RISK FACTORS OF VITAMIN D DEFICIENCY AND THE EFFECTS OF SUNLIGHT EXPOSURE AND VITAMIN D SUPPLEMENTATION ON SERUM VITAMIN D LEVEL, ADIPONECTIN, CARDIOMETABOLIC RISK FACTORS AND METABOLIC SYNDROME AMONG ADULTS IN KELANTAN

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by

NORLIYANA BINTI ARIS

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LIST OF SYMBOLS AND ABBREVIATIONS

1,25(OH)D	1,25-dihydroxyvitamin D_3
25(OH)D	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
AFA	Area Fishermen’s Associations
ALT	Alanine Transaminase
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CVD	Cardiovascular diseases
DM	Diabetes mellitus
FBG	Fasting blood glucose
FFA	Free fatty acid
HDL-C	High-density lipoprotein cholesterol
HMW	High molecular weight
HOMA	Homeostasis model assessment
HOMA-B	HOMA of β-cell function
HOMA-IR	HOMA of insulin resistance
IDF	International Diabetes Federation
IL	Interleukins
IOM	Institute of Medicine
IPAQ	International physical activity questionnaire
IQR	Interquartile range
IR	Insulin resistance
MCP-1	Monocyte Chemoattractant Protein 1

MD	Mean difference
MED	Minimal Erythema Dose
MET	Metabolic equivalent task
MetS	Metabolic syndrome
MOH	Ministry of Health
NCCFN	National Coordinating Committee on Food and Nutrition
NEA	National Environment Agency
PA	Physical activity
PPSK	Pusat Pengajian Sains Kesihatan
PPSP	Pusat Pengajian Sains Perubatan
SD	Standard deviation
SED	Standard erythemal dose
SZA	Solar Zenith Angle
T2DM	Type 2 diabetes mellitus
TG	Triglycerides
TNF	Tumor necrosis factor
USM	Universiti Sains Malaysia
UVB	Ultraviolet B
UVR	Ultraviolet radiation
VD	Vitamin D
VDD	Vitamin D deficiency
VDI	Vitamin D insufficiency
VDS	Vitamin D sufficiency
WC	Waist circumference
WHO	World Health Organization
WMO	World Meteorological Organization

FAKTOR-FAKTOR RISIKO BAGI KEKURANGAN VITAMIN D DAN KESAN PENDEDAHAN CAHAYA MATAHARI SERTA SUPLIMENTASI VITAMIN D TERHADAP ARAS VITAMIN D SERUM, ADIPONEKTIN, FAKTOR RISIKO KARDIOMETABOLIK DAN METABOLIK SINDROM DALAM KALANGAN ORANG DEWASA DI KELANTAN

ABSTRAK

Walaupun dilimpahi dengan cahaya matahari yang banyak untuk penghasilan vitamin D (VD) melalui kulit, aras VD yang rendah telah dilaporkan dalam kalangan rakyat Malaysia. Walau bagaimanapun, kesan pendedahan matahari berkaitan pekerjaan dan musim monsun ke atas VD masih belum cukup dikaji. Selain itu, aras VD yang rendah turut dikaitkan dengan beberapa penemuan yang tidak berkait dengan tulang atau kalsium termasuklah sindrom metabolik (MetS), yang mana mungkin diperantara oleh adiponektin. Kajian ini terbahagi kepada dua fasa. Fasa 1, kajian keratan rentas perbandingan telah dilaksanakan untuk mencirikan kesan pendedahan cahaya matahari berkaitan pekerjaan dan musim monsun ke atas 25-hydroxyvitamin D (25(OH)D) serum serta mengenalpasti faktor yang mempengaruhi aras 25(OH)D. Selain itu, hubungan di antara aras 25(OH)D dengan adiponektin berat molekul tinggi (adiponektin BMT) dan faktor risiko kardiometabolik turut dikaji. Fasa 2, kajian eksperimental berbentuk kuasi telah dijalankan untuk tempoh 12 minggu bagi menilai kesan pendedahan cahaya matahari secara sederhana [kumpulan pendedahan cahaya matahari; pendedahan cahaya matahari untuk 15 minit, 2 kali seminggu pada bahagian muka, tangan dan lengan serta kaki; n=19), pengambilan suplemen VD [kumpulan suplementasi VD, 50,000 IU cholecalciferol satu kali seminggu, n=15] dan placebo (kumpulan placebo, n=15) ke atas 25(OH)D serum, adiponektin BMT dan faktor risiko kardiometabolik dalam kalangan wanita dengan ketidakcukupan VD (serum 25(OH)D

< 50 nmol/l). Kedua-dua fasa dilaksanakan dalam kalangan Melayu dewasa yang bekerja di Kelantan pada 2012 sehingga 2015. Pengukuran hasil kajian merangkumi pengukuran antropometri (tinggi, berat dan ukurlilit pinggang), penilaian peratus lemak tubuh dan tekanan darah, ujian darah berpuasa (25(OH)D, adiponektin BMT, profil lipid, glukosa, insulin dan hs-CRP) serta borang soal selidik (sosio-demografi, sejarah perubatan, pendedahan dan perlindungan cahaya matahari, penilaian aktiviti fizikal serta pengambilan VD). Dalam fasa 1, sebanyak dua kali pengumpulan data dilakukan ke atas 138 pekerja luar (*outdoor workers*) dan 143 pekerja dalam (*indoor workers*), pertama ketika musim bukan-monsun (Mei-Jun 2012) dan kedua ketika musim monsun (Jan-Feb 2013). Hasil kajian fasa 1 mendedahkan bahawa aras 25(OH)D serum adalah lebih tinggi secara signifikan dalam kalangan pekerja luar berbanding pekerja dalam tanpa mengira musim dan jantina ($p<0.001$). Malah hubungan yang signifikan antara status VD dan jenis pekerjaan turut dilihat ($p<0.001$). Perbezaan musim monsun ke atas 25(OH)D hanya dilihat dalam kalangan pekerja luar lelaki (perbezaan min (MD) = 10.39 nmol/l, $p<0.001$). Walau bagaimanapun, secara keseluruhan, tiada hubungan yang signifikan ditemui antara status VD dan musim monsun ($\chi^2(1)=0.076$, $p=0.783$). Aras 25(OH)D serum adalah diramal secara langsung dengan jumlah jam pendedahan cahaya matahari ($\beta=0.38$, $p=0.010$) dan secara berlawanan arah oleh skor perlindungan cahaya matahari ($\beta=-4.64$, $p=0.001$), indeks jisim tubuh ($\beta=-1.02$, $p=0.002$), pekerja dalam ($\beta=-42.72$, $p<0.001$) dan jantina wanita ($\beta=-19.46$, $p<0.001$). Tiada hubungan yang signifikan di antara VD dengan adiponectin BMT dan faktor risiko kardiometabolik ditemui dalam kajian ini ($p>0.05$). Dengan mengambil kira keputusan faktor penentu VD dari fasa 1, pekerja dalam wanita dengan ketidakcukupan VD pada musim monsun dan bukan monsun telah direkrut untuk kajian fasa 2. Dalam fasa 2, selepas 12 minggu intervensi, aras 25(OH)D meningkat secara signifikan dalam kumpulan pendedahan cahaya matahari (perbezaan min, MD=14.27 nmol/l, p<0.001) dan suplimen VD (MD=14.30 nmol/l, p<0.001) tetapi tidak dalam kumpulan placebo (MD=1.63 nmol/l, p=0.067). Peningkatan adiponektin BMT hanya diilihat dalam kumpulan suplementasi VD (MD=0.43 ng/ml, p=0.024). Dalam kalangan faktor risiko kardiometabolik, hanya aras glukosa menurun secara signifikan selepas 12 minggu terdedah kepada cahaya matahari (MD=-0.27 mmol/l, p<0.001) namun tiada perubahan ditemui dalam kumpulan lain. Tiada perubahan yang signifikan bagi ukurlilit pinggang, tekanan darah, trigliserida dan faktor risiko kardiometabolik yang lain dalam semua kumpulan selepas 12 minggu. Walau bagaimanapun, HDL-C didapati meningkat secara signifikan dalam semua kumpulan selepas intervensi (p<0.05). Sebagai kesimpulan, kedua-dua intervensi pendedahan cahaya matahari secara sederhana dan suplimentasi VD meningkatkan aras VD, tetapi keberkesanan VD ke atas adiponektin BMT dan faktor risiko kardiometabolik adalah masih belum meyakinkan.

