# THE ASSOCIATION OF METABOLIC SYNDROME RISK FACTORS WITH SERUM HIGH-MOLECULAR WEIGHT ADIPONECTIN AND URINARY METABOLITES AMONG THE ORANG ASLI IN MALAYSIA

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# THE ASSOCIATION OF METABOLIC SYNDROME RISK FACTORS WITH SERUM HIGH-MOLECULAR WEIGHT ADIPONECTIN AND URINARY METABOLITES AMONG THE ORANG ASLI IN MALAYSIA

**by**

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## LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMNS

| | |
|---|---|
| AA | Amino acid |
| AAs | Amino acids |
| Ab | Antibody |
| ACC | Acetyl-coenzyme A carboxylase |
| ACE | Angiotensinogen-converting enzyme |
| ACN | Acetonitrile |
| ACR | Albumin/creatinine ratio |
| AdipoR1 | Adiponectin receptor 1 |
| AdipoR2 | Adiponectin receptor 2 |
| ADP | Adenosine 5′-diphosphate |
| AHA/NHLBI | American Heart Association/National Heart, Lung, and Blood Institute |
| AMPK | Adenosine monophosphate-activated protein kinase |
| ANGPTL2 | Angiopoietin-like protein 2 |
| ATP | Adenosine 5′-triphosphate |
| ATP III | Adult Treatment Panel III |
| AUC | Area under the curve |
| AUROC | Area under ROC curve |
| BA | Bile acid |
| BAs | Bile acids |
| BAT | Brown adipose tissue |
| BIA | Bioelectrical impedance analysis |
| BMI | Body mass index |

| | |
|---|---|
| BUN | Blood urea nitrogen |
| CAD | Coronary artery disease |
| cDNA | Complementary deoxyribonucleic acid |
| CI | Confidence interval |
| CPDRL | Centre for Pathology Diagnostic and Research Laboratories |
| CRP | C-reactive protein |
| CV | Coefficients of variability |
| CVD | Cardiovascular disease |
| CVDs | Cardiovascular diseases |
| DDAH | Dimethylarginine dimethylaminohydrolase |
| DXA | Dual energy x-ray absorptiometry |
| EGP | Endogenous glucose production |
| ELISA | Enzyme-linked immunosorbent assay |
| ESI | Electrospray ionization |
| FA | Fatty acid |
| FAs | Fatty acids |
| FFA | Free fatty acid |
| FFAs | Free fatty acids |
| FCR | Fractional catabolic rate |
| FDR | False discovery rate |
| FFA | Free fatty acid |
| G6PDH | Glucose-6-phosphate dehydrogenase |
| GIR | Glucose infusion rate |
| GLUT4 | Glucose transporter 4 |
| $H_2O_2$ | Hydrogen peroxide |

| | |
|---|---|
| $H_2SO_4$ | Sulfuric acid |
| HbA1c | Glycated hemoglobin |
| HDL | High-density lipoprotein |
| HDL-C | High-density lipoprotein cholesterol |
| HK | Hexokinase |
| HMDB | Human Metabolome Database |
| HMW | High-molecular weight |
| HOMA-IR | Homeostasis model assessment of insulin resistance |
| HRP | Horse radish peroxidase |
| hs-CRP | High-sensitivity C-reactive protein |
| HSD | Honestly significant difference |
| HSL | Hormone-sensitive lipase |
| IAF | Intra-abdominal fat |
| IASO | International Association for the study of Obesity |
| IDF | International Diabetes Federation |
| IFG | Impaired fasting glucose |
| IGT | Impaired glucose tolerance |
| IL-6 | Interleukin-6 |
| IOTF | International Obesity Task Force |
| IPH | Institute for Public Health |
| IQR | Interquartile range |
| IR | Insulin resistance |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| L/A | Leptin to adiponectin |
| LC | Liquid chromatography |

| | |
|---|---|
| LCFA | Long chain fatty acids |
| LC-MS | Liquid chromatography-mass spectrometry |
| LC-QTOF-MS | Liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry |
| LDL | Low-density lipoprotein |
| LDL-C | Low-density lipoprotein cholesterol |
| LIPID MAPS | Lipid Metabolites and Pathways Strategy |
| LMW | Low-molecular weight |
| LPL | Lipoprotein lipase |
| MCFA | Medium chain fatty acids |
| MCP-1 | Monocyte chemoattractant protein-1 |
| MetS | Metabolic syndrome |
| MFE | Molecular Feature Extraction |
| MMW | Middle-molecular weight |
| MRI | Magnetic resonance imaging |
| NEFA | Non-esterified fatty acid |
| NCEP ATP III | National Cholesterol Education Program Adult Treatment Panel III |
| NGT | Normal glucose tolerant |
| NHANES | National Health and Nutrition Examination Survey |
| NHMS | National Health and Morbidity Survey |
| OA | Orang Asli |
| OPD | O-phenylenediamine |
| PAD | Peripheral artery disease |
| PAI-1 | Plasminogen activator inhibitor-1 |

| | |
|---|---|
| PCA | Principal component analysis |
| PEG | Polyethylene glycol |
| PMI | Planned myocardial infarction |
| POD | Peroxidase |
| PPAR | Peroxisome proliferator activated receptor |
| QC | Quality control |
| QTOF | Quadrupole-time-of-flight |
| RBP4 | Retinol binding protein 4 |
| Rf | Relative flotation index |
| ROCCET | ROC Curve Explorer & Tester |
| SAA | Serum amyloid A |
| SAT | Subcutaneous adipose tissue |
| SCAT | Subcutaneous abdominal fat |
| SCFA | Small chain fatty acids |
| SD | Standard deviation |
| SPSS | Statistical Package for the Social Sciences |
| SUA | Serum uric acid |
| T2DM | Type 2 diabetes mellitus |
| TAG | Triacylglycerol |
| TC | Total cholesterol |
| TCA | Tricarboxylic acid |
| TG | Triglyceride |
| THM | Tetrahydrometabolites |
| TNF-α | Tumor necrosis factor-α |
| UAE | Urinary albumin excretion |

| | |
|---|---|
| UiTM | Universiti Teknologi MARA |
| USM | University Sains Malaysia |
| UPLC-qTOF-MS | Ultra performance liquid chromatography coupled to electrospray ionization quadrupole time of flight mass spectrometry |
| VAT | Visceral abdominal fat |
| VAT | Visceral adipose tissue |
| VEGF | Vascular endothelial growth factor |
| VLCFA | Very long chain fatty acids |
| VLDL | Very low density lipoprotein |
| VLDL B | Very low density lipoprotein B |
| VLDL–TAG | Very low density lipoprotein triacylglycerols |
| VIF | Variance inflation factor |
| WAT | White adipose tissue |
| WC | Waist circumference |
| WHO | World Health Organization |
| WHR | Waist hip ratio |
| WHtR | Waist height ratio |

**PERKAITAN FAKTOR-FAKTOR RISIKO SINDROM METABOLIK DENGAN ADIPONEKTIN BERAT MOLEKUL TINGGI SERUM DAN METABOLIT-METABOLIT URIN DALAM KALANGAN ORANG ASLI DI MALAYSIA**

