THE ROLE OF MATERNAL SERUM AND BREAST MILK ADIPOKINES IN DETERMINING POSTPARTUM AND INFANT ADIPOSITY DEVELOPMENT

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

Acrp30	Adipocyte complement-related protein of 30 kDa
AdipoQ	Adiponectin, C1Q and collagen domain containing
AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
ANOVA	Analysis of variance
apM1	Adipose most abundant gene transcript-1
BAZ	Body mass index-for-age Z-scores
BMI	Body mass index
CRP	C-reactive protein
CV	Coefficients of variability
DR	Diet recalls
ELISA	Enzyme-linked immunosorbent assay
FH	Food hypersensitive
GBP28	Gelatin-binding protein of 28 kDa
GDM	Gestational diabetes mellitus
GOD	Glucose oxidase
GK	Glucokinase
GPO	Glycerophosphate oxidase
GWG	Gestational weight gain
H_2O_2	Hydrogen peroxide
HDL-C	High density lipoprotein cholesterol
HRP	Horseradish peroxidase
HSD	Honest significant difference
HUSM	Hospital Universiti Sains Malaysia
HMW	High-molecular-weight
IGF-1	Insulin-like growth factor 1

IOM	Institute of Medicine
IPAQ	International Physical Activity Questionnaire
IPH	Institute for Public Health
IQR	Interquartile range
LAZ	Length-for-age Z-scores
LDL-C	Low density lipoprotein cholesterol
LGA	Large-for-gestational age
LMP	Last menstrual period
LMW	Low-molecular-weight
MET	Metabolic equivalent task
MLR	Multiple linear regression
MUAC	Mid-upper arm circumference
NASH	Nonalcoholic steatohepatitis
NCCFN	National Coordinating Committee on Food and Nutrition
NCEP	National Cholesterol Education Program
NFH	Non-food hypersensitive
NHMS	National Health and Morbidity Survey
O_2	Oxygen
Ox-LDL	Oxidized-low density lipoprotein
PAP	Phenol aminophenazone
PBST	Phosphate buffered saline tween-20
PIH	Pregnancy-induced hypertension
POD	Peroxidase
PPWR	Postpartum weight retention
PSQ	Pregnancy Symptoms Questionnaire
RNI	Recommended Nutrient Intakes
SD	Standard deviation
SGA	Small-for-gestational-age

SOB	Shortness of breath
SPSS	Statistical Package for Social Sciences
SSAZ	Subscapular skinfold-for-age Z-scores
TC	Total cholesterol
TG	Triglycerides
TMB	Tetramethylbenzidine
TNF-α	Tumour necrosis factor alpha
TWG	Total gestational weight gain
UNSCN	United Nations System Standing Committee on Nutrition
USDA	U.S. Department of Agriculture
USM	Universiti Sains Malaysia
VLDL-C	Very low density lipoprotein cholesterol
WAZ	Weight-for-age Z-scores
WLZ	Weight-for-length Z-scores
WGR	Weight gain rate
WHO	World Health Organization

PERANAN ARAS ADIPOKIN SERUM DAN SUSU IBU DALAM MENENTUKAN ADIPOSITI IBU SELEPAS BERSALIN DAN BAYI

ABSTRAK

Obesiti menjadi kebimbangan di Malaysia berikutan peningkatan prevalen yang merisaukan kebelakangan ini. Selain menyimpan lemak, tisu lemak juga merupakan organ endokrin yang berfungsi merembes sejumlah besar adipokin. Kehamilan, iaitu keadaan di mana terjadinya perubahan metabolik, dikenalpasti sebagai tempoh kritikal bermulanya adipositi dalam kalangan ibu dan bayi akibat ketidakseimbangan penghasilan adipokin. Justeru, Kajian Kohort Kehamilan Universiti Sains Malaysia telah dijalankan untuk mengkaji peranan aras adipokin (adiponektin dan leptin) serum dan susu ibu dalam menentukan pengekalan berat badan ibu selepas bersalin (PPWR) dan adipositi bayi dalam tempoh setahun pertama kelahiran. Kajian ini dijalankan dari April 2010 hingga Disember 2012. Seramai 155 ibu hamil yang sihat berusia di antara 19 hingga 40 tahun telah direkrut pada trimester pertama dan kedua kehamilan bertempat di negeri Kelantan, Malaysia. Pengumpulan data meliputi sosio-demografi, sejarah perubatan, antropometri, pemakanan, aktiviti fizikal, ketidakselesaan fizikal dan analisis biokimia klinikal ibu; serta antropometri dan pola pemberian makanan bayi. Sampel serum berpuasa diambil semasa tempoh kehamilan untuk analisa aras glukosa darah, profil lipid, adiponektin dan leptin. Sampel susu ibu diambil sejurus selepas bersalin dan 2 bulan selepas bersalin. Pengumpulan data telah dilakukan pada trimester kedua dan ketiga kehamilan, diikuti pengumpulan data susulan sejurus selepas bersalin, 2 bulan, 6 bulan dan 12 bulan selepas bersalin. Analisa regresi linear berganda (MLR) telah dijalankan untuk mengkaji; 1) perkaitan di antara aras adiponektin serum ibu semasa kehamilan dengan aras adiponektin susu ibu dalam tempoh 2 bulan selepas bersalin; 2) perkaitan di antara faktor pranatal dan aras adiponektin dan leptin serum ibu dengan PPWR 12 bulan selepas bersalin; dan 3) perkaitan di antara aras adiponektin dan

leptin serum dan susu ibu dengan adipositi bayi. Model MLR menunjukkan aras adiponektin susu ibu sejurus selepas bersalin meningkat dengan peningkatan aras adiponektin serum ibu pada trimester ketiga ($p=0.006$), manakala aras adiponektin susu ibu semasa 2 bulan selepas bersalin meningkat dengan peningkatan aras adiponektin serum ibu pada trimester kedua dan ketiga ($p=0.035$, $p=0.006$). Semasa 12 bulan selepas bersalin, PPWR meningkat dengan peningkatan kadar penambahan berat badan (WGR) pada trimester ketiga ($p<0.001$), dan menurun dengan peningkatan aras kolesterol HDL pada trimester kedua ($p<0.001$). Dalam tempoh usia setahun pertama, berat badan, skor-Z BMI-untuk-umur, lilitan abdomen dan ketebalan tricep bayi menurun dengan peningkatan aras adiponektin serum dan susu ibu ($p<0.05$). Kesimpulannya, 1) aras adiponektin serum ibu semasa kehamilan dikaitkan dengan aras adiponektin susu ibu dalam tempoh 2 bulan selepas bersalin; 2) WGR dan aras kolesterol HDL semasa kehamilan dikaitkan dengan PPWR 12 bulan selepas bersalin; dan 3) aras adiponektin serum dan/atau susu ibu dikaitkan dengan adipositi bayi dalam tempoh setahun pertama kelahiran. Dapatan yang memberangsangkan bagi adiponektin susu ibu menunjukkan potensi amalan penyusuan susu ibu dalam pencegahan obesiti sejak usia bayi.

