THE RELATIONSHIP OF NICOTINE DEPENDENCE WITH SERUM LEPTIN CONCENTRATION AND TOTAL CALORIE INTAKE AMONG HEALTHY MALE SMOKERS IN KOTA BHARU

by

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LIST OF ABREVIATIONS

ANOVA	Analysis of variance
BF	Body fat
BMI	Body mass index
BP	Blood pressure
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DSM-5	Diagnostic and Statistical Manual of Mental Disorder Fifth Edition
ELISA	Enzyme-linked immuno assay
F	Ratio of the variance between groups to the the variance within groups
FBS	Fasting blood sugar
FTND	Fagerstrom Test for Nicotine Dependence
GATS	Global Adult Tobacco Survey
HDL-C	High density lipoprotein cholesterol
HRP	Horseradish peroxidase
HUSM	Hospital University Sains Malaysia

IPAQ	International physical activity questionnaire
IS	Internal standard
kDa	Kilodalton
LDL-C	Low-density lipoprotein cholesterol
LEP	Leptin
LOD	Limit of detection
LOQ	Limit of quantification
LPL	Lipoprotein lipase
LR	Leptin receptor
M	Molar
MET	Metabolic equivalent
NaOH	Sodium hydroxide
NHMS	National Health and Morbidity Survey
NPY	Neuropeptide Y
OD	Optical density
p	*p* value
pH	Negative loq 10 of hydrogen concentration
pKa	Acid dissociation constant

QCS	Quality control sample
RIA	Radioimmunoassay
SBP	Systolic blood pressure
SD	Standard deviation
TC	Total cholesterol
TG	Triglyceride
TMB	Tetramethylbenzidine
USDHHS	United States Department of Health and Human Services
VF	Visceral fat
VLCD	Very low caloric diet
WC	Waist circumference
WHO	World Health Organization
WHR	Waist-hip ratio

HUBUNG KAIT ANTARA KEBERGANTUNGAN NIKOTIN DENGAN KEPEKATAN SERUM LEPTIN DAN JUMLAH PENGAMBILAN KALORI DALAM KALANGAN PEROKOK LELAKI SIHAT DI KOTA BHARU

ABSTRAK

Merokok didapati mempunyai perkaitan dengan penurunan berat badan manakala berhenti merokok pula mempunyai kaitan dengan peningkatan berat badan. Ianya dipercayai berlaku akibat kesan perantaraan nikotin. Pendedahan terhadap nikotin melalui asap rokok boleh mempengaruhi penghasilan leptin dalam badan. Peningkatan kepekatan hormon leptin dalam badan boleh menurunkan selera makan dan mengurangkan jumlah pengambilan makanan. Ia seterusnya menghasilkan hubungan berkadar songsang antara kepekatan nikotin dan berat badan. Fungsi leptin sebagai pengawal selera dikatakan sebagai penyebab kepada perubahan berat badan dalam kalangan perokok. Tujuan kajian ini adalah untuk menentukan hubung kait antara kebergantungan nikotin dengan kepekatan serum leptin dan jumlah pengambilan kalori dalam kalangan perokok lelaki sihat.

Kajian keratan rentas ini telah dijalankan di Universiti Sains Malaysia, Kampus Kesihatan, Kelantan. Perokok lelaki berumur 20-50 tahun telah dipilih secara terarah. Manakala kadar aktiviti merokok telah diukur melalui dua kaedah iaitu analisis nikotin dalam rambut dan soal selidik melalui kertas soalan ujian terhadap kebergantungan nikotin Fagerstrom. Selain itu, sampel darah para peserta yang berpuasa juga telah diambil bagi mengukur kepekatan serum leptin dengan menggunakan kaedah ELISA. Manakala jumlah pengambilan kalori harian para peserta turut dikira melalui temu duga ingatan pengambilan makanan 24 jam.

Seramai 107 peserta telah terlibat dalam kajian ini. Purata umur dan BMI para peserta adalah masing-masing 37.0 (9.42) tahun dan 24.59 (4.33) kg/m^2. Purata bagi kepekatan leptin serum dan aras nikotin dalam rambut dalam setiap kumpulan kebergantungan nikotin rendah, sederhana dan tinggi adalah tidak berbeza secara signifikan. Terdapat korelasi berkadar songsang antara markah Fagerstrom dengan kepekatan leptin serum ($r=-0.198$, $p=0.048$). Manakala tiada korelasi yang signifikan antara aras nikotin dalam rambut dengan kepekatan leptin serum. Purata bagi jumlah pengambilan kalori dalam kumpulan kebergantungan nikotin rendah, sederhana dan tinggi adalah berbeza secara signifikan ($F(2,46)=3.688$, $p=0.03$).Walau bagaimanapun, tiada korelasi yang signifikan antara markah Fagerstrom dengan jumlah pengambilan kalori.

