STUDY OF OBESITY: METABOLITE PROFILING, GENE EXPRESSION AND GENOTYPING

ERIC CHONG TZYY JIANN

FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2018

STUDY OF OBESITY: METABOLITE PROFILING, GENE EXPRESSION AND GENOTYPING

ERIC CHONG TZYY JIANN

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
2018

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: **STUDY OF OBESITY: METABOLITE PROFILING, GENE EXPRESSION AND GENOTYPING**

IJAZAH: **DOKTOR FALSAFAH (BIOTEKNOLOGI)**

Saya **ERIC CHONG TZYY JIANN**, Sesi **2015-2018**, mengaku membenarkan tesis Doktoral ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis ini adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/):

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☐	TIDAK TERHAD	

Disahkan Oleh,

_____ _____

ERIC CHONG TZYY JIANN (Tandatangan Pustakawan)
DS1511004T

Tarikh : 08 Ogos 2018

_____

(Prof. Madya Dr. Lee Ping Chin)
Penyelia

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

08 August 2018

_____
Eric Chong Tzyy Jiann
DS1511004T

CERTIFICATION

NAME : **ERIC CHONG TZYY JIANN**

MATRIC NO. : **DS1511004T**

TITLE : **STUDY OF OBESITY: METABOLITE PROFILING, GENE EXPRESSION AND GENOTYPING**

DEGREE : **DOCTOR OF PHILOSOPHY (BIOTECHNOLOGY)**

VIVA DATE : **03 AUGUST 2018**

CERTIFIED BY

1. SUPERVISOR
Assoc. Prof. Dr. Lee Ping Chin Signature

ACKNOWLEDGEMENT

I would like to express my deepest gratitude and appreciation to my supervisor, Assoc. Prof. Dr. Lee Ping Chin for her advices, guidance and supports in this research work that lead to the completion of this thesis. Apart from that, experiences and skills shared by her were also helpful in solving problems encountered throughout this study. I am really grateful for having her as my supervisor throughout my doctoral study.

Besides that, I would like to thank the Ministry of Higher Education, Malaysia for selected me as one of the recipients for MyPhD scholarship under MyBrain15. In addition, financial supports from the Ministry of Higher Education, Malaysia through Translational Research Grant Scheme (TRGS0006-SG-2/2014) and from Universiti Malaysia Sabah through University Research Grant Scheme (SBK0180-SG-2014) are also greatly appreciated.

Furthermore, special thanks to the journal editors for their kindness in allowing me to reuse and reproduce the published contents in this thesis.

Last but not least, I would also like to express my sincere appreciation to my family members and all my friends for their supports while I am doing this study. Their caring and understanding encourage me to complete this study in time.

Thank you.