THE ROLE OF MATERNAL SERUM AND BREAST MILK ADIPOKINES IN DETERMINING POSTPARTUM AND INFANT ADIPOSITY DEVELOPMENT

ABSTRACT

Obesity has been a great concern in Malaysia since there is an alarming increase in its prevalence. Besides storing fat, adipose tissue is also an endocrine organ that secretes a large number of adipokines. Pregnancy, a state with metabolic changes, has been recognized as a critical period for the development of maternal and infant adiposity as a result of imbalanced adipokines production. Hence, the Universiti Sains Malaysia Pregnancy Cohort Study was established to investigate the role of maternal serum and breast milk adipokines (adiponectin and leptin) in determining the first year postpartum weight retention (PPWR) and infant adiposity development. This study was conducted from April 2010 until December 2012. A total of 155 healthy pregnant mothers aged 19 to 40 years were recruited at first and second trimester of pregnancy in Kelantan, Malaysia. Data collection were consisted of maternal socio-demography, medical history, anthropometry, dietary, physical activity, physical discomforts and clinical biochemistry analysis; and infant's anthropometry and feeding patterns. Fasting serum samples were taken during pregnancy for the serum glucose, lipid profile, adiponectin and leptin levels analyses. Breast milk samples were collected at birth and 2 months postpartum. Data collection was performed at second and third trimester of pregnancy, continued with follow-up visits at birth, 2 months, 6 months and 12 months postpartum. Multiple linear regression (MLR) analyses were performed to examine; 1) the associations of maternal serum adiponectin with breast milk adiponectin within 2 months postpartum; 2) the associations of prenatal factors and maternal serum adiponectin and leptin on 12 months PPWR; and 3) the associations of maternal serum and breast milk adiponectin and leptin on infant adiposity development. MLR models showed that breast milk adiponectin at birth increased with increasing maternal serum adiponectin at

third trimester ($p=0.006$), while breast milk adiponectin at 2 months postpartum increased with increasing maternal serum adiponectin at second and third trimesters respectively ($p=0.035$, $p=0.006$). At 12 months postpartum, PPWR increased with increasing weight gain rate (WGR) at third trimester ($p<0.001$), and decreased with increasing HDL-cholesterol at second trimester ($p<0.001$). In the first year of age, as maternal serum and breast milk adiponectin increased, infant weight, BMI-for-age Z-scores, abdominal circumference and triceps skinfold significantly decreased ($p<0.05$). In conclusion, 1) maternal serum adiponectin during pregnancy was associated with breast milk adiponectin within 2 months postpartum; 2) WGR and HDL-cholesterol during pregnancy were related with 12 months PPWR; and 3) maternal serum and/or breast milk adiponectin were associated with the first year infant adiposity development. The favourable results of breast milk adiponectin indicated the potential role of breastfeeding practice in the prevention of obesity since infanthood.

CHAPTER 1: INTRODUCTION

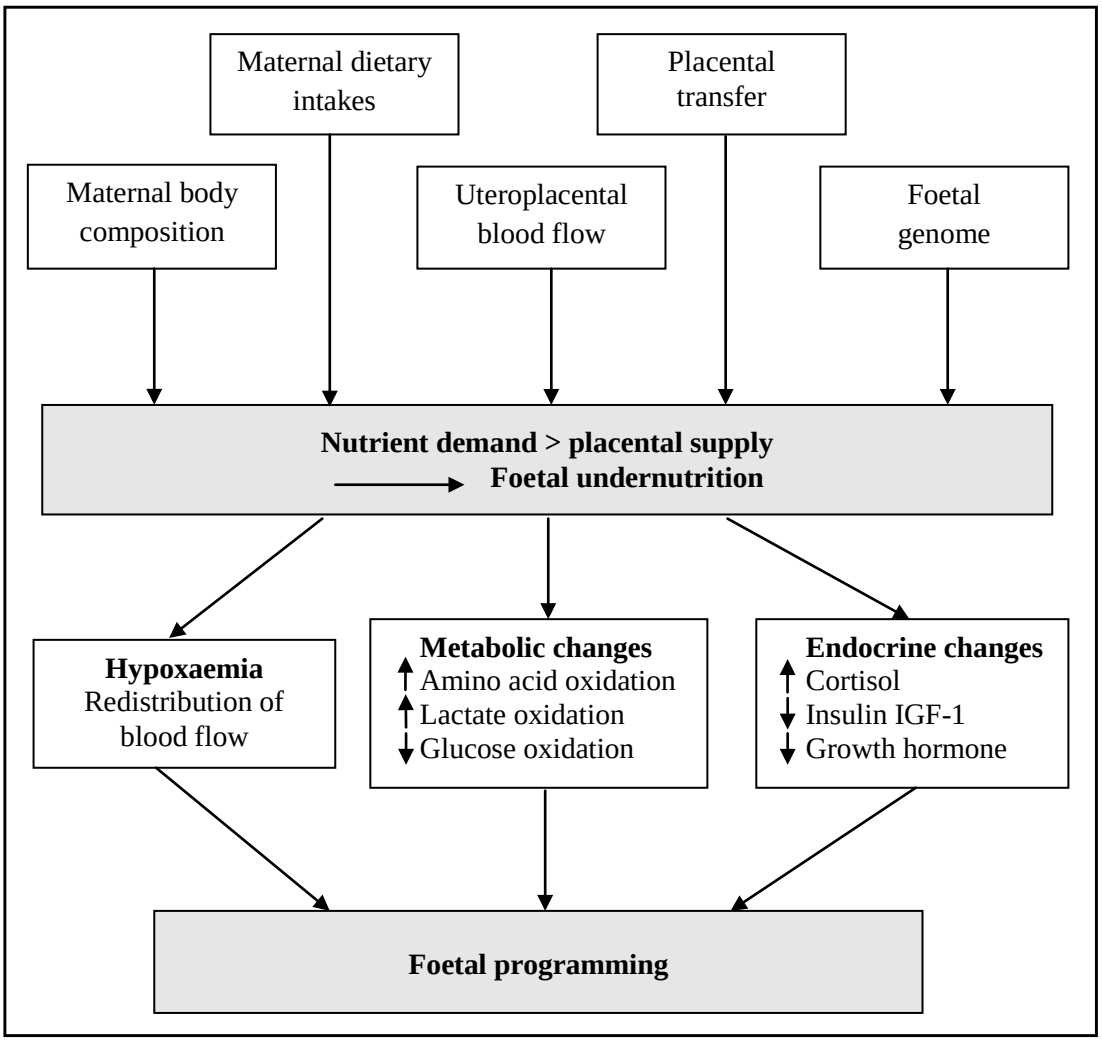
1.1 Background

Obesity has become a serious concern due to the sharp increasing rates worldwide. In fact, obesity is considered as a root cause for global mortality and disease burden, in which it was associated with well-known comorbidities such as cardiovascular disease, type 2 diabetes mellitus, hypertension, osteoarthritis, cancers, psychological distress (Basen-Engquist and Chang, 2011; Pedersen *et al.*, 2012; Bastien *et al.*, 2014), as well as cognitive impairment and risk of dementia in later life (Whitmer *et al.*, 2008).

Nowadays, obesity among population groups differentiated by gender and age are at alarming rate. In 2014, 40% of women worldwide aged 18 years and over were overweight compared to 38% of men (WHO, 2015). As reported in NHMS 2015, 20.6% of women in Malaysia were obese compared to 15.0% of men (IPH, 2015). In recent years, overweight among women in most developing countries was found to exceed the underweight (UNSCN, 2010). In the meantime, childhood obesity has also shown an escalating rate in which in the year of 2013 over 42 million children under the age of 5 were overweight or obese (WHO, 2015). While the prevalence of overweight/obese among Malaysian school-aged children was increased from 20.7% in 2002 to 26.4% in 2008 (Ismail *et al.*, 2009).

