.., 2000). Sprague Dawley rats is a standard type of rodents used for HFD induce obesity due to its higher susceptibility to obesity development (Marques et al., 2016). Despite promoting adipogenesis, prolonged HFD

administration also caused several metabolic complications such as dyslipidaemia, hypertension, hyperglycaemia, insulin resistant and hyperleptinaemia in rats, thus mimicking the pathophysiology of human obesity (Brahmanaidu et al., 2014).

Several drugs have been used to treat obesity such as orlistat, sibutramine, phentermine, and diethylpropion that have been shown to reduce the BMI. However, some of the patients developed cardiovascular effects such as tachycardia, palpitation, and increased in the blood pressure after consumption of sibutramine and phentermine (Arias et al., 2009; James et al., 2010). Hence the usage of these drugs was withdrawn by Food and Drug Administration (FDA). In addition, other drug such as diethylpropion has also been shown to be effective in reducing BMI. However, the consumer was reported to develop few neurological effects such as dry mouth, constipation, and insomnia (Arias et al., 2009). Therefore, these drugs are not advice for the long-term usage. This illustrates some difficulties encountered throughout the process of anti-obesity drug development especially for long-term usage in the treatment of obesity. Orlistat is the most widely available prescription pharmaceutical drug for reducing body fat mass. Additionally, it has also concomitantly caused positive effect on the reduction of cardiometabolic risk factors such as blood pressure, lipid profiles, atherogenic index, insulin, and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) levels in the obese patients treated with orlistat (Al-Kuraishy and Al-Gareeb, 2016; Kujawska- Łuczak et al., 2018). Nevertheless, orlistat has also side effects such as abdominal bloating, increase flatulence, and diarrhoea (Lee and Dixon, 2017).

Hence, this has led an interest to assess the potential role of natural products in protecting and treating CVDs. Several experimental studies have demonstrated that supplementation of natural products containing active bioingredients have

reduced the peroxidation products and therefore reduced the obesity related complications in the obese animal models. For example, Morin which is found in natural products such as guava leaves and almond has been significantly shown to improve vascular endothelial dysfunction by reducing the body weight gain, Lee obesity index, lipid profile, and improving the endothelium-dependent vasorelaxation in HFD-induced obese rats (Madkhali, 2020). The antioxidant capacity of natural products was not only contributed by increasing the activities of enzymatic antioxidants, but also by the presence of non-enzymatic antioxidants such glutathione and vitamin C. This, in turn, is able to ameliorate the obesity related complications (Putakala et al., 2017). Previous study showed that administration of bee bread at 0.5 g/kg/day for 6 weeks significantly improved Lee obesity index, serum levels of TC and LDL, aortic oxidative stress markers such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) as well of histology of myocardium suggestive of some cardioprotective effects of bee bread against the risk of CVDs in HFD-induced obese rats (Othman et al., 2020).

However, to date, it is not known whether bee bread may also have therapeutic effect on cardiovascular changes in obese male rats. Moreover, the assessment on vitamins, minerals, and fatty acids in the bee bread which could contribute to enhancement of biological activities is also limited. Hence, in this study, bee bread was administered to the obese rats to explore its potential therapeutic effects on the cardiovascular changes in the obese rats emphasising on the oxidative stress, inflammation, and apoptosis pathways in addition to the evaluation on minerals, vitamins, and fatty acid contents in bee bread. The findings of this study may provide a new knowledge to further a clinical study on the potential use of bee bread as adjuvant therapy among obese patients. Besides, this study may

also provide as a new resource that could help the stakeholders to explore the new biological potential of bee bread as well as may help to increase the economic profits or income of the local beekeepers.

1.3 General objective

The aim of this study was to determine the effect of bee bread on cardiovascular changes in the obese rat model emphasising on oxidative stress, inflammation, and apoptosis pathways.

1.4 Specific objectives

1. To determine the composition of minerals, vitamins, and fatty acids in the bee bread.
2. To determine the effect of bee bread on body and nutritional compositions and serum metabolic profiles in obese male rats.
3. To determine the effect of bee bread on oxidant-antioxidants markers, pro- and anti-inflammatory markers as well as apoptotic markers in the cardiac and aortic tissues of obese male rats.
4. To determine the effect of bee bread on cardiac mRNA transcripts level of selected genes for oxidant-antioxidant, pro-and anti-inflammatory, as well as apoptosis markers in obese male rats.
5. To determine the effect of bee bread on adipocyte tissue and lipid metabolism profiles in obese male rats.
6. To determine the effect of bee bread on aortic vascular reactivity and histological changes of the heart and aorta in the obese male rats.