RISK FACTORS OF VITAMIN D DEFICIENCY AND THE EFFECTS OF SUNLIGHT EXPOSURE AND VITAMIN D SUPPLEMENTATION ON SERUM VITAMIN D LEVEL, ADIPONECTIN, CARDIOMETABOLIC RISK FACTORS AND METABOLIC SYNDROME AMONG ADULTS IN KELANTAN

ABSTRACT

In spite of abundant sunshine for cutaneous vitamin D (VD) synthesis, low levels of VD have been documented among Malaysian population. However, the effects of occupational sunlight exposure and monsoon seasons on VD are not well established. Besides, low VD levels has been associated with several non-bone or calcium-related outcomes including metabolic syndrome (MetS), which may mediated by adiponectin. This study was divided into two phases. Phase 1, a comparative cross-sectional study was conducted to characterize the effect of occupational sunlight exposure and monsoon season on serum 25-hydroxyvitamin D (25(OH)D) and identify factors modifying the serum 25(OH)D levels. Besides, the relationship between 25(OH)D levels with high molecular weight (HMW) adiponectin and cardiometabolic risk factors were also observed. Phase 2, a quasi-experimental study was carried out for 12 weeks to evaluate the effects of moderate sunlight exposure (sunlight exposure group; 15 minutes sunlight exposure biweekly on face, arms, hands and feet; n=19), VD supplementation (VD supplement group, 50 000 IU cholecalciferol weekly, n=15] and placebo (placebo group, n=15) on serum 25(OH)D, HMW adiponectin and cardiometabolic risk factors in women with VD deficiency (serum 25(OH)D < 50 nmol/l). Both phases were carried out among Malay working adults in Kelantan from 2012 to 2015. Outcome measures comprises of anthropometric measurements (height, weight and waist circumference), body fat percentage and blood pressure assessment, fasting blood test (25(OH)D, HMW adiponectin, lipid profile, glucose, insulin and hs-CRP) and questionnaire (socio-demographic, medical history, sunlight exposure and sun protection use, physical activity assessment and VD intake). In phase 1, two-point data were collected in 138 outdoor and 143 indoor workers, first in non-monsoon (May-June 2012) and second in monsoon season (Jan-Feb 2013). Results of phase 1 study revealed that serum 25(OH)D levels were significantly higher in outdoor workers compared to indoor workers irrespective of season and sex ($p<0.001$). Furthermore, significant association between VD status and occupation was observed ($p<0.001$). Monsoonal differences of 25(OH)D was observed only in male outdoor workers (mean difference, MD = 10.39 nmol/l, $p<0.001$). However, in overall, no significant association was found between VD status and monsoon season ($\chi^2(1)=0.076$, $p=0.783$). Serum 25(OH)D level was directly predicted by hours of sunlight exposure ($\beta=0.38$, $p=0.010$) and inversely by sun protection score ($\beta=-4.64$, $p=0.001$), body mass index ($\beta=-1.02$, $p=0.002$), indoor occupation ($\beta=-42.72$, $p<0.001$) and female sex ($\beta=-19.46$, $p<0.001$). No significant relationship between VD with HMW adiponectin and cardiometabolic risk factors were found in this study ($p>0.05$). Considering the results of determinants of VD in Phase 1, female indoor workers with VD deficiency in both monsoon and non-monsoon seasons were recruited for phase 2 study. In phase 2, after 12 week of intervention, serum 25(OH)D increased significantly in sunlight exposure (MD = 14.27 nmol/l, $p<0.001$) and VD supplement group (MD = 14.30 nmol/l, $p<0.001$) but not in placebo group (MD = 1.63 nmol/l, $p=0.067$). Significant increase in HMW adiponectin was observed only in VD supplement group (MD=0.43 ng/ml, $p=0.024$). Among the MetS components, only glucose decreased significantly after 12 weeks of sunlight exposure (MD=-0.27 mmol/l, $p<0.001$) but no changes seen in the other groups. No significant changes were found in waist circumference, blood pressure, triglycerides and other cardiometabolic risk factors in all groups after 12 weeks. However, HDL-C was found increased significantly in all groups after the intervention ($p<0.05$). In conclusion, both moderate sunlight exposure and VD supplement interventions improved the VD levels, but the effects of VD on HMW adiponectin and cardiometabolic risk factors are still inconclusive.

CHAPTER 1

INTRODUCTION

1.1 Background

Vitamin D, the sunshine vitamin is a fat soluble secosteroids (Cesari *et al.*, 2011) occurring naturally in five forms (D1 – D5). The predominant forms of vitamin D are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Both vitamin D2 and vitamin D3 can be obtained from the diet (foods or supplements) but only vitamin D3 can be produced naturally by endogenous photosynthesis in the skin after solar ultraviolet B (UVB) radiation (wave length 280-315 nm) (Lehmann and Meurer, 2010). Sunlight exposure is the major source of vitamin D and its covers 90% of vitamin D production in human (Holick, 2003; Stroud *et al.*, 2008). Endogenous (skin pigmentation and thickness) and exogenous factors (weather, time of day and lifestyle) influence the cutaneous (skin) production of vitamin D (Azizi *et al.*, 2009; Holick, 2004).