## ABSTRAK

Orang Asli (OA) merupakan orang asal di Semenanjung Malaysia. Secara keseluruhan, terdapat lapan belas suku kaum OA yang dikategorikan di bawah tiga kaum utama iaitu Senoi, Melayu Proto, dan Negrito. Perkaitan faktor-faktor risiko sindrom metabolik dengan adiponektin berat molekul tinggi serum dan metabolit-metabolit urin dalam kalangan OA jarang dilaporkan. Tujuan kajian ini adalah untuk menentukan perkaitan antara faktor-faktor risiko sindrom metabolik dengan adiponektin berat molekul tinggi serum dan metabolit-metabolit urin dalam kalangan OA di Semenanjung Malaysia. Kaedah kajian yang dijalankan adalah keratan rentas dan pemilihan lokasi kajian adalah menerusi kaedah persampelan rawak mudah. Enam lokasi suku kaum OA telah dipilih iaitu Che Wong, Kensiu, Semai, Orang Kanaq, Lanoh, dan Bateq. Kaedah persampelan bertujuan dan bola salji telah digunakan untuk memilih 185 responden yang berumur 18 tahun dan ke atas. Ukuran berat, ketinggian, ukur lilit pinggang, dan tekanan darah direkodkan. Sampel darah subjek yang berpuasa semalaman telah dianalisis untuk mengkaji profil lipid, glukosa plasma, dan adiponektin berat molekul tinggi serum manakala sampel urin untuk mengkaji profil metabolit dengan menggunakan pendekatan metabolomiks. Secara keseluruhan prevalens sindrom metabolik adalah 29.7% (55/185). Prevalens sindrom metabolik secara signifikan lebih tinggi dalam kalangan responden wanita (36.2%)

berbanding lelaki (21.3%). Sindrom metabolik juga adalah lebih tinggi di kalangan suku kaum yang tinggal di kawasan pinggir bandar iaitu Orang Kanaq (81.8%) dan Kensiu (36.4%) dan lebih rendah di kawasan pedalaman iaitu Semai (23.8%) dan Bateq (8.0%) ($p$<0.001). Responden wanita dilaporkan secara signifikan mempunyai kadar peningkatan ukur lilit pinggang (45.7% vs. 2.5%, $p$<0.001) dan penurunan aras lipoprotein berdensiti tinggi (69.5% vs. 31.3%, $p$<0.001) yang lebih tinggi berbanding dengan lelaki. Analisis hasil kawasan bawah ROC lengkung menunjukkan 22 metabolit dikenalpasti sebagai penanda bio metabolit urin bagi penyakit sindrom metabolik dengan kawasan di bawah lengkung sekurang-kurangnya 0.7. Model analisis regresi linear berganda menunjukan adiponektin berat molekul tinggi serum mempunyai korelasi secara negatif dengan ukur lilit pinggang (β=-0.07; $p$=0.001) dan jantina (β=-1.53; $p$<0.001) tetapi mempunyai korelasi secara positif dengan umur (β=0.05; $p$=0.004). Selain daripada itu, asid 3-ethyl-3-methyl-tridecanoic ($C_{16}H_{32}O_2$) urin mempunyai korelasi secara positif dengan tekanan darah sistolik (β=0.06; $p$=0.031). Metabolit urin ini tidak mempunyai korelasi dengan adiponektin berat molekul tinggi serum dalam model analisis regresi linear berganda tetapi mempunyai korelasi dalam analisis korelasi Spearman. Kajian ini dapat memberi petunjuk dan maklumat tambahan bagi mekanisma patogenik sindrom metabolik dalam kalangan OA.

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## ABSTRACT

Orang Asli (OA) are the indigenous people of Peninsular Malaysia. Overall, there are 18 subtribes of OA which are categorised under three main tribes namely Senoi, Proto Malay, and the Negrito. The association of metabolic syndrome (MetS) risk factors with serum high-molecular weight (HMW) adiponectin and urinary metabolites among OA tribes are scantly reported. The purpose of this study was to determine the association of MetS risk factors with serum HMW adiponectin and urinary metabolites among the OA population in Peninsular Malaysia. This cross-sectional study was conducted according to the geographical locations of OA subtribes namely Che Wong, Kensiu, Semai, Orang Kanaq, Lanoh, and Bateq by simple random sampling method. The purposive and snow-ball sampling methods were used to select 185 respondents aged 18 years and above. The respondents were measured for their weight, height, waist circumference (WC), and blood pressure. Overnight fasting venous blood samples were analysed for lipid profiles, plasma glucose, and HMW adiponectin while urine samples were analysed for metabolite profiles using metabolomics approach. The overall prevalence of MetS was 29.7% (55/185). MetS prevalence was significantly higher in female (36.2%) compared to male (21.3%) respondents. MetS was also higher among the suburban Orang Kanaq (81.8%) and Kensiu (36.4%) subtribes and lower among rural Semai (23.8%) and Bateq (8.0%) subtribes ($p$<0.001). Females had significantly higher rates of high WC

(45.7% vs. 2.5%, $p$<0.001) and low HDL-C (69.5% vs. 31.3%, $p$<0.001) compared to males. Area under ROC curve (AUROC) analysis showed that 22 metabolites were determined as potential urinary metabolite biomarkers of MetS with area under the curve (AUC) of at least 0.7. Multiple linear regression models revealed that HMW adiponectin were negatively associated with WC (β=-0.07; $p$=0.001) and sex (β=-1.53; $p$<0.001) but positively associated with age (β=0.05; $p$=0.004). Besides, urinary 3-ethyl-3-methyl-tridecanoic acid ($C_{16}H_{32}O_2$) level was positively associated with systolic blood pressure (β=0.06; $p$=0.031). This urinary metabolite was not associated with HMW adiponectin in multiple linear regression models but it was correlated with HMW adiponectin in Spearman correlation analysis. This study could provide clues and additional insight into the pathogenic mechanism of MetS among OA population.

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of the study

Metabolic syndrome (MetS) is a constellation of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) risk factors namely hyperglycemia, elevated triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL-C) levels, raised blood pressure, and obesity (particularly central adiposity) (Alberti *et al.*, 2009). It can be defined using several definitions including World Health Organization (WHO) (Alberti and Zimmet, 1998), National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) (NCEP ATP III, 2001), International Diabetes Federation (IDF) (Alberti *et al.*, 2005) and the recently proposed Harmonized IDF criteria (Alberti *et al.*, 2009).

MetS is a complex disorder (Kassi *et al.*, 2011) that associated with adverse health outcomes and become one of growing problem worldwide (Schlaich *et al.*, 2015). National Health and Nutrition Examination Survey (NHANES) data from 2009 to 2010 indicated that the prevalence of MetS in United States was 22.9% based on Harmonized criteria (Beltran-Sanchez *et al.*, 2013). Ten population based cohort studies in seven countries in Europe observed that the MetS rate in obese subjects ranged from 43% to 78% and 24% to 65% in men and women, respectively (Van Vliet-Ostaptchouk *et al.*, 2014).