Kajian ini menunjukkan terdapat korelasi berkadar songsang antara kepekatan leptin serum dengan markah Fagerstrom. Selain itu, terdapat juga perbezaan yang signifikan antara purata jumlah pengambilan kalori antara tiga kumpulan yang berlainan kadar kebergantungan nikotin. Kumpulan yang mempunyai kadar kebergantungan nikotin tinggi mempunyai purata jumlah pengambilan kalori yang paling tinggi berbanding dengan kumpulan lain.

THE RELATIONSHIP OF NICOTINE DEPENDENCE WITH SERUM LEPTIN CONCENTRATION AND TOTAL CALORIE INTAKE AMONG HEALTHY MALE SMOKERS IN KOTA BHARU

ABSTRACT

Cigarette smoking has been demonstrated to be associated with lower body weight and its cessation leads to weight gain. This action of smoking on body weight is believed to be nicotine mediated. Exposure to nicotine via tobacco smoking may disrupt leptin release and decrease feeding by increasing leptin concentration, thereby contributing to the inverse relationship between nicotine and body weight. The role of leptin as an appetite suppressor is believed to be responsible for weight changes among smokers. The aim of this study was to determine the association between nicotine dependence with serum leptin concentration and total calorie intake among healthy male smokers.

This cross-sectional study was conducted in Universiti Sains Malaysia, Health Campus, Kelantan. Male smokers aged 20–50 years were purposively recruited. Smoking activity was measured by two methods which are through hair nicotine analysis and Fagerstrom Test for Nicotine Dependence (FTND) Questionnaire. Smokers were divided into low, moderate and high dependence according to Fagerstrom score. Fasting blood samples were collected from the subject and serum leptin concentration was measured by ELISA (a solid-phase two-site enzyme immunoassay). The total calorie intake of the subjects was assessed by 24-hour diet recall interview.

A total of 107 subjects were enrolled. The mean age and BMI of the subjects were 37.00 (9.42) years and 24.59 (4.33) kg/m², respectively. The mean concentration of serum leptin and mean level of hair nicotine among low, moderate and highly nicotine dependent groups were not significantly different. There was a significant inverse correlation between the Fagerstrom score and serum leptin concentration ($r=-0.198$, $p=0.048$). However, there was no significant correlation between hair nicotine level and serum leptin concentration. The mean of total calorie intake among low, moderate and highly smoking dependence groups were significantly different ($F(2,46)=3.688$, $p=0.03$). However, there was no significant correlation between Fagerstrom score and hair nicotine level with total calorie intake.

The present study showed that serum leptin concentration has a significant inverse correlation with Fagerstrome score. There was also a significant difference in the mean of total calorie intake between three groups of nicotine dependent where the highly dependent groups has the highest total calorie intake.

CHAPTER 1

INTRODUCTION

Cigarette smoking is a leading preventable cause of death and disability, causing illnesses including cancer and cardiovascular diseases (WHO, 2011b). It is estimated that there are more than 1.3 billion smokers globally and this number is expected to increase to 1.6 billion by 2025 (Jha, 2012). Based on the data from the 2008-2010 Global Adult Tobacco Survey for adults aged 15 years and older, it indicated that the proportion of adult men smoker ranges from about 20.0% in Brazil, and to over 60.0% in Russia (Jha, 2012). While among adult women, the proportion of smokers ranged from 0.5% in Egypt to 24.0% in Poland (Jha, 2012). The number of global death due to smoking related diseases is expected to reach 8.3 million by 2030, up from 5.4 million in 2005 and 6.4 million in 2015 (Mathers and Loncar, 2006; Alwan, 2010).

Most Asian countries confronted this epidemic as well. Multi-country reports and reviews showed the prevalence of smoking among adult males ranged from 24.3% in India (WHO, 2010b) to 52.9% in China (WHO, 2010a). The prevalence of smoking among adult males in other Asian countries were 43.3% in Bangladesh (WHO, 2009a), 45.6% in Thailand (WHO, 2009b) and 47.7% in the Philippines (WHO, 2010c). These variations may be due to differences in the tobacco control initiative and legislation implemented in those countries (WHO, 2011b).

As for Malaysia, the prevalence of smoking especially among adult male is still high in spite of several campaigns over the past decade. According to the report of the second and third National Health and Morbidity Surveys in 1996 and 2006, respectively, there was only 2.7% reduction in smoking prevalence among males aged 18 years and older which decreased from 49.2% to 46.5% (Lim *et al.*, 2013). In addition, the Global Adult Tobacco Survey (GATS) 2011 showed the prevalence of Malaysian men who currently smoke tobacco which was 43.9% (WHO, 2011a).