Vitamin D deficiency (VDD), reflected by circulating 25-hydroxyvitamin D (25(OH)D) levels < 50 nmol/l or 20ng/ml is a global health problem affecting all age groups (Holick and Chen, 2008; Lhamo *et al.*, 2017) even in low latitude countries where abundant UVB rays for cutaneous vitamin D synthesis. Besides, poor vitamin D status were also reported in industrialized countries where fortification of food with vitamin D has been implemented (Palacios and Gonzalez, 2014). In Malaysia, despite close proximity to the equator and plentiful sunlight, high prevalence of VDD (more than 70%) has been reported in Malaysian adults (Chin *et al.*, 2014; Moy, 2011; Nurbazlin *et al.*, 2013; Rahman *et al.*, 2004).

The classic role of vitamin D for normal skeletal development and maintenance in preventing bone diseases is well established (Christodoulou *et al.*, 2013; Holick, 2006). In the recent decades, increasing evidence suggests that vitamin D plays an important role of various non-classical actions that may be important in the pathogenesis of many chronic diseases (Holick 2007; Muscogiuri *et al.*, 2014; Wang *et al.*, 2017). The non-classical effect of low vitamin D levels has been associated with muscle weakness, cancer, multiple sclerosis, cardiovascular disease (CVD), diabetes mellitus (DM) and all-cause mortality (Muszkat *et al.*, 2010; Zittermann and Gummert, 2010a). This is due in part to widespread expression of vitamin D receptor (VDR) in most tissues and cells (Bikle, 2014) such as heart, kidney, immune cells, brain and muscle (Gonzalez-Parra *et al.*, 2012).

Metabolic syndrome (MetS) is comprised of several abnormalities that occur together in which represents a clustering of risk factors for CVD and type 2 diabetes. Based on the harmonized definition of MetS by International Diabetes Federation (IDF) 2009, five risk factors were identified. These risk factors include raised blood pressure, raised triglycerides (TG), lowered high-density lipoprotein cholesterol (HDL-C), raised fasting glucose, and elevated waist circumference. Existence of any three of these risk factors would qualify a person for the MetS (Alberti et al., 2009). In Malaysia, 25 to 40% adults were affected by MetS (Lim and Cheah, 2016) and the risk increases with age, sex (female), ethnicity (Indian) and abdominal obesity (Ashari *et al.*, 2016; Mohamud *et al.*, 2011).

Altered function of adipose tissues impact significantly on whole-body metabolism and represent a key driver of MetS development (Armani *et al.*, 2017). Adipokines (biologically active substances secreted by adipose tissue) were recently proposed as novel biomarkers and regulators of MetS (Deng and Scherer, 2010).

Among the different adipokines, adiponectin is seen to be “protective” adipokine and highly significant in the development of MetS (Esfahani *et al.*, 2015; Fisman and Tenenbaum, 2014).

Adiponectin is the most abundant circulating adipokine and is recognized as a key component in regulating insulin sensitivity, glucose and lipid metabolism besides anti-inflammatory and anti-atherogenic properties (Ahima, 2006; Esfahani *et al.*, 2015). Adiponectin exists in three forms: a low molecular weight trimer (LMW), a medium molecular weight hexamer (MMW) and a larger multimeric high molecular weight (HMW) form (Aso *et al.*, 2006). Consistent findings showed that low level of adiponectin is associated with most of the cardiometabolic risk factors (Calton *et al.*, 2013; Ding *et al.*, 2015) and, furthermore decreased HMW Adiponectin is suggested as predictor of the progression to MetS (Seino et al., 2009).

Emerging data suggest that vitamin D is the novel risk markers for MetS. It has been hypothesized that vitamin D may act through adiponectin to modulate MetS (Calton *et al.*, 2013; Nimitphong *et al.*, 2009). Recent evidence showed that vitamin D concentration are positively associated with circulating adiponectin level, and this association varies across body mass index (BMI) classification among adults (Bidulescu *et al.*, 2014; Nimitphong *et al.*, 2009; Vaidya *et al.*, 2012). Since both vitamin D and adiponectin have a favorable effects on cardiometabolic risk factors (Bidulescu *et al.*, 2014; Calton *et al.*, 2013; Kardas *et al.*, 2013), the interaction between vitamin D–adiponectin may be an indicator of MetS.

In view of that, this study was embarked to understand the associated factors of vitamin D and to investigate the association between vitamin D with HMW adiponectin and cardiometabolic risk factors. The research work of this study comprised of two phases, Phase 1: a cross-sectional study and Phase 2: intervention study. Both studies were carried out among Malay outdoor and indoor workers in Kelantan state, Malaysia from 2012 to 2015.

1.2 Problem statement

Known as ‘sunshine vitamin’, sunlight exposure is a major and natural source of vitamin D. Cutaneous synthesis of vitamin D provide 90% of vitamin D production in human. Vitamin D level significantly associated by occupational sunlight exposure and seasonal variations (Azizi *et al.*, 2009; Devgun *et al.*, 1981). It has been demonstrated that indoor workers, shiftworkers and healthcare workers were at high risk of VDD (Sowah *et al.*, 2017). Besides, the influence of seasonal variation in vitamin D status has been acknowledged and high prevalence of VDD during winter months was recognized (Bolland *et al.*, 2007; Mithal *et al.*, 2009).

Although some research has been carried out on the relationship between vitamin D with occupations and seasonal variations, the effects of prolonged periods of rain during monsoon season on vitamin D levels is not well documented. To the best of researchers' knowledge, there is no previous study that has investigated simultaneously the effects of occupation and dry/rainy season on vitamin D in Malaysia and other countries. In Malaysia particularly, the knowledge on the effects of different occupational groups on vitamin D level are still scarce. Earlier studies of vitamin D among Malaysian focused on ethnic comparison (Chin *et al.*, 2014; Green *et al.*, 2008; Rahman *et al.*, 2004), Malay employee (Moy, 2011), pregnant women (Jan Mohamed *et al.*, 2014) and urban-rural differences (Nurbazlin *et al.*, 2013). Therefore, this study aims to contribute to research on vitamin D in Malaysia by exploring the effects of different occupation and monsoon season on vitamin D level.

A number of researchers have examined the effects of high dose vitamin D supplementation either globally (De Niet *et al.*, 2018; Mozaffari-Khosravi *et al.*, 2015; Sollid *et al.*, 2014; Vieth *et al.*, 2004) or locally (Ramly *et al.*, 2014). However, little is known about the efficacy of modest direct sunlight exposure in increasing vitamin D level particularly among Malay adults. Experimental studies on the effects of UV exposure on vitamin D were mostly conducted by using artificial sunlight such as sunbed or UV light cabin (Bogh *et al.*, 2012; Lagunova *et al.*, 2013). Studies in Thailand (Watcharanon *et al.*, 2018), UAE (Dawodu *et al.*, 2011) and Finland (Karppinen *et al.*, 2017) have investigated the effects of natural sunlight exposure on vitamin D level, but the finding of these studies are inconclusive. Therefore, this study aims to contribute to this growing area of research by exploring simultaneously the effects of two vitamin D sources (modest direct sunlight exposure and vitamin D supplement) on vitamin D levels among Malay female indoor workers.