The high prevalence of MetS not only affects developed countries but also some developing countries. Using the ATP III criteria which was same as Europe, a higher rate of MetS was observed among males (47.2%) compared to females (40.3%) in Saudi Arabia population (Al-Daghri *et al.*, 2014). In a cross-sectional study in China, the prevalence of MetS was also higher in males (27.6%) than females (24.4%) based on Harmonized definition (Xu *et al.*, 2014). Meanwhile in Malaysia, 42.5% of Malaysian adults were diagnosed with MetS where 43.9%, 42.1%, and 51.9% were reported in Malay, Chinese, and Indian ethnic groups respectively (Mohamud *et al.*, 2011). Moreover, the prevalence of MetS among Malaysians aged 15 years and older was higher among Indians (35.6%) compared to Indigenous Sarawakians (30.5%), Malays (26.4%) and Chinese (26.2%) (Rampal *et al.*, 2012).

Another study among Malay adults in Malaysia observed that the prevalence was reported around 31.9% on the basis of the modified NCEP ATP III criteria. (Chu and Moy, 2014). The prevalence rates of MetS in Malaysian T2DM patients were 95.8%, 96.1%, 84.8%, and 97.7% according to the WHO, NCEP ATP III, IDF, and the Harmonized definitions respectively (Tan *et al.*, 2013). The MetS is not only emerge among main population of Malaysia but also minority population which is Orang Asli (OA). A study conducted in 2010 reported that the prevalence of MetS among OA based on IDF criteria was 22.7% (Mohamud and Suraiami, 2010).

Adiponectin is an adipokines that has association with the MetS risk factors. Low serum adiponectin concentrations are associated with multiple phenotypic traits of MetS including decreased insulin sensitivity, increased abdominal fat distribution,

and reduced HDL-C levels (Lara Castro *et al.*, 2008). As a result, low circulating adiponectin level has been proposed as a biomarker for the MetS (Ryo *et al.*, 2004; Trujillo and Scherer, 2005). Previous study indicated that high-molecular weight (HMW) adiponectin is the active form of the protein (Hara *et al.*, 2006) and might be a more useful predictor than total adiponectin for assessing T2DM risk (Nakashima *et al.*, 2006). According to Hara *et al.* (2006), the HMW ratio values has better predictive power than total adiponectin for prediction insulin resistance (IR) and MetS in humans (Hara *et al.*, 2006). The HMW adiponectin complex is the most active form of adiponectin in depressing blood glucose levels in mice (Pajvani *et al.*, 2004). Moreover, HMW adiponectin that has antiatherogenic factors can prevent endothelial cells from apoptosis (Kobayashi *et al.*, 2004).

Besides adiponectin, there is association of MetS risk factors with endogenous metabolites. Urinary nicotinuric acid metabolite that correlated with MetS risk factors (Blood pressure, TG, and HDL-C), body mass index (BMI), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), glycated hemoglobin (HbA1c), and high-sensitivity C-reactive protein (hs-CRP) could represents a potential biomarker of MetS (Huang *et al.*, 2013). The presence of metabolic risk factors including IR, obesity, high blood pressure, and dyslipidemia are significantly associated with serum metabolites such as branched-chain amino acids, other hydrophobic amino acids, tryptophan breakdown products, and nucleotide metabolites (Cheng *et al.*, 2012).

Bile acid (BA) metabolite in serum shows several positive and statistically significant correlations with single components of the MetS, namely BMI, glucose,

TG, homeostasis model assessment of insulin resistance (HOMA-IR), and HbA1c (Steiner *et al.*, 2011). Further, branched-chain and aromatic amino acid metabolites in serum are linked with hyperglycemia in the general population study (Würtz *et al.*, 2012). The association of adiponectin can also be observed with urinary metabolite. Increased urinary tetrahydrometabolites (THM) not only correlated with dyslipidemia, IR, β cell dysfunction, weight, and WC but it also correlated with hypoadiponectinemia (Baudrand *et al.*, 2011).

There have been many health studies conducted on OA focusing on nutritional status (Haemamalar *et al.*, 2010; Hian and Leng, 1998; Lin, 1988; Wong *et al.*, 2015), parasitic infections (Al-Mekhlafi *et al.*, 2013; Anuar *et al.*, 2012), anemia (Al-Mekhlafi *et al.*, 2008) and cardio-metabolic factors (Phipps *et al.*, 2015). A study on MetS has been conducted among female OA and the focus was mainly on prevalence of the disease (Mohamud and Suraiami, 2010). To date, there is no study yet on the association of MetS risk factors with serum HMW adiponectin and urinary metabolites among the OA tribes in Peninsular Malaysia.

## 1.2 Rationale of the study

The large increase in incidence of obesity in both developed and developing countries has led to an escalating incidence of T2DM and CVD risk factors which collectively termed as MetS (Schlaich *et al.*, 2015). The scant report on MetS prevalence among OA makes it impossible to provide trend of the disease in this population. However, this prevalence is expected to increase for the next decade as most OA population undergo resettlement programme which directly exposed them

to urbanised lifestyle. According to Kaur (2014), the increasing of the MetS are due to surplus energy intake, increasing obesity, urbanization, and sedentary life habits. Therefore it is important to search for greater insight about mechanisms contributing to the MetS development in this population.

Adiponectin, a fat cell-secreted hormone is one of the factors contribute to the MetS development. Low circulating levels of adiponectin have been linked to several components of the MetS such as elevated WC, elevated TG, reduced HDL-C, and elevated blood pressure (Yun *et al.*, 2011). Adiponectin that is abundantly expressed in adipose tissue has inverse association with IR (Mente *et al.*, 2010), MetS (Kim *et al.*, 2013), and T2DM (Jee *et al.*, 2013; Ley *et al.*, 2008).

A detailed understanding of the MetS pathophysiology could be achived by using metabolomics technology. The measurement of small molecule metabolites provides insight into changes in chemical ‘‘signature’’ due to specific cellular processes and environmental exposures. Profiles of urinary metabolites in individual that has MetS trait are useful in eluciding the potential roles of specific metabolites and the pathways underlying MetS disease. An earlier studies using metabolomics have identified urinary metabolite biomarkers and its mechanism for T2DM (Jankevics *et al.*, 2009) and atherosclerosis (Zhang *et al.*, 2009a).

Many recent studies on the associations between MetS risk factors with HMW adiponectin and urinary metabolites come mainly from South America (von Frankenberg *et al.*, 2014), Asia such as Japan (Saisho *et al.*, 2013), Taiwan (Huang *et al.*, 2013), and China (Yu *et al.*, 2014). However, to the best of my knowledge there

are no studies and data available in Malaysia especially among OA population. Therefore, the study need to be established as ethnic-specific differences could modify the relationship between MetS risk factors with HMW adiponectin and urinary metabolites.

## 1.3 Significance of the study

This study provides an updated figure of health status and fundamental database for serum of adults OA population. Important parameters related to nutritional status and MetS was examined throughout the study including anthropometry and biochemical parameters such as glucose, HDL-C, TG, and HMW adiponectin levels. Besides, urinary metabolites from various classes such as fatty acids (FAs), amino acids (AAs), bile acids (BAs), and eicosanoids have been measured and profiled by using metabolomics technology. The profiling of metabolites provides curation database, novel biomarkers, and biochemical pathways for diagnosis and prognosis of MetS and related disorders in the study population. The discovery of urinary metabolite may add an useful and crucial information on MetS in molecular level. Its association with MetS risk factors and HMW adiponectin may provide greater pathophysiological understanding of disease.