One of the main concerns among smoking cessation advocators are the body weight changes among smokers. Cigarette smoking has been demonstrated to be associated with lower body weight and its cessation leads to weight gain particularly in the first several weeks after quitting (Perkins, 1993; Pisinger and Jorgensen, 2007). An inverse relationship between nicotine and body weight exists that supports these concern, where body weight tends to be lower among moderate and heavy smokers as compared to non-smokers (Klesges *et al.*, 1991). This action of smoking on body weight appears to be nicotine mediated as indicated by Chen *et al*.(2004). However, it is also believed that the influence of cigarette smoking on food intake and to a lesser extent energy expenditure account for these weight influences, but specific mechanism by which smoking affect energy balance are not completely clear (Perkins, 1993). On the other hand, other researcher speculate that there was a clustering factors involved in smoking and body weight relationship (Chiolero *et al.*, 2008). The consumption of cigarette smoke, low

physical activity and unhealthy diet will contribute to lower body weight as shown in Figure1.1.

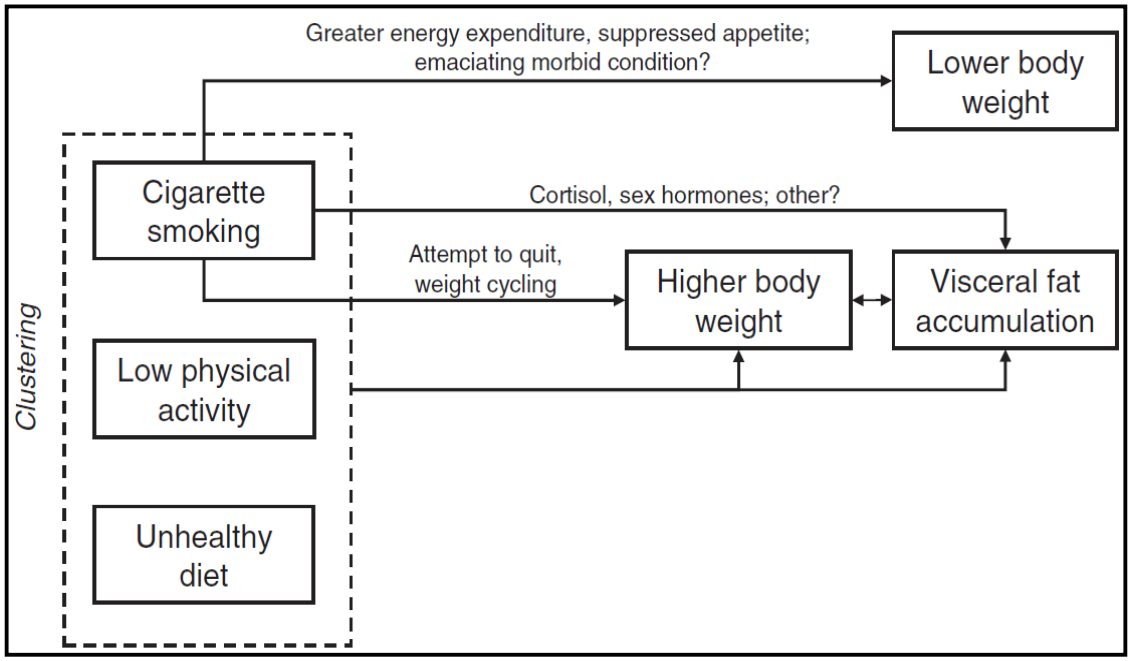

Figure 1.1 Hypothetical factor linking smoking and body weight (Chiolero *et al.* 2008)

Previous studies in animals have reported that nicotine administration decreases body weight and caloric intake (Chen *et al.*, 2004; Bellinger *et al.*, 2009; Bura *et al.*, 2010). On the other hand, chronic smoking has also been reported to modify body weight by decreasing the appetite and increasing metabolic rate through an enhancement of lipid metabolism. It has been suggested that nicotine might act directly on the hypothalamus which is important in the regulation of feeding and energy expenditure (Frankish *et al.*, 1995).

Furthermore, there were other postulations which explained the effect of smoking on body weight. Leptin hormone may be candidate mechanism that contributes to the inverse nicotine-body weight relationship (Oeser *et al.*, 1999; Miyata and Meguid, 2000). The leptin acts as a signaling molecule that communicates the level of adipose stores in the body to the hypothalamus, thus acting centrally to regulate metabolic activity and body weight (Himms-Hagen, 1999). Exposure to nicotine via tobacco smoking may disrupt leptin release, thereby contributing to the inverse relationship between nicotine and body weight. Nicotine as an appetite suppressant may decrease feeding by increasing leptin level or by enhancing steps along the leptin-receptor-mediated signaling cascade. Several studies have attempted to test these hypotheses and all of them found that nicotine induced changes in leptin concentration (Jo *et al.*, 2005).