In recent decades, there has been an increasing interest in non-skeletal role of vitamin D in protecting other systems and preventing chronic diseases including MetS. The mechanisms in which vitamin D may protect against cardiometabolic risk factors include activation of the renin-angiotensin-aldosterone systems, increase arterial intimae thickness and enhancement in insulin secretion and insulin sensitivity (Holick, 2011). The role of vitamin D in regulating MetS may partly mediated by adiponectin. However, the evidence from available research is insufficient and inconsistent in findings (Baziar *et al.*, 2014; Nimitphong *et al.*, 2009; Vaidya *et al.*, 2012). Thus, it is hoped that this study can provide a better understanding of the relationship between these variables (vitamin D, adiponectin, cardiometabolic risk factors and MetS) through observational and experimental study.

1.3 Significance of study

This study addressed the role of occupation, sunlight exposure and monsoon season on vitamin D level. Moreover, the effects of sensible, moderate sunlight exposure and vitamin D supplement on serum 25(OH)D were investigated. The results of this finding would be very useful to society and policy makers in terms of improving systemic vitamin D level. It could be applied in practice as an alternative approach to improve vitamin D status, in which to reduce VDD and ensure improved population health outcomes.

This study too investigates the relationship between vitamin D with adiponectin and cardiometabolic risk factors. For researcher, this finding will contribute to knowledge and understanding of the causal relationship between vitamin D with adiponectin in relation to cardiometabolic risk factors which could help to set new strategies for the improvement of the condition in the future. Thus, the findings will redound to the benefit of society considering that MetS is a major public health problem, which has close association with adiposity, diabetes and CVD. With vitamin D and adiponectin are emerging as potential biomarkers for MetS, both could be a promising target for prevention and treatment of MetS and related diseases.

1.4 Research question

a. Phase 1

i. Is there any difference in serum level of 25(OH)D between outdoor and indoor workers during non-monsoon and monsoon season?

ii. What are the potential determinants of serum 25(OH)D level during non-monsoon and monsoon season?

iii. Does the serum 25(OH)D level have an influence on HMW Adiponectin and the cardiometabolic risk factors?

b. Phase 2

i. Is there mean 25(OH)D difference between or within treatment groups (sunlight exposure, vitamin D supplementation and placebo) based on time?

ii. Are there any changes in mean HMW adiponectin and cardiometabolic risk factors within treatment groups (sunlight exposure, vitamin D supplementation and placebo) based on time?

1.5 Study Objectives

1.5.1 General objective

This study aimed to identify the risk factors of vitamin D deficiency and to investigate the effects of sunlight exposure and vitamin D supplementation on serum vitamin D concentration, adiponectin and cardiometabolic risk factors among Malay adults in Kelantan.

1.5.2 Specific objective

a. Phase 1

i. To compare serum 25(OH)D between outdoor and indoor workers during monsoon and non-monsoon season among Malay adults in Kelantan.

ii. To examine the determinants of serum 25(OH)D level during non-monsoon and monsoon season among Malay adults in Kelantan.

iii. To determine the relationship between serum 25(OH)D with HMW adiponectin, cardiometabolic risk factors and MetS among Malay adults in Kelantan.

b. Phase 2

i. To determine and compare the changes in serum 25(OH)D level following 12 weeks intervention by different interventions (sunlight exposure, vitamin D supplementation or placebo) among Malay female indoor workers in Kelantan.
ii. To determine and compare the changes in HMW adiponectin and cardiometabolic risk factors following 12 weeks intervention by different treatments (sunlight exposure, vitamin D supplementation or placebo) among Malay female indoor workers in Kelantan.

1.6 Alternative hypothesis

a. Phase 1

i. The mean serum 25(OH)D are significantly difference between outdoor indoor workers during non-monsoon and monsoon season.
ii. There will be a significant prediction of serum vitamin D by occupation, sex, sun exposure, sun protection, physical activity, dietary vitamin D intake & BMI (at least 1 independent variable).
iii. Serum 25(OH)D significantly related with HMW Adiponectin and cardiometabolic risk factors.

b. Phase 2

i. At least one of the mean serums 25(OH)D are significantly difference between or among treatment groups.
ii. Vitamin D supplementation or sunlight exposure significantly improve the HMW Adiponectin and cardiometabolic risk factors (at least one risk factor).

1.7 Conceptual framework

The primary outcome measure for this study is vitamin D. In the first phase, the study was focused on the determinants of vitamin D levels. The significant predictors from the outcome of phase 1 were used as the foundation for the study design in phase 2. Meanwhile, adiponectin, cardiometabolic risk factors and MetS were the secondary outcome measures of this study.

Figure 1.1 shows the overall concept of the study. Vitamin D can be obtained from two sources. First, from direct sunlight exposure of which covers 90% of vitamin D production in human through cutaneous synthesis after solar UVB radiation. Second, from dietary sources either from natural foods, vitamin D fortified foods or vitamin D supplements. Low sunlight exposure usually observed among women, those who use sun protection, people with indoor occupation, older individuals and during season where there is very little daylight and sunlight available such as winter or rainy season. Low serum vitamin D levels reflect decrease in cutaneous synthesis, poor dietary intake of vitamin D and low physical activity. Lack of physical activity result in poor anthropometric status in which leads to adiposity and, therefore, decrease the serum vitamin D levels.

Greater adipose tissue leads to greater circulating fatty acids, hypo-secretion of adiponectin and decrease the bioavailability of vitamin D. This condition may trigger chronic low-grade inflammation of metabolic tissues, including skeletal muscle and liver, which results in insulin resistance. Insulin resistance plays a key role in the pathophysiology of MetS, in which leads to increased blood glucose, blood pressure and triglyceride as well as reduced circulatory HDL-C. Additionally, the roles of vitamin D may mediated by adiponectin to modulate MetS through renin-angiotensin system by vitamin D metabolites.