In future, the biomarkers discovered through metabolomics technology could be used by doctors as a routine clinical practice to treat the patients. Furthermore, this study may help scientist and researchers to stimulate multiple research ideas especially in the aspect of epidemiology, public health, nutrition, metabolomics, and biomarker discovery towards Malaysian population specifically OA.

## 1.4 Objectives

### 1.4.1 General objective

To determine the association of MetS risk factors with serum HMW adiponectin and urinary metabolites among several subtribes of OA in Peninsular Malaysia.

### 1.4.2 Specific objectives

i. To assess the prevalence of MetS among the OA.
ii. To assess individual risk factors of MetS among the OA.
iii. To profile the urinary metabolites in MetS and non-MetS of OA.
iv. To identify potential urinary metabolites of MetS.
v. To investigate the association between MetS risk factors and HMW adiponectin.
vi. To investigate the association between MetS risk factors and urinary metabolite.
vii. To investigate the association between HMW adiponectin and urinary metabolite.

## 1.5 Research questions

i. What is the prevalence of MetS among the OA in Peninsular Malaysia?
ii. What are the individual risk factors of MetS among the OA?
iii. What are the urinary metabolite profiles in MetS and non-MetS of OA?

iv. What is the potential urinary metabolites of MetS?

v. Is there any association between MetS risk factors and HMW adiponectin?

vi. Is there any association between MetS risk factors and urinary metabolite?

vii. Is there any association between HMW adiponectin and urinary metabolite?

## 1.6 Null hypotheses

i. There are no difference in the urinary metabolite profiles in MetS and non-MetS of OA tribes.

ii. There are no association between MetS risk factors and HMW adiponectin.

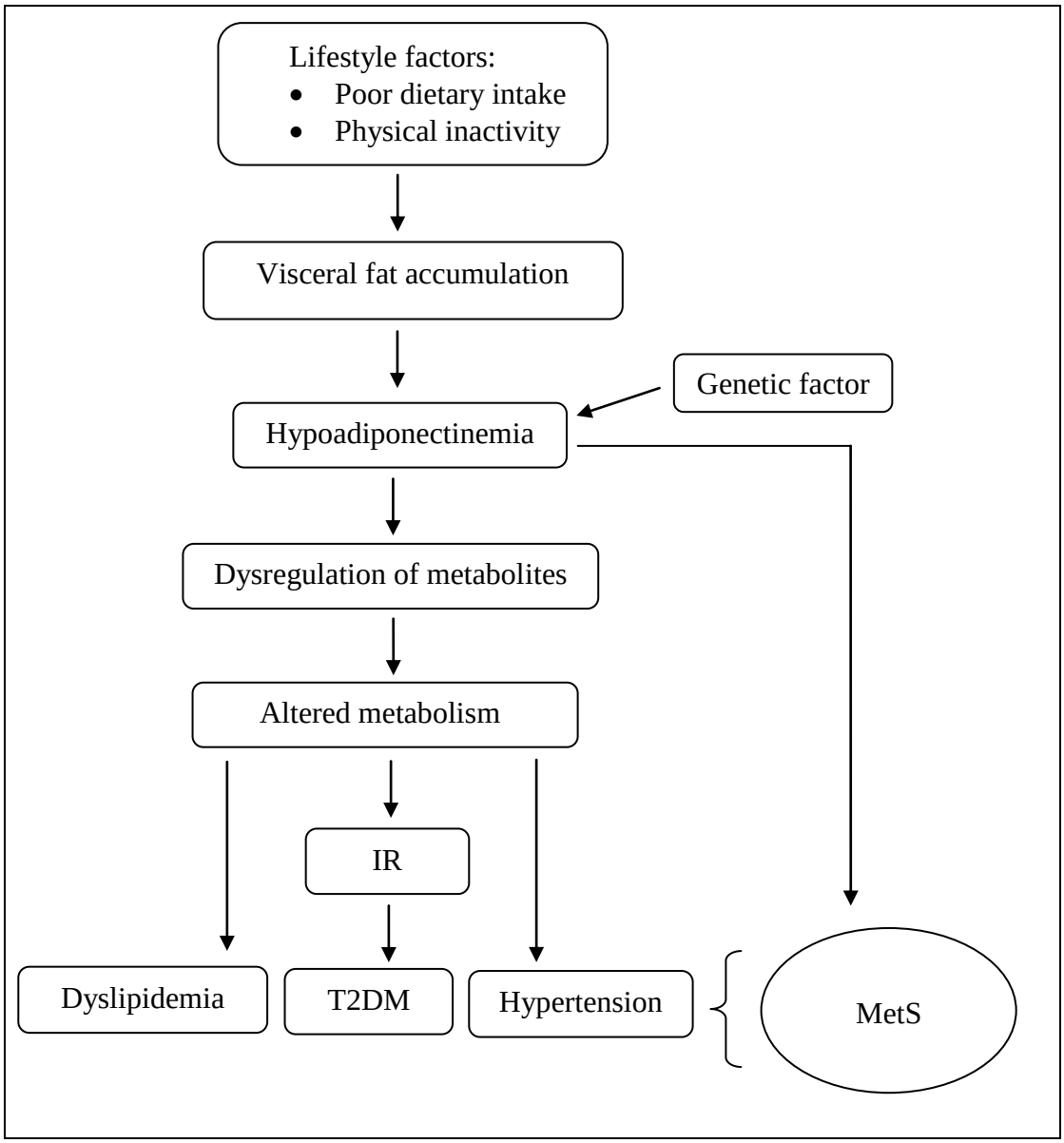
iii. There are no association between MetS risk factors and urinary metabolite.

iv. There are no association between HMW adiponectin and urinary metabolite.

## 1.7 Conceptual framework

The conceptual framework of the study is presented in Figure 1.1. Poor dietary intake and physical inactivity are lifestyle factors that induce visceral fat accumulation and low plasma adiponectin level (hypoadiponectinemia). Besides, genetic factors such as I164T SNP is also associated with hypoadiponectinemia. Hypoadiponectinemia may lead to dysregulation of urinary metabolites and alterations in FAs and AAs

metabolism. Visceral adiposity will lead to hypoadiponectinemia and an increase in free fatty acid (FFA) concentrations (Cnop *et al.*, 2003). These conditions enhance a cluster of T2DM, hypertension (elevated blood pressure), and dyslipidemia (high TG and low HDL-C) termed as MetS.




IR: Insulin resistance; T2DM: Type 2 diabetes mellitus; MetS: Metabolic syndrome

Figure 1.1 Conceptual framework of the study

(Adopted from Okamoto *et al*., 2006; current study)

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Orang Asli

OA is a Malay term which means original people are the indigenous inhabitants of Peninsular Malaysia (Khor and Zalilah, 2008). The Orang Asli Development Department (JAKOA) reported that the total population of OA in the year 2013 was 178,197 (JAKOA, 2013) representing 0.6% of national population. The OA is classified into three main tribes namely Senoi, Proto Malay or Aboriginal Malay, and the Negrito. The categorisation of the tribes were based on morphology, culture, language, and geographical locations for the convenience of administration (Masron *et al.*, 2013). The population distribution and characteristics of the study population is shown in Table 2.1.