Nevertheless, among the few human and animal studies that have examined the effects of nicotine and leptin levels among smokers results are inconclusive (Eliasson and Smith, 1999; Miyata and Meguid, 2000). In light of these inconsistent findings, the present study was conducted to investigate the relationship of nicotine dependence with serum leptin concentration and total calorie intake among healthy male smokers.

The present study looked at local data in studying the effect of nicotine dependence and nicotine level on serum leptin concentration and total calorie intake among smokers. A largely available data in literature have reported different results regarding the concentration of leptin hormone among smokers. Some studies found that the

concentration is increased while other studies found that it has decreased. On the other hand, there were studies which reported that leptin concentration was not affected by smoking. Therefore, a study that investigate the exact result of smoking and nicotine effect on leptin concentration is necessary by controlling the factors that might interfere the results such as age, gender, race, health status and physical activity.

On the other hand, the previous studies were not looking into nicotine dependence and nicotine level. There were only two groups involved which are smokers and non-smokers. Dependency and nicotine level might be a factor which affects the final result of leptin concentration. Minimal dependence and low nicotine level could be the answer why leptin concentration was not affected by smoking as reported in some studies. The effect of smoking on leptin concentration must be studied on different group of smokers regarding their dependency and nicotine level to ensure the exact results is obtained.

1.1 OBJECTIVES OF THE STUDY

1.1.1 General Objective

To study the relationship of nicotine dependence with serum leptin concentration and total calorie intake among healthy male smokers in Kota Bharu.

1.1.2 Specific Objectives

1 To determine the concentration of serum leptin among low, moderate and high nicotine dependence group in healthy male smokers.

2 To determine the level of hair nicotine among low, moderate and high nicotine dependence group in healthy male smokers.

3 To determine the total calorie intake among low, moderate and high nicotine dependence group in healthy male smokers.

4 To determine the relationship between hair nicotine level and serum leptin concentration in healthy male smokers.

5 To determine the relationship between hair nicotine level and total calorie intake in healthy male smoker.

1.2 RESEARCH HYPOTHESES

1 The concentration of serum leptin is high in highly nicotine dependence smokers compared to moderate and low nicotine dependent smokers.

2 The level of hair nicotine is high in highly nicotine dependence smokers compared to moderate and low nicotine dependent smokers.

3 The total calorie intake is high in low nicotine dependence smokers compared to moderate and high nicotine dependence.

4 High hair nicotine level is associated with high concentration of serum leptin in smoker

5 High hair nicotine level is associated with lower total calorie intake in smokers.

1.3 DEFINITION OF TERMS

1.3.1 Leptin

Leptin is a hormone that plays a key role in regulating energy intake and energy expenditure include appetite and metabolism. It works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP) and by increasing the activity of neurons expressing α-melanocyte-stimulating hormone (α-MSH). The elevation of leptin hormone reduces appetite and food intake (Dardeno *et al.*, 2010)

1.3.2 Nicotine

Nicotine is an alkaloid derived from the tobacco plant. It is a substance in tobacco to which smokers can become addicted. It also provides pleasure, more less irrespective of the behavioral context. It can induce muscular relaxation, suppressing appetite and body weight, enhancing learning, memory and sustain mental concentration (United Nation Office of drug Control and Crime Prevention Studies, 2000).

1.3.3 Nicotine dependence

Nicotine dependence is defined as a compulsive behavior to consume nicotine on a continuous or periodic basis in order to experience its psychic effect and to avoid discomfort of its absence (WHO, 1993). According to the 4th edition of Diagnostic and Statistical Manual of Mental Disorder (DSM-IV), nicotine dependence is classified under substance dependence and diagnosed when the smoker manifested at least three of the following criteria within the same 12 months period:

1. Development of tolerance :
   - a need for markedly increased amounts of the substance to achieve intoxication or desired effect.
   - markedly diminished effect with continued use of the same amount of the substance.
2. Withdrawal symptoms :
   - the characteristic withdrawal syndrome for the substance.
   - the same substance is taken to relieve or avoid withdrawal symptom.
3. Substance use is longer or is in larger quantities than intended.
4. Permanent wish or failure to control substance use.
5. Time-consuming procurement, use and recovery from substance.
6. Important social, professional or recreational activities are given up or limited due to substance use.
7. Continued substance use despite physical or psychic problems.

(American Psychiatric Association, 2013)

1.3.4 Ever Smokers

Ever smoker is a person who has smoked at least 100 cigarettes in his lifetime. Ever smoker can be a currently daily smoker, reducer or ex-smoker:

- Daily smoker is a person who smokes any tobacco product at least once a day.
- Reducer is a person who used to smoke daily but now does no longer smoke every day.
- Ex-smoker is a person who was formerly a daily smoker but currently does not smoke at all.

(WHO, 2008b)

1.3.5 Current smoker

Adult who have smoked 100 cigarette in their lifetime and currently smoke cigarette everyday or some days (nondaily).