1.5 Hypothesis

Bee bread plays beneficial role in cardiovascular changes by attenuating the increased oxidative stress, inflammatory and apoptosis markers in the heart and aorta, and improving the aortic relaxation which could be attributed to the improvement in antioxidant status and lipid metabolism in the obese male rats.

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of obesity and its prevalence

Obesity has affected a large fraction of the population worldwide. Last decades, there was a spark of controversy whether should obesity be labelled as a disease. According to Scottish Intercollegiate Guideline, 'Obesity is defined as a disease process characterised by excessive body fat accumulation with multiple organ-specific consequences' (Logue, 2010). By years, numbers of formal organisations have finally recognised obesity as a disease pandemic such as The Obesity Society, The American College of Cardiology, The Society for Cardiovascular Angiography and Interventions, The American Association of Clinical Endocrinologists, The Endocrine Society, The American Academy of Family Physicians, The American College of Surgeons (Mechanick et al., 2013). To date, the World Health Organization, the Food and Drug Administration and the National Institutes of Health have also recognised obesity as a disease pandemic (Garvey et al., 2014; Garvey et al., 2016).

Defining obesity is non-arbitrary and not only by categorical and numerical itself. However, the criteria of obesity need a thorough standardised approach and regulations in order to develop an accurate diagnosis. There might be new consideration raise up and should be take into account. In a normal living adult, the healthcare workers are using weight and height values to calculate body mass index (BMI) as the indicator for adiposity mass. The scoring of BMI is further divided into five different classes i.e., normal range: 18.5-24.9 kg/m^2, overweight: 25-29.9 kg/m^2, Class 1 obesity: 30.0-34.9 kg/m^2, Class 2 obesity: 35.0-39.9 kg/m^2, Class 3 obesity:

equal or greater than 40 kg/m^2. Obese individuals from Class 2 and 3 are tend to develop significant higher-all cause obesity-related diseases which further classified as morbid obesity (Ashwell et al., 2014). Few other methodologies are used to measure obesity which include measurement of skinfold thickness, bioimpedance assessments and determination using Dual-energy X-ray absorptiometry (DXA) scan (Fakhrawi et al., 2009). These methods aim to measure the percentage of body fat mass. Skinfold thickness was measured at different sites of the body to obtain subcutaneous fat percentage. This method might be difficult for some operators and the results might be differ depending on the operator (Scherf et al., 1986).

Obesity prevalence has been doubled since 1980s and it is continuously expanding in most of the countries worldwide. In 2015-2016, the United States has declared their obesity prevalence has approaching 39.8 % that includes adults in the age range of 40-59 (42.8%), exceeding adults in age range of 20-39 (35.7%) meanwhile older aged adults accounted for 41% (Hales et al., 2017). However, the obesity prevalence is constantly increased to the level of 42.4% in the year 2017-2018, giving 40 % of younger adults aged 20-39, 44.8% of middle adults aged 40-59, and 42.8% of older adults aged 60 and above (Hales et al., 2020). These rates were extremely terrifying as the rate of obesity among all ranges of adults was on the rise.

In Malaysia, a cross sectional study involving 16, 127 Malaysian aged fifteen years and above were subjected to determine the obesity prevalence. The national prevalence of obesity reported as 11.7% with higher predominant in females compared to male participants (Rampal et al., 2007). The prevalence of obesity among adult Malaysians was found to be 19.5% in a study conducted from 2007 to 2008 which accounted for one fifth of the population (Wan Mohamud et al., 2011). In a similar methodology study, data were obtained from National Health and

Morbidity Survey (NHMS) 2015 involving the elderly population aged 60 years and above. The study reported that obesity prevalence was accounted for 30.2% with a higher predominance in the female gender (Ariaratnam et al., 2020). These data suggested an upward increasing trend in the prevalence of obesity among Malaysian irrespective of their age group.