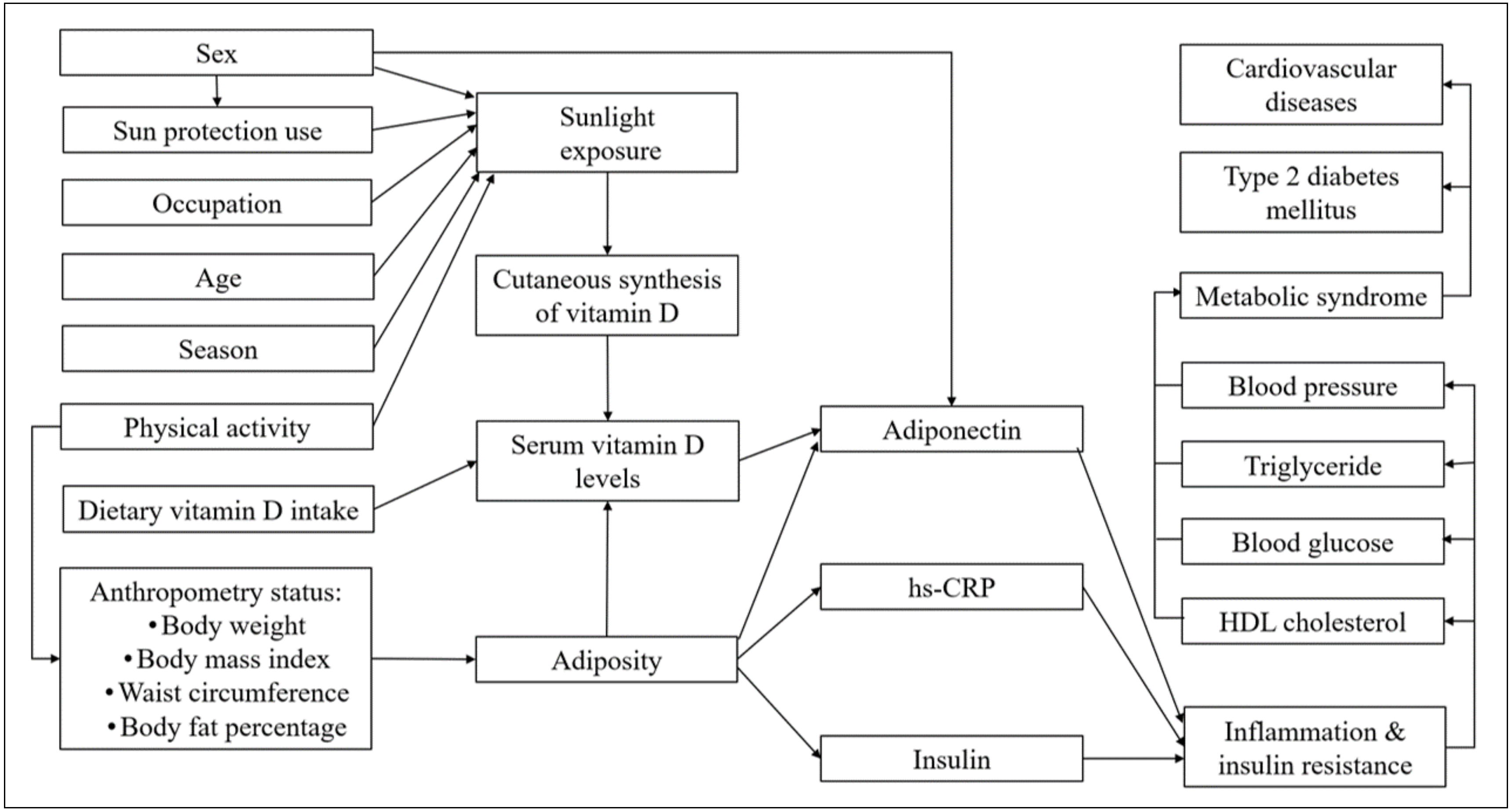

Figure 1.1 Conceptual framework of the study

1.8 Operational terms definition

Adiponectin – a protein hormone produced and secreted by adipose tissues (fat cells). In this study the adiponectin was determine by measuring the high molecular weight isoform which is known as HMW adiponectin.

Cardiometabolic risk factors – risk factors that increase the likelihood of experiencing vascular events or developing diabetes (Chatterjee *et al.*, 2012). In this study the cardiometabolic risk factors consist of metabolic syndrome related biomarkers such as glucose, insulin, lipid profile, hs-CRP, blood pressure and waist circumference.

HMW Adiponectin – an active form of Adiponectin protein and a good predictor of metabolic abnormalities.

Indoor workers – indoor workers in this study were defined as those who stayed indoors more than half of their working hours (office staff) for phase 1, and at least ¾ of working hours in phase 2.

Metabolic syndrome (MetS) – is a group of conditions that often occurs together and can increase the risk of having diabetes or CVD. The definition of MetS in this study was in accordance with the Harmonized International Diabetes Federation (IDF) for MetS 2019 (Alberti *et al.*, 2009).

Monsoon season - the periods when northeast monsoon (NEM) occurred at the study area (November 2012 to February 2013).

Non-monsoon season - the periods when the study area was not affected by northeast monsoon (April 2012 to July 2012).

Occupational sunlight exposure – exposure to ultraviolet radiation (UVR) from the sunlight through skin or eyes that may result from the performance of an employee's duties.

Outdoor workers – in this study outdoor workers were defined as those who experienced occupational sunlight exposure for more than 2 hours/day between 8.00 am to 2.00 pm (fishermen).

Seasons – season in this study refer to the period of non-monsoon and monsoon takes place.

Serum 25(OH)D – serum level/concentration of vitamin D.

Ultraviolet (UV) radiation – specific portion of the sun's energy reaching the earth's surface (the electromagnetic spectrum between 100–400 nm).

Vitamin D status – for this thesis, vitamin D status refer to the classification of vitamin D level [25(OH)D], which based on the Endocrine Society Clinical Practice Guideline (Holick *et al.*, 2011), as follows: sufficient (≥75 nmol/l), insufficient (50 – 74 nmol/l) and deficient (< 50 nmol/l).

CHAPTER 2

LITERATURE REVIEW

2.1 The climate of Malaysia

2.1.1 Monsoon seasons

Malaysia including others Southeast Asian countries are influenced by a monsoon (Loo *et al.*, 2015). The word "monsoon" comes from the Arabic word "mausim", which means season. Monsoon is defines as the large-scale seasonal reversal of the wind direction (prevailing wind) over a region. It is caused by a difference in thermal circulations between a landmass and the adjacent ocean (Ahrens and Henson, 2016).

Monsoon always blows from cold to warm region. The Asian monsoon results from a reversal of the winds between the winter and summer. During winter, the air over landmass colder than the air over the adjacent ocean. The surface winds blow from land to ocean leading to clear skies and generally dry weather. When summer arrives, the wind direction shift itself as air over landmass becomes much warmer than air above the ocean. The surface winds blowing from ocean to land and bring large amounts of water vapor to produce heavy rainfall over land. Monsoon cause wet and dry seasons over tropical and near-tropical regions and greatly affect these region's climate (Aguado and Burt, 2015b; Ahrens and Henson, 2016).

Malaysia's climate and weather are governed by the regime of two monsoon seasons, namely, the Northeast Monsoon (NEM) from November to March (winter monsoon), and the Southwest Monsoon (SWM) from late May to September (summer monsoon). The transition period between the monsoons is known as the inter-monsoon period (MMD, 2017b).

The NEM is characterized as a wet season and associated with cloudy conditions and frequent afternoon shower. It is caused by the steady strengthening of easterly or northeasterly winds (10 to 20 knots) blowing from the South China Sea which originate from cold air of Siberia. During this season, four to five episodes of monsoon surges are normally expected (MMD, 2017b). Monsoon surges refer to strong outbursts of cold air due to sudden increase in wind speed (may reach 30 knots or more) into the South China Sea. It could bring a continuous, moderate to heavy rainfall lasting two to five days, occasionally windy conditions and a few days of cooler temperatures (NEA, 2015b). Lack of sunlight and less solar radiation are expected during this period due to overcast and cloudy conditions with extensive cloud cover (MMD, 2017b).