As the current trend of obesity continues, it has become an alarming concern particularly among women since obesity increases the risk of obstetric complications (Kulie *et al.*, 2011) as well as contributes to high postpartum weight retention (Haugen *et al.*, 2014). As a result, maternal distressing condition will subsequently expose their offspring to adiposity thus a higher risk of health problems in adulthood (Yu *et al.*, 2006). The ‘developmental origins of chronic adult disease’ hypothesis proposed by Barker (2004)

emphasizes the crucial role of intrauterine environment in the early development and subsequent life-long health of the foetus. The hypothesis states that the adverse prenatal environment may influence the physiology and metabolism of the foetus, thus results to 'programming' described as the condition in which the foetus changes the body's structure, function and metabolism as a way to adapt to the scarce environment.



(Source: Barker, 1998)

Figure 1.1 Foetal adaptations to undernutrition: a framework

Figure 1.1 shows the foetal programming as proposed by Barker (1998). In limited supply of nutrients foetus responds to adverse intrauterine environment by altering metabolic processes and synthesis of growth factors as well as redistributing blood flow, which eventually result to disproportionate growth of foetus. Based on this hypothesis and the present trend of

obesity, the classic view that the adulthood diseases are associated with adult lifestyles and genetic factors has been projected to another important factor that plays a vital role to the underlying cause of the health problems. The wider parameters and better understanding of the mechanisms would probably provide more comprehensive and effective prevention programmes of obesity in the future.

Defining as the consequence of an energy imbalance, obesity is associated with myriad interrelated factors that increase the positive energy balance such as dietary intake, physical activity level, environmental, societal, cultural, psychological, genetic, non-genetic biological susceptibility and medical (WHO, 2000; Rankinen *et al.*, 2006; Lopez, 2007). The complex and multi-factorial interactions in a long term would subsequently lead to weight gain, or particularly the increment of adipose tissues. Given that the role of adipose tissue as an endocrine organ, the changes of the volume and number of adipocytes due to obesity may result to the changes in endocrine and metabolic functions of adipose tissue including the secretion of adipokines (Weisberg *et al.*, 2003).

Adipokines are a number of adipocyte-derived secretory factors, which are responsible for the regulation of multiple metabolic pathways (Badman and Flier, 2007). Adiponectin and leptin are among the most potent and most studied adipokines in relations to obesity. As a major circulating protein in blood, adiponectin modulates glucose and fatty acid metabolism (Chandran *et al.*, 2003), and was associated with insulin sensitivity (Tschritter *et al.*, 2003). Two distinct human adiponectin receptors were abundantly expressed in skeletal muscle, heart, kidney and lung (AdipoR1) as well as in the liver (AdipoR2) (Yamauchi *et al.*, 2003; Zhou *et al.*, 2005). Given that AdipoR1 is also expressed in small intestine of neonatal mouse (Zhou *et al.*, 2005), it is possible that oral adiponectin particularly lactation has direct role in infants. The presence of adiponectin in human breast milk suggests its protective effects on infants (Martin *et al.*, 2006). On the other hand, serum

leptin plays a vital role in energy balance regulation and acts in the hypothalamus to supress appetite and increase energy expenditure (Stocker and Cawthorne, 2008). Yet, the functional role of serum and breast milk adiponectin and leptin on maternal and childhood obesity among local mothers and infants remain uncertain, thus warrants further investigations.

Proposed model of intrauterine imbalance of adipokines level based on foetal programming hypothesis was presented in Figure 1.2. The imbalance maternal adipokines level in the intrauterine may induce the foetal programming, wherein it may permanently change the foetal metabolism and appetite regulation. Consequently, it may lead to the childhood obesity and the development of health problems in later life. Knowing that obesity is a difficult to reverse condition and the treatment result is often frustrating, the strategy of early prevention particularly at the intrauterine stage is critically needed in order to stop the intergenerational cycle of obesity.

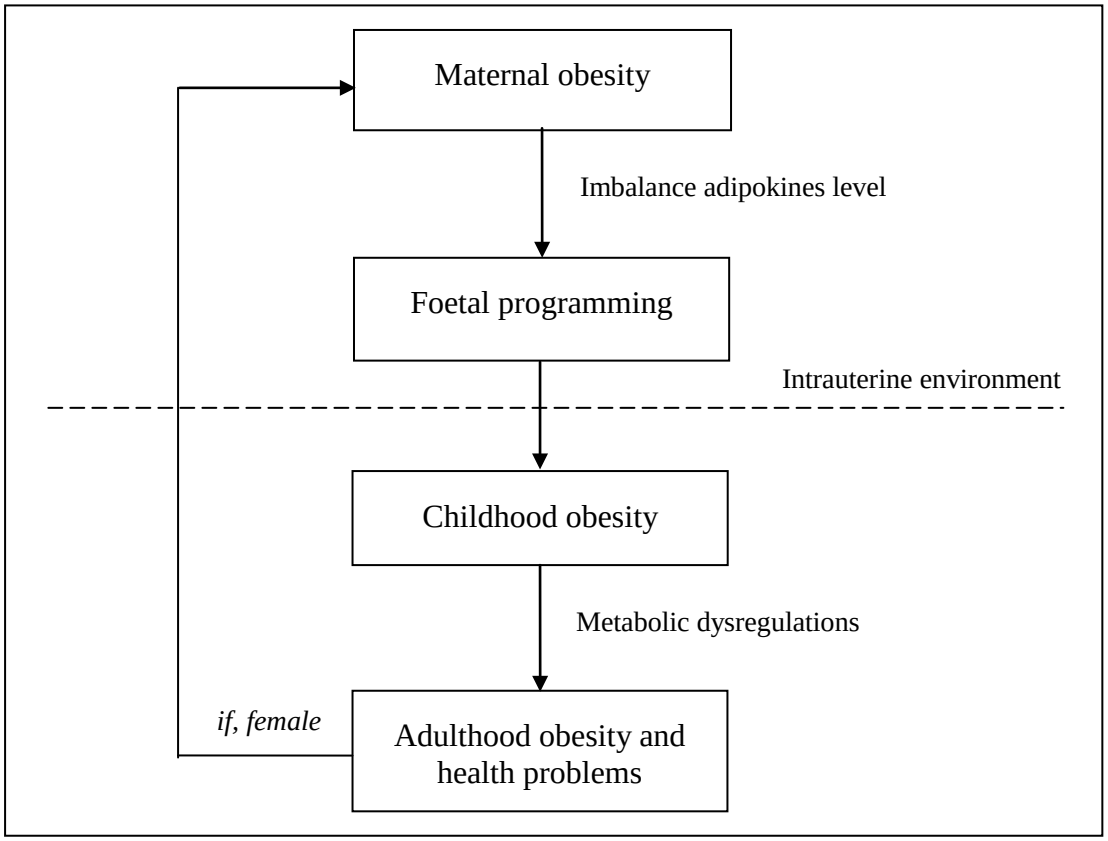


Figure 1.2 Proposed model of imbalance adipokines level and obesity programming

1.2 Rationales

The steep increase of obesity among women and the subsequent effects of maternal adiposity on the offspring health demand an urgent attention to elucidate the significant factors that contribute to obesity. The retention of gestational weight gain is one of the potential way of developing obesity among childbearing age women (Siega-Riz *et al.*, 2004) and the offspring who exposed to maternal adiposity are at higher risk of being obese and developing metabolic syndromes in later life (Hochner *et al.*, 2012; O'Reilly and Reynolds, 2013). In the meantime, pregnancy is the condition in which profound physiological and endocrinological alterations take place to meet the increased demands of maternal body and growing foetus, as well as to prepare the mothers for parturition and lactation (Picciano, 2003; Carlin and Alfirevic, 2008). It is of great interest to investigate the relationship of maternal and infant adiposity with adiponectin and leptin, which were known to play a major role in energy metabolism and weight control (Meier and Gressner, 2004; Qi *et al.*, 2004).