2.2 Obesity – hallmark manifestation of cardiovascular disease

In developed countries, almost 5.5 million deaths were reported pertaining to coronary vascular disease, stroke, cancer, and diabetes. Despite that, ischemic heart disease is projected to be the leading cause of disease burden in 2020 and the prevalence rate is expected to exceed by the year 2030 (Khan et al., 2020). Obesity is the primary risk factor for cardiovascular morbidity and mortality in the presence of cardiovascular risk factors such as hypertension, dyslipidaemia, and abnormal glucose tolerance. The prevalence of cardiovascular disease (CVD) increased in a parallel manner with the increase in BMI. It has been previously found that increase in 5 kg/m^2 of BMI was associated with 40% of higher risk for ischemic heart disease mortality (Prospective Studies Collaboration, 2009).

The effect of obesity on cardiac structure and function has been reported in the previous study. Obesity causes increased stroke volume and cardiac output which eventually results in elevation of ventricular filling pressures and volume. These changes may further cause structural dilatation and left ventricular hypertrophy (Parto and Lavie, 2017). The Framingham study reported that increasing BMI is associated with increase in left ventricular thickness and mass which attributed to cardiac hypertrophy. In chronic long-standing condition, cardiac hypertrophy, and enlargement lead to subsequent cardiac failure (Lauer et al., 1991).

Obesity-related cardiovascular complications have received major attention in worldwide countries. In a cohort study involving Asian people, it is estimated that those who have BMI of 25-29.9 kg/m^2 also have the lowest risk of death. Casanueva et al., (2010) reported that 20% men and 12% women with BMI more than 30 mg/kg presented with CVD. Within the obese group, 47% of men and 43% of women have developed dyslipidaemia. In another report, nearly 70% of the deaths that were related to high BMI were due to CVD, and more than 60% of those deaths occurred among obese persons (Tabarés Seisdedos, 2017).

In a cross-sectional study involving 238 Malaysian aged 16 and above who lived in rural areas, obesity prevalence was reported as 49%. The authors also found a significant association between obesity and cardiovascular risks such as hypertension and hyperglycaemia (Cheah et al., 2011). In a study involved overweight and obese Malaysian subjects, hypertension and heart failure are the highest significant diseases burden related to obesity accounted for 31.7% and 31.6%, respectively (Peng et al., 2018). Generally, hyperlipidaemia, hyperglycaemia, hyperinsulinemia, hyperleptinemia, and increased pro-inflammatory markers were the proposed factors that lead to endoplasmic reticulum stress and mitochondrial dysfunction in obesity-related CVD. To a great extent, these cardiovascular risk factors cause structural and functional changes of the cardiovascular system and lead to cardiomyopathy, endothelial dysfunction, and atherosclerosis (Van Gaal et al., 2006). Atherosclerosis is one of the primary causes leading to myocardial infarction as well as stroke, and in severe cases can lead to sudden death (Joshi et al., 2015).

2.3 Animal model of obesity

Animal model for human disease has been widely used in conducting research and investigation for better understanding of the particular disease and also to evaluate the efficiency of newly developing therapeutic drug without risking the human being. Murine models are more preferable by many researchers as they are small and easy to be handled compared to larger animals such as rabbits, pigs, and monkeys even though these large animals are also closely related to humans. The normal anatomical and developmental events in murine models are remarkably similar to humans (Wessels and Sedmera, 2003). In addition, murine models are genetically reproducible which give an extra score to choose them as obesity models. Furthermore, the time for generation or inbred period is short (Plump et al., 1992).

Diet-induced model of obesity is the most commonly used method by researcher. The diet formula is richer in cholesterol or fat compositions compared to protein and carbohydrate, which can contribute to high calorie, therefore, helps to increase fat mass and lipid levels of the animal. Obesity was established in male Sprague Dawley rats after administration of high-fat diet (HFD) containing 60% fat, 20% protein, and 20% carbohydrate for 8 weeks followed by validation of a significant increase in leptin level compared to control (Si et al., 2017). Development of obesity characteristics was observed in Wistar rats fed with HFD contained predominantly 20% casein, 15% corn starch, 27.5% sucrose and added with vitamin mixtures for 40 days weeks which caused significant increase in Lee's index and adiposity level in association to derangement in lipid profile compared to control group (Kaveripakam et al., 2017).