Eric Chong Tzyy Jiann
08 August 2018

ABSTRACT

Globally, there are 1.9 billion overweight (OW) adults and more than 650 million of them are obese (OB). In Malaysia, about 17.7% of the population are OB and more than 0.6 million OB adults are reported in East Malaysia. Worryingly, obesity is associated with several severe health disorders such as cardiovascular diseases, type 2 diabetes and cancers. Even though the aetiology of obesity is still not fully understood, an effective treatment to combat and reduce the seriousness of the obesity endemic is critically needed. Therefore, this study aims to utilize metabolite profiling, gene expression and genotyping approaches to further understand this particular perspective. Blood samples from 30 OB and 30 normal (NR) subjects were collected for blood biochemistry tests. Non-polar metabolites were extracted from 40 OB and 40 NR subjects, and profiled using a liquid chromatography-Orbitrap mass spectrometry for metabolite and pathway analyses. Total RNA was isolated from the peripheral blood of 30 OB and 30 NR subjects to investigate the expression of the genes in the involving pathway. Besides that, a total of 1030 blood samples were collected from age-matched subjects in the East Malaysian population for genotyping analysis. In this study, a higher mean of weight, body mass index, systolic blood pressure, haemoglobin concentration, red and white blood cell counts and triglyceride (TG) concentration was observed in the OB subjects. In metabolite profiling, most of the metabolites that were significantly up-regulated in the OB subjects were lipid species including ceramide, ceramide phosphoethanolamine, phosphatidylcholine (PC), TG, phosphatidylserine, and phosphoethanolamine. Pathway analysis was performed using the identified metabolites and this showed that the sphingomyelin-ceramide metabolic pathway in sphingolipid metabolism was highly impacted in the OB subjects. The two main genes that regulate this pathway are the sphingomyelin phosphodiesterase 1 (*SMPD1*) and the sphingomyelin synthase 2 (*SGMS2*) genes. Gene expression studies targeting the *SMPD1* and *SGMS2* genes revealed that the expression of the *SGMS2* was significantly down-regulated by 0.825-fold in the OB group. Among the 1030 subjects recruited for genotyping analysis, 21.6% were OW and 11.0% were OB. Genotyping of the rs4246445, rs2229425, rs2228305 and rs2229422 single nucleotide polymorphisms (SNPs) in the fatty acid synthase gene showed no significant evidence to associate these SNPs with the risk of being OW and obesity in the East Malaysian population, including in the haplotype analysis. In addition, these SNPs were demonstrated to be independent of each other in the linkage disequilibrium analysis. Interestingly, genotyping of the rs3751723 SNP in the iroquois homeobox 3 gene revealed that the variant G/G genotype was associated with the risk of obesity in the East Malaysian population, especially in subjects who did not consume fast food. Moreover, East Malaysian females who carried the variant G allele of this SNP also showed a higher risk of being OW but this allele had protective effects against being OW and OB among smokers in the population. A further assessment of this SNP using a meta-analysis revealed no significant links with the risk of obesity in all comparison models including the allelic, homozygous, heterozygous, dominant and recessive, but the meta-analysis concisely suggested that the variant G allele as a reduce risk factor for obesity overall. In conclusion, this is the first study to report that the expression of the *SGMS2* gene is inhibited in the OB subjects and consequently promoted the accumulation of the ceramide and PC metabolites, which was further validated in the metabolite profiling.

Interestingly, both blood biochemistry and metabolite profiling analyses also constantly observed an increased concentration of TG in the OB subjects. These findings provide an insight into obesity treatment. The genotyping data of this study can be beneficial in future personal- or population-based study designs in obesity management and prevention.

