METABOLIC, OXIDATIVE STRESS AND INFLAMMATORY MARKERS IN ADULT WOMEN EXPOSED TO SECONDHAND SMOKE

by

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LIST OF ABBREVIATIONS

SHS group	Secondhand smoke is exposure to secondhand smoke for 15 minutes in 2 days within a week
Non-SHS group	Non-secondhand smoke is non-exposed to SHS
ETS	Environmental tobacco smoke
CVD	Cardiovascular disease
CHD	Coronary heart disease
T2D	Type 2 diabetes
FBC	Fasting blood count
FLP	Fasting lipid profile
BMI	Body mass index
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
IR	Insulin resistance
OxLDL	Oxidized LDL
VLDL	Very low-density lipoprotein
HMW	High molecular weight
LMW	Low molecular weight
MMW	Medium molecular weight
CRP	C-reactive protein
hsCRP	High-sensitive C-reactive protein
NEFA	Non-estrified fatty acid
FFA	Free fatty acid
IL-6	Interleukin-6

HOMA-IR	Homeostatic model assessment insulin resistance
TNF-α	Tumor necrosis factor alpha
TAG	Triacylglyceride
PPARα	Peroxisome proliferator-activated receptor alpha
PI3K	Phosphatidylinositol 3-kinase
IRS	Insulin receptor substrate
GLUT	Glucose transporter type
GCMS	Gas chromatography–mass spectrometry
ELISA	Enzyme-linked immunosorbent assay
EIA	Enzyme Immunoassay
SD	Standard deviation
WHO	World Health Organization
US	United States

PENANDA METABOLIK, TEKANAN OKSIDATIF DAN KERADANGAN DALAM KALANGAN WANITA DEWASA YANG TERDEDAH KEPADA ASAP ROKOK

ABSTRAK

Pengenalan: Pendedahan kepada asap rokok (perokok pasif) terjadi hasil daripada pembakaran rokok dan produk tembakau lain dan dari hembusan asap rokok oleh perokok. Kebanyakan kematian dan morbiditi yang disebabkan oleh pendedahan kepada asap rokok dalam kalangan orang dewasa adalah berkaitan dengan penyakit kardiovaskular.

Objektif: Kajian ini bertujuan untuk menentukan kesan pendedahan asap rokok pada penanda metabolik, tekanan oksidatif dan keradangan pada wanita dewasa yang sihat.

Metodologi: Kajian ini adalah kajian perbandingan secara keratan-rentas di antara 101 melaporkan diri sebagai perokok pasif dan 91 bukan perokok pasif dalam kalangan wanita yang tiada mempunyai sejarah klinikal penyakit kardiovaskular. Perokok pasif didefinisikan sebagai pendedahan kepada asap rokok selama sekurang-kurangnya 15 minit dalam 2 hari dalam tempoh seminggu, manakala bukan perokok pasif didefinasikan bebas daripada terdedah kepada asap rokok. Sampel darah diambil daripada kesemua peserta yang layak untuk mengukur kadar penanda metabolik (HMW adiponektin, insulin, HOMA-IR dan NEFA), tekanan oksidatif (LDL teroksida dan 8-isoprostane) dan hsCRP sebagai penanda keradangan. Analisis nikotin pada rambut dijalankan untuk mengesahkan status pendedahan pada peserta.

Hasil kajian: Tahap nikotin pada rambut adalah lebih tinggi pada kumpulan perokok pasif berbanding dengan bukan perokok pasif [0.22 (0.62) vs 0.04 (0.11) ng / mg; p =

0.009]. Tiada perbezaan yang signifikan pada HMW adiponektin, insulin dan rintangan insulin (HOMA-IR), 8-isoprostane, LDL teroksida dan NEFA di antara kedua-dua kumpulan perokok pasif dan bukan perokok pasif dalam kalangan wanita dewasa, walaupun selepas mengawal beberapa faktor perancu yang berpotensi. Serum HMW adiponektin berkait secara negatif dengan tahap insulin dan rintangan insulin (HOMA-IR). Tiada perkaitan yang signifikan dilihat di antara HMW adiponectin dan NEFA, 8-isoprostane, LDL teroksida dan hsCRP pada wanita yang terdedah kepada asap rokok.

Kesimpulan: Penemuan kami menunjukkan bahawa pendedahan kepada asap rokok tidak memberi sebarang kesan terhadap penanda metabolisme, tekanan oksidatif dan keradangan dalam kalangan wanita dewasa yang sihat. Penurunan serum HWM adiponektin adalah berkaitan dengan peningkatan insulin dan rintangan insulin dalam kalangan mereka yang terdedah kepada asap rokok.

METABOLIC, OXIDATIVE STRESS AND INFLAMMATORY MARKERS IN ADULT WOMEN EXPOSED TO SECONDHAND SMOKE

ABSTRACT

Introduction: Secondhand smoke (SHS) is formed from the burning of cigarettes and other tobacco products and from smoke exhaled by smokers. Most of the mortality and morbidity attributable to exposure of adults to SHS is related to cardiovascular diseases.

Objectives: This study aims to determine the effects of SHS exposure on metabolic, oxidative stress and inflammatory markers in healthy adult women.

Methods: This is a comparative cross-sectional study between the group of self-reported SHS (n=101) and non-SHS exposure (n=91) in healthy women without clinical evidence of cardiovascular disease. Exposure to SHS is defined as exposed to secondhand smoke for at least 15 minutes in 2 days within a week, while non-SHS is people not exposed to SHS. Blood was drawn from all eligible subjects to measure metabolic (High molecular weight (HMW) adiponectin, insulin, homeostasis model assessment (HOMA-IR) and non-estrified fatty acids (NEFA), oxidative stress (oxidized low density lipoprotein (oxidized LDL) and 8-isoprostane) and inflammatory (hsCRP) markers. Hair nicotine analysis was performed to validate the exposure status in subjects.