Attempts were also conducted on chemically induced obesity research in rodents using gold thioglucose and monosodium glutamate. Two-weeks single

intraperitoneal injection of gold thioglucose at a range dose of 30-40 mg/kg was administered to adult mice as for induction of obesity (Hahm et al., 2002). Gold thioglucose injection is believed to produce necrotic lesion at ventromedial portion of the hypothalamus hence cause hyperphagia and subsequent development of obesity (Debons et al., 1977). A similar research attempt was also conducted on rats and mice using an injection of 2g/kg monosodium-L- glutamate for five consecutive days. This scheduled injection has been shown to induce damage to ventromedial hypothalamic and arcuate nuclei hence, lead to poor control between nutrient absorption, energy expenditure, and subsequent obesity development (Iwase et al., 1998). Few classes of drugs could also influence weight gain such as antidepressant and antipsychotic medications. The drugs are believed to interfere the central appetite-regulating neurotransmitter hence cause changes in energy expenditure (Wolden-Hansen et al., 1998). Advancement in technologies have provided various genetically modified murine models in multi research areas. This has become the most popular method in recent decades due to their genetic link which is easily susceptible to obesity progression and popularly described as monogenic or polygenic model. Mutation of single gene in monogenic method aims to target the damage of MC4R, LEP, LEPR, PCI and POMC genes through a leptinergic-melanocortinergic pathway. In the polygenic model, multiple genes are activated simultaneously to obtain the disease model. For instance, diffract coding regions of MC4R, FTO and INSIG2 are used to develop obesity in rodents (Hinney et al., 2010).

2.4 Management of obesity

Obesity management can be categorised into behavioural therapy, dietary therapy, physical therapy, pharmacotherapy, and surgical therapy.

2.4.1 Behavioural therapy

In the current setting of obesity management, behavioural treatment is the first-line approach. This involves few key components described as self-monitoring of eating, goal setting, stimulus control, problem solving and cognitive restructuring (Biswas et al., 2020). These components are derived in order to target maladaptive eating behaviours which are believed to be the domain cause of obesity. Self-monitoring activity includes the engagement of participants to record their meal intake including type, amount, and calorie contents as well as their physical activity and weight throughout the treatment (Forman et al., 2016). Studies have shown that participant who consistently keep their food records lose weight significantly compared to those who inconsistently doing it (Thomas and Wing, 2013). Stimulus-control applies the principle of creating a positive and conducive environment for healthy eating. Participants are advised to store food out of their sight and restrain from engaging with food while doing activities such as watching television or reading (Epstein et al., 2004). Problem solving skill teach on how to solve problem by creating behaviour chain and identifying possible solutions. Researchers suggested that by improving problem solving skill could also improve the weight loss. Cognitive restructuring method helps participants to monitor their thoughts and replace negative thinking to more rational responses. In order to prepare participants to effectively maintain weight loss in long term, participants are taught to perform strategies to deal with a high-risk situation such as how to deal with stress at work

and preparation of meals during a long vacation. This strategy is defined as relapse prevention which enables participants to navigate future situations more effectively (Perri et al., 2001).

2.4.2 Dietary and physical therapies

It is important to design a nutritionally balanced diet and create meals that contain less calories or energy depending on the individual's height, weight, age, sex, and activity level. Researchers have developed a few meal strategies for successful meal replacement diets such as portion-controlled diets, low-carbohydrate diets, low glycaemic and protein rich meal diet in order to produce desired weight loss (Halle et al., 2021). The aim of physical therapy is to increase energy expenditure hence increases the basal metabolic rate. Physical therapy intervention is believed to reduce the risk of several chronic diseases related to obesity, and improve emotional well-being, sleep, and self-confidence. This therapy includes light to moderate activities ranging from brisk walking, therapeutic gymnastic, aerobics, and breathing exercise (Kalmykova et al., 2018). For better treatment efficacy, the principle of behavioural techniques is applied in dietary and physical therapies. However, to achieve greater performance on behavioural, dietary, and physical therapies, the participant must also be assessed according to their flexibility, desired goal, and health status.