The Senoi is the largest OA tribe constituting about 55% (Khor and Zalilah, 2008) of the population and scattered from the middle to northern part of the Peninsular Malaysia (Masron *et al.*, 2013). The Senoi subtribes including Semai, Che Wong, Temiar, Jah Hut, Semoq Beri, and Mah Meri. The physical characteristics of Senoi are having variety of skin color and wavy hair, living as both hunter-gatherers and traders, and descend from an admixture of the Negrito and an East Asian population (Ang *et al.*, 2012). Senoi speak Austro-Asiatic languages of the Mon-Khmer subgroup, indicating their ancient connection with the mainland Southeast Asia (Nicholas, 2000).

Table 2.1 Population distribution and characteristics of the study population of Orang Asli subtribe

| Tribe | Subtribe | Population of 2013[a] | Name of the area | Location type |
|---|---|---|---|---|
| Negrito | | 5,009 | | |
| | Kensiu[b] | 237 | Kg. Lubuk Legong, Baling, Kedah | Suburban |
| | Kintak | 194 | | |
| | Lanoh[b] | 382 | Kg. Air Bah, Lenggong, Perak | Rural |
| | Jahai | 2,387 | | |
| | Mendriq | 362 | | |
| | Beteq[b] | 1,447 | Kg. Aring 5, Gua Musang Kelantan | Rural |
| Senoi | | 97,856 | | |
| | Temiar | 31,038 | | |
| | Semai[b] | 51,437 | Pos Tual, Kuala Lipis, Pahang | Rural |
| | Semoq Beri | 5,313 | | |
| | Che Wong[b] | 651 | Kg. Sungai Enggang, Lanchang, Pahang | Suburban |
| | Jah Hut | 5,618 | | |
| | Mah Meri | 3,799 | | |
| Proto Malay | | 75,332 | | |
| | Temuan | 27,590 | | |
| | Semelai | 7,727 | | |
| | Jakun | 34,722 | | |
| | Orang Kanaq[b] | 148 | Kg. Sungai Selangi, Kota Tinggi, Johor | Suburban |
| | Orang Kuala | 3,525 | | |
| | Orang Seletar | 1,620 | | |
| Total | | 178,197 | | |

[a]Data obtained from the Orang Asli Museum, Gombak, viewed 15 April 2015.
[b] Study population

The second largest tribe of OA is Proto Malay or Aboriginal Malay constituting around 42% of the population (Khor and Zalilah, 2008). They can be found mainly in the middle and southern states of Pahang, Johor, Negeri Sembilan, and Selangor (Khor and Zalilah, 2008) and are similar to Deutero-Malays from the morphological aspect, culture, and languages (Lim *et al.*, 2010). Proto Malays consist of Jakun, Temuan, Semelai, Orang Kanaq, Orang Seletar, and Orang Kuala.

The migration of Proto Malay from Yunnan to Peninsular Malaysia happened around 4,000 years ago after the arrival of Negrito and Senois (Fix, 1995). The Proto Malay, who work as farmer-traders have a lighter skin color and straight hair (Fix, 1995).

The Negrito is the least tribes which has approximately 3% of the population (Khor and Zalilah, 2008). The settlements of this tribe are isolated and scattered but mostly distributed in the northern and central region of the Peninsular Malaysia (Masron *et al.*, 2013). They can be classified into six subtribes, mainly Bateq, Kensiu, Kintaq, Jahai, Lanoh, and Mendriq. The Negrito is the earliest OA tribes arrived in the Peninsular Malaysia which was around 25, 000 years ago (Masron *et al.*, 2013). The Negrito population is the first occupants of South-East Asia, has dark skin, curly hair, and live as hunter-gatherers (Ang *et al.*, 2012). Generally, the Jahai subtribe is physically similar to negro in Africa, Andaman islanders, and Aeta in the Philippines (JAKOA, 2015b). The distribution map of the OA subtribes is shown in Figure 2.1.

The government of Malaysia through JAKOA had commenced an inclusive development programmes since 1954 as efforts to transform and develop the OA community (Yahaya *et al.*, 2011). Three main development programme by JAKOA includes structured settlements development, economic development, and social development (JAKOA, 2015a). One of the development programmes that has been implemented for remote and scattered settlement of the OA community was The Resettlement Scheme or RPS (Kamaruddin, 2008). This scheme provided basic facilities including housing, kindergarten, community halls, electricity, water, and

access roads and currently 17 RPS has been implemented and benefited 3,015 families (Kamaruddin, 2008).