(Centers for Disease Control and Prevention, 2009)

1.3.6 Smoker (in term of hair nicotine level)

Smoker who has level of hair nicotine more than 2.77 ng/mg.

(Kim *et al,*. 2014)

CHAPTER 2

LITERATURE REVIEW

2.1 NICOTINE

Nicotine is a natural compound called alkaloid, which is the major psychoactive substance in tobacco. It has been found that nicotine has both stimulant and relaxing effects. It alters certain hormone in human body and increased the capacity to focus on attention. In other words, it reduces anxiety and irritability (United Nation Office of drug Control and Crime Prevention Studies, 2000).

Nicotine is also known as a key neuroregulatory component in cigarette smoke. It has been suggested that nicotine might act directly on the hypothalamus which is important in the regulation of feeding and energy expenditure (Frankish *et al.*, 1995). Nicotine as an appetite suppressant may decrease feeding by increasing leptin level or by enhancing steps along the leptin-receptor-mediated signaling cascade. Several studies have attempted to test these hypotheses and overall of it appear that nicotine induced changes in leptin levels (Jo *et al.*, 2005). Nicotine also stimulates the dopaminergic pathways of the mesolimbic system in the brain, an area that involved in reinforcement for the drug abuse (Tuesta *et al.*, 2011).

2.1.1 Absorption of nicotine

Nicotine is entered into human body through different ways including inhalation of cigarette smoke, chewing tobacco, oral snuff and nicotine gum will result in different total absorption (Benowitz *et al.*, 2009). However, researchers had also found that inhalation of nicotine in the form of smoke provides the quickest delivery (Benowitz, 1996b; Matta *et al.*, 2007) with nicotine reaching the brain approximately in 7 seconds (Stephen A. Maisto *et al.*, 2010).

Nicotine absorption took place in a few parts of the body include in the mouth and inside the lung (Benowitz *et al.*, 2009). However, its absorption across biological membranes depends on pH. Nicotine is a weak base with a pKa of 8.0 (Benowitz *et al.*, 2009). Nicotine does not rapidly cross membranes in its ionised state such as in acidic environment. In the mouth, only small amount nicotine from cigarette smoke is absorbed (Gori *et al.*, 1986). This is due to the pH of smoke from flue-cure tobaccos which is found in most cigarettes which is acidic (pH 5.5-6.0) as nicotine is ionised at this pH. In contrary, nicotine from air-cured tobaccos which is available in most of tobacco used like in pipes, cigars and some other cigarettes, is well absorbed due to it alkaline (pH 6.5) property and nicotine which is unionised at this pH (Armitage *et al.*, 1978).

However, when cigarette smoke reaches the lung, nicotine is quickly absorbed. Dissolution of nicotine in the fluid at pH 7.4 in the lung facilitates nicotine transfer

across membranes (Benowitz *et al.*, 2009). In addition, nicotine concentration in the blood circulation also rises rapidly during a smoke and peaks at the completion of smoking due to the large surface area of the alveoli and small airways (Benowitz, 1990). High levels of nicotine can reach the brain approximately within 10-20 second after a puff. This is faster than intravenous administration and could produce a rapid behavioral reinforcement (Benowitz, 1990). Therefore, the rapid rise of nicotine level permits the smoker to titrate the level of nicotine and related effects during smoking. This also makes smoking to be considered as the most reinforcing and dependence producing form of nicotine administration (Henningfield and Keenan, 1993).

In addition, a smoker also can manipulate the dose of nicotine and brain nicotine levels on a puff-by-puff as the intake of nicotine during smoking depends on puff volume, depth of inhalation, the extent of dilution with room air and the rate and intensity of puffing (U.S Department of Health and Human Service, 2001). In this regard, a machine-determined nicotine yield of cigarettes is not an accurate tool to estimate the dose of nicotine by a smoker because of this reason (Jarvis *et al.*, 2001). In order to gain more nicotine, cigarette smoker will change their smoking pattern which is switching from a higher to a lower yield cigarette for compensation (USDHHS, 2001).

Chewing tobacco and snuff are buffered to alkaline pH in order to facilitate absorption of nicotine through oral mucosa. Although absorption through cell membranes is rapid for these more alkaline tobacco products, the rise in the brain

nicotine level is slower when compared with smoking. Concentration of nicotine in the blood rises gradually with the use of smokeless tobacco and plateau at about 30 minutes with the levels persisting and declining slowly over 2 hours or more (Benowitz *et al.*, 1988).