Results: Hair nicotine levels were significantly higher in SHS exposure group compared to non-SHS exposure group [0.22 (0.62) vs. 0.04 (0.11) ng/mg; p=0.009]. No significant differences were observed in HMW adiponectin, insulin and insulin resistance (HOMA-IR), NEFA, 8-isoprostane, oxidized LDL and hsCRP between the two groups, even after controlling for several potential confounders. Serum HMW

adiponectin was negatively correlated with insulin level and insulin resistance (HOMA-IR) in the group of women exposed to SHS. No significant correlation was seen between HMW adiponectin and NEFA, 8-isoprostane, oxidized LDL and hsCRP in SHS women.

Conclusion: This study did not show any significant differences in metabolic, oxidative stress and inflammatory markers in healthy adult women exposed and non-exposed to SHS. Low serum HWM adiponectin is associated with increased insulin level and resistance in SHS exposed women.

CHAPTER ONE

INTRODUCTION

Secondhand smoke is also called as environmental tobacco smoke (ETS) or passive smoke, is produced from the sidestream smoke that released into the environment from the burning end of a cigarettes or other tobacco products, and from the mainstream smoke which is smoke exhaled by the smoker (WHO, 2010a). Evidence indicates that SHS leads to coronary heart disease (CHD) in adults (U.S. Department of Health Human Services, 1986). Moreover, it has been estimated that about 46,000 cardiac deaths are attributable to SHS exposure in the United States (California Environmental Protection Agency, 2005). Around the world, it has been shown that about one third of adults are regularly exposed to tobacco smoke (WHO, 2010a). Whereas, in Malaysia, approximately 40% adults are exposed to SHS at home and workplace, while 71% adults are exposed to SHS inside of restaurants (WHO, 2011).

There are approximately 7000 chemicals found in cigarette smoke that are liberated as gases, vapours and particles during combustion (Öberg *et al.*, 2010a). Many crucial responses of the cardiovascular system are very sensitive to the toxins in SHS (Barnoya and Glantz, 2005). For example, prior study demonstrated that free radicals contained in cigarette smoke decreased the release of nitric oxide from the

endothelium and enhanced the production of highly reactive intermediates, hence causing endothelial injury (Jaimes *et al.*, 2004). The chemical toxicity present in SHS exerts adverse effects on cardiovascular system such as platelet activation, increased oxidative stress, inflammation, increased insulin resistance, endothelial dysfunction, increased arterial stiffness, atherosclerosis, increased risk of coronary disease events and decreased energy metabolism (Barnoya and Glantz, 2005).

Nicotine is one of the important elements present in large amounts in the cigarette smoke, forming approximately 7-8 mg per cigarette (IARC, 2004). Atmospheric concentration of nicotine is identified as a sensitive and specific indicator for SHS (U.S. Department of Health and Human Services, 2006a). Recently, hair nicotine biomarker has been used as a validated indicator for SHS exposure (Al-Delaimy *et al.*, 2002, Woodruff *et al.*, 2003, Avila-Tang *et al.*, 2012). Moreover, hair nicotine has been recommended by several studies as a potential marker of long-term exposure to cigarette smoke (Al-Delaimy, 2002, Pichini *et al.*, 1997)

Adiponectin is a beneficial protein secreted by white adipose tissue and its function is important in lipid and glucose metabolism. The protein of adiponectin exists in plasma in three forms; low molecular weight (LMW) form is adiponectin trimmer, medium molecular weight (MMW) is hexamer and high molecular weight (HMW) is a complex form of adiponectins (Waki *et al.*, 2003). Total adiponectin measurement comprises these three forms which include LMW, MMW and HMW adiponectin (Beltowski *et al.*, 2008). Studies suggested that HMW adiponectin form may be the most biologically active form and represent a better marker for metabolic syndrome and insulin resistance than total adiponectin (Hara *et al.*, 2006, Nakano *et al.*, 2006). In contrast to other adipocytokines, low levels of adiponectin have been

related with cardiovascular disease (CVD) risk factors including obesity and type 2 diabetes (T2D) (Arita *et al.*, 1999, Hotta *et al.*, 2000, Weyer *et al.*, 2001). To our knowledge, there are no studies available that examined the effects of SHS exposure on HMW adiponectin concentration in women. However, among both healthy subjects and diseased patients, adiponectin levels are lower in active smokers compared to non-smokers (Takefuji *et al.*, 2007, Miyazaki *et al.*, 2003, Iwashima *et al.*, 2005).

Insulin resistance is defined as a reduced response of tissues (such as the skeletal muscle, liver, and adipocytes) to the insulin action in transporting glucose (DeFronzo and Tripathy, 2009). Persons with insulin resistance generally have elevated glucose and insulin levels in their blood at the same time (NIDDK, 2008). Insulin resistance is not simply a complication of impaired glucose uptake in response to insulin, but a multifaceted syndrome that raises significantly the risk for CVD (Ginsberg, 2000). Furthermore, a prior study also found that women with SHS exposure are associated with low insulin sensitivity (Henkin *et al.*, 1999).

Non-esterified fatty acids (NEFA) or also known as free fatty acids (FFA) are a marker for the lipolysis effects (lipid mobilization). NEFA is important in modulating insulin and has good correlation with insulin resistance (Eliasson *et al.*, 1994). Previous studies reported that increase in NEFA levels are associated with diabetes (Wilding, 2007) and sudden death in middle-aged men (Jouven *et al.*, 2001). Kershbaum *et al* revealed that smoking elevates the levels of NEFA in active smokers (Kershbaum *et al.*, 1961). However, so far no study examined the effect of SHS exposure on NEFA levels.