As a number of international studies reported the association of serum adiponectin and leptin during gestation and breast milk adiponectin with postpartum and infant adiposity (Stein *et al.*, 1998; Woo *et al.*, 2009; Wang *et al.*, 2010; Josefson *et al.*, 2013; Kew *et al.*, 2014), however in Malaysia there is a dearth of scientific data regarding that. This limitation demonstrates the need for comprehensive longitudinal study targeting mothers and infants, in order to obtain the greater insight of the factors that make up the whole obesity issue in both populations in Malaysia.

In light of that, it is hoped that this study would provide meaningful information on the contribution of maternal serum adipokines, breast milk adiponectin and other prenatal factors to the postpartum weight retention (PPWR) and the development of infant adiposity, particularly among Malay mothers and children. A better understanding of the characteristics

related to the pathogenesis of obesity would be a strong foundation in formulating and implementing the effective and successful obesity prevention programs.

Furthermore, the breastfeeding rates in Malaysia drop rapidly after two or three months after delivery, which is when the maternity leave ends and working mothers return to work (MOH, 2009). In addition to the breastfeeding campaigns organized by government and non-government institutions (e.g. Baby-friendly Hospital Initiative, Mother's Smart Choice Programme, MyNutriBaby etc), it is hoped that the expanding of breast milk adiponectin potential role in the prevention of childhood obesity from this study, may also provide an avenue towards the development of obesity prevention strategies through the practice of breastfeeding.

1.3 Objectives

1.3.1 General objective

To investigate the associations of maternal serum and breast milk adipokines with postpartum weight retention (PPWR) and infant adiposity development.

1.3.2 Specific objectives

i. To assess the maternal nutritional status, dietary intake, physical activity, physical discomforts, lipid profiles and serum adiponectin and leptin levels during pregnancy.
ii. To assess the maternal breast milk adiponectin levels within 2 months postpartum.
iii. To investigate the associations of maternal serum adiponectin levels during pregnancy with maternal breast milk adiponectin levels within 2 months postpartum.

iv. To investigate the associations of prenatal factors and maternal serum adiponectin and leptin levels during pregnancy with 12 months PPWR.

v. To investigate the associations of maternal serum adiponectin and leptin levels during pregnancy with infant adiposity development during the first year of life.

vi. To investigate the associations of maternal breast milk adiponectin levels within 2 months postpartum with infant adiposity development during the first year of life.

1.4 Research questions

i. What are the maternal nutritional status, dietary intake, physical activity, physical discomforts, lipid profiles and serum adiponectin and leptin levels during pregnancy?

ii. What are the maternal breast milk adiponectin levels within 2 months postpartum?

iii. Is there any association of maternal serum adiponectin levels during pregnancy with maternal breast milk adiponectin levels within 2 months postpartum, after controlling the confounders?

iv. Is there any association of prenatal factors and maternal serum adiponectin and leptin levels during pregnancy with 12 months PPWR, after controlling the confounders?

v. Is there any association of maternal serum adiponectin and leptin levels during pregnancy with infant adiposity development during the first year of life, after controlling the confounders?

vi. Is there any association of maternal breast milk adiponectin levels within 2 months postpartum with infant adiposity development during the first year of life, after controlling the confounders?

1.5 Alternative hypotheses

i. H_{α}: Maternal serum adiponectin levels during pregnancy are significantly associated with maternal breast milk adiponectin levels within 2 months postpartum.

ii. H_{α}: Prenatal factors and maternal serum adiponectin and leptin levels during pregnancy are significantly associated with 12 months PPWR.

iii. H_{α}: Maternal serum adiponectin and leptin levels during pregnancy are significantly associated with infant adiposity development during the first year of life.

iv. H_{α}: Maternal breast milk adiponectin levels within 2 months postpartum are significantly associated with infant adiposity development during the first year of life.

1.6 Significance of study

This pregnancy cohort study was comprised of measurements of diverse parameters among mothers and infants throughout gestational period until the first year postpartum. Nutritional status, lifestyle indicators such as dietary intake and physical activity as well as blood and breast milk analysis were among the main studied variables that were incorporated into the study. Therefore, it is hoped that all those information would provide crucial longitudinal baseline data on the maternal-infant obesity particularly via the intrauterine interaction, provided that it would elucidate the important prenatal factors that contributed to obesity. Essentially, these data could somewhat benefit other researchers for better comprehension, wider perspective and further investigation in the existing or new studies of obesity.

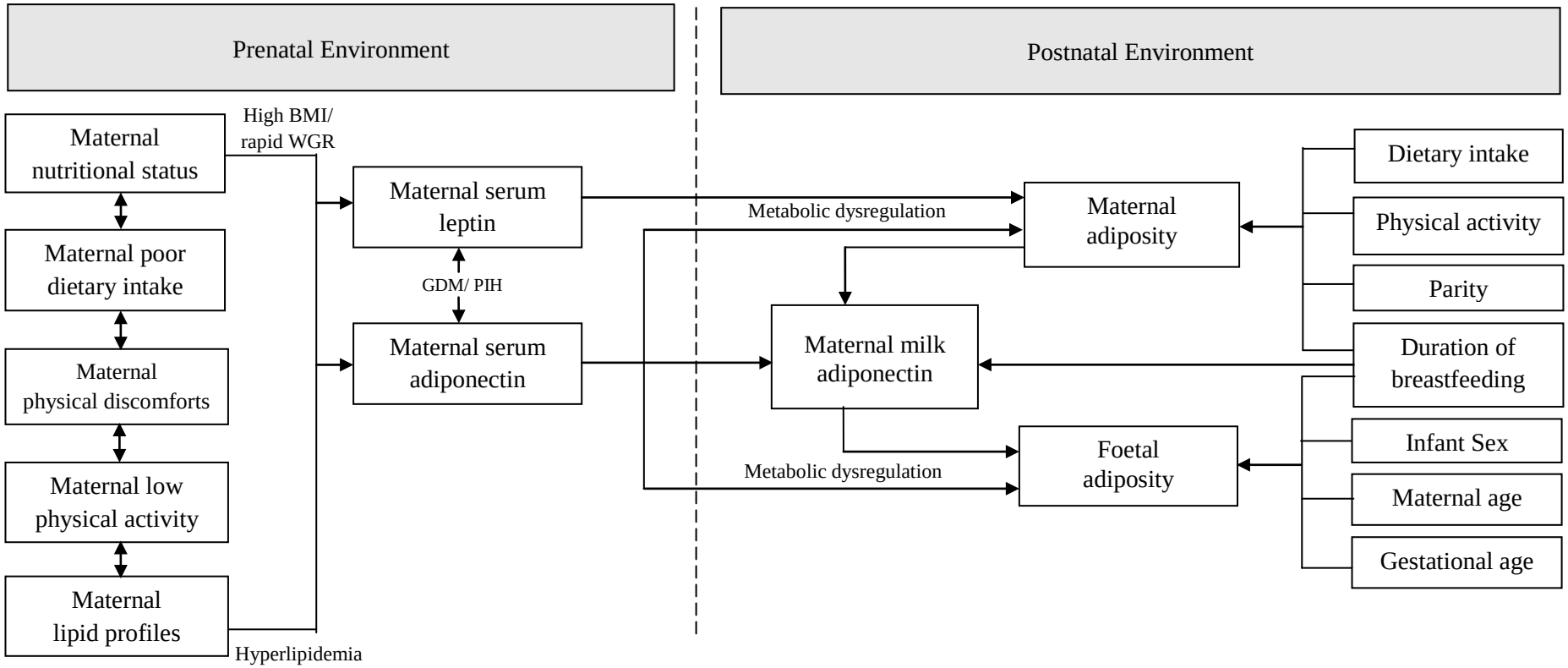
In spite of various obesity awareness campaigns targeting women were held in Malaysia either at the national, state or community levels, unfortunately so far they have had no visible effect on reducing women obesity rates. Hence, hopefully the data on obesity

development among pregnant mothers obtained from this study would enable the improvement of maternal and infant healthcare, particularly in the prevention and treatment of obesity. Thus, it would help to the successful implementation of community prevention programs or clinical therapies for obesity in future. In addition, the data also support the protective effect of breastfeeding practice against obesity, thus it appears that promoting breastfeeding is one of important parts of this study.