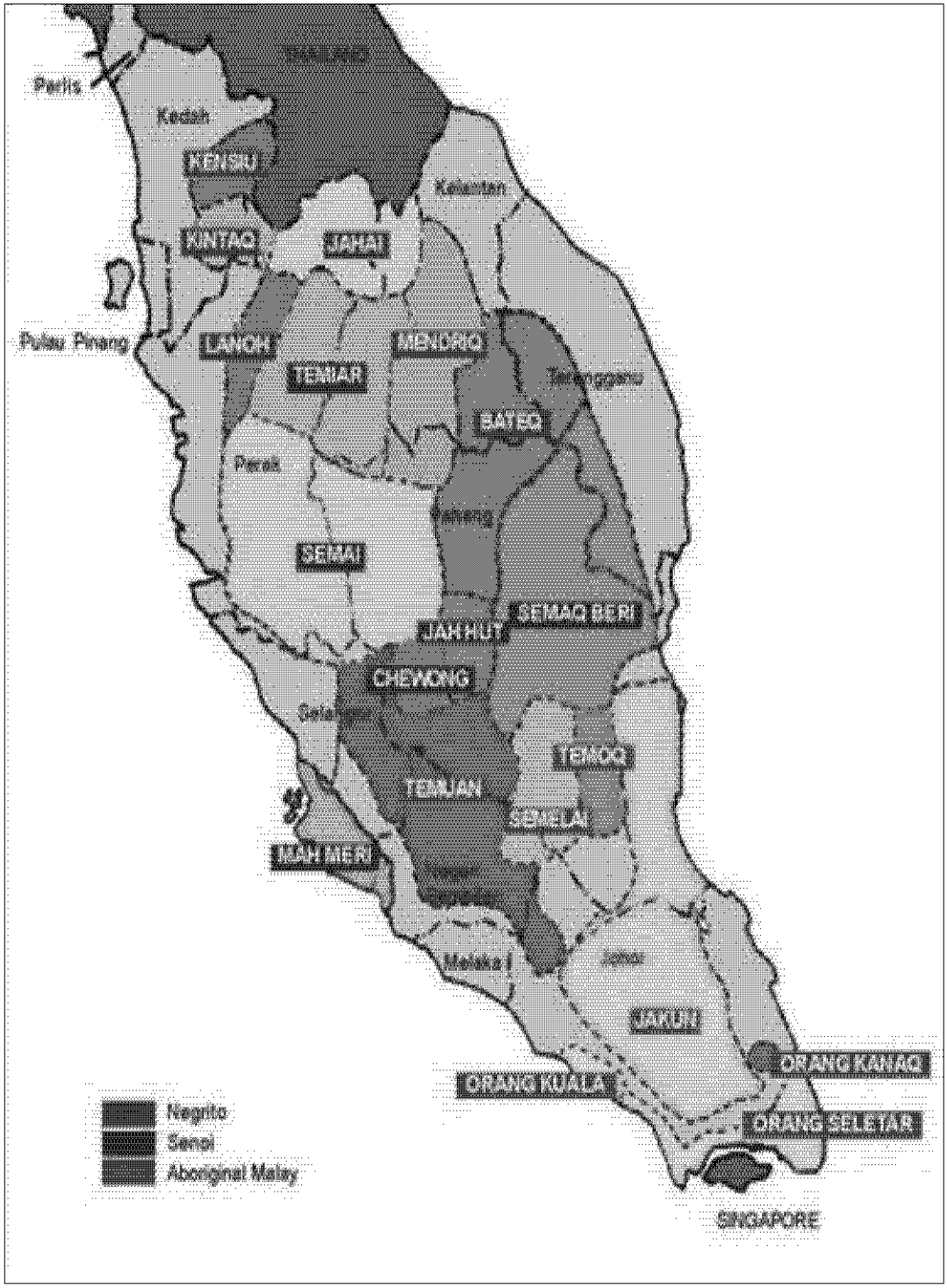



Figure 2.1 Distribution map of the Orang Asli subtribes in Peninsular Malaysia (Source: Center for Orang Asli Concerns, 2015)

Based on JAKOA record, until 31 Disember 2010, there are 852 OA villages that has been classified into rural, suburban, and urban (Yahaya *et al.*, 2011). These classifications were based on location, phase of economic development, and basic facilities and infrastructures (Yahaya *et al.*, 2011). The classification of OA village is shown in Table 2.2.

Table 2.2 Classification of Orang Asli village

| Category | Number of village | Criteria |
|---|---|---|
| Rural | 327 | a. Can be reached by dirt roads, trail roads, or waterways<br>b. Do not have access to clean water, 24 hours electricity, and other basic amenities<br>c. Do not have fix incomes |
| Suburban | 519 | a. Near with Malay village<br>b. Can be reached by paved roads<br>c. Have basic facilities, clean water supply, 24 hours electricity supply<br>d. Have land development projects and fix incomes |
| Urban | 6 | a. Has a complete facilities<br>b. There are no land development projects |

(Source: Yahaya *et al*., 2011)

## 2.2 Nutritional status

Nutritional status refer to the person health as it relates to how well diet meets that person's individual nutrient requirements (Mcguire and Beerman, 2007). Generally, the nutritional status can be assessed using four components namely anthropometric measurements (height, weight, circumferences, and body composition), biochemical measurements (blood and urine), clinical assessment (medical history and physical examination), and dietary assessment (24-hour recall method, food frequency questionnaire, and diet record) (Mcguire and Beerman, 2007). Anthropometric measurements were widely used because it can be easily performed with appropriate

training, does not require sophisticated machine or equipment, can be performed in field settings, and economical (Yusof *et al.*, 2007).

Globally, the overweight and obesity have affected both developed and developing countries, men and women, adults and children (Yatsuya *et al.*, 2014) and the prevalence of this epidemic is on the rise. The survey conducted on more than 100 countries around the world observed that the proportions of overweight and obesity increased between the year 1980 and 2013 from 28.8% to 36.9% in males and from 29.8% to 38.0% in females (Ng *et al.*, 2014). Ng *et al.* (2014) also reported that the estimated rate of obesity exceeded 50% in male in Tonga and female in Kuwait, Kiribati, the Federated States of Micronesia, Libya, Qatar, Tonga, and Samoa.

The findings of National Health and Morbidity Survey (NHMS) 2011 indicated that the rate of overweight and obesity (29.4% and 15.1%) among adults Malaysian was comparable to that reported in NHMS III 2006 (28.6% and 14.0%) based on the WHO (1998) classification (IPH, 2011). However, more than 60% of Malaysian adults were estimated pre-obese and obese according to the Malaysian Clinical Practice Guidelines on Management of Obesity (2004) classification (IPH, 2011). Another study in 2011 observed that the rate of overweight and obesity among adult Malaysians were 33.6% and 19.5% respectively (Wan Mohamud *et al.*, 2011). The updated in NHMS 2015 indicated that the national prevalence of overweight and obesity were 30.0% and 17.7% respectively according to the WHO (1998) classifications while the rate of overweight and obesity were 33.4% and 30.6% respectively according to the Malaysian Clinical Practice Guidelines of Obesity

(2004) classifications (IPH, 2015). Besides, the national prevalence of abdominal obesity or central obesity was 48.6% according to the International Diabetes Institute/Western Pacific World Health Organization/International Association for the study of Obesity/International Obesity Task Force (WHO/IASO/IOTF, 2000) (IPH, 2015). The emergence of obesity is believed to cause a number of risk factors for T2DM and cardiovascular diseases (CVDs) known as MetS. Underweight and obesity epidemics, particularly higher levels of obesity were found associated with increased mortality among American adults (Flegal *et al.*, 2005).

### 2.2.1 Metabolic syndrome

MetS is defined as a constellation of interconnected physiological, biochemical, clinical, and metabolic factors that directly augments the risk of CVD, T2DM, and all cause mortality (Kaur, 2014). Overweight, obesity, and physical inactivity are modifiable factors that closely linked with MetS (Chu and Moy, 2014). Many international organizations and expert groups had attempted to propose the definitions of MetS.

The first definition of MetS by WHO was proposed in 1998 which highlight IR as the major underlying risk factor and required evidence of IR for diagnosis (Alberti and Zimmet, 1998). A diagnosis of the syndrome by WHO criteria could thus be made on the basis of several markers of IR plus two additional risk factors, including obesity, hypertension, reduced HDL-C level, high TG level, or microalbuminuria (Alberti and Zimmet, 1998). In 2011, the other definition of MetS was proposed by NCEP ATP III (NCEP ATP III, 2001). NCEP ATP III criteria did

not require demonstration of IR and no single factor was required for diagnosis (NCEP ATP III, 2001). However, NCEP ATP III made the presence of 3 of the 5 factors including elevated WC (highly correlated with IR), reduced HDL-C, elevated TG, elevated fasting glucose (impared fasting glucose or T2DM), and elevated blood pressure as the basis for establishing the diagnosis (NCEP ATP III, 2001). This definition was presented as part of an educational programme for the prevention of coronary heart disease (CHD) (Alberti *et al.*, 2006).

In 2005, another definition of MetS was proposed by IDF (Alberti *et al.*, 2005) in an effort to define the syndrome more precisely and therefore it could be used by different clinical and research groups (Kassi *et al.*, 2011). IDF introduced abdominal obesity as a prerequisite of the diagnosis plus any two of risk factors (elevated WC, reduced HDL-C, elevated TG, elevated fasting plasma glucose, and elevated blood pressure) as the basis for establishing the diagnosis (Alberti *et al.*, 2005). Ethnic-specific values for WC is shown in Table 2.3. The IDF guidelines stressed adoption of different values for WC in different ethnic groups based on the relationship of WC either to the other MetS components or to longer-term outcome studies such as those on the risk of T2DM and CVD (Alberti *et al.*, 2009).