Free radicals from tobacco smoke may promote oxidative stress by promoting the generation of reactive oxygen species through enhancing

inflammatory cells activation (Burke and Fitzgerald, 2003). Oxidative stress is a disruption in the balance between reactive oxygen species generation and endogenous antioxidant defences, thereby resulting to oxidation of lipids, proteins, and DNA in ways that decrease cellular function (Burke and Fitzgerald, 2003). 8-isoprostane or F2-isoprostane is an important oxidative stress marker, comprised of prostaglandin-like compounds which are derived from arachidonic acid via lipid peroxidation (Morrow *et al.*, 1990). 8-isoprostane is shown to be increased in diabetes (Sampson *et al.*, 2002), hypertension (Hozawa *et al.*, 2004), obesity (Davi *et al.*, 2002), atherosclerosis (Oguogho and Sinzinger, 2000) as well as in cigarette smoking (Morrow *et al.*, 1995). Besides, oxidation of low-density lipoprotein (LDL) from native form into oxidized form (oxidized LDL) plays a significant role in the pathogenesis of atherosclerosis (Yoshida and Kisugi, 2010). Evidence suggests that smoking significantly increased oxidized LDL levels in men (Linna *et al.*, 2008). Moreover, exposure to SHS in non-smokers is related with the elevated levels of oxidized LDL (Panagiotakos *et al.*, 2004b) and 8-isoprostane (Kato *et al.*, 2006b).

Inflammation is important in mediating atherosclerosis formation and progression in the circulatory system (Libby *et al.*, 2002). C-reactive protein (CRP) is an acute phase plasma protein that synthesised in the liver in response to general inflammatory episodes within the body. Serum CRP levels have been used as a predictive marker for determining CVD risk (Tonstad and Cowan, 2009). Study has shown that a high CRP concentration is associated with risk of CVD in women (Bermudez *et al.*, 2002). Prior studies also showed, CRP levels were significantly higher in people who reported exposure to SHS than those whose were non-exposed and low SHS exposure (Panagiotakos *et al.*, 2004b, Hamer *et al.*, 2010).

Importance of study

The use of tobacco is the most important preventable cause of disease and death in the world today (WHO, 2008b). Globally, about 12% of all deaths in adults aged above 30 years are attributable to tobacco (WHO, 2012). Smoking prevalence is extremely high, especially among men. In Malaysia, almost 43.9% of men smoke (WHO, 2011). An increase in number of smokers definitely increased the prevalence of SHS exposure, especially among women and children. In Malaysia it is difficult to avoid exposure to cigarette smoke even in non-smoking areas such as airports, restaurants, mosques, schools or hospitals. In addition, there is also no law in placed to protect women and children from SHS exposure in the home. Although there is an awareness of the danger of SHS exposure in adults (WHO, 2011), but it is pointless if smokers still ignored it. Furthermore, active smokers inhaled only 15% from the entire cigarette smoke, while the remaining 85% of smoke is usually liberated into the surroundings, thus inhaled by people around the smokers (Division of Periodontology, 2003).

Majority of smokers in Malaysia are among the Malay ethnic group (27.9%) followed by Chinese (19.2%) and Indians (16.2%) (Tarmizi *et al.*, 2007). About 55.6% of children (aged 10-12 years) from Kota Bharu were reported to be living together with at least one smoker, particularly their father at home (Sharina, 2007). This shows that it is a serious phenomenon that needs to addressed in order to protect never smokers from the health risk of SHS exposure.

Metabolic, inflammatory and oxidative stress contribute to increased CVD risk. Subjects with SHS exposure also have increased risk for diabetes mellitus. So far, there are no studies on the chronic effects of SHS exposure to the metabolic markers NEFA and HMW adiponectin. There is also no large study that measures

the biomarkers of oxidative stress, inflammatory and metabolic markers among SHS exposed subjects and women in one single study. A study that simultaneously measures these parameters in a single study would be valuable in giving information to understand more about the mechanisms whereby secondhand smoke exposure contributes towards increasing the risk for CVD. This knowledge would be useful to educate the population, specifically smokers and SHS exposed individuals, and to institute preventive measures to prevent CVD complications from exposure to SHS.

General objective

To determine the effects of secondhand smoke exposure on metabolic, oxidative stress and inflammatory markers in healthy adult women.

Specific objectives

1. To compare serum HWM adiponectin levels in SHS and non-SHS women.
2. To compare serum insulin levels and insulin resistance (HOMA-IR) in SHS and non-SHS women.
3. To compare serum non-esterified fatty acid (NEFA) levels in SHS and non-SHS women.
4. To compare the oxidative stress markers (serum 8-isoprostane and oxidized LDL levels) in SHS and non-SHS women.
5. To compare inflammatory marker of plasma high sensitive C-reactive protein levels in SHS and non-SHS women.
6. To examine the relationship between HMW adiponectin and insulin, insulin resistance (HOMA-IR), NEFA, 8-isoprostane, oxidized LDL and hsCRP in the group of women with SHS exposure.

Hypothesis

1. Serum HMW adiponectin levels are lower in SHS women compared to non-SHS.
2. Serum insulin and insulin resistance (HOMA-IR) levels are higher in SHS women compared to non-SHS.
3. The serum of non-esterifies fatty acid (NEFA) is higher in SHS women compared to non-SHS.
4. Oxidative stress markers (both serum 8-isoprostane and oxidized LDL) are higher in SHS women compared to non-SHS.
5. Inflammatory marker of plasma high sensitive C-Reactive protein (hsCRP) is higher in SHS women compared to non-SHS.
7. HMW adiponectin is negatively associated with insulin, insulin resistance (HOMA-IR), NEFA, oxidative stress markers (8-isoprostane and oxidized LDL) and inflammatory marker (hsCRP) in the group of women exposed to SHS.

Operational definition

Secondhand smoke (SHS) exposure is defined as exposure to secondhand smoke for at least 15 minutes in 2 days within a week (WHO, 1986). Non-SHS is defined as people who are free from SHS exposure.

CHAPTER TWO

LITERATURE REVIEW

2.1 Secondhand smoke exposure

2.1.1 Definition of secondhand smoke exposure

Secondhand smoke (SHS) exposure or passive smoking is formed from the sidestream smoke and mainstream smoke from cigarette smoking. Sidestream smoke is smoke that is liberated into the surroundings from smouldering cigarette between puffs (primary contributor to SHS) and mainstream smoke is smoke that is exhaled by smokers (Öberg *et al.*, 2010a). Elements in sidestream smoke is more toxic compared to element contained in mainstream smoke (Öberg *et al.*, 2010a). Moreover, sidestream smoke have greater concentration of the most toxins contained in tobacco smoke than mainstream smoke, may be because sidestream smoke is produced at lower temperatures and with dissimilar burning conditions compared to mainstream smoke (U.S. Department of Health Human Services, 1986). The complex reactive substances contained in tobacco smoke are usually liberated as gases, vapors and particles during combustion (Öberg *et al.*, 2010a). The United States National Toxicology Program identified about 250 constituent found in SHS are toxic or carcinogenic (U.S. Department of Health and Human Services, 2006a).