ABSTRAK

KAJIAN TERHADAP OBESITI: PEMPROFILAN METABOLIT, EKSPRESI GEN DAN PENGGENOTIPAN

Secara global, terdapat 1.9 bilion orang dewasa yang berlebihan berat badan (OW) dan lebih 650 juta daripada mereka adalah obes (OB). Di Malaysia, kira-kira 17.7% dalam populasi adalah OB dan lebih daripada 0.6 juta orang dewasa yang OB dilapor di Malaysia Timur. Lebih membimbangkan, obesiti dikaitkan dengan beberapa masalah kesihatan yang teruk seperti penyakit kardiovaskular, diabetes jenis 2 dan kanser. Walaupun etiologi bagi obesiti masih belum difahami sepenuhnya, rawatan yang berkesan dalam memerangi dan mengurangkan keseriusan endemik obesiti adalah sangat diperlukan. Oleh itu, kajian ini bertujuan untuk menggunakan pemprofilan metabolit, ekspresi gen dan penggenotipan untuk lebih memahami perspektif tertentu ini. Sampel darah telah dikumpul daripada 30 subjek OB and 30 subjek biasa (NR) bagi ujian darah biokimia. Metabolit-metabolit bukan polar telah diekstrak daripada 40 subjek OB dan 40 subjek NR, dan diprofil dengan menggunakan kromatografi cecair-spektrometri jisim Orbitrap untuk analisis metabolit dan laluan. Jumlah RNA diasingkan daripada darah periferal 30 subjek OB dan 30 subjek NR untuk mengaji ekspresi gen-gen dalam laluan yang terlibat. Selain itu, sejumlah 1030 sampel darah telah dikumpul daripada subjek-subjek yang dipadankan dengan usia dalam populasi Malaysia Timur untuk analisis penggenotipan. Dalam kajian ini, purata yang lebih tinggi bagi berat badan, indeks jisim badan, tekanan darah sistolik, kepekatan hemoglobin, bilangan sel darah merah dan darah putih dan kepekatan trigliserida (TG) diperhatikan bagi subjek-subjek OB. Dalam pemprofilan metabolit, kebanyakan metabolit yang meningkat secara signifikan bagi subjek-subjek OB adalah spesies lipid termasuk ceramida, ceramida fosfoetanolamin, fosfatidilkolin (PC), TG, fosfatidilserin dan fosfoetanolamin. Analisis laluan dilakukan dengan menggunakan metabolit-metabolit yang dikenalpasti dan ia menunjukkan bahawa laluan metabolik spingomielin-ceramida dalam metabolisme spingolipid sangat terkesan dalam subjek-subjek OB. Dua gen yang mengawal laluan ini adalah spingomielin fosfodiesterase 1 (SMPD1) dan spingomielin sintase 2 (SGMS2). Kajian ekspresi gen yang mensasarkan gen SMPD1 dan SGMS2 menunjukkan bahawa eskpresi SGMS2 telah menurun secara signifikan dengan 0.825-kali ganda bagi kumpulan OB. Di antara sejumlah 1030 subjek yang direkrut dalam analisis penggenotipan, 21.6% adalah OW dan 11.0% adalah OB. Penggenotipan rs4246445, rs2229425, rs2228305 dan rs2229422 polimorfisme nukleotida tunggal (SNP) dalam gen sintase asid lemak menunjukkan tiada bukti yang nyata untuk mengaitkan empat SNP tersebut dengan risiko OW dan obesiti dalam populasi Malaysia Timur, termasuk dalam analisis haplotip. Di samping itu, empat SNP tersebut menunjukkan bebas daripada satu sama lain dalam analisis ketidaksamaan hubungan. Yang menariknya, analisis penggenotipan bagi SNP rs3751723 dalam gen iroquois homeobox 3 menunjukkan bahawa genotip G/G dikaitkan dengan risiko obesiti dalam kalangan populasi Malaysia Timur, terutamanya bagi subjek yang tidak mengambil makanan segera. Selain itu, wanita-wanita Malaysia Timur yang membawa alel G bagi SNP ini juga menunjukkan risiko yang lebih tinggi terhadap OW tetapi alel ini melindungi daripada menjadi OW dan OB bagi perokok-perokok dalam populasi ini. Penilaian lanjut bagi SNP ini dengan menggunakan meta-analisis

tidak menunjukkan hubungan yang signifikan dengan risiko obesiti dalam semua model perbandingan termasuk alelik, homozigot, heterozigot, dominan dan resesif, tetapi meta-analisis ini menyarankan bahawa alel G adalah faktor pengurangan risiko terhadap obesiti secara keseluruhan. Kesimpulannya, kajian ini merupakan kajian pertama yang melaporkan bahawa ekspresi gen SGMS2 dihalang bagi subjek-subjek OB dan seterusnya menggalakkan pengumpulan metabolit-metabolit ceramida dan PC, yang juga telah disahkan dalam pemprofilan metabolit. Yang menariknya, kedua-dua analisis darah biokimia and pemprofilan metabolit sentiasa menunjukkan peningkatan TG bagi subjek-subjek OB. Penemuan-penemuan ini memberi petunjuk dalam rawatan obesiti. Data penggenotipan dalam kajian ini juga bermanfaat bagi reka bentuk kajian sama ada secara peribadi atau populasi pada masa depan dalam pengurusan dan pencegahan obesiti.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADA	-	American Diabetes Association
BMI	-	Body mass index
CI	-	Confidence interval
DG	-	Diacylglycerol
ECM	-	Extracellular matrix
FASN	-	Fatty acid synthase
FFAs	-	Free fatty acids
FTO	-	Fat mass and obesity-associated
GAPDH	-	Glyceraldehydes 3-phosphate dehydrogenase
GQN	-	Genomic quality number
GWAS	-	Genome wide association studies
HWE	-	Hardy-Weinberg equilibrium
IRX3	-	Iroquois homeobox 3
LC-MS	-	Liquid chromatography-mass spectrometry
LD	-	Linkage disequilibrium
MAF	-	Minor allele frequency
MASO	-	Malaysian Association for the Study of Obesity
NADPH	-	Nicotinamide adenine dinucleotide phosphate
NR	-	Normal
OB	-	Obese
OR	-	Odd ratio
OW	-	Overweight
PC	-	Phosphatidylcholine
PE	-	Phosphoethanolamine
PS	-	Phosphatidylserine
qPCR	-	Quantitative real-time polymerase chain reaction
R^2	-	Amplification correlation
RIN	-	RNA integrity number
ROS	-	Reactive oxygen species
RQ	-	Relative fold change
SGMS2	-	Sphingomyelin synthase 2
SMPD1	-	Sphingomyelin phosphodiesterase 1
SNPs	-	Single nucleotide polymorphisms
TG	-	Triglyceride
WHO	-	World Health Organization
XCMS	-	Various Forms of Chromatography Mass Spectrometry