2.4.3 Pharmacological therapy

Pharmacological therapy can induce modest weight loss and significant improvement in obesity associated diseases. Few medications have been approved by FDA for the treatment of overweight and obese individuals. These include orlistat, phentermine and diethylpropion. Recent data on pharmacological studies have shown that orlistat

works by dose-dependent effect in fecal loss by inhibiting the breakdown and absorption of dietary fat. This potent inhibitor of gastropancreatic lipase works selectively and causes as maximum inhibition as 30% of fat from dietary meals. Gastrointestinal symptoms are common including oily spotting, flatulence, fecal incontinence, steatorrhea, and fecal urgency. Orlistat causes small but significant effect on fat absorbable vitamins and does not seem to affect absorption of drugs (Hauptman et al., 2001). Phentermine and diethylpropion are absorbed orally and acted by inhibiting dopamine and norepinephrine reuptake at nerve endings. Its common side effects include dry mouth, insomnia, and constipation (Arias et al., 2009). However, there is a consistent report following monotherapy of phentermine in which the patient had experienced undesirable cardiovascular side effects such as tachycardia, palpitation, increased blood pressure and ischemic event. Hence, phentermine is contraindicated in patients with underlying CVD (Cosentino et al., 2013). Sibutramine in the past has shown a promise for obesity treatment but it was eventually removed by the FDA after deleterious side effects among the consumers. Sibutramine is a selective inhibitor towards norepinephrine, serotonin and to a lesser degree, dopamine reuptake at the nerve endings. Although it causes significant loss of weight, sibutramine promotes increases in heart rate and blood pressure to the greatest, increase in incidence of myocardial infarction and stroke thus, the safety concern was issued (James et al., 2010). There are few drugs that were not awarded license by FDA during clinical trial phase namely lorcaserin and liraglutide due to behavioural side effects although they do show significant weight loss (Briand et al., 2020). This illustrates some difficulties encountered throughout the process of anti-obesity drug development especially for long term usage in the treatment of obesity.

2.4.4 Surgical therapy

Surgical treatment is increasingly important in severe obese individuals who have met the criteria of BMI equal or more than 40 kg/m^2, or with 35 kg/m^2 who have significant obesity-related comorbidity, provided that these groups have demonstrated dietary attempts which turn to be ineffective. The procedures include Roux-en-Y gastric bypass (RYGB) and biliopancreatic bypass, which induce weight loss by limiting gastric capacity and causing mild malabsorption. Laparoscopic gastric band, a common technique, is positioned around the uppermost portion of the stomach, hence restricting its capacity. The band is adjustable to allow tailoring of the gastric pouch size depending on an individual's need, and the weight is lost through restriction of meal volume (Snow, 2005).

2.4.5 Preventive measure of obesity

Habitual diet plays an important chain in the interplay between diet, genes, and obesity. The major driven to obesity occurrence was due to increased food availability and affordability in concordance to the increase in intense marketing of high calorie foods and sweet beverages. In this era of modernisation and urbanisation, there is an increase in lifestyle trend of urban workers who were more contained to gain ready-made processed food rather than freshly prepared meals. On the other hand, the build social and environment factors would also contribute to the reduction in opportunity for physical activity (Brantley et al., 2005). Researchers have proposed few alternatives in order to reduce the prevalence of obesity. Among such alternatives are prohibiting the advertisements on unhealthy foods to children, formulating and inventing healthy foods for school meals, charging the taxes for any expenditure of unhealthy foods, providing subsidies for consumption of healthy

foods, and granting incentives for mass production of healthy foods (Hawkes et al., 2015).

2.5 Complication of obesity

Complication of obesity can be divided into three important fields involving systemic, psychosocial, and economical factors. Obesity contributes to development of numbers of chronic diseases which lead to increased morbidity and mortality. Hyperlipidaemia, manifested as increases in total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL), is closely associated with obesity (Xu et al., 2016b), and in human, most lipid abnormalities are related to elevated TG and reduced HDL-cholesterol, particularly in central obesity (Walatara et al., 2016; Barzi et al., 2010). This is linked to the increase in hepatic synthesis of free fatty acids, hyperinsulinemia, and impaired lipoprotein lipase (Howard et al., 2003). Recently, there is an increase in epidemic proportion for the incidence of diabetes mellitus which is closely parallel to the increase in obesity prevalence (Ariaratnam et al., 2020). This association might attribute to increased insulin resistance. Some factors related to this increment are due to defect in insulin receptor and post-receptor in obese individuals hence impair the glucose transport, oxidation, and storage (Al-Goblan et al., 2014). Additionally, central obesity in the existence of insulin resistance subsequently may also results in alteration of lipid and glucose metabolism, which appear to be the basis for metabolic syndrome (Anderson et al., 2001). Individuals with metabolic syndrome manifest any 3 of 5 following features as defined according to the Asian populations: (1) waist circumference $\geq$ 90 cm for men and $\geq$ 80 for women, (2) TG $\geq$ 1.7 mmol/L (3) HDL $<$ 1.0 mmol/L for men and $<$ 1.3 mmol/L for women, (4) blood pressure above 130/85 mmHg, and (5) fasting