Apart from the terms secondhand smoke, “environmental tobacco smoke” or “passive smoking” or involuntary smoking” are also frequently used in studies to describe tobacco smoke exposure. The terms “secondhand” reflects the involuntary nature of the exposure. This study uses the term secondhand smoke following the official position from the Tobacco Free Initiative (Öberg *et al.*, 2010a). Moreover, the term “environmental” reflects more on ambient pollutant which is not specifically caused by smoking but can be contributed by other pollutant such as those emitted from factories and vehicles such as lorry and cars. Furthermore, exposure to cigarette smoke may happen in various locations, such as at home, workplace, public place and in car.

The SHS exposure definition of at least 15 minutes in 2 days within a week exposure that was used in this study originated from World Health Organization (WHO) (1986). Nearly similar definition as ours has been applied in several epidemiological studies (Yang *et al.*, 1999, Samet and Yang, 2001, Yang *et al.*, 2010, Panagiotakos *et al.*, 2004b, Nor *et al.*, 2008). For example, in China they demonstrated that SHS exposure for at least 15 minutes daily for more than one day every week were well-correlated with hair nicotine concentration among women exposed to SHS at home (Yang *et al.*, 2010).

2.1.2 Prevalence of secondhand smoke exposure

In the United States, exposure to SHS remains a serious public health hazard, although progress has been made to protect non-smokers (U.S. Department of Health and Human Services, 2006a). A Global Adult Tobacco Survey (GATS) Malaysia 2011 was conducted among persons age 15 and above reported that about half of

non-smokers in this study were exposed to SHS in their homes, while 70.3% non-smokers reported exposed to SHS in public places (Ministry of Health, 2003).

Across the globe, men are the biggest tobacco user (Shafey, 2009). For example, in Malaysia, it is estimated that about 43.9% of men and 1.0% of women are currently smoking (WHO, 2011). In China, smoking prevalence rates for men and women were 63% and 3.8%, respectively (Yang *et al.*, 1999). In developed countries, however, smoking among men and women are nearly equal which is about 32% and 18%, respectively (Shafey, 2009). High prevalence of smoking among men placed women and children at the risk for SHS exposure especially in developing countries (Gilmore *et al.*, 2004). A survey in China showed that prevalence of SHS exposure were much higher in women rather than in men (Yang *et al.*, 1999, Gu *et al.*, 2004). However, in high income country such as Spain, exposure prevalence rate is similar, with 70% for men and 63% for women (Twose *et al.*, 2007).

Home and workplace are classified as predominant places for SHS exposure in the United States (U.S. Department of Health and Human Services, 2006a). According to several surveys conducted in Malaysia, it is estimated that 78% smokers smoked in the homes (Wipfli *et al.*, 2008). Another survey in Malaysia also indicated that 33.3% women were exposed to SHS at home (WHO, 2011). Furthermore, Marbury *et al.* (1993) showed that concentration of ambient nicotine in the homes where smoking is restricted were lower than that in the homes where smoking is allowed (Marbury *et al.*, 1993). According to a probe on the behaviour of smoking among spouses at home, a report indicated that the level of hair nicotine in women whose spouses smoked at home is higher compared to women with non-smoking spouses. Moreover, no difference was reported in hair nicotine level between the group of women whose spouses were smoking outside the home versus those with

spouses smoking inside the home (Yoo *et al.*, 2010). Another report shows that the increase in serum cotinine level is in parallel with an increase in the number of tobacco smoked by smokers at home (Nondahl *et al.*, 2005).

Workplaces also has been identified as primary contributor to SHS exposure (Perez-Rios *et al.*, 2012). United States' workers reported to have higher exposure to SHS in workplaces rather than at home (Hammond, 1999). A survey conducted in Malaysia estimated that 30.1% women had SHS exposure at workplace during the past 30 days (WHO, 2011). The highest prevalence of SHS exposure in the workplace was in southern and central Europe (Janson *et al.*, 2001). Serum cotinine levels in non-smoking workers have been identified to increase with increased hours per day of SHS exposure at workplace; 0 h/day exposure (0.08 ng/mL), <1 h/day of exposure (0.18 ng/mL), 1-4 h/day of exposure (0.34 ng/mL) and >4 h/day of exposure (0.30 ng/mL) (Nondahl *et al.*, 2005). Even though the ventilation systems was used inside the buildings to minimize soiled air, it is identified as incapable to completely remove the hazardous compounds from the smoke (Kotzias *et al.*, 2006).

Exposure in the car or other public places is also a crucial source of SHS exposure. About 38% students reported having been exposed to cigarette smoke in the car over the last 7 days (Wolfson *et al.*, 2009). Concentration of nicotine in confined spaces such as cars also can be higher (Danzon *et al.*, 2000). Restaurants are also important locations for contributing to SHS exposure in women.According to the Global Adult Tobacco Survey among 5112 households in Malaysia, 68.4% adult women were exposed to SHS in restaurants over the past 30 days (WHO, 2011).

2.1.3 Implications of secondhand smoke exposure

Based on scientific evidence, WHO Framework Convention on Tobacco Control established an articulation that SHS exposure can cause death, disease and disability, and there is no safe level of SHS exposure (U.S. Department of Health and Human Services, 2006a). It was estimated that about 603 000 deaths were caused by SHS worldwide and ischaemic heart disease has been recorded as the highest contributor to mortality among adults caused by SHS exposure (Öberg *et al.*, 2010b). Besides, more than 79,000 adults die each year as a result of SHS in the 25 European countries (Jamrozik, 2006).