In 2009, IDF and American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) representatives held discussions to resolve the remaining differences between definitions of MetS and to unify the criteria (Alberti *et al.*, 2009). This latest harmonized definition proposed that MetS was diagnosed by the presence of any 3 of 5 risk factors which abdominal obesity is not a prerequisite risk factor (Alberti *et al.*, 2009). The definitions of MetS by organization is shown in

Table 2.4. In Harmonized definition, the population and country-specific values for WC was used (Alberti *et al.*, 2009). According to Alberti *et al.* (2009), the IDF cut points of WC were recommended to be used for non-Europeans and either the IDF or AHA/NHLBI cut points were used for European origin. Table 2.5 presents WC threshold by several organization.

Table 2.3 Ethnic-specific cut points for waist circumference by International Diabetes Federation organization

| Ethnic group[a] | WC (as measure of central obesity) |
|---|---|
| Europids | Men ≥94 cm<br>Women ≥80 cm |
| South Asians | Men ≥90 cm<br>Women ≥80 cm |
| Chinese | Men ≥90 cm<br>Women ≥80 cm |
| Japanese | Men ≥85 cm<br>Women ≥90 cm |
| Ethnic south and central Americans | Use south Asian recommendations until more specific data are available |
| Sub-Saharan Africans | Use European data until more specific data are available |
| Eastern Mediterranean and Middle East (Arab) | Use European data until more specific data are available |

WC: Waist circumference

[a]Ethnicity should be basis for classification, not country of residence

(Source: Alberti *et al.*, 2005)

Table 2.4 Definitions of metabolic syndrome by organization

| Risk factors | WHO (1998) | NCEP ATP III (2001) | IDF (2005) | Harmonized IDF (2009) |
|---|---|---|---|---|
| Obesity (BMI) or abdominal obesity (WC) | BMI >30kg/ $m^2$ and/or WHR > 0.90 (M), > 0.85 (F) | WC M >102 cm, F >88 cm | WC (Ethnic-specific) | WC (Population- and country-specific )[a] |
| High BP (Systolic/ Diastolic) | ≥ 160/90 mmHg | ≥130/85 mmHg | ≥130/85 mmHg or on Rx | Systolic ≥ 130 and/or diastolic ≥ 85 mmHg or on Rx |
| High FPG | IGT or DM and/or IR | ≥ 6.1 mmol/L or DM | ≥5.6 mmol/L or DM | ≥5.6 mmol/L or DM or on Rx |
| Microalbuminuria | UAE ≥ 20 µg/min or ACR ≥ 20 mg/g | - | - | - |
| Elevated TG | ≥ 1.7 mmol/L | ≥1.7 mmol/L | > 1.7 mmol/L | ≥1.7 mmol/L or on Rx |
| Reduced HDL-C | <0.9 mmol/L (M), < 1.0 (F) | <1.0 mmol/L (M), <1.3 (F) | <1.03 mmol/L (M), <1.29 (F) | <1.0 mmol/L (M), <1.3 (F) or on Rx |
| MetS | IGT or DM and/or IR + any 2 or more RF | At least 3 RF | WC + 2 more RF | At least 3 RF |

WHO: World Health Organization; NCEP ATP III: National Cholesterol Education Program Adult Treatment Panel III; IDF: International Diabetes Federation; BMI: Body Mass Index; WHR: Waist hip ratio; WC: Waist circumference; BP: Blood pressure; FPG: Fasting plasma glucose; UAE: Urinary albumin excretion; ACR: Albumin/creatinine ratio; TG: Triglyceride; HDL-C: High-density lipoprotein cholesterol; Rx: On medication; IGT: Impaired glucose tolerance; DM: Diabetes mellitus; IR: Insulin resistance; MetS: Metabolic syndrome; M: Male; F: Female; RF: Risk factors

[a]It is recommended that the IDF cut points be used for non-Europeans and either the IDF or AHA/NHLBI cut points used for European origin until more data are available.

(Source: Alberti *et al*., 2009; Alberti *et al*., 2005; Alberti and Zimmet, 1998; NCEP ATP III, 2001)

Table 2.5 Current recommended cut points for waist circumference by organization

| Population | Organization | WC cut points | |
|---|---|---|---|
| | | Men | Women |
| Europid | IDF | ≥94 cm | ≥80 cm |
| Caucasian | WHO | ≥94 cm (increased risk) ≥102 cm (still higher risk) | ≥80 cm (increased risk) ≥88 cm (still higher risk) |
| United States | AHA/NHLBI (ATP III) | ≥102 cm | ≥88 cm |
| Canada | Health Canada | ≥102 cm | ≥88 cm |
| European | European Cardiovascular Society | ≥102 cm | ≥88 cm |
| Asian (including Japanese) | IDF | ≥90 cm | ≥80 cm |
| Asian | WHO | ≥90 cm | ≥80 cm |
| Japanese | Japanese Obesity Society | ≥85 cm | ≥90 cm |
| China | Cooperative Task Force | ≥85 cm | ≥80 cm |
| Middle East, Mediterranean | IDF | ≥94 cm | ≥80 cm |
| Sub-Saharan African | IDF | ≥94 cm | ≥80 cm |
| Ethnic Central and South American | IDF | ≥90 cm | ≥80 cm |

WC: Waist circumference; IDF: International Diabetes Federation; WHO: World Health Organization; AHA/NHLBI: American Heart Association/National Heart, Lung, and Blood Institute; ATP III : Adult Treatment Panel III

(Source: Alberti *et al*., 2009)

## 2.3 Adipokines

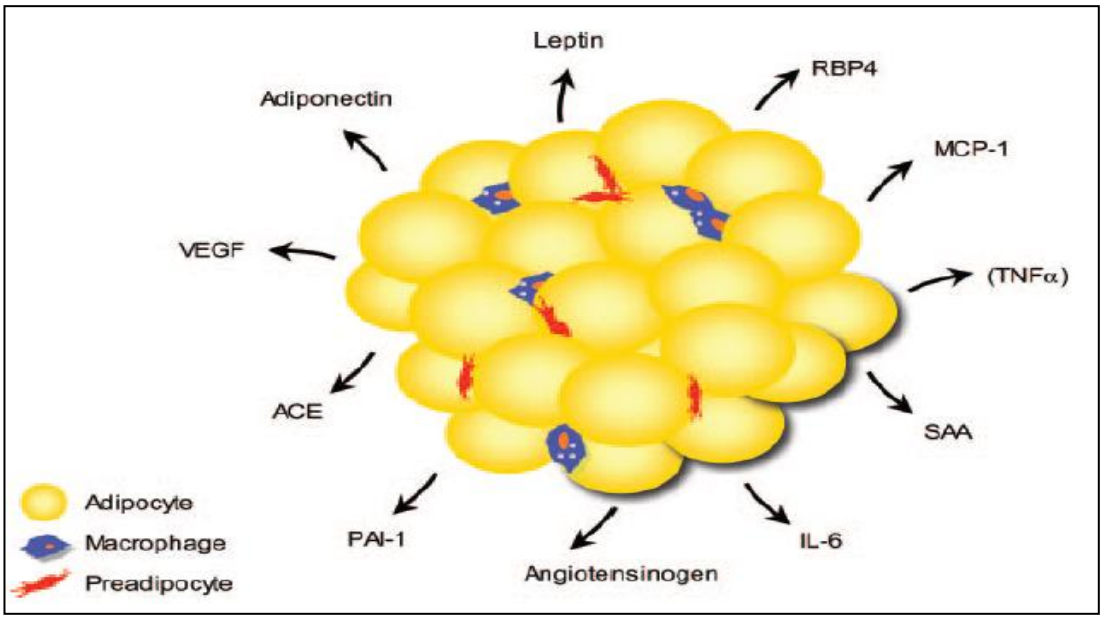
Adipose tissue can be classified into two major types namely white adipose tissue (WAT) and brown adipose tissue (Fantuzzi, 2005). The distribution of brown adipose tissue in humans are in cervical-supraclavicular area and the shape of this tissue is polygonal with multi-ocular lipid droplets (Kwon and Pessin, 2013). It can be found in newborn humans but almost absent in adults (Coelho *et al.*, 2013). The function of brown adipose tissue (BAT) is to dissipate energy and generate heat (Berry *et al.*,