Areas affected by the NEM includes east coast of Peninsular Malaysia, Western Sarawak and the northeast coast of Sabah (MMD, 2017b). The west coast region of Peninsular Malaysia is less influenced by this monsoon due to the existence of the Titiwangsa Range which blocks the region from receiving heavy rainfall. This region denoted as the driest area but still receive 41% of total rainfall from the northeast monsoonal flow. The NEM contribute 55% of the total annual rainfall of the east coast region of Peninsular Malaysia and strongly influenced the rainfall characteristics (e.g. total amount of rainfall, rainfall intensity and frequency of wet days) of this region (Suhaila *et al.*, 2010; Wong *et al.*, 2009). Besides, this region considered as the wettest area during the NEM season (Deni *et al.*, 2009).

The SWM caused by light southwesterly winds (below 15 knots) blowing from Sumatra, originating from the Southern Indian Ocean. Except for Sabah, the period of this monsoon is relatively drier throughout the country, especially Peninsular Malaysia since it sheltered by Sumatra’s mountain ranges (MMD, 2017b). The SWM contribute

37% and 31% of the total annual rainfall of the west coast and east coast region of Peninsular Malaysia, respectively (Wong *et al.*, 2009). In term of rainfall characteristics, the west coast region of Peninsular Malaysia is greatly affected by the SWM where the northwest region considered as the wettest area during this season (Suhaila *et al.*, 2010).

2.1.2 Malaysia weather and climate

Malaysia's climate is described by uniform temperature (max. 33ºC, min. 23ºC), high humidity (70 – 90%), copious rainfall and usually light wind. Located in equatorial region, Malaysia is granted with abundant of sunlight with an average of six hours per day. The amount of sunlight and solar UVR are influenced by cloud cover which consequently affects temperature (MMD, 2017a). The flux of solar UVR maximum in March and September. During clear days, the solar UV radiation is high or extreme for about 5 hour, starting at 10.30 a.m. throughout the year (Ilyas *et al.*, 1999).

As maritime country, a full day with completely clear sky is extremely rare even during severe drought but during the days with clear skies, the effects of land and sea breeze on the general wind flow pattern is very marked (MMD, 2017a). The rainfall distribution of the country is determined by seasonal wind direction together with local topographic characters. The country experience more than 170 rainy days with rainfall commonly in the afternoon or early evening (Azhari *et al.*, 2008). A total of 81% of the mean annual rainfall is originated from monsoon rainfall (Wong *et al.*, 2009).

2.2 Overview of vitamin D

Vitamin D exists in nature in two major forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is made by invertebrates, some plants and fungi in response to UV radiation (Nair and Maseeh, 2012). It synthetically prepared from radiating a compound (ergosterol) from the mold ergot or from UV treated mushrooms (Moyad, 2009). According to Houghton and Vieth (2006), vitamin D2 was first produced and patented in the early 1920s by exposing foods to UV exposure and since then it has been licensed to pharmaceutical companies for use in prescription vitamins. Vitamin D3 is the most "natural" form of vitamin D. It is synthesized in the skin following exposure to ultraviolet B radiation (UVB, spectrum 280 to 315 nm) (Chen *et al.*, 2007). Since it can be formed in the body by direct sunlight, Vitamin D is often referred to as the 'sunshine vitamin'.

2.2.1 Sources of vitamin D

Vitamin D may be obtained from two sources: sunlight and dietary intake. Sunlight is the best and major source of vitamin D. Cutaneous synthesis of vitamin D involving UVB radiation which naturally initiates the conversion of 7-dehydrocholesterol (7-DHC) in the skin to vitamin D3 (Chen *et al.*, 2007). Sunlight induced vitamin D synthesis largely influenced by season, time of day, duration of exposure, latitude, air pollution, skin pigmentation, sunscreen use, aging (Babaria and Watson, 2013; Nair and Maseeh, 2012) and passing through glass and plastic (Wacker and Holick, 2013). According to Holick (2001), exposure of hands, arms, and face to a half of a minimal erythema dose (1MED, time for skin to get a light pinkness or develop a mild sunburn 24 hours after exposure) between the hours of 10 a.m. to 3 p.m.

for two to three times a week is more than adequate to satisfy the body's requirement of vitamin D.

The dietary source of vitamin D include natural food, vitamin D fortified food and supplements. The combination of these dietary source of vitamin D reflects the 'total vitamin D intake' of individuals (IOM, 2011). The natural food sources of vitamin D are limited. Vitamin D2 is found only in yeast and mushroom. Vitamin D3 is primarily found in fish liver oils and fatty fish such as herring, tuna, salmon and mackerel. Small amounts of vitamin D are found in meat, offal, and egg yolks. Human milk and unfortified cow's milk are poor sources of vitamin D (Ovesen *et al.*, 2003; Schlenker, 2015). Vitamin D content of foods is given in Table 2.1.

Table 2.1 Vitamin D content of foods

Food	**Vitamin D ug/100 g (1ug = 40 IU)**
Poultry, Meat, Fish	
Fish, salmon, pink	10.9
Fish, mackerel, cooked	7.3
Fish, sardines, cooked	4.8
Egg, whole	2.0
Beef, liver	1.2
Fish, catfish, farmed	0.2
Beef, Meat	0.1
Chicken, Meat	0.1
Lamb, meat	0.1
Dairy	
Milk, cow fortified, low fat	1.3
Yogurt, fortified, low fat	1.2
Cheese, cheddar	1.0
Vegetables	
Mushroom, oyster	0.7
Potatoes, mashed	0.3

(Source: NCCFN, 2017)

It has become a practice in many countries to fortify certain frequently consumed foods with vitamin D in order to increase vitamin D in the diet. In the United

States (US) and Canada, vitamin D is routinely fortified in milk, baked goods, orange juices, cereals, yogurts and cheeses while in European countries, fortified margarine is the major dietary source of vitamin D (Holick, 2010a). In Malaysia, milk powder for children and adults are recently fortified with vitamin D on a voluntary basis by manufactures (Jan Mohamed, 2017). Vitamin D is also added in many formulated nutritional supplements as other possible source of Vitamin D. Both vitamin D2 and vitamin D3 are use in foods fortification and supplement products. Multivitamin supplements often contain 400 IU (10 mg) vitamin D, and pharmaceutical preparations of vitamin D contain as much as 50,000 IU (1250 mg) vitamin D2 per capsule or tablet (Combs Jr and McClung, 2016).

2.2.2 Vitamin D metabolism

Figure 2.1 illustrates a basic metabolism of vitamin D in human. Upon exposure to UVB radiation, previtamin D_3 is synthesized by the photolytic cleavage of 7-DHC in the skin, primarily in epidermal keratinocytes. Then through thermal isomerization reactions, previtamin D_3 isomerizes to form vitamin D_3 (Dusso *et al.*, 2005; Holick *et al.*, 1980).