Cardiovascular disease (CVD) is the major contributor for morbidity and mortality among Malaysian women. The deaths caused by CVD among Malaysian female patients in the government hospitals remained constant over 7 years (from 1999 to 2006) (MOH, 2008). In the United States, cardiovascular-related medical care is more costly compared to any other diseases (NIH, 2011), whilst, for developing country particularly Malaysia it gives a huge economic burden to health sector. Thus, it is necessary to identify the risk factors contributing to CVD in people who have been exposed to SHS (Glantz and Parmley, 1991, He *et al.*, 1999).

During the past two decades, studies reported evidence concerning SHS exposure and CVD risk factors and other health implications including CHD (U.S. Department of Health, 1972, He *et al.*, 1999, Steenland, 1992, U.S. Public Health Service, 1983), atherosclerosis (Yuan *et al.*, 2007, Nagel *et al.*, 2009) and lung cancer (U.S. Department of Health and Human Services, 2006b). The effect of even a short SHS exposure on the cardiovascular system could reach around 80 to 90% as in chronic active smoking (Barnoya and Glantz, 2005). In children, SHS exposure can cause respiratory symptoms (Sharina, 2007). Although smoke inhaled by a

person with SHS exposure is much less than smoke inhaled by smokers, but it increases the risk of CHD by approximately 30% (Barnoya and Glantz, 2005). Regrettably, the risks associated with SHS exposure were not as often reported as compared to risks associated with active smokers.

2.2 Nicotine exposure

Nicotine is a chemical constituent that is present in tobacco and has a mild stimulant and relaxing effects to smokers. Nicotine acts by affecting the sympathetic nervous systems and adrenal glands, which enhances releasing epinephrine and norepinephrine (Yusoff, 2010). In addition, nicotine also acts on human adipose tissue, where it is previously demonstrated that incubation of mouse adiposities media culture with nicotine caused reduction in secretion of adiponectin into the media (Iwashima *et al.,* 2005). Nicotine is released from sidestream smoke particle and evaporates as soon as it is diluted into ambient, and it is found highly concentrated (919 µg per cigarette) in sidestream cigarettes smoke (Fowles *et al.,* 2000). Nicotine has been identified as a specific to cigarette combustion and is present in high amount in SHS. Hence, nicotine is appropriate to be used as an indicator for SHS (U.S. Environmental Protection Agency, 1992). Nicotine in the blood has a relatively short half-life (2 hours), hence blood nicotine level is suitable to use as an indicator for exposure during the prior hours. Recently hair nicotine has been measured as a reliable biomarker for SHS exposure over the previous 1-2 months (Öberg *et al.*, 2010a). Over the past several years, nicotine measurement has been used extensively as a reasonably sensitive biomarker indicative of exposure to SHS exposure in various population studies (Man *et al.*, 2009, Al-Delaimy *et al.,* 2002, Al-Delaimy *et al.,* 2000, Woodruff *et al.,* 2003).

Previously urine cotinine is widely used to measure SHS exposure and has been recommended as the “gold standard” biomarker of SHS exposure. However this biomarker has a relatively short half life of 20 hours and it is easily affected by inter-individual variability in cotinine excretion levels for identical exposures (Al-Delaimy *et al.*, 2002). Therefore, the more recent method has been developed to measure nicotine in the hair. Hair nicotine is less affected by the daily fluctuation of SHS exposure because hair has a slow growth rate. Moreover, the hair nicotine levels can provide a long term exposure history because each one centimetre (cm) of hair is equivalent to about one month’s exposure (Al-Delaimy *et al.*, 2002, Uematsu *et al.*, 1995). Hair nicotine concentration reflects the amount of nicotine accumulated in the hair and it is sustained in the hair shaft throughout the hair life. Nicotine assembles in the hair mainly by direct absorption from the environment (Eisner *et al.*, 2005). Report revealed that hair nicotine concentration is linearly associated with the duration and concentration of exposure to ambient nicotine (Eliopoulos *et al.*, 1994). Advantages of doing hair nicotine assay is hair sample is easy for sample collection, inexpensive for storage and transport, less variability and capable to describe the exposure over a long-term period compared to other biological samples. Moreover, hair nicotine shows a better correlation with variables of reporting SHS exposure than urine cotinine levels (Al-Delaimy *et al.*, 2002).

Kim *et al.* (2009) demonstrated that women exposed to SHS have higher levels of hair nicotine than those who were non-exposed (Kim *et al.*, 2009). Moreover, women from Asia who lived together with household smokers showed the highest hair nicotine levels compared to women from Europe and Latin America living with smokers at home (Wipfli *et al.*, 2008).

2.3 Cardiovascular biomarkers

2.3.1 High molecular weight adiponectin

Adiponectin is a beneficial protein that is released abundantly from white adipose tissue (Hotta *et al.*, 2001). Adiponectin is also known as ACRP30 (Scherer *et al.*, 1995), AdipoQ (Hu *et al.*, 1996) or gelatine-binding protein-28 (Nakano *et al.*, 1996). It consists of 247 amino acids and four domains; an amino-terminal signal sequence, a variable region, a collagen region and a carboxyl-terminal global region. Adiponectin is regulated by an autocrine or paracrine manner and endocrine manner within distal tissue. According to Arita *et al.*, (1999), plasma adiponectin is abundantly present in healthy subjects ranging from 1900 to 17000 µg/ml (Arita *et al.*, 1999). In contrast to other adipocytokines that are higher in obesity, adiponectin is significantly decreased in obese compared to lean subjects. Adiponectin is beneficial in several activities such as increasing insulin sensitivity, stimulating fatty acid oxidation, inhibiting inflammatory reaction and inducing endothelium-dependent nitric oxide-mediated vasorelaxation (Beltowski *et al.*, 2008). In addition, adiponection level is also reported to be low in cases of metabolic syndrome (Hara *et al.*, 2006, Matsushita *et al.*, 2006), T2D (Zhu *et al.*, 2010, Almeda-Valdes *et al.*, 2010) and arteriosclerosis (Ouchi *et al.*, 2001, Matsuda *et al.*, 2002). A study suggested that the rise of adiponectin concentration may be a useful novel therapeutic strategy in a number of CVD. Because, adiponectin has a protective role in some cardiovascular pathologies including endothelial dysfunction, atherogenesis, myocardial ischemia-reperfusion injury and myocardial hypertrophy. Deficiency of this protein may contribute to complications in patients with metabolic syndrome (Beltowski *et al.*, 2008).