1.7 Conceptual framework

Figure 1.3 shows the conceptual framework as the background of the study. The imbalance in prenatal environment that includes maternal prepregnancy adiposity, poor dietary intake, low physical activity level, greater physical discomforts and hyperlipidaemia as well as rapid gestational weight gain rate may relate with the concentrations of maternal serum adipokines (adiponectin and leptin) during pregnancy and subsequently are responsible for the maternal breast milk adiponectin levels.

On the other hand, the duration of breastfeeding as one of the factors in early postnatal environment may also associate with the levels of adiponectin in breast milk. Given the importance of adipokines in human physiological and metabolic processes such as fatty acid metabolism and energy balance, the impaired levels of maternal serum adipokines (adiponectin and leptin) during pregnancy as well as breast milk adiponectin are somewhat considered as among the factors that influence the postpartum adiposity and infant adiposity development in later life.



Note. BMI = Body Mass Index; WGR = Weight Gain Rate; GDM = Gestational Diabetes Mellitus; PIH = Pregnancy-Induced Hypertension

Figure 1.3 Conceptual framework of the study

1.8 Operational definitions

Adipokines – proteins that are secreted from (and synthesised by) adipocytes (include adiponectin and leptin).

At birth – a follow-up visit, within (median=3, IQR=1-4) days after delivery; for the milk collection, within (median=3, IQR=1-5) days after delivery.

Caesarean delivery – a delivery by a surgical procedure, in which one or more incisions are made through mother's abdomen and uterus.

Complementary food – food other than breast milk and infant formula given to the child to provide nutrients.

Excess weight (prepregnancy BMI category) **–** mothers with overweight and obese prepregnancy BMI status.

Gestational age – a measure of the age of a pregnancy in weeks, from the first day of the mother's last menstrual cycle to the current date.

Gravidity – the number of pregnancy, including miscarriage and term delivery.

Healthy pregnancy – a pregnancy in the absence of pre-existing or currently diagnosed chronic medical condition and pregnancy complications.

High birth weight (HBW) – birth weight of more than 4000g.

Low birth weight (LBW) – birth weight of less than 2500g.

Normal delivery – a delivery through the vagina.

Parity – the number of >20 weeks birth, including stillborn.

Postpartum/ postnatal period – a period after delivery or birth (postpartum is the term used for the mothers, while postnatal is the term used for the infants).

Prenatal factors – include maternal nutritional status, dietary intake, physical activity, physical discomforts and lipid profiles.

Prenatal period – a period during pregnancy before delivery or birth.

Term delivery – a delivery between 37 and 42 weeks of gestation.

Trimesters of pregnancy – comprised of three stages of pregnancy period; the first trimester (0-13 weeks of gestation), second trimester (14-26 weeks of gestation) and third trimester (week 27 to the end of gestation) (APA, 2015).

Visceral fat rating – amount of visceral fat (fat in the abdominal cavity surrounding the vital organs), in which visceral fat rating for optimal health should stay under 13.

Weaning age – the age at which the child is firstly introduced to complementary food.

CHAPTER 2: LITERATURE REVIEW

2.1 Obesity

In adults, obesity refers to adipocyte hypertrophy (Kadowaki and Yamauchi, 2005), in which the enlargement of adipocyte size is predominant in adipose tissue cellularity (Hirsch and Batchelor, 1976; Van Harmelen *et al.*, 2003). The imbalance of energy intake and energy expenditure often results to adipocyte hypertrophy (Bays *et al.*, 2008). When energy consumed exceeds energy expended in prolonged period, it results to the impairment of adipogenesis or adipocyte hyperplasia, defined as the failure of adipose organ to recruit and proliferate new adipocytes from preadipocytes (Dubois *et al.*, 2006). Consequently, individuals with distortion in fat proliferation and differentiation are susceptible to adipocyte hypertrophy (Heilbronn *et al.*, 2004). When fat is predominantly deposited through hypertrophy and the large adipocyte reaches its limit expansion, human body is exposed to adipocyte dysfunction (Rutkowski *et al.*, 2015) and escalated ectopic fat storage (Heilbronn *et al.*, 2004; Dubois *et al.*, 2006).

It was revealed that dietary intake and physical activity level play significant role in the development of obesity (Stubbs and Lee, 2004). Higher intake of dietary fat that does not match the rate of fat oxidation would subsequently lead to fat imbalance or obesity. While along with the composition of fatty acids in the diet, physical activity is also known as one of the most effective ways to reduce the transient delay in adaptation to high fat diet (Krishnan and Cooper, 2014). Other than the frequently studied poor diet and sedentary lifestyle factors, there is a wide range of factors or combination of risk factors that are related to obesity. Aging factor was associated with the incidence of obesity regarding the declining function of adipose tissue, in which the proliferation and lipolysis were increased among younger person, whereas the tendency of lipid accumulation in nonadipose tissue was increased among older individual (Schipper *et al.*, 2008). It was also revealed that there is

strong link of genetic factor with obesity (Ramachandrappa and Farooqi, 2011), in which the inter-individual genetic variations and mutations determine the susceptibility or resistance to develop obesity. Other than biological and genetic, psychological factor of depression was also associated with obesity through changes in behaviour as it intervenes the behaviour of eating and engaging in physical activities (Blaine, 2008).

On the other hand, built environment or obesogenic environment which involves human-made factors such as income, density of population and employment, food safety and fast food availability has created a vulnerable environment to the risk of developing obesity (Lopez, 2007). While among potential risk factors that were recognized to associate with childhood obesity are including birth weight, catch-up growth, weight gain within the first year of age, adiposity development at early age, parental obesity, television viewing and sleep duration (Reilly *et al.*, 2005).

2.2 Prevalence of obesity

Globally, almost 2 billion adults aged 18 years and above were overweight, with more than 600 million were obese in 2014 (WHO, 2015). Global prevalence of obesity in 2014 was more than doubled the prevalence in 1980 (WHO, 2015). Considering the escalating trends of obesity continue, it was estimated that up to 3.3 billion of the world's adult population could be overweight by 2030 (Kelly *et al.*, 2008). In Malaysia, the national prevalence of overweight has raised from 16.6% in 1996 to 29.1% in 2006, while the national prevalence of obesity has escalated from 4.4% in 1996 to 14.0% in 2006, as reported in the Third National Health and Morbidity Survey (NHMS III) (Nor *et al.*, 2008). Recently from NHMS 2015, it was reported that the national prevalence of overweight continued to increase to 30.0%, while national obesity prevalence of 17.7% has shown four-time increase from the level in 1996 (IPH, 2015).