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

An individual is defined as obese (OB) when his or her body mass index (BMI) is equal to or more than 30 kg/m^2. Obesity is currently a global health issue as more than 650 million adults are reported to be OB (World Health Organization, 2017). The trend of obesity is continuously increasing and it is projected that a total of 1.12 billion of adults in the world will be OB by the year 2030 (Kelly *et al.*, 2008). In Malaysia, the National Health and Morbidity Survey Report 2015 reported that the trend of the obesity is gradually rising since early 20th century and it is estimated that 3.31 million (17.7%) of the Malaysian population was OB in 2015 (Malaysian Institute for Public Health, 2015). In addition, the survey also revealed that more than 0.6 million adults (15.6%) are OB in East Malaysia (Malaysian Institute for Public Health, 2015). This unpromising condition should be monitored seriously since obesity has been associated with a number of life-threatening health disorders such as cardiovascular diseases, type 2 diabetes, cancers, arthritis and mental illness (Wolin *et al.*, 2010; Boffetta *et al.*, 2011; Chen *et al.*, 2013a; Aune *et al.*, 2014; Mannan *et al.*, 2016; Pérez-Pevida *et al.*, 2018). In fact, a recent study reported that the global mortality related to high BMI has escalated by an appalling 28.3% from a mortality rate of 41.9 per 100,000 individuals in 1990 to a mortality rate of 53.7 per 100,000 individuals in 2015 (Afshin *et al.*, 2017). Therefore, a profound understanding of the aetiology of obesity is undoubtedly essential in combating and reducing the obesity rate in the world's population. To date, the factors that contribute to the susceptibility of obesity remain debatable but they appear to involve both genetic and environmental aspects, either independently or by the interaction between them (Brantley *et al.*, 2005; Qi and Cho, 2008; Herrera *et al.*, 2011).

With the advancements in technology, biochemical changes in the body caused by a disease can be analyzed more readily, including the obesity endemic.

The liquid chromatography-mass spectrometry (LC-MS) approach is frequently used in metabolite profiling and pathway prediction in obesity research. Xie *et al*. (2012) has systematically compiled previous studies that comprehensively report the discoveries of potential metabolites and the related metabolic pathways in obesity research using the LC-MS method. However, most of these studies utilized blood and tissue samples from animal models. Studies that utilize peripheral blood samples in humans were rarely reported. Moreover, most of the studies did not further assess the gene expression profile of the metabolic pathways involved to further verify their results. Therefore, a study that integrates the metabolite profiling and gene expression analyses in obesity research is conspicuously needed for an insightful understanding of the obesity endemic.

DNA sequencing is another ideal method to identify the genetic variants that are linked to obesity. Fatty acids that are encoded by the fatty acid synthase (*FASN*) gene have been known to be associated with the development of obesity (Bray *et al*., 2002; Boden, 2008). Interestingly, sequencing of the *FASN* gene reveals several single nucleotide polymorphisms (SNPs) that are associated with the risk of obesity in Pima Indian and German populations, including the rs4246445, rs2229425, rs2228305 and rs2229422 SNPs (Kovacs *et al*., 2004; Schleinitz *et al*., 2010). However, the association of these SNPs with the risk of obesity in the Southeast Asian population, including the East Malaysian population, is yet to be determined.