Adiponectin level is relatively 65% greater in women compared in men (8.0±4.5 µg/ml vs. 4.9±2.6 µg/ml; $p<0.001$) (Ahonen *et al.*, 2008, Kern *et al.*, 2003). Lower adiponectin in men may be related to divergence in fat distribution and higher testosterone levels. Men have more visceral fat and less subcutaneous fat compared to women (Matsushita *et al.*, 2014, Ludescher *et al.*, 2007). Adiponectin is mainly synthesized in subcutaneous fat cells in adipose tissues. Moreover, testosterone in men inhibits secretion of adiponectin from fat cells (Xu *et al.*, 2005). On the other hand, adiponectin was negatively associated with BMI, where increased in weight reflects to the accumulation of visceral fat that causes for insulin resistance and inflammation, this could reduce adiponectin level (Kern *et al.*, 2003, Komatsu *et al.*, 2012, Krakoff *et al.*, 2003).

Adiponectin exists in adipocytes and plasma in three different forms, low-molecular weight (LMW) adiponectin (three native molecules connected hydrogen bonds between their globular domains), medium-molecular weight complexes (MMW) adiponectin (2 trimers connected with the disulfide bond between Cys^{22} residues) and high-molecular weight forms (HMW) adiponectin (non-covalently bound 2 or 3 hexamers contain 12 or 18 single monomer molecules) (Waki *et al.*, 2003). Quantifying LMW, MMW and HMW by gel filtration sub-fractionation accounted for approximately 20%, 34% and 46%, respectively in serum (Lo *et al.*, 2011). Different forms of adiponectin varies in biological function, however studies suggested that HMW adiponectin complex is the most active form of adiponectin because it has the ability to reduce blood glucose (Pajvani *et al.*, 2004).

A number of studies have suggested that HMW adiponectin is the best marker to determine adiponectin rather than total adiponectin (comprise of LMW, MMW and HMW protein complexes). Previous studies revealed that HMW

adiponectins have a greater statistical power for diabetes estimation (Hara *et al.*, 2006, Seino *et al.*, 2007, Heidemann *et al.*, 2008, Nakashima *et al.*, 2006), coronary artery disease (Aso *et al.*, 2006), and metabolic syndrome (Hara *et al.*, 2006, Seino *et al.*, 2007) than total adiponectin. Hence, evaluations of HMW adiponectin is likely more relevant in determining and evaluating CVD mechanisms than total adiponectin.

Recently, an ELISA specific monoclonal antibody system was developed to quantify human HMW adiponectin (Nakano *et al.*, 2006). ELISA is more affordable and less time consuming compared to using western blotting (Arita *et al.*, 1999) or gel filtration chromatography (Aso *et al.*, 2006), which is more expensive and difficult to perform in large population studies.

Smoking inhibits adiponectin in healthy subjects and in patients with chronic disease (Takefuji *et al.*, 2007, Miyazaki *et al.*, 2003, Iwashima *et al.*, 2005). Adiponectin is significantly lower in active smokers compared to non-smokers (Iwashima *et al.*, 2005, Takefuji *et al.*, 2007). Furthermore, heavy smokers (smoking more than 25 cigarettes per day) have a lower adiponectin level compared to moderate and light smokers even after the age, BMI, alcohol intake, physical activity and total calorie intake were controlled (Takefuji *et al.*, 2007). Adiponectin was found to be inversely associated with Brinkman index (number of cigarettes smoked per day X number of years of the habit) in current smokers (Miyazaki *et al.*, 2003). Iwashima *et. al.*, (2005) demonstrated that 12 hours of acute smoking exposure in healthy non-smokers markedly reduced adiponectin level. Besides, a laboratory experiment showed that incubation of adipocytes cell culture with nicotine decreased the mRNA (messenger ribonucleic acid) expression and secretion of adiponectin (Iwashima *et al.*, 2005). A study revealed that heavy smokers have the lowest level

of serum HMW adiponectin levels compared to non-smokers in the group of subject with high insulin resistance (subjects with age ≥ 60 years, BMI ≥ 22 kg/m^2 and HOMA-IR ≥ 1.6) (Kawamoto *et al.*, 2010). However, there are contradictory results arise, where one study reported no difference in HMW adiponectin levels between current smokers and non-smokers among Japanese male subjects who have normal glucose tolerance (Watanabe *et al.*, 2011). This contradictory finding between Kawamoto *et al.* and Watanabe *et al.* may be because the difference in subject population, where subjects from Kawamoto's study have higher insulin resistance compared to subjects in Watanabe's study with mean of HOMA-IR was 0.90 ± 0.18. This suggests that low levels of HMW adiponectin in smokers may be related to insulin resistance response, since insulin resistance is increased in smokers compared to non-smokers (Facchini *et al.*, 1992, Abbasi *et al.*, 2006).

2.3.2 Insulin

Insulin was recognized in the early 1920's as the major hypoglycaemic hormone (Kumar and O'Rahilly, 2005). Insulin is produced in the islets of Langerhans with a molecular weight of 5808 Da and consists of 51 amino acid (Srinivasa Nageswara Rao *et al.*, 2011). Insulin is generated from proinsulin through the action of tryptic and carboxypeptidaselike enzymes. Proinsulin is a single polypeptide chain comprised with subunits of insulin joined by the C-peptide region (Orci *et al.*, 1986). Generally, insulin is well-known as an important regulator in glucose homeostasis. In high glucose condition insulin production is increased, increasing in insulin levels ultimately enhances for glucose uptake and glycogen synthesis activities, and inhibits glycogenolysis and gluconeogenesis process, thus preserving a normoglycaemia status in humans. Besides, insulin also exerts in a

number of other important metabolic effects. For example, it controls the genes expression involved in lipid metabolism in the muscle and adipose tissues, amino acid uptake and cell growth, development and survival (Rhodes and White, 2002).