The prevalence of women obesity has increased and higher than that of men from 1980 to 2008, as reported in 16 of 21 regions (Stevens *et al.*, 2012). In fact, it was observed that between 1980 and 2008, women BMI had increased by 0.5 kg/m^2 per decade worldwide (Finucane *et al.*, 2011). While in 2014, 15.0% of women worldwide were obese compared to 11.0% of men (WHO, 2015). Rapid rising trend of obesity was linked to nutrition transition particularly in the developing countries and Asia region (Baker and Friel, 2014). In most low-income and middle-income regions, women were observed to have higher BMI than men (Finucane *et al.*, 2011). According to the report of NHMS 2015, the prevalence of women obesity (20.6%) and abdominal obesity (35.4%) in Malaysia were higher than that of men (15.0% and 11.8%, respectively) (IPH, 2015). Compared to NHMS 2006, women obesity (17.4%) and abdominal obesity (26.0%) prevalence were also significantly greater than that of men (10.0% and 7.3%, respectively) (Nor *et al.*, 2008).

The trend of childhood obesity is soaring upward, wherein by the year 2020 it was estimated that the global prevalence of overweight and obesity in children aged 0-5 years could reach 9.1% (De Onis *et al.*, 2010). The projection of this trend was based on the relative increase of worldwide childhood obesity prevalence from 4.2% in 1990 to 6.7% in 2010 (De Onis *et al.*, 2010). According to the Nutrition Survey of Malaysian Children in 2011-2012 among children aged 6 months to 12 years old, it was reported that the prevalence of overweight and obese were 9.8% and 11.8%, respectively (Poh *et al.*, 2013). While the national prevalence of overweight in children aged less than 5 years from NHMS 2015 has reported an increase to 7.6% (IPH, 2015), as compared to the prevalence of 6.4% from NHMS 2006 (Khor *et al.*, 2009).

2.3 Dietary intake during pregnancy and obesity

According to the ‘developmental origins of chronic adult disease’ hypothesis, Barker (2004) proposed that intrauterine environment is responsible for the changes in foetal body’s

structure, physiological and metabolic regulation. Given that intrauterine is a critical period of when the cells divide rapidly, hence any exposure to stimulus or insult may trigger foetal adaptation by permanently altering the physiology and metabolism that later may predispose the infant to chronic diseases (De Boo and Harding, 2006). As a stimulus to the intrauterine environment, it was proposed that foetal overnutrition or over supply of energy in prenatal period is associated with the childhood and adulthood obesity (McMillen *et al.*, 2009; Desai and Ross, 2011). Foetal overnutrition is mostly attributable to maternal adiposity particularly of greater prepregnancy weight status and gestational weight gain, in which it may result to permanent changes in the function of neuroendocrine, appetite control and energy metabolism of the foetus (Armitage *et al.*, 2008).

During pregnancy, the quantity and quality of dietary intake are very important for both mothers and infants (Guelinckx *et al.*, 2008). Nutrient intake during the first trimester of pregnancy would benefit the process of cells differentiation and the development of organs, while in later gestation the nutrients are mostly required for the rapid growth of the foetus (Godfrey and Barker, 2001; Burton *et al.*, 2002). The changes in dietary intake during this critical period may greatly influence maternal physiological and nutritional metabolism (King, 2000).

Excessive and imbalance food intake may pose a greater risk to excess maternal weight gain throughout pregnancy as reported in previous studies (Lagiou *et al.*, 2004; Olafsdottir *et al.*, 2006; Gardner *et al.*, 2011). A prospective cohort study among multi-ethnic mothers in the Netherlands showed that there were 1.13-4.49 times higher risk of gaining excess gestational weight among those with higher consumption of total energy, carbohydrate, protein and fat during pregnancy (Gaillard *et al.*, 2013). The same study also reported that excessive gestational weight gain was associated with 1.51 times greater risk of childhood overweight as compared to the groups of mothers with recommended and less than the recommended gestational weight gain.

In terms of quality of food intake, an adjusted multivariate regression model from a study among pregnant mothers reported that fried food and dairy intake were the predictors for excessive gestational weight gain (Stuebe *et al.*, 2009). While an animal study demonstrated that rats fed with junk food diet high in fat, sugar and salt during gestation and lactation were more likely to have offspring with escalated adiposity until adulthood (10 week postpartum) (Bayol *et al.*, 2008). In addition, a prospective cohort study of Project Viva in eastern Massachusetts, USA showed that the higher maternal consumption of omega-3 polyunsaturated fatty acids (PUFAs) during pregnancy was associated with lower childhood adiposity at 3 years of age (Donahue *et al.*, 2011).

On the other hand, it was also revealed that the quality of dietary intake during pregnancy was attributed to the prepregnancy weight status. A study showed that fibre and folate intake during pregnancy were decreased with the increased of prepregnancy BMI (Laraia *et al.*, 2007). In another study, obese pregnant mothers were reported to have lower score for diet quality, in which their meal pattern and intake of fruits and vegetables were unlikely to meet the recommendation (Derbyshire *et al.*, 2006). While a dietary pattern study demonstrated that mothers with greater prepregnancy BMI were prone to the Western diet which is of high-fat dairy, processed and red meats, rather than the Healthy and Intermediate diet (Knudsen *et al.*, 2008).

Fruits and vegetables intake are of high interest and greatly recommended in diet as it poses a protective effect on chronic diseases (Boeing *et al.*, 2012; Slavin and Lloyd, 2012). In pregnancy, maternal consumption of fruits and vegetables was significantly associated with birth size (Rao *et al.*, 2001; Loy *et al.*, 2011) and glucose tolerance of infants (Yajnik, 2004). In addition, a study revealed that overweight mothers with suboptimal weight gain during gestation had increased fibre intake at late pregnancy, which was contrary to overweight mothers with excess weight gain who were reported to decrease the intake of fibre in advancing gestation (Olafsdottir *et al.*, 2006). In spite of its benefits, the intake of

fruits and vegetables was shown to less likely to meet recommended amount of consumption during pregnancy, with only a small percentage of mothers reported to consume enough portion per day (Bang and Lee, 2009; Wen *et al.*, 2010; Cooper *et al.*, 2012).

2.4 Obesity in pregnancy and implications

Both maternal prepregnancy weight status and gestational weight gain is closely related to adverse effects on mothers and birth outcomes. Mothers with excess weight were prone to experience pregnancy health complications such as gestational diabetes (GDM), pregnancy-induced hypertension and preeclampsia (Rode *et al.*, 2005; Abenhaim *et al.*, 2007; Poston *et al.*, 2011). Overweight mothers with GDM were revealed to have higher risk to develop type 2 diabetes within years of postpartum (Bellamy *et al.*, 2009). On top of that, the risk to induced labour and caesarean delivery were increased among overweight mothers during labour (Doherty *et al.*, 2006). Previous studies also reported that overweight mothers had higher chance to miscarriage (Lashen *et al.*, 2004) and postpartum haemorrhage (Liu *et al.*, 2011; Fyfe *et al.*, 2012) as compared to mothers with normal BMI.

Obesity in pregnancy was associated with deleterious effects on pregnancy outcomes in a way it may increase the risk of health complications to the infant. Maternal obesity was demonstrated as a risk factor for infant mortality (Aune *et al.*, 2014) and preterm birth (Madan *et al.*, 2010), including induced preterm birth (McDonald *et al.*, 2010). Significant link between congenital malformations with overweight mothers was reported in previous studies, involving a wide range of birth defect categories (Blomberg and Källén, 2010; Gilboa *et al.*, 2010). The incidence of neural tube defects was related with maternal obesity (Waller *et al.*, 2007; Agopian *et al.*, 2013) even after supplementation of folic acid (Ray *et al.*, 2005). In addition, excess weight in mothers was also shown to influence infant overgrowth which particularly result to large-for-gestational age (LGA) and macrosomic

newborns (Athukorala *et al.*, 2010; Kerrigan and Kingdon, 2010), as well as increased birth weight (Surkan *et al.*, 2004).