blood glucose ≥ 5.6 mmol/L (Alberti et al., 2009). There is also considerable evidence showing that obesity is closely related to the incidence of hypertension directly. The underlying pathogenesis would be related to chronic increases in sympathetic nervous system activation and renin-angiotensin-aldosterone system (RAAS), partly as consequence of blood volume expansion (Schütten et al., 2017). Moreover, a previous study showed that there is also a possible link between an increase in abdominal obesity with the increase in renal filtration, intra-renal pressure and endothelin-1 vasoconstrictor releasing factor (Schinzari et al., 2018).

It is now established that obesity is associated with an increased risk of death notably from cancer of stomach, oesophagus, liver, colorectum, kidney, breast, and gallbladder (Berrigan et al., 2014; Akinyemiju et al., 2018). Several hypotheses have been proposed for a pathological link between obesity and cancer. The most recent mechanism is related to the increased production of endogenous hormones like insulin-like growth factor (IGF-1), abnormal secretion of adipokines, as well as chronic inflammation and oxidative stress (Hopkins et al., 2016). Obese individuals who have developed insulin resistance and hyperglycaemia may accelerate the growth of tumour cells by providing a nutritional conducive environment for its mitotic activity. Carcinogenesis may also relate to an activation of PI3-kinase/Akt pathway in the presence of IGF-1 and insulin (Luey and May, 2016). Substantial studies have shown a close association between obesity and obstructive sleep apnoea. This could be due to the anatomical reason related to widening neck circumference which causes impingement and collapse of the airway (Kim et al., 2014). Additionally, obese individuals are prone to develop asthmatic symptoms. Hyper-responsiveness of airway decreased tidal volume and chronic systemic inflammation are considered as the mechanisms linking obesity and asthma (Bates et al., 2021).

Other systemic complications which appear to increase with obesity are gastroesophageal reflux disease, polycystic ovarian syndrome, and osteoarthritis (Segula, 2014). Instead of systemic illness, individuals with obesity are observed to develop untoward outcomes in the aspect of psychosocial and socioeconomic wellbeing. Following psychiatric evaluation, patients with obesity develop psychopathologies described as personality disorders, anxiety, and depression (Rajan and Menon, 2017).

2.6 Pathological crosstalk between obesity and hyperlipidaemia

Obesity is closely related to an imbalance in lipid metabolism. Hyperlipidaemia is defined as isolated elevation of total cholesterol (TC) concentration or isolated elevation of triglyceride concentration or both and may be associated with elevation of LDL cholesterol (De Costa and Park, 2017). Dyslipidaemia, a common phenomenon associated with obesity, is characterised as more than one of the following criteria i.e., increase shift of low-density lipoprotein (LDL) into pro-atherogenic type (small dense LDL), elevated triglyceride (TG), and decreased high-density lipoprotein (HDL) cholesterol levels (National Cholesterol Education Program (US), 2001). These lipid fractions are differentiated based on the presence of lipoproteins and their densities.

Constant and prolonged intake of HFD has led to increased adipocyte sizes. Subsequent to this, insulin resistance develops and enhances the release of more fatty acids into circulation. The circulating lipids tend to deposit into other organs such as heart, liver, muscle, and vessels. These fatty acids are carried by membrane lipid transporters known as fatty acid transporter protein (FAT/CD36), fatty acid binding protein of plasma membrane fraction as well as fatty acid transport protein 1,4, and 6

(Glatz et al., 2010; Wu et al., 2014). Abundance of lipid accumulation in the heart is an indicator of lipotoxic cardiomyopathy. Studies conducted have shed light on the reasons of increased lipid accumulation in the heart. Such work has highlighted the remarkable importance of human analogue fatty acid transporter (FAT/CD36), plasma membrane fatty acid binding protein, and fatty acid transport protein in carrying the fatty acids into cardiac cells which further being stored as myocardial triacylglycerol (Glatz et al., 2016). This in turn will be utilised for subsequent energy formation through mitochondrial β-oxidation (Kienesberger et al., 2013). This vicious cycle process can last longer in chronic form and cause cardiomyopathy which is highly associated with cardiac dysfunction (Fillmore et al., 2014).