Maintenance of normoglycaemia needs a strong co-ordinated control of both insulin action and secretion. Because loss of glycaemic control usually causes reduction in both insulin action (example is peripheral insulin resistance) and insulin secretion (example is β-cell dysfunction) activities. Insulin action is mediated through a multi-component signalling complex that is strongly conserved across a wide range of species. Binding of insulin to its receptor activates tyrosine kinase, resulting in autophosphorylation of tyrosine residues on the receptor β-subunit and leads to phosphorylation of insulin receptor substrate (IRS) proteins. This IRS protein importantly trigger multiple downstream signalling molecules, such as phosphatidylinositol 3-kinase (PI3K). In skeletal muscle and adipose tissues, activation of the PI3K pathway promotes glucose utilization by regulating the expression or subcellular localization of glucose transporters (GLUT) and stimulates the glucose storage as glycogen or fat. Whereas, in pancreatic β cells, the PI3K cascade possibly promotes survival of β cells (Rhodes and White, 2002).

Glucose is the main regulator of insulin secretion. Briefly, exposure to glucose elevates the ATP:ADP ratio and leads to closure of ATP-sensitive potassium channels. This in turn causes membrane depolarization and consequently stimulates the opening of the voltage-dependent Ca^{2+} (calcium) channels. The resultant Ca^{2+} influx leads to increased cytosolic Ca^{2+} concentrations and results in exocytosis of insulin secretory particles from the storage granule. Figure 2.1 shows the mechanisms of insulin secretion from β-cells that are located in the islets of Langerhans in the pancreas in response to blood glucose changes.

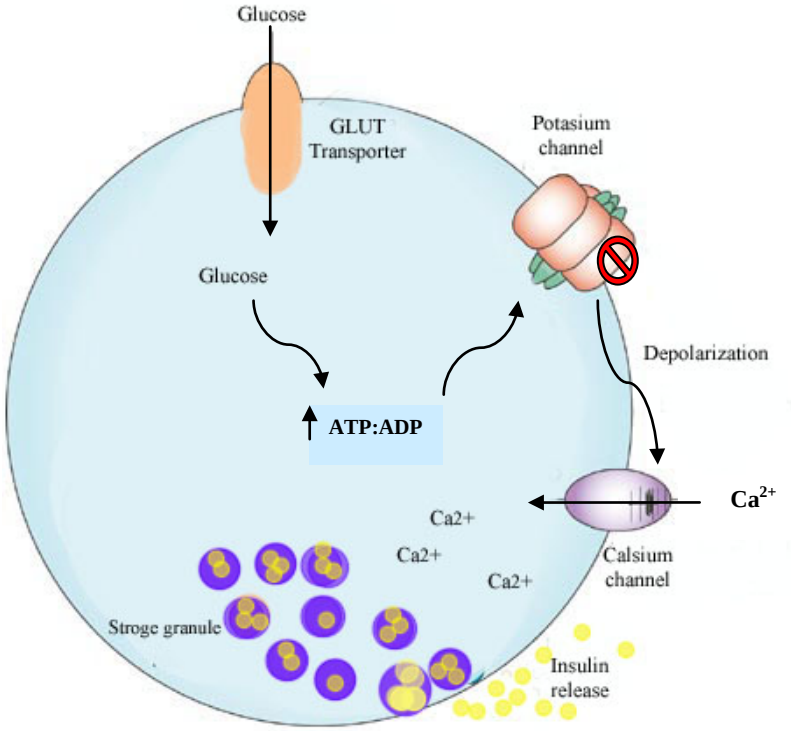

Figure 2.1 Glucose-stimulated insulin secretions in pancreatic β-cells at the islets of Langerhans in the pancreas. Transportation of glucose into β cell increases the ATP:ADP ratio, thus triggers the opening the calcium channels through closure of ATP-sensitive potassium channels. This allows the flow inward of Ca^{2+} (calcium ions) into the β cells and yields in exocytose of insulin secretion from storage granules (modified figure from (De Leon and Stanley, 2007))

2.3.3 Insulin resistance

Insulin resistance (IR) is a state where body secretes insulin but does not use it properly (NIDDK, 2008). Low insulin sensitivity or insulin resistance always been noticed in CVD such as diabetes, metabolic syndrome, obesity and stroke (McFarlane *et al.*, 2001, Ginsberg, 2000). Fasting insulin level is capable of determining insulin resistance (Laakso, 1993). Abnormalities in insulin signalling in the muscle, adipose tissue, liver and pancreas lead to IR and β-cell dysfunction in T2D patients (Rhodes and White, 2002)

A number of methods have been developed to measure insulin sensitivity or resistance which ranges from complex procedures to simple indices (Muniyappa *et al.*, 2008, Borai *et al.*, 2011). The measurement of glucose clamp method (DeFronzo *et al.*, 1979) is a gold standard for direct measurement of insulin sensitivity. It is a steady-state technique which needs a constant insulin infusion in order to measure the sensitivity of insulin action. However, this technique is time consuming, expensive and requires expertise to handle, while not suitable to be appllied in large samples. A quite simple, non-invasive alternative to the clamp method is the homeostasis model assessment (HOMA). A simple index of insulin resistance is categorized as an index that does not need the intravenous administration of exogenous insulin or glucose. It can be assessed either from a fasting specimen alone or fasting specimen along with other blood samples taken following an oral glucose load. Simple indixes, not like dynamic techniques which does not demand on steady state conditions (Borai *et al.*, 2011). Besides, using the simple indexes required inexpensive spending because it simply to use in nearly every study's setting including epidemiological studies, large clinical trials, clinical research investigations, and clinical practice (Muniyappa *et al.*, 2008). The simple index used in our present study is homeostasis model assessment derived for insulin resistance (HOMA-IR) (Matthews *et al.*, 1985). HOMA-IR was first developed by Matthews *et al.* in 1985 (Matthews *et al.*, 1985) and has widely been used in estimation of insulin resistance. Moreover, HOMA-IR has a reasonable correlation against glucose clamp method in several different population studies (Radziuk, 2000, Wallace *et al.*, 2004).