Other than that, maternal obesity may affect the health and nutritional status of infants during their childhood, such as the development of adiposity (Crozier *et al.*, 2010), cognitive function (Smith *et al.*, 2011), blood pressure (Gaillard *et al.*, 2014) and atopic diseases (Mihrshahi *et al.*, 2003). In long-term effect, the development of obesity in childhood was associated with adulthood obesity and higher risk of developing metabolic syndrome (Catalano and Ehrenberg, 2006). A birth cohort study revealed that the greater BMI, waist circumference and other cardiometabolic risk factors among young adults at 32 years later were associated with higher maternal prepregnancy weight and gestational weight gain (Hochner *et al.*, 2012).

2.5 Obesity and adipokines

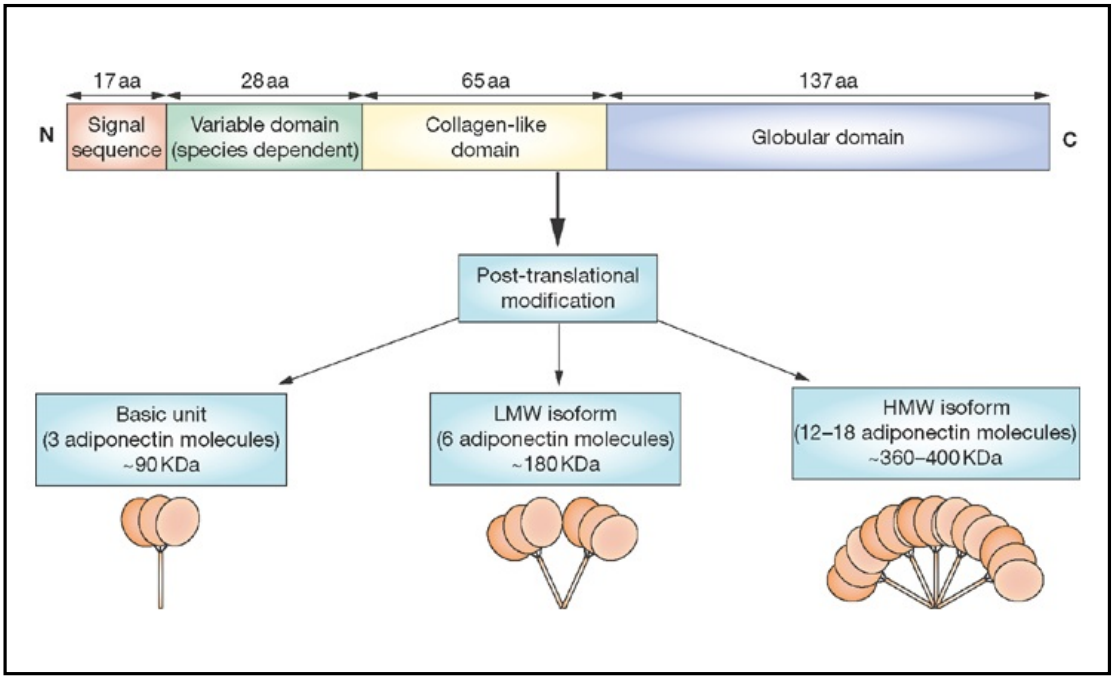
Apart from being the fat storage depot and energy source, the adipose tissue was also recognized as an endocrine organ which secretes a large number of cytokines and hormones (adipokines) (Galic *et al.*, 2010). Adipokines refer to bioactive proteins that are synthesised by or expressed from adipocytes (Trayhurn and Wood, 2004). As adipokines were known to play critical roles in lipid and glucose metabolism (Hauner, 2005; Matsuzawa, 2006; Halberg *et al.*, 2008), therefore individuals with distorted function of adipocytes would experience severe alterations in adipokines secretion and are thus highly predisposed to the development of metabolic syndrome and other adipocyte-related clinical consequences (Hajer *et al.*, 2008; Vázquez-Vela *et al.*, 2008).

The production of adipokines in obese adult is influenced by adipocyte hypertrophy (Skurk *et al.*, 2007). However, in newborns higher proportion of fat tissue accumulate in the subcutaneous area and most of the internal fat is nonabdominal (Harrington *et al.*, 2004),

while the size of adipocytes is smaller than the adults (Escofet *et al.*, 1996). Therefore, the differences in regional deposition and size of fat tissue might have accounted for the amount of adipokines in infants (Kotani *et al.*, 2004).

2.5.1 Adiponectin

Adiponectin is a hormonal protein primarily expressed in adipocytes (Hu *et al.*, 1996; Maeda *et al.*, 1996). Firstly identified and described by Scherer *et al.* (1995) as Acrp30 (adipocyte complement-related protein of 30 kDa), adiponectin was reported in the literature by different names; AdipoQ (Hu *et al.*, 1996), apM1 (Maeda *et al.*, 1996) and GBP28 (Nakano *et al.*, 1996). As the most abundantly expressed gene in adipocytes (Maeda *et al.*, 1996; Matsuzawa *et al.*, 2004), adiponectin is structurally composed of an N-terminal signal sequence at one end, followed by a collagen-like domain in the middle region and globular domain at C-terminal (Berg *et al.*, 2002) (Figure 2.1).



(adopted from Goldstein *et al.*, 2009)

Figure 2.1 Structure of adiponectin

There are at least three forms of adiponectin in human plasma; a trimer, a hexamer, known as low-molecular-weight (LMW) oligomer, and a high-molecular-weight (HMW) multimer (Whitehead *et al.*, 2006), which have variable ratios in individual human thus suggested that there are no correlation between total adiponectin and HMW adiponectin (Nakano *et al.*, 2006). To activate the signalling molecules, there are two main adiponectin receptors involved, AdipoR1 and AdipoR2 (Yamauchi *et al.*, 2003; Vasseur, 2006) as well as T-cadherin for hexameric and HMW adiponectin (Hug *et al.*, 2004; Thundyil *et al.*, 2012).

Besides adipocytes, the expression of adiponectin and its receptors were also found in the bone-forming cells (osteoblasts) (Berner *et al.*, 2004), endothelial cells and hepatocytes in liver (Kaser *et al.*, 2005), myocardium (Fujioka *et al.*, 2006), skeletal muscle (Yamauchi *et al.*, 2003; Staiger *et al.*, 2004), pancreas (Kharroubi *et al.*, 2003), placenta (Caminos *et al.*, 2005; Chen *et al.*, 2006), pituitary gland and the brain (Psilopanagioti *et al.*, 2009) in mice and human. Due to androgens that reduce the expression of adiponectin (Nishizawa *et al.*, 2002), men was shown to have a significantly lower level of total serum adiponectin as compared to women (Nakano *et al.*, 2006).

Adiponectin plays a central role in the modulation of lipid and glucose metabolism (Tsao *et al.*, 2002; Yamauchi *et al.*, 2002; Lin *et al.*, 2013). When adiponectin binds with its receptor, the AMP-activated protein kinase (AMPK) phosphorylation is activated. The phosphorylation of AMPK inhibits acyl-CoA carboxylase (ACC), which subsequently reduces the production of malonyl-CoA thus enhances mitochondrial β-oxidation, a process of which energy balance is restored in human body. Other than regulating fatty acids oxidation, the activation of AMPK also promotes catabolic reactions- including glucose uptake, and inhibits anabolic reactions. Additionally, adiponectin also acts on the activation of peroxisome proliferator-activated receptor (PPARα) to increase energy metabolism in the body. (Lafontan and Viguerie, 2006). As the ‘starvation hormone’, adiponectin level increases in the state of caloric restriction/fasting/starvation to suppress energy expenditure

and stimulate energy intake, and the level of adiponectin decreases after feeding (Lee and Shao, 2014). Therefore, hypoadiponectinaemia and reduced expression of adiponectin receptors are associated with dysregulation of lipid and glucose metabolism, as well as development of obesity-linked insulin resistance (Yamauchi and Kadowaki, 2013).