Cutaneously synthesized vitamin D_3 or vitamin D from the diet enters the systemic circulation bound to vitamin D-binding protein (DBP). Once in circulation, the inert vitamin D2 or vitamin D3 is converted to 25-hydroxyvitamin D (25(OH)D, calcidiol) by a hepatic 25-hydroxylase (CYP2R1, mitochondrial enzyme). The 25(OH)D is the main circulatory vitamin D metabolites, with a serum half-life is approximately 15 days (Keane *et al.*, 2017; Mostafa and Hegazy, 2015). The 25(OH)D is further hydroxylated by mitochondrial CYP27B1 (1α-hydroxylase) in the proximal tubule of the kidney producing 1,25-dihydroxyvitamin D (1,25(OH)D, calcitriol), the active hormonal form of vitamin D (Nair and Maseeh, 2012). This active 1,25(OH)D has a short serum half-life of approximately 6 hours (Keane *et al.*, 2017).

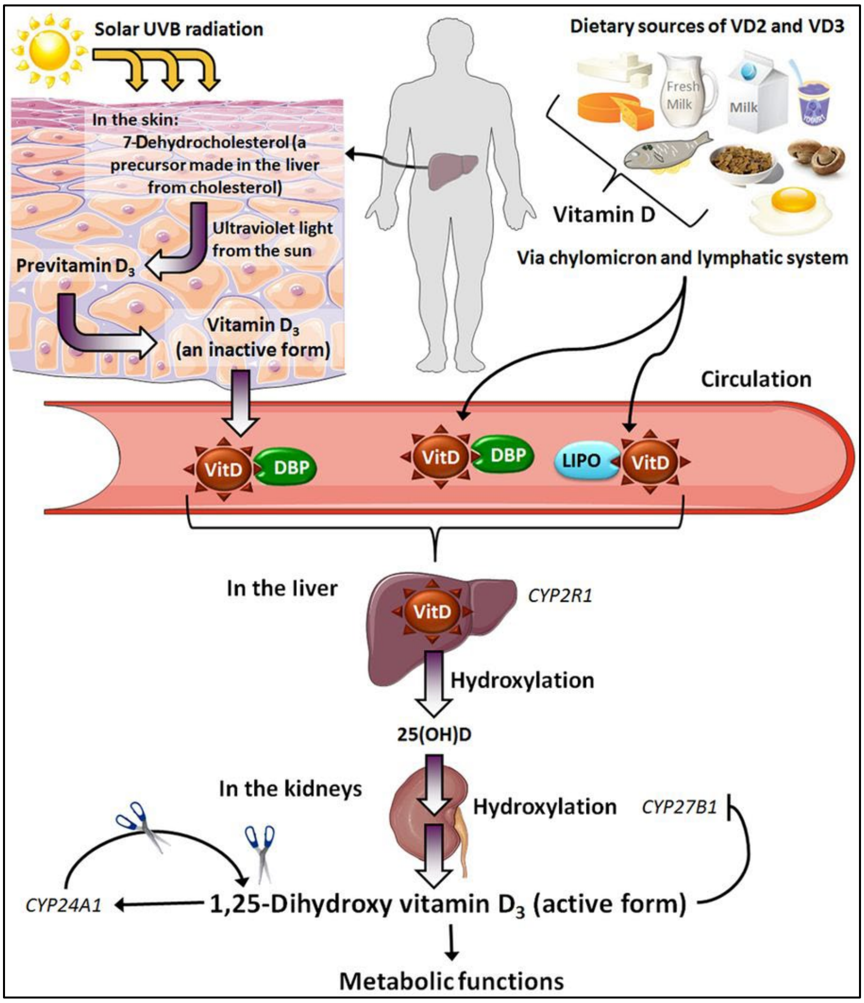

Figure 2.1 Vitamin D metabolism
(Source: Keane *et al.*, 2017)

The biologic actions of 1,25(OH)D are mediated by a soluble receptor protein termed the vitamin D receptor (VDR) (Dowd and MacDonald, 2010). The VDR binds 1,25(OH)D to form a hormone-receptor complex in target cells (Kreutz et al., 1993), which has its biological effect through gene transcriptions (Bikle, 2014; Dowd and MacDonald, 2010; Kreutz et al., 1993). To avoid toxicity, the 1,25(OH)D induces its own destruction (deactivation process) by transcriptionally promote CYP24A1 expression (24-hydroxylase) that catalyzed the conversion of 25(OH)D and 1,25(OH)D to calcitroic acid, an inactive water-soluble vitamin D metabolites (Jones *et al.*, 2012; Keane *et al.*, 2017).

2.2.3 Assessing vitamin D status

The status of vitamin D is evaluated by measuring the prohormone 25(OH) D, which is the most stable and abundant vitamin D metabolite (Thacher and Clarke, 2011). It is recognized as the optimal indicator of vitamin D status (Jassil et al., 2017). The circulating concentration of 25(OH)D reflects both UV exposure and dietary intake of vitamin D (Mostafa and Hegazy, 2015). Assay methods to measured 25(OH)D includes radioimmunoassay (RIA), electrochemiluminescence (ECL), ELISA, HPLC-UV, and HPLC-mass spectrometry (LC/MS). The non-HPLC methods typically detect both vitamin D2 and D3 and thus the results produce are referred to as ‘total’ 25(OH)D (Heaney, 2011).

Defining vitamin D deficiency or insufficiency based on 25(OH)D values is still debated. Although different opinions exist on how to classify vitamin D status, the vast majority of vitamin D researchers agree that 25(OH)D levels below 50 nmol/l (20 ng/ml) are insufficient (Zittermann and Gummert, 2010b). The US Institute of Medicine (IOM) has stated that 25(OH)D levels below 30 nmol/l (12 ng/ml) and between 30 - 50 nmol/l as deficient and insufficient, respectively. Meanwhile, the 25(OH)D levels at or above 50 nmol/l is defined as sufficient. This statement was set based on beneficial vitamin D effects with regard to prevention of rickets and/or symptomatic osteomalacia (IOM, 2011). In strong opposition to this classification, the US Endocrine Society Task Force has defined vitamin D deficiency as a serum 25(OH)D below 50 nmol/l while vitamin D sufficiency has set above 75 nmol/l (30 ng/ml). The recommendation is set higher in order to maximize the effect of vitamin D on calcium, bone and muscle metabolism (Holick *et al.*, 2011). Vitamin D toxicity occurs at 25(OH)D levels above 500 nmol/l (Kimball and Vieth, 2008).

2.2.4 Recommended nutrient intake for vitamin D

The recommended nutrient intake (RNI) for vitamin D is established to meet the body's needs when a person has inadequate exposure to sunlight, and the tolerable upper intake levels (UL) are set at those considered to pose no risk of adverse effects. The preferred units for quantification of vitamin D are micrograms (ug), in which 1 ug equals 40 IU of vitamin D (Gallagher, 2008).