Recently, obesity researchers were astonished by the discovery that the intron region of the fat mass and obesity-associated (*FTO*) gene is physically connected with the promoter region of the iroquois homeobox 3 (*IRX3*) gene, and this interaction is linked with the development of obesity. *FTO* has been previously reported to be an independent gene that causes obesity (Dina *et al*. 2007; Frayling *et al*., 2007; Scuteri *et al*. 2007; Smemo *et al*. 2014). Multi-locus linkage and haplotype analysis showed that the rs3751723 SNP in the promoter region of the *IRX3* gene was in perfect linkage disequilibrium with several SNPs in the intron region of the *FTO* gene and was significantly associated with the risk of obesity in the Pima Indian population (Srivastava *et al*., 2016). The finding highlights that the *IRX3* rs3751723 SNP is a potential predictable biomarker in the obesity endemic.

However, the association of this SNP with the risk of obesity in the East Malaysian population remains unclear and a study investigating this association is required.

1.2 Problem Statement

Despite many studies, blood biochemistry tests between the OB and normal subjects remain inconclusive. Besides that, metabolite profiling by utilizing peripheral blood samples in humans are rarely reported in obesity research. Moreover, the association of *FASN* and *IRX3* SNPs with the risk of overweight and obesity is unknown in the East Malaysian population.

1.3 Hypothesis

This study hypothesizes that there are significant blood biochemistry and metabolite differences between the OB and normal subjects. Metabolites that are up-regulated in the OB subjects could be used for pathway analysis and subsequently gene expression in the impacted pathway could be performed. In addition, this study postulates that the selected *FASN* and *IRX3* SNPs are significantly associated with the risk of being overweight and obesity in the East Malaysian population.

1.4 Objectives

The aims of this study are:

(i) To compare the blood biochemistry parameters between the OB and normal subjects.

(ii) To analyze the metabolites that are up-regulated in the OB subjects when compared to normal subjects in metabolite profiling.

(iii) To investigate the gene expression profile of the metabolic pathway involved in the OB subjects using real-time PCR.

(iv) To examine the association of the *FASN* rs4246445, rs2229425, rs2228305 and rs2229422 SNPs with the risk of being overweight and obesity.

(v) To examine the association of the *IRX3* rs3751723 SNP with the risk of being overweight and obesity.

CHAPTER 2

LITERATURE REVIEW

2.1 The Definition of Obesity

Obesity is defined as excessive fat accumulated in the body as a result of energy expenditure that is perceptibly less than energy consumption. There are many approaches to measure obesity including body mass index (BMI), waist circumference, waist-to-hip ratio, skin fold thicknesses and bioelectrical impedance. Among these measures, BMI is the most widely applied method in obesity measurement. BMI is calculated by dividing a person's weight in kilograms with the square of the person's height in meters (kg/m^2). According to the international classification reported by the World Health Organization (WHO) (Table 2.1), an individual is categorized as underweight when his or her BMI is less than 18.5 kg/m^2. In addition, an individual is considered as normal (NR) in weight when his or her BMI ranges from 18.5 to 24.9 kg/m^2. If the BMI is between 25.0 and 29.9 kg/m^2, the individual is overweight (OW) whereas an individual with a BMI of more than or equal to 30.0 kg/m^2 is classified as obese (OB) (WHO, 1995).