Barnoya and Glantz (2005) concluded SHS exposure might also lead to an increase in heart disease by increasing insulin resistance (Barnoya and Glantz, 2005). Previous studies have extensively discussed the relationship between passive

smoking and the incidence of diabetes (Hayashino *et al.*, 2008b, Kowall *et al.*, 2010, Houston *et al.*, 2006). For example, the significance of diabetes cases were reported in those exposed to SHS at workplace over 4 years (Hayashino *et al.*, 2008a). Similarly, the cumulative 7 year incidence of diabetes was lower in non-SHS (7.1%) compared to SHS (11.4%) (Kowall *et al.*, 2010). A cohort study showed that the 15 year prevalence of glucose intolerance was highest in non-smokers with exposure to SHS compared to non exposure (Houston *et al.*, 2006). Furthermore, Henkin et al (1999) also reported that the level of insulin sensitivity is lower in people exposed to tobacco smoke than those who were free from exposure (Henkin *et al.*, 1999).

2.3.4 Non-estrified fatty acid (NEFA)

Non-esterified fatty acids were recognized in 1950s by Robert Gordon (Gordon and Cherkes, 1956). Non-esterifies ("free" or unsaturated) fatty acids (NEFAs) is also known as free fatty acids (FFAs). NEFA are important energy source for several organs and also associated in promoting insulin resistance in the human body. Higher level of NEFA usually causes pathogenesis of metabolic disturbances such as insulin resistance and metabolic syndrome (Stich and Berlan, 2004). Adipose tissue releases fatty acids for the muscle and liver by hydrolysis of triacylglycerol, through the action of the hormone-sensitive lipase (HSL) enzyme and other lipases, to liberate NEFA into the plasma (Frayn *et al.*, 2006, Thompson *et al.*, 2012).

The half-life of plasma NEFA is about 3 minutes before being secreted into the urine (Bonadonna *et al.*, 1990). The plasma NEFA level varies considerably with time. For example, in overnight fasting state NEFA ranges about 300-600 µmol/L and increased approximately to 1300 µmol/L after 72 hours fasting (Frayn *et al.*,

1996). During the fasting state, hydrolysis (lipolysis) of stored (intracellular) triacylglycerol occurrs in the adipocytes to liberate NEFA into the circulation (Frayn *et al.*, 1996). After taking any meal containing carbohydrates, plasma NEFA concentrations significantly decrease because carbohydrates stimulate insulin release (Dole, 1956).

Smoking is responsible for NEFA increase and glycerol fluxes due to nicotine induced lypolysis. Early on 1961, a study by Kershbaum *et al.* revealed that a minimum of two cigarettes smoking elevate the FFA level (Kershbaum *et al.*, 1961). Nicotine may induce lipolysis by stimulating the release of catecholamines (norepinephrine and epinephrine) from the sympathetic nervous system. These catecholamines rapidly act on adipose tissues and lipolysed triglycerides to liberate NEFA into the circulation (Andersson and Arner, 2001, Cryer *et al.*, 1976b).

An increase in NEFA leads to metabolic syndrome and insulin resistance in humans (Stich and Berlan, 2004). NEFA that is produced from the adipose tissue enhances the level of triglycerides, glucose and secretion of very low-density lipoprotein (VLDL) in the liver, as well as it decreases insulin sensitivity in the muscle through inhibiting insulin-mediated glucose uptake (Eckel *et al.*, 2005). Previous studies recognized that NEFA level is higher in obese (Opie and Walfish, 1963) and insulin resistant subjects (Jouven *et al.*, 2001). This is because obesity is linked with increased accumulation of adipose tissue mass; enhanced triglycerides (TG) production, thereby increased NEFA levels. Otherwise, excess NEFA leads to acute hyperinsulineamia by inhibiting insulin binding to hepatocytes, hence reduces approximately 40% of insulin secretion (Svedberg *et al.*, 1990, Karpe *et al.*, 2011). In addition, there are studies showing that higher level of NEFA is related with coronary heart disease incidences (Frayn *et al.*, 1996, Jouven *et al.*, 2001).

2.3.5 8-isoprostane

Oxidative stress is defined as a imbalance between production of free radicals and antioxidant defences, causing oxidation of proteins, lipids and DNA which potentially impair cellular function (Burke and Fitzgerald, 2003). Oxidative stress is linked with many acute and chronic diseases such as lung disease, CVD, neurodegenerative disease, cancer and aging process (Montuschi *et al.*, 2004).

8-isoprostane, also referred to F2-isoprostane, is a prostaglandin-like compound derived from the peroxidation of arachidonic acid (lipid). Over the past decade, 8-isoprostane has appeared as the "gold standard" to determine of oxidative stress *in vivo* and has been applied broadly to assess lipid peroxidation (Morrow, 2005). Measurement of 8-isoprostane has several advantages over other markers (Montuschi *et al.*, 2004); 1) specific products of lipid peroxidation, 2) chemically stable compound, 3) formed in vivo, 4) present in detectable amounts in all normal tissues and biological fluids, thus allowing definition of a normal range, 5) not affected by lipid content in the diet (Gopaul *et al.*, 2000, Richelle *et al.*, 1999) and 6) their levels increase significantly in animal models of oxidant injury. Urinary 8-isoprostane concentration is reportedly unaffected by the daily variability in healthy subjects (Montuschi *et al.*, 2004).

Gas choromatography-mass spectrophotometer (GCMS) is the gold standard method for measuring 8-isoprostane, but it is time consuming and unreliable to apply in large sample population as our present study. Thus, currently an *in vivo* enzyme immunoassay (EIA) has been developed and reliable to use in large sample study population. There is evidence that isoprostanes measured by EIA has a good correlation with GCMS (r=0.99) (Devaraj *et al.*, 2001, Wang *et al.*, 1995). Moreover, serum isoprostane has an advantage over urinary isoprostane, because urinary