2013). WAT is located throughout the body (Kwon and Pessin, 2013). Subcutaneous and visceral adipose tissues are the major adipocyte depots of WAT with additional adipose depots distributed at various organs including heart, lung, and kidney (Kwon and Pessin, 2013). WAT is composed of variety cell types such as adipocytes, macrophages, lymphocytes, fibroblasts, and endothelial cells (Kwon and Pessin, 2013). It is designed as energy storage (Berry *et al.*, 2013) and endocrine organ because of its capacity to secrete hormones and cytokines (Vázquez-Vela *et al.*, 2008).

Adipokines are proteins that produced mainly by adipocytes (Fantuzzi, 2005). Subcutaneous or visceral adipose tissue play a role in secretion of adipokines (Kwon and Pessin, 2013). Adipokines can act centrally to regulate appetite and energy expenditure, and peripherally affect insulin sensitivity, oxidative capacity, and lipid uptake (de Oliveira Leal and Mafra, 2013). The release of adipokine by either adipocytes or adipose tissue macrophages could induce a low-grade chronic inflammatory state that play a central role in obesity related cardiovascular complications and IR (Antuna-Puente *et al.*, 2008). The inflammatory functions of adipokines are responsible for mediating obesity-induced insulin resistance (Kwon and Pessin, 2013). On this point, adipokines are categorized as pro- and anti-inflammatory adipokines according to their effects on inflammatory responses in adipose tissues (Kwon and Pessin, 2013).

Example of pro-inflammatory adipokines are leptin, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), retinol binding protein 4 (RBP4), resistin, CC-chemokine ligand 2 (CCL2), CC-chemokine receptor type 5 (CCR5), angiopoietin-

like protein 2 (ANGPTL2), and chemerin while anti-inflammatory adipokines are adiponectin, secreted frizzled-related protein 5, visceral adipose tissue-derived serine protease inhibitor, omentin-1, and apelin (Kwon and Pessin, 2013). Figure 2.2 shows the adipokines that most clearly associated with obesity and MetS.




ACE: Angiotensinogen-converting enzyme; MCP-1: Monocyte chemoattractant protein-1; PAI-1: Plasminogen activator inhibitor-1; RBP4: Retinol binding protein 4; SAA: Serum amyloid A; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-α; VEGF: Vascular endothelial growth factor

Figure 2.2 Different type of adipokines released by the adipose tissue that associated with obesity and metabolic syndrome

(Source: Gustafson *et al*., 2007)

Of these adipokines, leptin is widely studied (Gustafson *et al.*, 2007) and has been the most thoroughly investigated (Avram *et al.*, 2005). Leptin is positively related to adipocyte size and obesity which is in contrast to adiponectin (Gustafson *et al.*, 2007). The metabolic function of leptin including repression of food intake, promotion of energy expenditure, stimulation of FA oxidation in liver, pancreas, and skeletal muscle, modulation of hepatic gluconeogenesis, and modulation of

pancreatic β cell function (Rabe *et al.*, 2008). TNF-α and IL-6 are important inflammatory molecules associated with obesity and IR (Gustafson *et al.*, 2007) which functioning as modulation of hepatic and skeletal muscle insulin signalling (Rabe *et al.*, 2008). Adiponectin is one of the most widely studied adipokine (Gustafson *et al.*, 2007; Turer and Scherer, 2012) and highly expressed by adipocyte cells with strong anti-inflammatory properties (Kwon and Pessin, 2013). Adiponectin (microgram per milliliter) circulates at the highest levels as compared to leptin (nanograms per millilitre) (Fantuzzi, 2005). It is a key mediator of systemic insulin sensitivity and glucose homeostasis (Turer and Scherer, 2012). These effects can be achieved by a diverse set of effects on several important targets including liver, pancreas, cardiac myocytes, the immune system, and even the adipose tissue itself (Turer and Scherer, 2012).

### 2.3.1 Adiponectin

Adiponectin was discovered during gene-expression profiling of human adipose tissue conducted by the human complementary deoxyribonucleic acid (cDNA) project (Di Chiara *et al.*, 2011). It is encoded by the ADIPOQ gene that can modulate insulin sensitivity and glucose homeostasis (Schwarz *et al.*, 2006). It is involved in regulating glucose levels as well as fatty acid (FA) breakdown (Al-Braich *et al.*, 2014). According to Kadowaki *et al.* (2006), adiponectin is an adipokine or adipocytokines that is specifically and abundantly expressed in adipose tissue and directly sensitizes the body to insulin (Kadowaki *et al.*, 2006). It possesses anti-diabetic, anti-atherogenic, and anti-inflammatory properties (Matsuzawa, 2010).

### 2.3.1 (a) Structure and circulating levels

Full-length adiponectin contains 244 amino acids, a signal peptide, a collagen like domain at its N-terminus and a globular domain at its C-terminus, which shares sequence similarities with collagens X and VIII as well as complement factor C1q (Okamoto *et al.*, 2006). It requires post-translational modifications for biological activity (hydroxylation and glycosylation) (Wang *et al.*, 2002).

Adiponectin circulates in plasma as a low-molecular weight (LMW) trimer (~90kDa), a middle-molecular weight (MMW) hexamer (~180 kDa), and high-molecular weight (HMW) 12- to 18-mer (400 kDa) and these forms were postulated to differ in biologic activity (Pajvani *et al.*, 2003; Waki *et al.*, 2003; Wang *et al.*, 2006). A smaller form of adiponectin consisted of globular domain also exists in plasma in a very small amount (Fruebis *et al.*, 2001). Figure 2.3 shows the domains and structure of adiponectin. Adiponectin is relatively abundant in plasma with a concentration range of 2-10 μg/ml (Arita *et al.*, 1999). Matsuzawa (2010) reported that the average concentrations of adiponectin in human plasma are extremely high up to 5-10 μg/ml.