Absorption of nicotine from most of nicotine replacement therapy (NRT) such as nicotine gum, transdermal patch, nasal spray, inhaler, sublingual tablets and lozenges, are also slower than smoking (West *et al.*, 2000). Only nasal spray provides a rapid delivery of nicotine that is closer to the rate of nicotine delivery achieved with smoking (Gourlay and Benowitz, 1997; Guthrie *et al.*, 1999). The absolute dose of nicotine absorbed from nicotine gum is much less than the nicotine content of the gum. This is because nicotine is swallowed with subsequent first-pass metabolism (Benowitz *et al.*, 1987). Some nicotine is also retained in chewed gum. The bioavailabilities for these products are mainly contributed by absorption through mucosa of the oral cavity where 50-80% of the nicotine is swallowed and metabolised.

Nicotine base is well absorbed through skin. This contributes to the occupational risk of nicotine poisoning (green tobacco sickness) in tobacco harvesters who are exposed to wet tobacco leaves (McBride *et al.*, 1998). Transdermal delivery technology is essentially based on this phenomenon. Nicotine patch is one of product that used this transdermal delivery technology. It is commonly used as an aid in nicotine replacement therapy (NRT) and effective for quitting smoking (Fiore et al., 1994). The rate of release of nicotine into skin is depend on the permeability of the skin, rate of diffusion through a

polymer matrix and the rate of passage through a membrane in the patches. Therefore, rates of nicotine delivery and plasma nicotine concentrations are vary among different transdermal systems (Fant *et al.*, 2000). In all cases, there is an initial lag time of about 1 hour before nicotine appears in the bloodstream and there is a continued systemic absorption (about 10% of the total dose) after the patch is removed, the latter due to residual nicotine in the skin (Fant *et al.*, 2000).

2.1.2 Distribution of nicotine in body tissues

About 69.0% ionised and 31.0% unionised nicotine enters the bloodstream at pH 7.4 after absorption. Binding to plasma proteins is less than 5% (Benowitz *et al.*, 1982). A steady-state volume of nicotine averaging 2.6 L/kg is distributed extensively to body tissues. Based on human autopsy samples from smokers, the highest affinity for nicotine is in the liver, kidney, spleen and lung and the lowest is in adipose tissue. In the skeletal muscle, concentration of nicotine and cotinine are close to that of whole blood. Nicotine also strongly bound to brain tissues and the receptor binding capacity is increased due to a higher number of nicotinic cholinergic receptors in the brain of the smokers (Perry *et al.*, 1984; Breese *et al.*, 1997). Nicotine also accumulates in gastric juice and saliva due to its ion-trapping capability (Lindell *et al.*, 1996). There is also an evidence that nicotine also accumulates in breast milk (Dahlstrom *et al.*, 1990), fetal serum and amnionic fluid (Dempsey and Benowitz, 2001).

The route and rate of dosing of nicotine will determine the time course of nicotine accumulation in the brain and other body organs and the resultant pharmacologic effect. Smoking a cigarette delivers nicotine rapidly to the pulmonary venous circulation, from which it moves quickly to the left ventricle of the heart and to the systemic arterial circulation and brain. The lag time between a puff of a cigarette and nicotine reaching the brain is 7 seconds (Stephen A. Maisto *et al.*, 2010).

2.1.3 Biomarker for nicotine exposure

Nicotine is metabolized to a number of metabolites in the liver including cotinine, nicotine *N*'-oxide, *N*-isomethonium ion, nicotine glucuronide, 4-oxo-4-(3-pyridyl)-butanoic acid and nornicotine. Notably, it is about 70.0% to 80.0% of nicotine is being converted to cotinine. This made it the highest amount of nicotine metabolite in human's body (Benowitz, 2009).

Nicotine and cotinine are the most common alkaloid being measured as a biomarker for nicotine exposure. Cotinine has been used as a biomarker for daily intake, both in cigarette smokers and in those exposed to second tobacco smoke because of its long half-life (Benowitz, 1996a). The presence of cotinine in biological fluids such as plasma, saliva and urine indicates exposure to nicotine (Benowitz, 1996a). Although there is a high correlation between steady state cotinine and nicotine intake, there are few individual variabilities that might influence the result including the difference

conversion percentages of nicotine to cotinine (usually range between 50% to 90%) and difference in cotinine metabolism rates (usual clearance range 20-75 ml min^{-1}) (Benowitz, 1996a). A limitation of measuring cotinine and nicotine from latter matrices as a marker for nicotine exposure is that it only reflects the short-term exposure to tobacco and not for chronic exposure because of its average half-life is 16 hours which is relatively short (Benowitz, 2009).

When measuring the long-term exposure to smoking, the most recommended way is to measure the nicotine by using hair or nails sample compared to other matrices (Al-Delaimy, 2002). This is because nicotine is incorporated into hair and nail as it grow over time. The average rate of hair growth is 1 cm per month while nails grow at a rate of 0.1 cm per month. Thus, measurement of nicotine level in hair and nails are a promising biomarker for long-term tobacco exposure similar to the measurement involving chronic smokers (Al-Delamy, 2002; Florescu *et al.*, 2007).