Table 2.1: International classification of BMI

BMI (kg/m^2)	WHO classification	Description
< 18.5	Underweight	Thin
18.5 – 24.9	Normal weight	Normal
25.0 – 29.9	Overweight (grade 1)	Overweight
30.0 – 39.9	Overweight (grade 2)	Obesity
≥ 40.0	Overweight (grade 3)	Morbid obesity

Source : World Health Organization, 1995

2.2 Global Obesity Statistics

Obesity is the leading public health problem worldwide. In 2016, WHO reported that the prevalence of obesity has tripled since 1975 as more than 1.9 billion adults

aged 18 years old and above were OW, and over 650 million of them were OB (WHO, 2017). In fact, the trend of the OW and obesity in adults is increasing alarmingly from 1980 to 2013 in both developed and developing countries (Figure 2.1) (Ng *et al.*, 2014). By 2030, the world's adult population is estimated to be a total of 2.16 billion OW and 1.12 billion OB individuals (Kelly *et al.*, 2008). In addition, this endemic also affecting younger individuals with more than 340 million children and adolescents aged between 5 and 19, and 41 million children under 5 years old reported to be OW or OB (WHO, 2017). Interestingly, the prevalence of obesity is correlated with an income level of the countries. For instance, obesity prevalence is roughly tripled in upper income countries when compared with low and middle income countries (WHO, 2018).

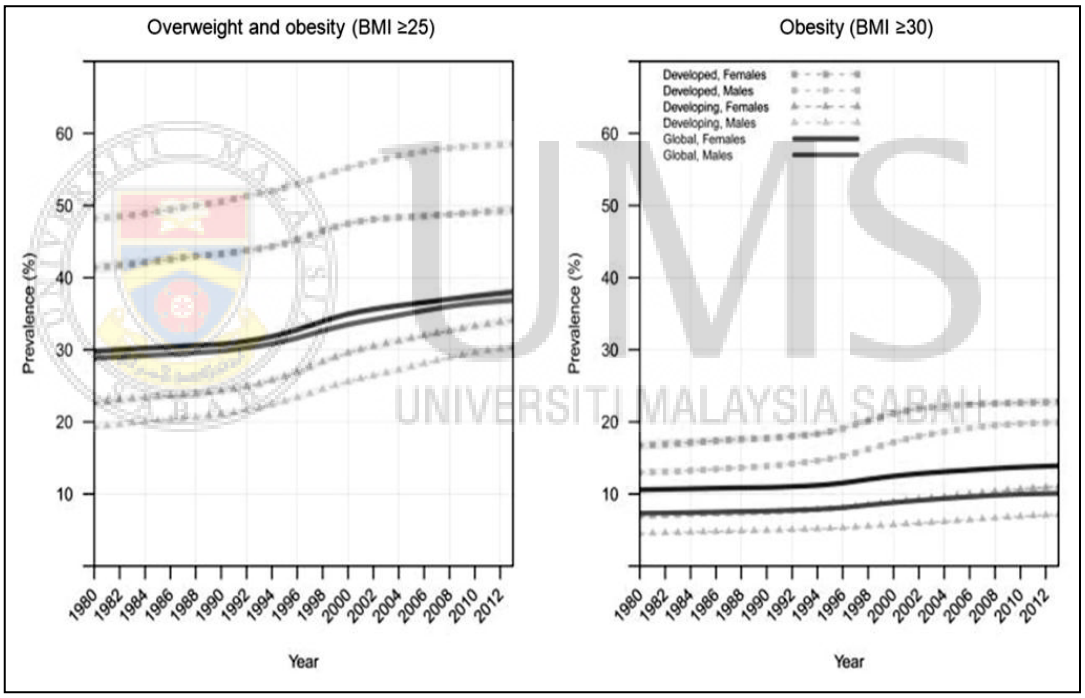

Figure 2.1 : Age-standardized prevalence of overweight/obesity and obesity in global adults from 1980 to 2013.

Source : Ng *et al.*, 2014

2.3 Obesity in Malaysia

Malaysia has been named as the top country with the highest number of OW and OB individuals among all the countries in Southeast Asia (Table 2.2) (WHO, 2011). Recent data from the National Health and Morbidity Survey Report 2015 revealed that nearly half (47.7%) of the Malaysian population are OW. The prevalence of OB

individuals in the population has continuously increased from 4.4% in 1996, 14.0% in 2006, 15.1% in 2011, to 17.7% in 2015 (Malaysian Institute for Public Health, 2015). In addition, studies reported from 1996 to 2015 also revealed a precarious situation of this endemic in which an increasing pattern of the obesity prevalence in the Malaysian population was observed (Figure 2.2) (Lim, 2016).