The RNI of vitamin D for Malaysian adults, age 19 to 65 years is 15 ug (600 IU) per day with an assumption of minimal sunlight exposure (NCCFN, 2017). This recommendation for adults is considered based on the relationship between calcium absorption and vitamin D levels, in which serum vitamin D levels between 30 and 50 nmol/l were consistent with maximal calcium absorption (IOM, 2011). Except for infants (0-11 months) and elderly (> 65 years), the RNI of vitamin D for others age groups are also recommended at 15 ug/day. The amount of 10 ug and 20 ug vitamin D per day are recommended for infant and elderly, respectively (NCCFN, 2017). The UL for vitamin D for children and adults are 100 ug/day (4000 IU/day) while for infants is below 37.5 ug/day (IOM, 2011).

2.2.5 Recommended sunlight exposure for cutaneous vitamin D synthesis

Solar UV exposure may provide an alternative to vitamin D supplement for prevention and treatment of VDD. Among UV spectrum, only UVB (280-315 nm) can initiates cutaneous synthesis of vitamin D. Results from earlier studies found that sunbathing in swimsuit to 1MED (skin slightly pink) increased vitamin D equivalent to 10 000 to 20 000 IU vitamin D taken orally (Holick, 2001). It has been estimated that exposing arms and legs (about 25% of body surface area, BSA) to ¼ and ½ MED provides approximately 2000 to 4000 IU vitamin D.

From these findings, the 'Holick Formula' was developed and have been widely used as a reference to get the sensible amount of sunlight exposure. The formula requires individuals to expose 25% of BSA to 25% of 1MED two to three times a week throughout a year whenever the sunlight is available. The 1MED time is based on the individuals skin type, and for adults with skin type II (white skin), about 5 minutes are required to get 25% of 1MED. The amount of vitamin D produced from this formula equivalent to 1000 IU oral vitamin D (Dowdy *et al.*, 2010; Holick and Jenkins, 2009; Holick, 2010b).

Another recommendation for sunlight exposure was based on the "shadow rule". The body is able to make vitamin D when a person's shadow is less than the person's height (Holloway, 1992) which happen between 10 a.m. to 3.0 p.m. (Holick, 2010b). UV exposure is well known as a major risk factor for skin cancer. Thus, it is important to avoid excessive UV exposure, particularly getting sunburned (Mason and Reichrath, 2013). Safety precaution includes wearing sunglasses and sunscreen if planning to be in direct sunlight for extended periods of time.

2.3 Determinants of vitamin D status

2.3.1 Determinants of cutaneous vitamin D synthesis

Any factors that absorbs and prevent UVB radiation will reduce the cutaneous synthesis of vitamin D3. The determinants of cutaneous synthesis of vitamin D3 are divided into two types: (1) endogenous factors affecting UVB responsiveness and (2) exogenous factors affecting sunlight exposure. The endogenous factors include melanin pigmentation and skin thickness. Meanwhile, the exogenous factors include latitude, season, time of day, pollution, weather conditions and lifestyle such as sunscreen use, clothing, and indoor living. (Chen *et al.*, 2010; Combs Jr and McClung, 2016; Holick, 1995; Lips *et al.*, 2014).

a) Melanin pigmentation and skin thickness

Epidermal cells are mostly keratinocytes, with melanocytes in the basal layer producing melanin that determines pigmentation of the skin (Cichorek *et al.*, 2013). Melanin is an efficient natural sunscreen that effectively absorbs solar UV radiation from 280 to 700 nm, including UV-B radiation (280-315 nm). Therefore, melanin pigmentation can reduce the efficiency of previtamin D3 synthesis in the skin by competing for UV-B photons with 7-DHC which is substrate for making vitamin D (Chen *et al.*, 2010; Holick, 1995). Dark-skinned individuals have high amounts of melanin, thus require greater UV doses and longer UVR exposure to photosynthesize the equal amount of vitamin D3 than light-skinned individuals (Chen *et al.*, 2010; Clemens *et al.*, 1982).

It has been known, that aging causes various dermatologic changes. According to Tan *et al.* (1982), skin thickness decline linearly with age after the age of 20 years. This is corresponded with decreased in 7-DHC content in the epidermis. A comparison of the amount of previtamin D3 produced in the skin of the young subjects (8- and 18-year-old) with the amount produced in elderly (77- and 82-year-old) revealed that aging decreased the skin's capacity by more than twofold of previtamin D3 production. Thus, advancing age decreases 7-DHC due to skin thinness and markedly diminish the capacity for cutaneous vitamin D synthesis (MacLaughlin and Holick, 1985).

b) Latitude, season and time of day.

The solar zenith angle (SZA) is the angle between the vertical line at a place on earth (known as zenith) and the line joining the place to the sun (Figure 2.2). When the solar is directly overhead and on the horizon, the SZA is at 0º and 90º respectively. This angle is affected by change in latitude, season of the year and time of day (Chen *et al.*, 2010; Webb *et al.*, 1988).

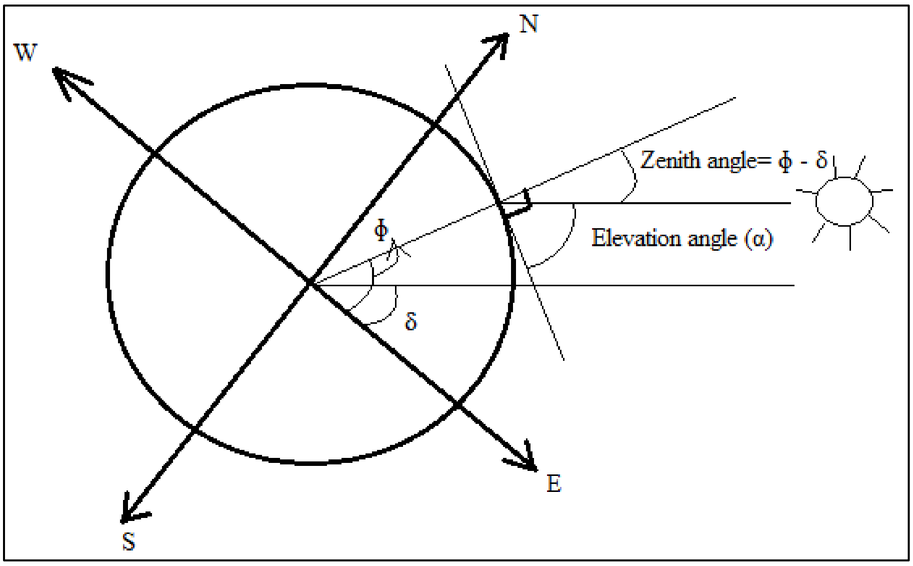

Figure 2.2 The solar zenith angle
(Source: Pal and Das, 2015)

An alteration in the SZA have a significant impact on the amount of UVB radiation available for cutaneous vitamin D synthesis (Engelsen, 2010). As the SZA increases or at a more oblique angle, the amount of UVB radiation reaching the earth's surface is decreased. This is because most of the UVB photons are absorbed by the stratospheric (10 to 50 km from earth) ozone layer (Holick, 2010b; Webb *et al.*, 1988). Thus, UVB radiation is the highest at noon (60% occurs between 10 a.m. and 3 p.m.), reaching an annual peak during the summer months, and declining with the distance from the earth's equator (Combs Jr and McClung, 2016; Holick, 2010b).