2.2 HAIR NICOTINE LEVEL MEASUREMENT

Hair nicotine level measurement has been proposed as one of the methods to determine the smoking exposure among smokers (Al-Delaimy, 2002; Appenzeller *et al.*, 2012). Assessment based on cigarettes per day might be inaccurate indicators for cigarette smoke exposure because of the differences in how smokers smoke their cigarettes and the brand of cigarettes itself (Patrick *et al.*, 1994; Al-Delaimy *et al.*, 2000; USDHHS, 2001). On the other hand, the hair has the capacity in providing more accurate long term information on an individual's exposure to cigarette smoke compared to other biological matrices (Al-Delaimy *et al.*, 2002; Appenzeller *et al.*, 2012). The usefulness of nicotine concentration in body fluids like in urine, saliva and blood serum, is restricted by the short half-life of these biomarkers. The half-life of nicotine in body fluid is approximately 2-3 hours (Jaakkola and Jaakkola, 1997). Other advantages of hair as a sample matrix include the non-invasiveness of its collection, easy to store and stable (Al-Delaimy, 2002; Appenzeller *et al.*, 2012). The nicotine compound in hair is not lost by storage of samples for a period of up to five years (Zahlsen and Nilsen, 1994).

It is known that hair at the scalp grows about 1 cm for one month (Myers and Hamilton, 1951; Zahlsen and Nilsen, 1990; Harkey, 1993; LeBeau *et al.*, 2011). Its growth is considered as the most uniform and faster than other hair in other parts of the body such as axillary and pubic. It is also noted that about 85-90% of scalp hair grows continuously (Harkey, 1993). Therefore, hair at the scalp is considered as the best site for sample collection. The continuous growth and minimum non-growing of hair scalp

can provide updated information on exposure to any related substances include nicotine (Al-Deilamy, 2002). In addition, the use of this method could avoid bias such as under reporting and lack of awareness of exposure usually faced during answering the questionnaire (Al-Delaimy, 2002).

Historically, the presence of nicotine in human hairs was first discovered by Ishiyama *et al.*, (1983) and further evidenced by other group of researchers from different countries (Mizuno *et al.*, 1993; Eliopoulus *et al.*, 1996; Al-Delaimy *et al.*, 2000; Kim *et al.*, 2008). The result from this measurement also had been further compared with a number of cigarettes per day in previous studies in order to show its usefulness and reliability in providing information on smoking exposure (Mizuno *et al.*, 1993; Eliopoulus *et al.*, 1996; Al-Delaimy *et al.*, 2000; Kim *et al.*, 2008). Hair nicotine can be analysed by using the radioimmunoassay (RIA) (Haley and Hoffman, 1985), gas or liquid chromatography method. Gas and liquid chromatography are the preferred analysis method since it provides more accuracy and cost-effective compared to RIA (Zahlsen and Nilsen, 1994; Chetiyanukornkul *et al.*, 2004; Kim *et al.*, 2008; Man *et al.*, 2009).

However, there is a potential problem with the use of hair as a biomarker includes a strong influence of hair pigmentation on nicotine binding and uptake where the nicotine is bound to melanin. This means a dark hair binds much more nicotine than does blonde and white hair (Dehn *et al.*, 2001). In addition, hair also is exposed to

nicotine from sweat, sebaceous gland secretion and nicotine from environmental tobacco smoke exposure. Therefore, washing the hair before analysis may reduce this environment contamination problem (Al-Delaimy, 2002). Apart from that, racial and ethnic difference also had influence on nicotine level in human hair. For example, Black smokers were found to have a higher hair nicotine level compared to White smokers with the same smoking exposure (Apelberg *et al.*, 2011).

2.3 LEPTIN

Leptin is a 16-kDa hormone, originated from the ob (obese) gene. It has been discovered and cloned in 1994. It is synthesised and secreted specifically from white adipose cells (Zhang *et al.*, 1994). Leptin has multiple central and peripheral actions in regulation of energy balance and metabolism, feeding behavior, fertility, and bone metabolism. These actions are mediated by specific cell surface leptin receptors (Schwartz *et al.*, 1996; Margetic *et al.*, 2002). The leptin receptor (LR), which possesses only one transmbrane domain, is known to be presented in the choroids plexus, cerebral cortex, hippocampus, thalamus, and hypothalamus (Tartaglia *et al.*, 1995). The Ob(Lep) gene (Ob for obese, Lep for leptin) is located on chromosome 7 in humans. Leptin interacts with six types of leptin receptors isoform which are *ObRa, ObRb, ObRc, ObRd, ObRe*, and *ObRf* (Lee *et al.*, 1996). These isoforms classified to two groups which are short and long leptin receptor isoform. The short leptin receptor isofrom *ObRa* and *ObR*c are responsible for transporting leptin across the blood-brain barrier while the long leptin receptor isoform *ObRb* plays it role in leptin signaling (Dardeno *et al.*, 2010). This ObRb leptin receptor is expressed in a variety of organs primarily in the hypothalamus, where it regulate energy homeostasis and neuroendocrine function (Dardeno *et al.*, 2010).