High level of TG has been shown to be a strong predictive value for cardiovascular risk (Tenenbaum et al., 2014). The TG has a potential to be shifted into the production of small dense LDL-cholesterol and further contributed to higher pro-atherogenic impact toward atherosclerosis progression (Kannel and Vasan, 2009). LDL is easily permeable to the membrane and its accumulation can be found in the arterial wall through facilitating diffusion, lipoprotein influx, and binding to the arterial wall (Alexander et al., 1991). LDL easily accumulates at predilection sites with anatomical geometries like arches, branches, and bifurcations due to flow stagnation and prolonged adherence between blood contents and the vascular endothelial cells (Vickers et al., 2009). Many substantial data in the recent study indicates that apolipoproteins may play as an important risk marker in the development of CVDs other than lipoprotein fractions (Tognon et al., 2012). Moreover, a study found that obese individuals who developed higher concentrations of plasma apolipoprotein have decreased apolipoprotein ratio after an intervention with healthy lifestyle (Alberga et al., 2015). Additionally, HDL cholesterol level

exhibits a strong inverse relationship with the incidence of CVD. HDL-cholesterol not only provides its role in clearance of cholesterol pathway, but also contributes to prevention against oxidation, inflammation and thrombosis (Ansell et al., 2005).

Obese rats fed with HFD for 20 weeks cause a detrimental effect on lipid concentrations as evidenced by significant increases in serum TC, TG, LDL, very low-density lipoprotein (VLDL) and FFAs with increased concentration of heart fatty acid binding protein (Kilany et al., 2020). HFD containing 60% of fat for 9 weeks and 33.4% of fat for 4 weeks led to significant inclination in serum TC, TG, and LDL cholesterols (Lee et al., 2014; Tung et al., 2018). In addition, hyperlipidaemia and obesity are co-existed in rats fed with HFD for 8 weeks and led to increases in body weight gain, final weight, adipose tissue, and levels of TC, LDL/VLDL, TG cholesterols in association with low level of HDL-cholesterol (Son et al., 2020).

2.7 Lipid metabolism pathway

Basic chemistry and physiology of lipoproteins metabolism is the most crucial aspects in the diagnosis and treatment of obesity. Lipid metabolisms can be divided into exogenous lipoprotein pathway, endogenous lipoprotein pathway, and reverse cholesterol transport pathway. The role of fatty acid synthase (FAS) in the synthesis of polyunsaturated fatty acid (PUFA)s also contributes to the mechanistic understanding of the lipid metabolism pathway.

2.7.1 Exogenous lipoprotein pathway and chylomicrons formation

The primary site for the exogenous lipoprotein pathway is located in the intestine. This pathway works to transfer dietary fatty acids to muscle, heart and adipose tissue

for energy utilisation and storage. The dietary fat rich TGs are hydrolysed into FFA and monoacylglycerol by intestinal lipase and emulsified by bile acids to form micelles (Abumrad and Davidson, 2012). The TGs and cholesterol ester are packaged into chylomicron (CM)s in the endoplasmic reticulum and transferred to circulation through lymphatic system. CMs consist of predominantly neutral core lipids including TG (more than 90%), cholesterol ester, shell of amphipathic lipid and Apolipoprotein B48. CMs rich TG load are metabolised by lipoprotein lipase (LPL) which is abundantly found at endothelial capillary of major tissue organs to form fatty acids (Olivecrona, 2016). These fatty acids are taken up for tissue utilisation or being stored depending on the tissues need. After hydrolysis of CM-rich TG, the particle becomes lighter and smaller, forming a CM-remnant. This remnant particle is taken up by the liver and the TG remaining in the particle is packaged into very low-density lipoprotein (VLDL) and then released to circulation (Dallinga-Thie et al., 2010). Exogenous lipoprotein pathway and CM synthesis are summarised in Figure 2.1.

2.7.2 Endogenous lipoprotein pathway and VLDL metabolism

The cholesterols are delivered to the liver, then the liver packages the TG and cholesterol ester into a newly synthesised lipoprotein package known as VLDL via endogenous lipid pathway. This newly synthesised VLDL particle contains a lipid core of Apo B-100, which can also be found in its downstream hydrolysation lipoprotein particles notably intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL). The VLDL containing TG are hydrolysed by LPL and the released FAs are transferred into peripheral tissues. The remnants of VLDL are also known as IDL (Tiwari and Siddiqi, 2012). These IDL particles are removed from