Table 2.2: Overweight and obesity prevalence in Southeast Asia

Country	Overweight and obesity (BMI ≥ 25 kg/m^2) prevalence rate
Malaysia	44.2%
Thailand	32.2%
Singapore	30.2%
Philippines	26.5%
Indonesia	21.0%
Myanmar	18.4%
Laos	13.3%
Cambodia	12.1%
Vietnam	10.2%

Source : World Health Organization, 2011

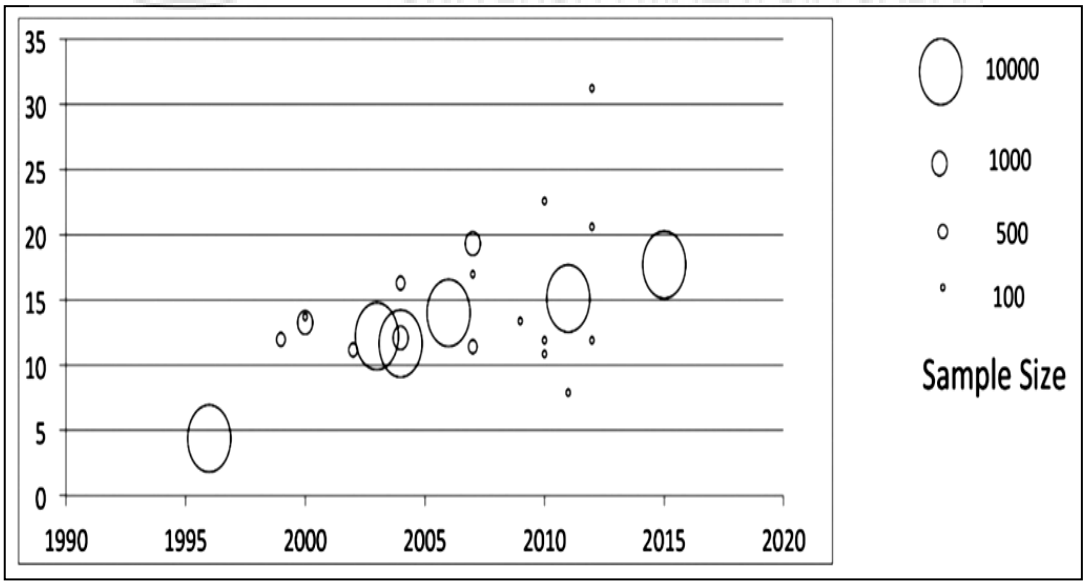

Figure 2.2 : Percentage of obesity prevalence in the Malaysian population in studies from 1996 to 2015.

Source : Lim, 2016

When stratified with the socio-demographic characteristics, more than 1.7 million (44.6%) adults are reported to be OW in East Malaysia and about 0.6 million (15.6%) of them are OB according to the National Health and Morbidity Survey Report 2015 (Malaysian Institute for Public Health, 2015). Interestingly, Malaysian females are more prevalent in obesity when compared with the Malaysian males. Obesity in the Malaysian population is more prevalent in age group of 35-64 years old and Indians (27.1%) are more OB than the Malays (21.1%) and Chinese (11.7%) in the country (Malaysian Institute for Public Health, 2015).

The Malaysian government is beginning to realize the seriousness of this condition in the country. Several efforts have been introduced to monitor the obesity rate in the country including a collaboration between the Ministry of Health and the Malaysian Association for the Study of Obesity (MASO) in 2004 to update the population's obesity rate every five years (MASO, 2005). Although various awareness programmes and guidelines for obesity prevention have been introduced, the obesity rate keeps on increasing with about 20% of the Malaysian population being OB today. This concerning situation may result in an increase in health risks in the Malaysian population as obesity has been associated with several chronic diseases which can lead to increased morbidity and mortality.

2.4 Obesity-Related Health Disorders

Obesity is a complex and multi-factorial disease which is greatly associated with a number of life-threatening health disorders including cardiovascular diseases, type 2 diabetes, cancers, and others.