Leptin secretion can be promoted by few conditions including excess energy stored as fat, overfeeding, the present of glucose, insulin, glucocorticoids, estrogens and inflammatory cytokines (Dardeno *et al.*, 2010). Leptin secretion can be inhibited by few factor including low energy states with decreased fat stored (leanness), fasting,

catecholamines and adrenergic agonists, thyroid hormones and androgens (Dardeno *et al.*, 2010).

Apart from being a biomarker for body fat, serum leptin levels also reflect individuals energy balance (Friedman and Halaas, 1998). Multiple studies have shown that fasting or following a very low caloric diet (VLCD) lower the leptin levels (Weigle *et al.*, 1997; HALUZÃ KOVÃ *et al.*, 2006; Morel *et al.*, 2011). It might be that on short term, leptin is an indicator of energy balance (Lin *et al.*, 2001). Additionally, other researchers have proposed that the fluctuation of leptin hormone is more sensitive to starvation than to overfeeding (Ahima *et al.*, 1996). For example, the leptin levels do not rise extensively after over feeding (Romon *et al.*, 1999). It might be that the dynamic of leptin due to an acute change in energy balance are related to appetite and eventually to food intake (Lin *et al.*, 2001).

Leptin binds to the ventromedial nucleus of the hypothalamus, known as the "appetite center". Thus, circulating leptin levels give the brain input regarding energy storage so it can regulate appetite and metabolism.

2.3.1 Leptin and dietary intake

Researchers have proposed that the level of leptin hormone can be influenced by various factors including dietary intake (Koutsari *et al.*, 2003, Murakami *et al.*, 2007). The level of leptin has been observed to be higher among healthy postmenopausal women after the

consumption of high-carbohydrate and high-sugar diet (Koutsari *et al.*, 2003). In other studies, Murakami *et al.* (2007) found the same result where the intake of dietary fiber increased serum leptin concentration among young Japanese women. The results showed the increasing of dietary fiber was associated with lower serum leptin concentration independent of potential confounding factors, including body mass index. In contrary, the level of circulating leptin is reduced among obese subjects in response to energy-restrictive diets as a consequence of body weight loss (Considine *et al.*, 1996).

2.3.2 Leptin and physical activity

Apart from the influence of dietary intake, researches have proposed that the level of circulating leptin is also influenced by physical activities. It has been proved by some researchers that both of short and long-term exercises have shown reduction or no change in leptin concentration (Koutsari *et al.*, 2003). Study showed the addition of daily moderate intensity exercise among postmenopausal women suppressed both fasting and postprandial plasma leptin (Koutsari *et al.*, 2003). This finding has been supported by the later study based on mildly obese women and men who underwent a supervised diet and exercise intervention. Both of women and men subjects showed a significant decreased in serum leptin level (Volpe *et al.*, 2008). Recently, Azizi (2011) found a lower serum leptin level among un-trained females after underwent a 8-weeks aerobic training (Azizi, 2011). This indicates that although the increase in serum leptin is indirect proportion with BMI in general, the major determinant of serum leptin level is the body fat as regular exercise reduces body fat rate, it also reduces serum leptin level.

In contrary, other studies revealed that both of short and long-term exercise did not affect leptin concentration (Weltman *et al*,. 2000; Kraemer *et al.*, 2002). Weltman *et al.* (2000) reported that 30 minutes of exercise at, above and below lactate threshold (an index of accelerated metabolism and exercise intensity) did not alter leptin concentration in young males during exercise or recovery when compared with control values. The same finding has been observed by Zafeiridis *et al.* (2003) which recruited 10 young lean men to examine the effect of maximum strength, muscular hypertrophy and strength endurance resistance exercise protocols on serum leptin. There was no change in serum leptin concentration when sampled immediately after exercise or 30 minutes of recovery.

2.3.3 Leptin and obesity

Although leptin is a circulating signal that reduces appetite, in general, obese people have an unusual high circulating concentration of leptin. These peoples are found to be resistant to the effect of leptin, in much the same way that people with type 2 diabetes are resistant to the effect of insulin (Enriori *et al.*, 2006; Myers *et al.*, 2008). The loses ability of elevated leptin to suppress feeding and increased energy expenditure also known as leptin resistance (Myer *et al.*, 2008). Leptin resistance can be promoted by a few mechanism including leptin-stimulated phosphorylation of Tyr985 on LRb and the suppressor of cytokine signaling 3 which attenuate leptin signaling (Myer *et al.*, 2008).