It was also revealed that adiponectin has anti-atherogenic properties (Matsuda *et al.*, 2002; Skrabal *et al.*, 2011) and anti-inflammatory effects (Weyermann *et al*., 2006a; Villarreal-Molina and Antuna-Puente, 2012). Low level of adiponectin in blood was shown to relate with metabolic syndromes, diabetes mellitus and cardiovascular diseases (Behre, 2007; Lara-Castro *et al.*, 2007). In a study by Koh *et al.* (2009), adiponectin level was demonstrated to have significant negative association with the number of metabolic syndrome components, which suggested the increased risk of developing coronary heart disease and diabetes with lower level of adiponectin (Sattar *et al.*, 2003).

It is important to note that adiponectin level was inversely associated with adiposity (Gavrila *et al.*, 2003; Fuglsang *et al.*, 2006) and visceral fat accumulation in adult (Cnop *et al.*, 2003) and children (Asayama *et al.*, 2003), despite the fact that it is abundantly and exclusively produced in adipose tissue. The inverse relationship was also revealed between cord blood adiponectin and birth weight among LGA newborns (Mazaki-Tovi *et al.*, 2005). On the contrary, cord blood adiponectin concentration was shown to have positive (Kotani *et al.*, 2004) or no relationship with birth weight in full-term newborns from uncomplicated pregnancies (Lindsay *et al.*, 2003; Kajantie *et al.*, 2004). The increased size of adipocytes (hypertrophy) was related with the dysfunction of adipocytes, in which consequently resulted to the decrease in adiponectin secretion (Chevillotte *et al.*, 2007).

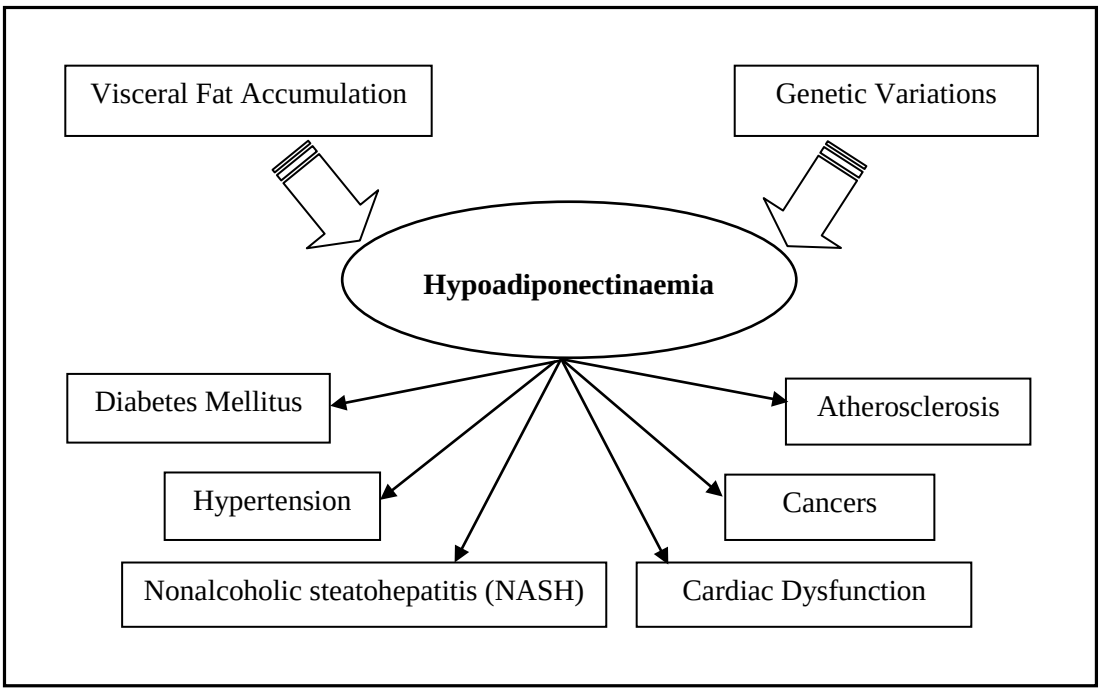
Given that hypertrophy and the inhibition of adipogenesis prevails in adiposity, hence it is possible to presume that hypertrophied adipocytes are may in part contribute to the distortion of adiponectin release as reported in previous studies on children and adults

with adiposity (Mathieu *et al.*, 2010). Whereas disruption of adiponectin release among infants with adiposity might be attributable to the abnormally higher number of adipocytes (Hammami *et al.*, 2001; Mazaki-Tovi *et al.*, 2005), as opposed to the healthy infants with substantially higher number of smaller adipocytes in the subcutaneous region which enhance the secretion of adiponectin (Kotani *et al.*, 2004).

On the other hand, it was shown that the release of adiponectin by abdominal visceral adipocytes decreased with the rise of BMI (Motoshima *et al.*, 2002), which might also explain the negative association of adiponectin with adiposity. A study among obese adolescents revealed that those with greater proportion of visceral fat and less subcutaneous fat had significantly lower concentration of adiponectin and were associated with other metabolic abnormalities, which proposed the higher risk of developing metabolic syndromes in later life (Taksali *et al.*, 2008). While a study that investigated the difference of adiponectin level between two subgroups of obese men with high and low visceral fat accumulation found that those with high visceral fat level have significantly lower level of adiponectin, in fact they were in the lowest margin of the adiponectin level for all subjects (Côté *et al.*, 2005). Multiple regression analysis from the study also reported that visceral fat accumulation was the only independent factor that explained the level of adiponectin.

Furthermore, it was revealed that the concentration of adiponectin was significantly correlated with central adiposity (Park *et al.*, 2004), and the results from multivariate analysis has also demonstrated that hypoadiponectinaemia was independently associated with abdominal visceral fat among type 2 diabetic Japanese men (Yatagai *et al.*, 2003). The findings from these various studies postulated that visceral fat accumulation might account for most of the adiponectin secretion in the blood. Considering that the accumulation of visceral adipose tissue has influenced the changes in the release of adiponectin (Steffes *et al.*, 2004) and is closely related to metabolic derangements (Asayama *et al.*, 2003; Lenchik *et al.*, 2003), it is clearly evident that the reduced release of adiponectin is deleterious in the

protection of human body from metabolic abnormalities and diseases. Taken together, a 'disease entity of hypoadiponectinaemia' was proposed by Matsuzawa (2010) to explain the relationship of low level of adiponectin with various major diseases (Figure 2.2).



(adopted from Matsuzawa, 2010)

Figure 2.2 A disease entity of hypoadiponectinaemia

Low level of adiponectin was related with adiposity (Comuzzie *et al.*, 2001; Nemet *et al.*, 2003), in which the significant reduction in body weight resulted to the increment of adiponectin level (Yang *et al.*, 2001). Metabolic changes along with the change of weight might lead to the alteration of adiponectin levels (Abbasi *et al.*, 2004a), given that the decline of insulin concentrations and increased of insulin sensitivity after weight loss (McLaughlin *et al.*, 2001) were associated with a rise in the level of adiponectin (Maeda *et al.*, 2001; Fasshauer *et al.*, 2002).

Despite that, there are several studies demonstrated that independent of adiposity, adiponectin level was strongly associated with insulin level (Möhlig *et al.*, 2001; Joseph *et al.*, 2002) and insulin resistance (Weyer *et al.*, 2001; Tschritter *et al.*, 2003). These findings