2.4.1 Obesity and Cardiovascular Diseases

Obesity has been prospectively associated with the risk of cardiovascular diseases for a few decades. A study involving a total of 5881 subjects from the Framingham Heart Study revealed that each unit increment of BMI was correlated with an increase in heart disease risk of 7% for women and 5% for men (Després and Lemieux, 2006). The findings are supported by a cohort study involving 1.8 million subjects where obesity is associated with a 44% and 69% increased risk for coronary heart disease and stroke, respectively (Lu *et al.*, 2014). A recent pooled

analysis has also concluded that obesity is a strong risk factor for mortality from cardiovascular diseases including coronary heart disease, ischemic stroke and hemorrhagic stroke in the Asian populations (Chen *et al*., 2013b). In addition, Arnlöv *et al*. (2010) reported that metabolically healthy OB subjects had the lowest probability of being cardiovascular event-free when compared to the metabolically healthy OW and NR subjects in a 30 year follow-up (Figure 2.3). More tragically, obesity is related with sudden cardiac death. Duflou *et al*. (1995) reported that patients with morbid obesity had a higher risk of sudden cardiac death, which is supported by a recent study reporting that 25% of young OB sudden death patients had underlying coronary artery disease to a certain degree (Finocchiaro *et al*., 2018).

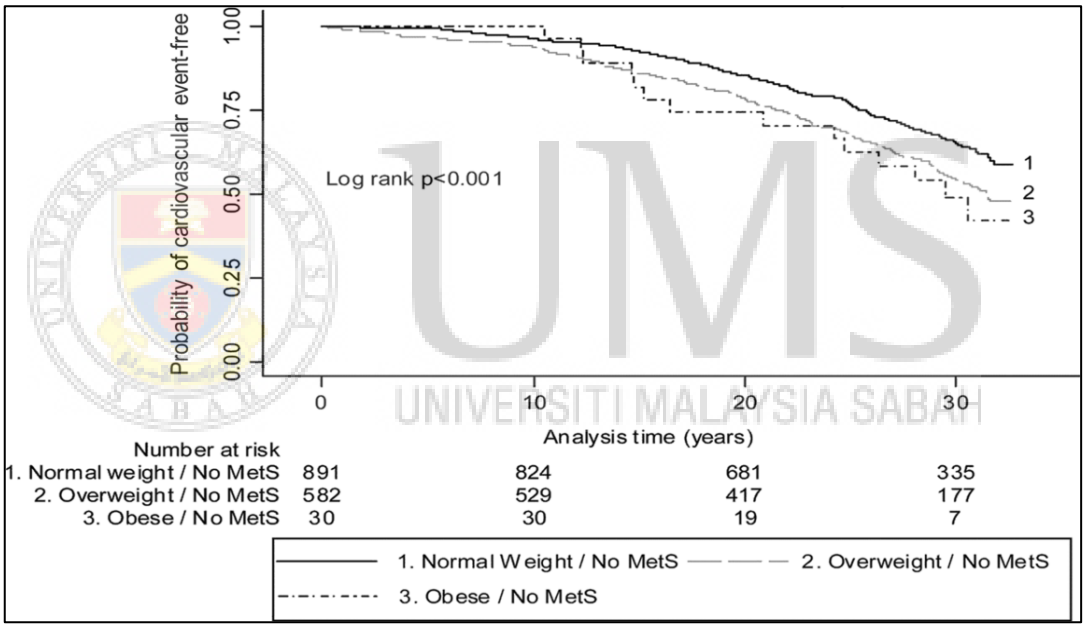

Figure 2.3 : Probability of cardiovascular events in a 30 years follow-up in different BMI without metabolic syndrome.

Source : Arnlöv *et al*., 2010

One of the underlying mechanisms between the obesity and cardiovascular diseases proposed by Kopelman (2000) was that OB individuals have an increase in both lean and fat tissue mass as well as the epidermis surface area, resulting in an increase of total oxygen consumption. This subsequently increases the stroke volume and cardiac output of the heart to fulfil the blood demand in the body, with an unchanged heart rate. In time, the left ventricle of the heart is dilated causing